

"INVESTIGATIONS INTO THE INTERRELATIONSHIPS BETWEEN
POTATO ROOT EELWORMS AND FUNGI ON THE ROOTS OF
SOLANACEAE"

by

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ABSTRACT.

The association between the causal fungus of Brown Root Rot of tomatoes and Heterodera rostochiensis Woll. was investigated, and it was demonstrated that the nematode did not increase the susceptibility of the roots to invasion by the fungus (G.S.F.). However, a complex relationship was revealed in which G.S.F. was shown to have an inhibitory effect on the hatch of the potato root eelworm, the invasion of the host plant by the nematode, and the number of new cysts subsequently produced.

All six species of *Lycopersicon* examined were shown to be attacked to a greater or lesser extent by the pathotype of potato root eelworm used in these experiments; and *L. hirsutum* and *L. glandulosum* - species used for the breeding of tomato varieties resistant to Brown Root Rot - were shown to be affected by the fungal disease.

An investigation on the effect of applying methyl bromide to a soil containing a mixture of both pathogens showed that, while the fumigant controlled the potato root eelworm attack, it had no effect on the fungus; furthermore, if methyl bromide was not applied, the population build up of the nematode was still limited by some factor. This factor was postulated to be the fungus.

The experimental part of the thesis is divided into three sections, and each section is discussed separately. However, as the sections are inter-related, all three are again discussed at the end and conclusions are drawn.

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GENERAL INTRODUCTION.

In 1881 Kühn, in Germany, observed a cyst forming nematode, which he recorded as Heterodera schachtii, attacking potatoes. Strachan and Taylor (1926) recorded its occurrence in Yorkshire in 1904; however, the disease was not reported again in Europe until 1913, when Massee reported the nematode as a common parasite of the potato crop in Scotland. The Potato root eelworm is also a serious pest of tomatoes under glass, and was first recorded as such in 1928 in Yorkshire (Thompson 1949 & 1959).

It was Wollenweber who, in 1923, observed that the cysts from potatoes were pyriform instead of lemon-shaped, and the larvae were slightly shorter than those attacking sugar beet. Further investigations suggested that it was not H. schachtii. The specific name 'rostochiensis' which Wollenweber proposed, was not generally accepted, however, and the nematode continued to be known as H. schachtii. It was not until Franklin (1940) described well marked morphological differences, between the potato strain and the sugar beet strain of H. schachtii, that the name H. rostochiensis was restored.

The life history of potato root eelworm is as follows:-

The first larval moult takes place within the encysted egg (Hegemeyer 1951), thus it is the second stage larva that infects the host plant. These larvae leave the cyst, either through the mouth or vulval

openings, and move through the soil to the host rootlets entering close to the tip.

A very striking feature of the bionomics is the way the nematode responds to the presence of the growing host. Larval hatch is very small or non-existent when no host plant roots are growing in the vicinity; however, in the presence of the host, the larvae hatch in large numbers. Considerable evidence is presented in the literature supporting the view that the stimulus is produced by the host plant; the activating principle produced by the host is referred to as root diffusate.

The larvae penetrate the root epidermis by the use of their protrusible stylet, and production of enzymes, such as invertase, amylase and pectinase (Goffart and Heiling, 1962) in the salivary secretions, enable the larvae to pass through the cortex to the endodermis, leaving a track of disrupted tissue (Fassuliotis and Willey, 1954). They come to rest next to the endodermis lying parallel with the long axis of the root, and here undergo three more moults to the adult stage. During the development through the larval stages, the female becomes progressively more pear-shaped, and eventually the posterior end bursts through the cortex of the roots. Soon after the fourth and final moult the males leave the root and become free living, in order to find and fertilise a mature female; the males then pass into the soil and die. After

fertilization the ovaries of the female develop rapidly, and ultimately fill the swollen body; within four weeks fully embryonated eggs result. Meanwhile the female has died and the cuticular body wall gradually becomes tougher, and changes colour from white through golden brown to a rich chestnut brown. Ellenby (1946) has shown that these colour changes are caused by the enzyme polyphenol oxidase, and are similar to the browning and hardening of insect cuticle. However, unlike insect cuticle, the cyst wall has no outer waxy layer which would render it impermeable to water and root diffusate. This cyst is a tough inanimate sac containing several hundred living eggs.

In 1953, the economic loss caused by Heterodera rostochiensis on potatoes was estimated to be £2,000,000 (Peters 1953). Southey (1965) regards it as the most serious plant nematode pest of temperate regions as it attacks a staple food crop, is very easily disseminated by any process that involves movement of soil and, furthermore, is extremely persistent and difficult to control.

Allowing for differences in the plants and in growing conditions, the effects of potato root eelworm on tomatoes are similar to those on potatoes (Anon 1963). The main effect is growth retardation; the host plant is rendered more prone to wilting; and the lower leaves have a tendency to die back (Southey, 1965). Feldmesser (1952) working on tomatoes reported that, in the early stages of attack, the roots develop

small swellings similar to the slight galling caused by low infections of the root-knot nematode, Meloidogyne spp., an effect not noticeable on potatoes.

It would appear, from the work of Wille and Bazan de Segura (1952), Bazan de Segura (1953), Bell and Segundo Alandia (1955), and Brücher (1960), that the nematode, H. rostochiensis Woll., occurs throughout the Andes in Argentina, Bolivia and Peru, where its chief host plants are also indigenous. Thus there is circumstantial evidence to suggest that the parasite was introduced when potatoes were brought to Europe in the early 17th century, and possibly again in the early 19th century when tomato culture was first regarded favourably (Sabine, 1822).

The presence on tomato roots of a sterile fungus with a mycelium which was white to grey in colour, was first recorded by Williams (1929); however he did not identify it as the fungus which produces brown root rot symptoms on the host. Previously Shapovalov (1925) and Johnson et al. (1926) had noted a brown root rot of tomatoes which they could not attribute to any one fungus. Subsequently Johnson (1939) made further studies on the nature of brown root rot on Tobacco and tomato, the symptoms of which were similar to those recognised for Tomato Brown Root Rot today. He reported that no single organism could be found constantly associated with the disease. Richardson and Berkeley (1944), in Canada, isolated cultures of a sterile grey fungus from what they called Basal Rot

of tomatoes. They found that they could reproduce the symptoms of rot in soil inoculation experiments with these cultures. They reported that this unidentified organism, took 3 to 4 weeks to cover the surface of a 10 cm. Petri dish of Potato dextrose agar. Their description of the cultures obtained was very similar to that reported in 1929 by Williams. Gram (1944) stated that Brown Root Rot was a glasshouse tomato disorder of obscure etiology, characterised in the early stages by a brown zonate discoloration of the fibrous roots finally involving the whole root system; and thus paves the way for decay by facultative fungal and bacterial parasites. He noted that the premature destruction of the roots cuts off the water supply to the aerial parts, resulting in poor development, wilting, and a reduction in yield to a third or even a half of normal. Further work by Williams in association with Ebben (1950, 1951 & 1952) led them to state that Colletotrichum atramentarium (B & Br.) Toub., was the causal fungus of Brown Root Rot. However, they found that, when tomato plants were inoculated with Colletotrichum atramentarium used in combination with Chaetomium cochlioides and the grey sterile fungus (Fungus K) - the latter two fungi also being isolates from the diseased roots - the damage to the root was greater than when Colletotrichum was used alone. Nevertheless, it was thought that the grey sterile fungus in diseased root tissue was present only as a secondary saprophyte. Roll-Hansen (1952) reported that the most

troublesome soil-borne tomato disease in Norway was Brown Root Rot. He thought that the fungal damage was secondary to some other cause, which had not been elucidated. In 1954 Thung reported that 'corky root' — synonymous with Brown Root Rot — caused severe injury to the tomato plant; the disease was thought to be soil borne virus and belonged, he believed, to the tobacco necrosis group, damage occurring only when the virus invaded the root through a wound. Strömme (1954) believed the disease might be caused by Cylindrocarpon radicicola Wollenw. Ebben & Williams (1956) reaffirmed that Colletotrichum atramentarium was the primary pathogen causing Brown Root Rot, though they noted that a succession of fungi appeared to play a part in the disease syndrome. In 1957, however, Noordam, Termohlen and Thung reported that they had produced the symptoms of Brown Root Rot in inoculation experiments with the grey sterile fungus (G.S.F.), and in 1958 Termohlen presented further proof of G.S.F.'s pathogenicity. From recent observations on the Brown Root Rot complex on tomatoes, Last and Ebben (1963) support the view of Termohlen (1962) that a fungus, which usually produces a grey sterile mycelium only in culture, is responsible for causing the Brown Root Rot symptoms, and Colletotrichum atramentarium, is an invader of old tissues and therefore is much less important than previously recorded.

There are wide differences in the pathogenicity of isolates of G.S.F., and some produce microsclerotia in culture (Termohlen 1962).

However, Preece (1964) would appear to be in agreement with Termohlen's observations that the ability of the fungus to produce microsclerotia bears no relation to its pathogenicity. Last and Ebben (1963) note that certain isolates of the grey sterile mycelium made by Ebben and Williams (1956) were, apart from the absence of pycnidia, morphologically similar to a Pyrenochaeta sp. also isolated from corky roots. Further to this, Gerlach and Schneider (1964) have found that certain isolates from corky roots produce pycnidia on tomato roots and were, in culture indistinguishable from Pyrenochaeta terrestris.

Termohlen (1962) observed that the fungus, causing Brown Root Rot penetrates straight into the epidermal cells and grows into deeper cell layers, both intra- and inter-cellularly. The resultant necrosis in the epidermis leads to an inability of the cells to keep pace with the secondary thickening of the root, thus causing cracks to develop in the infected cortical tissue, which subsequently becomes suberized.

The symptoms of Brown Root Rot are as follows:-

- a) Small brown cortical lesions on young or thin roots which lead to their dying back.
- b) Larger lesions on medium sized laterals which often girdle the entire root causing the cortex to separate from the central cylinder.
- c) A suberisation of larger roots characterised by the development of brown furrowed bark.

- d) The development of lesions at the base of the stem sometimes completely girdling it and causing a basal stem rot.

The effect of Brown Root Rot on the host plant, tomato, is to cause increasing debility throughout the season, though rarely killing the plant. The infected plants are usually shorter and, during sunny weather, have a tendency to wilt - indicative of a poor root system. The lower leaves are shed earlier and, in the case of a heavy infection, flower drop occurs. The eventual yield can be decreased by as much as 75% (Van de Linden 1951; Roll-Hansen 1952; Hainsworth & Lloyd 1958).

Morgan (1925, 1926) and Morgan and Peters (1929) reported the presence of Rhizoctonia solani Kühn, an imperfect stage of Corticium solani, (Prill & Dal) Bourd & Galz. together with Heterodera rostochiensis (then H. schachtii) in a crop suffering from 'Potato sickness'. Miles (1930) noted the presence of Colletotrichum tabificum on the roots of potato suffering from this pathological condition known as 'Potato sickness'. It was Triffitt (1931) who was the first to report a definite association between the potato root eelworm, Rhizoctonia solani and Colletotrichum tabificum. Millard et al. (1932), from a series of pot experiments involving the two fungal pathogens noted above and Heterodera, showed that the disease incidence was increased by the addition of both these fungi to a soil already containing potato root eelworms; however, they concluded that the nematode pathogen was the primary cause of 'Potato sickness'. More recently Koltermann (1953) registered his doubt as to

whether 'Potato sickness' was due entirely to Heterodera rostochiensis. With inocula of Rhizoctonia solani and Heterodera rostochiensis, Grainger and Clark (1963) obtained a reduction in potato tuber yield only when the pathogens were added in combination and not singly. Dunn and Hughes (1964) investigated the interrelationship of potato root eelworms, Rhizoctonia solani and Colletotrichum atramentarium on the growth of tomato plants. They found that combined inoculation with Heterodera rostochiensis and Rhizoctonia solani, Colletotrichum atramentarium and Rhizoctonia, and all three pathogens together checked growth significantly. Inoculation with Rhizoctonia alone only caused a slight growth reduction; but Heterodera, at the low level of inoculum used, alone or with Colletotrichum, or the latter pathogen alone did not.

The genera, Meloidogyne Goeldi 1887 and Heterodera Schmidt 1871, belong to the same family, Heteroderidae Skarbilovich 1947; however, the associations of many members of the former genus with both fungi and bacteria causing plant diseases have been extensively reported, whilst, it can be seen from the literature survey above, similar information about Heterodera is scant. Nevertheless, it must be recognised, as Steiner pointed out in his paper in 1953, that soil borne diseases are, in most cases, of complex character, and that nematodes frequently are members of such complexes. Therefore, nematodes in relation to plants should no longer be considered exclusively as single or detached entities, but as

members of a complex biotic community. In such a light the present work, on potato root eelworms and grey sterile fungus (G.S.F.), common pathogenic concomitants in the soil of commercial greenhouses used for tomato production, was initiated.

Section A.TECHNIQUES AND BIOLOGICAL MATERIAL.1. Source of Cyst Inoculum.

The cysts, used in the first series of pot experiments, were isolated from the soil of a field plot at Ashurst. For the second series, pot raised cysts were used, and were obtained from the treatments that had received a cyst inoculum only in the first series of experiments.

(a) Recovery of cysts from the soil.

Fenwick's method (1940) of water flotation was used for the separation of cysts from air-dried soil; and the hand-rolling technique (Goodey 1963) was used to separate the cysts from the dried 'float' collected on bolting cloth. Perspex trays (Johnson 1957) were used for counting the cysts.

(b) Determination of egg content.

The egg content of the cysts was determined by the technique used by Reid (1955). The crushed cysts were washed from the channelled metal slide to a macerator bottle. The blades of the macerator were replaced by rubber flails (Bijloo 1954). The suspension was then agitated for a $\frac{1}{2}$ minute, in three bursts of 10 seconds each, thus ensuring that most of the eggs and larvae were separated from the cyst walls (Goodey 1963). The contents of the macerator bottle were then transferred to a measuring cylinder making sure that the rubber flails were

washed free of any adhering eggs or larvae. The volume was then adjusted so as to yield a count of between 100-200 eggs or larvae per c.c. (Jones 1955). The counts were done in 1 cc. perspex counting slides similar in design to Fenwick's (1951).

2. Sources of Fungus inoculum.

Tilehurst, Reading.

The fungus was isolated from young roots showing the first symptoms of Brown Root Rot (Last, personal communication); and such isolates were used for inocula in the first series of pot experiments (1964).

Glasshouse Crops Research Institute.

Culture number M105.

Sub-cultures from this isolate were used in all the experiments in the second series of pot experiments (1965).

(a) Isolation and culture of G.S.F.

Roots showing the small brown lesions characteristic of early Brown Root Rot were first cut into lengths of about 0.5 cm. and then surface sterilised by first immersing in 75% Ethyl Alcohol for a $\frac{1}{2}$ minute followed by 0.1% Mercuric chloride for one minute, and washed 5 times in sterile water in McCartney vials (Last, personal communication). The root pieces were then placed on plates of Malt Extract Agar (Oxoid CM60). As soon as G.S.F. appeared on the plate, it was sub-cultured onto further plates of agar. The pH of this medium was

approximately 5.4 caused by the abnormally low pH of the distilled water used to make up the agar. Fortunately such a pH is near the optimum for the growth of G.S.F. (Termohlen 1962).

(b) Media used in inoculation experiments.

i. Grain spawn (Last, personal communication)

50 grammes of a large grained wheat variety were soaked in 75 cc. of water in a 250 cc. Erlenmeyer flask. The flask was closed with a non-absorbent cotton-wool plug, and autoclaved for 30 minutes at 121°C. Three cubes of agar cut from a stock culture of G.S.F. were placed on the surface of the medium, and incubated at 25°C. Thereafter the Erlenmeyer flask was shaken to distribute the growing inoculum evenly. After 3-4 weeks it was clear that the fungus had a tendency to autolyse, and, therefore the culture period on this medium was never allowed to exceed $2\frac{1}{2}$ weeks.

ii. Liquid culture.

Many liquid media were tried initially but Malt Extract Broth (Oxoid CM57) was chosen because it was the easiest to prepare.

250 cc. Erlenmeyer flasks, closed with non-absorbent cotton-wool plugs and containing 30 cc. of malt extract broth, were autoclaved for 10 minutes at 115°C. Three cubes of malt extract agar cut from stock cultures of G.S.F. were 'sown' on the surface of the liquid medium, and then incubated for two weeks at 25°C.

To prepare a hyphal suspension of G.S.F., the liquid medium was first poured away and the mycelial mat washed 3 times in the conical flask with 60 cc. of sterile water. The fungus was then transferred to a sterile homogeniser bottle, and a further 60 cc. of sterile water were added after which the mycelial mat was rendered down at 14000 r.p.m. to a hyphal suspension by the use of an M.S.E. homogeniser, kept in an aseptic cabinet periodically sterilised by formaldehyde gas (Smith 1954). The resultant suspension was tested for contaminants by streaking a small quantity on the surface of a plate of malt extract agar. No secondary infections did, in fact, occur throughout the series of experiments using hyphal suspensions as inoculum.

iii. Culture on Vermiculite.

In a 250 cc. Erlenmeyer flask 20 grammes of Vermiculite (10-14 mesh/inch) were allowed to soak in 80 cc. of malt extract broth. The flask was closed with a plug and then autoclaved for 10 minutes at 115°C. The medium was inoculated as before and incubated at a temperature of 25°C. The culture was shaken daily to ensure that the fungus grew evenly throughout. At the end of two weeks the vermiculite was washed with sterile water to remove the liquid medium leaving the vermiculite and fungus only.

3. Preparation of the soil mixture for pot experiments.

Sieved loam and sand were mixed in the proportions 3:2 respectively. For the first series of pot experiments this potting medium was sterilised

in an autoclave at 121°C for 30 minutes. An electrically heated soil steriliser, in which the soil was heated for $1\frac{1}{4}$ hours at a temperature of 180°C, was used for the second series. After sterilization, the soil was spread thinly on a clean concrete floor and turned daily to aerate it. This was done to free the mixture of accumulated ammonia, which otherwise inhibits the hatch of the nematode (Johnson and Townsend 1949).

4. Production of Tomato seedlings for pot experiments.

Seeds were sown in polystyrene boxes of sterilised potting mixture (3 loam:2 sand and 1 pulverised peat), and grown until one pair of true leaves had been produced. They were then transferred singly to small plastic pots ($2\frac{1}{2}$ " diameter x $2\frac{1}{2}$ " deep) filled with sterilised loam/sand mixture (3:2) plus fertiliser (Tomorite) and after 5 weeks were ready to be transplanted.

5. Inoculation methods for both pathogens in pot experiments.

(a) The Nematode.

Cysts were first graded by sieving to the following size ranges:-

235 μ : 235-422 μ : 422-599 μ : $> 599 \mu$.

In the first series of pot experiments (1964), the size grade of cysts used for inoculum was 235-599 μ containing 69 eggs per cyst; in the second series (1965) all experimental inoculations were made with

cysts 422-599 μ grade containing 160 eggs per cyst.

The number of cysts added to a pot was varied to give particular inocula in terms of eggs per gramme of soil (e/g).

In certain pot experiments an egg suspension was added : the eggs were extracted from the cysts by the technique described above, and the final volume of egg suspension was adjusted to give a particular infestation per gramme of soil. The suspension was then poured on to the potting mixture and thoroughly mixed before planting.

(b) The Fungus.

G.S.F., when cultured on either the wheat grain spawn or vermiculate, was mixed thoroughly with the potting medium at a predetermined rate, before the tomato seedling was planted. When the fungus was raised in liquid culture it was incorporated with the potting medium as a hyphal suspension.

6. Apparatus and techniques used for the sterile culture of tomato seedlings.

Before surface sterilisation the seeds were first soaked in water for about 4 hours, and then transferred to a solid watch glass containing 'Milton' - a 1% sodium hypochlorite solution. Two drops of 'Triton X100' were added to the sterilant in the watch glass to act as a surfactant. The above technique was used because it was found that, if a stock solution of 'Milton' and 'Triton X100' was made, the sodium hypochlorite was very quickly denatured. The seeds were left in this

Fig. 1.

Apparatus for surface sterilisation and
germination of tomato seed.



liquid for 4 minutes, and then washed twice in sterile water to prevent the delay in germination caused by the surface sterilant (Fig. 1).

The seeds were then transferred to plates of malt extract agar where they were allowed to germinate; any surface contaminant left on the seed soon became obvious here. An additional check on the surface sterility of the seeds was made by placing random samples in thioglycolate broth. When germination had taken place, usually in about 5 days, the seeds were aseptically planted in tubes of sterile sand and nutrient.

7. Surface sterilising potato root eelworm eggs and larvae.

The eggs and larvae were extracted from cysts as described above for the determination of egg contents. After the crushed cysts had been agitated for 30 seconds in the macerator, they were transferred to a 100 cc. beaker where they were stirred by a vibro-mixer. This ensured that most of the eggs were separated from the pieces of cyst wall; and subsequently the suspension was washed through a fine nylon screen (92 meshes/inch) to remove the pieces of cyst. The filtrate containing the eggs and larvae was collected in a 30 cc. centrifuge tube, and spun down at 1500 r.p.m. for 5 minutes. The volume of the suspension was reduced to 5 cc., and then transferred to a stoppered 100 cc. Erlenmeyer flask containing 25 cc. of Hydrogen peroxide - 20 volumes (Fenwick 1956, Shepherd, personal communication). The suspension of eggs and larvae was agitated on a wrist action shaker for 2 hours; and then poured into

Fig. 2.

Apparatus for the extraction of Heterodera eggs
from cysts; and the surface sterilisation of the
eggs.



a capped, sterile polypropylene centrifuge tube, and spun down at 1500 r.p.m. for 5 minutes. The supernatant liquid was removed by a sterile siphon tube, leaving approximately 5 cc., to which 20 cc. of sterile water were added. The process was repeated, and the concentration of the suspension of surface sterile eggs and larvae was estimated. The suspension was then diluted with sterile water, so that 1 cc. would contain the required number of eggs and larvae to add to the culture tubes in which the tomato seedlings were growing. Tubes of thioglycollate broth were used to test the asepsis of the suspension (Fig 2)

8. Technique for the estimation of nematode attack.

The roots were preserved in Formalin-acetic-alcohol, and so the infested roots were carefully washed in water before putting them into boiling tubes containing 0.05% cotton blue in lactophenol kept at 100°C in a water bath.

After staining for 15 minutes, the contents of the tube were allowed to cool and then poured through a fine nylon screen. The stained roots, retained on the screen, were washed in water and differentiated in plain lactophenol for 12 hours. Pieces of root were squashed between two microscope slides for examination.

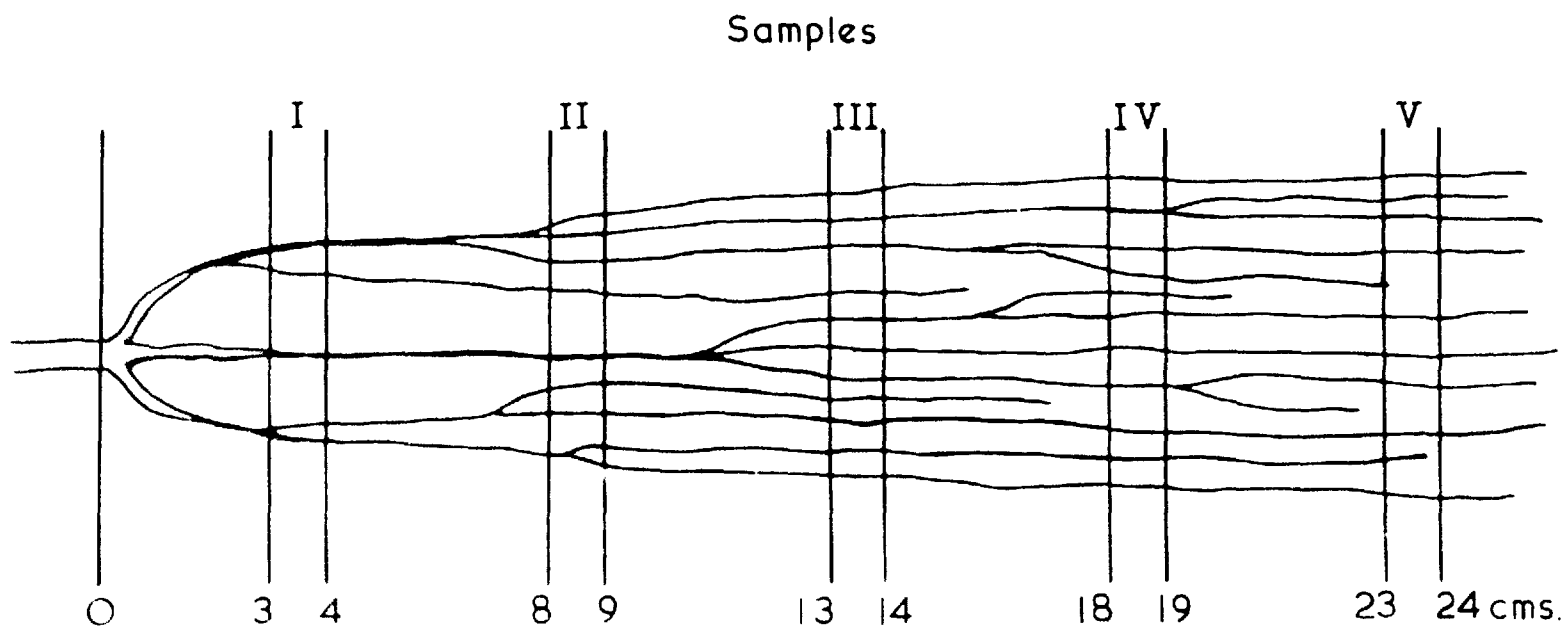
9. Techniques used for the estimation of fungal attack.(a) Visual score on the whole root system.

This is an arbitrary scoring system adapted from Key No. 10
(N.A.A.S. Conference of Plant Pathologists. Disease Assessment Committee)

Table 1.

Score	Percentage	Description of roots.
0.0 to 1.0	0-5%	Roots white. Small chocolate brown or corky patches stand out. Slight distortion on a few of the larger roots.
1.1 to 2.0	5-20%	Larger brown or corky lesions - the roots have a tendency to break at these points.
2.1 to 3.0	20-40%	Less than half the root surface affected. Stem lesions frequent.
3.1 to 4.0	40-60%	Clearly more than half the roots chocolate brown or corky. Marked deformity. Stem lesions.
4.1 to 5.0	60-80%	All the root system vaguely brownish. Stem lesions. Tendency for the cortex to separate from the central cylinder.
5.1 to 6.0	80-100%	All the roots chocolate brown or corky. Base of stem girdled by lesions. Gross distortion of root system - often very fragile. Cortex strips easily.

Fig. 3 Root Piece Technique



(b) Root piece technique (Last & Ebben 1963)

The root systems were carefully lifted and washed free of soil and other adhering debris, and laid on a cutting board. 1 cm. bands of roots were cut at various distances or depths from the level of the first lateral root (Fig. 3).

The root pieces at each depth were first separated into Clean roots and those with Brown Root Rot symptoms; they were then graded for size (0-1 mm., 1-2 mm., 2-4 mm., 4+ mm.); and the numbers in each size grade were recorded. The total fresh weight in the two symptom categories was recorded for each depth.

(c) The use of homogenised root tissues after surface sterilisation (Termohlen 1962).

The same technique was used for surface sterilisation as described above for the isolation of G.S.F. after which the roots were washed 3 times in 20 cc. of sterile water and eventually transferred to a small sterile masticator bottle containing 5 cc. of sterile water. The root material was then rendered to a suspension and a 1 cc. aliquot was pipetted into a petri dish. 20 cc. of malt extract agar and Rose Bengal (0.004%) were then poured at approximately 40°C, and the whole dish swirled carefully to mix the contents. The plate was then incubated at 25°C and the resultant G.S.F. colonies counted after 1 week.

10. The Treatment of plants in pot experiments.

(a) Growth records.

On transplanting the seedlings the height of the plant was recorded; thereafter, at weekly intervals, measurements were taken of the heights of the plants, and subsequently the cumulative extension growth for each plant was recorded.

(b) Yield records.

These were taken by weighing the ripe crop produced by each plant, and also including crop that had fallen prematurely. At the end of the experiment the remaining crop, both ripe and unripe, was weighed. Thus the total yield per plant was recorded.

(c) Post-cropping records.

The stem was cut at the level of the first lateral root, and the height and fresh weight of the aerial parts were recorded.

The entire ball of soil was carefully removed from the pot and soaked in a bucket of water, any debris overflowing was collected on a large sieve (60-100 mesh). The roots were separated from the soil and washed clean; the water in the bucket was then poured through the sieve. The wet soil, together with the debris collected on the sieve, was then transferred to a plastic plant pot and allowed to drain. No loss of soil occurred because the bottom of these pots were lined with filter paper. When this soil was dry and thoroughly mixed, the population of cysts in a 200 g. sample was estimated.

S E C T I O N B I .

Section B. I.EXPERIMENTS AND RESULTS.1a. The rate of invasion of the host plant by the grey sterile fungus.

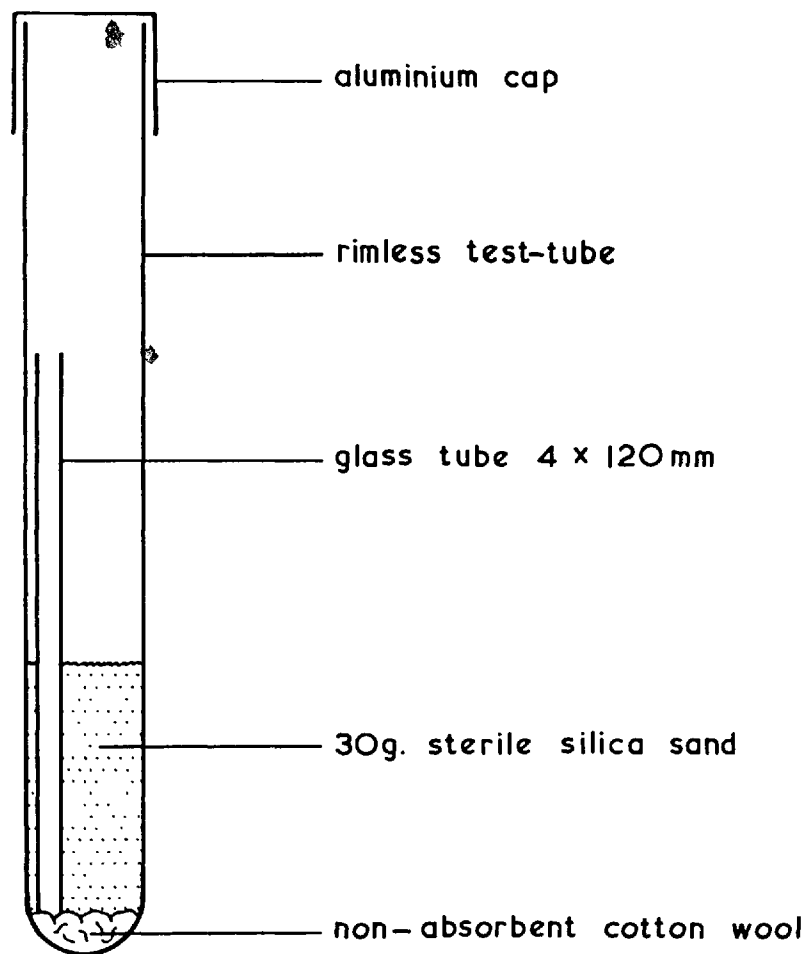
After preliminary investigations involving tomato plants grown in pots, it was decided to use plants grown under sterile conditions.

(i) The apparatus used for the sterile culture of tomato seedlings (Townshend 1963).

Non-absorbent cotton wool was put at the bottom of a 25 x 200 mm. test-tube, and a 4 x 120 mm. glass tube was placed vertically in the test-tube. 30 grammes of white silica sand were then added, and 5 cc. of nutrient solution (Hoagland and Arnon 1950) were pipetted into the tubes. The nitrogen in this nutrient solution was in the form of 'nitrate' not 'ammonium', because Johnson and Townshend (1949) showed that free ammonia in Ammonium carbonate inhibited hatch of Heterodera rostochiensis Woll., at 100 p.p.m. The culture-tubes (Fig. 4), closed with Oxoid aluminium caps, were autoclaved for 20 minutes at 121°C.

Tomato seeds, surface sterilised in a 1% solution of sodium hypochlorite (Section A, page 21) were germinated on plates of malt extract agar, and then transferred singly, in an inoculation chamber, to the culture tubes of sterile sand and nutrient solution and placed in the illuminated test-tube racks : growth was allowed to proceed for

Fig.4 Culture Tube



two weeks at a temperature of 20°C in a constant temperature room. Infestation with either the nematode or fungal pathogen was done in the inoculation chamber (Fig. 5).

(ii) The illuminated test-tube rack.

This apparatus (Fig. 6) was a shallow wooden box in the top of which holes were cut to fit the 25 mm. diameter culture tubes. The sand level in the culture tubes, was the same as the top of the box, and so light was excluded from the growing roots. The switches etc., for the six neon lights were placed at the front of the box; and a Venner time switch regulated the length of photoperiod to 16 hours (Nicolaisen 1962). As the red end of the spectrum is necessary for good growth of tomato plants (Vince, D. 1956; Dunn, S. & Went, F.W. 1959; De Lint, P.J.A.L. 1961), Philips "Warm White" (TL AK 40w/29) was the light source used, and each culture tube received approximately 630 foot candles.

The row of holes adjacent to the switchgear was left to allow the heat developed by the chokes to escape. The differences in temperature recorded by thermocouple readings both in and above the sand in the culture tubes, and also on the outside of the tubes was no greater than 1°C.

(iii) Experiment and Results.

Two G.S.F. mycelial mats were washed with sterile water and macerated (Section A, page 19), and 4 cc. of the hyphal suspension were

Fig. 5. Apparatus for the inoculation of pathogens into culture tubes.

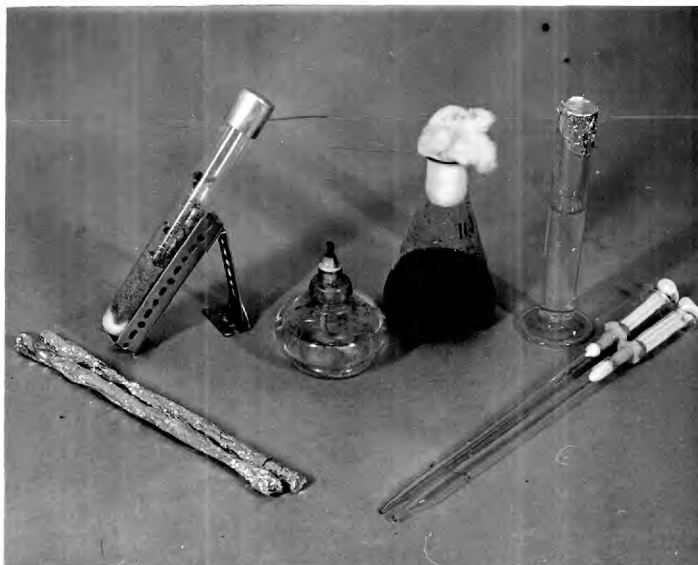


Fig. 6. Illuminated Test-Tube Rack.



added to the sand in the culture tubes. Samples were taken at 2; 4; 8; and 16 days after inoculation : replication was seven-fold.

The amount of the fungus in the roots was estimated by the technique of root homogenisation described in Section A (page 28), and the mean number of G.S.F. colonies isolated for each sample date is shown in Table 2.

Table 2.

(Mean of 7 replicates.)

Results.

Day	Mean no. of colonies
2	0
4	0.3
8	3.3
16	10.6

Symptoms of Brown Root Rot were first recorded on the eighth day.

Termohlen (1962) found that the rate of growth of G.S.F. was slow in sterilised soil; and the above results corroborate this observation.

The inter-replicate variation in the number of colonies isolated from the roots on each sampling date was high. This reveals a lack of sensitivity in the technique which can only be alleviated by increasing the replication i.e. the numbers of degrees of freedom for the residual term.

- 1b. To establish the time of attack of the host plant by the potato root eelworm.

In this experiment the illuminated "test-tube rack" was used. Tomato seedlings were grown for two weeks when nematode eggs (and/or larvae), surface sterilised with hydrogen peroxide using the technique described in Section A (page 23), were added to the tubes of sand at a rate of 40 e/g., (1200 eggs per culture tube).

Four times of sampling were chosen namely 2; 4; 8 and 16 days after the addition of the nematode to the sand; with seven-fold replication.

The roots were stained in 0.05% cotton blue in lactophenol as described in Section A (page 25), and the number of nematodes in each root system counted (Table 3).

Table 3. The mean number of nematodes per root system.

Results.

(Mean of 7 replicates)

Day	Mean no. of nematodes
2	0.4
4	4.0
8	9.3
16	26.9

Table 3 shows that the nematode entered the roots of tomatoes within two days.

1c. The effect of each of the pathogens upon the invasion by the other of the host plant roots.

(i) The influence of the nematode upon the rate of invasion by the fungus.

Seedlings of the tomato variety Eurocross A were grown in culture tubes for two weeks before inoculation with nematodes at a rate of 40 e/g. of sand. After the addition of the nematode to the culture tubes, the plants were left for a further fortnight before the fungus inoculum was added at a rate of 4 cc. of a hyphal suspension per tube.

The plants were then sampled 2; 4; 8; 16 and 32 days after the fungal inoculation, the roots of the seedlings were surface sterilised, and the attack of G.S.F. was estimated by the homogenised root technique described in Section A (page 28).

Table 4. Number of G.S.F. colonies isolated.

Results. (Mean of 5 replicates)

Day	Mean no. of colonies
2	0
4	0.6
8	3.8
16	15.2
32	21.0

Comparing the above table with Table 2 of Experiment 1a., it can be seen that the nematode does not influence the rate of entry of the fungus.

(ii) The influence of the fungus upon the time of entry of the nematode.

Again Eurocross A seedlings were grown in culture tubes standing in an illuminated "test-tube rack". After the two week establishment period for the seedlings, the G.S.F. inoculum was added at a rate of 4 cc. of hyphal suspension per tube; the plants were left for a further fortnight before the nematode inoculum was added at a rate of 40 e/g. of sand (1200 eggs per culture tube).

The plants were sampled 2; 4; 8; 16 and 32 days after inoculation, and the seedling roots were stained in 0.05% cotton blue in lactophenol (Section A, page 25).

Table 5. Mean number of nematodes per root system.

Results. (Mean of 5 replicates)

Day	Mean no. of nematodes
2	0
4	0.6
8	0.8
16	2.2
32	2.8

Comparing this table with Table 3, Experiment 1b., it can be seen that G.S.F. inoculum influences the rate of attack of the host plant roots by the Heterodera larvae.

These two experiments demonstrate that the time of entry of the fungus appears to be independent of the nematode, but the entry of the nematode is greatly influenced by the presence of G.S.F. in the roots.

2a. The effect of different levels of G.S.F. inoculum on the tomato plant.

The inoculum rates chosen were 0g; 0.5g; 1.0g; and 2.0g. of fungus inoculum, incubated on wheat grain spawn per 10g. of soil; the 1.0g. per 10g. rate was based on a recommendation by Last (personal communication).

The plants were grown for 4 weeks in size '54' pots, as it was thought that an adequate watering of the small five week-old seedlings, in pots large enough to take 7 kg. of soil, would be difficult. After this period they were transferred to the large pots, and additional G.S.F. inoculum was incorporated at the relevant rates with the extra soil necessary to fill the pots. The four treatments were replicated four times, and the pots were randomised for position on the greenhouse bench.

(i) Crop yield.

Results.

Table 6.

Weight of crop in grammes.

Treatment	Rep. I	Rep. II	Rep. III	Rep. IV
0g.	682	662	716	694
0.5g.	824	1013	1028	909
1.0g.	1272	889	808	1290
2.0g.	891	846*	655	1170

(* By 'Missing plot' technique - plant died)

An analysis of the above figures showed no significant difference between treatments.

(ii) Growth recording.

Weekly measurements of the heights of the plants were taken as described in Section A (page 29), and the cumulative increase in height over the initial was recorded for each treatment (Table 7).

These data could lead one to the spurious conclusion that plants infected with fungus grew better than the control plants. More recent experiments (Ketudat, personal communication) have shown that grain spawn has a manurial effect on the soil; and Preece (1964) has demonstrated that the inclusion of organic material, such as saw dust, grass clippings and compost with the inoculum causes suppression of Brown Root Rot symptom development. Nevertheless, the results show that G.S.F. has an effect on the extension growth in the first month, but by the ninth week all fungal treatments grew better than controls i.e. a manurial effect (Fig. 7).

(iii) Post cropping samples.

Increase in height over the initial, fresh weight of aerial parts, length and fresh weight of roots.

When all the plants were washed free of soil twenty two weeks after planting, the above mentioned measurements were taken and the data for increase in height and fresh weight of aerial parts showed that the plants in the presence of all 3 rates of fungal inoculum had grown better than the controls. No difference was revealed between treatments from

Table 7.

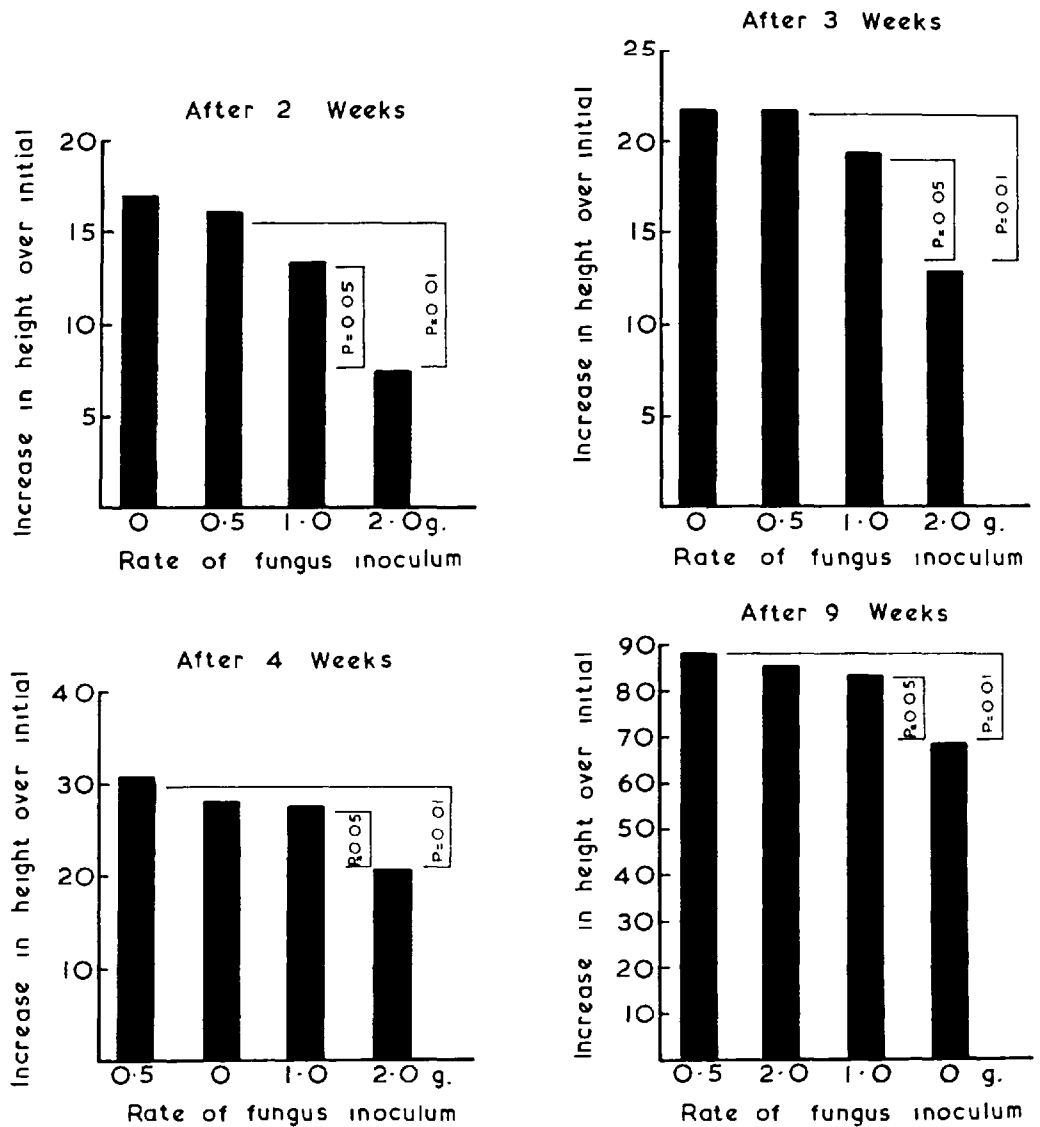
The mean height of the plants in each treatment measured weekly (in centimetres).

Rates of Fungus Inoculum.	<u>Weeks.</u>							
	1	2	3	4	5	6	7	8
0g/10g	7.4	17.0	21.7	28.1	29.1	37.6	44.6	53.1
0.5g/10g	7.7	16.1	21.7	30.9	35.2	43.2	56.6	68.5
1.0g/10g	7.0	13.4	19.4	27.6	31.2	43.7	53.6	67.0
2.0g/10g	3.6	7.5	12.9	20.7	20.7	39.6	51.8	68.2

10	11	12	13	14	15	16	17
74.5	81.7	85.0	87.9	89.5	91.6	94.4	94.5
94.7	103.6	105.9	110.0	112.0	113.4	115.7	116.7
90.9	97.2	103.5	111.7	114.1	117.5	122.9	124.9
88.0	94.0	99.6	108.8	111.1	113.1	117.8	118.4

Experiment 2a.

Fig 7 Cumulative Growth (Mean of 4)



the results for both the length and fresh weight of roots (Appendix Table 1).

Root fragment data.

No treatment difference was shown when the data of the total number of root pieces sampled from each treatment were examined. If the root fragments 0-1 mm. in diameter expressed as a percentage of the total were similarly examined no significant difference was demonstrable (Appendix Table 2). The size grade 0-1 mm. was chosen because such roots are of a size important to the nutrition of the plant, as they are feeder roots or at least feeder bearers.

Brown Root Rot.

Visual assessment of Brown Root Rot on the whole root systems.

The system described in Section A (page 26) was used, and revealed no difference between the treatments in which fungus inoculum was added to the soil (Table 8).

Table 8. Visual score of Brown Root Rot symptoms.

Treatment	Rep.	Score
0.5g.	I	2.7
"	II	3.1
"	III	2.7
"	IV	2.5
1.0g.	I	2.5
"	II	2.7
"	III	2.6
"	IV	2.7
2.0g.	I	2.7
"	II	Dead
"	III	2.3
"	IV	2.7

Percentage of root fragments with Brown Root Rot symptoms.

The differences that occurred between the means for each treatment were not shown to be significant (Table 9).

Table 9.

Treatments	Rep. I	Rep. II	Rep. III	Rep. IV	Mean
0.5g.	61.3	55.5	69.5	79.4	66.4
1.0g.	67.0	72.6	66.1	75.4	70.3
2.0g.	68.0	68.0	80.3	72.7	71.2

The results of this experiment are in agreement with the observations by Last (personal communication); it would appear that it is the early attacks of the G.S.F. that are damaging to the host plant.

In order that the increased nutritional status should not eclipse any effects on the plant ascribable to the fungus when wheat grain spawn was included with the soil, inoculations of pot experiments in the following year were done with the fungus cultured on Vermiculite (Section A, page 19).

2b. The effect of different levels of potato root eelworm inoculum on the tomato variety Eurocross A.

The inoculum rates chosen were 0; ~~33~~ 67 and 134 eggs and/or larvae per gramme of soil (e/g). The nematode was added as cysts to the sterilised soil, and the above rates corresponded to $\frac{1}{2}$, 1 and 2 cysts per gramme.

The plants were grown for 4 weeks in size '54' pots, and then were transferred to the larger pots with additional cyst inoculum. The replication was fourfold, and the pots were randomised for position on the greenhouse bench.

Results.

(i) Growth recording.

Weekly measurements of the heights of the plants were taken, and the cumulative increase in height over initial was recorded for each treatment (Table 10).

The effect of the nematode invasions on the extension growth of the host plant became statistically measurable in the first month. At the end of four weeks all treatments were significantly less than the controls; however, with time, the difference that occurred between the controls and those plants receiving ~~33~~ e/g. became smaller (Fig. 8). No significant difference occurred between the three rates of nematode inoculum at the end of the first month. By the sixteenth week the difference between the means of the treatment receiving ~~33~~ e/g and the other

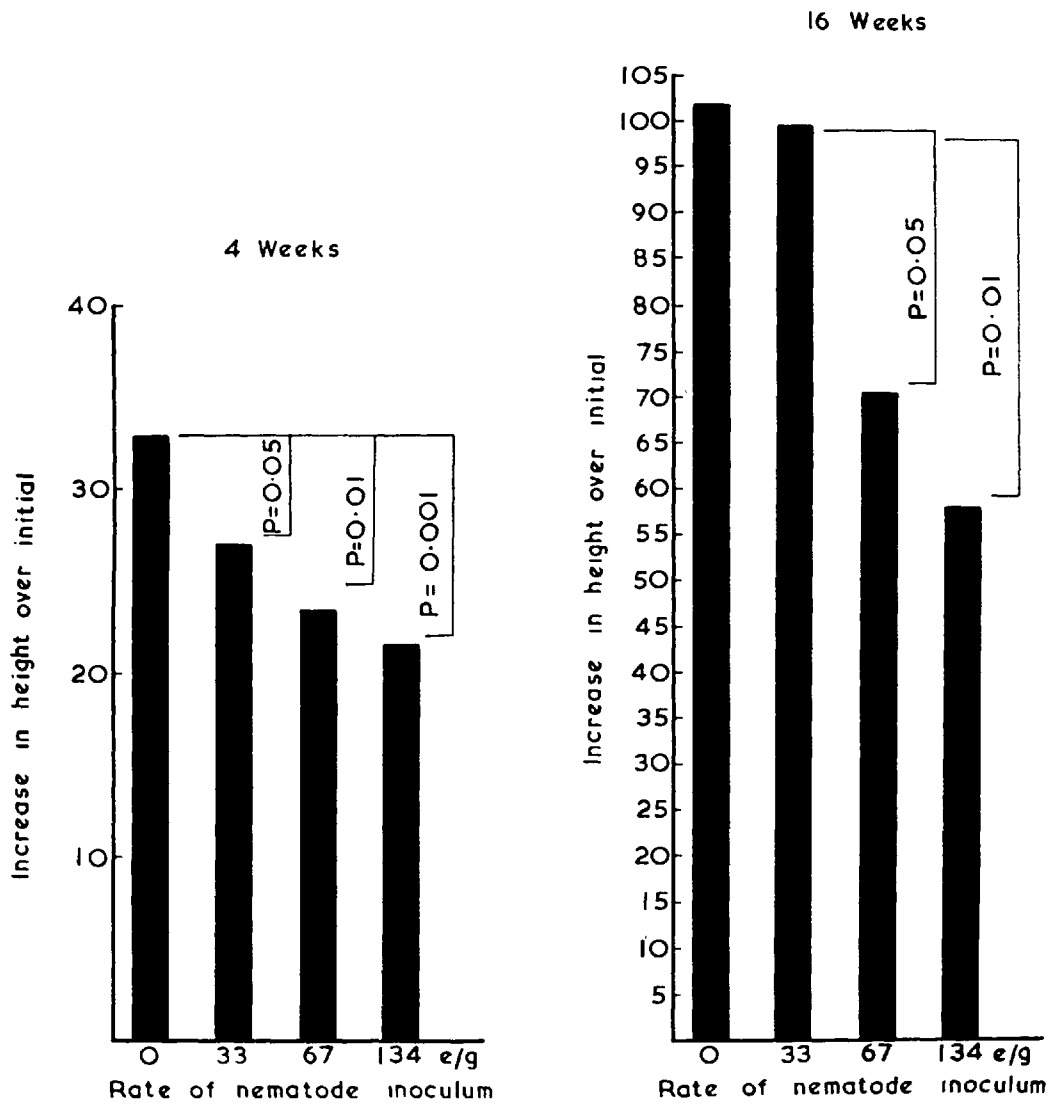
Table 10.

Mean height of plants in each treatment measured weekly
(in centimetres).

Rate of Nematode Inoculum.	<u>Weeks.</u>																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
0 e/g	9.0	18.5	27.0	32.9	35.4	42.7	51.9	61.6	78.5	84.4	96.0	97.9	99.1	99.4	100.0	101.9	105.7
34 e/g	8.9	17.7	22.7	27.0	29.0	37.4	47.5	59.2	74.6	82.1	90.5	93.6	95.6	96.6	97.2	99.5	101.1
67 e/g	9.2	16.4	19.9	23.4	24.9	25.4	27.9	34.7	48.0	53.5	60.7	65.6	66.4	68.0	68.9	70.5	70.9
134 e/g	9.2	14.4	17.0	21.5	22.2	24.6	26.9	30.2	36.2	39.2	47.5	54.4	57.2	57.5	57.7	58.0	59.0

Experiment 2b.

Fig 8 Cumulative Growth (Mean of 4)



two nematode treatments was within the 5% fiducial limits. It would appear from these results that a nematode inoculum rate of 30 e/g of soil has little effect on the host plant Eurocross A., when extension growth is considered; but an inoculum of over 60 e/g results in an obvious set back to host growth.

(ii) Yield.

The data for crop yield substantiate the last observation, but it would appear that the low rate of nematode inoculum (~~35~~ e/g) depresses yield, as the difference between the mean for this treatment and the control is almost significant at the 5% level (Fig. 9).

(iii) Post cropping samples.

Increase in height over the initial, and the fresh weight of aerial parts.

The data for both the above again substantiate the hypothesis that the level of nematode inoculum necessary to cause perceptible damage to the tomato variety Eurocross A is between 30 and 60 e/g of soil (Fig. 10).

Length and Fresh weight of roots.

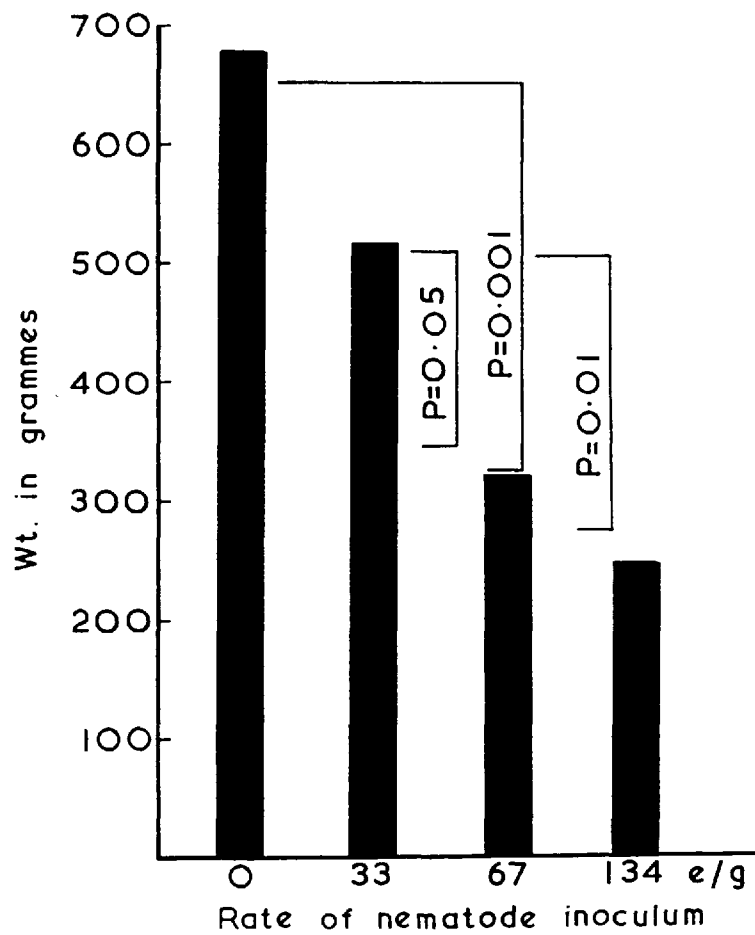
Measurements of the lengths of the root systems and their fresh weights revealed no significant differences (Appendix Table 3).

Root fragment data.

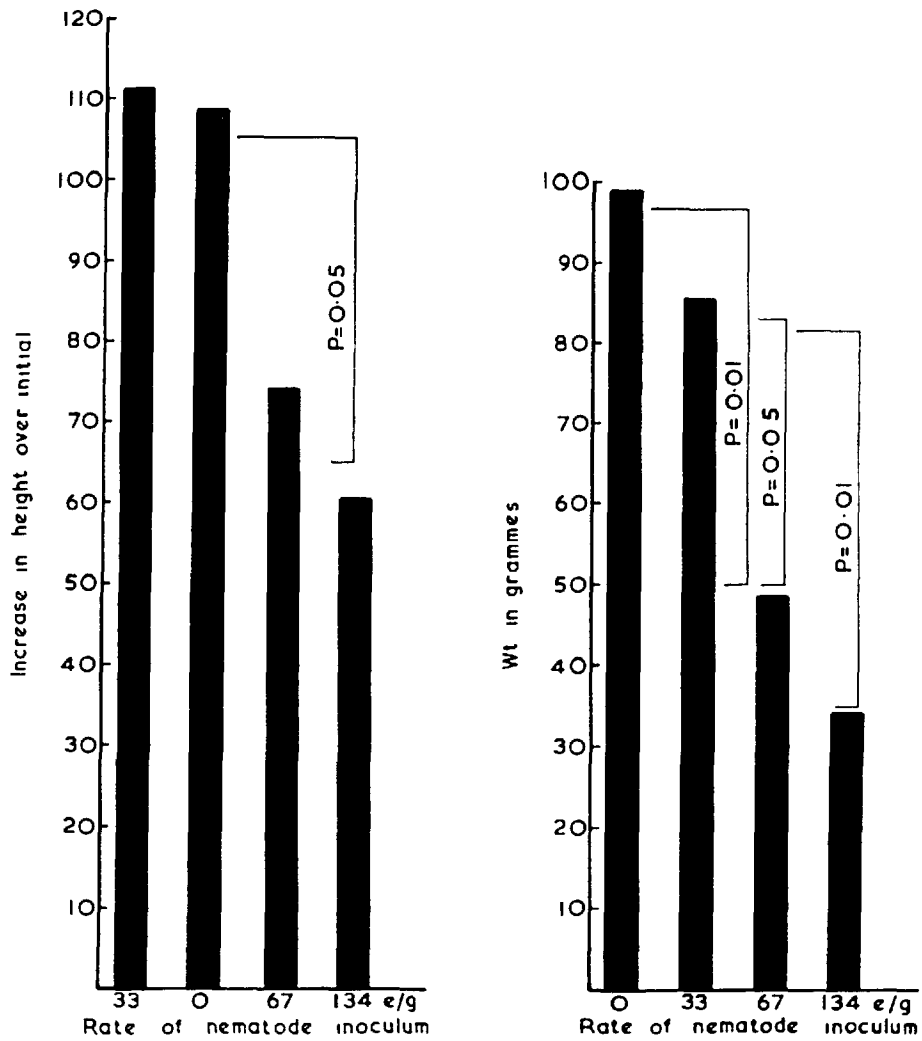
The total number of root pieces yielded by each of the plants showed that the plants growing in the presence of 134 e/g of soil

Experiment 2 b.

Fig.9 Yield (Mean of 4)



Experiment 2 b.

Fig. 10 Increase in Height Over Initial and Weight of Aerial Parts
(Mean of 4)

produced less root pieces than the controls ($P = 0.01$). Similarly there were significantly fewer root fragments from the plants receiving an inoculum of 67 e/g when compared to the controls ($P = 0.05$). The difference between the medium (67 e/g) and low (33 e/g) treatments was almost significant at this level (Fig. 11).

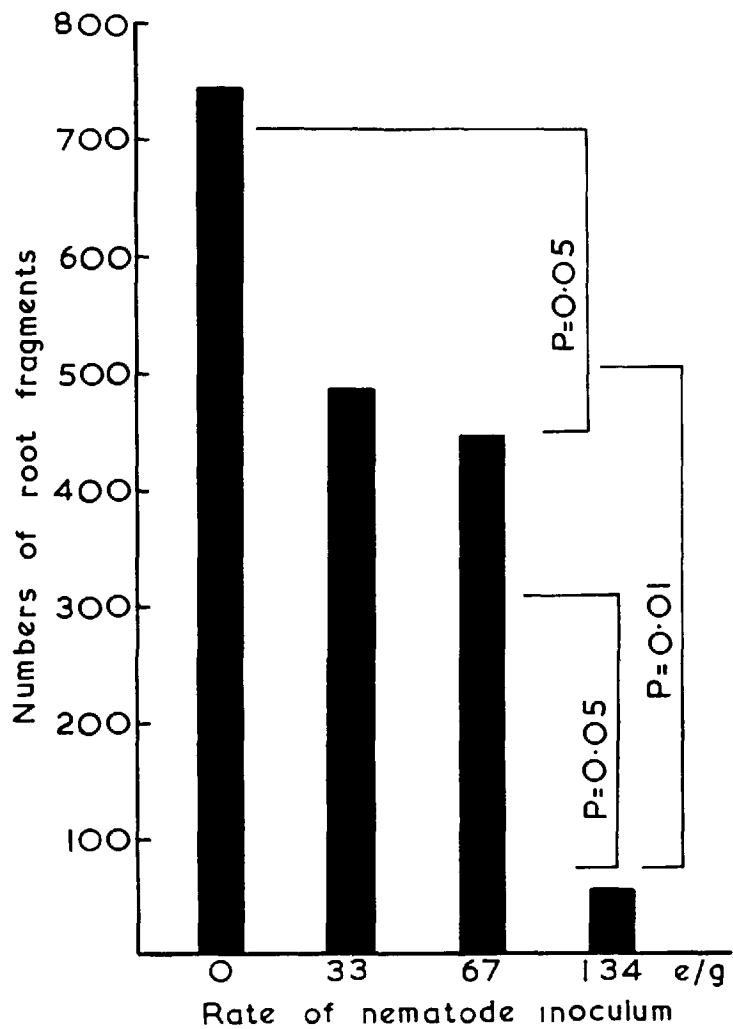
It is interesting to note that such a reduction in root numbers results in a parallel decrease in crop, demonstrating the pathogenic effect of the nematode.

Table 11. Final number of cysts per 200 g. of soil.
(Mean of 2 estimations)

Treatment	Initial cyst density/200g.	Final cyst density per 200 g.			
		Rep.I	Rep.II	Rep.III	Rep.IV
0.5 cysts /g.	100	773.5	496.5	656.5	785.0
1.0 cysts /g.	200	677.0	568.0	492.0	611.0
2.0 cysts /g.	400	927.5	760.0	646.5	578.0
Treatment	Log. FINAL - Log. INITIAL				Total.
	Rep.I	Rep.II	Rep.III	Rep.IV	
0.5 c/g.	0.89	0.70	0.82	0.89	3.30
1.0 c/g.	0.53	0.45	0.39	0.48	1.85
2.0 c/g.	0.36	0.28	0.21	0.16	1.01
	1.78	1.43	1.42	1.53	6.16

Experiment 2 b.

Fig.II Total Numbers of Root Fragments
(Mean of 4)



An analysis of the results in Table 11 shows that the multiplication rates for the 3 inoculum levels of the nematode are significantly different (Fig. 12), and also reveals that the lower the initial infestation of the nematode the higher is the multiplication rate.

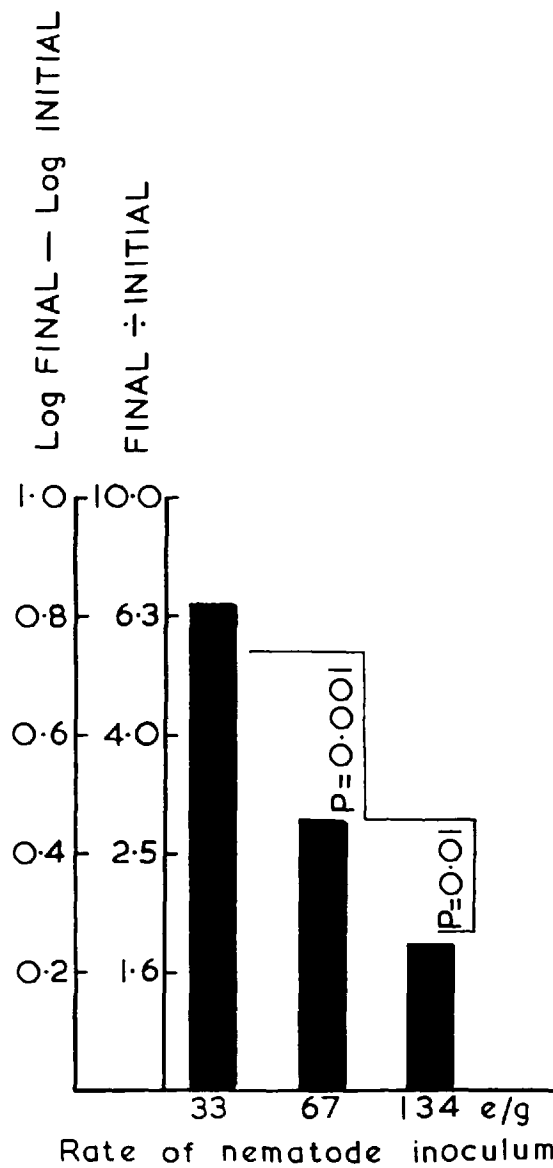
Table 12. Final number of eggs and larvae per gramme of soil.
 (Mean of 2 estimations)

	Initial egg density /g.	Final egg Density/g.			
		Rep. I	Rep. II	Rep. III	Rep. IV
	35	532.5	93.0	305.5	547.0
	67	434.5	291.5	269.5	567.5
	134	310.5	311.5	265.0	334.5

Treatment.	Log. FINAL - Log. INITIAL				Total
	Rep. I	Rep. II	Rep. III	Rep. IV	
35 e/g.	1.19	0.44	0.95	1.21	3.79
67 e/g.	0.81	0.64	0.60	0.93	2.98
134 e/g.	0.36	0.37	0.30	0.40	1.43
	2.36	1.45	1.85	2.54	8.20

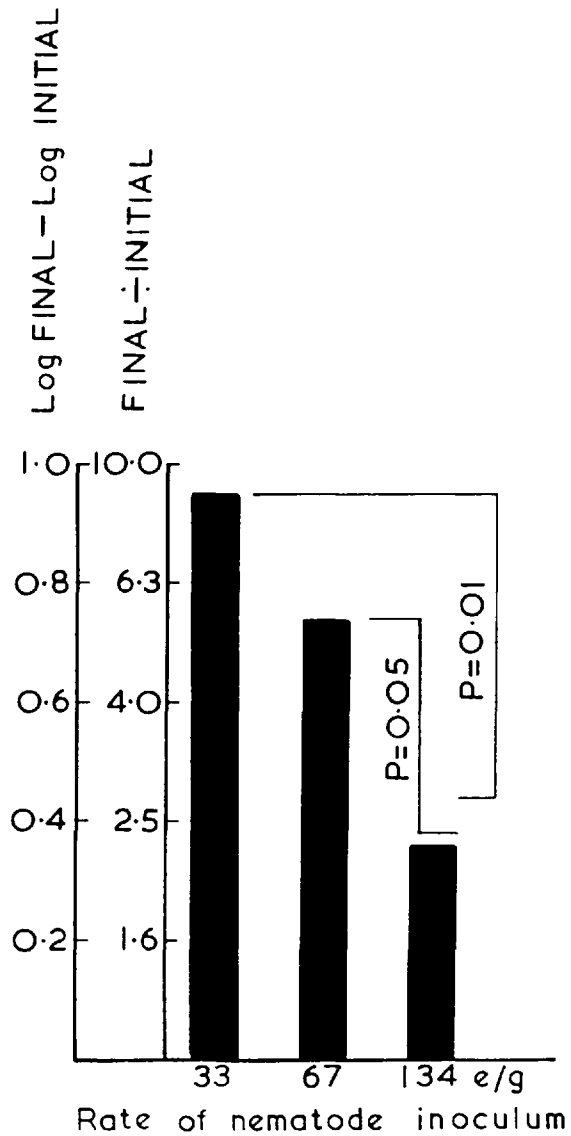
The results in Table 12 show that the multiplication rate for the highest level of inoculum of the nematode (134 e/g) was significantly lower than either of the other two rates (Fig. 13). The higher the

Experiment 2b

Fig 12 Log FINAL — Log INITIAL
Cysts /200g. (Mean of 4)

Experiment 2b

Fig 13 Log FINAL – Log INITIAL
Egg Density per g. (Mean of 4)



initial inoculum the nearer the ratio, Final/Initial egg density per gramme of soil, approaches unity (Jones, 1956; and Hague & Hesling, 1958).

From these observations it would appear that an inoculum rate of 30 e/g. of soil has little or no effect on the tomato variety Eurocross A, and it is not until the number of eggs per gramme of soil is increased to 60 that perceptible damage is caused to the host. Recent work by Palo has shown that the multiplication rate of Heterodera rostochiensis Woll. is much lower on tomato than on potato, indicating that it is not as good a host to the nematode as potato (personal communication).

3. To investigate the effects of different rates of fungus and nematode inocula separately upon the host plant grown aseptically.

The fungus a(i)

The range of inocula chosen was 0.5; 1.0; 2.0; and 4.0 cc. of hyphal suspension per culture tube. A complementary series of fungal inocula, which had been subsequently autoclaved for 10 minutes at 115°C in order to discover whether the pathogen produced any heat stable substance deleterious to plant growth, was added at a similar rate. The volume of liquid pipetted into each tube was made up to 4 cc. by the addition of sterile water, and a control was included to which only 4 cc. of sterile water was added. After inoculation the seedlings were allowed to grow for one month.

These nine treatments were replicated 4 times, and the culture tubes randomised for position.

Results.

At the end of the experiment the plants were carefully washed out of the tubes and the following records taken.

(i) Brown Root Rot assessment.

Two methods were used for the assessment of G.S.F. attack; they were the Visual scoring technique on the whole root system (Section A, page 26), and the isolation of G.S.F. colonies from homogenised root tissue (Section A, page 28).

Table 13.

Visual score of Brown Root Rot.

Treatments		Rep. I	Rep. II	Rep. III	Rep. IV	Total
Control		0.1	0.0	0.0	0.0	0.1
Auto Fung. Inoc.	0.5cc	0.0	0.0	0.0	0.0	0.0
	1.0cc	0.0	0.0	0.0	0.0	0.0
	2.0cc	0.0	0.0	0.0	0.0	0.0
	4.0cc	0.1	0.0	0.0	0.0	0.1
Fung. Inoc.	0.5cc	3.4	3.4	3.3	3.1	13.2
	1.0cc	3.5	3.1	2.5	3.2	12.3
	2.0cc	3.5	3.6	3.4	3.4	13.9
	4.0cc	5.4	3.1	3.6	3.8	15.9
		15.8	13.2	12.8	13.5	55.3

Table 13 shows that the range of fungal inoculum chosen was too high to give an increasing intensity of symptoms that could be assessed.

Isolation of G.S.F. colonies.

A 1cc. aliquot of homogenised root tissue from all the treatments was plated with malt extract agar and the number of colonies that developed in one week were counted (Table 14).

Table 14.

Treatment		Rep. I	Rep. II	Rep. III	Rep. IV
Fung. Inoc.	0.5cc	37	29	11	3
	1.0cc	46	1	4	10
	2.0cc	12	2	0	5
	4.0cc	32	16	22	67

No treatment produced significantly more G.S.F. colonies than any other. It would appear that the concentrations of hyphal suspensions used in this experiment were above the threshold value of the plant.

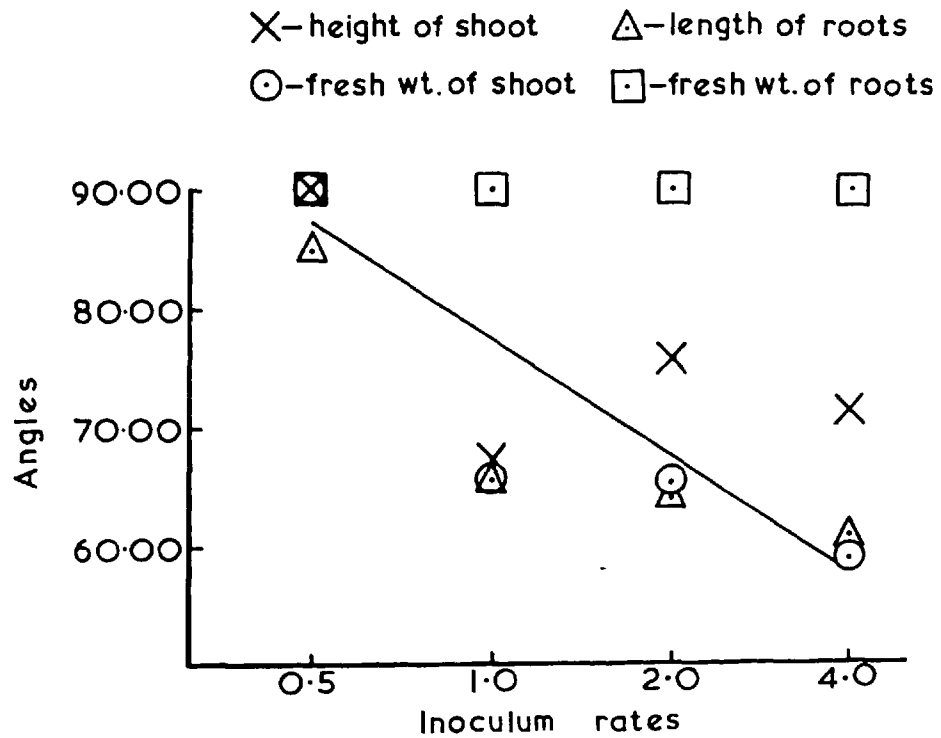
(ii) Height and fresh weight of shoot, and length and fresh weight of roots.

The above data are shown in Appendix Table 4. The mean values for each treatment were expressed as a percentage of their respective controls and then transformed to angles (Appendix Table 5).

From Fig. 14 and Fig. 15, it is clear that both the fungus inoculum and autoclaved fungus inoculum have some effect on growth factors; the shoots being more sensitive to the presence of both factors. Fig. 14 shows that the autoclaved fungus inoculum had no effect upon the fresh weight of roots. When the same four factors are considered for the fungus inoculum, one line can be drawn to fit 3 of them, but for

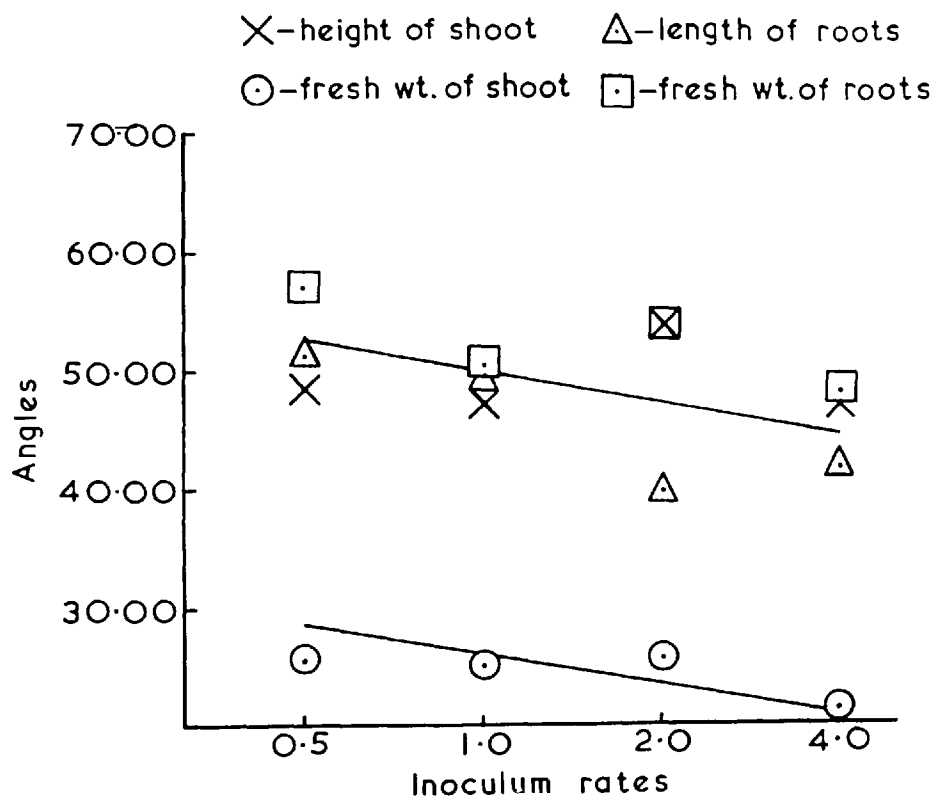
Experiment 3

Fig.14 Growth of Eurocross A Seedlings in the Presence of Autoclaved G.S.F Inoculum



Experiment 3

Fig.15 Growth of Eurocross A Seedlings in the Presence of G.S.F Inoculum



the fourth a separate line, of similar slope to the first, has to be fitted (Fig. 15). As the slope of these regression lines is small, no measurable difference can be shown between the increasing rates of fungus inoculum. The displacement of the two lines shows that the fungus inoculum had a greater depressant effect on the fresh weight of the shoot than on the other three factors.

3 a(ii) The effect of G.S.F. filtrates on the growth of the host plant tomato.

The G.S.F. filtrate was prepared by filtering a homogenised hyphal suspension through a sterile sintered glass Buchner funnel of pore size 1-1.5 μ . The filtrate was added to 12 two week-old seedlings growing in culture tubes at the following rates:- 0; 0.5; 1.0; and 4.0 cc.per tube. The volume of liquid added to each tube was made up to 4 cc. with sterile water. The replication of each treatment was three-fold.

Results.

At the end of four weeks the plants were washed from the tubes and measurements of the height and dry weight of the shoot, and length and fresh weight of the roots were taken (Table 15).

Table 15.

MEASUREMENT	Rate of Inoculum			
	0 cc.	0.5 cc.	1.0 cc.	4.0 cc.
Height (Shoot)	8.90 cm.	7.77 cm.	8.30 cm.	6.97 cm.
Dry wt. (Shoot)	8mg.	18mg.	13mg.	10mg.
Length (Root)	6.33 cm.	6.43 cm.	6.40 cm.	5.50 cm.
Fresh wt. (Root)	99mg.	109mg.	94mg.	103mg.

This data shows that the fungus filtrate, though having no effect on the dry weight of the shoot or the fresh weight of the roots, inhibits the other two factors - height of shoot and length of roots. The fungus filtrate caused no browning of the host plant roots.

3b. The Nematode.

The range of nematode inocula added was 0; 10; 20; 40; and 80 e/g in 1cc. of sterile water to the 30 g. of sand in the culture tubes. The replication was five-fold, and the treatments were fully randomised.

Results.

At the end of one month, the plants were washed from the culture tubes, and the following data was recorded.

- (i) Height, fresh weight and dry weight of shoots, and the length and fresh weight of roots.

The measurements of the above, shown in Appendix Table 6, showed no significant differences between the rates of nematode inoculum.

- (ii) Number of nematodes per root system.

The root systems were stained with 0.05% cotton blue in lactophenol and the number of nematodes counted (Table 16).

Table 16. Number of nematodes per root system.

Treatment	Rep. I	Rep. II	Rep. III	Rep. IV	Mean
0 e/g	0	0	0	0	0
10 e/g	9	11	13	11	11.0
20 e/g	12	19	22	15	17.0
40 e/g	13	19	24	18	18.5
80 e/g	17	23	27	12	19.7

The above table shows that the number of nematodes entering the roots increases as the inoculum level rose; conversely, the proportion of larvae in the roots falls with increasing inoculum rate (Table 17). These results corroborate those of Rao and Peachey (1965).

Table 17.

Number of eggs/tube	Proportion of inoculum in roots.	
300	3.9%	(Mean of 4)
600	2.8%	"
1200	1.5%	"
2400	0.8%	"

The amount of space available for shoot growth in the culture tubes limited the length of the experiment, and the results show that the one month allowed was not long enough for the pathogenic effects of the nematode parasite on the host to be exhibited.

4. A preliminary examination of the nematode and fungus complex.

This experiment was done to investigate whether tomato plants invaded by the nematode are predisposed to attacks by the fungus responsible for Brown Root Rot.

Two rates of inocula were chosen for each pathogen:-

The nematode 0; and 67 e/g of soil (N_0 and N_1 respectively).

The fungus 0; and 1g. of wheat grain spawn per 10 g. of soil (F_0 and F_1 respectively).

The plants were grown in size '54' earthenware pots for four weeks and then transferred to the larger pots with additional inoculum and soil.

The four treatments $N_0 F_0$, $N_1 F_0$, $N_0 F_1$ and $N_1 F_1$ were replicated four times, and the pots were placed in a factorial design on the greenhouse bench.

Results.

At the end of the experiment, when all the root systems were washed from the soil, a visual assessment of Brown Root Rot was made on the whole root system using the technique described in Section A (page 26).

(i) Table 18.

Visual Assessment of Brown Root Rot.

Treatment	Replicates	Score
$N_0 F_0$	I	0
$N_0 F_0$	II	0
$N_0 F_0$	III	0
$N_0 F_0$	IV	0
$N_1 F_0$	I	0
$N_1 F_0$	II	0
$N_1 F_0$	III	0
$N_1 F_0$	IV	0
$N_0 F_1$	I	2.5
$N_0 F_1$	II	3.2
$N_0 F_1$	III	2.5
$N_0 F_1$	IV	2.6

Table 18 cont.Visual Assessment of Brown Root Rot.

Treatment	Replicates	Score
N ₁ F ₁	I	2.6
N ₁ F ₁	II	2.8
N ₁ F ₁	III	2.4
N ₁ F ₁	IV	2.2

No difference can be demonstrated by this method between the plants in those treatments receiving fungus inoculum alone and those receiving both G.S.F. and potato root eelworm inocula.

An assessment of Brown Root Rot was made on the root fragments samples from the whole root system using the technique described in Section A (page 23)

(ii) Table 19. Percentage of root pieces showing Brown
Root Rot symptoms.

Treatment	Rep. I	Rep. II	Rep. III	Rep. IV	Mean
N ₀ F ₀	0	0	0	0	0
N ₁ F ₀	0	0	0	0	0
N ₀ F ₁	32.4	58.2	60.5	66.8	53.6
N ₁ F ₁	37.5	53.5	50.6	47.8	49.3

Again no difference can be shown between the two treatments $N_0 F_1$ and $N_1 F_1$. It would therefore appear that the invasion of the G.S.F. is independent of the nematode.

(iii) Cyst population.

The assessment of the cyst population in the pots was done by the method described in Section A (page 16).

Table 20.

Final Cyst Population per 200 g. soil (Mean of 2)					
Treatment	Initial Cyst Density	Final Cyst Density			
		Rep. I	Rep. II	Rep. III	Rep. IV
$N_1 F_0$	200	* 909.5	404.5	550.5	633.0
$N_1 F_1$	200	256.0	212.5	322.0	287.0

Treatment	Number of New Cysts/200 g.				Mean
	Rep. I	Rep. II	Rep. III	Rep. IV	
$N_1 F_0$	795.9	204.5	350.5	433.0	424.4
$N_1 F_1$	56.0	12.5	122.0	87.0	69.4

The number of new cysts produced at the end of the experiment is obtained from:-

$$\text{FINAL CYST DENSITY} - \text{INITIAL CYST DENSITY.}$$

It can be seen that the fungus inoculum has a great effect on the nematode.

(iv) Egg Density per gramme of soil.

The number of eggs per cyst was estimated using the technique described in Section A (page 16), and from this the final egg density per gramme of soil was calculated.

Table 21.

Final Population of eggs/g. of soil.					
Treatment	Initial Egg Density	Final Egg Density			
		Rep. I	Rep. II	Rep. III	Rep. IV
$N_1 F_0$	67	684	225	277	450
$N_1 F_1$	67	158	65	116	123
Treatment	Number of New eggs/g. of soil				Mean
	Rep. I	Rep. II	Rep. III	Rep. IV	
$N_1 F_0$	617	158	210	393	344.5
$N_1 F_1$	91	0	49	56	49.0

The fungus inoculum is shown to have had a depressant effect on the multiplication of the nematode. This is contrary to what would be expected if the wheat grain spawn merely increased the nutrient status of the soil. Ketudat (personal communication) found that the addition to the soil of wheat grain, on which no fungus had been cultured, also had an effect on the final number of Heterodera cysts. The multiplication rate of the nematode was decreased by approximately half. There could be two reasons for this, either the autoclaved wheat grain produces substances that are deleterious to the hatch of the nematode, or the addition of the wheat grain to the soil changes its structure and aggregation. Rode (1952) found that a change in soil type could have an effect on the migration of the nematode towards the host plant roots, and the subsequent number of cysts produced at the end of the season. However, the fungus inoculum in this experiment had a greater effect on the multiplication of the nematode than Ketudat found; it would appear, therefore, that G.S.F. influences in some way, the hatch or development of the nematode.

(v) Weekly Growth Recordings.

These data were recorded throughout the period of the experiment (Appendix Table 7). As Ketudat (personal communication) has shown, wheat grain spawn has a manurial effect when added to the soil, thus the effects of both pathogens on the host plant will be eclipsed to a

greater or lesser extent. The fungal pathogen was shown to have an effect on the extension growth of the tomato in the first month. This depression of growth by the fungal inoculum alone decreased in the second month; and eventually it was the nematode factor that had become important (Fig. 16).

There was a positive interaction between the two inocula factors in the third, fourth and fifth weeks at the 5% level of confidence.

Table 22.

(Mean of 4 observations)

3rd. WEEK	m N ₀	m N ₁	
m F ₀	24.50	20.25	22.37
m F ₁	12.62	19.37	16.00
	18.56	19.81	Total mean 19.19
4th WEEK	m N ₀	m N ₁	
m F ₀	33.00	24.75	28.87
m F ₁	19.37	25.87	22.62
	26.19	25.31	Total mean 25.75
5th WEEK	m N ₀	m N ₁	
m F ₀	35.37	27.62	31.50
m F ₁	24.75	30.75	27.75
	30.06	29.19	Total mean 29.62

Experiment 4

Fig 16 Cumulative Growth (Mean of 4)

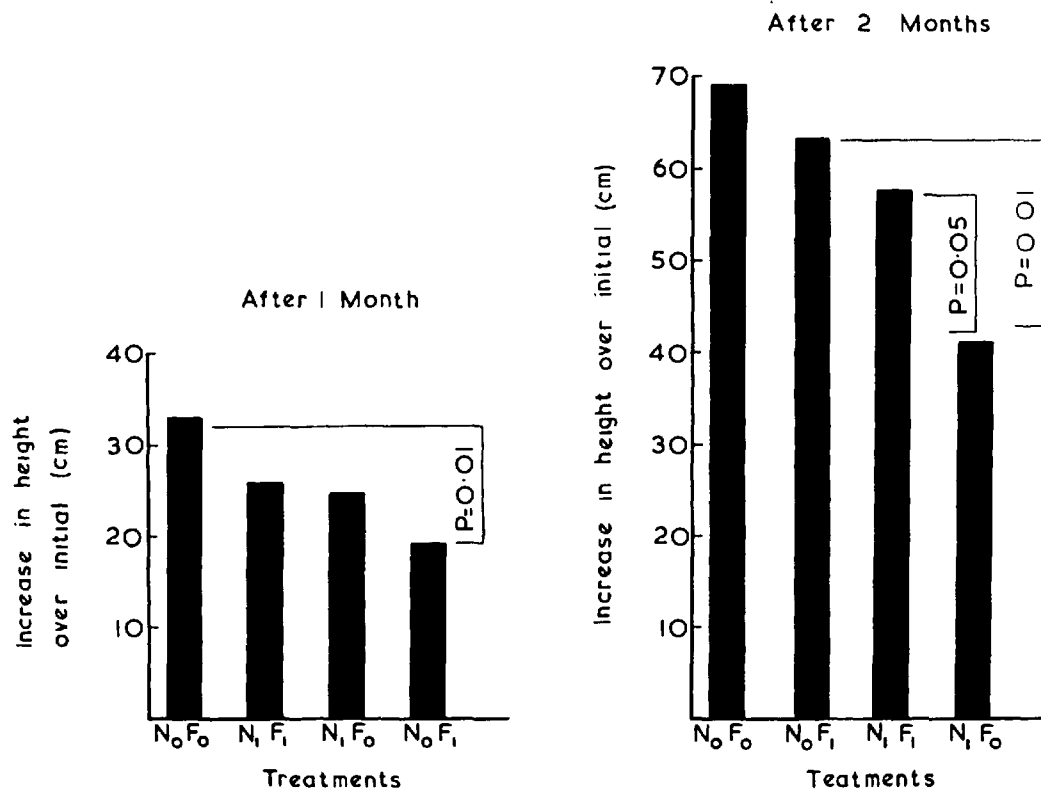


Table 22 shows that the nematode inoculum alters the effect of the fungal inoculum over this 3 week period, possibly by stimulating the host plant to grow more roots (Coursen & Jenkins 1958; Peters 1961).

It is interesting to note that later in the experiment the inclusion of fungal and nematode inocula together in the soil modifies the growth depression caused by the nematode alone. Undoubtedly this is due in part to the manurial effect of the wheat grain inoculum. It was therefore unfortunate that sterile wheat grain spawn was not added to the soil mixture for the control plants. This was because there was not enough wheat grain; a large percentage of the initial inoculum prepared for this experiment was lost because of autolysis of the fungus.

As the results for yield and post-cropping samples (e.g. Increase in height over Initial; Fresh weight of aerial parts; Length and fresh weight of roots; and the data for root fragment numbers) are influenced by the manurial effect of the wheat grain spawn, no great importance has been attached to the differences in growth results (Appendix Table 8).

5. The nematode and fungus complex on tomato seedlings grown in sterile conditions.

The sterile tomato seedlings, variety Eurocross A, were grown for two weeks in the culture tubes before the inocula were added.

The range of inoculum chosen for the fungus was 0; 0.5; 1.0; 2.0; and 4.0 cc. of hyphal suspension per tube; and for the nematode 0; 10; 20; 40 and 80 e/g. of sand in the culture tube (i.e. 0; 300; 600; 1200 and 2400 eggs per tube). These inocula rates are represented by the symbols F₁; F₂; F₃; F₄; F₅ and N₁; N₂; N₃; N₄; N₅ respectively. When the inocula had been added, the volumes of liquid pipetted in each tube were adjusted to 5 cc. in order that the water status in each tube was the same.

Each treatment was replicated four-fold and the whole experiment fully randomised. After inoculation the seedlings were allowed to grow for one month before the following data were taken.

Results.

Two replicates were used for the fungus estimations and two for the counts of the number of nematodes per root system.

(i) Brown Root Rot.

Each of the root systems sampled from the fifty tubes - the total for two replicates - were first allotted a score for the symptoms of Brown Root Rot using the schedule shown in Section A (page 26). The

scores are shown in Appendix Table 9. All the roots were surface sterilised and homogenised, using the technique described in Section A (page 28), and the number of colonies of G.S.F. which developed on plates of malt extract agar at the end of one week were counted (Appendix Table 10).

If the mean number of G.S.F. colonies isolated for each inoculum rate are plotted against the mean of the Brown Root Rot scores (Table 23) a linear relationship is revealed between the two methods of fungus estimation (Fig. 17). The angle of inclination of this line is an indication of the sensitivity of the two techniques of assessing the invasions by G.S.F. on the roots; and it shows that the homogenised root technique is the more sensitive.

Table 23.

Techniques of Assessment	Rates of fungal inoculum				
	0.0cc.	0.5cc.	1.0cc.	2.0cc.	4.0cc.
No. of G.S.F. colonies	0	11.30	15.70	21.40	30.00
Brown Root Rot score	1.89	2.68	3.23	3.81	4.27

Fig.17a shows the number of G.S.F. colonies isolated from the roots receiving the five rates of fungus inoculum. The plates were selected at random from the experiment.

Experiment 5

Fig.17 Relationship Between the Two Methods of
Assessment of Brown Root Rot

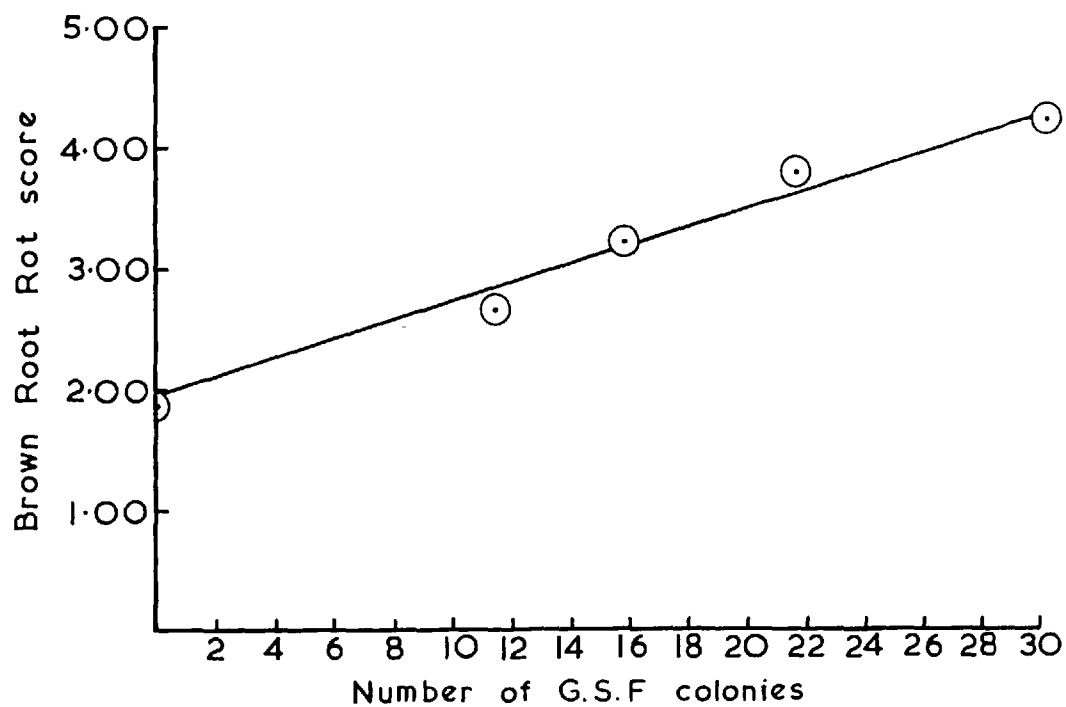
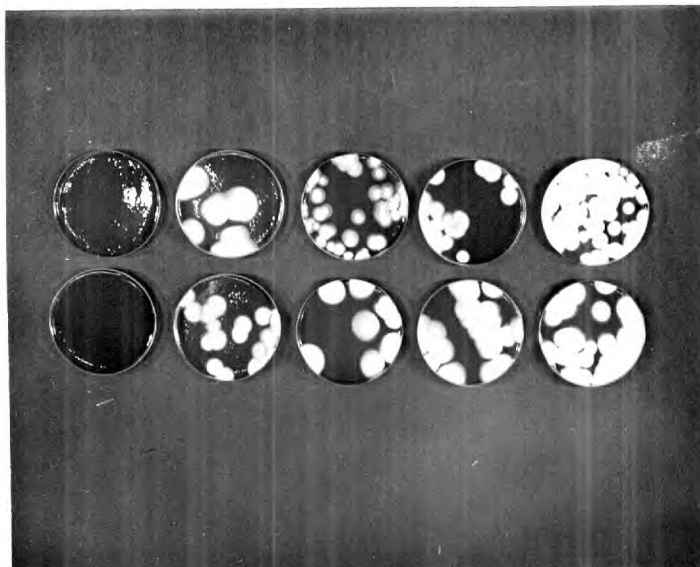


Fig. 17a. Colonies of G.S.F. isolated from roots.



N₄ F₁ N₂ F₂ N₂ F₃ N₁ F₄ N₅ F₅

N₁ F₁ N₄ F₂ N₅ F₃ N₄ F₄ N₂ F₅

(ii) The number of nematodes per Root system.

The roots of the seedlings from the two replicates remaining were stained in $0\frac{9}{5}\%$ cotton blue in lactophenol using the technique described in Section A (page 25). The number of nematodes in each root system was counted (Table 24)

Table 24. Number of nematodes per root system.

Treatment	Rep. I	Rep. II	Total	
N ₁ F ₁	0	0	0	
N ₂ F ₁	1	1	2	
N ₃ F ₁	3	1	4	
N ₄ F ₁	4	2	6	
N ₅ F ₁	9	7	16	28
N ₁ F ₂	0	0	0	
N ₂ F ₂	1	0	1	
N ₃ F ₂	0	5	5	
N ₄ F ₂	0	4	4	
N ₅ F ₂	4	6	10	20
N ₁ F ₃	0	0	0	
N ₂ F ₃	0	0	0	
N ₃ F ₃	0	0	0	
N ₄ F ₃	2	4	6	
N ₅ F ₃	0	4	4	10

Table 24 cont.

Treatment	Rep. I	Rep. II	Total	
N ₁ F ₄	0	0	0	
N ₂ F ₄	0	0	0	
N ₃ F ₄	0	0	0	
N ₄ F ₄	0	0	0	
N ₅ F ₄	0	0	0	0
N ₁ F ₅	0	0	0	
N ₂ F ₅	0	0	0	
N ₃ F ₅	0	0	0	
N ₄ F ₅	0	0	0	
N ₅ F ₅	0	0	0	0

The above table shows that increasing the inoculum rate of G.S.F. in the culture tubes has an influence on the number of potato root eelworms entering the roots of the host plant.

(iii) Height of shoot and length of roots.

All four replicates were used for these measurements, and the data are shown in Appendix Tables 10 and 11. Analysis of these data (Appendix Tables 11 and 12) reveal that the fungus inoculum has a significant effect upon the extension growth of both shoot and root. The means of the results for both height of shoot and length of root for each fungus inoculum were expressed as a percentage of their relevant controls, and these percentages were transformed to angles (Appendix Table 13).

These angular transformed data were then graphed (Fig. 18), and calculated lines fitted to the points. The significance of the slopes of the two Regression lines was assessed; and a comparison between the two lines was made (Appendix Table 14) showing a significant difference between them. This indicates that the height of the shoot was a more sensitive measure of host response to increasing fungus inoculum rates than was root length.

Fig. 19 shows the effect of increasing rates of G.S.F. inoculum upon the growth of the tomato seedlings in the culture tubes.

(iv) Dry Weight of Shoot.

The data for the dry weight of the shoot showed no significant difference between treatments (Appendix Table 15).

(v) Fresh weight of roots.

Two methods were used for determining the fresh weight of the roots. For the first, the weighing was incorporated into the schedule for the surface sterilising of the roots from the two replicates used for the assessment of fungal attack. The excess 0.1% mercuric chloride was allowed to drain from the roots before they were transferred to McCartney vials of known weight, containing a known volume of water. The vials were then weighed and the fresh weight of the roots determined. In the second method, the roots, which were used for the assessment of nematode attack, were dried between sheets of absorbent tissue before they were weighed.

Experiment 5

Fig.18 Growth of Shoot and Root

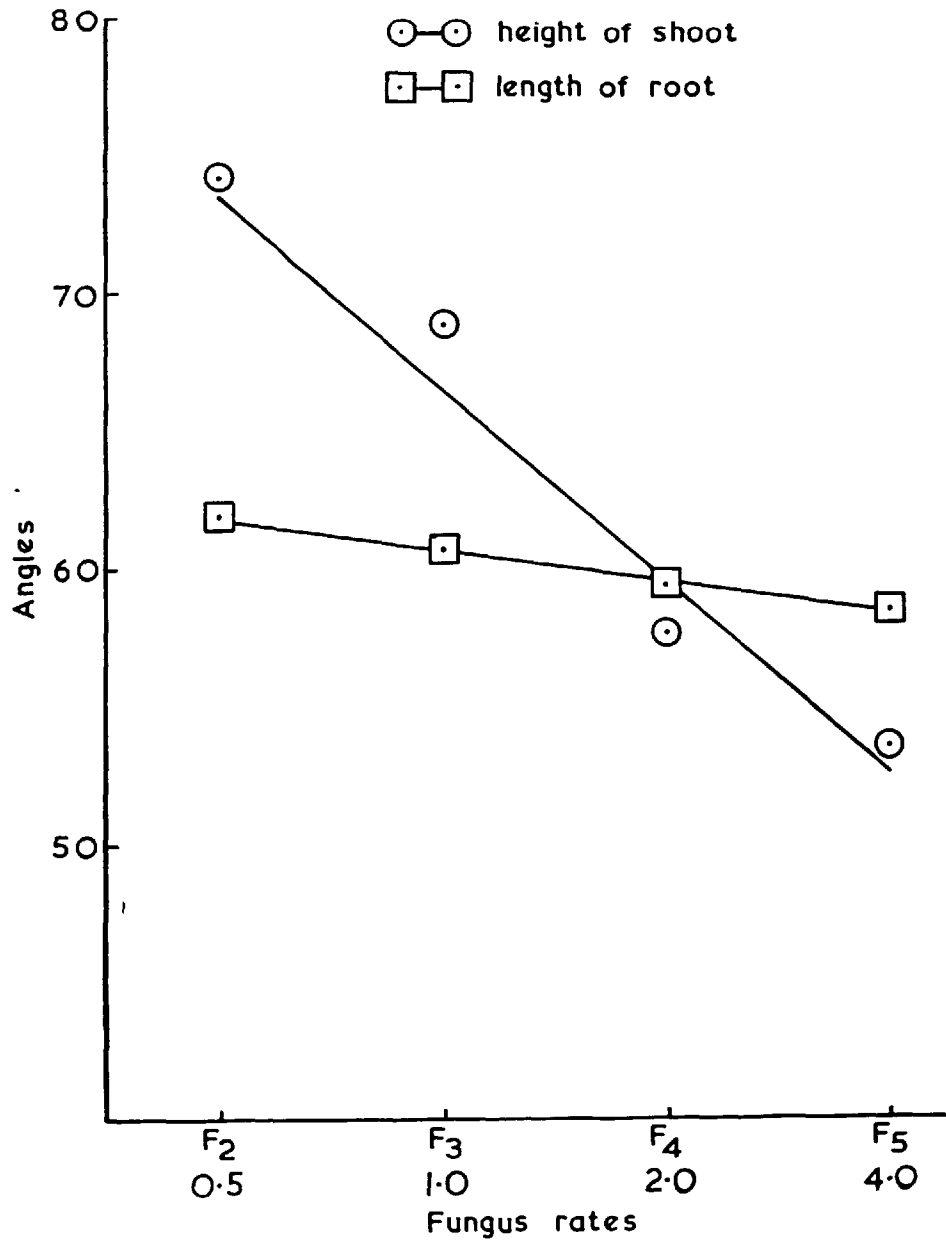
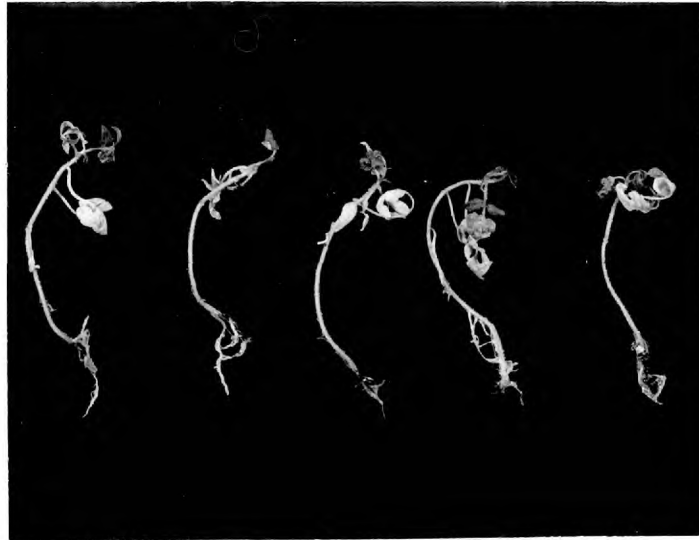


Fig. 19. The effect of increasing G.S.F. inoculum
on seedling growth.

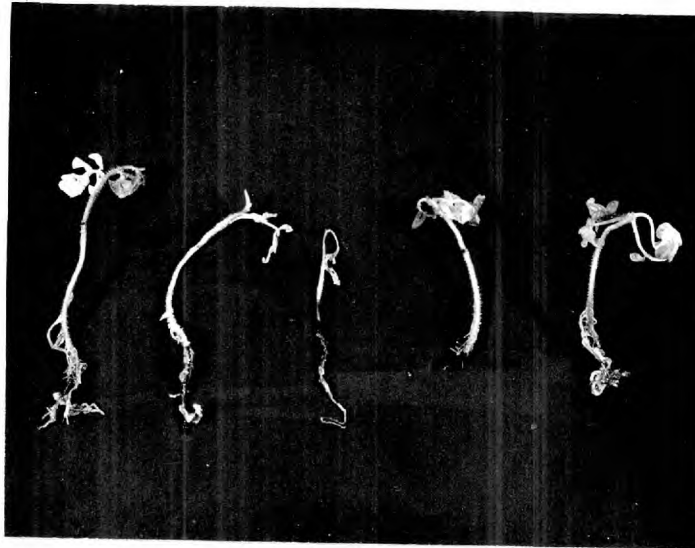


F₁

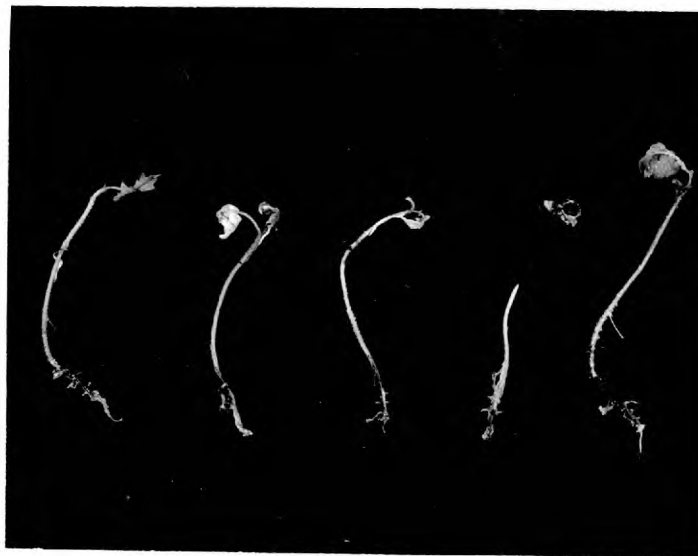


F₂

82a.



F₃



F₄

82b.



F5

The data for the former method are recorded in Appendix Table 16, and an analysis revealed no significance for any factor. An analysis of the data from the latter method, however, revealed that the nematode inoculum had an effect on the fresh weight of the roots (Appendix Table 17).

Table 25. Fresh weight of roots in milligrammes.

(Mean of 10)

Rates of nematode inoculum.

N ₁ 0 e/g.	N ₂ 10 e/g.	N ₃ 20 e/g.	N ₄ 40 e/g.	N ₅ 80 e/g.
69	61	44	82	34

(Critical difference when $P = 0.05$, 25)

However it is thought that this is a spurious result.

6a. To investigate the effect of the fungus inoculum on the nematode invasion of the host plant.

Eighty tomato seedlings of the variety Eurocross A were grown in a sand; brick dust; pulverised peat mixture (proportions 11:5:1) for about four weeks when one pair of true leaves had been produced. These seedlings were then transferred to small plastic pots containing 150 g. of the same potting medium, in which 50 cysts of Heterodera rostochiensis Woll. (containing 150 eggs per cyst) were incorporated. To forty of the pots G.S.F. inoculum on vermiculite (Section A, page 19) was also mixed with the soil at a rate of 1g. per 10g. of soil : to the remaining forty pots sterilised vermiculite was added at the same rate. The pots were then thoroughly randomised on the greenhouse bench, and 10 samples were taken for both treatments 4; 8; 16 and 32 days after inoculation.

Results.

Table 26. Number of nematodes per gramme fresh weight of roots.

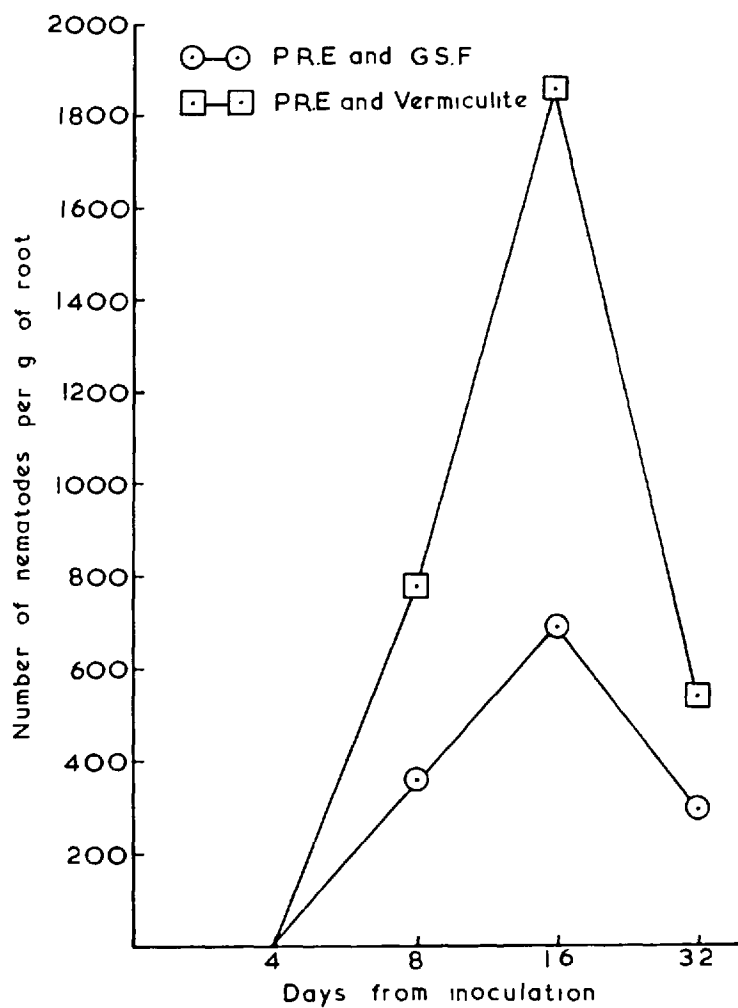
Sampling Time	Treatment	Nematodes (Mean of 10)
4 Days	P.R.E. & G.S.F. Inoculum A	0
	" " Vermiculite B	0
8 Days	P.R.E. & G.S.F. Inoculum A	357.1
	" " Vermiculite B	774.6
16 Days	P.R.E. & G.S.F. Inoculum A	681.8
	" " Vermiculite B	1853.1
32 Days	P.R.E. & G.S.F. Inoculum A	286.6
	" " Vermiculite B	537.8

Appendix Table 18 shows the full data of the number of nematodes per gramme fresh weight of root, and an analysis of the data on the eighth day reveals that there were less nematodes in these roots growing in the presence of both inocula than those where only potato root eelworm had been added ($P = 0.05$). By the sixteenth and thirty second days the significance of the difference between the two treatments had increased ($P = 0.01$); Fig. 20 illustrates this.

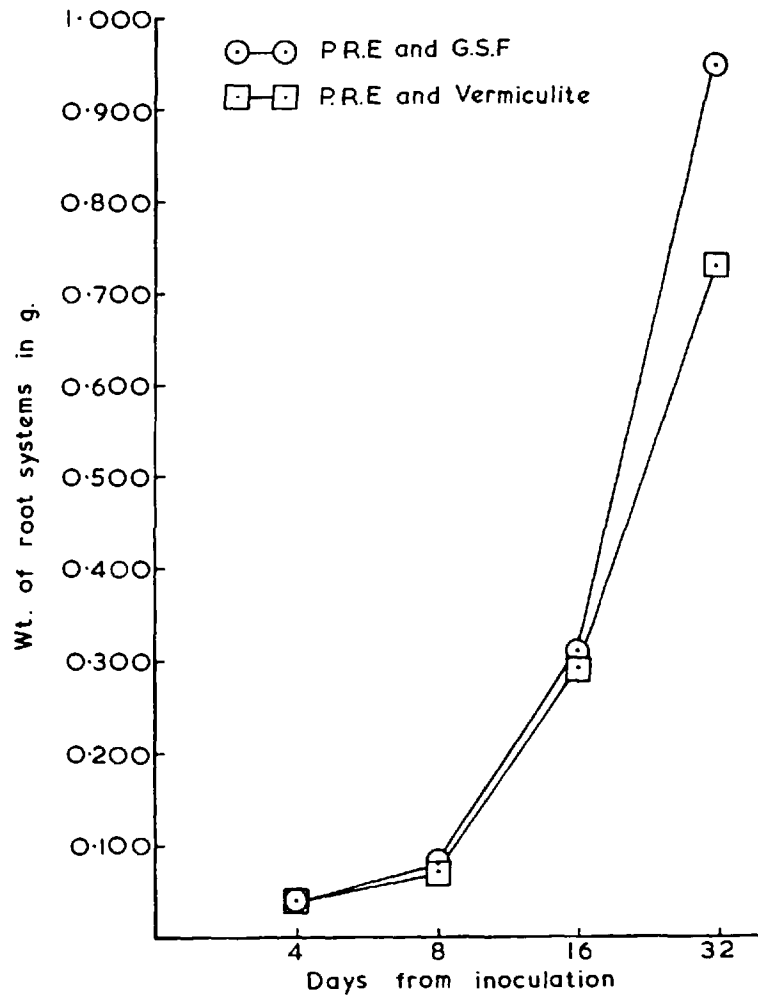
As examination of the fresh weight data of the root systems for both treatments (Appendix Table 19) shows that there was no statistical difference between them until the thirty second day, by which time the plants receiving both inocula had much heavier root systems than those growing in the presence of nematodes alone (Fig. 21).

The apparent fall in the number of nematodes per gramme fresh weight of root system on the thirty second day can be explained. As the majority of the nematode larvae are reported to enter the roots within a few days of inoculation of the soil (Dropkin 1957), the proportion - nematodes per gramme - will decrease as the roots grow. Furthermore, many of the developing females will have burst through the root cortex by the thirty second day, and are easily dislodged when the roots are washed from the potting medium.

Experiment 6a

Fig. 20 Number of Nematodes per g. Fresh Weight
of Root (Mean of 10 replicates)

Experiment 6

Fig. 21 Fresh Weight of Root Systems in g.
(Mean of 10 replicates)

6b. The effect of different times of inoculation of the soil with the two pathogens on:-

- (i) The final nematode population in the soil.
- (ii) The attack of the roots by G.S.F.
- (iii) The host.

One month old seedlings of the tomato variety Eurocross A grown in sterilised soil (3:2 loam:sand mixture), were transferred, with their soil ball, from small plastic pots, $2\frac{1}{2}" \times 2\frac{1}{2}"$, to earthenware pots large enough to take 5 kg. of soil. The seedlings were then allowed to become established for a week, by which time the relevant treatments were added.

The four treatments chosen were:-

- I. Nematode first.
- II. Fungus first.
- III. Both together.
- IV. Control.

The period between adding the first and second inocula in treatments I and II was two weeks.

The nematode was added to the soil as a suspension of eggs (and larvae) prepared as described in Section A (page 21). The rate of inoculum for P.R.E. was 40 e/g. of soil.

The fungus was added as a hyphal suspension prepared as described in Section A (page 19). 5 cultures in 250 cc. were added to each pot. The volumes of liquid added to each pot were made up to a constant by the addition of water.

Each treatment was replicated 4 times, and the pots were set out in a randomised block design. The experiment lasted two months.

Results.

(i) The final nematode population in the soil.

The cysts were isolated from the soil in the pots using the techniques described in Section A (page 16).

Table 27. Number of cysts per 200 g. of soil.

Treatment	Rep. I	Rep. II	Rep. III	Rep. IV	Total
Nematode 1st.	44	42	36	30	152
Fungus 1st.	48	51	93	65	257
Both	32	40	37	26	135
	124	133	166	121	544

An analysis of this data (Appendix Table 20) reveals that there were significantly more cysts produced at the end of the experiment in those pots in which the fungus inoculum was added first ($P = 0.05$).

It is difficult to see why this should be so. The number of eggs per cyst was then estimated (Section A, page 16)

Table 28.Number of eggs per cyst.

Treatment	Rep. I	Rep. II	Rep. III	Rep. IV	Total
Nematode 1st.	64.1	106.4	102.9	53.7	347.1
Fungus 1st.	72.7	63.5	135.5	128.0	399.7
Both	8.4	19.1	46.5	90.8	164.8
	165.2	189.0	284.9	272.5	911.6

An analysis of this data (Appendix Table 21) revealed no treatment significance, however, presumably because of the high inter-replicate variation. Nevertheless, it would seem that those cysts, from the pots receiving both inocula at the same time, had appreciably fewer eggs in them, indicating that the fungus possibly influences the development and subsequent reproduction of the nematode in some way. Subsequently the number of eggs per gramme of soil was calculated.

Table 29.Number of eggs per g. of soil.

Treatment		Initial Pop'n	Final Pop'n
Nematode 1st.	Rep. I	40 e/g	92.5 e/g
	Rep. II	40 "	111.5 "
	Rep. III	40 "	92.5 "
	Rep. IV	40 "	40.0 "
Fungus 1st.	Rep. I	40 "	87.0 "
	Rep. II	40 "	81.0 "
	Rep. III	40 "	315.0 "
	Rep. IV	40 "	208.0 "

Table 29 cont.

Treatment		Initial Pop'n	Final Pop'n
Both.	Rep. I	40 e/g	6.5 c/g
	Rep. II	40 "	19.0 "
	Rep. III	40 "	43.0 "
	Rep. IV	40 "	59.0

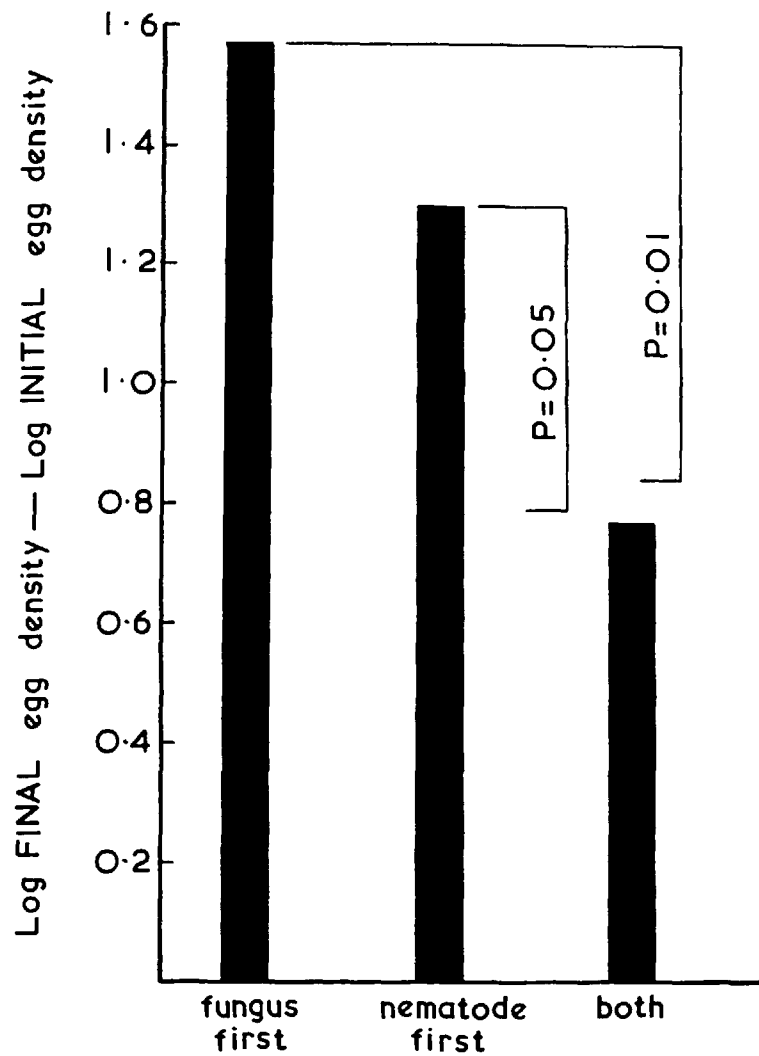
An analysis on the ratios of Final egg density/Initial egg density per gramme of soil (Log. FINAL - Log. INITIAL) demonstrates a treatment significance (Appendix Table 22). Fig. 22 shows the mean values of these ratios for the three treatments indicating the critical differences. The timing of inoculation of the two pathogens is, therefore, important.

(ii) Brown Root Rot.

The assessment of G.S.F. attack was done by the visual scoring technique on the whole root system using the schedule shown in Section A (page 26). These data are shown in Appendix Table 23. There is no difference of significance between the levels of Brown Root Rot for each of the treatments receiving the pathogens (Fig. 23). It would appear, therefore, that the timing of inoculation of the two pathogens has little influence upon the severity of the fungus attack on the host roots.

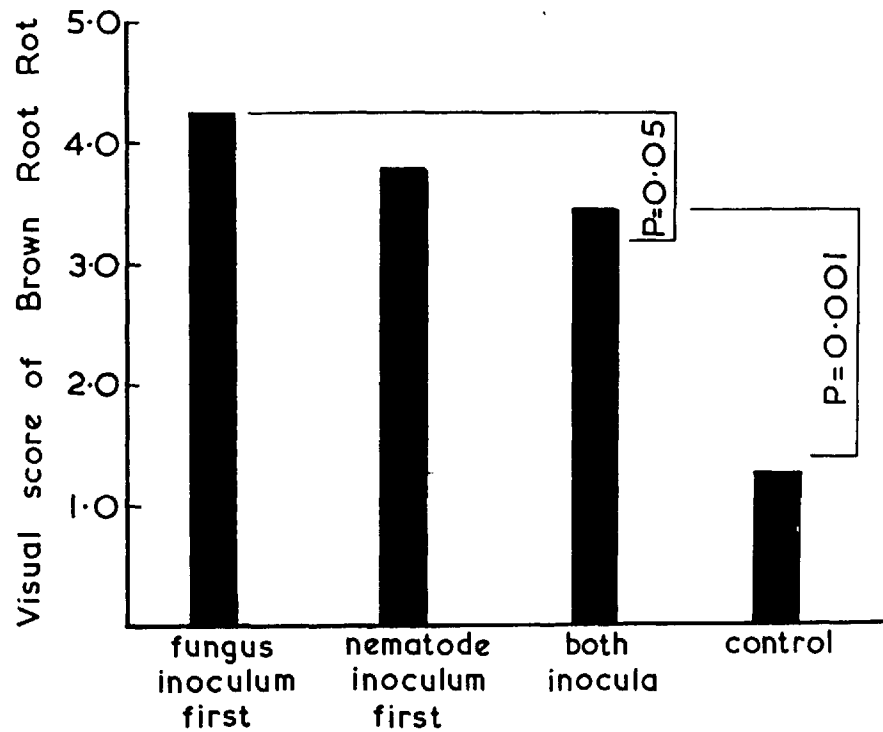
Experiment 6a.

Fig.22 Log FINAL — Log INITIAL
Egg Density per g.



Experiment 6a.

Fig.23 Brown Root Rot Scores (Mean of 4)



(iii) The host plant.

The data for crop yield, increase in height over initial, root length and fresh weight (Appendix Table 24) revealed no difference between treatments.

Fig. 24 shows the results of the analysis of the data for the fresh weight of aerial parts (Appendix Table 25). The difference between the mean fresh weight of those plants receiving both inocula concurrently and those receiving the nematode two weeks prior to the inoculation of the soil with G.S.F. was significant within the 1% fiducial limit. The fungus, therefore, mitigates the effects of the nematode inoculum upon the host, when they are added at the same time to the soil. Together with the data for the multiplication of the nematode in terms of eggs per gramme of soil, these results reveal that there must be a competition between the two pathogens when they are both actively colonising the roots. This competition appears to be to the disadvantage of the nematode. It is interesting to note that when the fungus had become established, i.e. when it was inoculated first, it appeared not to have the same effect on the nematode.

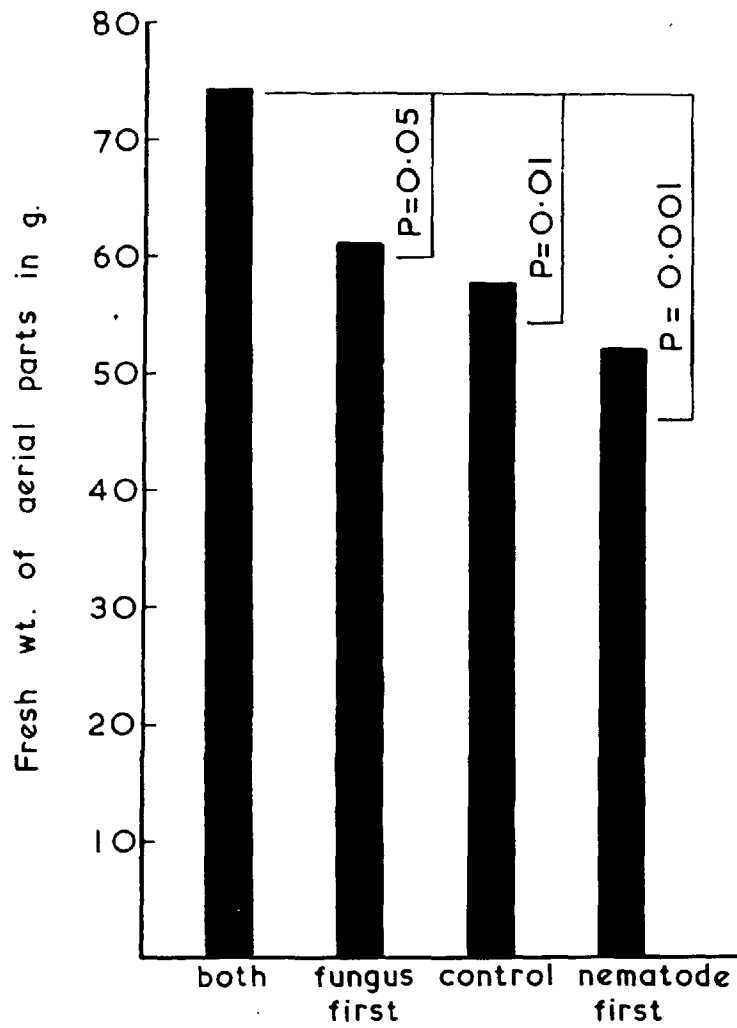
6c. The effect of different times of inoculation with the two pathogens on the number of nematodes entering the host plant roots.

This experiment was done on seedlings growing in culture tubes, and is complementary to the previous experiment 6b.

The sterile seedlings of the tomato variety Eurocross A were allowed

Experiment 6a.

Fig. 24 Fresh Weight of Aerial Parts in g.
(Mean of 4)



to grow for two weeks after the pregerminated, surface sterilised seeds were sown before any inoculation with the pathogens was done.

The treatments were:-

I. Nematode inoculum first.

II. Fungus inoculum first.

III. Both inocula together.

The rate of inoculum for the nematode was 40 e/g. of sand (1200 eggs per culture tube); and the fungus was added at the rate of 4 cc. of a hyphal suspension per culture tube, prepared as described in Section A (page 19).

The period between the addition of the second inoculum in treatments I and II was two weeks. One month after the inoculation of the culture tubes with nematodes, the seedlings were washed out and the roots stained with 0.05% cotton blue in lactophenol (Section A, page 25).

Results.

Table 30. Total number of nematodes per root system.

Treatment	Rep. I	Rep. II	Rep. III	Rep. IV	Rep. V	Total
Nematode 1st.	11	17	16	9	2	55
Fungus 1st.	19	8	7	4	9	47
Both	3	6	2	1	0	12
	33	31	25	14	11	114

The analysis of the data in Table 30 (Appendix Table 26) shows that the addition of the fungus inoculum simultaneously with the nematodes inhibited the invasion of the host roots by Heterodera larvae. However, if the fungus was added to the culture tubes two weeks previous to the addition of potato root cecidworms, this inhibition is not shown. These results, therefore, substantiate the data recorded for experiment 6b.

6d. The effect of exudates produced by G.S.F. upon the hatch of Heterodera rostochiensis Woll.

The fungus was incubated at 25° C. on malt extract broth in Erlenmeyer flasks for 2 weeks; the resultant mycelial mat was removed, and the liquid medium filtered through a sintered glass filter (1-1.5 μ pore size). Malt extract broth on which no fungus had been cultured was similarly filtered. Root diffusate, collected from growing potato plants (Goodey 1963) was also sterilised by filtering through a sintered glass filter of similar pore size.

A suspension of Heterodera eggs, extracted from cysts and surface sterilised with H₂ O₂ (Section A, pages 16 and 23 respectively) was made up to a known volume with sterile water, and 1 cc. was aseptically pipetted into 18 solid watch glasses kept in large petri dishes (1 petri dish contained 6 watch glasses). An estimate was made of the number of larvae added to each watch glass.

To each watch glass one of the following six treatments was added.

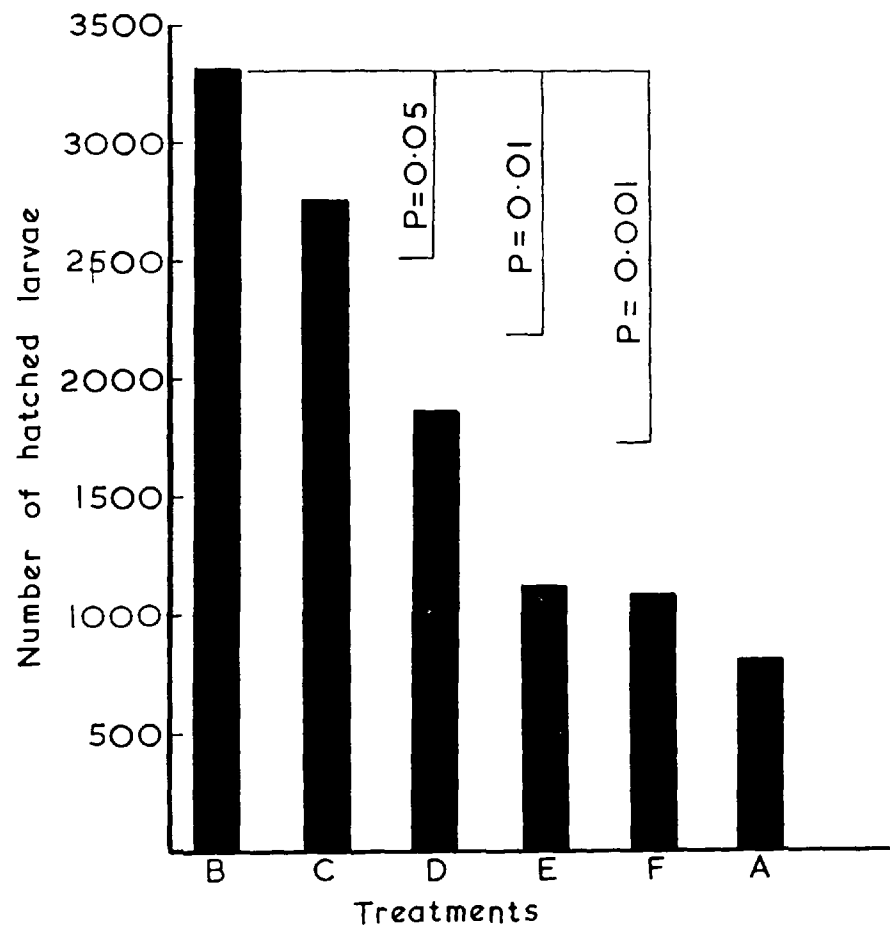
- A. Filter sterilised malt extract broth on which G.S.F. had been cultured for 2 weeks + Potato root diffusate also filter sterilised (1 cc. of both).
- B. Filter sterilised malt extract broth on which no fungus had been cultured + filter sterilised Potato root diffusate (1 cc. of both).
- C. Filter sterilised Potato root diffusate + Sterile water (1 cc. of both).
- D. Unsterilised Potato root diffusate + water (1 cc. of both).
- E. Water (2 cc.)
- F. Filter sterilised malt extract broth + sterile water (1 cc. of both).

Results.

At the end of one week the number of larvae present in each watch glass was counted. From this was subtracted the number of larvae per cc. added initially, and the hatch for the eggs in each treatment was calculated and analysed (Appendix Table 51).

Fig. 25 shows that the fungus produced exudates in the culture medium which very significantly inhibited the hatch of P.R.E. It would also appear that the malt extract broth enhanced the 'hatching power' of the root diffusate when treatments B and D are compared.

Experiment 6d
Fig.25 Hatch (Mean of 3)



Discussion.

Visual symptoms of Brown Root Rot were observed approximately one week after the inoculation of the sand in the culture tubes with the fungus. The rate of invasion by G.S.F. was not influenced by the presence of potato root eelworm whether added to the sand before or at the same time as the fungus. Furthermore, the degree of infestation of the roots was not influenced by the increasing rates of Heterodera inoculum. It would appear, therefore, that the potato root eelworm does not assist G.S.F. in its invasion of tomato roots.

Investigations involving seedlings growing in culture tubes showed early attacks of the fungus were damaging, corroborating Last's observations (personal communication). In practice, the ability of tomatoes to withstand Brown Root Rot is related to the size of the seedling transplanted into infected soil, and also the amount of sterile soil transferred with the seedling - the larger the sterile soil ball around the roots, the less the plant will be affected by the initial G.S.F. attacks, and, thus the plant will become established quicker.

The effects of Heterodera larval invasions on the host plant are at first insidious (see Experiment 3b); however, the effect of eelworm on the inhibition of host growth can be seen eventually, the final result being the severely decreased root system of the host paralleled by a low yield (see Experiment 2b).

In an attempt to achieve a more quantitative estimation of fungus within plant tissue, a small amount of G.S.F. was exposed to a Periodic acid - Schiff reaction (P.A.S.). The hyphae stained red indicating a positive reaction. Pillsbury & Klignan (1950) state that the P.A.S. positive substance observed in various fungi is possibly chitin or a chitin-like substance.

Chitin has long been considered to be a component of the fungal mycelial wall. Since the first identification of chitin in fungi by Braconnot in 1811 (reported by Tracey 1955) many qualitative microchemical tests have been developed to prove the presence of chitin (Foster 1949). The occurrence of fungal chitin was investigated by Ulrich (1943), who found chitinous cell walls to be characteristic of the Plasmodiophoraceae, Synchytriaceae, numerous Zygomycetes, Endomycetaceae and the majority of Ascomycetes and Basidiomycetes. Blumenthal and Roseman (1957) estimated the chitin content of 25 species of fungi, and reported that it varied from 2-26% in fungal mycelia.

Therefore, it is envisaged that the colorimetric technique, which was used to estimate the amount of chitin present in wheat leaves infected with Puccinia graminis (Bassett 1958), could be used as a quantitative method of estimating the amount of G.S.F. within tomato roots.

S E C T I O N B I I .

Section B II.7. The effect of different levels of G.S.F. and P.R.E. inocula on six species of *Lycopersicon*.

The species of *Lycopersicon* chosen were *L. esculentum*, Hort.var. Eurocross A (Sp. 1), *L. pimpinellifolium* (Sp. 2), *L. hirsutum* (Sp. 3), *L. glandulosum* (Sp. 4), *L. peruvianum* (Sp. 5), and *L. hirsutum* var. *glabratum* (Sp. 6).

The seedlings were grown in polystyrene boxes of autoclaved soil (a loam, sand and pulverised peat mixture in the proportions 3:2:1) After three weeks they were transferred to plastic pots ($2\frac{1}{2}" \times 2\frac{1}{2}"$) of heat sterilised soil, in which they were allowed to grow for seven weeks. The reason for this exceptionally long period in the small pots was because the seedlings were very slow growing at the time of year the experiment was started.

The three rates of nematode inoculum used were 0; 10; and 40 e/g of soil. The nematode inoculum was added as cysts at the rate of 312.5 and 1250 per kg. of soil.

The G.S.F. was cultured on vermiculite (Section A, page 19), and added to the soil at a rate of 0; 0.2; and 0.4 g. per 10g. of soil.

Table 31.

Table of treatments.

	0g. = F ₁	0.2g. = F ₂	0.4g. = F ₃
0 c/g. = N ₁	N ₁ F ₁	N ₁ F ₂	N ₁ F ₃
10 c/g. = N ₂	N ₂ F ₁	N ₂ F ₂	N ₂ F ₃
40 c/g. = N ₃	N ₃ F ₁	N ₃ F ₂	N ₃ F ₃

The nine treatments were replicated twice for each of the six species of *Lycopersicon*.

Results.

a. The assessment of G.S.F. attack.

The arbitrary scoring schedule, set out in Section A (page 26), was used to assess the percentage of the whole root system affected by Brown Root Rot. There was a significant difference in the levels of Brown Root Rot between the species of *Lycopersicon* (Appendix Table 27). *L. hirsutum* was the only species showing significantly less Brown Root Rot symptoms than *L. esculentum* ($P = 0.05$). Termohlen (1962) found that all cultivated varieties of the tomato (*L. esculentum*) were susceptible to 'corky root' (synonymous with Brown Root Rot). Of the wild species he examined *L. pimpinellifolium* seemed as susceptible as *L. esculentum*, while *L. peruvianum*, *L. glandulosum* and *L. hirsutum* showed a high degree of resistance. Bravenboer & Pet (1962) also state that *L. hirsutum* is

resistant to 'corky root'.

No significance in the degree of symptoms was revealed for the three rates of fungus inoculum; nor was there any significant interaction between the nematode and fungus factors.

b. The final nematode population.

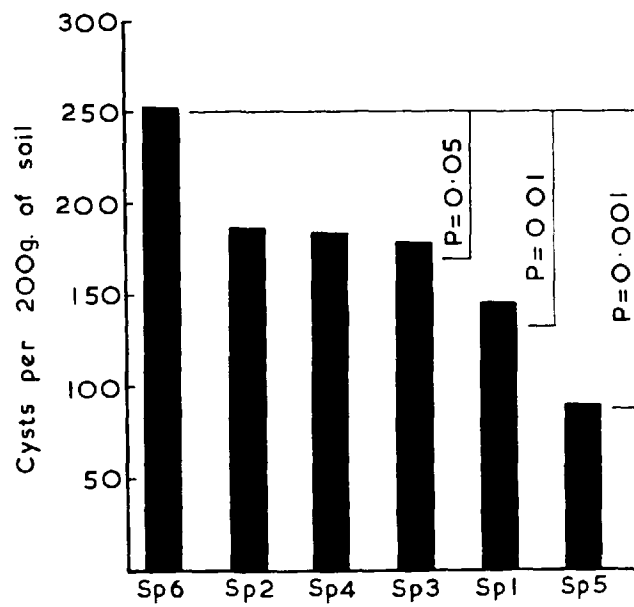
(i) The cyst density per 200 g. of soil.

Estimations were made of the number of Heterodera cysts in two samples of 200 g. of soil taken from each pot, and from these results (Appendix Table 28) the number of new cysts produced was calculated. (Final number of cysts - Initial number of cysts). There was a significant difference between the species of Lycopersicon in the number of new cysts produced at the end of the experiment. Fig. 26 shows that L. hirsutum var glabratum produced significantly more new cysts than L. esculentum ($P = 0.05$) or L. peruvianum ($P = 0.001$); however, the differences between the number of new Heterodera cysts produced on L. pimpinellifolium, L. glandulosum, L. hirsutum, L. esculentum, and L. peruvianum were not great enough to be within the accepted fiducial limits.

If, however, an estimation is made of the multiplication rate of the nematode, by subtracting the logarithm for the initial cyst density per 200 g. of soil from log. final cyst density, no difference is demonstrated between the 6 species (Appendix Table 29). This was because the cysts of the initial inoculum still present in the soil confused the final

Experiment 7

Fig 26 New Cysts per 200g. of Soil
(Mean of 12)

Sp1 *L. esculentum*Sp2 *L. pimpinellifolium*Sp3 *L. hirsutum*Sp4 *L. glandulosum*Sp5 *L. peruvianum*Sp6 *L. hirsutum* var. *glabratum*

result. Therefore the assessment of the new cysts produced at the end of the experiment gives a much clearer picture of pathogen success on a particular host.

An examination of the number of new cysts produced for each rate of nematode inoculum reveals that significantly more ($P = 0.001$) cysts were produced by the high rate of nematode inoculum (40 c/g) than by the low rate (10 c/g). If the multiplication rate of the potato root eelworms is considered then N_2 (10 c/g) was greater than N_3 (40 c/g) at the 1% level. This is to be expected, however, as it is known that the multiplication rate for low initial densities of cysts may be greater than that for high initial densities (Rao & Peachey 1965; Peters 1961, and Hague & Hesling, 1958).

The rates of G.S.F. inoculum have a significant depressant effect ($P = 0.01$) on the number of new cysts produced (Table 32).

Table 32.

Critical difference when $P = 0.01$,	101.25
Critical difference when $P = 0.001$,	133.30
$m F_1 = 263.54$	
(Mean of 24)	127.08 **
$m F_2 = 136.46$	
	3.96
$m F_3 = 132.50$	

The first order interaction between the nematode and the fungus factors was shown to be significant at the 0.1% level (Appendix Table 28). Table 33, compiled from the means of all the values determined for the two way table of N against F calculated from the data in Appendix Table 28, shows that the interaction between the two factors decreased the numbers of new cysts produced at the end of the experiment.

Table 33.

	$\bar{m} F_1$	$\bar{m} F_2$	$\bar{m} F_3$
$\bar{m} N_2$	102	93	126
$\bar{m} N_3$	425	180	139

Both treatments, $N_3 F_2$ and $N_3 F_3$, were much lower than could be expected, if the fungus inoculum only was responsible for depressing the number of nematode cysts. It is interesting to note that the number of new cysts produced by the low rate of nematode inoculum (10 e/g) seemed to be unaffected by the interaction.

(ii) The final number of eggs per gramme of soil.

The eggs were extracted from the cysts using the techniques described in Section A (page 16); and, from the estimations of the number of eggs per cyst, calculations were made of the number of eggs per gramme of soil. An analysis on the number of new eggs produced per g. (Appendix Table 30) revealed no difference between the six species of Lycopersicon.

Significantly more eggs were produced by the high rate of nematode inoculum (40 e/g) than the low (10 e/g), ($P = 0.05$).

The fungus inoculum had a depressant effect on the numbers of new eggs produced per gramme. (Table 34)

Table 34.

Critical difference, when $P = 0.05$,	61.40
Critical difference, when $P = 0.01$,	82.34
$\bar{m} F_1 =$	119.52
(Mean of 24)	54.60
$\bar{m} F_2 =$	64.92
	42.40
$\bar{m} F_3 =$	22.52.

The analysis also revealed that the first order interaction between the nematode and fungus factors was significant within the 1% confidence limits. Table 35, compiled from the means of all the values determined for the two way table N against F calculated from the data in Appendix Table 30 shows that this interaction decreased the number of new eggs produced at the end of the experiment.

Table 35.

	$\bar{m} F_1$	$\bar{m} F_2$	$\bar{m} F_3$
$\bar{m} N_2$	15.1	60.7	31.6
$\bar{m} N_3$	223.9	69.1	13.4

c. Growth Recording.

The data of the extension growth over the initial height for the eight weeks of the experiment are shown in Appendix Tables 31-37 inclusive. The salient features of the analyses of these data are shown in Appendix Table 38.

The G.S.F. inoculum inhibited the growth of the six species of Lycopersicon to a greater or lesser degree throughout the period of the experiment. In the first week, both rates of inoculum of the fungus depressed the growth of the plants significantly when compared to the control (Table 36).

Table 36.

Critical difference when $P = 0.01$, 0.63 cms.

Critical difference when $P = 0.001$, 0.82 cms.

	$\bar{m} F_1 = 5.7$ cm.	
		0.80 cm.*
(Mean of 36)	$\bar{m} F_3 = 4.97$ cm.	
		0.07 cm.
	$\bar{m} F_2 = 4.90$ cm.	

The nematode inoculum did not affect the extension growth of the host plant until the second week, and then it was only the low rate (10 e/g) which depressed the extension growth significantly (Table 37).

Table 37.

	Critical difference when $P = 0.05$,	1.39 cm.
	Critical difference when $P = 0.01$,	1.85 cm.
	$\bar{m} N_1 =$	17.80 cm.
(Mean of 36)		0.76 cm.
	$\bar{m} N_3 =$	17.04 cm.
		0.99 cm.
	$\bar{m} N_2 =$	16.05 cm.

This was the case throughout the period of the experiment. The pathogenic effect on the host of the high rate of nematode inoculum (40 e/g) was therefore modified by some factor. Earlier in this experiment it was shown that the G.S.F. inoculum affected the multiplication of the nematode, when both the number of new cysts and eggs produced at the end of the experiment were considered (Tables 32 and 34). Furthermore, a significant interaction between the nematode and fungus factors was demonstrated, which appeared to affect the high inoculum rate of the nematode decreasing the final population (Tables 33 & 35). However, the number of new cysts produced at the end of the experiment by the low rate of potato root eelworm inoculum (10 e/g) did not appear to be affected by this interaction.

In earlier experiments (Section BI, page 78) it was found that the presence of G.S.F. could decrease the numbers of potato root eelworms entering the roots. Christie and Perry (1959) observed that many decay-promoting organisms are incompatible with plant parasitic nematodes.

The obligate plant parasitic nematode, therefore, seems especially vulnerable to the inevitable competition that occurs between the components of a nematode/fungus interaction when they seek nourishment from the same individual host plant, and often from the same organ of that host.

The analyses (Appendix Table 39) showed the first order interaction, NF, to be significant throughout the experiment. 3×3 tables of the nematode factor against the fungus factor were constructed for each week from Appendix Tables 31-37, and the mean value for each treatment was then expressed as a percentage of the Grand Total mean so that the results for each week could be more easily compared (Appendix Table 34). The NF interaction throughout the period of the experiment was negative, i.e. both pathogen factors exacerbated the effect of the other in decreasing the extension growth of the host plant.

(i) The effect of the two inocula on the extension growth of the six *Lycopersicon* species considered separately.

The analysis of these data are shown in Appendix Tables 40-44 inclusive.

The extension growth of *L. pimpinellifolium* was not affected by either of the pathogens; and *L. peruvianum* was only affected in the eighth week by the interaction of the nematode and fungus factors ($P = 0.05$). Table 38 shows that this interaction was negative i.e. each pathogen aggravated the effect of the other.

Table 38. (Total of 2 replicates)

	N ₁	N ₂	N ₃
F ₁	154.0	240.5	238.5
F ₂	248.0	172.0	239.5
F ₃	242.0	238.5	228.0

The extension growth of L. osculentum was also shown to be affected by the interaction of the nematode and fungus factor (Appendix Table 40); Appendix Table 45 revealed that this interaction was negative.

In the third and fourth week L. glandulosum was shown to be affected by the NF interaction, each factor aggravated the other in depressing the growth (Appendix Table 46).

The extension growth of L. hirsutum is shown (Appendix Table 41) to have been affected by the G.S.F. inoculum. In the first week only the high rate of inoculum (0.4g. per 10g. soil) inhibited growth significantly (P=0.01), and the difference between the low (0.2g. per 10g. soil) and high rate was significant at the 5% level (Table 39).

Table 39.

Critical difference when P = 0.05, 0.8 cm.

" " when P = 0.01, 1.2 cm.

" " when P = 0.001, 1.7 cm.

(Mean of 6)

Table 39 cont.

$\bar{m} F_1$	=	4.2 cm.
		0.5 cm.
$\bar{m} F_2$	=	3.7 cm.
		0.8 cm. *
$\bar{m} F_3$	=	2.9 cm.

However, by the second week both rates of G.S.F. inoculum depressed the extension growth of this species significantly; and this effect continued until the end of the experiment.

Appendix Table 42 shows that initially L. hirsutum var glabratum was unaffected by either pathogen, but by the second week both rates of the fungus inoculum were demonstrated to have inhibited the growth (Table 40).

Table 40.

	Critical difference when $P = 0.05$,	2.3 cm.
	" " when $P = 0.01$,	3.4 cm.
	$\bar{m} F_1$	= 12.5 cm.
(Mean of 6)		2.6 cm. *
	$\bar{m} F_2$	= 9.9 cm.
		0.3 cm.
	$\bar{m} F_3$	= 9.6 cm.

The extension growth was shown to be affected by both inocula in the third week (Table 41).

Table 41.

Critical difference when $P = 0.05$,		3.3 cm.
"	"	when $P = 0.01$, 4.8 cm.
"	"	when $P = 0.001$, 7.0 cm.
(Mean of 6)		
$\bar{m} F_1 = 27.7$ cm.		$\bar{m} N_1 = 26.6$ cm.
	7.5 cm. **	5.9 cm. **
$\bar{m} F_2 = 20.2$ cm.		$\bar{m} N_2 = 20.7$ cm.
	0.3 cm.	2.1 cm.
$\bar{m} F_3 = 19.9$ cm.		$\bar{m} N_3 = 18.6$ cm.

The two pathogens continued to inhibit extension growth significantly until the end of the experiment; however, no interaction was detected between the two i.e. they affected the plant independently.

d. Final records.

At the end of the experiment the plants were carefully washed from the pots, and measurements of the height and fresh weight of the shoot, and length and fresh weight of the roots were taken.

(i) Increase in height over initial.

Appendix Table 47 shows that the species factor was significant at the 0.1% level. (Fig. 27)

The low rate of G.S.F. inoculum (0.2g. per 10g. of soil) inhibited extension growth ($P = 0.01$) when compared to the control (Table 42).

Experiment 7

Fig.27 Increase in Height Over Initial

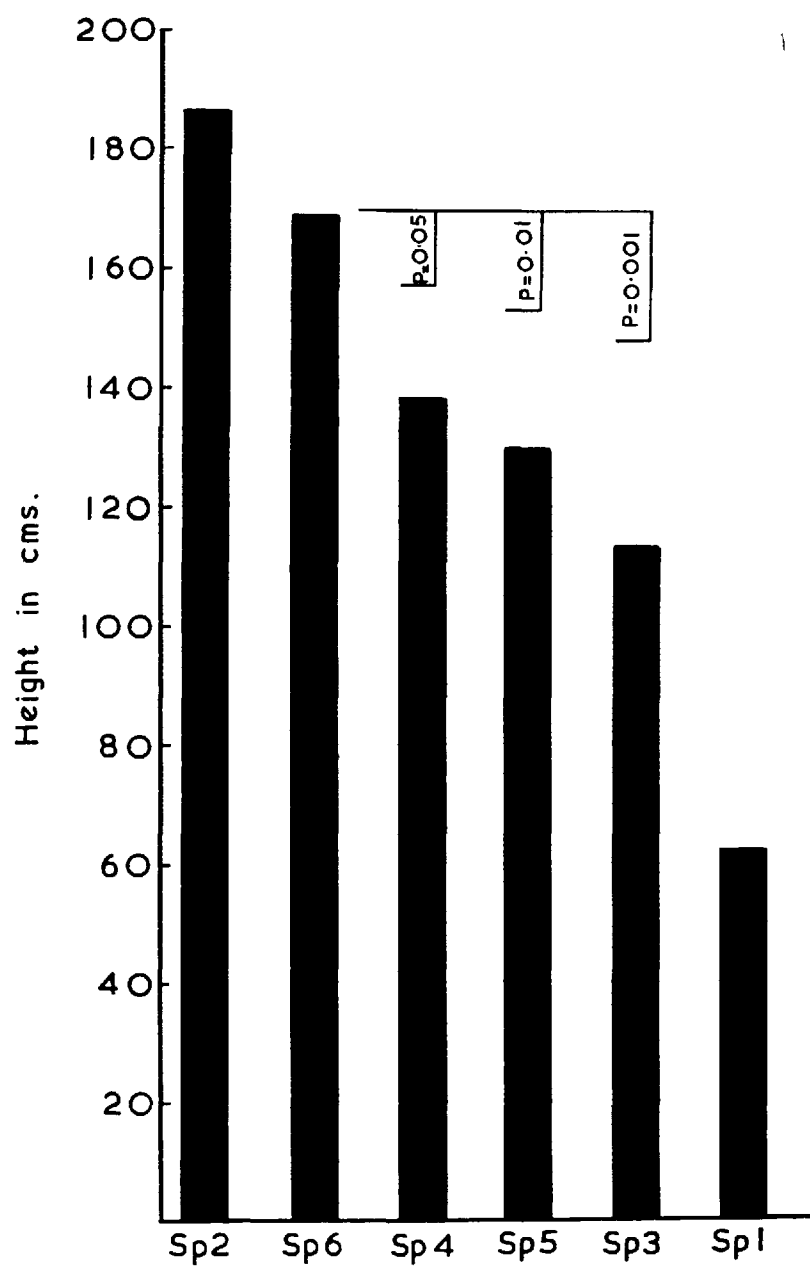


Table 42.

Critical difference when $P = 0.05$, 8.9 cm.

" " when $P = 0.01$, 11.8 cm.

" " when $P = 0.001$, 15.4 cm.

(Mean of 36)

$$\bar{m} F_1 = 139.7 \text{ cm.}$$

7.0 cm.

$$\bar{m} F_3 = 132.7 \text{ cm.}$$

5.7 cm.

$$\bar{m} F_2 = 127.0 \text{ cm.}$$

Table 43 shows that the first order interaction was negative ($P = 0.05$) i.e. each pathogen factor aggravated the effects of the other in inhibiting extension growth.

Table 43.

(Total of 12 replicates)

	N_1	N_2	N_3
F_1	1572.0	1698.5	1758.5
F_2	1623.5	1391.5	1559.0
F_3	1651.5	1577.5	1548.0

(ii) Fresh weight of the shoot.

Analysis of the results in Appendix Table 48 shows that L. osculentum was significantly heavier than the other species. As the entire aerial parts were weighed, the fruit of this species made up a high percentage of the weight; many of the other species did not produce any crop.

(iii) Root length and fresh weight.

Neither the root length nor the fresh weight of the roots were shown to be affected by either pathogen : there was significance between the species only (Appendix Tables 49 and 50).

Discussion.

Although Lycopersicon hirsutum is used as a source of resistance in the breeding of tomato varieties resistant to Brown Root Rot (Bravenboer & Pet 1962, Maxon Smith & Proctor 1965), and the root systems of this species in this experiment showed significantly less visual symptoms of G.S.F. attack than L. esculentum, it was demonstrated that the extension growth of this species was inhibited by the fungus inoculum. The absence of visual symptoms of G.S.F. attack on the roots does not indicate that the species has resistance to Brown Root Rot, but the pathogenic effect on the host may be expressed differently i.e. suppression of extension growth.

The interaction between the two pathogens, P.R.E. and G.S.F., was shown to inhibit the growth of L. glandulosum. This species is also used as a genetic source of resistance to Brown Root Rot (Szteyn 1962).

In addition to these two species of Lycopersicon, Termohlen (1962) stated that L. peruvianum showed a high degree of resistance to G.S.F., however, in this experiment it was demonstrated that the interaction between the nematode and fungus pathogens also inhibited the growth of this species.

Although L. pimpinellifolium is reported to be susceptible to Brown Root Rot (Termohlen 1962), this experiment showed that neither pathogen inhibited the extension growth. However, it must be remembered that the fragmentation of the total data into six parts will decrease the

sensitivity of the analysis,

Only the interaction between the pathogens was shown to affect the extension growth of L. esculentum hort. var. Eurocross A.; the fungus alone had no effect.

Both pathogens suppressed the growth of L. hirsutum var glabratum independently, throughout the period of the experiment.

Mai (1952) and Sembner (1963) report that L. peruvianum, L. glandulosum, L. pimpinellifolium and L. hirsutum are all susceptible to potato root eelworm attack, and Sembner has observed that the roots of these species are galled by P.R.E. (See also Feldmesser 1952). Mai, however, noticed that the susceptibility of L. peruvianum to Heterodera was very variable. McFarlane et al. (1946) noted that L. peruvianum varied in its degree of resistance to Meloidogyne spp.. Mai also observed that L. hirsutum was as heavily attacked as L. esculentum, and that the numbers of nematodes on the roots of L. pimpinellifolium was intermediate between L. hirsutum and L. esculentum. Palo (personal communication) examining the population changes of P.R.E. in relation to Lycopersicon species, found that L. hirsutum was a better host to the nematode than L. esculentum. He showed that the multiplication of the nematode on L. glandulosum was very similar to that on L. esculentum. Finally he found that L. pimpinellifolium and L. peruvianum were not good hosts to Heterodera rostochiensis Woll. The results from my experiment corroborate the observations that all the species of Lycopersicon

used are susceptible to potato root eelworm to a greater or lesser degree. If the results for both Palo's experiment and mine are compared with those of Mai, it would appear that the selection of L. peruvianum we were using was a resistant strain to Heterodera. The discrepancies between my observations and those of Palo are possibly caused by the effects of the fungus on potato root eelworm.

Young (1939); Nusbaum and Chaplin (1952); Jenkins and Coursen (1957); Sasser, Lucas and Powers (1953); and Cohn and Minz (1960) have all found that plant parasitic nematodes can bring about a break-down in resistance to such fungal diseases as Fusarium wilt of tomatoes. Heterodera rostochiensis Woll., therefore, may act in a similar role when the effect of the interaction between it and G.S.F. is observed on L. peruvianum and L. glandulosum.

It is not known which of the pathotypes of Heterodera rostochiensis Woll. postulated by Jones & Parrott (1965) was used in this experiment. Furthermore, information in the literature about the performance of these pathotypes on species of Lycopersicon is lacking. It may well be that the variability in resistance of L. peruvianum to H. rostochiensis, noted by Mai (1952), is explained by differing pathotypes of the nematode infecting L. peruvianum.

122.

S E C T I O N B I I I .

Section B III.

8. Methyl bromide fumigation of soil containing a mixture of G.S.F. and potato root eelworms.

A preliminary experiment was carried out in a commercial glass-house at Reading, Berkshire.

After the experimental areas had been thoroughly cultivated, the fumigations were carried out under polythene sheets (500 gauge). The plots, 17 ft. $\times$ 4.5 ft., were covered by a sheet supported on a longitudinal ridge wire carried on light wooden posts about 2 ft. high, and the edges of the sheet were dug into the soil in order to obtain a gas-tight seal (Fig. 28).

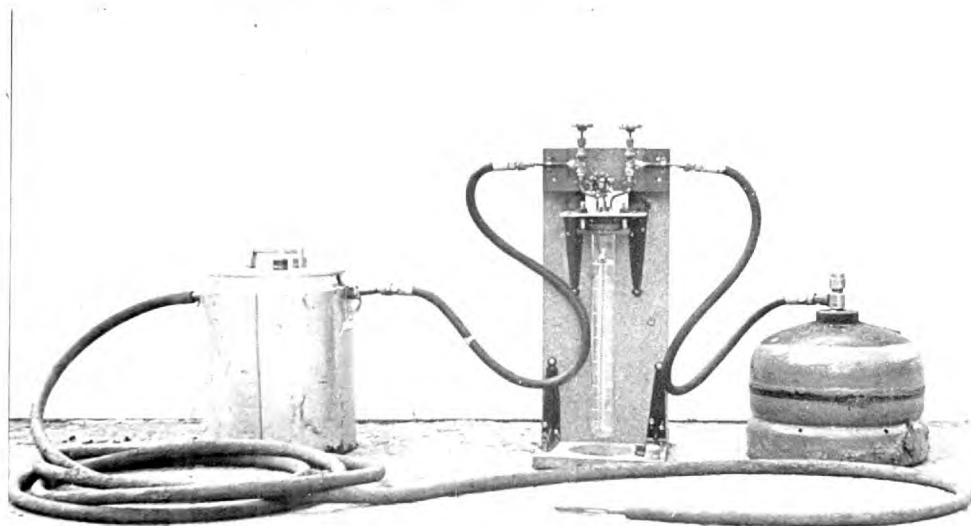
The methyl bromide was measured and vapourised by the apparatus shown in Fig. 29; and consists of a vaporizer (A), a volumetric dosing device (B), and a gas cylinder (C) containing liquid methyl bromide. A full description of this apparatus is given by Hague et al. (1964).

The methyl bromide vapour passes through a length of half-inch neoprene hose to the distributing tubes sited under the sheet. These distributing tubes consist of lengths of $\frac{1}{4}$ inch galvanized gas pipe perforated with 1/16 inch holes at intervals of 3ft., and the pipes are joined by rubber sleeves. To ensure an even distribution of the fumigant in the space enclosed by the sheet equal numbers of lengths of gas pipe joined by rubber sleeves are connected to the two arms of the T-piece. Fig. 30 shows the layout of the apparatus diagrammatically.

Fig. 28. Areas under fumigation in the glasshouse.



Fig. 29. Fumigation apparatus.

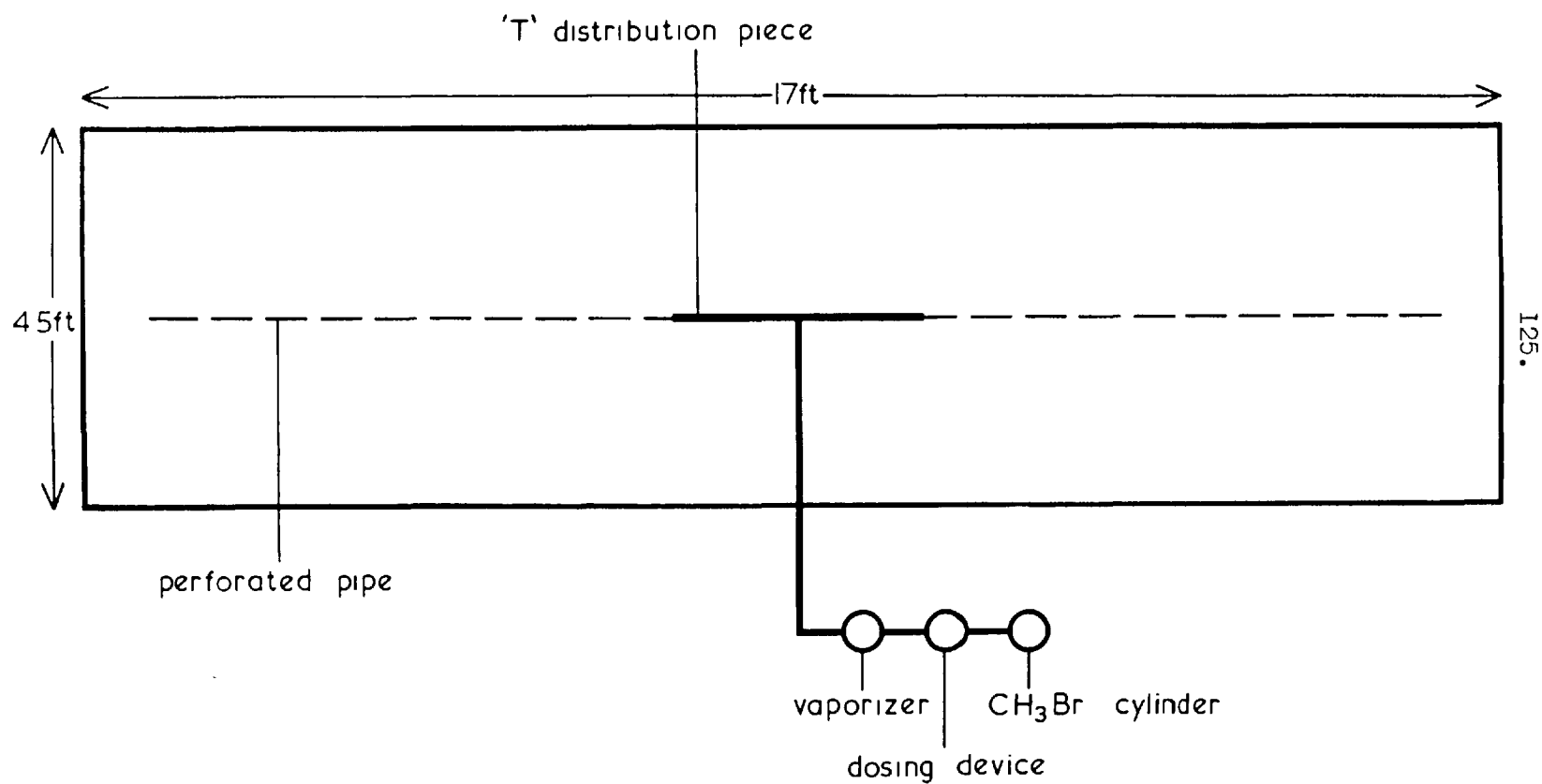


A

B

C

Fig. 30 Plan of fumigation area



The dosage rates are shown in Table 44.

Table 44.

Plot no.	Concentration Ozs/10 cu. ft.	Time of Application Hours
1	16	24
2	8	48
3	2	48
4	4	96
5	2	96
6	8	96
7	Untreated Control	

When the treatments had been completed the polythene sheets were removed, and the soil was left to air for two weeks; then 4 rows of tomatoes were planted, two rows were the variety Eurocross A 'approach-grafted' on to rootstock K - resistant to Brown Root Rot, and the other two rows variety Eurocross A.

An additional twelve tomato plants, variety Eurocross A, were inter-planted in each of the experimental plots for lifting at monthly intervals after planting to assess the degree of both nematode and fungal infestations. Every month two of these plants were lifted with a small spade; the root systems carefully washed free of soil; and a visual estimation was made of the percentage of the whole root system affected by Brown Root Rot, taking the symptoms of chocolate brown lesions and corkiness as evidence of fungal attack. An arbitrary scoring system

was devised as follows:-

0 = 0% : 1 = 1-5% : 2 = 5-25% : 3 = 25-50% : 4 = 50-100%

The root systems were then dried between absorbent tissues and the fresh weight recorded. Each root system was laid out on a cutting board and bands of root pieces cut (Last & Ebben 1963). Unlike the technique described in Section A (page 28), the length of root pieces sampled were 1 inch : the first band of root fragments were taken 3 inches below the first lateral root, and further samples were taken at 2 inch intervals. The thickness of the root pieces were classified into the following size grades:-

0-2 mm., 2-4 mm., 4-6 mm., and >6 mm.

and counted. The root pieces were stored in Formalin-acetic-alcohol as "Clean" or "Brown Root Rot" in their depth grades.

For the assessment of nematode invasion the root pieces were stained in 0.05% cotton blue in lactophenol, using the technique described in Section A (page 25).

Results.

Brown Root Rot.

The visual scoring technique showed that the disease was not controlled by Methyl bromide (Appendix Table 52); and graphing the data of percentage of root pieces showing Brown Root Rot symptoms for each treatment throughout the experiment confirmed the above observations (Fig. 31).

Experiment 8

Fig. 31 Percentage of Root Pieces Affected by Brown Root Rot

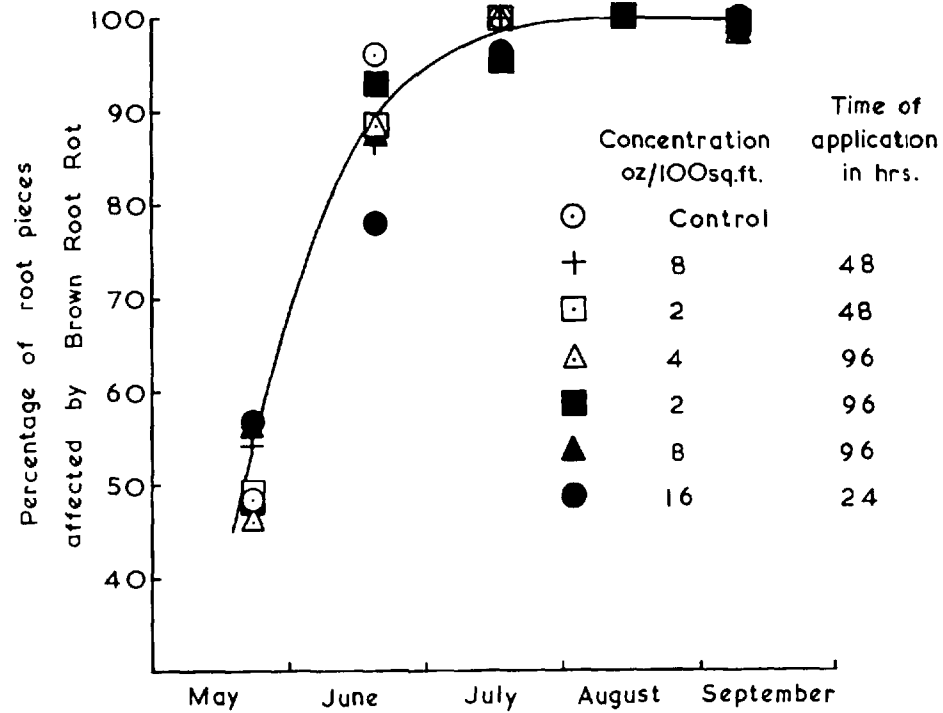


Table 45.

Treatment	Total Cysts Per Pot		Multiplication Rate	
	Before Fumigation	After Fumigation	Before Fumigation	After Fumigation
CONTROL	1223	—	4.9	—
16 oz/24 hr.	804	146	3.2	0.6
8 oz/48 hr.	825	163	3.3	0.6
2 oz/48 hr.	1163	505	4.6	2.0
4 oz/96 hr.	221	159	0.9	0.6
2 oz/96 hr.	437	136	1.7	0.5
8 oz/96 hr.	944	201	3.8	0.8

Estimations were also made of the number of Heterodera cysts per 200 gramme of soil before fumigation and after the tomatoes had finished growing in the greenhouse plots (Table 46).

Table 46.

Treatment	Nematode Cysts/200 g.		Eggs per g. soil	
	Before Fumigation	After Cropping	Before Fumigation	After Cropping
CONTROL	244	188	39	49
16 oz/24 hr.	237	192	14	25
8 oz/48 hr.	244	152	37	35
2 oz/48 hr.	168	144	27	37
4 oz/96 hr.	213	83	30	50
2 oz/96 hr.	233	110	30	37
8 oz/96 hr.	160	79	31	47

Root invasion by the Nematode.

The estimations of the number of nematodes per gramme of roots showed that this pathogen was controlled by Methyl bromide, except in the 2 ozs for 48 hours treatment (Appendix Table 53). As there are many records of 0 nematodes, analysis would yield spurious significance between treatments, therefore no analysis was done on these data. With time the number of nematodes per gramme of root decreases, reflecting the maturation of the Heterodera females (Fig. 32).

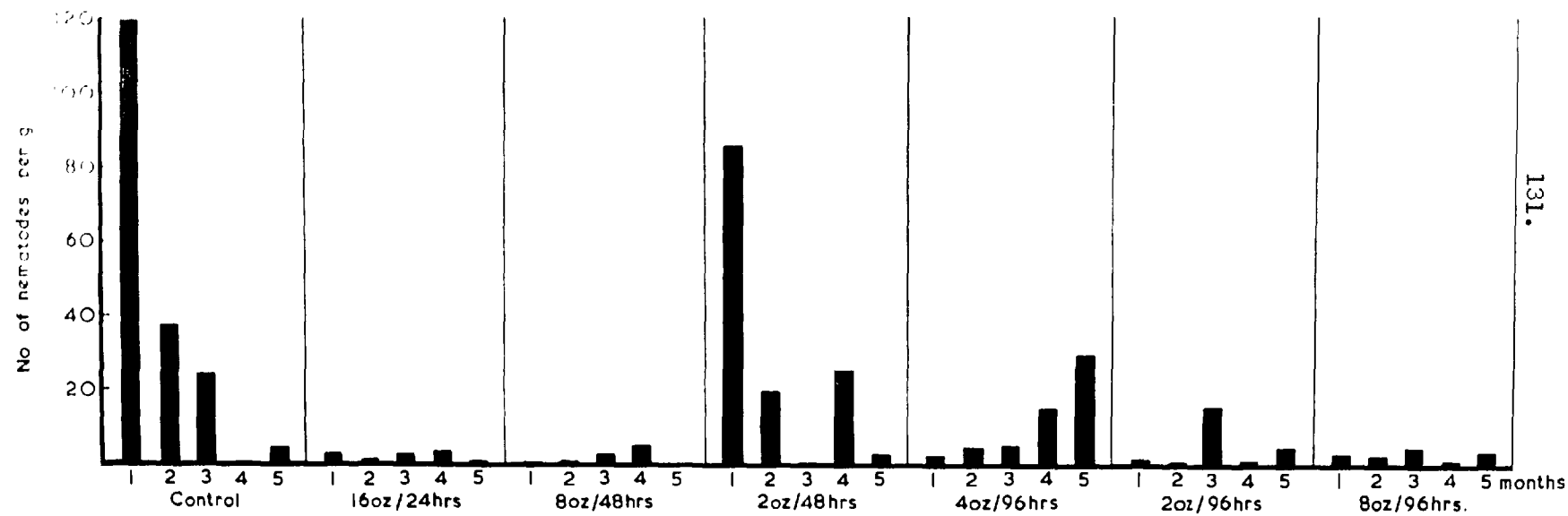
Root Systems.

Analyses of the fresh weights of the root systems taken monthly did not reveal any significant difference between treatments (Appendix Table 54), nor did analyses of the total number of root pieces (Appendix Table 55). Root fragments of the size grade 0-2 mm. are important to the plant, as they are feeder roots or feeder bearers; and an analysis of the number of roots in this grade showed a significant treatment difference in the first month (Fig. 33). This possibly was the result of the increased nutritional status of the soil brought about by Methyl bromide fumigation (Peachey et al. 1963; Nezer 1962; Bald et al. 1958).

Soil samples were taken from each plot both before and after fumigation and the cysts were extracted (Section A, page 16). Batches of 250 cysts were mixed with 800 grammes of sterilised soil in size '48' pots. One potato chit was planted in each pot; and when the haulms had died the cyst population in each pot was estimated (Table 45).

Experiment 8

Fig 32 Number of Nematodes per g of Tomato Root



Experiment 8

Fig.33 Number of Root Pieces 0-2 mm in Thickness

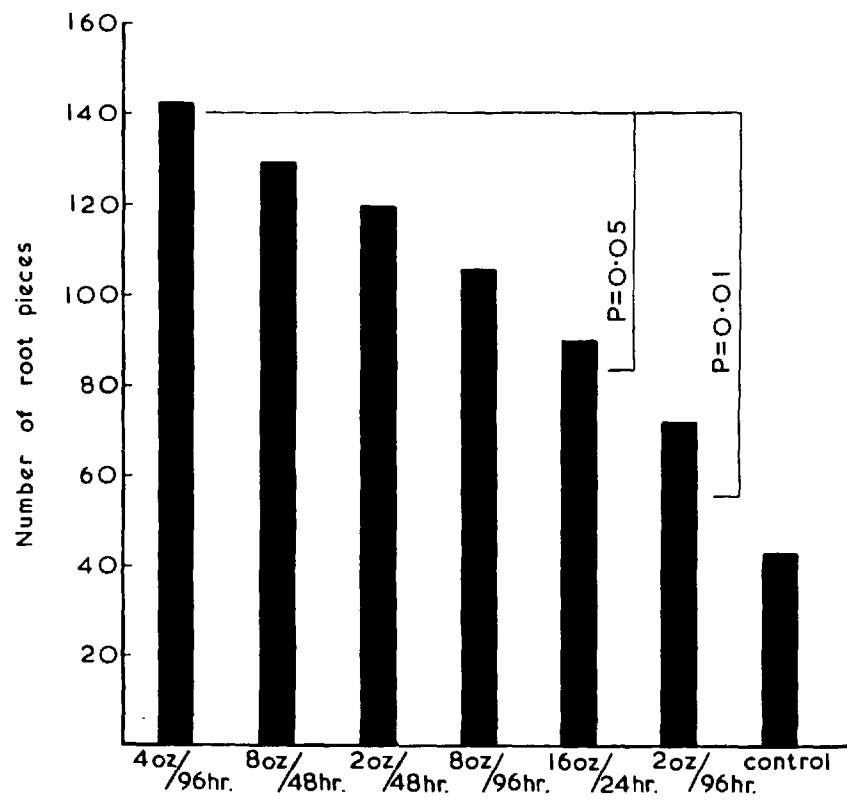


Table 46

~~The previous table~~ shows that potato root eelworms did not increase in number in the glasshouse soil whether Methyl bromide had been applied or not. Comparing this table to table 45 it is shown that, when the cysts were removed from the soil containing a mixture of both the pathogens, development of the nematode carried on as would be expected where Methyl bromide had not been effective.

Such evidence suggests that the fungus inhibits the development of the nematode in some way.

Discussion.

The literature on the control of Heterodera rostochiensis Woll. by fumigation with Methyl bromide is quite comprehensive (Hague 1959 a & b; Peachey et al. 1963; Hague & Sood 1963; and Hague et al. 1964); however, the observations on the control of soil borne fungi are not so complete, and in certain cases are contradictory.

Methyl bromide used as a soil fumigant has been shown to be effective in the control of certain Fusarium sp.. (Newhall & Lear 1948; Swank & Perry 1953; Munnecke & Lindgren 1954; Ciccarone 1955; Cox 1957; Hodges 1960; Munnecke et al. 1960; and Dzidzariya 1962); however, Apt & Gould (1961) demonstrated that Fusarium basal rot of narcissus increased with Methyl bromide treatment of the nursery soil. Munnecke & Lindgren (1954) showed that Verticillium albo-atrum was resistant to Methyl bromide; whilst Thomson & Mcleod (1959) demonstrated that this fumigant controlled a wilt in tobacco seedlings caused by V. dahliae.

Lester (personal communication) has observed that the main success of methyl bromide as a fungicide has been against the Phycomycetes (see also Gustafson 1955; and Segall & López-Matos 1956).

Methyl bromide, has been used against Armillaria mellea - a Basidiomycote (Larue et al. 1962) against anthracnose of tobacco (Colletotrichum tabacum) - Fungi Imperfecti (McDonald et al. 1958), and against Plasmodiophora brassicae (Clubroot) - Myxomycophyta (Winstead & Garriss 1960).

It is, therefore, difficult to see why this fumigant does not control Brown Root Rot of tomatoes, unless the microsclerotia (Termohlen 1962; Preece 1964) are resistant to Methyl bromide.

GENERAL DISCUSSION
AND
CONCLUSIONS

GENERAL DISCUSSION AND CONCLUSIONS.

Although the experiments in this thesis have been reported in separate sections, the results obtained are so inter-related that they are now discussed together.

The invasion of the tomato roots by potato root eelworm did not predispose the host plant to attack by the causal fungus of Brown Root Rot; however, a complex relationship between the nematode and fungal pathogens was recorded.

If the host plant roots were infected with G.S.F. two weeks prior to the addition of a nematode inoculum, the fungus substantially decreased the rate of invasion of the potato root eelworm. Increasing the rate of fungus inoculum resulted in a proportionally significant restriction of the number of nematode larvae entering the roots. This had a marked adverse effect on the number of new cysts and new eggs produced at the end of the experiments. There was also a certain amount of evidence to suggest that the number of new eggs per cyst was reduced by the fungus.

The interaction between the two pathogens restricted the production of new cysts (and new eggs). Why this should be so is difficult to explain; however, the result of this interaction, and also the effect of the fungus inoculum alone on the nematode, is that an apparent 'ceiling' is placed on the final number of cysts produced.

In the Methyl bromide experiment it was shown that the multiplication of the nematode was restricted by some factor (possibly the fungus),

even when no treatment had been applied. Graham (in litt.) has observed that, in commercial glasshouse soils used continually for the production of tomatoes, where both G.S.F. and Heterodera rostochiensis Woll. are present, the population of the nematode pathogen remains at a low level.

The pathogenic effects of the nematode were moderated by the fungus inoculum; however, it was also shown that the interaction of the two pathogens significantly inhibited the extension growth of the host plant.

The reasons for the restriction of nematode reproduction and symptom expression could be accounted for by:-

- a. The fungus producing some substance which affects the development of the nematode in the root.
- b. The fungus making the root less attractive to the nematode.
- c. The fungus producing some substance which affects Heterodera hatch.

It is interesting to note that Laan (1953) reported a fungus parasitizing Heterodera cysts. These cysts, from a sandy soil in Holland, showed black spots consisting of pycnidia of what he thought might be Phoma or Pyrenochaeta. The fungal hyphae could be seen within the cysts intertwining and mummifying the eggs. He recorded that few larvae hatched from the infected eggs. Ebben & Williams (1956) have isolated G.S.F. cultures that were morphologically identical to Pyrenochaeta sp., apart from the absence of pycnidia. Gerlach & Schneider (1964) have

isolated cultures of Pyrenochaeta, which have produced pycnidia both in culture and on tomato roots, and they identify the fungus as P. terrestris.

The causal fungus of Brown Root Rot appears to produce some substance (or substances) in culture which inhibit the hatch of Heterodera rostochiensis eggs.

Although the above evidence is circumstantial, one may conclude that a factor inhibitory to the hatch of potato root eelworm is produced by G.S.F. when it is growing in the soil. The presence of such a substance would explain the low pathogenicity of the potato root eelworm on the growth of the host plant. If the fungus is established on the roots first, there is no moderating action on the nematode's pathogenic effect. It would therefore appear that it is only when G.S.F. is actively colonising the roots of tomato plants that the substance causing a reduction in hatch is produced in quantity.

In nematode/fungal interactions two factors must be considered; firstly, the effect of the competition which is inevitable when two types of pathogen compete for nourishment from the same individual host plant and, secondly, the possible synergistic effects on the two pathogenic components. Pitcher (1965) points out that the obligate plant-parasitic nematode seems especially vulnerable to competition.

The literature on the mechanisms of competition between the two components of a nematode/fungal interaction is somewhat sparse. Christie & Perry (1959) refer to the incompatibility of plant-parasitic nematodes

and many decay promoting organisms. The destruction of giant cells by fungal hyphae (Powell & Nusbaum 1960) obviously undermines nematode nutrition and also prevents maturation of female Meloidogyne spp. (Davis 1962; and Davis & Jenkins 1963). Du Charme (1958) found that secondary fungal invaders decreased the severity of the damage caused by Radopholus similis in the roots of citrus. When the citrus seedlings were grown and inoculated under aseptic conditions, Radopholus remained in the roots, and even after causing extensive damage, they continued to feed and reproduce until extremely large populations were built up. As soon as a nematode lesion was invaded by bacteria or fungi, the nematodes began leaving the root. Populations comparable to those that built up under aseptic conditions were never obtained when seedlings were grown in untreated field soil. Neither was the injury caused to the roots of seedlings grown in field conditions as great as that caused under aseptic conditions. Du Charme concluded that the damage inflicted by this nematode to citrus trees would be much greater were it not for the mitigating effects of other soil organisms.

Henry (1961) observed that the microbial associates of root disease fungi, which abound in the soil, are now recognised to have a marked influence on them and in turn on the development of the diseases caused by them - the most pronounced and common effect being an antagonistic or detrimental one (Dobbs & Hinson 1953; Garrett 1956). If it were not for this widespread fungistatic and/or fungicidal action, root diseases would be more destructive than they are.

Mai (1954), noting that fungi and Actinomycetes produce these substances which are non-phytotoxic yet are lethal to other fungi and bacteria, tested the effect of these substances on the hatch of Heterodera rostochiensis Woll. However, with the techniques he employed and the fungal filtrates used, he noticed no significant inhibition of hatch.

Pitcher (1965) postulates that some nematodes may have an anti-biotic mechanism which helps to keep competitors at bay, leaving them "to enjoy the fruits of their enzymes in peace". It would appear from the results of the experiments reported here that root infecting fungi may have similar substances which enhance their success in infecting host tissues.

It is therefore concluded that examinations of the effects on each other of the component pathogens of an interaction are necessary for the elucidation of the disease syndrome shown by the host plant.

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APPENDIX

APPENDIX.

The following are the abbreviations and symbols used in the Appendix.

df	=	Degrees of Freedom.
S.S	=	Sums of Squares.
M.S	=	Mean Square.
F	=	Variance ratio.
P	=	Level of Probability.
*	=	Significant when $P = 0.05$
**	=	Significant when $P = 0.01$
***	=	Significant when $P = 0.001$
N.S	=	Not significant.
C.D	=	Critical difference.

APPENDIX.Table 1.Experiment 2a.

Treatment	Rep. I	Rep. II	Rep. III	Rep. IV	Mean.
Increase in height over initial (cm.)					
0g	101.0	109.5	94.5	92.5	99.4
0.5g	116.0	114.5	133.5	112.5	119.1
1.0g	127.5	138.0	151.5	158.0	143.7
2.0g	108.0	118.0 *	114.0	127.0	116.7
Fresh weight of aerial parts (g.)					
0g	95.5	70.0	83.5	70.0	79.7
0.5g	177.0	197.0	120.0	183.5	169.4
1.0g	222.0	204.0	237.5	176.5	210.0
2.0g	129.0	152.0 *	115.5	195.0	147.9
Length of roots (cm.)					
0g	37.0	25.0	22.0	38.0	30.5
0.5g	37.0	28.0	29.0	34.0	32.0
1.0g	38.0	34.0	41.0	33.0	36.5
2.0g	32.0	23.0 *	18.0	36.0	27.2
Weight of roots (g.)					
0g	64.0	13.0	19.5	34.0	32.6
0.5g	68.0	37.5	47.5	54.0	51.7
1.0g	103.5	61.0	65.0	91.0	80.1
2.0g	38.0	30.0 *	34.5	88.5	47.7

(* By 'missing plot' technique - plant dead)

Table 2.Experiment 2a.

Treatment	Rep. I	Rep. II	Rep. III	Rep. IV	Mean
Total number of root pieces.					
0g	158	146	358	970	408
0.5g	714	619	510	918	690
1.0g	723	1082	831	1208	961
2.0g	402	396	999	431	557
Percentage of root pieces 0-1 mm in diameter.					
0g	95.7	91.1	87.7	94.7	92.3
0.5g	93.6	91.2	90.6	84.3	89.9
1.0g	88.2	88.5	92.1	92.1	90.2
2.0g	86.3	89.3	91.2	92.5	89.8

Table 3.Experiment 2b.Length of roots in centimetres.

Treatment	Rep. I	Rep. II	Rep. III	Rep. IV
0 e/g	36	50	36	36
34 e/g	50	13	30	26
67 e/g	10	24	28	27
134 e/g	28	33	31	13
<u>Weight of roots in grammes.</u>				
Treatment	Rep. I	Rep. II	Rep. III	Rep. IV
0 e/g	44	34	52	56
34 e/g	31	22	56	38
67 e/g	11	30	46	34
134 e/g	18	47	12	8

Table 4.

Experiment 3a.

Height of shoot in centimetres						
Treatment		Rep. I	Rep. II	Rep. III	Rep. IV	Mean
Control		9.3	9.5	9.9	8.5	9.3
Auto. Fungal Inoc.	0.5 cc	10.1	9.0	9.9	8.2	9.3
	1.0 cc	8.1	8.3	7.1	8.2	7.9
	2.0 cc	8.5	8.6	8.6	9.3	8.7
	4.0 cc	7.1	8.0	9.7	8.6	8.3
Fungal Inoc.	0.5 cc	4.2	5.8	5.4	5.4	5.2
	1.0 cc	3.1	5.9	5.6	5.3	5.0
	2.0 cc	3.9	7.1	7.4	5.9	6.1
	4.0 cc	3.0	8.0	4.2	4.7	5.0
Fresh weight of shoot in milligrammes						
Treatment		Rep. I	Rep. II	Rep. III	Rep. IV	Mean
Control		491	350	401	388	407
Auto. Fungal Inoc.	0.5 cc	490	388	433	377	422
	1.0 cc	383	261	303	403	337
	2.0 cc	341	322	336	343	335
	4.0 cc	167	305	336	387	299
Fungal Inoc.	0.5 cc	127	48	87	42	76
	1.0 cc	101	41	71	80	73
	2.0 cc	133	47	68	58	76
	4.0 cc	39	81	59	34	53
Length of roots in centimetres.						
Treatment		Rep. I	Rep. II	Rep. III	Rep. IV	Mean
Control		6.1	6.5	7.8	8.2	7.1
Auto. Fungal Inoc.	0.5 cc	7.1	7.5	6.1	7.4	7.0
	1.0 cc	4.6	6.4	7.7	5.0	5.9
	2.0 cc	6.1	7.2	3.8	6.0	5.8
	4.0 cc	4.6	6.8	3.5	6.9	5.4
Fungal Inoc.	0.5 cc	5.1	4.0	4.2	4.1	4.3
	1.0 cc	3.8	3.5	4.9	4.4	4.1
	2.0 cc	2.5	1.8	3.7	3.8	2.9
	4.0 cc	1.4	3.8	3.6	4.0	3.2
Fresh weight of roots in milligrammes.						
Treatment		Rep. I	Rep. II	Rep. III	Rep. IV	Mean
Control		371	306	324	329	332
Auto. Fungal Inoc.	0.5 cc	325	355	340	410	357
	1.0 cc	360	362	351	330	351
	2.0 cc	362	462	398	344	391
	4.0 cc	454	451	505	393	451
Fungal Inoc.	0.5 cc	395	190	179	174	234
	1.0 cc	152	134	324	183	198
	2.0 cc	297	276	149	142	216
	4.0 cc	55	227	208	271	187

Table 5.Experiment 3a

Values of the mean as a % of the Controls.				
Treatment	Height of shoot.	Fresh wt. of shoot.	Length of roots.	Fresh wt. of roots.
0.5 cc	100.0	100.0	98.2	100.0
Auto. 1.0 cc	85.2	82.8	82.9	100.0
Fung. 2.0 cc	94.1	82.3	80.8	100.0
Inoc. 4.0 cc	89.8	73.3	76.2	100.0
0.5 cc	55.9	18.6	60.8	70.5
Fung. 1.0 cc	53.5	18.0	58.0	59.6
Inoc. 2.0 cc	65.3	18.8	41.3	65.0
4.0 cc	53.5	13.1	44.7	55.6
Angular transforms.				
Treatment	Height of shoot.	Fresh wt. of shoot.	Length of roots.	Fresh wt. of roots.
0.5 cc	90.00	90.00	84.93	90.00
Auto. 1.0 cc	67.37	65.50	65.57	90.00
Fung. 2.0 cc	75.94	65.12	64.01	90.00
Inoc. 4.0 cc	71.37	58.89	60.80	90.00
0.5 cc	48.39	25.55	51.24	57.10
Fung. 1.0 cc	47.01	25.10	49.60	50.53
Inoc. 2.0 cc	53.91	25.70	39.99	53.73
4.0 cc	47.01	21.22	41.96	48.22

Table 6.Experiment 3b.

ITEM	0 e/g	10 e/g	20 e/g	40 e/g	80 e/g
Height of shoot.	7.3 cm.	7.8 cm.	7.3 cm.	7.8 cm.	7.1 cm.
Fresh wt. shoot.	443 mg.	429 mg.	403 mg.	424 mg.	407 mg.
Dry wt. shoot.	21 mg.	24 mg.	24 mg.	22 mg.	22 mg.
Root length.	6.2 cm.	6.0 cm.	5.5 cm.	5.1 cm.	5.1 cm.
Dry wt. root.	121 mg.	92 mg.	80 mg.	91 mg.	76 mg.

Appendix Table 7.Experiment 4.

Mean height of plants in each Treatment measured every
week (in centimetres).

Treatments	<u>Weeks.</u>																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
$N_0 F_0$	8	17	24	33	35	43	55	69	83	92	100	102	104	106	107	110	114
$N_1 F_0$	9	16	20	25	28	33	38	41	47	52	58	62	66	67	68	70	72
$N_0 F_1$	4	8	13	19	25	41	49	63	83	92	97	100	102	103	104	107	108
$N_1 F_1$	7	13	19	26	31	38	47	58	65	68	74	76	82	85	87	89	91

Table 8.Experiment 4.

Item.	Treatment (mean of 4)			
	N ₀ F ₀	N ₁ F ₀	N ₀ F ₁	N ₁ F ₁
Weight of crop.	701 gm.	146 gm.	1055 gm.	721 gm.
Increase of height over initial.	117 cm.	71 cm.	114 cm.	92 cm.
Fresh wt. aerial parts.	117 gm.	39 gm.	204 gm.	112 gm.
Length of roots.	36 cm.	18 cm.	33 cm.	43 cm.
Fresh wt. of roots.	52 gm.	24 gm.	78 gm.	63 gm.
Total number of root fragments.	906	133	1181	691

Table 9.Experiment 5.Brown Root Rot - Visual Score.

Treatment	Rep. I.	Rep. II.
N ₁ F ₁	0.0	1.2
N ₂ F ₁	1.5	2.5
N ₃ F ₁	2.8	2.9
N ₄ F ₁	3.0	0.0
N ₅ F ₁	2.5	2.5
N ₁ F ₂	3.0	2.3
N ₂ F ₂	2.3	3.1
N ₃ F ₂	2.1	3.1
N ₄ F ₂	2.5	2.9
N ₅ F ₂	2.4	3.1
N ₁ F ₃	2.8	3.9
N ₂ F ₃	2.8	3.9
N ₃ F ₃	2.8	3.6
N ₄ F ₃	2.9	3.3
N ₅ F ₃	3.1	3.2
N ₁ F ₄	4.1	3.2
N ₂ F ₄	3.9	2.5
N ₃ F ₄	3.5	5.5
N ₄ F ₄	3.2	5.1
N ₅ F ₄	3.9	3.2
N ₁ F ₅	4.2	5.8
N ₂ F ₅	5.0	3.9
N ₃ F ₅	3.9	3.5
N ₄ F ₅	4.3	3.0
N ₅ F ₆	3.1	6.0

Table 10.Experiment 5.Number of G.S.F. colonies developing in one week.

Treatment	Rep. I.	Rep. II.
N ₁ F ₁	0	0
N ₂ F ₁	0	0
N ₃ F ₁	0	0
N ₄ F ₁	0	0
N ₅ F ₁	0	0
N ₁ F ₂	22	5
N ₂ F ₂	3	19
N ₃ F ₂	3	5
N ₄ F ₂	9	22
N ₅ F ₂	5	20
N ₁ F ₃	3	29
N ₂ F ₃	9	8
N ₃ F ₃	4	8
N ₄ F ₃	6	51
N ₅ F ₃	1	38
N ₁ F ₄	24	2
N ₂ F ₄	13	4
N ₃ F ₄	63	1
N ₄ F ₄	46	23
N ₅ F ₄	30	8
N ₁ F ₅	12	26
N ₂ F ₅	86	27
N ₃ F ₅	30	32
N ₄ F ₅	12	18
N ₅ F ₅	41	16

Table 11.Experiment 5.Height of shoot in centimetres.

Treatment	Rep. I	Rep. II	Rep. III	Rep. IV	Total
N ₁ F ₁	9.0	8.3	9.2	11.6	38.1
N ₂ F ₁	5.7	8.1	7.6	7.6	29.0
N ₃ F ₁	9.2	7.1	7.3	7.1	30.7
N ₄ F ₁	10.5	9.3	7.8	4.9	32.5
N ₅ F ₁	7.2	8.2	7.0	6.9	29.3
N ₁ F ₂	8.5	7.2	7.3	5.8	28.8
N ₂ F ₂	9.0	7.4	7.3	8.1	31.8
N ₃ F ₂	7.6	7.5	7.0	6.1	28.2
N ₄ F ₂	8.1	6.7	7.1	6.7	28.6
N ₅ F ₂	9.5	6.1	7.5	7.4	30.5
N ₁ F ₃	7.0	6.8	7.3	9.0	30.1
N ₂ F ₃	6.4	5.7	7.7	7.6	27.4
N ₃ F ₃	7.5	5.0	6.8	6.5	25.8
N ₄ F ₃	8.1	4.4	5.9	7.3	25.7
N ₅ F ₃	8.1	7.5	8.3	6.2	30.1
N ₁ F ₄	6.8	5.5	5.6	5.7	23.6
N ₂ F ₄	4.6	6.8	6.0	6.8	24.2
N ₃ F ₄	5.9	7.5	4.9	5.4	23.7
N ₄ F ₄	6.6	5.1	4.8	5.6	22.1
N ₅ F ₄	4.4	6.0	5.4	4.6	20.4
N ₁ F ₅	4.5	4.5	6.3	6.1	21.4
N ₂ F ₅	4.1	3.8	4.7	4.6	17.2
N ₃ F ₅	5.2	3.5	6.2	6.1	21.0
N ₄ F ₅	5.3	5.1	3.2	5.2	18.8
N ₅ F ₅	10.6	4.2	5.0	5.2	25.0
	179.4	157.3	163.2	164.1	664.0

Correction factor = 4408.96

Table 11 cont.

	N ₁	N ₂	N ₃	N ₄	N ₅	Total F.
F ₁	38.1	29.0	30.7	32.5	29.3	159.6
F ₂	28.8	31.8	28.2	28.6	30.5	147.9
F ₃	30.1	27.4	25.8	25.7	30.1	139.1
F ₄	23.6	24.2	23.7	22.1	20.4	114.0
F ₅	21.4	17.2	21.0	18.8	25.0	103.4
Total N.	142.0	129.6	129.4	127.7	135.3	664.0

Items	df	S.S.	M.S.	F.	P.
N	4	6.93	1.73	1.14	N.S.
F	4	110.19	27.55	18.15	***
NF	16	25.16	1.57	1.03	N.S.
Residual.	75	113.82	1.52		

Total. 99

Table 12.Experiment 5.Length of root in centimetres.

Treatments	Rep. I	Rep. II	Rep. III	Rep. IV	Total
N ₁ F ₁	5.0	3.4	3.7	7.5	19.6
N ₂ F ₁	7.4	6.3	3.7	3.5	20.9
N ₃ F ₁	3.5	4.5	3.8	5.1	16.9
N ₄ F ₁	2.2	6.0	5.8	4.5	18.5
N ₅ F ₁	5.5	3.8	3.9	4.6	17.8
N ₁ F ₂	3.8	3.4	3.3	2.5	13.0
N ₂ F ₂	3.1	4.1	2.7	2.5	12.4
N ₃ F ₂	5.5	5.5	2.8	2.5	16.3
N ₄ F ₂	4.7	4.1	3.8	4.6	17.2
N ₅ F ₂	3.3	4.0	3.7	3.0	14.0
N ₁ F ₃	4.5	2.6	3.0	4.0	14.1
N ₂ F ₃	6.1	2.0	2.1	3.7	13.9
N ₃ F ₃	4.2	3.5	3.2	3.2	14.1
N ₄ F ₃	3.5	6.9	2.9	3.1	16.4
N ₅ F ₃	2.8	3.2	2.1	2.9	11.0
N ₁ F ₄	2.0	6.9	3.0	5.0	16.9
N ₂ F ₄	4.4	3.7	3.3	2.0	13.4
N ₃ F ₄	3.3	2.0	3.4	2.5	11.2
N ₄ F ₄	4.3	3.1	3.7	2.5	13.6
N ₅ F ₄	5.7	2.4	3.0	5.1	16.2
N ₁ F ₅	5.2	2.8	2.7	2.0	12.7
N ₂ F ₅	4.1	3.4	1.8	2.7	12.0
N ₃ F ₅	3.5	6.5	2.7	3.5	16.2
N ₄ F ₅	3.8	5.3	6.3	2.6	18.0
N ₅ F ₅	2.5	2.1	3.1	1.6	9.3
	103.9	101.5	83.5	86.7	375.6

Correction factor = 1410.75

Table 12 cont.

	N ₁	N ₂	N ₃	N ₄	N ₅	Total F.
F ₁	19.6	20.9	16.9	18.5	17.8	93.7
F ₂	13.0	12.4	16.3	17.2	14.0	72.9
F ₃	14.1	13.9	14.1	16.4	11.0	69.5
F ₄	16.9	13.4	11.2	13.6	16.2	71.3
F ₅	12.7	12.0	16.2	18.0	9.3	68.2
Total N.	76.3	72.6	74.7	83.7	68.3	375.6

Items	df	S.S.	M.S.	F.	P.
N	4	6.41	1.60	< 1	N.S
F	4	22.21	5.55	3.32	*
NF	16	21.37	1.34	< 1	N.S
Residual.	75	125.60	1.67		

Total. 99 175.59

Table 13.Experiment 5.Height of shoot.

	Measurement	% of Control	Angle.
F ₁	7.98		
F ₂	7.39	92.6	74.21
F ₃	6.95	87.1	68.95
F ₄	5.70	71.4	57.67
F ₅	5.17	64.8	53.61

Length of roots.

	Measurement	% of Control	Angle
F ₁	4.68		
F ₂	3.64	77.8	61.89
F ₃	3.56	76.1	60.73
F ₄	3.47	74.1	59.41
F ₅	3.41	72.9	58.63

Table 14.Experiment 5.Regression Analysis.Length of root.

	1	2	3	4	Σ
y	61.89	60.73	59.41	58.63	240.66
x	0.50	1.00	2.00	4.00	7.50
xy	30.94	60.73	118.82	234.62	

Sum of squares accountable by regression line

$$= \frac{\left[\Sigma (y - \bar{y})(x - \bar{x}) \right]^2}{\Sigma (x - \bar{x})^2}$$

$$= 5.40$$

$$\text{Total sum of squares} = \Sigma (y - \bar{y})^2 = 6.22$$

Items	df	S.S.	M.S.	F.	P.
Regression	1	5.40	5.40	38.57	***
Residual	6	0.82	0.14		
Total.	7	6.22			

Height of shoot.

	1	2	3	4	Σ
y	74.21	68.95	57.67	53.61	254.44
x	0.50	1.00	2.00	4.00	7.50
xy	37.10	68.95	115.34	214.44	

Sum of squares accountable by regression line

$$= \left[\frac{\sum (y - \bar{y})(x - \bar{x})}{(x - \bar{x})^2} \right]^2$$

$$= 236.54$$

$$\text{Total sum of squares} = \sum (y - \bar{y})^2 = 276.16$$

Items	df	S.S.	M.S.	F.	P.
Regression	1	236.54	236.54	35.84	***
Residual	6	39.62	6.60		
Total.	7	276.16			

Comparison of Regression lines.

Fungus rates.

Lines	0.50	1.00	2.00	4.00	7.50
Length of root.	61.89	60.73	59.41	58.63	240.66
Height of shoot.	74.21	68.95	57.67	53.61	254.44

Items	df	S.S.	M.S.	F.	P.
Common Regression line	1	156.90	156.90	15.52	*
Difference between Common line and Separate line	1	85.04	85.04	8.41	*
Difference in Intercepts	1	23.74	23.74	2.35	N.S.
Residual	4	40.44	10.11		

Total.

7. . .

Table 15.Experiment 5.Dry weight of shoot in milligrammes.

Treatment	Rep. I	Rep. II	Rep. III	Rep. IV	Total
N ₁ F ₁	11	10	16	11	48
N ₂ F ₁	10	15	16	11	52
N ₃ F ₁	11	14	18	3	46
N ₄ F ₁	11	11	14	50	86
N ₅ F ₁	17	17	12	22	68
N ₁ F ₂	13	18	6	23	60
N ₂ F ₂	27	5	6	17	55
N ₃ F ₂	16	9	4	0	29
N ₄ F ₂	9	10	3	27	49
N ₅ F ₂	14	10	20	13	57
N ₁ F ₃	7	6	8	8	29
N ₂ F ₃	6	3	5	6	20
N ₃ F ₃	11	6	1	25	43
N ₄ F ₃	38	23	8	17	86
N ₅ F ₃	28	17	7	74	126
N ₁ F ₄	25	11	7	8	51
N ₂ F ₄	3	34	14	18	69
N ₃ F ₄	3	11	3	3	20
N ₄ F ₄	8	3	8	12	31
N ₅ F ₄	1	9	8	10	28
N ₁ F ₅	6	1	0	10	17
N ₂ F ₅	1	5	1	28	35
N ₃ F ₅	3	1	0	6	10
N ₄ F ₅	1	14	3	8	26
N ₅ F ₅	26	2	0	19	47
	306	265	188	429	1188

Correction factor = 14113.44

Items	df	S.S.	M.S.	F.	P.
N	4	929.06	232.26	2.24	N.S.
F	4	1023.66	255.91	2.47	N S.
NF	16	2090.84	130.68	1.26	N.S.
Residual	75	7761.00	103.48		
Total.	99	11804.56			

Table 16.Experiment 5. Fresh weight of roots in milligrammes.

(The 2 replicates used for the assessment of fungus invasion of the roots.)

Treatment	Rep. I	Rep. II	Total
N ₁ F ₁	193	129	322
N ₂ F ₁	162	152	314
N ₃ F ₁	78	346	424
N ₄ F ₁	86	119	205
N ₅ F ₁	224	147	371
N ₁ F ₂	138	153	291
N ₂ F ₂	175	112	287
N ₃ F ₂	207	165	372
N ₄ F ₂	206	171	377
N ₅ F ₂	172	188	360
N ₁ F ₃	384	64	448
N ₂ F ₃	156	106	262
N ₃ F ₃	163	142	305
N ₄ F ₃	271	234	505
N ₅ F ₃	238	111	349
N ₁ F ₄	169	268	437
N ₂ F ₄	143	292	435
N ₃ F ₄	125	42	167
N ₄ F ₄	1367	64	1431
N ₅ F ₄	253	247	500
N ₁ F ₅	261	128	389
N ₂ F ₅	204	140	344
N ₃ F ₅	99	276	375
N ₄ F ₅	205	272	477
N ₅ F ₅	242	34	276
	5921	4102	10023

Correction factor = 2009210.0

Items	df	S.S.	M.S.	F.	P.
N	4	127904.3	3197.61	< 1	N.S.
F	4	120774.7	3019.37	< 1	N.S.
NF	16	391068.5	24441.78	< 1	N.S.
Residual	25	1025459.5	41018.38		
Total.	49	1665207.0			

Table 17.Experiment 5. Fresh weight of roots in milligrammes.

(The 2 replicates used for nematode determinations.)

Treatment	Rep. I	Rep. II	Total
N ₁ F ₁	59	42	101
N ₂ F ₁	63	72	135
N ₃ F ₁	65	55	120
N ₄ F ₁	134	72	206
N ₅ F ₁	73	66	139
N ₁ F ₂	66	45	111
N ₂ F ₂	66	52	118
N ₃ F ₂	43	56	99
N ₄ F ₂	53	139	192
N ₅ F ₂	67	95	162
N ₁ F ₃	111	107	218
N ₂ F ₃	50	36	86
N ₃ F ₃	47	90	137
N ₄ F ₃	69	80	149
N ₅ F ₃	44	102	146
N ₁ F ₄	143	69	212
N ₂ F ₄	84	114	198
N ₃ F ₄	10	43	53
N ₄ F ₄	83	48	131
N ₅ F ₄	145	80	225
N ₁ F ₅	21	31	52
N ₂ F ₅	19	56	75
N ₃ F ₅	23	13	36
N ₄ F ₅	101	44	145
N ₅ F ₅	87	86	173
	1726	1693	3419

Correction factor = 233791.2

Items	df	S.S.	M.S.	F.	P.
N	4	10764.7	2691.17	3.67	*
F	4	6243.1	1560.77	2.13	N.S.
NF	16	17333.5	1083.34	1.48	N.S.
Residual	25	18314.5	732.58		
Total.	49				

Appendix Table 18.Experiment 6a.The number of nematodes per gramme fresh weight of roots.

TIME	I	II	III	IV	V	VI	VII	VIII	IX	X	TOTAL
8 days A	404	361	340	333	524	487	375	167	167	413	3571
B	280	344	408	324	922	758	370	1500	1678	1162	7746
16 days A	787	721	502	538	739	585	879	920	683	464	6818
B	3253	1854	1240	961	1826	2848	2012	2102	1191	1244	18531
32 days A	271	340	428	227	274	145	289	294	235	303	2866
B	631	610	489	412	698	384	435	480	447	792	5378

A = P.R.E. + G.S.F.

B = P.R.E. + Vermiculite

Appendix Table 19.

Experiment 6a.

Fresh weight of root systems in grammes.

TIME		I	II	III	IV	V	VI	VII	VIII	IX	X	XI
4 days	A	0.053	0.047	0.049	0.039	0.048	0.048	0.036	0.031	0.042	0.050	0.443
	B	0.047	0.036	0.053	0.051	0.039	0.042	0.031	0.032	0.040	0.048	0.419
8 days	A	0.075	0.096	0.103	0.111	0.077	0.062	0.073	0.036	0.090	0.074	0.797
	B	0.047	0.036	0.053	0.039	0.042	0.113	0.080	0.156	0.078	0.080	0.724
16 days	A	0.353	0.262	0.313	0.398	0.383	0.253	0.206	0.275	0.316	0.304	3.063
	B	0.269	0.335	0.379	0.435	0.340	0.158	0.161	0.254	0.361	0.225	2.917
32 days	A	1.066	0.911	0.769	1.012	1.059	1.328	0.862	0.833	0.882	0.817	9.539
	B	0.689	0.779	0.755	0.871	0.625	0.797	0.825	0.783	0.646	0.539	7.309

32nd day.

Correction factor = 14.19

Items	df	S S.	M.S.	F.	P.
Treatments	1	0.25	0.25	12.50	**
Residual	18	0.35	0.02		
Total	19				

Table 20.Experiment 6b.Number of cysts per 200 g. of soil.

Correction factor = 24661.3

Items	df	S.S.	M.S.	F.	P.
Treatments	2	2183.2	1091.60	6.55	*
Residual	9	1499.5	166.61		
Total.	11	3682.7			

Table 21.Experiment 6b.Number of eggs per cyst.

Correction factor = 69251.2

Items	df	S.S.	M.S.	F.	P.
Treatments	2	7598.2	3799.10	3.45	N.S.
Residual	9	9920.5	1102.28		
Total	11	17518.7			

Table 22.Experiment 6b.Log. FINAL EGG DENSITY - Log. INITIAL EGG DENSITY.

Treatment		Log F - Log i	Add 1 Throughout
Nematode Inoculum First.	Rep. I	0.37	1.37
	Rep. II	0.45	1.45
	Rep. III	0.37	1.37
	Rep. IV	0.00	1.00
Fungus Inoculum First.	Rep. I	0.34	1.34
	Rep. II	0.31	1.31
	Rep. III	0.90	1.90
	Rep. IV	0.72	1.72
Both Inocula Together.	Rep. I	1.21	0.21
	Rep. II	1.68	0.68
	Rep. III	0.03	1.03
	Rep. IV	0.17	1.17

Correction factor = 17.64

Items	df	S.S.	M.S.	F.	P.
Treatments	2	1.31	0.655	6.42	*
Residual	9	0.92	0.102		
Total.	11				

Critical difference when P = 0.05, 0.51.

when P = 0.01, 0.73

when P = 0.001, 1.08

Table 23.Experiment 6b.Visual score of Brown Root Rot symptoms.

Treatment	Rep. I	Rep. II	Rep. III	Rep. IV
Control	1.3	0.8	0.5	2.5
Nematode 1st.	4.5	2.9	4.5	3.3
Fungus 1st.	3.9	4.5	4.1	4.5
Both	3.9	3.0	3.0	4.0

Correction factor = 163.8

Items	df	S.S.	M.S.	F.	P.
Treatments	3	21.0	7.00	14.8	***
Residual	12	5.6	0.47		
Total.	15	26.6			

Critical difference when $P = 0.05$, 1.0
 when $P = 0.01$, 1.5
 when $P = 0.001$, 2.1

Table 24.

Experiment 6b.

Crop yield in grammes.				
Treatments	Rep. I	Rep. II	Rep. III	Rep. IV
Control	244	344	407	256
Nematode 1st.	347	299	169	377
Fungus 1st.	164	248	287	259
Both	294	326	355	246
Increase in height over initial in centimetres.				
Treatments	Rep. I	Rep. II	Rep. III	Rep. IV
Control	79	62	61	72
Nematode 1st.	79	76	79	75
Fungus 1st.	81	62	65	79
Both	97	84	72	66
Root length in centimetres.				
Treatments	Rep. I	Rep. II	Rep. III	Rep. IV
Control	60	37	43	35
Nematode 1st.	41	33	39	35
Fungus 1st.	31	49	26	54
Both	30	42	41	44
Fresh weight of roots in grammes.				
Treatments	Rep. I	Rep. II	Rep. III	Rep. IV
Control	41	46	41	43
Nematode 1st.	35	43	37	29
Fungus 1st.	35	46	20	27
Both	42	52	29	36

Table 25.Experiment 6b.Fresh weight of Aerial parts in grammes.

Treatment	Rep. I	Rep. II	Rep. III	Rep. IV	Total
Control	66.9	55.2	57.4	52.3	231.8
Nematode 1st.	50.4	50.5	56.8	50.8	208.5
Fungus 1st.	63.4	61.9	54.2	65.2	244.7
Both	83.6	64.7	57.2	92.1	297.6
	264.3	232.3	225.6	260.4	982.6

Correction factor = 60343.9

Items	df	S.S.	M.S.	F.	P.
Treatments	3	1067.9	355.97	4.25	*
Residual	12	1007.3	83.94		
Total	15	2075.2			

Critical difference when $P = 0.05$, 14.1when $P = 0.01$, 19.8when $P = 0.001$, 28.0

Table 26.Experiment 6c.Total number of nematodes per root system.

Correction factor = 866.4

Items	df	S.S.	M.S.	F.	P.
Treatments	2	209.2	104.60	4.23	*
Residual	12	296.4	24.70		
Total.	14	505.6			

Critical difference when $P = 0.05$, 6.85
 when $P = 0.01$, 9.60
 when $P = 0.001$, 13.57

$\bar{m}$ Nematode 1st. = 11.00
 $\bar{m}$ Fungus 1st. = 9.40
 $\bar{m}$ Both = 2.40

Table 27.Experiment 7.Root Rot Assessment.

Species	Treatment	Rep. I	Rep. II	Total
L. esculentum Eurocross A. Sp. 1.	N ₁ F ₁	0.5	3.0	3.5
	N ₂ F ₁	0.3	0.1	0.4
	N ₃ F ₁	0.1	0.5	0.6
	N ₁ F ₂	2.0	1.0	3.0
	N ₂ F ₂	1.8	4.0	5.8
	N ₃ F ₂	2.1	2.5	4.6
	N ₁ F ₃	1.2	2.5	3.7
	N ₂ F ₃	2.2	1.0	3.2
	N ₃ F ₃	4.2	3.2	7.4
L. pimpinellifolium Sp. 2.	N ₁ F ₁	0.0	0.1	0.1
	N ₂ F ₁	0.1	0.3	0.4
	N ₃ F ₁	0.1	0.2	0.3
	N ₁ F ₂	0.3	0.5	0.8
	N ₂ F ₂	0.9	0.9	1.8
	N ₃ F ₂	1.0	0.5	1.5
	N ₁ F ₃	0.0	0.8	0.8
	N ₂ F ₃	0.5	0.9	1.4
	N ₃ F ₃	0.6	0.8	1.4
L. hirsutum Sp. 3.	N ₁ F ₁	0.1	0.1	0.2
	N ₂ F ₁	0.0	0.1	0.1
	N ₃ F ₁	0.1	0.0	0.1
	N ₁ F ₂	0.8	0.1	0.9
	N ₂ F ₂	0.2	0.2	0.4
	N ₃ F ₂	0.1	1.0	1.1
	N ₁ F ₃	0.1	0.1	0.2
	N ₂ F ₃	0.2	0.1	0.3
	N ₃ F ₃	1.0	0.3	1.3

Table 27 cont.

Species	Treatment	Rep. I	Rep. II	Total
L. glandulosum	N ₁ F ₁	0.2	0.3	0.5
	N ₂ F ₁	0.0	0.2	0.2
	N ₃ F ₁	0.3	0.2	0.5
	N ₁ F ₂	1.0	0.1	1.1
	N ₂ F ₂	0.1	0.5	0.6
	N ₃ F ₂	3.0	0.5	3.5
	N ₁ F ₃	0.1	0.2	0.3
	N ₂ F ₃	0.0	0.9	0.9
	N ₃ F ₃	1.0	0.3	1.3
L. peruvianum Sp. 5.	N ₁ F ₁	4.2	0.3	4.5
	N ₂ F ₁	0.0	0.3	0.3
	N ₃ F ₁	0.3	0.0	0.3
	N ₁ F ₂	1.5	0.3	1.8
	N ₂ F ₂	1.0	0.6	1.6
	N ₃ F ₂	0.5	0.2	0.7
	N ₁ F ₃	0.0	0.3	0.3
	N ₂ F ₃	2.8	0.1	2.9
	N ₃ F ₃	1.8	0.1	1.9
L. hirsutum var glabratum. Sp. 6.	N ₁ F ₁	0.0	0.0	0.0
	N ₂ F ₁	0.2	0.2	0.4
	N ₃ F ₁	0.3	0.1	0.4
	N ₁ F ₂	0.2	0.3	0.5
	N ₂ F ₂	0.0	0.1	0.1
	N ₃ F ₂	0.5	0.8	1.3
	N ₁ F ₃	0.5	0.0	0.5
	N ₂ F ₃	0.5	0.2	0.7
	N ₃ F ₃	2.8	1.3	4.1
		43.3	33.2	76.5

Table 27 cont.

Items	df	S.S.	M.S.	F.	P.
Species	5	27.92	5.58	3.62	*
N	2	1.94	0.97	< 1	N.S.
F	2	6.75	3.37	2.18	N.S.
NF	4	7.39	0.45	< 1	N.S.
Sp x N	10	4.53	0.78	< 1	N.S.
Sp x F	10	7.77	1.85	1.20	N.S.
Sp x N x F	20	12.44	0.62	< 1	N.S.
Pairs	53	68.74			
Error	54	83.33	1.54		
Total.	107	152.07			

Critical difference when $P = 0.05$, 1.44

$\bar{m}$ Sp 1 = 1.79

$\bar{m}$ Sp 5 = 0.79

$\bar{m}$ Sp 4 = 0.49

$\bar{m}$ Sp 2 = 0.47

$\bar{m}$ Sp 6 = 0.44

$\bar{m}$ Sp 3 = 0.25

Table 28.

Experiment 7.New cysts.

Treatment		Initial Cyst Inocu. per 200 g.	Final Cyst No/200g.		New Cysts		Total
			Rep. I	Rep.III	Rep. I	Rep. II	
L. esculentum Sp. 1	F ₁	12.5	65	40	52.5	27.5	80.0
	F ₁	50	315	185	265.0	135.0	400.0
	F ₂	12.5	105	215	92.5	202.5	295.0
	F ₂	50	85	500	35.0	450.0	485.0
	F ₃	12.5	95	210	82.5	197.5	280.0
	F ₃	50	250	65	200.0	15.0	215.0
L.pimpinellifolium Sp2	F ₁	12.5	95	230	82.5	217.5	300.0
	F ₁	50	565	375	515.0	325.0	840.0
	F ₂	12.5	195	110	182.5	97.5	280.0
	F ₂	50	75	310	25.0	260.0	285.0
	F ₃	12.5	35	285	22.5	272.5	295.0
	F ₃	50	85	260	35.0	210.0	245.0
L. hirsutum Sp. 3	F ₁	12.5	30	130	17.5	117.5	135.0
	F ₁	50	265	615	215.0	565.0	780.0
	F ₂	12.5	75	260	62.5	247.5	310.0
	F ₂	50	190	110	140.0	60.0	200.0
	F ₃	12.5	95	60	82.5	47.5	130.0
	F ₃	50	235	465	185.0	415.0	600.0

Table 28 cont.

Treatment		Initial Cyst Inocu. per 200 g.	Final Cyst No/200 g.		New Cysts		Total
			Rep. I	Rep. II	Rep. I	Rep. II	
<i>L. glandulosus</i> Sp. 4	F ₁	12.5	150	50	137.5	37.5	175.0
	F ₁	50	500	575	450.0	525.0	975.0
	F ₂	12.5	90	95	77.5	82.5	160.0
	F ₂	50	300	335	250.0	285.0	535.0
	F ₃	12.5	100	135	87.5	122.5	210.0
	F ₃	50	130	125	80.0	75.0	155.0
<i>L. peruvianum</i> Sp. 5	F ₁	12.5	105	95	92.5	82.5	175.0
	F ₁	50	120	360	70.0	310.0	380.0
	F ₂	12.5	25	20	12.5	7.5	20.0
	F ₂	50	55	175	5.0	125.0	130.0
	F ₃	12.5	35	95	22.5	82.5	105.0
	F ₃	50	200	160	150.0	110.0	260.0
<i>L. hirsutum</i> var <i>glabratum</i> Sp. 6	F ₁	12.5	65	315	52.5	302.5	355.0
	F ₁	50	650	1180	600.0	1130.0	1730.0
	F ₂	12.5	20	55	7.5	42.5	50.0
	F ₂	50	215	410	165.0	360.0	525.0
	F ₃	12.5	280	235	267.5	222.5	490.0
	F ₃	50	60	235	10.0	185.0	195.0
					4830.0	7950.0	12780.0

Items	df	S.S.	M.S.	F.	P.
Sp.	5	230058	46011.6	2.77	*
N	1	359835	359835.0	21.64	***
F	2	266702	133351.0	8.02	**
NF	2	315617	157808.5	9.49	***
Sp x N	5	85698	17139.6	1.03	N.S.
Sp x F	10	278302	27830.2	1.67	N.S.
Sp x N x F	10	276588	27658.8	1.66	N.S.
Pairs	35	1812800			
Error	36	598600	16627.8		
Total	71	2411400			

Experiment 7.

Multiplication of the nematode (cysts).

Treatment		Final Cyst Count per 200 g.		Replicate I Log. Final - Log. Initial	Replicate 2. Log. Final - Log. Initial	Total
		I	II			
L. esculentum.	N ₂ F ₁	65	40	0.71	0.50	1.21
	N ₃ F ₁	315	185	0.80	0.57	1.37
	1 N ₂ F ₂	105	215	0.92	1.23	2.15
	N ₃ F ₂	85	500	0.23	1.00	1.23
	N ₂ F ₃	95	210	0.88	1.22	2.10
	N ₃ F ₃	250	65	0.70	0.11	0.81
L. pipinellifolium.	N ₂ F ₁	95	230	0.88	1.26	2.14
	N ₃ F ₁	565	375	1.05	0.87	1.92
	N ₂ F ₂	195	110	1.19	0.94	2.13
	2 N ₃ F ₂	75	310	0.17	0.79	0.96
	N ₂ F ₃	35	285	0.44	1.35	1.79
	N ₃ F ₃	85	260	0.23	0.71	0.94
L. hirsutum.	N ₂ F ₁	30	130	0.38	1.01	1.39
	N ₃ F ₁	265	615	0.72	1.08	1.80
	N ₂ F ₂	75	260	0.77	1.31	2.08
	3 N ₃ F ₂	190	110	0.58	0.34	0.92
	N ₂ F ₃	95	60	0.88	0.68	1.56
	N ₃ F ₃	235	465	0.67	0.97	1.64

Table 29 cont.

Treatment		Final Cyst Count per 200 g.		Replicate 1 Lor. Final - Log. Initial	Replicate 2 Log. Final - Log. Initial	Total
L. glandulosum. 4	N ₂ F ₁	150	50	1.08	0.60	1.68
	N ₃ F ₁	500	575	1.00	1.06	2.06
	N ₂ F ₂	90	95	0.85	0.88	1.73
	N ₃ F ₂	300	335	0.78	0.82	1.60
	N ₂ F ₃	100	135	0.90	1.03	1.93
	N ₃ F ₃	130	125	0.41	0.40	0.81
L. peruvianum. 5	N ₂ F ₁	105	95	0.92	0.88	1.80
	N ₃ F ₁	120	360	0.38	0.86	1.24
	N ₂ F ₂	25	20	0.30	0.20	0.50
	N ₃ F ₂	55	175	0.04	0.54	0.58
	N ₂ F ₃	35	95	0.44	0.88	1.32
	N ₃ F ₃	200	160	0.60	0.50	1.10
L. hirsutum var. glabr. 6	N ₂ F ₁	65	315	0.71	1.40	2.11
	N ₃ F ₁	650	1180	1.11	1.37	2.48
	N ₂ F ₂	20	55	0.20	0.64	0.84
	N ₃ F ₂	215	410	0.63	0.91	1.54
	N ₂ F ₃	280	235	1.35	1.27	2.62
	N ₃ F ₃	60	235	0.08	0.67	0.75
				23.98	30.85	54.83

Items	df	S.S.	M.S.	F.	P.
Sp	5	0.78	0.16	2.00	N.S.
N	1	0.75	0.75	9.37	**
F	2	0.56	0.28	3.50	*
Sp x N	5	0.21	0.04	< 1	N.S.
Sp x F	10	1.01	0.10	1.25	N.S.
N x F	2	0.70	0.35	4.37	*
Sp x N x F	10	1.36	0.14	1.75	N.S.
Pairs	35	5.37			
Error	36	3.03	0.08		
Total	71	8.40			

Table 30.

Experiment 7.

New eggs per g.

Treatment		INITIAL e/g.	FINAL e/g.		New eggs/g.		Total
			Rep. I	Rep. II	Rep. I	Rep. II	
L. esculentum Sp. 1	F ₁	10	34.0	0.5	24.0	0.0	24.0
	F ₁	40	232.5	147.5	192.5	107.5	300.0
	F ₂	10	475.0	77.0	465.0	67.0	532.0
	F ₂	40	79.5	370.0	39.5	330.0	369.5
	F ₃	10	74.5	66.5	64.5	56.5	121.0
	F ₃	40	118.0	5.0	78.0	0.0	78.0
L. pimpinellifolium Sp. 2	F ₁	10	35.5	25.0	25.5	15.0	40.5
	F ₁	40	619.5	85.5	579.5	45.5	625.0
	F ₂	10	37.0	24.0	27.0	14.0	41.0
	F ₂	40	12.0	10.0	0.0	0.0	0.0
	F ₃	10	0.5	133.5	0.0	123.5	123.5
	F ₃	40	25.0	64.0	0.0	24.0	24.0
L. hirsutum Sp. 3	F ₁	10	12.0	8.5	2.0	0.0	2.0
	F ₁	40	202.0	248.5	162.0	208.5	370.5
	F ₂	10	5.5	51.0	0.0	41.0	41.0
	F ₂	40	115.5	48.0	75.5	8.0	83.5
	F ₃	10	30.0	18.0	20.0	8.0	28.0
	F ₃	40	40.0	66.5	0.0	26.5	26.5

Table 30 cont.

Treatment		INITIAL e/g.	FINAL e/g.		New eggs/g.		Total
			Rep. I	Rep. II	Rep. I	Rep. II	
L. glandulosum Sp. 4	F ₁	10	49.5	12.0	39.5	2.0	41.5
	F ₁	40	168.5	240.5	128.5	200.5	329.0
	F ₂	10	46.5	65.5	36.5	55.5	92.0
	F ₂	40	123.5	189.0	83.5	149.0	232.5
	F ₃	10	30.0	34.5	20.0	24.5	44.5
	F ₃	40	54.0	56.0	14.0	16.0	30.0
L. peruvianum Sp. 5	F ₁	10	2.0	5.0	0.0	0.0	0.0
	F ₁	40	33.5	8.5	0.0	0.0	0.0
	F ₂	10	3.0	4.5	0.0	0.0	0.0
	F ₂	40	21.0	9.0	0.0	0.0	0.0
	F ₃	10	13.0	4.0	3.0	0.0	3.0
	F ₃	40	24.0	15.5	0.0	0.0	0.0
L. hirsutum var glabratum Sp. 6	F ₁	10	27.5	66.0	17.5	56.0	73.5
	F ₁	40	342.5	800.0	302.5	760.0	1062.5
	F ₂	10	5.5	32.0	0.0	22.0	22.0
	F ₂	40	133.5	91.0	93.5	51.0	144.5
	F ₃	10	48.0	31.5	38.0	21.5	59.5
	F ₃	40	29.5	42.5	0.0	2.5	2.5
Totals.					2531.5	2435.5	4967.0

Items	df	S.S.	M.S.	F.	P.
Sp	5	117068.3	23413.66	2.13	N.S.
N	1	79268.3	79268.30	7.21	*
F	2	13504.1	56752.05	5.16	*
NF	2	184718.3	92359.15	8.40	**
Sp x N	5	58461.5	11692.30	1.06	N.S.
Sp x F	10	214529.9	21452.99	1.95	N.S.
Sp x N x F	10	101113.3	10111.33	< 1	N.S.
Pairs	35	868663.7			
Error	36	395944.3	10998.45		
Total.	71	1264608.0			

Table 31.Experiment 7.Weekly Growth Recordings (Week 1)

Species	Treatment	Rep. I	Rep. II	Total
L. esculentum Eurocross A. Sp. 1.	N ₁ F ₁	7.0	6.0	13.0
	N ₂ F ₁	7.5	6.0	13.5
	N ₃ F ₁	9.0	7.5	16.5
	N ₁ F ₂	5.0	6.5	11.5
	N ₂ F ₂	5.5	6.5	12.0
	N ₃ F ₂	7.5	6.0	13.5
	N ₁ F ₃	5.0	6.5	11.5
	N ₂ F ₃	7.5	8.5	16.0
	N ₃ F ₃	6.0	4.5	10.5
L. pimpinellifolium Sp. 2.	N ₁ F ₁	9.5	9.5	19.0
	N ₂ F ₁	8.5	7.0	15.5
	N ₃ F ₁	8.5	7.5	16.0
	N ₁ F ₂	9.0	6.0	15.0
	N ₂ F ₂	5.0	5.0	10.0
	N ₃ F ₂	9.0	6.5	15.5
	N ₁ F ₃	9.0	8.0	17.0
	N ₂ F ₃	6.0	8.5	14.5
	N ₃ F ₃	6.5	6.5	13.0
L. hirsutum Sp. 3.	N ₁ F ₁	4.5	4.5	9.0
	N ₂ F ₁	3.5	4.0	7.5
	N ₃ F ₁	4.5	4.0	8.5
	N ₁ F ₂	3.5	3.0	6.5
	N ₂ F ₂	4.5	3.0	7.5
	N ₃ F ₂	5.0	3.0	8.0
	N ₁ F ₃	3.5	3.0	6.5
	N ₂ F ₃	2.5	2.5	5.0
	N ₃ F ₃	3.0	3.0	6.0

Table 31 cont.

Species	Treatment	Rep. I	Rep. II	Total
L. glandulosum Sp. 4.	N ₁ F ₁	6.5	5.5	12.0
	N ₂ F ₁	7.0	6.5	13.5
	N ₃ F ₁	7.0	9.0	16.0
	N ₁ F ₂	5.0	5.0	10.0
	N ₂ F ₂	4.5	6.0	10.5
	N ₃ F ₂	7.0	7.0	14.0
	N ₁ F ₃	11.5	7.0	18.5
	N ₂ F ₃	5.0	5.0	10.0
	N ₃ F ₃	7.5	5.0	12.5
L. peruvianum Sp. 5.	N ₁ F ₁	2.5	4.0	6.5
	N ₂ F ₁	5.0	6.5	11.5
	N ₃ F ₁	3.0	5.0	8.0
	N ₁ F ₂	5.5	5.0	10.5
	N ₂ F ₂	4.5	2.5	7.0
	N ₃ F ₂	4.0	3.5	7.5
	N ₁ F ₃	3.0	4.5	7.5
	N ₂ F ₃	3.5	3.5	7.0
	N ₃ F ₃	3.0	4.0	7.0
L. hirsutum var glabratum. Sp. 6.	N ₁ F ₁	5.0	3.5	8.5
	N ₂ F ₁	2.5	3.5	6.0
	N ₃ F ₁	3.5	4.0	7.5
	N ₁ F ₂	4.0	2.0	6.0
	N ₂ F ₂	2.5	3.0	5.5
	N ₃ F ₂	3.0	3.0	6.0
	N ₁ F ₃	3.5	3.5	7.0
	N ₂ F ₃	2.5	2.0	4.5
	N ₃ F ₃	3.0	2.0	5.0
Total.		290.0	273.5	563.5

Table 32.Experiment 7.Weekly Growth Recordings (Week 2)

Species	Treatment	Rep. I	Rep. II	Total
L. esculentum Eurocross A.	N ₁ F ₁	20.5	18.0	38.5
	N ₂ F ₁	21.5	16.0	37.5
	N ₃ F ₁	27.5	23.5	51.0
	N ₁ F ₂	17.0	20.0	37.0
	N ₂ F ₂	18.0	19.5	37.5
	N ₃ F ₂	19.0	19.0	38.0
	N ₁ F ₃	18.0	17.5	35.5
	N ₂ F ₃	23.0	25.0	48.0
	N ₃ F ₃	17.5	14.0	31.5
L. pimpinellifolium Sp. 2.	N ₁ F ₁	34.0	26.5	60.5
	N ₂ F ₁	30.5	25.5	56.0
	N ₃ F ₁	25.5	27.5	53.0
	N ₁ F ₂	33.5	24.0	57.5
	N ₂ F ₂	17.5	17.0	34.5
	N ₃ F ₂	26.5	27.5	54.0
	N ₁ F ₃	32.5	27.0	59.5
	N ₂ F ₃	21.5	32.0	53.5
	N ₃ F ₃	21.5	21.5	43.0
L. hirsutum Sp. 3.	N ₁ F ₁	14.5	15.5	30.0
	N ₂ F ₁	11.0	14.0	25.0
	N ₃ F ₁	15.5	14.0	29.5
	N ₁ F ₂	10.5	12.0	22.5
	N ₂ F ₂	12.5	10.5	23.0
	N ₃ F ₂	13.0	8.5	21.5
	N ₁ F ₃	12.0	10.5	22.5
	N ₂ F ₃	10.0	10.0	20.0
	N ₃ F ₃	9.0	10.5	19.5

Table 32 cont.

Species	Treatment	Rep. I	Rep. II	Total
L. glandulosum Sp. 4.	N ₁ F ₁	21.0	16.0	37.0
	N ₂ F ₁	22.5	21.0	43.5
	N ₃ F ₁	20.5	30.0	50.5
	N ₁ F ₂	15.5	16.0	31.5
	N ₂ F ₂	13.0	19.0	32.0
	N ₃ F ₂	24.0	21.0	45.0
	N ₁ F ₃	32.0	25.5	57.5
	N ₂ F ₃	17.0	13.0	30.0
	N ₃ F ₃	24.0	17.0	41.0
L. peruvianum Sp. 5.	N ₁ F ₁	5.0	14.0	19.0
	N ₂ F ₁	13.5	20.0	33.5
	N ₃ F ₁	11.5	16.0	27.5
	N ₁ F ₂	17.0	16.5	33.5
	N ₂ F ₂	13.5	11.5	25.0
	N ₃ F ₂	11.5	12.5	24.0
	N ₁ F ₃	11.0	14.0	25.0
	N ₂ F ₃	11.0	11.5	22.5
	N ₃ F ₃	10.5	12.5	23.0
L. hirsutum var glabratum. Sp. 6.	N ₁ F ₁	15.0	12.5	27.5
	N ₂ F ₁	9.0	12.5	21.5
	N ₃ F ₁	12.0	14.0	26.0
	N ₁ F ₂	13.5	8.5	22.0
	N ₂ F ₂	9.0	9.5	18.5
	N ₃ F ₂	8.0	11.0	19.0
	N ₁ F ₃	12.0	12.5	24.5
	N ₂ F ₃	8.0	8.5	16.5
	N ₃ F ₃	8.0	8.5	16.5
		921.5	911.0	1832.5

Table 33.Experiment 7.Weekly Growth Recordings (Week 3.)

Species	Treatment	Rep. I	Rep. II	Total
L. esculentum Eurocross A. Sp. 1.	N ₁ F ₁	34.0	29.5	63.5
	N ₂ F ₁	32.5	24.5	57.0
	N ₃ F ₁	40.0	35.5	75.5
	N ₁ F ₂	28.5	30.0	58.5
	N ₂ F ₂	27.5	30.5	58.0
	N ₃ F ₂	30.5	32.5	63.0
	N ₁ F ₃	28.0	28.0	56.0
	N ₂ F ₃	35.0	38.0	73.0
	N ₃ F ₃	30.0	22.0	52.0
L. pimpinellifolium Sp. 2.	N ₁ F ₁	65.5	48.5	114.0
	N ₂ F ₁	62.5	52.0	114.5
	N ₃ F ₁	55.0	49.5	104.5
	N ₁ F ₂	60.5	43.5	104.0
	N ₂ F ₂	41.5	37.5	79.0
	N ₃ F ₂	52.0	48.0	100.0
	N ₁ F ₃	60.0	53.0	113.0
	N ₂ F ₃	43.5	61.0	104.5
	N ₃ F ₃	42.5	44.0	86.5
L. hirsutum Sp. 3.	N ₁ F ₁	32.0	29.0	61.0
	N ₂ F ₁	20.5	28.0	48.5
	N ₃ F ₁	26.5	24.5	51.0
	N ₁ F ₂	21.0	21.0	42.0
	N ₂ F ₂	29.0	19.0	48.0
	N ₃ F ₂	23.5	18.5	42.0
	N ₁ F ₃	22.5	21.0	43.5
	N ₂ F ₃	19.5	17.5	37.0
	N ₃ F ₃	17.5	19.5	37.0

Table 33 cont.

Species	Treatment	Rep. I	Rep. II	Total
L. glandulosum Sp. 4.	N ₁ F ₁	41.5	31.5	73.0
	N ₂ F ₁	46.0	40.5	86.5
	N ₃ F ₁	42.5	51.0	93.5
	N ₁ F ₂	33.5	30.5	64.0
	N ₂ F ₂	27.5	34.0	61.5
	N ₃ F ₂	40.5	39.5	80.0
	N ₁ F ₃	59.0	48.0	107.0
	N ₂ F ₃	32.0	21.0	53.0
	N ₃ F ₃	36.0	29.5	65.5
L. peruvianum Sp. 5.	N ₁ F ₁	8.5	28.5	37.0
	N ₂ F ₁	28.0	38.0	66.0
	N ₃ F ₁	28.5	36.5	65.0
	N ₁ F ₂	32.0	34.5	66.5
	N ₂ F ₂	27.5	22.0	49.5
	N ₃ F ₂	27.5	28.0	55.5
	N ₁ F ₃	28.5	31.0	59.5
	N ₂ F ₃	26.0	25.5	51.5
	N ₃ F ₃	25.0	23.0	48.0
L. hirsutum var glabratum Sp. 6.	N ₁ F ₁	32.0	28.0	60.0
	N ₂ F ₁	22.0	25.5	47.5
	N ₃ F ₁	24.5	22.5	47.0
	N ₁ F ₂	27.0	18.5	45.5
	N ₂ F ₂	21.0	19.0	40.0
	N ₃ F ₂	17.5	18.5	36.0
	N ₁ F ₃	27.5	26.5	54.0
	N ₂ F ₃	17.5	19.5	37.0
	N ₃ F ₃	15.0	13.5	28.5
		1775.0	1689.5	3464.5

Table 34.

Experiment 7.Weekly Growth Recordings (Week 4)

Species	Treatment	Rep. I	Rep. II	Total
L. esculentum Eurocross A. Sp. 1.	N ₁ F ₁	42.5	40.5	83.0
	N ₂ F ₁	43.5	34.5	78.0
	N ₃ F ₁	49.5	48.5	98.0
	N ₁ F ₂	37.0	39.0	76.0
	N ₂ F ₂	39.5	38.5	78.0
	N ₃ F ₂	39.0	41.5	80.5
	N ₁ F ₃	37.5	39.0	76.5
	N ₂ F ₃	48.5	48.5	97.0
	N ₃ F ₃	41.5	30.5	72.0
L. pimpinellifolium Sp. 2.	N ₁ F ₁	90.5	68.0	158.5
	N ₂ F ₁	82.5	72.5	155.0
	N ₃ F ₁	79.0	67.5	146.5
	N ₁ F ₂	82.5	60.5	143.0
	N ₂ F ₂	61.5	57.5	119.0
	N ₃ F ₂	71.5	77.0	148.5
	N ₁ F ₃	82.5	77.0	159.5
	N ₂ F ₃	58.0	85.5	143.5
	N ₃ F ₃	66.5	71.0	137.5
L. hirsutum Sp. 3.	N ₁ F ₁	42.5	43.0	85.5
	N ₂ F ₁	30.5	40.5	71.0
	N ₃ F ₁	42.0	37.5	79.5
	N ₁ F ₂	35.5	30.0	65.5
	N ₂ F ₂	40.5	30.5	71.0
	N ₃ F ₂	34.5	28.0	62.5
	N ₁ F ₃	31.0	31.5	62.5
	N ₂ F ₃	27.0	26.0	53.0
	N ₃ F ₃	28.5	32.0	60.5

Table 34 cont.

Species	Treatment	Rep. I	Rep. II	Total
L. glandulosum. Sp. 4.	N ₁ F ₁	60.5	51.0	111.5
	N ₂ F ₁	60.0	65.5	125.5
	N ₃ F ₁	61.5	72.0	133.5
	N ₁ F ₂	53.0	50.5	103.5
	N ₂ F ₂	45.0	50.5	95.5
	N ₃ F ₂	58.5	58.5	117.0
	N ₁ F ₃	83.0	71.5	154.5
	N ₂ F ₃	51.5	34.0	85.5
	N ₃ F ₃	53.5	46.5	100.0
L. peruvianum Sp. 5.	N ₁ F ₁	12.5	46.0	58.5
	N ₂ F ₁	43.5	51.5	95.0
	N ₃ F ₁	42.0	53.5	95.5
	N ₁ F ₂	44.5	52.0	96.5
	N ₂ F ₂	37.5	34.0	71.5
	N ₃ F ₂	38.5	44.5	83.0
	N ₁ F ₃	46.5	44.5	91.0
	N ₂ F ₃	41.0	42.5	83.5
	N ₃ F ₃	40.0	43.0	83.0
L. hirsutum var glabratum. Sp. 6.	N ₁ F ₁	56.5	51.0	107.5
	N ₂ F ₁	36.0	46.0	82.0
	N ₃ F ₁	42.5	39.5	82.0
	N ₁ F ₂	45.5	34.5	80.0
	N ₂ F ₂	38.0	40.5	78.5
	N ₃ F ₂	30.5	26.5	57.0
	N ₁ F ₃	46.5	43.0	89.5
	N ₂ F ₃	28.5	36.5	65.0
	N ₃ F ₃	22.5	18.5	41.0
		2584.0	2543.5	5127.5

Table 35.Experiment 7.Weekly Growth Recordings (Week 6)

Species	Treatment	Rep. I	Rep. II	Total
L. esculentum Eurocross A. Sp. 1.	N ₁ F ₁	48.0	48.0	96.0
	N ₂ F ₁	49.0	37.0	86.0
	N ₃ F ₁	54.0	62.5	116.5
	N ₁ F ₂	43.5	45.5	89.0
	N ₂ F ₂	45.5	45.5	91.0
	N ₃ F ₂	43.5	44.5	88.0
	N ₁ F ₃	47.0	47.0	94.0
	N ₂ F ₃	49.5	49.0	98.5
	N ₃ F ₃	53.5	36.0	89.5
L. pimpinellifolium Sp. 2.	N ₁ F ₁	119.5	103.5	223.0
	N ₂ F ₁	125.5	120.0	245.5
	N ₃ F ₁	120.0	114.0	234.0
	N ₁ F ₂	120.0	109.5	229.5
	N ₂ F ₂	97.5	103.0	200.5
	N ₃ F ₂	111.0	124.0	235.0
	N ₁ F ₃	128.5	127.0	255.5
	N ₂ F ₃	104.0	134.5	238.5
	N ₃ F ₃	109.0	121.5	230.5
L. hirsutum Sp. 3.	N ₁ F ₁	71.5	69.5	141.0
	N ₂ F ₁	50.0	68.5	118.5
	N ₃ F ₁	61.5	63.0	124.5
	N ₁ F ₂	62.0	52.0	114.0
	N ₂ F ₂	59.5	49.0	108.5
	N ₃ F ₂	57.0	51.5	108.5
	N ₁ F ₃	46.5	48.5	95.0
	N ₂ F ₃	43.5	45.5	89.0
	N ₃ F ₃	52.0	52.0	104.0

Table 35 cont.

Species	Treatment	Rep. I	Rep. II	Total
L. glandulosum Sp. 4.	N ₁ F ₁	89.0	79.5	168.5
	N ₂ F ₁	65.0	96.5	161.5
	N ₃ F ₁	85.5	102.0	187.5
	N ₁ F ₂	87.0	75.0	162.0
	N ₂ F ₂	69.0	77.0	146.0
	N ₃ F ₂	83.5	89.5	173.0
	N ₁ F ₃	115.0	105.5	220.5
	N ₂ F ₃	81.5	61.0	142.5
	N ₃ F ₃	82.5	75.5	158.0
L. peruvianum Sp. 5.	N ₁ F ₁	19.5	69.0	88.5
	N ₂ F ₁	73.5	72.5	146.0
	N ₃ F ₁	75.5	79.0	154.5
	N ₁ F ₂	60.0	90.0	150.0
	N ₂ F ₂	56.5	48.5	105.0
	N ₃ F ₂	59.0	62.5	121.5
	N ₁ F ₃	82.0	74.5	156.5
	N ₂ F ₃	77.5	74.5	152.0
	N ₃ F ₃	70.0	67.5	137.5
L. hirsutum var glabratum Sp. 6.	N ₁ F ₁	109.5	101.5	211.0
	N ₂ F ₁	75.0	92.5	167.5
	N ₃ F ₁	89.0	86.5	175.5
	N ₁ F ₂	92.0	77.0	169.0
	N ₂ F ₂	76.0	83.5	159.5
	N ₃ F ₂	67.5	46.0	113.5
	N ₁ F ₃	91.0	87.0	178.0
	N ₂ F ₃	60.5	77.5	138.0
	N ₃ F ₃	47.5	36.0	83.5
		4011.5	4058.5	8070.0

Table 36.Experiment 7.Weekly Growth Recordings (Week 7)

Species	Treatment	Rep. I	Rep. II	Total
L. esculentum Eurocross A. Sp. 1.	N ₁ F ₁	51.5	51.0	102.5
	N ₂ F ₁	50.0	40.5	90.5
	N ₃ F ₁	59.5	67.5	127.0
	N ₁ F ₂	46.5	48.0	94.5
	N ₂ F ₂	48.0	47.5	95.5
	N ₃ F ₂	49.5	48.0	97.5
	N ₁ F ₃	48.5	47.0	95.5
	N ₂ F ₃	55.5	56.0	111.5
	N ₃ F ₃	56.0	41.0	97.0
L. pimpinellifolium Sp. 2.	N ₁ F ₁	142.5	137.0	279.5
	N ₂ F ₁	156.0	151.5	307.5
	N ₃ F ₁	147.5	149.0	296.5
	N ₁ F ₂	149.5	142.5	292.0
	N ₂ F ₂	126.0	138.0	264.0
	N ₃ F ₂	139.0	150.0	289.0
	N ₁ F ₃	150.0	163.0	313.0
	N ₂ F ₃	128.5	150.5	279.0
	N ₃ F ₃	138.5	147.5	286.0
L. hirsutum Sp. 3.	N ₁ F ₁	89.5	87.5	177.0
	N ₂ F ₁	66.0	85.5	151.5
	N ₃ F ₁	79.5	84.5	164.0
	N ₁ F ₂	79.0	68.0	147.0
	N ₂ F ₂	77.5	62.0	139.5
	N ₃ F ₂	80.0	68.0	148.0
	N ₁ F ₃	68.5	62.5	131.0
	N ₂ F ₃	62.0	68.5	130.5
	N ₃ F ₃	68.0	73.5	141.5

Table 36 cont.

Species	Treatment	Rep. I	Rep. II	Total
L. glandulosum Sp. 4.	N ₁ F ₁	110.0	92.0	202.0
	N ₂ F ₁	73.0	119.0	192.0
	N ₃ F ₁	105.5	123.5	229.0
	N ₁ F ₂	113.5	94.0	207.5
	N ₂ F ₂	87.5	91.5	179.0
	N ₃ F ₂	103.0	112.0	215.0
	N ₁ F ₃	130.5	128.0	258.5
	N ₂ F ₃	106.0	82.0	188.0
	N ₃ F ₃	98.5	100.5	199.0
L. peruvianum Sp. 5.	N ₁ F ₁	33.5	91.5	125.0
	N ₂ F ₁	106.0	93.5	199.5
	N ₃ F ₁	101.0	107.0	208.0
	N ₁ F ₂	86.0	118.0	204.0
	N ₂ F ₂	74.5	66.0	140.5
	N ₃ F ₂	83.0	82.0	165.0
	N ₁ F ₃	82.0	95.5	177.5
	N ₂ F ₃	102.5	94.5	197.0
	N ₃ F ₃	99.5	86.5	186.0
L. hirsutum var glabratum Sp. 6.	N ₁ F ₁	146.0	136.0	282.0
	N ₂ F ₁	109.5	127.0	236.5
	N ₃ F ₁	123.5	128.5	252.0
	N ₁ F ₂	127.0	116.5	243.5
	N ₂ F ₂	104.5	116.5	221.0
	N ₃ F ₂	99.5	77.0	176.5
	N ₁ F ₃	126.0	124.0	250.0
	N ₂ F ₃	88.5	113.0	201.5
	N ₃ F ₃	87.5	54.5	142.0
		5120.0	5205.5	10325.5

Table 37.Experiment 7.Weekly Growth Recordings (Week 8)

Species	Treatment	Rep. I	Rep. II	Total
L. esculentum Eurocross A. Sp. 1.	N ₁ F ₁	63.5	54.0	117.5
	N ₂ F ₁	61.5	48.0	109.6
	N ₃ F ₁	64.5	73.0	137.5
	N ₁ F ₂	47.5	48.5	96.0
	N ₂ F ₂	51.0	49.0	100.0
	N ₃ F ₂	59.0	50.5	109.5
	N ₁ F ₃	52.0	51.5	103.5
	N ₂ F ₃	57.5	56.0	113.5
	N ₃ F ₃	62.5	47.0	109.5
L. pimpinellifolium Sp. 2.	N ₁ F ₁	161.5	170.0	331.5
	N ₂ F ₁	191.5	178.0	369.5
	N ₃ F ₁	173.0	172.5	345.5
	N ₁ F ₂	171.0	171.5	342.5
	N ₂ F ₂	140.5	166.5	307.0
	N ₃ F ₂	177.0	166.0	343.0
	N ₁ F ₃	175.5	185.5	361.0
	N ₂ F ₃	149.0	170.5	319.5
	N ₃ F ₃	151.5	160.5	312.0
L. hirsutum Sp. 3.	N ₁ F ₁	108.0	90.0	198.0
	N ₂ F ₁	88.5	99.0	187.5
	N ₃ F ₁	95.0	97.0	192.0
	N ₁ F ₂	87.5	80.0	167.5
	N ₂ F ₂	97.5	72.0	169.5
	N ₃ F ₂	106.5	85.0	191.5
	N ₁ F ₃	88.5	82.5	171.0
	N ₂ F ₃	79.5	86.0	165.5
	N ₃ F ₃	84.5	89.5	174.0

Table 37 cont.

Species	Treatment	Rep. I	Rep. II	Total
L. glandulosum Sp. 4.	N ₁ F ₁	123.0	105.5	228.5
	N ₂ F ₁	92.5	132.0	224.5
	N ₃ F ₁	120.0	141.0	261.0
	N ₁ F ₂	133.5	108.0	241.5
	N ₂ F ₂	109.5	102.0	211.5
	N ₃ F ₂	117.0	131.0	248.0
	N ₁ F ₃	153.0	148.0	301.0
	N ₂ F ₃	124.5	97.0	221.5
	N ₃ F ₃	100.0	119.5	219.5
L. peruvianum Sp. 5.	N ₁ F ₁	53.0	101.0	154.0
	N ₂ F ₁	127.5	113.0	240.5
	N ₃ F ₁	115.0	123.5	238.5
	N ₁ F ₂	105.0	143.0	248.0
	N ₂ F ₂	88.5	83.5	172.0
	N ₃ F ₂	109.0	100.5	209.5
	N ₁ F ₃	127.5	114.5	242.0
	N ₂ F ₃	123.0	115.5	238.5
	N ₃ F ₃	120.0	108.0	228.0
L. hirsutum var glabratum Sp. 61	N ₁ F ₁	167.0	162.0	329.0
	N ₂ F ₁	139.5	154.0	293.5
	N ₃ F ₁	144.0	161.5	305.5
	N ₁ F ₂	161.0	131.0	292.0
	N ₂ F ₂	128.0	131.5	259.5
	N ₃ F ₂	120.0	99.0	219.0
	N ₁ F ₃	152.5	154.0	306.5
	N ₂ F ₃	106.0	128.0	234.0
	N ₃ F ₃	107.5	88.5	196.0
		6112.0	6095.5	12207.5

Table 38.Experiment 7.Analysis of Weekly Growth Records.

	Items	df	S.S.	M.S.	F.	P.
Week 1	Sp	5	311.3	62.26	62.26	***
	N	2	5.2	2.60	2.60	N.S.
	F	2	17.0	8.50	8.50	**
	Sp x N	10	18.4	1.84	1.84	N.S.
	Sp x F	10	7.5	0.75	0.75	N.S.
	N x F	4	11.2	2.80	2.80	*
	S x N x F	20	40.7	2.03	2.03	*
	Error	54	5.3	1.00		
Week 2	Sp	5	3359.2	671.84	78.03	***
	N	2	55.4	27.70	3.22	*
	F	2	134.0	67.00	7.78	***
	Sp x N	10	182.0	18.20	2.11	*
	Sp x F	10	73.6	7.36	< 1	N.S.
	N x F	4	141.0	36.25	4.21	**
	Sp x N x F	20	413.3	20.66	2.40	*
	Error	54	465.1	8.61		
Week 3	Sp	5	10917.3	2183.46	85.06	***
	N	2	192.7	96.35	3.75	*
	F	2	508.2	254.10	9.90	***
	Sp x N	10	391.6	39.16	1.52	N.S.
	Sp x F	10	221.4	22.14	< 1	N.S.
	N x F	4	517.7	129.42	5.04	**
	Sp x N x F	20	1217.1	60.85	2.37	*
	Error	54	1386.1	25.67		
Week 4	Sp	5	19422.4	3884.48	81.15	***
	N	2	375.5	187.75	3.92	*
	F	2	791.5	395.75	8.27	***
	Sp x N	10	1000.7	100.07	2.09	*
	Sp x F	10	544.0	54.40	1.14	N.S.
	N x F	4	670.2	167.55	3.50	*
	Sp x N x F	20	1917.8	95.89	2.00	*
	Error	54	2585.1	47.87		

Table 38 cont.

	Items	df	S.S.	M.S.	F.	P.
Week 6	Sp	5	54017.1	10803.42	115.84	***
	N	2	973.4	486.70	5.22	**
	F	2	1139.5	569.75	6.11	**
	Sp x N	10	2986.4	298.64	3.20	**
	Sp x F	10	2755.1	275.51	2.95	**
	N x F	4	1449.6	362.40	3.89	**
	Sp x N x F	20	3334.6	166.73	1.79	N.S.
	Error	54	5036.0	93.26		
Week 7	Sp	5	94954.5	18990.90	143.51	***
	N	2	942.6	471.30	3.56	*
	F	2	1412.1	706.05	5.33	**
	Sp x N	10	4197.3	419.73	3.17	**
	Sp x F	10	2683.8	268.38	2.03	*
	N x F	4	1928.4	482.10	3.64	*
	Sp x N x F	20	4334.4	216.72	1.64	N.S.
	Error	54	7145.9	132.33		
Week 8	Sp	5	134756.4	26951.28	185.63	***
	N	2	1237.1	618.55	4.26	*
	F	2	1683.5	841.75	5.80	**
	Sp x N	10	4082.4	408.24	2.81	*
	Sp x F	10	3629.5	362.95	2.50	*
	N x F	4	3361.8	840.45	5.79	***
	Sp x N x F	20	5007.0	250.35	1.72	N.S.
	Error	54	7840.4	145.19		

Table 39.Experiment 7.NF Interaction.

Week 1	N ₁	N ₂	N ₃
F ₁	109.6	107.7	115.4
F ₂	96.1	84.6	103.8
F ₃	109.6	90.4	86.5
Week 2	N ₁	N ₂	N ₃
F ₁	104.1	106.5	116.5
F ₂	100.0	83.5	98.8
F ₃	110.0	93.5	85.3
Week 3	N ₁	N ₂	N ₃
F ₁	105.9	109.0	113.4
F ₂	98.7	87.2	97.8
F ₃	112.5	92.5	82.5
Week 4	N ₁	N ₂	N ₃
F ₁	106.1	106.3	111.4
F ₂	98.9	90.1	96.2
F ₃	111.2	92.6	86.7
Week 6	N ₁	N ₂	N ₃
F ₁	103.5	103.2	110.7
F ₂	101.9	90.4	93.7
F ₃	111.5	95.7	89.6

Table 39 cont.

Week 7	N ₁	N ₂	N ₃
F ₁	101.8	102.6	111.3
F ₂	103.6	90.6	95.1
F ₃	106.8	96.5	91.6
Week 8	N ₁	N ₂	N ₃
F ₁	100.2	105.0	109.1
F ₂	102.3	89.9	97.3
F ₃	109.5	95.3	91.3

Table 40.Experiment 7.Analysis of Weekly Growth Records for Species of Lycopersicon.Week 1.L. esculentum.

Items	df	S.S.	M.S.	F.	P.
N	2	2.9	1.45	1.58	N.S.
F	2	3.5	1.75	1.90	N.S.
NF	4	10.3	2.57	2.79	N.S.
Error	9	8.3	0.92		

Week 2.

Items	df	S.S.	M.S.	F.	P.
N	2	13.3	6.65	1.49	N.S.
F	2	20.0	10.00	2.25	N.S.
NF	4	117.6	29.40	6.61	**
Error	9	40.1	4.45		

Week 3.

Items	df	S.S.	M.S.	F.	P.
N	2	14.6	7.30	<1	N.S.
F	2	27.8	13.90	1.30	N.S.
NF	4	205.4	51.35	4.80	*
Error	9	96.3	10.70		

Table 40 cont.Week 4.

Items	df	S.S.	M.S.	F.	P.
N	2	29.9	14.95	1.22	N.S.
F	2	50.2	25.10	2.05	N.S.
NF	4	261.1	65.27	5.33	*
Error	9	110.3	12.25		

Week 6.

Items	df	S.S.	M.S.	F.	P.
N	2	32.2	16.10	< 1	N.S.
F	2	77.7	38.85	1.32	N.S.
NF	4	232.2	58.05	1.98	N.S.
Error	9	263.8	29.31		

Week 7.

Items	df	S.S.	M.S.	F.	P.
N	2	80.1	40.05	1.86	N.S.
F	2	88.1	44.05	2.05	N.S.
NF	4	346.4	86.60	4.03	*
Error	9	193.3	21.48		

Week 8.

Items	df	S.S.	M.S.	F.	P.
N	2	151.1	75.55	2.05	N.S.
F	2	298.1	149.05	4.04	N.S.
NF	4	130.4	32.60	< 1	N.S.
Error	9	332.3	36.92		

Table 41.Experiment 7.L. hirsutum.Week 1.

Items	df	S.S.	M.S.	F.	P.
N	2	0.6	0.30	< 1	N.S.
F	2	4.8	2.40	6.0	*
NF	4	1.1	0.27	< 1	N.S.
Error	9	3.6	0.40		

Week 2.

Items	df	S.S.	M.S.	F.	P.
N	2	4.2	2.10	< 1	N.S.
F	2	46.6	23.30	9.71	**
NF	4	6.5	1.62	< 1	N.S.
Error	9	21.6	2.40		

Week 3.

Items	df	S.S.	M.S.	F.	P.
N	2	25.2	12.60	1.11	N.S.
F	2	159.5	79.75	7.01	*
NF	4	44.6	11.15	< 1	N.S.
Error	9	102.3	11.37		

Table 41 cont.Week 4.

Items	df	S.S.	M.S.	F.	P.
N	2	28.9	14.45	< 1	N.S.
F	2	305.5	152.75	8.97	**
NF	4	67.8	16.95	< 1	N.S.
Error	9	153.3	17.03		

Week 6.

Items	df	S.S.	M.S.	F.	P.
N	2	98.1	49.05	1.48	N.S.
F	2	770.8	385.40	11.62	**
NF	4	104.7	26.17	< 1	N.S.
Error	9	298.5	33.17		

Week 7.

Items	df	S.S.	M.S.	F.	P.
N	2	119.4	59.70	1.03	N.S.
F	2	687.0	343.50	6.04	*
NF	4	103.4	25.85	< 1	N.S.
Error	9	511.5	56.83		

Week 8.

Items	df	S.S.	M.S.	F.	P.
N	2	103.4	51.70	< 1	N.S.
F	2	400.7	200.35	2.11	N.S.
NF	4	120.3	30.07	< 1	N.S.
Error	9	855.1	95.01		

Table 42.Experiment 7.L. hirsutum var glabratum.Week 1.

Items	df	S.S.	M.S.	F.	P.
N	2	2.5	1.25	2.50	N.S.
F	2	2.9	1.45	2.90	N.S.
NF	4	0.9	0.25	< 1	N.S.
Error	9	4.5	0.50		

Week 2.

Items	df	S.S.	M.S.	F.	P.
N	2	27.1	13.55	4.23	N.S.
F	2	30.6	15.30	4.78	*
NF	4	7.5	1.87	< 1	N.S.
Error	9	28.8	3.20		

Week 3.

Items	df	S.S.	M.S.	F.	P.
N	2	205.5	102.75	15.86	**
F	2	128.8	64.40	9.94	**
NF	4	40.1	10.02	1.55	N.S.
Error	9	58.3	6.48		

Table 42 cont.Week 4.

Items	df	S.S.	M.S.	F.	P.
N	2	785.1	392.55	18.86	***
F	2	517.3	258.65	12.43	**
NF	4	185.4	46.35	2.23	N.S.
Error	9	187.3	20.81		

Week 6.

Items	df	S.S.	M.S.	F.	P.
N	2	2867.5	1433.7	16.57	***
F	2	2123.4	1061.7	12.27	**
NF	4	799.7	199.93	2.31	N.S.
Error	9	778.6	86.51		

Week 7.

Items	df	S.S.	M.S.	F.	P.
N	2	3523.8	1761.90	10.99	**
F	2	2797.5	1398.75	8.73	**
NF	4	1100.0	275.00	1.72	N.S.
Error	9	1442.5	160.28		

Week 8.

Items	df	S.S.	M.S.	F.	P.
N	2	3722.8	1861.40	12.22	**
F	2	3479.6	1739.80	11.42	**
NF	4	1092.7	273.17	1.79	N.S.
Error	9	1371.0			

Table 43.Experiment 7.L. glandulosum.Week 3.

Items	df	S.S.	M.S.	F.	P.
N	2	184.3	92.15	3.08	N.S.
F	2	189.6	94.80	3.17	N.S.
NF	4	824.1	206.02	6.88	**
Error	9	269.5	29.94		

Week 4.

Items	df	S.S.	M.S.	F.	P.
N	2	348.1	174.05	4.15	N.S.
F	2	248.7	124.35	2.97	N.S.
NF	4	1217.6	304.40	7.26	**
Error	9	377.3	41.92		

Table 44.Experiment 7.L. peruvianum.Week 8.

Items	df	S.S.	M.S.	F.	P.
N	2	94.3	47.15	< 1	N.S.
F	2	664.0	332.00	1.33	N.S.
NF	4	3840.7	960.17	3.84	*
Error	9	2248.5	249.83		

Table 45.Experiment 7.(Total of 2 replicates.)NF Interaction for L. esculentum.

Week 2	N ₁	N ₂	N ₃
F ₁	38.5 cm.	37.5 cm.	51.0 cm.
F ₂	37.0 cm.	37.5 cm.	38.0 cm.
F ₃	35.5 cm.	48.0 cm.	31.5 cm.
Week 3	N ₁	N ₂	N ₃
F ₁	65.5 cm.	57.0 cm.	75.5 cm.
F ₂	58.5 cm.	58.0 cm.	63.0 cm.
F ₃	56.0 cm.	73.0 cm.	52.0 cm.
Week 4	N ₁	N ₂	N ₃
F ₁	83.0 cm.	78.0 cm.	98.0 cm.
F ₂	76.0 cm.	78.0 cm.	80.5 cm.
F ₃	76.5 cm.	97.0 cm.	72.0 cm.
Week 7	N ₁	N ₂	N ₃
F ₁	102.5 cm.	90.5 cm.	127.0 cm.
F ₂	94.5 cm.	95.5 cm.	97.5 cm.
F ₃	95.5 cm.	111.5 cm.	97.0 cm.

Table 46.Experiment 7.(Total of 2 replicates).NF Interaction for L. glandulosum.

Week 3	N ₁	N ₂	N ₃
F ₁	73.0 cm.	86.5 cm.	93.5 cm.
F ₂	64.0 cm.	61.5 cm.	80.0 cm.
F ₃	107.0 cm.	53.0 cm.	65.5 cm.
Week 4	N ₁	N ₂	N ₃
F ₁	111.5 cm.	125.5 cm.	133.5 cm.
F ₂	103.5 cm.	95.5 cm.	117.0 cm.
F ₃	154.5 cm.	85.5 cm.	100.0 cm.

Table 47.Experiment 7.Final Records.Increase in height over initial.

Species	Treatment	Rep. I	Rep. II	Total
L. esculentum	N ₁ F ₁	79.0	69.0	148.0
	N ₂ F ₁	69.5	55.5	125.0
	N ₃ F ₁	67.0	80.0	147.0
Eurocross A.	N ₁ F ₂	51.5	53.5	105.0
	N ₂ F ₂	60.5	50.0	110.5
Sp. 1.	N ₂ F ₂	63.5	58.0	121.5
	N ₁ F ₃	54.0	68.0	122.0
	N ₂ F ₃	61.0	56.5	117.5
	N ₃ F ₃	67.5	56.5	124.0
L. pimpinellifolium	N ₁ F ₁	180.0	197.5	377.5
	N ₂ F ₁	220.5	207.5	428.0
	N ₃ F ₁	188.0	202.5	390.5
	N ₁ F ₂	190.5	207.0	397.5
	N ₂ F ₂	155.0	190.5	345.5
	N ₃ F ₂	191.5	180.0	371.5
	N ₁ F ₃	113.0	205.0	318.0
	N ₂ F ₃	168.5	193.5	362.0
	N ₃ F ₃	160.5	180.5	341.0
L. hirsutum	N ₁ F ₁	125.0	120.5	245.5
	N ₂ F ₁	119.0	122.0	241.0
	N ₃ F ₁	110.0	131.0	241.0
	N ₁ F ₂	94.0	106.5	200.5
	N ₂ F ₂	116.5	96.0	212.5
	N ₃ F ₂	125.0	105.5	230.5
	N ₁ F ₃	105.0	119.5	224.5
	N ₂ F ₃	102.5	116.0	218.5
	N ₃ F ₃	100.5	127.0	227.5

Table 47 cont.

Species	Treatment	Rep. I	Rep. II	Total
L. glandulosum Sp. 4.	N ₁ F ₁	139.0	119.0	258.0
	N ₂ F ₁	113.5	157.5	271.0
	N ₃ F ₁	130.5	161.5	292.0
	N ₁ F ₂	157.5	131.5	289.0
	N ₂ F ₂	113.0	115.5	228.5
	N ₃ F ₂	134.5	161.0	295.5
	N ₁ F ₃	143.0	181.0	324.0
	N ₂ F ₃	143.0	119.5	262.5
	N ₃ F ₃	133.0	145.5	278.5
L. peruvianum Sp. 5.	N ₁ F ₁	67.0	131.0	198.0
	N ₂ F ₁	144.0	139.0	283.0
	N ₃ F ₁	133.5	155.5	289.0
	N ₁ F ₂	124.0	157.5	281.5
	N ₂ F ₂	99.5	80.5	180.0
	N ₃ F ₂	130.0	122.5	252.5
	N ₁ F ₃	147.0	136.5	283.5
	N ₂ F ₃	148.0	148.0	296.0
	N ₃ F ₃	142.0	136.0	278.0
L. hirsutum var glabratum Sp. 6.	N ₁ F ₁	186.0	159.0	345.0
	N ₂ F ₁	157.5	193.0	350.5
	N ₃ F ₁	171.0	228.0	399.0
	N ₁ F ₂	183.5	166.5	350.0
	N ₂ F ₂	153.5	161.0	314.5
	N ₃ F ₂	140.5	147.0	287.5
	N ₁ F ₃	162.0	217.5	379.5
	N ₂ F ₃	150.5	170.5	321.0
	N ₃ F ₃	140.0	159.0	299.0
		6925.0	7455.0	14380.0

Table 47.Experiment 7.0 Increase in height over initial.

Items	df	S.S.	M.S.	F.	P.
Sp	5	170211.5	34042.30	97.01	***
N	2	664.5	332.25	< 1	N.S.
F	2	2886.5	1443.25	4.11	*
Sp x N	10	2234.7	223.47	< 1	N.S.
Sp x F	10	4640.7	464.07	1.32	N.S.
N x F	4	3710.0	927.50	2.64	*
Sp x N x F	20	7429.0	371.45	1.06	N.S.
Error	54	18949.8	350.92		

Critical difference when $P = 0.05$, 12.53 cm.
when $P = 0.01$, 16.69 cm.
when $P = 0.001$, 21.77 cm.

Table 48.

Experiment 7.Final Records.Fresh weight of shoot.

Species	Treatment	Rep. I	Rep. II	Total
L. esculentum. Eurocross A. Sp. 1.	N ₁ F ₁	450.2	336.2	786.4
	N ₂ F ₁	421.2	445.4	866.6
	N ₃ F ₁	366.2	434.9	801.1
	N ₁ F ₂	420.6	394.0	814.6
	N ₂ F ₂	445.5	377.8	823.3
	N ₃ F ₂	369.2	446.6	815.8
	N ₁ F ₃	217.2	429.3	646.5
	N ₂ F ₃	386.3	454.7	841.0
	N ₃ F ₃	426.2	351.1	777.3
L. pimpinellifolium Sp. 2.	N ₁ F ₁	180.7	187.6	368.3
	N ₂ F ₁	214.9	240.0	454.9
	N ₃ F ₁	180.9	221.9	402.8
	N ₁ F ₂	193.3	221.0	414.3
	N ₂ F ₂	174.1	188.3	362.4
	N ₃ F ₂	170.7	212.9	383.6
	N ₁ F ₃	148.0	175.4	323.4
	N ₂ F ₃	189.1	192.9	382.0
	N ₃ F ₃	227.6	149.6	377.2
L. hirsutum Sp. 3.	N ₁ F ₁	166.6	175.1	341.7
	N ₂ F ₁	159.2	136.7	295.9
	N ₃ F ₁	180.4	146.7	327.1
	N ₁ F ₂	176.7	156.6	333.3
	N ₂ F ₂	160.4	122.4	282.8
	N ₃ F ₂	170.2	172.9	343.1
	N ₁ F ₃	202.7	191.0	393.7
	N ₂ F ₃	201.2	162.8	364.0
	N ₃ F ₃	193.0	150.2	343.2

Table 48 cont.

Species	Treatment	Rep. I	Rep. II	Total
L. glandulosum Sp. 4.	N ₁ F ₁	85.8	81.4	167.2
	N ₂ F ₁	123.8	141.8	265.6
	N ₃ F ₁	108.6	117.3	225.9
	N ₁ F ₂	121.1	94.8	215.9
	N ₂ F ₂	111.3	74.0	185.3
	N ₃ F ₂	142.3	161.9	304.2
	N ₁ F ₃	145.9	126.6	272.5
	N ₂ F ₃	130.1	122.4	252.5
	N ₃ F ₃	105.7	141.8	247.5
L. peruvianum Sp. 5.	N ₁ F ₁	67.8	76.8	144.6
	N ₂ F ₁	130.1	86.9	217.0
	N ₃ F ₁	125.8	85.2	211.0
	N ₁ F ₂	118.1	142.3	260.4
	N ₂ F ₂	104.6	107.3	211.9
	N ₃ F ₂	112.7	91.8	204.5
	N ₁ F ₃	174.6	115.1	289.7
	N ₂ F ₃	135.8	76.8	212.6
	N ₃ F ₃	119.1	93.7	212.8
L. hirsutum var glabratum	N ₁ F ₁	137.3	151.5	288.8
	N ₂ F ₁	172.9	98.2	271.1
	N ₃ F ₁	156.3	134.1	290.4
	N ₁ F ₂	168.6	109.7	278.3
	N ₂ F ₂	127.8	137.9	265.7
	N ₃ F ₂	167.7	144.7	312.4
	N ₁ F ₃	173.3	208.7	382.0
	N ₂ F ₃	168.9	173.2	342.1
	N ₃ F ₃	192.9	147.9	340.8
		10421.2	10117.8	20539.0

Items	df	S.S.	M.S.	F.	P.
Sp	5	1023696.6	204739.30	164.47	***
N	2	655.7	327.85	< 1	N.S.
F	2	1094.7	547.35	< 1	N.S.
Sp x N	10	10287.4	1028.74	< 1	N.S.
Sp x F	10	14622.4	1462.24	1.17	N.S.
N x F	4	5483.9	1370.97	1.10	N.S.
Sp x N x F	20	13327.9	666.39	< 1	
Error	54	67221.4	1244.84		

Critical difference when $P = 0.05$, 23.60g.
when $P = 0.01$, 31.44g.
when $P = 0.001$, 41.01g.

(Mean of 18) $\bar{m}$ Sp 1 = 398.48g.
205.76g. ***
 $\bar{m}$ Sp 2 = 192.72g.
24.68g. *
 $\bar{m}$ Sp 3 = 168.04g.
14.06g.
 $\bar{m}$ Sp 6 = 153.98g.
35.28g. **
 $\bar{m}$ Sp 4 = 118.70g.
9.56g.
 $\bar{m}$ Sp 5 = 109.14g.

Table 49.Experiment 7.Final Records.Root length.

Species	Treatment	Rep. I	Rep. II	Total
L. esculentum Eurocross A. Sp. 1.	N ₁ F ₁	43.5	36.0	79.5
	N ₂ F ₁	38.0	38.0	76.0
	N ₃ F ₁	37.5	69.0	106.5
	N ₁ F ₂	27.0	60.0	87.0
	N ₂ F ₂	43.0	40.0	83.0
	N ₃ F ₂	43.5	33.5	77.0
	N ₁ F ₃	27.0	33.0	60.0
	N ₂ F ₃	28.0	36.0	64.0
	N ₃ F ₃	37.5	32.0	69.5
L. pimpinellifolium Sp. 2.	N ₁ F ₁	52.5	49.0	101.5
	N ₂ F ₁	50.5	46.0	96.5
	N ₃ F ₁	56.0	40.0	96.0
	N ₁ F ₂	62.0	38.0	100.0
	N ₂ F ₂	39.0	73.0	112.0
	N ₃ F ₂	62.0	41.0	103.0
	N ₁ F ₃	29.0	69.0	98.0
	N ₂ F ₃	43.5	74.0	117.5
	N ₃ F ₃	71.0	49.0	120.0
L. hirsutum Sp. 3.	N ₁ F ₁	35.5	54.0	89.5
	N ₂ F ₁	50.5	54.0	104.5
	N ₃ F ₁	54.0	54.5	108.5
	N ₁ F ₂	58.0	42.0	100.0
	N ₂ F ₂	69.0	68.0	137.0
	N ₃ F ₂	52.0	37.5	89.5
	N ₁ F ₃	54.5	52.0	106.5
	N ₂ F ₃	31.0	54.5	85.5
	N ₃ F ₃	46.0	58.0	104.0

Table 49 cont.

Species	Treatment	Rep. I	Rep. II	Total
L. glandulosum Sp. 4.	N ₁ F ₁	40.0	46.0	86.0
	N ₂ F ₁	48.5	40.5	89.0
	N ₃ F ₁	29.0	37.0	66.0
	N ₁ F ₂	32.0	42.0	74.0
	N ₂ F ₂	51.0	37.0	88.0
	N ₃ F ₂	35.5	40.0	75.5
	N ₁ F ₃	43.5	50.5	94.0
	N ₂ F ₃	46.0	30.0	76.0
	N ₃ F ₃	39.0	31.0	70.0
L. peruvianum Sp. 5.	N ₁ F ₁	26.5	30.5	57.0
	N ₂ F ₁	65.0	63.0	128.0
	N ₃ F ₁	54.5	52.5	107.0
	N ₁ F ₂	46.0	50.0	96.0
	N ₂ F ₂	45.0	37.5	82.5
	N ₃ F ₂	51.0	54.0	105.0
	N ₁ F ₃	67.0	68.0	135.0
	N ₂ F ₃	54.0	41.0	95.0
	N ₃ F ₃	37.0	49.5	86.5
L. hirsutum var glabratum Sp. 6.	N ₁ F ₁	38.0	46.0	84.0
	N ₂ F ₁	61.0	52.0	113.0
	N ₃ F ₁	44.0	33.0	77.0
	N ₁ F ₂	32.0	31.0	63.0
	N ₂ F ₂	88.0	49.0	137.0
	N ₃ F ₂	35.0	38.0	73.0
	N ₁ F ₃	33.0	48.0	81.0
	N ₂ F ₃	64.5	37.0	101.5
	N ₃ F ₃	45.0	37.5	82.5
		2492.0	2502.5	4994.5

Items	df	S.S.	M.S.	F.	P.
Sp	5	3053.1	610.62	4.89	***
N	2	620.1	310.05	2.48	N.S.
F	2	18.0	9.00	< 1	N.S.
Sp x N	10	1479.7	147.97	1.18	n.S.
Sp x F	10	750.8	75.08	< 1	N.S.
N x F	4	742.1	185.52	1.48	N.S.
Sp x N x F	20	3140.3	157.01	1.26	N.S.
Error	54	6746.1	124.93		

Critical difference when $P = 0.05$, 7.48 cm.

when $P = 0.01$, 9.96 cm.

when $P = 0.001$, 12.99 cm.

(Mean of 18)	$\bar{m}$ Sp 2	= 52.47 cm.	1.08 cm.
	$\bar{m}$ Sp 3	= 51.39 cm.	1.84 cm.
	$\bar{m}$ Sp 5	= 49.55 cm.	4.44 cm.
	$\bar{m}$ Sp 6	= 45.11 cm.	5.19 cm.
	$\bar{m}$ Sp 4	= 39.92 cm.	0.89 cm.
	$\bar{m}$ Sp 1	= 39.03 cm.	

Table 50.

Experiment 7.Final Records.Root weight.

Species	Treatment	Rep. I	Rep. II	Total
L. esculentum Eurocross A. Sp. 1.	N ₁ F ₁	46.4	32.1	78.5
	N ₂ F ₁	50.95	62.3	113.2
	N ₃ F ₁	44.9	63.9	108.8
	N ₁ F ₂	58.7	53.2	111.9
	N ₂ F ₂	46.2	36.4	82.6
	N ₃ F ₂	31.2	57.2	88.4
	N ₁ F ₃	33.8	51.3	85.1
	N ₂ F ₃	39.7	53.6	93.3
	N ₃ F ₃	53.6	36.6	90.2
L. pimpinellifolium Sp. 2.	N ₁ F ₁	37.1	43.3	80.4
	N ₂ F ₁	48.3	120.4	168.7
	N ₃ F ₁	99.1	76.4	175.5
	N ₁ F ₂	62.5	69.5	132.0
	N ₂ F ₂	118.1	53.6	171.7
	N ₃ F ₂	46.4	61.4	107.8
	N ₁ F ₃	28.4	82.9	111.3
	N ₂ F ₃	74.4	58.9	133.3
	N ₃ F ₃	68.8	59.5	128.3
L. hirsutum Sp. 3.	N ₁ F ₁	68.0	118.9	186.9
	N ₂ F ₁	127.1	88.3	215.4
	N ₃ F ₁	57.0	117.2	174.2
	N ₁ F ₂	71.4	90.3	161.7
	N ₂ F ₂	207.6	74.2	281.8
	N ₃ F ₂	67.4	150.2	217.6
	N ₁ F ₃	35.6	111.7	147.3
	N ₂ F ₃	34.3	87.1	121.4
	N ₃ F ₃	38.4	87.3	125.7

Table 50 cont.

Species	Treatment	Rep. I	Rep. II	Total
L. glandulosum Sp. 4.	N ₁ F ₁	24.1	31.8	55.9
	N ₂ F ₁	44.4	70.3	114.7
	N ₃ F ₁	52.8	52.7	105.5
	N ₁ F ₂	49.7	47.0	96.7
	N ₂ F ₂	19.0	31.1	50.1
	N ₃ F ₂	25.7	38.6	64.3
	N ₁ F ₃	64.9	63.4	128.3
	N ₂ F ₃	44.8	41.0	85.8
	N ₃ F ₃	29.9	41.8	71.7
L. peruvianum	N ₁ F ₁	24.3	34.4	58.7
	N ₂ F ₁	258.4	102.6	361.0
	N ₃ F ₁	121.1	142.8	263.9
	N ₁ F ₂	99.2	63.6	162.8
	N ₂ F ₂	111.2	108.0	219.2
	N ₃ F ₂	200.3	115.3	315.6
	N ₁ F ₃	83.7	90.7	174.4
	N ₂ F ₃	93.2	95.8	189.0
	N ₃ F ₃	188.7	64.2	252.9
L. hirsutum var glabratum Sp. 6.	N ₁ F ₁	37.5	59.8	97.3
	N ₂ F ₁	105.8	46.3	152.1
	N ₃ F ₁	64.5	38.3	102.8
	N ₁ F ₂	35.2	53.9	89.1
	N ₂ F ₂	48.7	76.5	125.2
	N ₃ F ₂	28.7	41.5	70.2
	N ₁ F ₃	54.9	87.2	142.1
	N ₂ F ₃	33.4	47.8	81.2
	N ₃ F ₃	43.4	37.9	81.3
		3682.8	3722.0	7404.8

Items	df	S.S.	M.S.	F.	P.
Sp	5	65893.3	13178.66	12.18	***
N	2	6280.6	3140.30	2.90	N.S.
F	2	2180.2	1090.10	1.01	N.S.
Sp x N	10	16711.1	1671.11	1.54	N.S.
Sp x F	10	5919.8	591.98	< 1	N.S.
N x F	4	9197.3	2299.32	2.12	N.S.
Sp x N x F	20	13561.9	678.09	< 1	N.S.
Error.	54	58436.9	1082.16		

Critical difference when $P = 0.05$, 22.01 g.
when $P = 0.01$, 29.31 g.
when $P = 0.001$, 38.24 g.

(Mean of 18)

$\bar{m}$ Sp 5 = 110.97 g.
20.30 g.

$\bar{m}$ Sp 3 = 90.67 g.
23.50 g.*

$\bar{m}$ Sp 2 = 67.17 g.
14.88 g.

$\bar{m}$ Sp 6 = 52.29 g.
4.96 g.

$\bar{m}$ Sp 1 = 47.33 g.
4.39 g.

$\bar{m}$ Sp 4 = 42.94 g.

Table 51.Experiment 6d.

1 cc. of the suspension of surface sterilised eggs and larvae
contained 2567 larvae (Mean of 3 counts).

Larval counts after 1 week.

Treatment	Rep. I	Rep. II	Rep. III
A	3255	3510	3360
B	6525	5880	5250
C	5400	5970	4590
D	4320	4560	4410
E	3450	3930	3690
F	4200	3450	3330

Actual hatch after 1 week.

Treatment	Rep. I	Rep. II	Rep. III	Total
A	688	943	793	2424
B	3958	3313	2683	9954
C	2833	3403	2023	8259
D	1753	1993	1843	5589
E	883	1363	1123	3369
F	1633	883	763	3279
	11748	11898	9228	32874

Correction factor = 60038882

Items	df	S.S.	M.S.	F.	P.
Treatments	5	15463750	3092750	15.48	***
Residual	12	2396700	199725		
Total	17	17860450			

Critical difference when $P = 0.05$, 795.11.
when $P = 0.01$, 1114.76.
when $P = 0.001$, 1575.63.

Appendix Table 52.Experiment 8.Visual score on whole root system.

Treatment		Month 1	Month 2	Month 3	Month 4	Month 5
Control	a)	2	4	4	4	4
	b)	2	3	4	4	4
16 ozs/24 hrs.	a)	2	3	4	4	4
	b)	2	3	4	4	4
8 ozs/48 hrs.	a)	1	4	3	4	4
	b)	2	3	4	4	4
2 ozs/48 hrs.	a)	3	4	4	4	4
	b)	2	3	4 ¹	4	4
4 ozs/96 hrs.	a)	2	3	4	4	4
	b)	2	3	4	4	4
2 ozs/96 hrs.	a)	2	3	4	4	4
	b)	2	3	4	4	4
8 ozs/96 hrs.	a)	2	3	4	4	4
	b)	2	3	4	4	4

Appendix Table 53.Experiment 8.Nematodes per gramme fresh weight of roots.

Treatment		Month 1	Month 2	Month 3	Month 4	Month 5
Control "	a)	170.1	40.4	6.0	0.0	5.8
	b)	69.6	34.0	42.8	1.4	3.9
16 ozs/24 hrs. "	a)	0.5	2.2	4.1	0.0	1.0
	b)	5.5	0.0	0.9	6.7	0.5
8 ozs/48 hrs. "	a)	1.1	0.0	0.0	10.0	0.0
	b)	0.0	1.4	5.2	0.0	0.1
2 ozs/48 hrs. "	a)	96.2	17.6	0.0	40.0	0.7
	b)	74.6	22.3	0.6	10.5	4.2
4 ozs/96 hrs. "	a)	0.2	7.4	7.9	30.0	57.1
	b)	4.3	2.2	2.2	0.3	1.7
2 ozs/96 hrs. "	a)	0.7	0.0	4.9	0.0	0.0
	b)	1.5	1.5	26.3	1.9	9.1
8 ozs/96 hrs. "	a)	5.2	0.4	1.8	0.0	5.0
	b)	0.4	4.4	5.1	3.8	1.5

Appendix Table 54.Experiment 8.Fresh weight of roots throughout the experiment in grammes.

Treatment		Month 1	Month 2	Month 3	Month 4	Month 5
Control "	a)	12.0	14.9	16.2	24.4	21.4
	b)	4.3	16.3	6.6	6.7	22.7
16 ozs/24 hrs. "	a)	13.0	12.0	10.8	4.0	15.0
	b)	10.2	12.4	14.9	18.5	16.0
8 ozs/48 hrs. "	a)	12.0	9.0	7.5	12.0	8.0
	b)	10.5	7.8	22.5	5.8	26.2
2 ozs/48 hrs. "	a)	5.5	11.7	2.5	2.4	19.2
	b)	11.0	7.8	9.4	15.7	15.5
4 ozs/96 hrs. "	a)	9.5	9.2	10.7	3.6	8.0
	b)	11.0	7.3	13.7	9.7	14.3
2 ozs/96 hrs. "	a)	9.5	6.8	13.7	5.2	5.8
	b)	7.0	11.8	6.8	14.0	21.9
8 ozs/96 hrs. "	a)	10.0	13.3	10.8	5.9	5.8
	b)	12.2	14.4	12.4	7.3	8.7

Appendix Table 55.Experiment 8.

Treatment	Root Pieces	Month 1	Month 2	Month 3	Month 4	Month 5
Control 0	Total 0-2 mm.	46 43	67 60	42 35	36 26	17 12
16ozs/24hrs. "	Total 0-2 mm.	100 92	65 61	49 40	34 25	28 19
8ozs/48hrs. "	Total 0-2 mm.	134 129	57 54	49 33	20 17	35 24
2ozs/48hrs. "	Total 0-2 mm.	148 119	62 61	21 17	31 25	35 29
4ozs/96hrs. "	Total 0-2 mm.	143 142	62 61	48 34	24 21	49 41
2ozs/96hrs. "	Total 0-2 mm.	74 72	46 44	46 38	37 32	29 20
8ozs/96hrs. "	Total 0-2 mm.	110 106	87 80	48 41	24 22	19 14