INVESTIGATIONS INTO THE INTERACTIONS OF *HETERODERA ROSTOCHIENSIS* WOLL., *RHIZOCTONIA SOLANI* KÜHN AND *COLLETOTRICHUM COCCODES* (WALLR.) HUGHES ON THE TOMATO PLANT AND THE EFFECTS OF *APHELENCHUS AVENAE* BASTIAN ON THESE FUNGI

by

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CONTENTS

SUMMARY

Investigations into the interrelationships of two nematodes, Heterodera rostochiensis and Aphelenchus avenae and two fungi, Rhizoctonia solani and Colletotrichum coccodes on tomato (var. - Alisa Craig) were undertaken.

The plants suffered greater growth check when H. rostochiensis attacked before the fungi than when the fungi preceded the nematode. Incidence of diseases due to the fungi and production of cysts of H. rostochiensis was also greater in the former treatment than the latter. Effects of simultaneous inoculation of fungi and nematode lay between these two treatments. The fungi retarded the formation of giant cells by H. rostochiensis and therefore, development of cysts decreased. The fungi depressed the hatching of larvae from cysts but did not affect entry of the larvae into roots. On the other hand, if the nematodes could produce giant cells before fungus invasion these were favoured by the fungi for colonization. The nematodes were found to possess some indole derivative/s in their bodies.

Though R. solani grew better at 27°C in P.D.A. and at 25°C in sterilized soil than at lower temperatures but the rate of growth at these higher temperatures dropped quickly. The survival capacity of R. solani in sterilized soil declined considerably at 25°C and was almost nil after 8 months. On the contrary, it perpetuated much better at 6 ± 2°C. Variability in the growth rate as well as in cultural characters were noticed among different

isolates of R. solani.

A. avenae multiplied more rapidly in R. solani than C. coccodes culture but no significant difference in any of the morphometric values was observed. Number of eggs laid per female was more in R. solani than C. coccodes but no significant difference was found in the length of egg laying period.

Incidence of diseases due to R. solani and C. coccodes was reduced by adding A. avenae in sterilized soil but not in unsterilized soil. Correspondingly the growth of the plants was also increased. A. avenae multiplied considerably in soil in the presence of fungus inoculum grown on sugarbeet seed but their number did not increase appreciably when the fungus added was grown on maize meal sand medium.

INTRODUCTION

Morgan (1925) stated that Rhizoctonia solani could be a contributing factor in "potato sickness" in the presence of Heterodera rostochiensis. This view was supported by Cheal (1929) and Edwards (1929) who obtained a greater growth check when the pathogens were inoculated together compared with either organism alone. Miles (1930) and Millard et al. (1932) on the other hand did not consider R. solani to be an important agent for this malady. Graham (1966) however, observed that predominence of soil inhabiting fungi adversely affected the production of cysts by potato cyst eelworm. Grainger and Clark (1963) and Dunn and Hughes (1964) showed that the combined effect of R. solani and potato cyst eelworm was greater than potato cyst eelworm alone on potato and tomato, respectively. Ketudat (1968) obtained similar results on the growth of tomato and an increase in male/female sex ratio in the eelworm; and also found that these fungi adversely affected the production of cysts and hatching of larvae of H. rostochiensis as was observed by James (1968) working with grey sterile fungus.

Miles (1930) and Triffitt (1931) emphasized that Colletotrichum coccodes could be a contributing factor for "potato sickness", but Millard et al. (1932) and Dunn and Hughes (1964) did not find any such effect on potato and tomato, respectively.

As both R. solani and C. coccodes generally occur with H. rostochiensis in glasshouse soils where tomatoes are grown in Scotland, it was decided to investigate in what way the one organism may affect the other. It is believed that R. solani

causes trouble in Scotland and the North of England but rarely in the South of England but from the studies of literature it appears that the temperature of the south of England should not be too high to limit its growth. Therefore, some detailed studies on the growth and survival of R. solani in relation to temperature requirements were investigated.

The ubiquitous, saprozoic and mycophagous nematode, Aphelenchus avenae is known to feed on a variety of fungi under laboratory conditions (Mankau and Mankau, 1963; Townshend, 1964; Pillai and Taylor, 1967a,b) and therefore, when it was observed in glasshouse soil infested with C. coccodes, some experiments were conducted to find out its effect on the inoculum potential of both these fungi with a view to elucidate whether its presence in the soil was a factor in the degree of occurrence of these fungal diseases in glasshouse.

The above workers have recorded differential multiplication rate of A. avenae on different fungi in vitro. Pillai and Taylor (1967b,c) have also recorded changes of morphometric values in them and therefore, some biological studies of this nematode were undertaken.

The thesis has been divided into the following four chapters:

I. Interrelationships between H. rostochiensis and (1) R. solani and (2) C. coccodes.

II. Growth of R. solani at different temperatures.

III. Morphology and biology of A. avenae in cultures of R. solani and C. coccodes.

IV. Effects of the presence of *A. avenae* on the growth of tomato plants and the incidence of diseases caused by *R. solani* and *C. coccodes*.

Each chapter has been discussed separately and at the end of the thesis a general discussion and conclusions have been given.

REVIEW OF LITERATURE

Our knowledge about interrelationships between hosts and soil inhabiting causal pathogens is scanty, Barkely (1944) aptly stated "this is not surprising when it is realized that roots grow in an inert environment of most complex physical and chemical nature, inhabited by countless number of species of living organisms in a dynamic equilibrium". In a complex medium, like soil, the single pathogen-host relationship does not usually exist. In addition to a direct effect there may be many indirect effects from such an interaction.

The necrosis on roots caused by ectoparasitic or cortical endoparasitic nematodes is accompanied by chemical changes which make the tissues a useful "food base" for soil microorganisms. Working on the peach replant problem in Ontario, Mountain and Patrick (1959) recorded that the main role of Pratylenchus penetrans (Cobb) Filip. and Stek. was to provide such infection courts for soil organisms. Steiner (1953) observed that nematodes are frequently members of disease complexes on roots "acting either as inhibitors, cooperators, synergists and aggravators, or otherwise."

Pitcher (1965) recorded that in interactions between nematodes and fungi, the role of each partner was usually to assist the other but only in one case, the association between Dilophosphora alopecuri (Fr.) Fr. and Anguina tritici (Steinbuch) Filipjev was found to be essential for the disease development. It is probable that in

such cases of complex associations instead of a single mechanism, a combination or succession of mechanisms may be involved. Recently, more attention has been focussed on this aspect of root diseases where besides nematodes, fungi, bacteria, viruses etc. occur together. Slack (1963) advocated more emphasis on this complex biological association and sought an integrated approach by workers in different disciplines of plant pathology in order to solve the problems of pathogenesis. The extent of interest in this statement aroused by plant pathologists can be judged by the fact that during the 7 years period (1956 to 1963) in the north eastern states of the U.S., 23 investigations on interrelationships between nematodes and other plant pathogens were conducted and positive reactions were obtained in 15. It was found that nematodes increased the severity of Fusarium wilt, shortened the time of disease expression or broke the resistance of plants to fungus diseases (Jenkins et al., 1963). McKeen and Mountain (1960) pointed out the possibilities of error when the possible effect of soil microorganisms was ignored while studying population dynamics of plant pathogenic soil nematodes. The disease syndrome produced from a complex association of more than one organism may be quite different than that produced by the individual pathogen separately.

Most of the works carried out so far have shown that the association between nematodes and other disease producing organisms is either obligatory or complementary but in a few cases the effect

of one on the other has been found to be inhibitory. This often happens in case of mycophagous, saprozoic or free living nematodes. The genera which contain the important mycophagous nematodes are Aphelenchus Bastian, Aphelenchoides Fischer, Ditylenchus Filipjev, Paraphelenchus (Micoletzky) Micoletzky and Bursaphelenchus Fuchs.

The term "disease complex" is most commonly used for the association of Fusarium spp. with the root knot syndrome. The first reference to such a disease complex was made by Atkinson (1892) when he observed that root knot nematodes appeared to increase the severity of wilt due to Fusarium in cotton. Since then many U.S. workers (Young, 1938; Smith, 1941; Martin et al., 1956; Hollis, 1958 and Paxman and Gerber, 1966) have made similar observation on this disease. Of the 5 spp. of nematodes used by Martin et al. (1956) only Meloidogyne incognita (Kofoid and White) Chitwood and M. incognita acrita Chitwood increased the incidence of wilt and the other 3 spp. - Trichodorus, Tylenchorhynchus and Helicotylenchus did not.

Young (1938) recorded that susceptibility of cotton to Fusarium wilt could be reduced by the application of potash but when root knot nematodes were present neither potash application nor varietal resistance was effective. Arndt and Christie (1937) failed to demonstrate any convincing evidence that any of the 4 spp. of nematodes (Aphelenchoides parientinus Bastian, Aphelenchus avenae Bastian, Cephalobus elongatus de Man and Acrobeles butschlii de Man) used was capable of producing soreshin lesions on cotton but recorded that severity of hypocotylar infection caused by several

fungi was aggravated in their presence. Newson and Martin (1953) observed that yield of cotton was increased in the fumigated fields in Louisiana when the soil was previously heavily infested with Meloidogyne, Trichodorus, Pratylenchus and Tylenchorhynchus along with the wilt fungus but fumigation failed to give any effect in areas where the wilt infection was low.

Pratylenchus pratensis (de Man) Filipjev has been reported also to increase severity of Fusarium wilt of cotton in North Carolina (Taylor et al., 1936) and Georgia (Smith, 1940), respectively. Belonolaimus gracilis Steiner was found to break the resistance of resistant varieties and increase the susceptibility of susceptible varieties of cotton to Fusarium wilt in South Carolina (Holdeman and Graham, 1952). A correlation between the number of nematode population and the loss of wilt resistance was demonstrated. Porter and Powell (1967) observed a distinct interrelationship between wilt due to Fusarium oxysporum Schlecht. f. nicotianae (Johnson) Snyd. and Hans. and root knot nematodes (M. incognita, M. arenaria (Neal) Chitwood, M. javanica (Treub.) Chitwood) in the development of disease in both wilt resistant and wilt susceptible varieties of tobacco in North Carolina. Vigorous colonisation by F. oxysporum f. nicotianae of giant cells caused by M. incognita was observed in both wilt resistant and wilt susceptible varieties of tobacco (Melendez and Powell, 1967).

Wilt of carnations caused by F. oxysporum Schlecht. f. dianthi (Prill. and Del.) Snyd. and Hans. showed a synergistic reaction with different root knot nematodes (M. hapla Chitwood, M. incognita,

M. incognita acrita, M. arenaria, M. arenaria thamesi Chitwood, M. javanica) but did not interact with Helicotylenchus nannus Steiner and H. buxophilus (Golden) Perry (Schindler et al., 1961). Similarly, Rankin (1957) observed that the incidence of okra wilt caused by F. oxysporum f. vasinfectum (Atk.) Snyd. and Hans. was increased in the presence of M. incognita acrita but not Pratylenchus brachyurus (Godfrey) Filip. and Stek. (=P. leicephalus Steiner). More than 100% increase of panama disease of bananas due to F. oxysporum f. cubense (Sm.) Snyd. and Hans. occurred in the presence of Radopholus similis (Cobb) Thorne in Panama but the Meloidogyne spp. did not increase the intensity.

F. solani (Appel and Wr.) Snyd. and Hans. was considered to be a weak pathogen not to cause damage to citrus plants (Citrus limon (L.) Burm.) in Arizona but O'Bannon et al. (1967) observed that in the presence of Tylenchulus semipenetrans Cobb reduction of growth was greater than that caused by either organism alone at 30°C. Similarly, Feder and Feldmesser (1961) noted that combined inoculation of grapefruit seedlings with Fusarium (F. oxysporum (Schlecht.) Snyd. and Hans. and F. solani) and R. similis caused more damage to seedlings than the fungus or nematode alone. An interesting observation of temperature effect on the host plants was recorded by Palmer et al. (1967) who observed that when corn seedlings were grown at 24°C with 16 hours light for 3 weeks and then inoculated with F. moniliforme Sheld. and Pratylenchus scribneri Steiner there was no difference in the growth reduction between nematode + fungus and only nematode inoculation but when grown at 30°C for two weeks preceded by 18°C for one week, there was more reduction in

the fungus + nematode treatment than either pathogen alone.

Jenkin and Coursen (1957) supported the observation of Young (1939) that the resistance of tomato varieties to wilt due to F. oxysporum f. lycopersici (Sacc.) Snyd. and Hans. was broken down by Meloidogyne spp. The former workers showed that the wilt resistant variety, Chesapeake developed no wilt when inoculated with the fungus alone but 100% wilt developed when M. incognita acrita was included and 60% when M. hapla was included. However, Binder and Hutchison (1959) could not develop wilt in Chesapeake in the presence of M. incognita acrita and suggested that the population of nematode they used might be relatively non-pathogenic or the inoculum was insufficient or the seedlot of Chespeake varied in susceptibility. Afterwards, Bowman and Bloom (1966) and Goode and McGuire (1967) have again shown the breaking of resistance of tomato varieties to Fusarium wilt by M. incognita. Similar observations had been made in Israel where two nematodes were involved - M. incognita and M. hapla (Cohn and Minz, 1960).

Few cases of interactions between Verticillium and root parasitic nematodes, especially Pratylenchus have been reported. De Segura and Pedro (1955) observed that growth of Peruvian cotton was checked significantly in soils where V. albo-atrum Reinke and Berth. was present with light infestation of root knot nematodes. McClellan et al. (1955) did not find any synergistic effect between V. albo-atrum and root knot nematodes on cotton. Parker (1959) could not find evidence to support that lesions made by nematodes (root knot on cotton or root lesion nematode on strawberry) provided

infection courts for Verticillium although damage caused by two pathogens together might be additive. McKeen and Mountain (1960) and Mountain and McKeen (1960) recorded a synergistic effect between Pratylenchus penetrans and V. albo-atrum on eggplant. They found that at low and intermediate levels of fungus inoculum, Pratylenchus increased wilt and that multiplication of the nematode was greater in the fungus infected than non-infected roots.

In a later trial, Mountain and McKeen (1962) observed that in soil infested with P. penetrans, inoculation with V. dahliae Kleb. resulted in increase in the multiplication rate of the nematode which was highest on the root of eggplant, less on tomato and least on Capsicum annuum L. The corresponding wilt incidence was 100% in eggplant, 60% in tomato and 15% in C. annuum. In another interrelationship between V. dahliae and P. minyus Sher and Allen on Mentha piperita L., Faulkner and Scotland (1955) observed that the nematode increased both prevalence and severity of wilt, reduced the incubation period of the fungus and that reproduction of Pratylenchus was almost double in presence of the fungus. Reduction of dry matter was much higher in the presence of both the pathogens than either organism alone. Significant interaction was also observed between V. albo-atrum and P. penetrans in reducing dry weight of tuber and tops of potato (Morsink and Rich, 1968).

Cylindrocarpon radicicola Wr. has been recorded to have a synergistic effect with P. pratensis in reducing plant growth of a number of horticultural crops in British Columbia (Hastings and Bosher, 1938). Langdon et al. (1961) from Oklahoma, recorded a

new disease of wheat and barley characterized by stunting, reduced tillering and yield due to Tylenchorhynchus brevidens Allen which was aggravated by the presence of Olpidium.

Slootweg (1956) observed that lily of the valley suffered badly from a root rot caused primarily by a Pratylenchus sp. or Hoplolaimus uniformis Thorne with C. radicicola as a secondary pathogen in Holland. In another interrelationship between Phoma solanicola Prill. and Del. and Ditylenchus dipsaci Kühn on potato from Holland, Hijink (1963) found a strong regression line of percentage of Phoma infected plants on preplanting D. dipsaci count in soil and also observed that the loss in yield due to the combined attack might be over 50%.

The ubiquitous and polyphagous fungus, Rhizoctonia solani Kühn has been found to interact with some nematodes in the expression of diseases. It was found to be constantly associated with P. minyus in inciting a root rot of wheat in Ontario. The combined effect was twice as great as either of the pathogen alone and the incidence of the fungus appeared to be correlated with the nematode population in soil (Mountain and Benedict, 1956; Benedict and Mountain, 1956). Taylor and Wyllie (1959) observed a greater increase of pre-emergence damping-off of Chippewa soybeans due to R. solani with M. javanica and M. hapla. Attack of R. solani on cotton was increased in the presence of M. incognita acrita in Arizona (Raynolds and Hanson, 1957). White (1962) also recorded an interaction between M. incognita and R. solani or Thielaviopsis basicola (Berk. and Br.) Ferr. on the same crop.

Aphelenchus avenae Bastian was considered to be a saprozoic nematode (Goodey, 1935 and Thorne and Price, 1935) but Steiner (1936) and Christie and Arndt (1936) showed it to have a limited parasitic ability on plants. Later, Goodey (1963) recorded it both as saprophytic and mycophagous. Barker (1963) has shown parasitism of A. avenae on callus tissue of tobacco (Nicotiana tabacum L. and N. glutinose L.), Kentuky bluegrass and also reduction of growth of bluegrass due to its attack. Mankau and Mankau (1963), Southerland (1967) and Southerland and Fortin (1968) recorded it to be mycophagous which did not damage plant roots. Its occurrence in the rhizosphere of diseased root was considered due to the presence of fungus mycelia. Soloveva (1964) in Russia, observed that in the diseased roots of Brassica capitata L. due to Plasmodiophora brassicae Wr. the number of nematodes were 3 times greater than the uninfected roots.

A. avenae has been found capable of feeding on a number of fungi including R. solani in artificial cultures (Mankau and Mankau, 1963; Townshend, 1964 and Pillai and Taylor, 1967 a,b). Its potentiality as a biological control agent against plant diseases had been demonstrated first by Rhoades and Linford (1959) who obtained control of root rot of corn due to Pythium arrhenomanes Drechsler by adding 125,000 nematodes per 6 in. pot. Control of root rot of bean due to R. solani was achieved by adding 100,000 A. avenae per 5 in. crock (Barker, 1964). Recently, Klink (1966) and Klink and Barker (1968) had shown that while 4,000 to 6,000 of

this nematode per ml. of fungal inoculum controlled root rot of Phaseolus vulgaris L. (due to R. solani) and Pisum sativum L. (due to F. oxysporum f. pisi (Linf.) Snyd. and Hans. isolates 1 and 2) any number more or less decreased the efficiency. They also demonstrated that the nematode destroyed the fungal mycelium of R. solani, F. solani f. pisi Jones, F. solani f. phaseoli Burk. in soil and prevented formation of sclerotia by R. solani and Sclerotium sp.Klink (1966) also included Pythium debaryanum Hesse, P. ultimum Thow and F. solani f. pisi which could be controlled on the same hosts with the same number of A. avenae.

Feeding of A. avenae on 7 mycorrhizal fungi and prevention of the formation of an ectoparasitic mycorrhizal relationship between red pine (Pinus resinosa Ait) and Suillus granulatus (Fr.) Gray were observed by Southerland and Fortin (1968). Another nematode, a Deladenus sp. on Rhododendron was considered to feed on the mycorrhiza of the root (Clark, 1964). Reduction of carnation wilt due to F. oxysporum f. dianthi was also obtained with the addition of 3,200 mycophagous Ditylenchus spp. per plant (Schindler and Stewart, 1956).

Association of R. solani with Heterodera rostochiensis Woll. in "potato sick" soil in U.K. was observed by a few workers in the thirties (Morgan, 1925 and 1926; Morgan and Peters, 1929 and Cheal, 1929). The last worker was of the opinion that though the combined effect of R. solani and H. rostochiensis was more than either of the pathogen alone, the main cause was the potato cyst eelworm (P.C.E.) Triffitt (1931) considered C. coccodes (Wallr.)

Hughes to be a contributing factor in "potato sickness". Millard et al. (1932) also found C. coccodes along with R. solani and P.C.E. in the affected potato soil but they concluded that the nematode was the primary cause of the malady. Thereafter, Grainger and Clark (1963) on potato and Dunn and Hughes (1964) on tomato showed that the combined attack of H. rostochiensis and R. solani was more severe than either of the pathogens alone. The latter workers had demonstrated significant growth check of tomato when combined inoculation of P.C.E. and R. solani or P.C.E., R. solani and C. coccodes was made simultaneously.

While the above effects of fungi and P.C.E. seem to be complementary, Graham (1966) on the other hand, reported that in a badly "tomato sick" soil final population of P.C.E. was decreased in the presence of brown root rot fungi (Fusarium sp., Thielaviopsis sp. and C. coccodes). In such soils when tomatoes were grown continuously, root rotting fungi had dominated and either rendered the roots unsuitable for P.C.E. to enter or incapable of supporting it. James (1966, 1968) continued his studies on the same direction and found that grey sterile fungus had an inhibitory effect on the hatching, invasion and production of cysts of H. rostochiensis on tomato root. The inhibitory effect of grey sterile fungus was found to affect in some way to stop the females of P.C.E. to form giant cells in the fungus infected roots (Roy, 1968).

Working with P.C.E. and root rotting fungi (R. solani, grey sterile fungus, V. albo-atrum) on tomato, Ketudat (1968) has shown that the plants exhibited more severe disease symptoms in the

combination of fungus and nematode, than either of the pathogens alone and that hatching of P.C.E. and M. incognita and final population of P.C.E. were depressed in the presence of the fungi. The male/female sex ratio of P.C.E. was also increased in the presence of the fungi (Ketudat, 1968 and 1969). Dunn and Hughes (1967) have shown another interrelationship among H. rostochiensis, Oospora pustulans Owen and Wakef. and R. solani on potato. Growth check in the presence of P.C.E. or R. solani alone was small but moderate in O. pustulans or O. pustulans + P.C.E. and severe in the presence of all the 3 organisms or the two fungi together.

All the above mentioned relationships concern the underground portions of plants. There are, however, a few instances where nematodes and fungi have been seen to act on the aerial parts. Dilophospora alopecuri depends on Anguina tritici for the spores to be carried to the growing points of wheat and other cereals to cause infection (Atanasoff, 1925 and Leukel, 1948). Nonaka (1959) had shown that the severity of rice stem rot due to Leptosphaeria salvanii Catt. was reduced when the plants were previously infected with Aphelenchoides besseyi Christie. Nematode attack resulted in increased respiration and activity of respiratory enzymes of the host.

Occurrence of R. solani and C. coccodes on tomatoes: Though Pythium and Phytophthora spp. are considered most responsible for the cause of damping-off, foot rot and root rot of tomatoes, R. solani may also be present along with these diseases, particularly in the north of England and Scotland (Anonymous, 1965). As early as 1921, Bewley

recorded Rhizoctonia along with Phytophthora, Fusarium and Verticillium to be the cause of damping-off and foot rot of tomatoes. Dunn (1967) reported that R. solani and C. coccodes were responsible for poor growth of tomatoes which occurred either alone or with P.C.E. In Netherlands, foot rot of tomato caused by inter alia R. solani was considered to be one of the most important diseases (Verhoeff, 1963 and 1967).

R. solani causes another trouble of green tomatoes, soil rot, when the fruits come in contact with the infested soil. This disease is prevalent to some extent in all the tomato growing tracts of the eastern U.S.A. (Barksdale, 1968). In Florida, this was considered to be a serious limitation to growing green tomatoes (Hayslip and Stall, 1959 and Gonzalez and Owen, 1963). Crossan et al. (1960) observed 26% loss of marketable fruits due to this disease in the unsprayed plots.

C. coccodes is a widespread pathogen on tomatoes in greenhouse causing brown rot, corky root and black dot. Bewley and Shearn (1924) considered it to be an important pathogen where tomatoes had been grown continuously for several years. A variety of fungi were isolated from the brown rot affected roots by Ebben and Williams (1956) and Ebben (1959) of which C. coccodes was found to be the most serious pathogen. McKay (1942) from Ireland, recorded that this had been known to occur on tomatoes either alone or accompanying other pathogens for almost 100 years. Occurrence of C. coccodes with other organisms has also been observed by Last

and Ebben (1963) and Graham (1966).

Though many of the above workers have mentioned C. coccodes as an important pathogen, others had contrary views (Last and Ebben, 1963 and Chesters and Hornby, 1965). They consider this fungus to be a pathogen of old plants.

Nomenclature of R. solani and C. coccodes: Though Rhizoctonia solani Kühn has been redesignated as Thanatephorus cucumeris (Frank) Donk, the former name is retained here on two grounds: firstly, all the experiments performed were with the 'mycelia sterilia' stage of the fungus and secondly, this name is more prevalent among the plant pathologists. In the text, R.solani will include the other names, Pellicularia filamentosa (Pat.) Rogers, Corticium solani Prill. and Del. and C. vegum Berk. and Curt.

To denote the cause of black dot of potatoes and brown rot and black dot of tomatoes, the binomial, Colletotrichum atramentarium (Berk. and Br.) Taubenh. was used in many parts of the world and until recently in U.K. In Canada and some other countries, C. coccodes (Wallr.) Hughes is used to denote the cause of tomato fruit anthracnose. Recently, by pathogenicity tests, Chesters and Hornby (1965) have shown that the isolates from potatoes, tomato fruits and tomato roots were the same and therefore, proposed the binomial, C. coccodes (Wallr.) Hughes after Hughes (1958). In this text as the cause of root rot and black dot C. coccodes will be used which will also include the names C. atramentarium Taubenh. and C. tabificum (Hallier) Pethybridge.

CHAPTER I

INTERRELATIONSHIPS BETWEEN *HETERODERA ROSTOCHIENSIS* and (1) *RHIZOCTONIA SOLANI* and (2) *COLLETOTRICHUM COCCODES*.

MATERIALS AND METHODS

Sterilization of cysts: As larvae of H. rostochiensis were used for inoculation, it was necessary to find out a method of obtaining sterile cysts to yield sterile larvae. For this purpose cysts were placed in different concentrations of copper sulphate and 1:1000 mercuric chloride for different periods of time as follows:

a - Copper sulphate 0·5% for 1 hour.

b - " " " " 4 "

c - " " " " 14 "

d - " " " " 24 "

e - " " 0·1% " 8 days

f - Mercuric chloride 0·1% for 1½ minutes.

After disinfection, cysts were kept on sterile filter paper soaked with sterile water for 7 days at 25°C and at room temperature (15·6° to 25·0°C, av. 21·6°C). In case of 'e' the cysts without transferring to filter paper were left in $CuSO_4$ solution for 8 days. Afterwards, 3 replicates of 10 cysts of more or less uniform size from each treatment including 'e' were placed in each solid watch glass with 1 c.c. of sterile root diffusate and 1 c.c. of sterile water.

First larval count was done after keeping the cysts in root diffusate for 7 days, a second and third count was made at 3 days interval i.e. counting was done up to 13 days. After each count fresh sterile root diffusate and water was added to the watch glasses.

To obtain uniform cysts, they were passed through sieves of 40, 30 and 20 mesh. Cysts which passed through 20 mesh but not 30 mesh (500 to 700 μ diam.) were used and any individual cyst which appeared too big or too small was discarded.

Sterilization of root diffusate: The diffusate after collection was filtered under pressure through a sterilized Zeiss filter with Carlson-ford filter sheet which was connected to a suction pump. The diffusate was collected in a sterile bottle and then stored in a refrigerator.

Maintenance of fungus culture: Both the cultures of R. solani and C. coccodes isolated from tomato roots were supplied by the Plant Pathology Department. R. solani agreed between B and C type of Houston (1945) and C. coccodes matched well with Blackman and Hornby's (1966) description except that the width of mycelial conidia sometimes measured more, up to 7·8 μ. The cultures were maintained on potato carrot agar (Anonymous, 1968) of the following constituents: potato - 20 g., carrot - 20 g., agar - 25 g., distilled water - 1 litre, in McCartny vial at 25°C. To avoid the risk of loss of pathogenicity by the fungi due to prolonged culturing, they were passed through tomato plants after every 4 to 5 months.

Preparation of fungus culture for inoculation: The culture for inoculation was prepared by growing on sugarbeet seeds after Dunn and Hughes (1967). The seeds were soaked overnight in water in one pint milk bottle. Next day the excess of water was poured off

and the bottles were sterilized at 15 lbs. pressure for one hour. Each bottle was inoculated with 2 discs of mycelial mat cut with a sterile cork borer from fungus culture grown on P.D.A. and then incubated at 25°C for 4 weeks.

Sterilization of soil and raising of seedlings: John Innes potting mixture was sterilized at 71°C for $1\frac{1}{4}$ hours in a Camplex electric soil sterilizer. Tomato seeds, variety - Alisa Craig, were surface sterilized with 0·1% $HgCl_3$ for $1\frac{1}{2}$ minutes and then washed in several changes of water. The seeds were sown in a wooden box containing sterilized soil. After one month, the seedlings were pricked out in a second box and 20 days later repotted in plastic pots of 9 cm. diam. containing 375 g. sterilized soil.

Production of larvae for inoculation: The cysts after surface sterilization with 0·5% $CuSO_4$ for overnight were transferred on sterile filter paper soaked in sterile water. After 7 days these were transferred from filter paper and kept in sterile diffusate and sterile water mixture. Hatching started after 2 to 3 days and was considerable from 5 days onwards.

Treatments and design of experiment: The following 11 treatments of inoculation were used with 5 replications in each.

a - control (uninoculated)

b - *R. solani* alone

c - *R. solani* first, then *H. rostochiensis*

d - *H. rostochiensis* first, then *R. solani*

e - R. solani + H. rostochiensis simultaneously

f - C. coccodes alone

g - C. coccodes first, then H. rostochiensis

h - H. rostochiensis first, then C. coccodes

i - C. coccodes + H. rostochiensis simultaneously

j - R. solani + C. coccodes + H. rostochiensis simultaneously

k - H. rostochiensis alone

The experiment was conducted simultaneously in two houses which will be numbered 1 and 2. In house No.1, the temperature of soil varied from 21° to 27°C (av. 22°C) at day time and 11·6° to 15·8°C (av. 14°C) at night. In house No.2 where the underground heating was switched on, the temperature varied from 25° to 31·4°C (av. 26°C) at day time and 17·7° to 23·9°C (av. 22·2°C) at night. The fluctuation of temperature was due to insolation of the soil which could not be controlled. The pots were arranged randomly in a sand bath in a tray sunk in the soil.

All the treatments of R. solani were separated from that of C. coccodes and analysis was done separately for each with the exception of 'a', 'j' and 'k' which were common to both.

Inoculation: First inoculation with both the fungi and nematode was done on 12th July in the treatments 'c', 'd', 'g', and 'h' and the second inoculation after 18 days. All the organisms in the treatments 'e', 'i' and 'j' and the fungi in 'b' and 'f' and H. rostochiensis in 'k' were also inoculated on 12th July. Rate of fungus inoculum was 3% of soil (with both the fungi)and that of

H. rostochiensis 18 larvae per g. of soil.

Drying of plants: Harvesting was done 5 weeks after final inoculation. After harvest, the shoots were kept in separate envelopes and dried at 90°C overnight in an electric drier fitted with an exhaust outlet to take out the water vapour during drying.

Counting of cysts: After harvesting the roots were carefully lifted out from the pots and thoroughly washed in a strong jet of water over a sieve to retain the dislodged cysts. The roots were then weighed after removing the excess of water by pressing between filter papers. Most of the cysts were detached from the roots during washing but a few still remained attached, especially the young ones. These roots were, therefore, then stained in boiling lactophenol (liquid phenol-500 ml., lactic acid-500 ml., glycerol-1000 ml. and distilled water-500 ml.) with 0·05% acid fuchsin for 4 to 5 minutes (Goodey, 1963). As the cysts might become detached in the lactophenol, the roots were tied in a piece of muslin cloth while in the boiling lactophenol. After staining, roots were washed in water acidified with a few drops of acetic acid and macerated in a homogenizer with 40 ml. of acidified water. Cysts were counted from 20 ml. of suspension and the number was doubled at the time of calculation.

The cysts which remained in the soil were extracted by a Fenwick can (Goodey, 1963) and counted by Dunn's method on a turn-table under a binocular microscope. The float from the sieve after

thorough washing was transferred to a filter paper, 32 cm. diam., in a funnel and water was added up to the brim. A drop of teepol was added to lower surface tension and cause the float to move to the periphery. The filter paper is overprinted with concentric circles at regular intervals of 3·5 mm. up to a distance of 3·7 cm. at the periphery but leaving a clean area of 2 cm. from the margin. Some straight lines converging at the centre are also overprinted dividing the circles. When the entire float adhered on the filter paper, the bottom was pierced allowing the sediment to run off. Afterwards, it was kept on a turntable and the cysts were counted.

Calculation of the number of cysts was made by adding the number from the soil, from the roots after staining and that which were retained on the sieve during washing and then expressed per g. of root.

Assessment of disease: Disease on the roots was recorded by classifying all the root pieces in 1 cm. band from different portions of the root system as healthy and diseased and then expressed in percentage (Last and Ebben, 1963). For this purpose, the following apparatus was devised where the root could be classified as healthy and diseased without cutting into pieces.

The "disease recording tray" was made from perspex sheet. It consisted of 3 parts: a rectangular tray, 14 cm. long, 8·5 cm. broad and 1·5 cm. high A; a cover, 13 cm. long and 7·5 cm. broad B; and a long piece of perspex, 7·85 cm. long and 1·3 cm. broad C (Fig. 1). On the side walls of the long axis of the tray, 2 long

APPARATUS TO ASSESS THE PERCENTAGE OF DISEASE ON ROOT

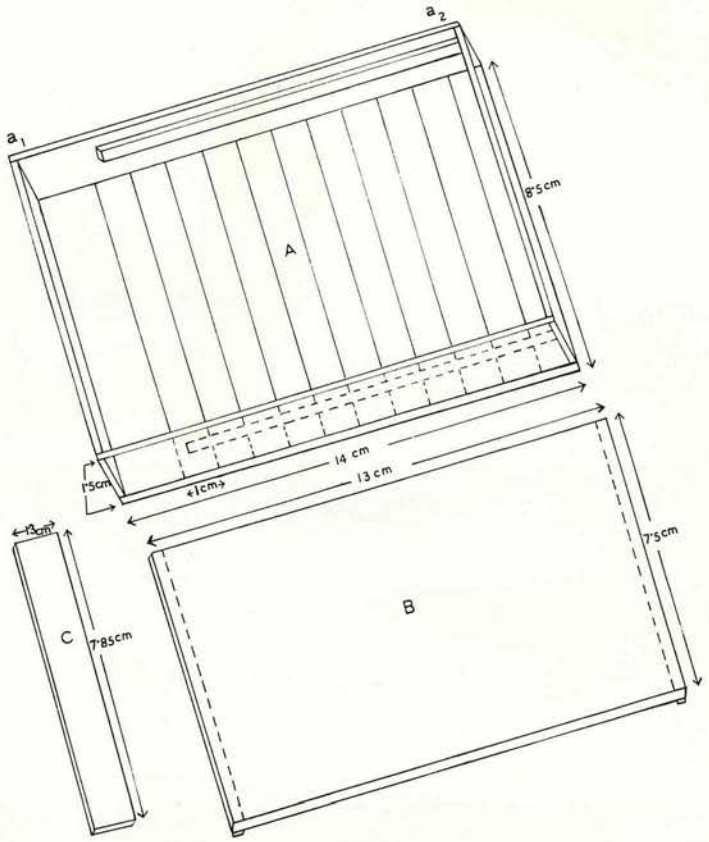


Fig. 1.

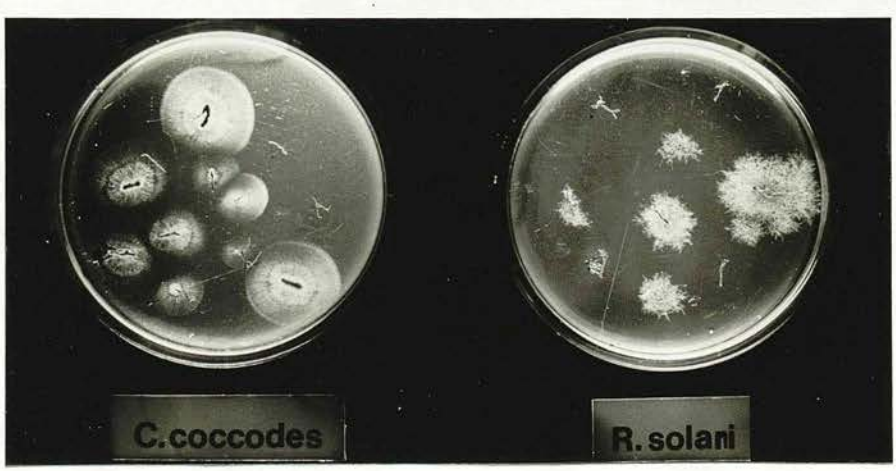


Fig. 2.

Fig. 2: Colonies of fungi from infected root pieces assessed by the above apparatus.

strips (10 cm.) were attached at the a_2 end. On the under-surface of the cover (B) too, 2 pieces of long perspex strips, 7·5 cm. long and about 1 mm. thick, were fixed at the edge of the broad axis one on each side. The bottom of the tray was marked with straight lines 1 cm. apart.

During observation, depending on the size of the root system, the whole root or a cut portion was placed on the tray in thin layer of water and after spreading the rootlet, the cover was put on this which helped to spread the roots further. Then the perspex piece C which could be moved into the tray at the a_1 end but not at a_2 end, was moved towards the opposite end in between the long strips on the side walls and top of the cover. The cover was thus fixed on the tray. Two thin strips on the undersurface of the cover at the two ends prevented the roots being pressed too much and at the same time helped in spreading the roots. The tray was then placed on the stage of a stereoscopic binocular microscope and assessment of infection was recorded by classifying all the roots in 1 cm. band as diseased and healthy. Similar observations were made from different portions of root. Where the root system was large it was cut into smaller parts and 2 to 3 such parts were taken at one time for observation.

To test whether any correlation existed between the observed infection assessed by the above method and the number of colonies developed from the diseased root pieces, both R. solani and C. coccodes infected roots after examination were cut into separate

root pieces in the corresponding 1 cm. band, sterilized with 1:10 Deosan (Na hypochlorite - 14 to 15% available Cl) and then put in petri dishes containing 10 c.c. Oxoid P.D.A. containing 50 ppm. Rose Bengal and incubated at 25°C (Fig. 2). Ten plates were used for each of the fungi. Roots on which even trace of mycelium of R. solani was found were counted as diseased. In treatment 'j' where both R. solani and C. coccodes were inoculated with H. rostochiensis attempt was not made to assess the incidence of diseases.

Hatching of larvae in the presence of fungus exudate: Both R. solani and C. coccodes were grown in potato dextrose broth for 21 days and R. solani was filtered through a Zeiss filter fitted with Carlsonford filter sheet under pressure. As C. coccodes produced some mucilagenous substance which clogged the filter sheet, the liquid was simply pipetted out by a sterile pipette after carefully removing the mycelial mat in the flask in the inoculation room. The following 5 treatments were used.

a - 1 c.c. sterile distilled water + 1 c.c. sterile root diffusate (control)

b - 1 c.c. R. solani exudate + 1 c.c. sterile distilled water

c - 1 c.c. " " " + 1 c.c. sterile root diffusate

d - 1 c.c. C. coccodes exudate + 1 c.c. sterile distilled water

e - 1 c.c. " " " + 1 c.c. sterile root diffusate

Hatching test was first tried by keeping the cysts in solid watch glasses in the above liquids according to James (1966) but as

considerable contamination occurred, this was repeated substituting the watch glasses by bijou bottles to minimise the risk.

Cysts of more or less uniform size (passed through 30 mesh but not 40 mesh - 380 to 500 μ diam.) after surface sterilization (0·5% $CuSO_4$ for 24 hrs.) were kept on sterile filter papers in sterile petri dishes for 6 days at 21 ± 1°C. The filter papers were soaked with 1 c.c. sterile water + 1 c.c. R. solani exudate for the treatment 'b' and 'c', 1 c.c. sterile water + 1 c.c. C. coccodes exudate for 'd' and 'e' and 2 c.c. sterile water for the control. If any contamination was observed the plate was discarded.

Six cysts were transferred to a bijou bottle containing the fungus exudate and root diffusate according to the treatments mentioned above with 5 replications in each and incubated at 21 ± 1°C. Counting of the hatched larvae was done after 8 days. To find out the effect of individual fungus, the treatments of R. solani were kept separate from that of C. coccodes at the time of calculation with the exception of 'a' which was common to both.

Invasion of tomato roots by H. rostochiensis larvae in the presence of the fungi: Tomato seedlings raised in sterilized soil were pricked out in a second wooden box after four weeks. Final planting out was done after another 18 days in plastic pots, 6·5 cm. diam. containing 150 g. John Innes compost. The pots were inoculated on the same day with cultures (on sugarbeet) of R. solani and C.coccodes at the rate of 3% of compost. The following treatments were used.

a - H. rostochiensis alone

b - R. solani followed by H. rostochiensis

c - C. coccodes followed by H. rostochiensis

The experiment was carried out at two temperatures in water baths, one at 15·5°C and the other at 26·6°C. Two 200 watt reflector lamps (Mazda) were hung over the water baths and connected with an automatic Timac switch to give 12 hr. light per day.

Eighteen days after the fungus inoculation, the pots were inoculated with H. rostochiensis larvae, obtained by the method already described, at the rate of 18 per g. of soil. The plants were uprooted after 15 days and the roots were stained by 0·05% acid fuchsin in lactophenol. Before staining the roots were weighed after drying the water between filter papers and macerated with 20 c.c. of water in a homogenizer by the method previously described. Nematodes in 1 c.c. aliquot of suspension were counted by a Hawksley nematode counting slide and repeated 6 times. The total number of nematodes in 20 c.c. was calculated and expressed as larvae per g. of root. At the time of calculation all the treatments with R. solani in both the temperatures were analysed separately from that of C. coccodes keeping 'a' in both the temperatures common for both.

Histological Studies: Portions of root after harvest were fixed in formalin acetic acid (50% ethyl alcohol 90 c.c. + glacial acetic acid 5 c.c. + commercial formalin 5 c.c.) and then Johansen's (1940) method was followed in dehydrating through grades of tertiary butyl alcohol and ethyl alcohol mixtures and embedding. The material was placed first for 2 hours in 50% alcohol, overnight in 70%, 1 hr. in 95% and 1 hr. in 100% and then transferred to a

mixture of equal parts of paraffin oil and tertiary butyl alcohol. One hr. later the material was transferred to solid but hot paraffin wax (melting pt. 55°C) in specimen tubes, covered with paraffin oil and butyl alcohol mixture and kept in a paraffin bath for overnight. Afterwards, the root pieces were removed to clean molten paraffin and again kept in the paraffin bath for overnight and then made into blocks.

The tissues were cut by a Cambridge rocking microtome at 12 μ thick sections, fixed to the slides by Gurr's glycerine albumin and then dipped in xylene to remove paraffin. The sections were brought back to 50% ethyl alcohol through different ethyl alcohol grades and stained in 0·5% safranin O (in 50% ethyl alcohol) for 12 to 24 hr. These were then brought to 90% ethyl alcohol through 70%, counterstained in 0·5% Fast Green FCF (in equal parts of absolute alcohol and clove oil) for nearly 1 min., differentiated in a mixture of equal parts of xylene and absolute ethyl alcohol (Jensen, 1962) and mounted in canada balsam after clearing in xylene.

Rosindole reaction to detect indole derivative/s was performed after Glenner (1957). Root tissues after 18 days of nematode inoculation were fixed in Ca acetate (commercial formalin - 10 ml., water - 90 ml. and Ca acetate - 2 g.) for 6 hr. and blocks were made as has been described above. The tissues were cut into 9 μ thick sections and treated as follows.

After removing the paraffin the slides were placed into

absolute alcohol and treated for 3 min. in a solution containing 1 g. p-dimethylaminobenzaldehyde, 5 ml. 60% perchloric acid, 1 ml. conc. HCl and 34 ml. glacial acetic acid and then for 1 min. in a solution of 5 ml. conc. HCl and 35 ml. glacial acetic acid which was poured in a Coplin jar previously layered with 500 mg. of $NaNO_2$. The slides were washed in 2 changes of glacial acetic acid, passed through 50% glacial acetic acid in xylene, 20% glacial acetic acid in xylene and then 2 changes of xylene. Mounting was done in cellulose tridecanoate (cellulose caprate) made after Lillie and Hanson (1955).

RESULTS

Sterilization of cysts: Except when the cysts were kept continuously for 8 days in 0·1% $CuSO_4$, no other treatment of this chemical had any adverse effect on the hatching of *H. rostochiensis* larvae (Appendix I). 0·1% $HgCl_3$ dip even for 1½ min. proved to be toxic. There were lots of fungal and bacterial contamination in 1 and 4 hr. treatment of 0·5% $CuSO_4$, especially at 25°C. Considerable number of larvae died after emergence, particularly at 25°C which can be seen from the mortality ratio (total/dead).

Effects of different treatments of nematode and fungus inoculation on the growth of plant, production of cysts and disease incidence.

Growth of plant: Fig. 4 shows that inoculation of *R. solani* did not retard the growth of either shoot or root of tomato plant in

any of the houses in this experiment. Results were similar when R. solani inoculation preceded nematode inoculation. Growth of shoot was checked significantly in other treatments in both the houses. However, growth check was most severe in the treatments where nematodes were inoculated alone and before the fungus.

In the treatments 1, 2 and 3 root growth was significantly more than 5 and 6 ($P = 0.01$) which was again more than 4 and 7 ($P = 0.001$) i.e. most severe reduction occurred in the nematode only and nematode preceded the fungus inoculated plants. In house 2 significant ($P = 0.05$) reduction occurred in two treatments - inoculation by nematodes alone and nematode followed by fungus.

With C. coccodes growth of shoot in all the fungus and nematode inoculations was significantly checked in house 1. The maximum retardation occurred in the plants grown in pots inoculated with nematode only and nematode followed by fungus. No significant difference was, however, found between 5 and 7. Similar was the result in house 2 but no significant difference could be found among the treatments 1, 2 and 3. The reduction of growth in the nematode alone and nematode followed by fungus inoculation was more than fungus alone and fungus followed by nematode inoculation. Growth check in the treatment of simultaneous inoculation lay between these two extreme groups.

Reduction of root growth was most severe in the treatments 4, 5 and 7 ($P = 0.01$) in both the houses. In house 2 no significant difference was found in the remaining 4 treatments but in house 1 treatment 6 and 2 differed significantly ($P = 0.05$). Though

WEIGHT OF SHOOT AND ROOT OF TOMATO PLANTS IN DIFFERENT TREATMENTS OF INOCULA-TIONS BY HETERODERA ROSTOCHIENSIS, RHIZOCTONIA SOLANI & COLLETOTRICHUM COCCODES

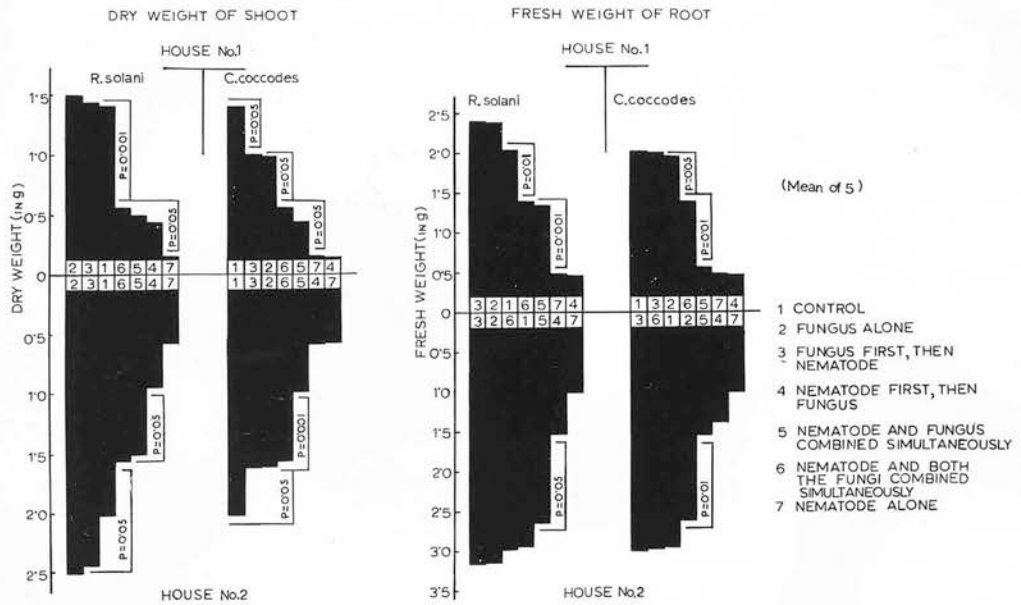


Fig. 4.

NUMBER OF CYSTS OF HETERODERA ROSTOCHIENSIS AND DISEASE INCIDENCE BY RHIZOCTONIA SOLANI & COLLETOTRICHUM COCCODES IN DIFFERENT TREATMENTS OF FUNGUS AND NEMATODE INOCULATIONS

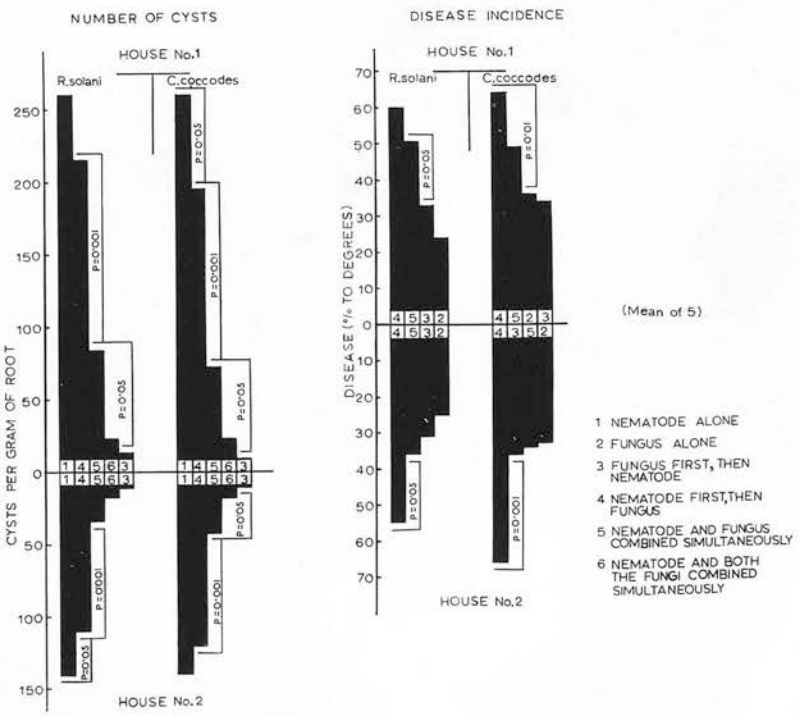


Fig. 5.

Fig. 6.

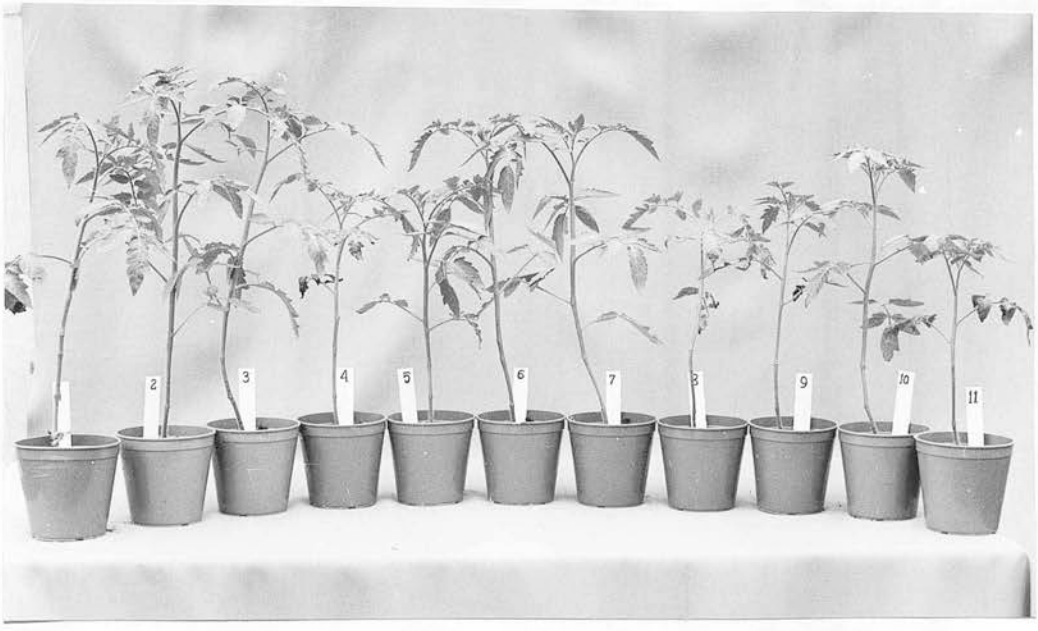


Fig. 7.

Figs. 6 and 7: Growth of tomato plants in different treatments of fungus and nematode inoculation in house 1 (Fig. 6) and 2 (Fig. 7). 1 - control, 2 - R. solani (R) alone, 3 - R first then nematode (N), 4 - N first then R, 5 - R + N simultaneously, 6 - C. coccodes (C) alone, 7 - C first then N, 8 - N first then C, 9 - C + N simultaneously, 10 - R + C + N simultaneously, and 11 - N alone.

there was no significant difference among the treatments 4, 5 and 7 growth in 4 and 7 was less than 5.

The details of observations with analysis of variance are given in the Appendices III (a), (b), (c) and (d) and IV (a), (b), (c) and (d).

Production of Cysts: Fig. 5 shows that with R. solani, in both the houses the production of cysts on roots inoculated with the nematode alone and when inoculated before the fungus was significantly greater ($P = 0{\cdot}001$) than other treatments. Also, in both the houses the number of cysts was smallest when fungus inoculation preceded nematode. Significant difference existed between 3 and 5 in house 1 ($P = 0{\cdot}05$) and between 1 and 4 in house 2 ($P = 0{\cdot}05$).

With C. coccodes, though there was significant difference between 1 and 4 in house 1, this was not so in house 2. Significant difference also existed between 3 and 5 in both the houses ($P = 0{\cdot}05$). On the whole, the picture was similar with that of R. solani, i.e. number of cysts was significantly higher in the only nematode and nematode followed by fungus inoculated than fungus followed by nematode inoculated plants. The number in 5 was between these two groups.

Disease incidence: As good correlation was found ($r = 0{\cdot}7699$ in R. solani and $= 0{\cdot}8733$ in C. coccodes) between the observed infections and colonies developed from them, the "disease recording tray" was used to assess infection on roots. Fig. 5 shows that the disease incidence (percentage converted into angles) due to R. solani in the

CORRELATION BETWEEN THE OBSERVED INFECTION OF THE FUNGI ON TOMATO ROOTS AND THE CORRESPONDING NUMBER OF COLONIES ON AGAR PLATES

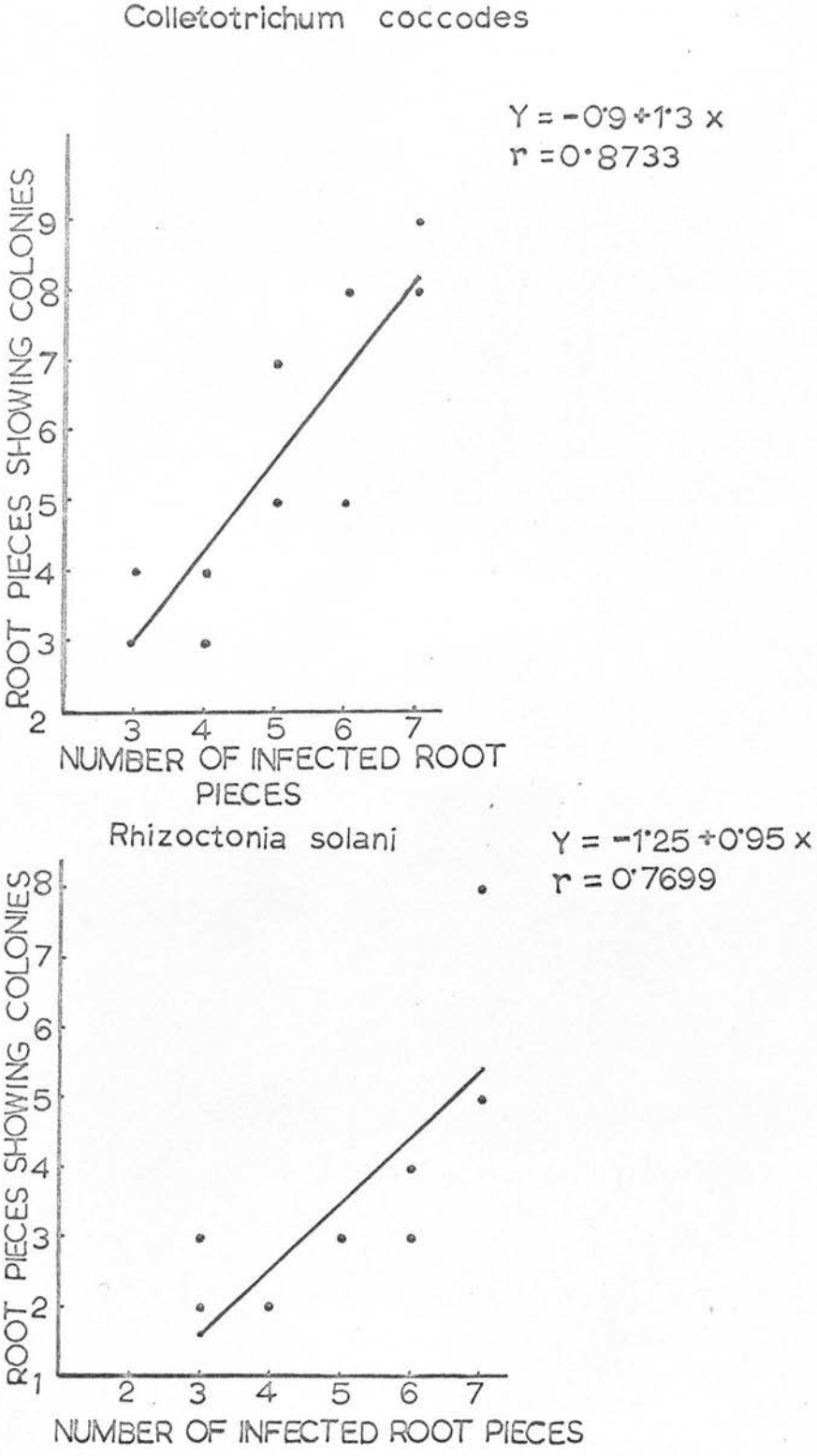


Fig. 3

nematode followed by fungus inoculation was significantly higher ($P = 0{\cdot}05$) than all other treatments in house 2. In house 1 also the disease incidence was maximum in the same treatment but the difference between 4 and 5 was not significant. These two treatments, however, were significant over other treatments ($P = 0{\cdot}05$).

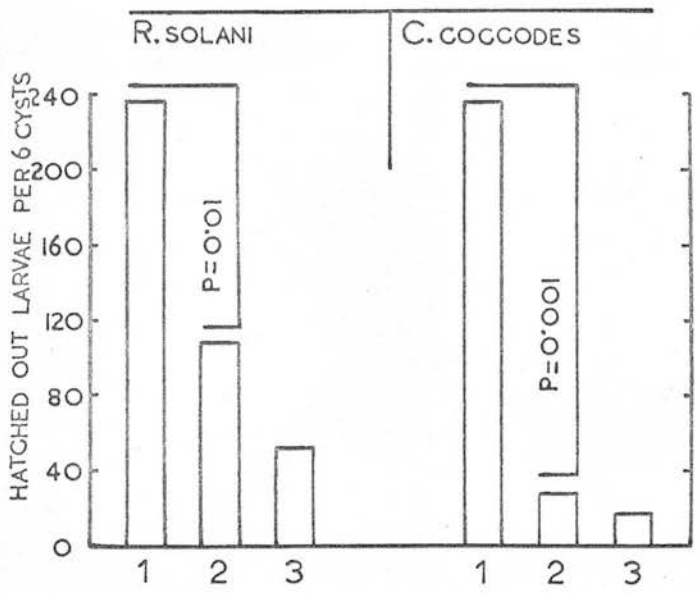
In house 2, with C. coccodes, maximum disease developed in the treatment 4 ($P = 0{\cdot}001$) but no differences were found among other treatments. In house 1, disease development in 4 was significant over 2 and 3 ($P = 0{\cdot}01$). No difference was found between 4 and 5 and similarly among the treatments 2, 3 and 5.

The details of observations with analysis of variance of disease incidence and number of cysts are given in the Appendix V (a), (b), (c) and (d).

Hatching of H. rostochiensis larvae in fungus exudate: Hatching of larvae from cysts was depressed significantly in all the treatments of R. solani ($P = 0{\cdot}01$) and C. coccodes ($P = 0{\cdot}001$) exudate (Fig. 8). The inhibitory effect of C. coccodes appears to be stronger than R. solani. The details of observations with analysis of variance are given in the Appendix VI.

Invasion of tomato roots by H. rostochiensis larvae in the presence of fungi: Fig. 9 shows that there was no difference in the rate of invasion of roots by H. rostochiensis larvae between the control (nematode without fungus) and fungus (both R. solani and C. coccodes)

EFFECT OF RHIZOCTONIA SOLANI & COLLETOTRICHUM COCCODES EXUDATE ON THE HATCHING OF HETERODERA ROSTOCHIENSIS LARVAE FROM CYSTS

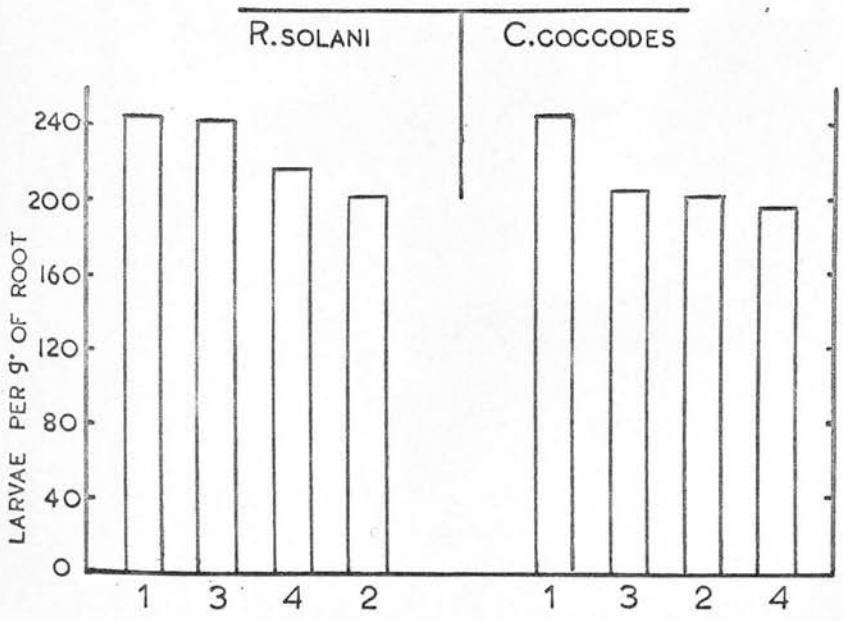


(MEAN OF 5)

1 ROOT DIFFUSATE + WATER
2 FUNGUS EXUDATE + ROOT DIFFUSATE
3 FUNGUS EXUDATE + WATER

Fig. 8

INVASION OF TOMATO ROOT BY HETERODERA ROSTOCHIENSIS LARVAE IN THE PRESENCE OF RHIZOCTONIA SOLANI & COLLETOTRICHUM COCCODES



(MEAN OF 5)

1 CONTROL AT 15·5°C
2 CONTROL AT 26·6°C
3 FUNGUS FIRST, THEN NEMATODE AT 15·5°C
4 FUNGUS FIRST, THEN NEMATODE AT 26·6°C

Fig. 9

followed by nematode inoculation in both the temperatures used. The detailed observations with analysis of variance are given in the Appendix VII.

Histological Studies: In treatments where fungi preceded nematode giant cells could not be observed or in a few cases were deformed. Fig. 11 shows undeveloped giant cells in the Rhizoctonia infected tissues and hyphae alongside. Fig. 12 shows that the nematode is unable to initiate the formation of giant cells at all in the Colletotrichum infected tissues - hyphae of the fungus and deeply stained area near the broken tissue, indicative of nematode attack, can be seen.

Figs. 13 and 14 show that the position is quite different where the nematode enters first and has stimulated the formation of giant cells. In such cases mycelia of the fungi develop much more profusely in the giant cells than the surrounding healthy tissues. It was also observed that where the fungus is followed by nematode inoculation, in tissues which are not invaded by the fungus mycelium, giant cells can be formed. Rosindole reaction gave positive results in the body of the invading nematode.

It was noted that R. solani (Fig. 16) is able on occasion to penetrate deep into the pericycle forming some sort of cushion like mass. Contrary to this, Fig. 17 shows that though the root was completely girdled by fungal mass the pathogen was unable to penetrate the tissues - only one cell of cortex was invaded with the fungus mycelium. Sections in the Figs. 15 and 17 were cut

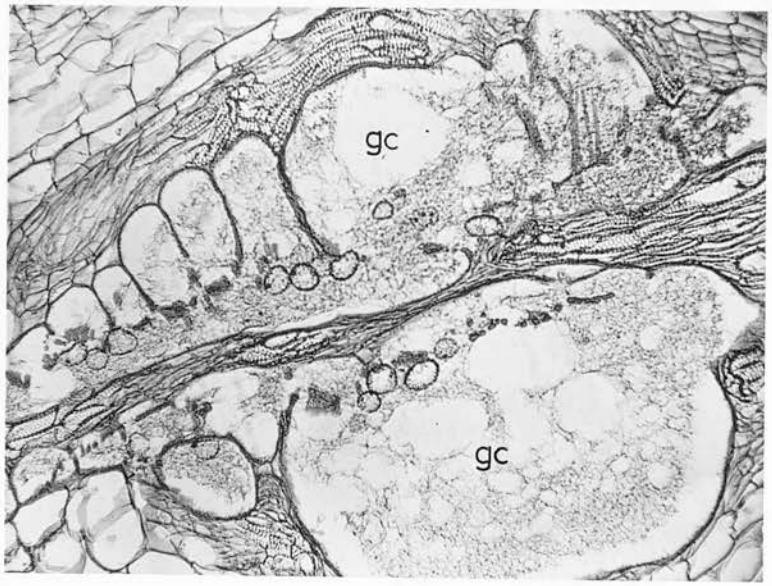


Fig. 10.

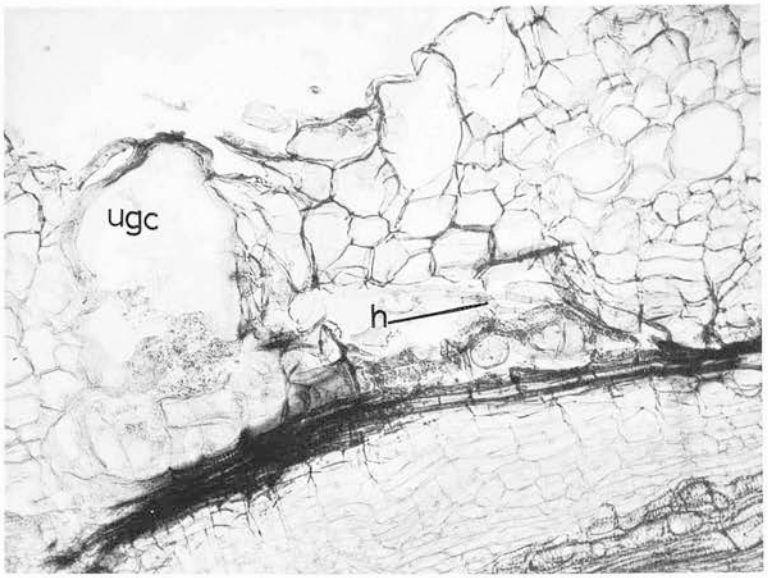


Fig. 11.

Fig. 10: L.S. of tomato root showing normal giant cells (gc) caused by H. rostochiensis. Fig. 11: L.S. of R. solani infected root showing undeveloped giant cells (ugc) and hyphae (h) of the fungus in the treatment fungus followed by nematode.

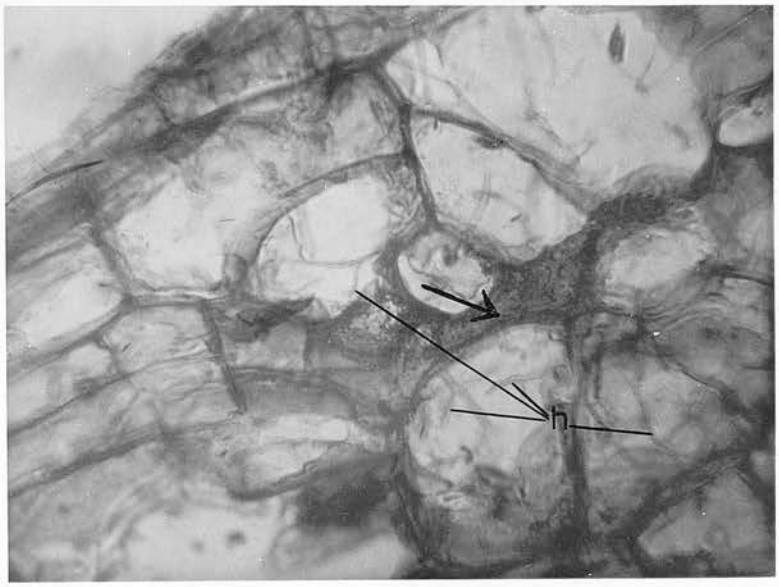


Fig. 12.

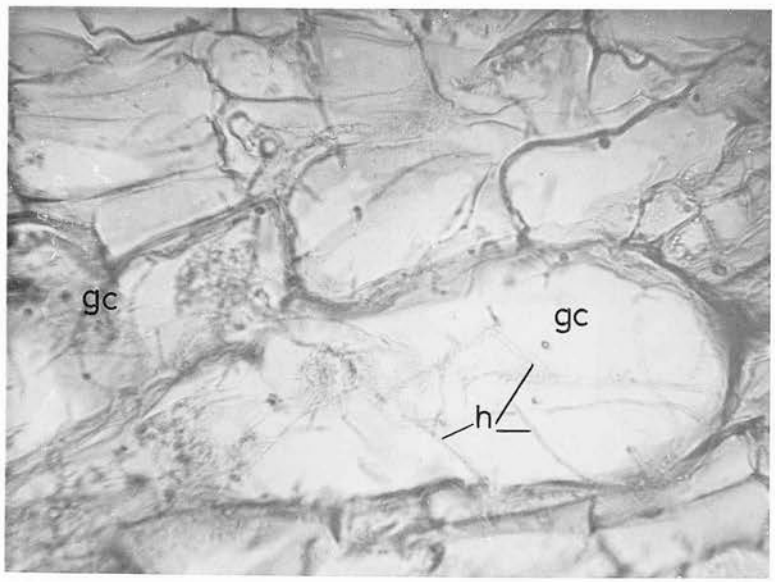


Fig. 13.

Fig. 12: L.S. of C. coccodes infected root showing no giant cell formation by H. rostochiensis in the treatment fungus followed by nematode - deeply stained area, indicative of nematode attack (arrowed) and hyphae (h) can be seen. Fig. 13: L.S. of giant cells (gc) showing colonization by C. coccodes hyphae in the treatment nematode followed by fungus - nearby cells are uninvaded.

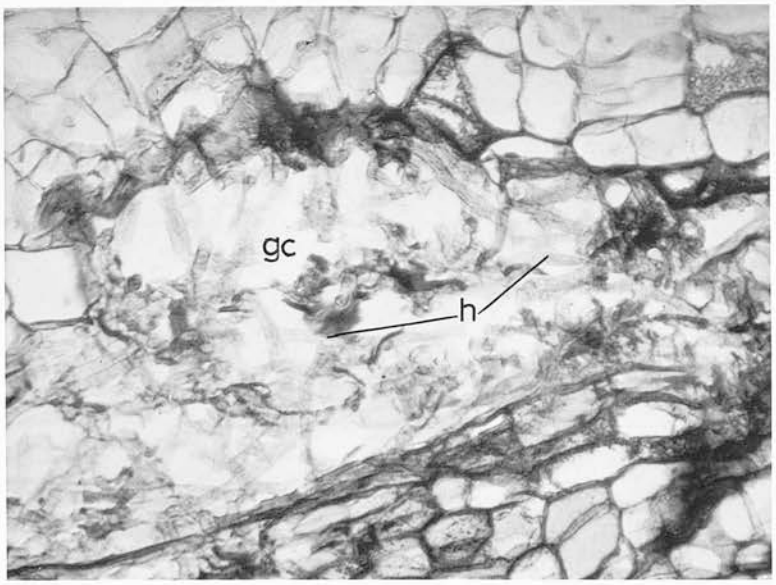


Fig. 14.

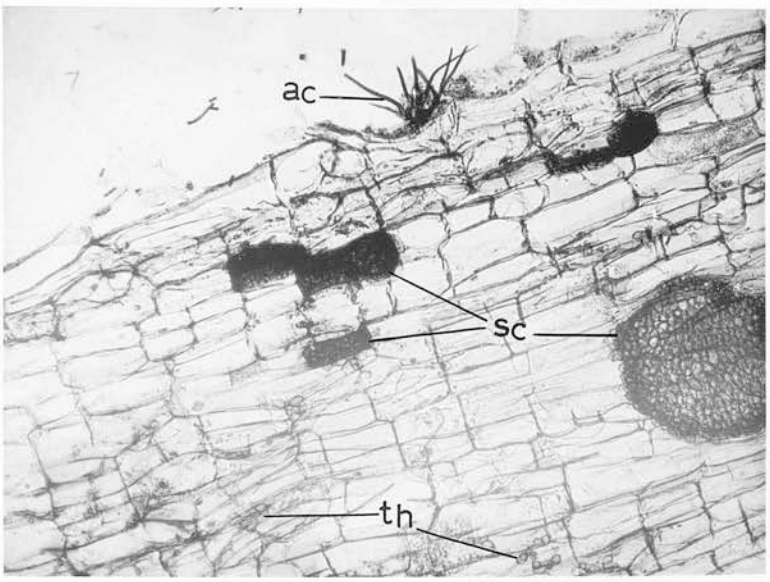


Fig. 15.

Fig. 14: L.S. of giant cells (gc) caused by H. rostochiensis on tomato root showing profuse colonization by R. solani hyphae (h) in the treatment nematode followed by fungus - nearby cells are uninvaded. Fig. 15: L.S. of C. coccodes infected root showing acervulus (ac) on the surface, sclerotia (sc) in the tissues and thick and constricted hyphae (th) indicative of formation of sclerotia.

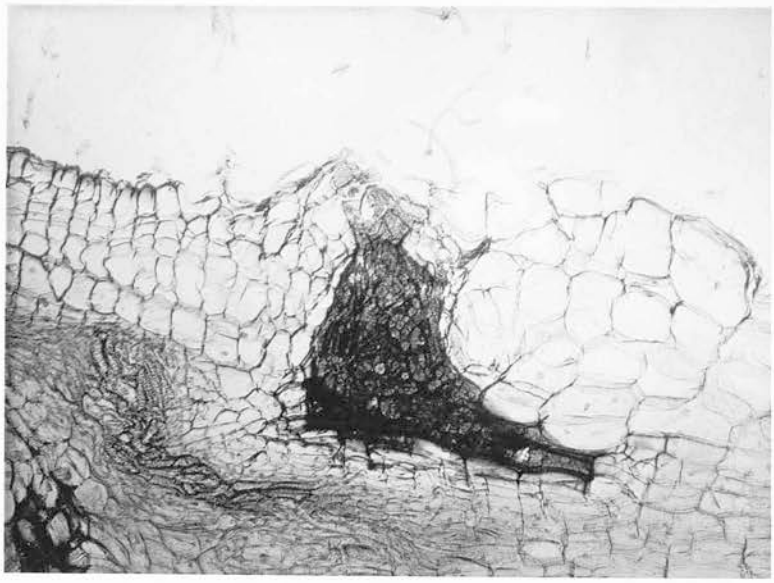


Fig. 16.

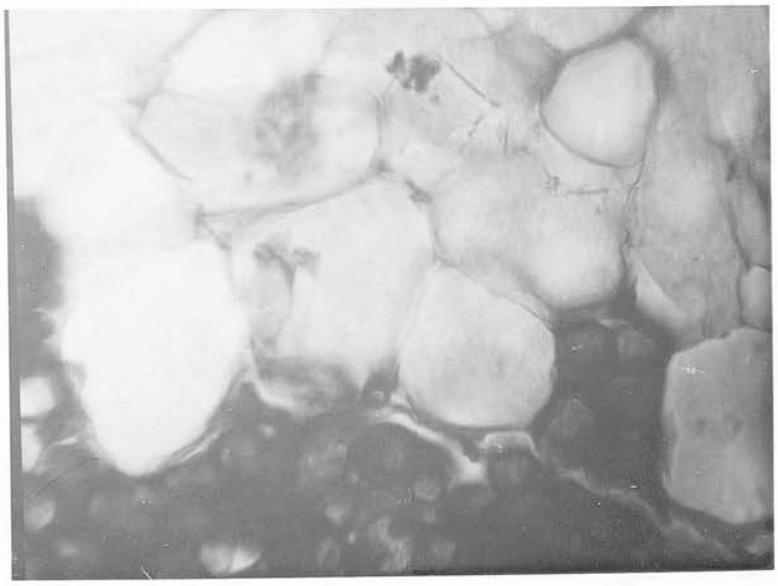


Fig. 17.

Attack of tomato root by R. solani. Fig. 16: L.S. showing invasion of the fungus deep into the pericycle. Fig. 17: T.S. showing only one cell of the cortex is attacked leaving the nearby cells uninvaded.

from roots which were harvested after 148 days of fungus inoculation.

Both acervuli and sclerotia were formed simultaneously on C. coccodes infected roots (Fig. 15). While acervuli with conidiophores and conidia were formed only on the surface, sclerotia could develop anywhere - on the surface as well as inside the root tissues up to the stele. Sclerotia which formed on the epidermis were setose but those formed inside asetose.

DISCUSSION

Most of the early investigators working on the interaction of H. rostochiensis and R. solani or C. coccodes considered H. rostochiensis as the main cause of the trouble. Grainger and Clark (1963), Dunn and Hughes (1964) and Ketudat (1968) recorded greater reduction of growth of the host plants when both pathogens (H. rostochiensis and R. solani) were present than in the presence of one only. In the present study shoot and root weight of tomato have been reduced significantly when the nematode inoculation precedes fungus or both pathogens are inoculated at the same time but not when fungus inoculation precedes the nematode.

Both fungi appear to act adversely on the hatching of H. rostochiensis and production of new cysts. James (1966) reported similar inhibitory effects of grey sterile fungus and Ketudat (1968) of V. albo-atrum and R. solani on potato cyst eelworm. The fungi, on the other hand, do not affect the penetration of roots by the

larvae. Therefore, it appears that if R. solani or C. coccodes attacks root first, the hyphae ramify the infected tissues and render them unsuitable for the females to form giant cells and thereby inhibit the development of the eelworms.

The absence of growth differences of plants between (i) nematode alone and (ii) any treatment of combined inoculation of fungus and nematode is in agreement with Miles (1930), Millard et al. (1932) and Goffart (1938). Dunn and Hughes (1964) and Ketudat (1968) on the other hand, obtained greater reduction of growth of tomato plants in the combined presence of R. solani and H.rostochiensis than H. rostochiensis alone. The reasons of these differences are, that in the present study larvae instead of cysts were used for inoculation as a result of which all the worms after entering the soil at the same time attacked the roots simultaneously causing severe damage. When cysts are added, larvae hatch out gradually over a period of time and therefore, concentration of eelworm is also built up gradually which is low at the beginning and during this period the plant continues to grow when it can resist the attack to some extent. Secondly, as 9 cm. pots instead of 10 in. ones as used by the above workers were used, there was more concentration of nematodes in the root zone. So, in the present work the damage due to the nematode alone caused such severe reduction of growth of plants that the difference of effect of this treatment and the combined inoculation of fungus and nematode was small.

The lack of adverse effect of R. solani on the growth of tomato plant is in agreement with the findings of Cheal (1929), Edwards (1929) and Miles (1930) on potato. They observed, however, that at the initial stage R. solani could check the growth to some extent but ultimately the plants recovered and produced normal yields. Miles (1930) in his paper quoted H.H. Stirrup who was of the opinion that R. solani is a normal fungus flora of potato root. This fungus was categorized as a "primitive parasite" by Garrett (1956) who stated that it constitutes a part of normal microbiological environment for the roots of higher plants and during the process of evolution root system develops certain power to resist its attack.

In this study growth of the plants has been found to be enhanced to some extent in R. solani inoculation than the control though the difference is not significant. The metabolic activities of R. solani seem to be very complex. It has been reported to secrete pectolytic enzymes (Batëman, 1963a; Sherwood, 1964), cellulolytic enzymes (Garrett, 1962) and both pectolytic and cellulolytic enzymes (Barker and Walker, 1962; Batëman, 1963b). Difference in enzyme activities in different pH and temperatures was recorded by Barker and Walker (1962). Both these workers and Batëman (1963b) observed that polygalacturonase activity of the fungus was primarily responsible for maceration of tissues. Recently, Batëman (1968) recorded a mechanism of induced resistance by R. solani infected excised bean to the action of polygalacturonase.

As a reaction of pathogenesis increase in the permeability of cell membrane after 14 to 18 hours of inoculation with R. solani has been observed by Lai et al. (1968). Therefore, considering all these complex biochemical and biophysical activities, it is not unlikely that R. solani may secrete some substance to stimulate growth of plants which is a common phenomenon in some fungi.

Though R. solani is capable of infecting plant tissues by producing infection hyphae independent of any sort of injury, frequently mycelia have been seen to run on the root surface without causing any apparent infection. Both R. solani and C. coccodes have been observed to enter through natural growth cracks or other type of injury. Therefore, besides biochemical changes, mechanical injury caused by the invading larvae appears to be a contributing factor for increased fungus attack on previously nematode inoculated roots. R. solani was found to penetrate sugarbeet roots wounded by H. schachtii Schmidt without forming infection structures (Polychronopoulos et al. 1969). Slootweg (1956) noted that Cylindrocarpon radicicola could only infect the roots of lily of the valley when some injury was made and therefore, suggested that the injuries caused by Pratylenchus or Hoplolaimus sp. provided infection courts for the fungus to enter.

The injuries of plant tissues caused by nematodes are somewhat different to those caused by any other mechanical means. Tissues surrounding the wound tend to suberize to give some protection against the attack of microorganisms but in the case of nematode attack as long as the parasite feeds it injects secretion

into the cells and may prevent certain changes taking place which may otherwise give the plant some sort of protection (Christie, 1960). Du Charme (1959) recorded that in citrus roots attacked by Radopholus similis the exposed tissues were neither suberized nor dried and that wound phallogen and wound cork were not formed in and about the lesions. In this regard, parasitism by H. rostochiensis provides a new line of thinking as the nematode feeds continuously from the same site during the whole period of parasitism.

Reduction of plant growth is accompanied by an increase in the number of cysts and incidence of disease on the root. It appears from the result that the greater the number of cysts the more the incidence of disease on the root. This is true in case of both the fungi. Increased disease incidence on the roots inoculated with the nematode first and then by the fungus can be explained in two ways. Firstly, the mechanical injuries caused by the larvae facilitate entry of the fungi. Secondly, the giant cells produced by the nematodes are favoured more by the fungi to colonize than the normal cells. Powell and Nusbaum (1960) recorded that Phytophthora parasitica var. nicotianae (Breda de Haan) Tucker had an affinity for hypertrophied and hyperplastic tissues of the galls on tobacco caused by Meloidogyne incognita acrita. In such areas mycelium was more extensive and vigorous than in non-galled tissues.

In Fusarium wilt-root knot complex of tobacco, Porter and

Powell (1967) reported that when the plants were inoculated with F. oxysporum f. nicotianae 2 to 4 weeks after root knot (M. incognita, M. arenaria and M. javanica) inoculation, wilt was much more severe than when inoculated with both the pathogens simultaneously. In another experiment working on the same problem, vigorous colonization by the fungus of giant cells caused by M. incognita on both wilt resistant and wilt susceptible varieties of tobacco was observed, especially when the nematode inoculation preceded fungus by 3 to 4 weeks (Melendez and Powell, 1967). Giant cells produced by H. schachtii on sugarbeet root were found to be very suitable substrate for R. solani to colonize (Polychronopoulos et al., 1969). R. solani usually does not cause root disease in Nicotiana tabacum once the plants pass the juvenile stage but Powell and Batten (1967) observed that when the root knot susceptible varieties were inoculated with it 3 weeks after M. incognita, extensive root necrosis occurred and when added after 1 week only a trace and no necrosis developed when inoculated simultaneously with both the pathogens.

Krusberg (1963) stated that galling in root tissues due to nematode attack is initiated by two mechanisms - directly by triggering a mechanism to form gall inducing principle and indirectly by releasing or injecting contained substances. Increased amount of proteins (Owens and Novotny, 1960; Bird, 1961), amides, amino acids (Owens and Novotny, 1960; Hanks and Feldman, 1963) and nucleic acids (Owens and Novotny, 1960) have been observed in the

infected root tissues by root knot nematodes. Kannan (1967) observed that M. incognita acrita secretes glucose dehydrogenase and endogenous reductase in the galled tissues of tomato root. Recently, Endo and Veech (1969) had demonstrated the presence of oxidoreductases in the roots of soybeans infected by the same nematode which were more intense in the giant cells. Production of pectinase and cellulase have been detected in H. trifolii Goffart by Morgan and McAllan (1962). Bird (1962) demonstrated that for the normal development of giant cells continual presence of root knot nematode is essential and if the nematode is killed the giant cells disintegrate.

Sayre (quoted by Mountain, 1960) stated that root knot nematodes secrete pectolytic enzymes which act on the splitting of peptide bonds of the protein chains in the host tissues releasing a number of amino acids. Tryptophan, an immediate precursor of indole acetic acid (I.A.A.) is one of them which is metabolized into I.A.A. in the host tissues. This I.A.A. together with plant metabolic conversion of tryptophan into I.A.A. result in accumulation of high level of I.A.A. in the host tissues which stimulate over-growth in that area. Besides demonstrating the presence of indole materials in the nematodes themselves and galled tissues, Yu and Viglierchio (1964) showed great difference in their kind and proportion among 3 spp. of Meloidogyne. Powell and Nusbaum (1960) and Melendez and Powell (1967) were of the opinion that these modified tissues caused by root knot nematode attack provide suitable

substrate for fungal colonization irrespective of whether the host is resistant to the fungus or not. So, considering the above facts it seems likely that the indole derivative/s found in the bodies of H. rostochiensis larvae may be a factor to act in some way for the increased fungus attack in the giant cells. Recently, Johnson and Viglierchio (1969) have demonstrated the presence of I.A.A. in the larvae of H. schachtii.

When the superficial mycelia of R. solani on the root come in contact with giant cells it can get ingress into the tissues. Normally its attack is not observed deeper than cortex but in the giant cells the mycelium reach up to the stele which may subsequently spread laterally. Similar observations have been made with C. coccodes. In the treatment when fungus precedes nematode and the tissues are ramified by the mycelium, giant cells either cannot be formed or are deformed as has been observed in the same nematode when it attacks tomato roots invaded by the grey sterile fungus (Roy, 1968).

However, it is observed that if the roots are not ramified by the fungus mycelium giant cells can be formed even in the treatment when fungus inoculation precedes nematode. Conversely, in the treatment when nematode inoculation precedes the fungus or both the pathogens are inoculated simultaneously, giant cells cannot be developed in the areas invaded by the fungus hyphae. That indicates, once the tissues are invaded by the fungus giant cells cannot be formed and if the nematode produce giant cells on healthy roots, the fungus infection is aggravated. The picture

is the same with both the fungi. The reason for the higher disease incidence and greater production of cysts in the treatments when nematode inoculation precedes fungus and both the pathogens are inoculated together than when fungus inoculation precedes nematode is that in the former two cases H. rostochiensis get more chance to attack healthy roots and therefore, the fungi could penetrate deep into the tissues through the giant cells causing extensive damage and decay. In the last treatment as the fungi colonize the roots first but without invading the tissues of the deeper layer, subsequent nematode development is checked and the effect of the disease is less severe.

Greater reduction of growth of plants and greater number of cysts in house 1 than in house 2 can be explained in two ways. Firstly, temperature in house 1 is more suitable for the development of H. rostochiensis as observed by Fenwick (1951) who stated that the number of cysts decreased progressively as the temperature was increased from 21°C to 32°C. Secondly, temperature in house 2 is more suitable for plant growth. Bewley (1950) recorded heaviest plucking of tomato at 65° to 70°F of day temperature and 65°F of night temperature. According to Reithmann (1933) maximum growth of stem and fruit of tomato largely depends on soil temperature and for the variety Schöne von Lothringen, the optimum root temperature is 33°C.

CHAPTER II

GROWTH OF *RHIZOCTONIA SOLANI* AT DIFFERENT TEMPERATURES

MATERIALS AND METHODS

Growth of the fungus on P.D.A.: Growth of R. solani was recorded on Oxoid P.D.A. at three temperatures - 13°, 20° and 27°C with 4 replications in each. Mycelial discs, 6·5 mm. diam., were cut with a sterile cork borer from 5 days old culture and inoculated at the centre of petri dish containing 10 c.c. medium (pH 5·6). Radial growth of mycelium was measured daily from the second up to the fifth day. Analysis of variance was done for each day separately.

In another experiment growth rate of tomato isolate of R. solani, T was compared with 6 potato isolates from fields in Scotland which were designated as P1, P2, P3, P4, P5 and P6, respectively. Growth of all the isolates was recorded up to 5 days except in P3 in which case it was only for 4 days as the mycelial growth covered the surface completely at 27°C. Analysis was done to find out the significance of isolates and temperatures separately as well as their interaction. Cultural characters of the isolates were recorded at 20° and 27°C.

Growth of R. solani in soil in vitro: Growth of R. solani in soil was measured following Mughogho's (1968) method but as the tubes with soil were autoclaved one end was capped with autoclave tape instead of cellophane and the other closed by non-absorbent cotton plug. Glass tubes, 25 cm. long with 1·9 cm. internal diam. were filled up with 70 g. of a mixture of John Innes compost and sand,

approx. in the proportion of 2·5:1, respectively (pH 5·8). The tubes were tapped gently on the table to bring down the level of soil column to 18 cm. Four c.c. of water was added in each tube to raise the moisture content of the soil to approx. 35%. The tubes were autoclaved at 15 lbs. pressure for 20 minutes and then each was inoculated with a 9 mm. diam. mycelial disc from 5 days old culture on P.D.A. The disc was placed upside down on the top of the soil. The following treatments were used with 6 replications in each.

a - Continuous 2 $\pm$ 1°C b - Continuous 10°C

c - Continuous 15°C d - Continuous 25°C

e - First 6 days at 10°C, then at 25°C

f - Continuous 10°C with 3 hrs. exposure at 25°C

g - Continuous 10°C with 6 hrs. exposure at 25°C

Growth measurement was recorded under a stereoscopic microscope with low magnification, 12·5x. The tube of the microscope was removed from the body and fixed on a stand which could be moved in any direction (Fig. 18). The soil tube was kept upright in a stand and the mycelial growth was measured by marks on the tube under reflected light. When the growth advanced considerably, a Zeiss binocular head magnifier could be used instead. Measurement was recorded up to 14 days at an interval of 2 days in all the treatments except at continuous 25°C which was recorded only up to 10 days as the mycelium reached the other end by that time.

Since no growth occurred at 2 $\pm$ 1°C, it was excluded for

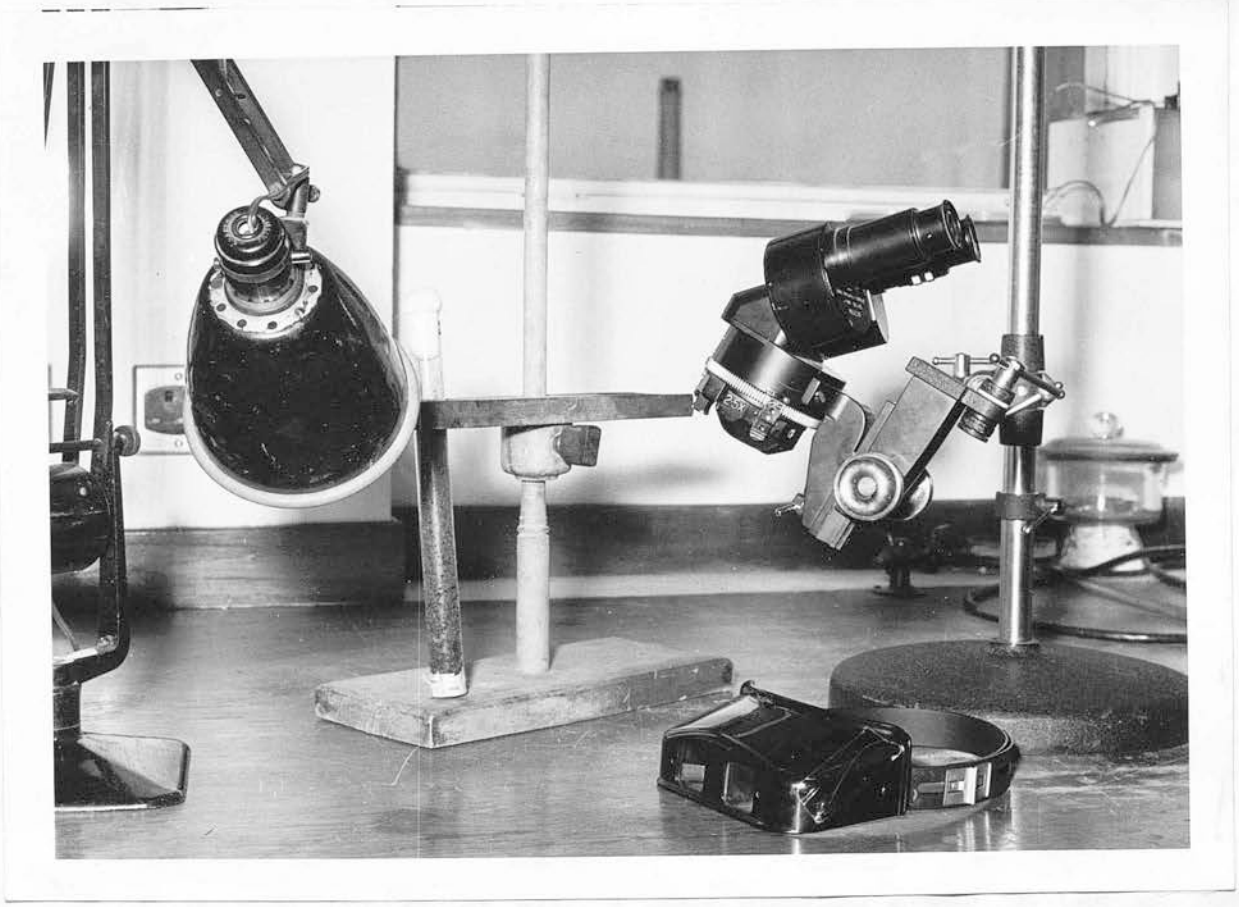


Fig. 18.

Apparatus to record growth of R. solani in soil in glass tube.

calculation. Calculations were done (1) to find out the regression lines at continuous 10°, 15° and 25°C separately and (2) to assess the growth rate at 10° and 25°C in the treatments 'b', 'd', 'e', 'f' and 'g' on alternate days.

Effect of temperature on the perpetuation of R. solani in soil in vitro: This experiment was done with the above tubes of the treatments 'a', 'b' and 'd' in the following way.

i - 2 ± 1°C for 23 days, at 25°C for 22 days, then in the refrigerator (6 ± 2°C)

ii - 2 ± 1°C for 23 days, at 10°C for 22 days, then at 25°C

iii - 10°C for 45 days, then in the refrigerator (6 ± 2°C)

iv - 25°C throughout

As the treatment 'a' of the previous experiment was split into 2, only 3 tubes could be used in the treatments 'i' and 'ii' but in the last two treatments all the tubes were kept but observations taken only from 4 tubes. Approximately 0·3 g. of soil was taken out of each of the tubes with a long sterile scoop in the inoculation room and mixed with 10 c.c. of P.D.A. containing 50 ppm. of Rose Bengal in a culture tube and then poured into a pair of petri dishes, 9 cm. diam. Two pairs of such petri dishes were used for each of the tubes. Number of colonies developed in each plate incubated at 25°C was counted after 2 days.

RESULTS

Growth of R. solani on P.D.A.: The following table shows that up to the fourth day the rate of mycelial growth varied directly with the rise in temperature i.e. decreasing progressively from the maximum at 27°C to the two lower temperatures of 20° and 13°C. Growth at 13°C was significantly lower in all the days. The difference between 27° and 20°C was significant up to the third day which, however, became insignificant on the fourth day. Linear contrast in the change in growth rate (on the mean over the days) showed difference between 27° and 20° or 13°C but not between 20° and 13°C. The details of observations with analysis of variance are given in the Appendix VIII.

Table: Growth rate of R. solani on P.D.A. at different temperatures in different dates (mm. per hr.)

Temperature	Days after inoculation			
	2	3	4	5
27°C	0·6125	0·6775	0·7575	0·7075
20°C	0·3600	0·5500	0·6900	0·7175
13°C	0·0500	0·3400	0·4175	0·4700

In the experiment with different isolates of R. solani it was observed (Fig. 19a) that after one day growth of majority of the isolates was nil at 13°C and scanty at 20°C but P3 grew well at both these temperatures. The same was true after 3 days except that at 20° and 13°C the difference between P3 and T became

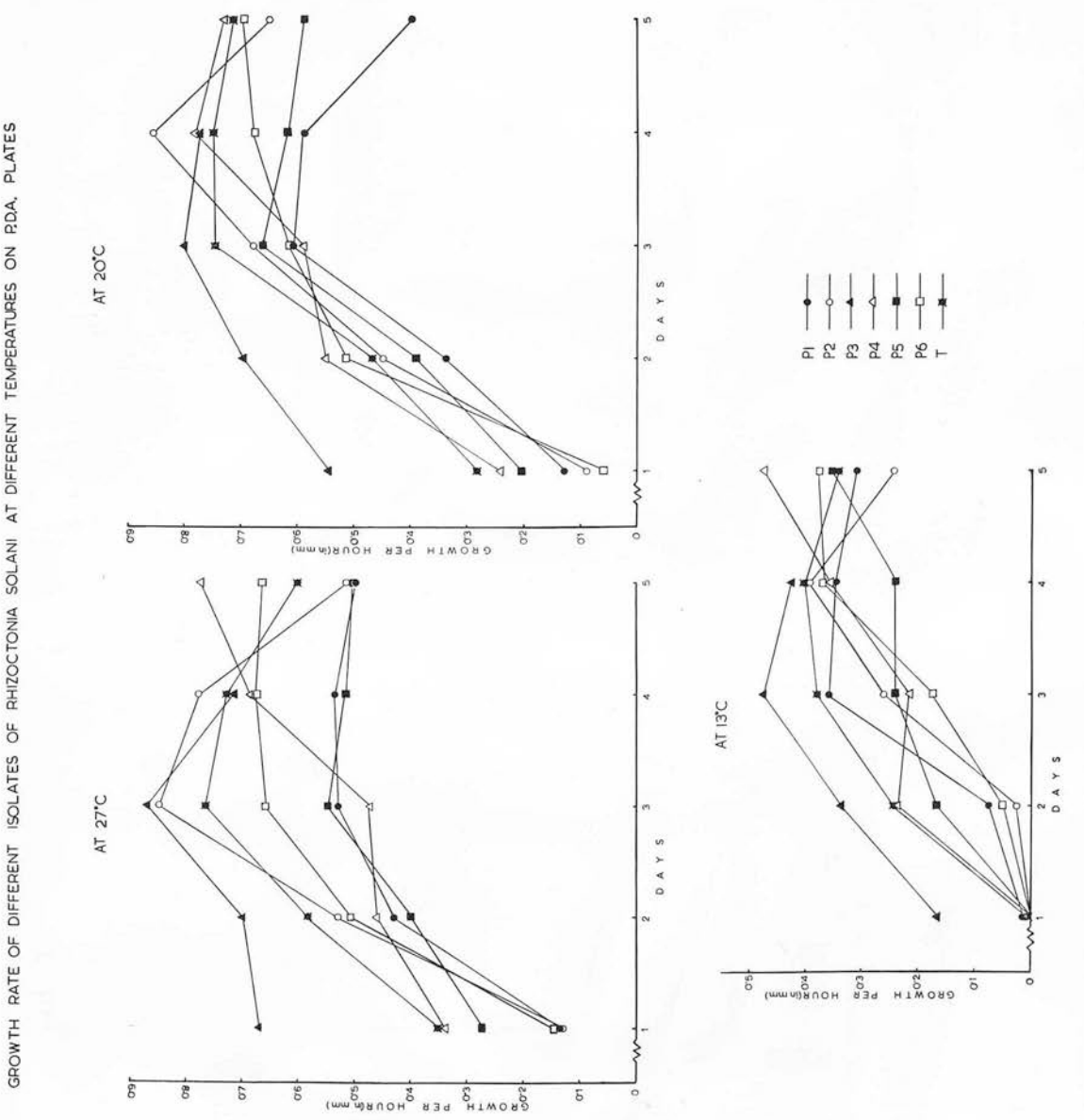

GROWTH RATE OF DIFFERENT ISOLATES OF RHIZOCTONIA SOLANI AT DIFFERENT TEMPERATURES ON PDA. PLATES
AT 27°C
AT 20°C
AT 13°C
GROWTH PER HOUR (in mm)
DAYS
P1
P2
P3
P4
P5
P6
T

Fig. 19a.

insignificant. The rate of growth at 13°C was poorest in all the isolates in all the days. Either 27° or 20°C supported the maximum growth of the isolates though in many cases the difference between them was not significant. There was great variation in the growth rate among the isolates and temperatures which is evident from the significant F ratio for isolate (I) and temperature (Temp) in every day. Growth of P3 at 13°C was almost the same as that of P4 at 27°C after 3 days. On the basis of mean values over the days together the effects of all the factors (I, Temp and I x Temp) were found significant.

The interaction between I and Temp was found to be significant up to the third day and then on the fifth day but not on the fourth day. Linear contrast in the change in growth rate over days showed significant effect of I, Temp and I x Temp but in quadratic contrast only was the effect of I significant. The details of observations and analysis of variance are given in the Appendix IX. Fig. 19b shows the growth of the colonies of all the isolates at different temperatures.

All the isolates had moderate aerial growth which crept along the side of the petri dish and produced tiny sclerotia. Colour of the colonies in P3 was snuff brown - shade 2, in P4 buff - shade 1 and in all others chamois - shade 1. Pigmentation in P.D.A. slant was light, chamois -shade 1 or 2 in all the isolates except in P1 where it was a bit darker, snuff brown - shade 1.* There was

* Colour standards taken from Repartoire de Couleurs by Societe Francaise des Chrysanthemistes et Rene Oberthür (1905).

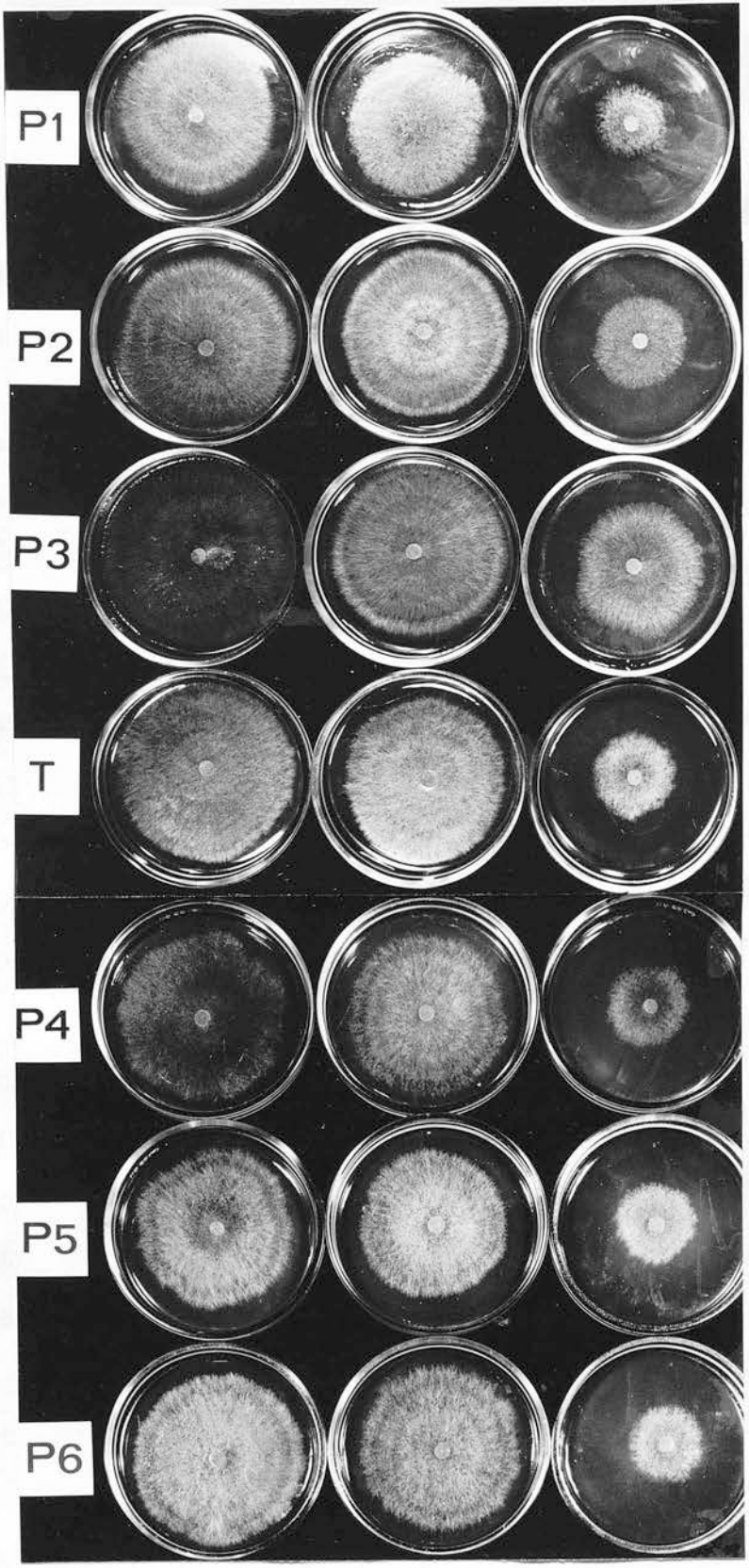


Fig. 19b.

Growth of different isolates of R. solani (P1 to P6 and T) on P.D.A. at (from left to right) 27°, 20° and 13°C.

thin stromatic layer on P.D.A. slants in P3, P6 and T which was thinner in others. Development of sclerotia was moderate in P1, P2, P4 and T, poor in P5 and almost nil in P6. Sclerotia in P6 were absent in 3 plates and only 2 formed in one plate. In all the isolates the size of sclerotia varied from less than 1 mm. to just over 2 mm. The colour of sclerotia in all the isolates was a shade of brown which varied slightly except in P2 where it was yellow ochre.

Growth of R. solani in soil in vitro: Two series of observations were made in this experiment. In the first, the growth rate of the fungus was recorded separately for 10°, 15° and 25°C in relation to time, in the second the effects of 10° and 25°C at different temperature treatments with 10° and 25°C were examined. Fig. 20 shows that at 10°C the growth rate shows a linear regression line but at 15° and 25°C quadratic curves. Linear contrast in the change in growth rate showed difference between 25° or 15°C and 10°C but not between 25° and 15°C. In quadratic contrast no difference was found between 15° and 10°C which differed significantly from 25°C. The details of observations with analysis of variance are given in the Appendix X.

In the second series of observations growth rate at 10°C was found to be very slow for the first 4 days and then increased (Fig. 21). Though the rate of growth at 10°C in the treatments alternated with 25°C for 3 and 6 hours was higher than continuous 10°C for the first few days but this difference became insignificant

GROWTH RATE OF RHIZOCTONIA SOLANI AT DIFFERENT TEMPERATURES IN SOIL IN VITRO

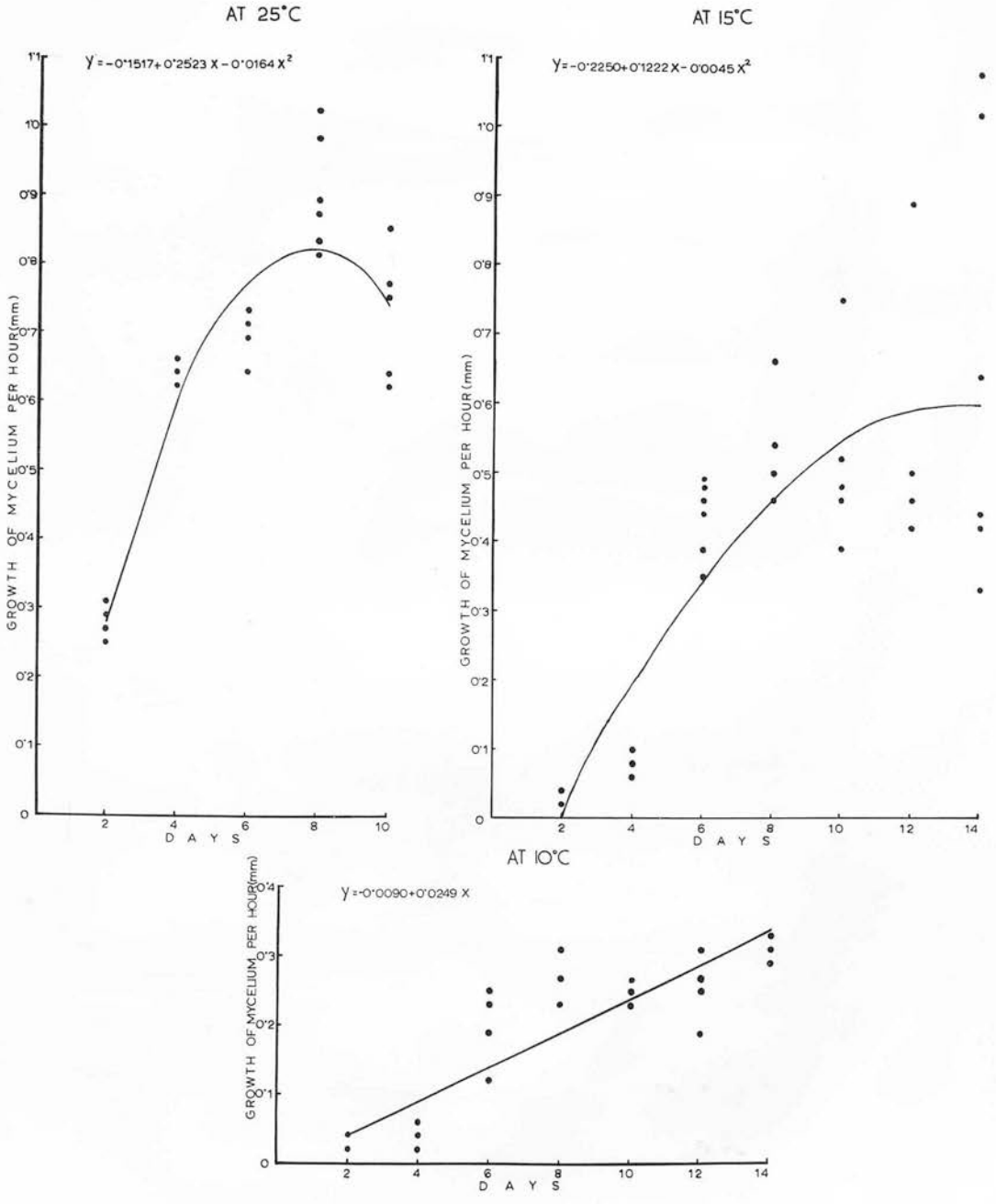


Fig. 20.

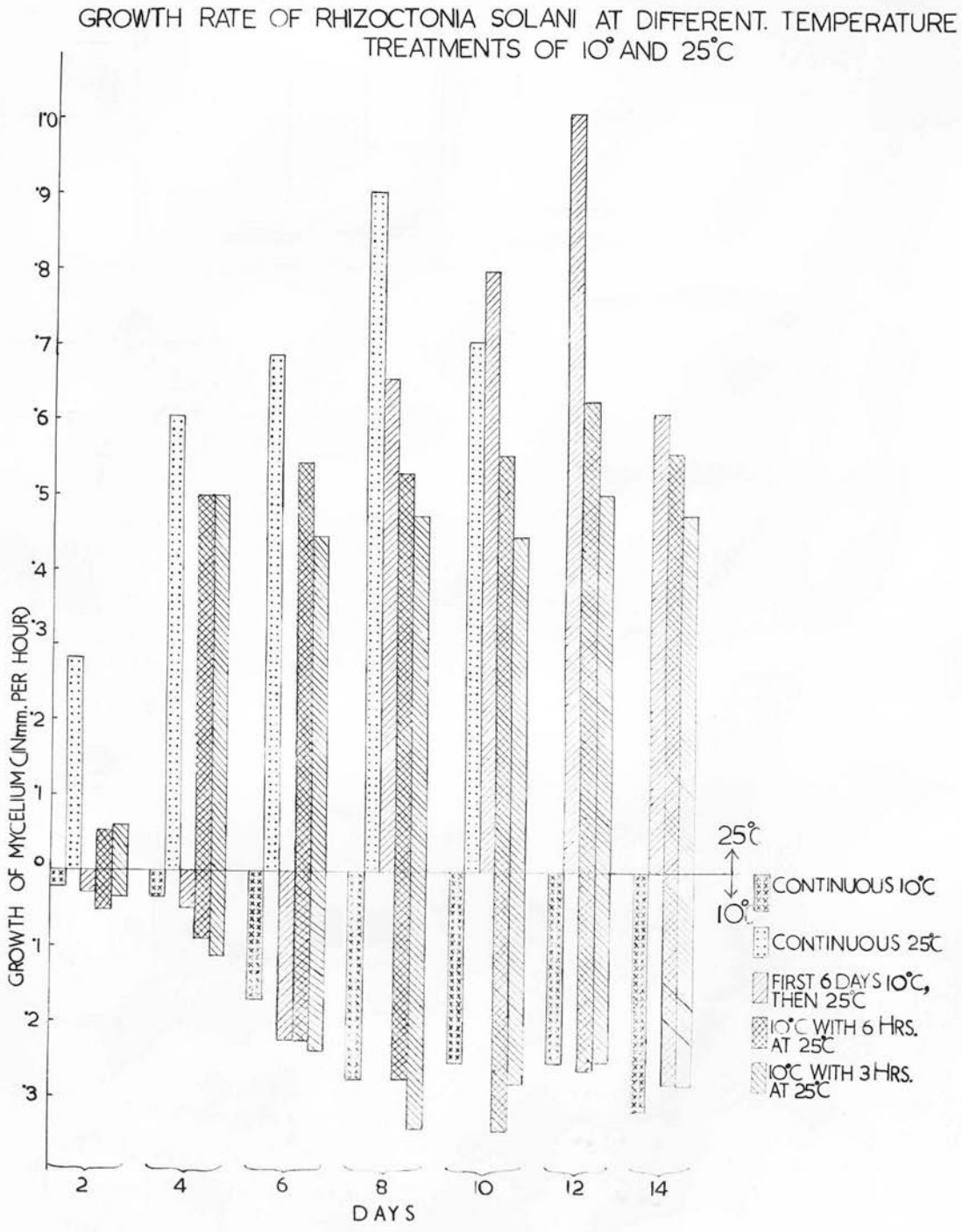

GROWTH RATE OF RHIZOCTONIA SOLANI AT DIFFERENT. TEMPERATURE TREATMENTS OF 10° AND 25°C
GROWTH OF MYCELIUM (N mm. PER HOUR)
1·0
·9
·8
·7
·6
·5
·4
·3
·2
·1
0
·1
·2
·3
2
4
6
8
10
12
14
DAYS
25°C
10°C
CONTINUOUS 10°C
CONTINUOUS 25°C
FIRST 6 DAYS 10°C, THEN 25°C
10°C WITH 6 HRS. AT 25°C
10°C WITH 3 HRS. AT 25°C

Fig. 21.

afterwards. The details of observations with calculations are given in the Appendix XI (b).

When the tubes were kept at 10°C for 6 days and then at 25°C, for the first 2 days the difference between the growth rate at 25°C in this treatment and that of the one at continuous 25°C was significant but no difference was found two days later. Appendix XI (a) shows that the rate of growth at 25°C was better than when alternated with 10°C but decreased with time, being significant from eighth to tenth day. However, the difference of growth rate between twelfth and fourteenth day at the same temperature when alternated with 10°C was insignificant.

Perpetuation of R. solani in soil in vitro: The table on p. 64 shows that after 3 months the number of colonies in each of the petri dishes was so numerous that it was impossible to count them separately. However, very striking differences were found after 6 and 8 months between the tubes kept at 25°C and 6 ± 2°C. At 25°C the number of colonies declined considerably irrespective of the fact that in the treatment 'ii'the tubes had been kept at lower temperatures for 45 days at the beginning of the experiment. After 8 months only 2 colonies developed in one petri dish in each of the treatments 'ii' and 'iv'. In the treatments 'i' and 'iii' the number of colonies were more numerous in the former than the latter.

Table: Average number of colonies of R. solani developed from 0·3 g. of soil in different temperature treatments (per petri dish)

Treatment	After 3 months	After 6 months	After 8 months
i - 2 ± 1°C for 23 days, at 25°C for 22 days, then at 6 ± 2°C	Innumerable	8·7	6·7
ii - 2 ± 1°C for 23 days, at 10°C for 22 days, then at 25°C	"	2·7	0·3
iii - 10°C for 45 days, then at 6 ± 2°C	"	6·0	5·2
iv - 25°C throughout	"	0·6	0·2

'i' and 'ii' - mean of 6 plates; 'iii' and 'iv' - mean of 8 plates.

DISCUSSION

The maximum growth rate of the tomato isolate of R. solani at 27°C is in close agreement with the findings of Walker (1928). Leach (1947) recorded maximum growth of this fungus at 30°C on P.D.A. The optimum temperature for the development of soil rot of tomatoes due to R. solani was found to be 24°C which was close to the optimum for the fungus growth in culture (Gonzalez and Owen, 1963). Houston (1945) classified the isolates of this fungus into 3 types - A, B and C and observed that while the types A and B had their optimum temperature for growth in culture at 28°C, C had a lower one at 25°C. Similarly, Elnur and Chesters (1967) found

that 2 isolates of R. solani from wheat differed in their temperature requirement - isolate 1 grew best in culture at 25°C and 2 at 21°C.

The optimum for growth of all the isolates of R. solani on P.D.A. lie between 20° and 27°C and minimum growth occurs at 13°C. Variations occur in isolates as regards colour of colonies, pigmentation of medium, and formation and colour of sclerotia etc. In general, growth rate of P2, P3 and T is greater than the other isolates. Regarding colour of colonies and pigmentation of medium P3 and P4 are darker than others. In P1 though the colony is of lighter colour pigmentation of medium is darker.

Interaction between isolate and temperature was significant up to the third day and again on the fifth day but not on the fourth day. The possible explanation is that with the exception of P4 and P6 growth was most active at 27°C and decreased with the advance of time, by the fourth day growth at 20°C surpassed that at 27°C. In P4 and P6 where growth was most active at 20°C to begin with, a similar pattern occurred. On the third day growth of P6 at 27°C surpassed that at 20°C but on the fourth day it was the same in both 20° and 27°C. P4 had the best growth at 20°C up to the fourth day. So, growth of all the isolates was better at 20°C on the fourth day. Conditions, however, changed again on the fifth day as the growth rate of P1 and P4 at 27°C surpassed that at 20°C.

R. solani grows better at 27°C on P.D.A. and at 25°C in soil, but the rate decreases with time. The regression analysis of 3 temperatures in soil shows a linear line for 10°C and quadratic curves for 25° and 15°C. However, the rate of decrease is

significantly more at 25° than 15°C. Accumulation of "staling product" may be a factor for the slowing down of the growth rate at these higher temperatures. A similar phenomenon was observed in Phoma lingum (Fr.) Desm. by Pound (1947) who observed that though some isolates of this fungus grew faster at 30°C than at room temperature but ultimately staled very severely when the growth was no better than at room temperature. Rapid decrease in the growth rate of R. solani at temperatures between 23·6° and 32·6°C was observed by Richards (1923).

From the second observation it appears that 3 or 6 hours exposure of 25°C of the fungus growing at 10°C does not have much effect on the growth rate. However, the rate of decrease at continuous 25°C seems to be faster than when alternated with 10°C.

Das and Western (1959) recorded that R. solani could persist in the natural soil for 3 months in the absence of host crops and then rapidly disappeared. Papavizas (1969) noted that the decline in the survival of R. solani was faster in natural soil than in autoclaved soil and that the pathogen could be recovered from soil 33 months after addition of 0·31% cornmeal inoculum to autoclaved soil and 0·62% inoculum to natural soil. Practically no information is available about the temperature effect on the perpetuation of this fungus except that of Richards' (1923) who propounded that R. solani remained in active state much longer at lower temperature than at higher. The present study supports this view on the evidence of the number of colonies recovered from the tubes kept at different

temperatures. Survival capacity, however, decreased with time in both higher and lower temperature which was almost nil at 25°C after 8 months.

CHAPTER III

MORPHOLOGY AND BIOLOGY OF *APHELENCHUS AVENAE* IN CULTURES OF *RHIZOCTONIA SOLANI* AND *COLLETOTRICHUM COCCODES*

MATERIALS AND METHODS

Rate of multiplication, morphometric values and male/female sex ratio: Rate of multiplication of A. avenae was recorded from progenies of single female grown in the cultures of the fungi in petri dishes (9 cm. diam.) containing 12 c.c. P.D.A. Nematodes were added as one day old larvae from eggs kept for a few hours before hatching in water. Four plates for each of R. solani and C. coccodes were used and incubated at 25°C. This experiment was duplicated to enable observations to be taken at two different dates - after 13 and 21 days, respectively.

When the incubation period was over, the cultures were removed, cut into small pieces, placed on "nematode filter" over a nylon strainer and extracted for 3 days by Baermann funnel technique. After removing the cultures the plates were washed with water and the remaining worms counted separately and added to the total count of each plate.

Observations on morphometric values and male/female sex ratio were made on progenies of this experiment. Morphometric values were recorded on 20 females in both R. solani and C. coccodes and on 10 males in C. coccodes. Males in R. solani could not be included as they occurred very rarely. Measurements were done on 22 eggs.

Egg laying capacity and vivipary: Few females, particularly in R. solani were found to be exceptionally robust containing a few eggs at a time or in rare cases even developing larva/e inside the

uterus and ovary. To find out whether those eggs and larvae were actually laid by the mother, 10 such females from Rhizoctonia culture were examined individually by keeping in water in solid watch glass at 25°C. During observation the worms were transferred frequently to new watch glasses with fresh water to check bacterial growth as well as for better aeration. Again, to test whether the eggs laid by such females were viable, 15 apparently normal eggs were kept individually in solid watch glasses and examined daily.

Hatching and egg laying behaviour: To study hatching, maturation, egg laying period, number of eggs laid per female and feeding habit, a single female was reared in 2% water agar in a small petri dish (4·7 cm. internal diam.) and examined daily under stereoscopic binocular microscope. Three c.c. of medium was poured in each petri dish and inoculated with the fungus: smaller amount dried up quickly in the incubator and larger amount made observation difficult. Each plate with the fungus colony was inoculated with a single freshly laid egg and incubated at 25°C. Twenty-four observations were taken in each of the fungi.

The number of eggs laid per female and the total egg laying period were recorded by transferring the nematode frequently from one plate to another to differentiate the mother from the offspring. Depending on the egg laying period, a single female was transferred 2 to 4 times or even more. When the frequency of egg laying

dropped considerably indicating the approach of the end of laying, observations were made daily to note the precise date of the termination of egg laying.

Desiccation capacity of A. avenae: Nine mm. discs were cut out from cultures of A. avenae on R. solani and C. coccodes in 9 cm. petri plates and kept at room temperature. Two plates were used of each of the fungus. After every 3 months 3 discs from each plate i.e. 6 discs each of Rhizoctonia and Colletotrichum were placed individually in solid watch glass with water and the extracted nematodes were counted for 4 days. Observations were taken up to 18 months.

RESULTS

Rate of multiplication of A. avenae in the fungi: Fig. 22 shows that the rate of multiplication of A. avenae was significantly higher in R. solani than in C. coccodes both after 13 and 21 days. Normally R. solani has good aerial growth but the nematodes grazed the colony to such an extent that no aerial mycelium could be seen after 21 days and the growth was very much suppressed. C. coccodes has little aerial mycelium as compared with R. solani which has also been found to be grazed by the nematode. The details of observations with analysis of variance are given in the Appendix XII.

MORPHOLOGICAL AND BIOLOGICAL CHARACTERS OF APHELENCHUS AVENAE IN RHIZOCTONIA SOLANI & COLLETOTRICHUM COCCODES

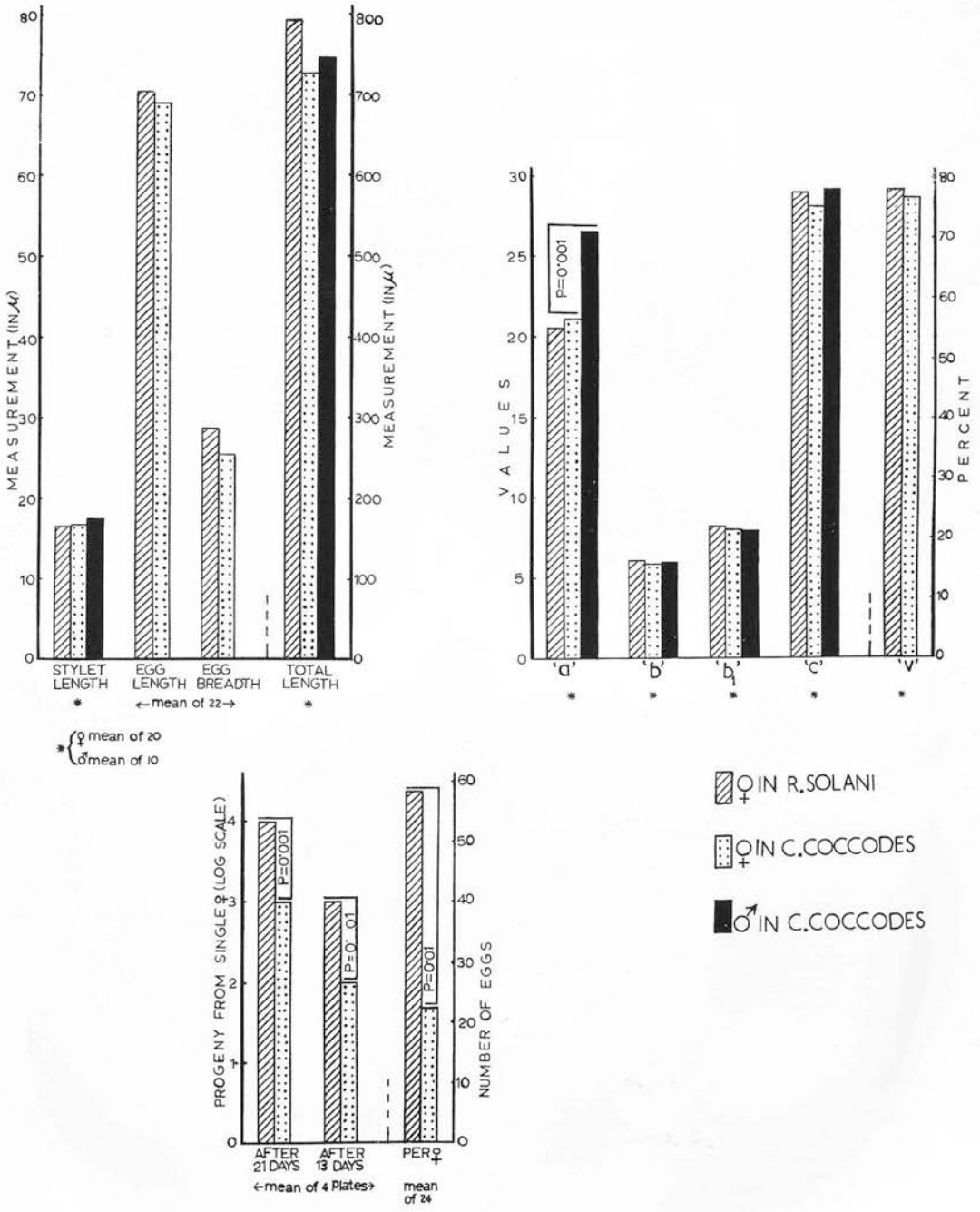


Fig. 22.

Morphometric values: The value of 'a' differed significantly ($P = 0{\cdot}001$) between the males in C. coccodes and females in either of the fungi but not between the females. No significant difference was found in any other morphometric values between the females grown in R. solani and C. coccodes or between the males in C. coccodes and females in either of the fungi (Fig. 22). Total length of the females in R. solani was found to be greater than both males and females in C. coccodes but the difference was not significant. Though no measurement of males in R. solani is included in the analysis, 4 males from its culture were measured subsequently and their morphometric values were found not to differ much from those in C. coccodes. The detailed observations with analysis of variance are given in the Appendix XIII.

Male/female sex ratio: From the population of different plates of C. coccodes random samples were examined to find the male/female sex ratio which was 1·32%, 1·06%, 0·73% and 0·43%, respectively. However, it was interesting to note that in the first culture of C. coccodes and A. avenae isolated from the infected roots, it was 7·60%.

Egg laying capacity and vivipary: Appendix XIV shows that several eggs along with developing larvae inside the uterus and ovary of the obese females were laid though in No.2 worm two eggs and in No.4 one egg was retained till the time of death. As many as 14 eggs were found inside a single female grown in R. solani though

some of them might not be viable. Fig. 23 shows two worms with several eggs - one with normal and the other with abnormal eggs including one in the post vulval sac. In C. coccodes culture up to 5 eggs in a single female were observed.

Observations were continued up to 18 days when all the worms except No.2 died (Appendix XIV). This nematode died after 24 days. One larva inside No.1 nematode was found to move very actively from mouth to the tail even after the death of the mother. The contour of the body wall of the dead worm changed with its movement. It emerged after 17 days as a L_2 larva with developed stylet and median oesophageal bulb. After emergence of the larva the mother was examined further when another dead larva was found inside.

Hatching and egg laying behaviour: In both R. solani and C. coccodes hatching of larvae from eggs took place after 2 to 3 days (more frequently after 2 days). Egg laying started after 5 to 6 days (more frequently after 5 days) of hatching. Only one nematode in Colletotrichum was found to start laying eggs after 4 days. From a few apparently normal eggs larvae failed to hatch. Again, in a few cases though the larvae grew into well developed females with normal genital organs, they did not lay eggs. Out of 15 eggs laid by these worms, 11 hatched after 2 days, 2 after 3 days and the remaining 2 did not hatch. No apparently abnormal egg was found to hatch.

Egg laying period varied from 5 to 19 days in R. solani and 3 to 12 days in C. coccodes (with the exception of only one day in

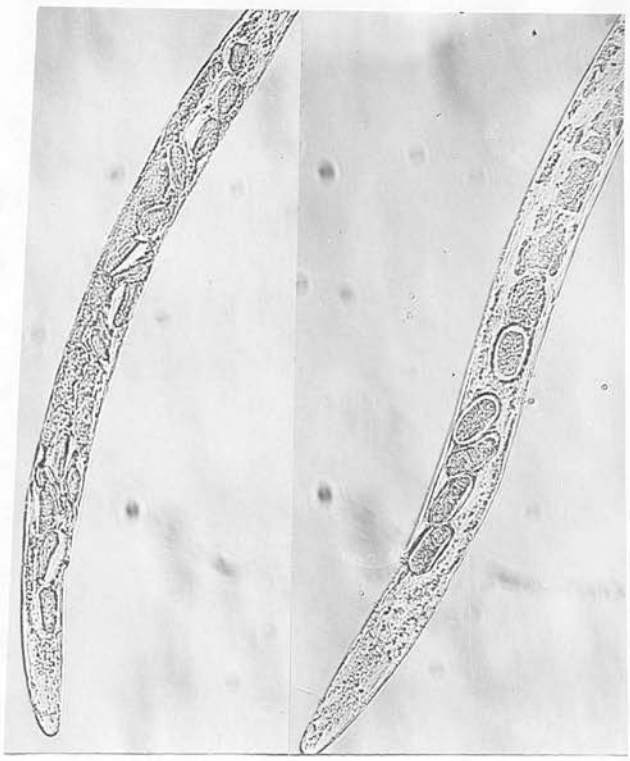


Fig. 23.

Fig. 27.

Fig. 23: Females of A. avenae containing many eggs - the left worm shows all abnormal eggs including one in the post vulval sac and the right one shows five normal eggs. Fig. 27: A avenae carrying conidia of C. coccodes.

2 cases). The rate of egg laying during the first few days was rapid and gradually slowed down with time, even as low as only one per day. At the later stage, in rare occasion, the regularity of egg laying might also be lost i.e. after a gap of one day of non-egg laying period, another egg might be laid the next day. Appendix XV shows that though the egg laying period of A. avenae in these two fungi did not vary significantly, the number of eggs laid per female differed significantly (P = 0·01) - the number was greater in R. solani than in C. coccodes. Great variation was found in the number of eggs laid per female within individual observations in both the fungi.

Feeding habit: Feeding habit of A. avenae was found to be almost the same as described by Linford (1937) and Rhoades and Linford (1959). The nematode pressed the mouth more or less at right angle of the hypha (Fig. 24), thrusted the stylet rapidly and during the process of feeding the median bulb pulsated continuously. The feeding period was brief: usually 6 to 50 seconds in Colletotrichum and up to 76 seconds in Rhizoctonia. Time of feeding appeared to be directly related with the thickness of hyphae. As R. solani hyphae are thicker than that of C. coccodes, it took longer time to feed on R. solani than on C. coccodes. Even in C. coccodes itself, the time taken was more on thicker than on thinner hyphae. Withdrawl of protoplasm could be seen at the time of feeding and at the end of the process the hyphae became empty (Fig. 25). Sometimes the empty cells might be shrivelled too, particularly the

Fig. 24.

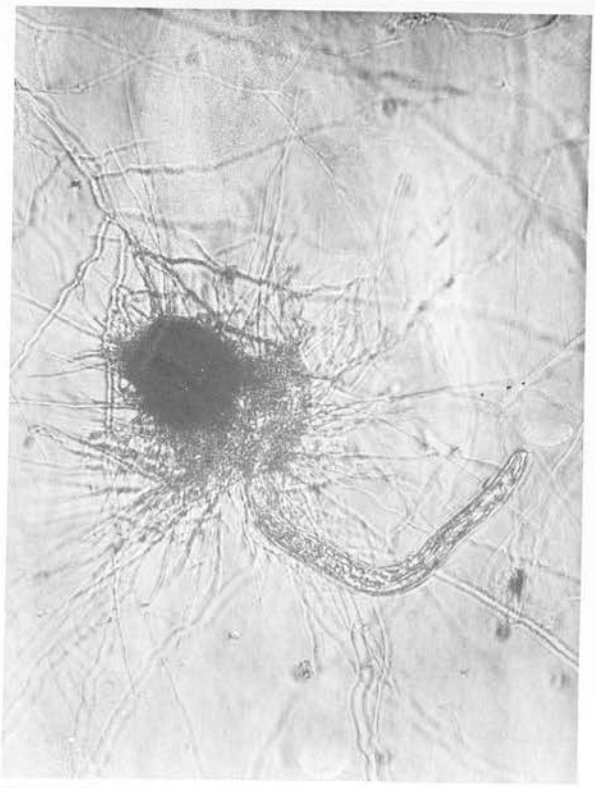


Fig. 26.

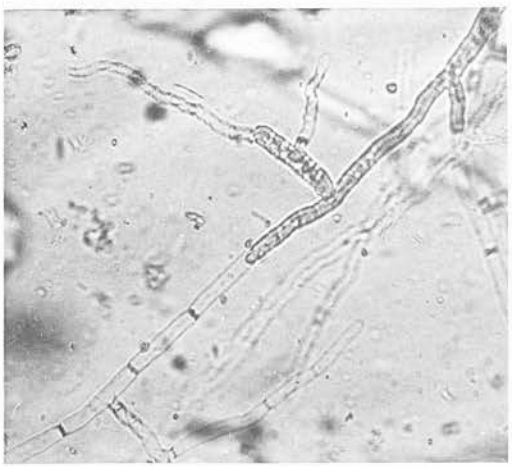


Fig. 25.

Figs. 24 and 25: Feeding of A. avenae on mycelium of R. solani. Fig. 24 shows the feeding process and Fig. 25 portions of hyphae emptied due to withdrawal of protoplasm. Fig. 26: A. avenae feeding on a sclerotium of C. coccodes.

terminal ones. As R. solani supported more worms, if the cultures (with more than 3 c.c. medium) were left for a long time, the progeny died of starvation sooner in it than in C. coccodes. In C. coccodes, the worms after finishing the mycelium invaded the micro-sclerotia (Fig. 26). Occasionally, conidia of this fungus were also found to be attacked. Large number of conidia adhered on the body of the nematode and carried with the movement (Fig. 27).

Desiccation capacity of A. avenae: It can be seen from the following table that A. avenae survived in both the fungus cultures up to 18 months but the survival capacity decreased to a great extent after 15 months. The rate of survival decreased progressively with time in both the fungi but more in R. solani.

Table: Survival of A. avenae in R. solani and C. coccodes culture in different months.

Fungus	After 3 months		After 6 months		After 9 months	
	No. of worms	% of survival	No. of worms	% of survival	No. of worms	% of survival
R.solani	252/194	77·0	201/130	64·7	136/70	51·5
C.coccodes	50/45	86·0	32/29	90·6	41/36	87·8

Fungus	After 12 months		After 15 months		After 18 months	
	No. of worms	% of survival	No. of worms	% of survival	No. of worms	% of survival
R.solani	94/32	34·0	80/12	15·0	52/1	1·9
C.coccodes	40/35	87·5	41/23	56·1	38/2	5·3

Figure of numerator denotes the number of total worms recovered from 9 mm. disc and that of denominator number of live worms.

DISCUSSION

Host preferences in A. avenae in cultures of different fungi have been recorded by many workers (Mankau and Mankau, 1963; Barker, 1964; Townshend, 1964; Pillai and Taylor, 1967a and b). Mankau and Mankau (1963) observed that plant parasitic soil fungi including R. solani were preferred more by A. avenae than soil saprophytes. Phytopathogenic pythiaceous fungi, however, did not support the nematode. Pillai and Taylor (1967a and b) classified R. solani as an excellent host of A. avenae. Barker (1964) noted that different isolates of R. solani varied greatly as hosts. No such studies, however, have been made with any species of Colletotrichum. In the present study though R. solani has been found to support a much higher population than C. coccodes, the latter is also a good host of this nematode.

None of the morphometric values has been found to differ significantly between the females grown in R. solani and C. coccodes. The value of 'a' of the males in C. coccodes culture was significantly higher than the females in both the fungi which means that the males are more slender than the females. While recording variation in the morphometric values of A. avenae in different fungi, Pillai and Taylor (1967b) observed that 'L' varied significantly in all the fungi except between R. solani and Fusarium solani and 'a', 'b', 'c' and 'v' varied in some but were insignificant in others.

Males in A. avenae have been recorded rarely and appear to vary from locality to locality. Hechler (1962) found males in the

ratio of 1:10,000 in one population and 1:100,000 in another. Out of 54 fungi recorded as hosts by Townshend (1964) males were found only in 5. Goodey and Hooper (1965) could not find males in the population with which they were working but stated that they occurred in other English population in the ratio of 1:80,000. Considering these facts the present population differ considerably as the percentage of males varies from 0·43 to 1·32.

In the first C. coccodes culture which was isolated from the diseased roots large number of males were found as the condition was less favourable for development causing a greater number of males. On the other hand, conditions in the cultures were optimum for growth and also due to sufficiency of food the worms became robust and thus resulting in a predominance of females. Goodey and Hooper (1965) also observed that well-fed mature females of A. avenae from mushroom culture were usually larger and more robust than topotypes. From the reproduction rate in R. solani and C. coccodes it is evident that the former fungus is more favoured than the latter which might be related with more males in C. coccodes. Greater number of eggs laid by the females seems to be a factor for the increased population of A. avenae in R. solani than C. coccodes. Pillai and Taylor (1967b) propounded several reasons for the population increase in A. avenae, such as, number of eggs laid per female, % of eggs hatched, % of larvae reaching maturity etc.

Usually one egg is developed inside the uterus of the female but Goodey and Hooper (1965) observed up to 4 eggs at a time in some old females which they thought was due to the loss of egg laying

capacity. Though it has been found true in some cases, in others the several eggs inside a single obese female were laid and also most of the apparently normal eggs hatched.

Pillai and Taylor (1967d) stated that both hatching and developing time of A. avenae decreased as the temperature was increased until a minimum was reached above which the time required again increased. On this consideration the generation time as observed in this experiment at 25°C, 7 to 8 days (egg laying to egg laying) is comparable with 11 to 12 days at 20°C (Goodey and Hooper, 1965), 6 days at 28°C (Hechler, 1962) and 6·75 days at 30°C (Pillai and Taylor 1967d). Hatching of majority of the eggs after 2 days at 25°C is in close agreement with Taylor (1962).

Though some workers have noted formation of acervuli of C. coccodes on the host but have questioned about their role to disseminate the conidia to incite infection as the activity of the fungus is linked up with antagonistic bacteria and actinomycites in soil (Ettig, 1955; Schmiedeknecht, 1956 and Gemeinhardt, 1957). Ettig (1955) though recorded relative resistance of acervuli to the antagonistic bacteria but could not isolate the fungus from soil. McKay (1942) observed numerous conidia in flooded greenhouse soil which according to him and also Blackman and Hornby (1966) could be formed from germinating sclerotia under suitable conditions. So, considering these facts dissemination of conidia adhering on the body of A. avenae on agar plate deserves consideration for their spread in soil.

Townshend (1964) recovered a large number of A. avenae from the dried culture of F. oxysporum f. lycopersici after 12 months. In this study it appears that they can survive in dried culture even longer and possibly the survival rate varies according to fungi.

CHAPTER IV

EFFECTS OF THE PRESENCE OF *APHELENCHUS AVENAE* ON THE GROWTH OF TOMATO PLANTS AND THE INCIDENCE OF DISEASES CAUSED BY *RHIZOCTONIA SOLANI* AND *COLLETOTRICHUM COCCODES*

MATERIALS AND METHODS

This experiment was first conducted in clay pots, 25 cm. diam. containing, in each, 4·5 kg. John Innes compost. It was repeated using two sets of plastic pots, 10·5 cm. diam., in the first set, containing in each pot 500 g. sterilized soil and 500 g. unsterilized soil in the second set. The soil mixture consisted of 4 parts of arable soil and 1 part John Innes compost.

Raising and planting out of seedlings: Tomato seedlings, var. - Alisa Craig, were raised in the same manner as described in the first chapter. In the first exp. before final planting the seedlings were pricked out in small poly-pots individually. In the second exp. where the soil was sterilized, it was done in the same way as described in the first chapter. Harvesting was done after 148 and 80 days of inoculation in the first and second exp., respectively.

Preparation of fungus culture and inoculation: In the first exp. culture was grown on sterilized sugarbeet seeds for 5 weeks at 25°C and inoculation of pots was done at the rate of 3% of soil. To provide the control and nematode inoculated pots with the same quantity of organic matter, a similar quantity of sterilized sugar-beet seeds were added in each.

In the second exp. the inoculum was grown on maize meal sand medium according to Papavizas and Davey (1962): 98% sand, 2% maize meal and water to 20% moisture w/v. The fungi were grown for

5 weeks at 25°C and used as inocula at the rate of 3% of soil.

Collection of nematodes and inoculation: Nematodes were raised on R. solani in 9 cm. petri dishes containing 15 c.c. P.D.A. at 25°C. After 3 to 4 weeks extraction of nematodes was carried out using a modified technique of Oostenbrink's (1960) cotton wool filter method. Mycelial mats were removed from the plates, cut into small pieces and placed on the "nematode filter" supported on a sieve in the extraction dish with water sufficient enough to cover the mats. Next day the water with the nematodes was removed from the dish and refilled with fresh water and extraction was continued for a second day. The rate of inoculum was 7 nematodes per g. of soil in the first exp. and 26 per g. in the second.

Inoculations with both the fungi and nematode were done simultaneously 12 days after planting in the first and on the same day of planting in the second exp.

Assessment of disease and counting of nematodes: Disease assessment was done with the "disease recording tray" as described in the first chapter. Nematodes from roots were extracted by Young's (1954) method but instead of mason jars, big glass jars were used. Roots after harvest were kept individually in each of the jars with a little water at the bottom and sealed to keep the atmosphere saturated. To minimise the risk of moulds or bacterial contamination, counting was done every 3 days up to the ninth day and after each count fresh water was added.

Extraction of nematodes from the soil was done by the same method as used to collect the nematodes from the fungus culture for inoculation. In addition, at the end of the first exp. 10 partially decayed sugarbeet seeds were placed separately in solid watch glasses and the nematodes were extracted.

Treatments: The following 4 treatments were used with 4 replications in the first and 5 in the second exp. (both in sterilized and unsterilized soil) in each treatment.

a - Control (uninoculated) b - Fungus only

c - Fungus + nematode d - Nematode only

Calculations were done separately for R. solani and C. coccodes inoculations keeping treatments 'a' and 'd' common for both.

Survival of fungi in the presence of A. avenae: After the harvest of plants in the first exp., 20 surface sterilized (0·1% $HgCl_3$ for $1\frac{1}{2}$ min.) tomato seeds were sown in each pot of the treatments 'b' and 'c' and the incidence of disease was recorded.

RESULTS

Growth of plants: Fig. 28 shows that in the first exp. heights of plants and dry weights of roots in the nematode and nematode + fungus treatments were greater than fungus inoculation but the differences were not significant. Results were similar in dry weights of shoots with C. coccodes inoculated plants but with

R. solani the observed differences between the treatments were insignificant (Fig. 29). Detailed observations and analysis of variance are given in the Appendix XVI (a) and (b).

In the second exp. (Fig. 30), in sterilized soil, height was significantly greater in the fungus + nematode incoulated than fungus inoculated plants (P = 0·01 in C.coccodes and = 0·05 in R. solani). Results were similar with dry weight of shoot and fresh weight of root but the differences were only significant with C. coccodes (P = 0·01 in shoot and = 0·05 in root). Figs. 31 and 32 show a comparison of the growth of root inoculated with the nematode and fungus and the disease incidence due to R. solani and C. coccodes, respectively.

In unsterilized soil no significant result was obtained with any of the fungi. The details of observations and analysis of variance are given in the Appendix XVII (a), (b), (c) and (d).

Number of A. avenae extracted from soil, roots and sugarbeet seeds: Fig. 33 shows that in the first exp. the number of nematodes increased both in the soil and roots (more in roots), this was greater in the nematode + fungus than nematode inoculation but the difference was only significant in soil in the presence of both the fungi (P = 0·001). Similarly, nematodes extracted from sugarbeet seeds (used as base of fungus inoculum) was significantly higher (P = 0·001) in the nematode + fungus inoculated than nematode inoculated pots. The details of observations and analysis of variance are given in the Appendix XVIII (a) and (b).

Fig. 28.

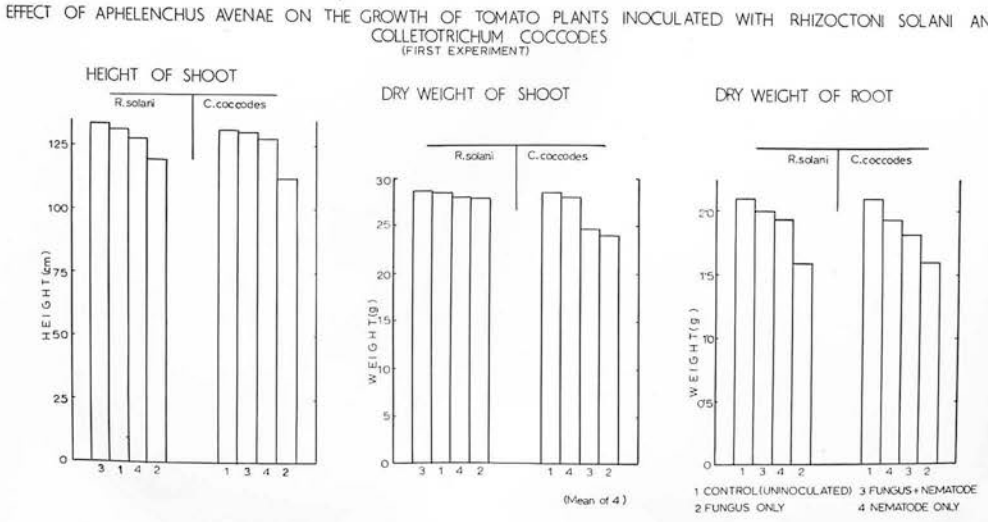


Fig. 29.

Fig. 28: Growth of tomato plants in different inoculations with A. avenae (N), R. solani (R) and C. coccodes (C) in the first exp. 1 - Control, 2 - R. alone, 3 - N + R, 4 - C alone, 5 - N + C, and 6 - N alone.

EFFECT OF APHELENCHUS AVENAE ON THE GROWTH OF TOMATO PLANTS INOCULATED WITH RHIZOCTONIA SOLANI AND COLLETOTRICHUM COCCODES
(SECOND EXPERIMENT)

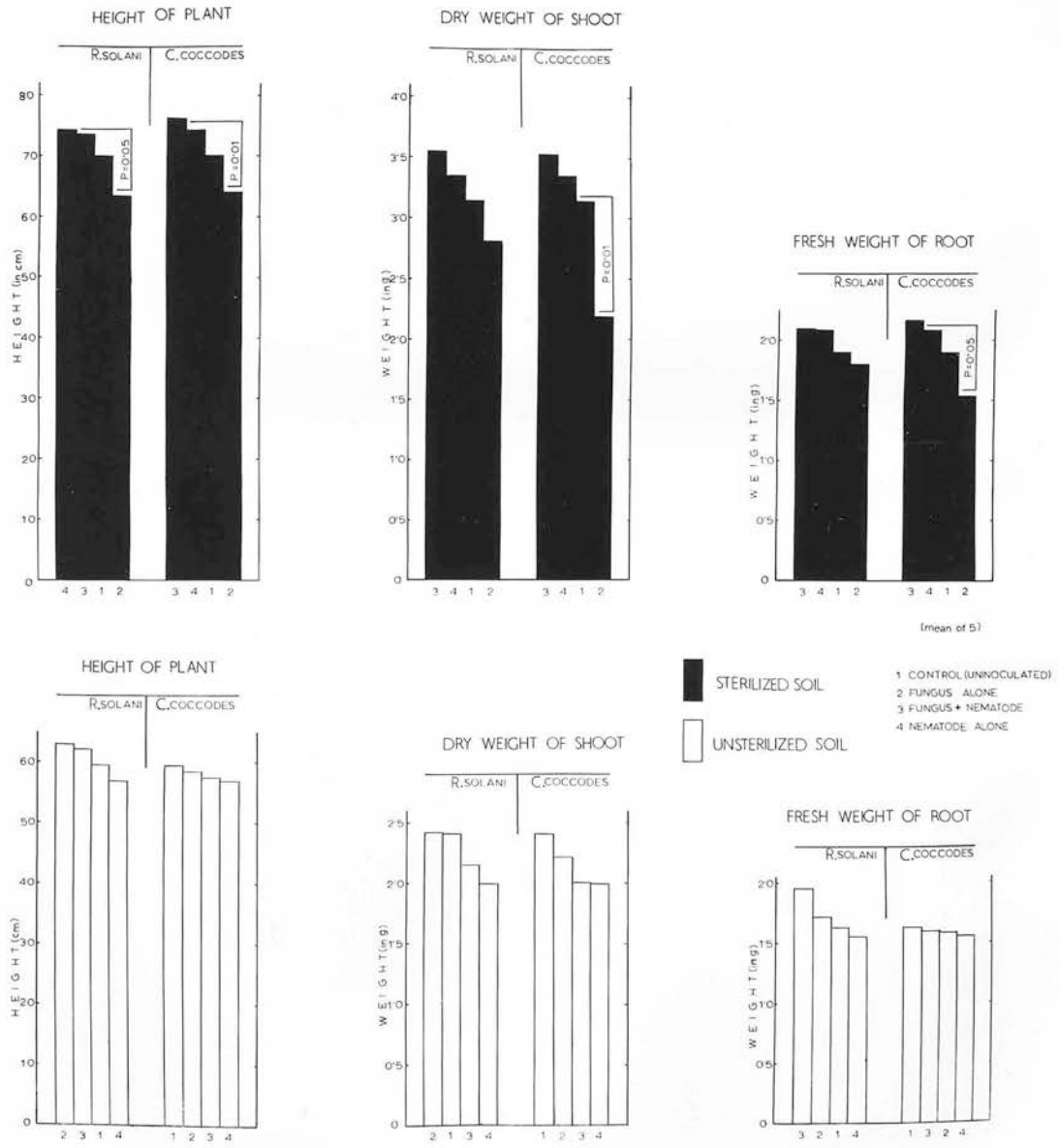


Fig. 30.

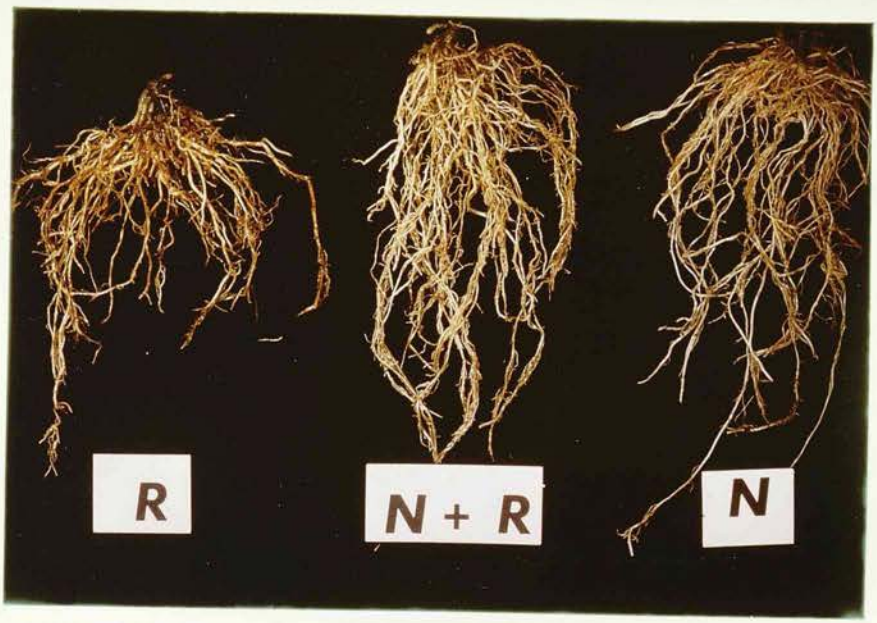


Fig. 31.

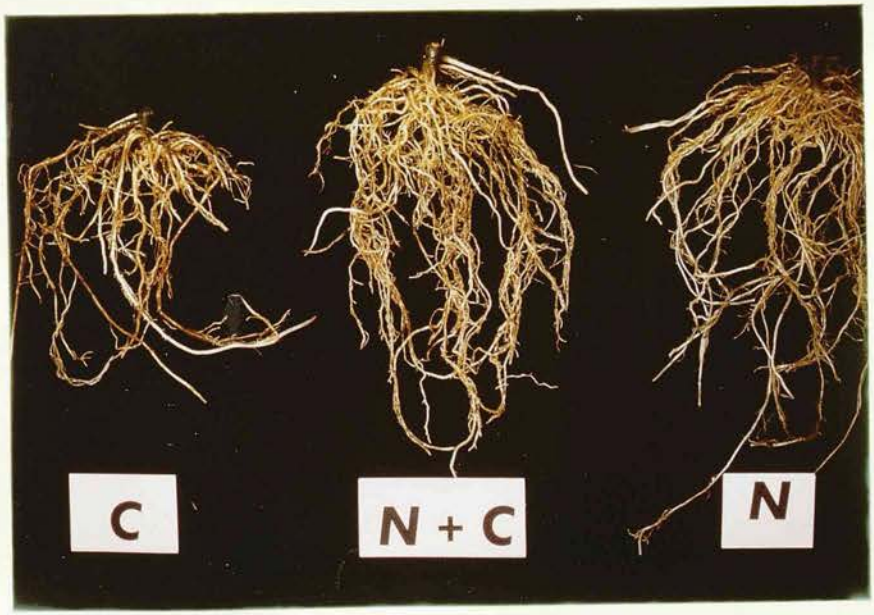


Fig. 32.

Growth of root of tomato inoculated with A. avenae (N), R. solani (R) and C. coccodes (C) in sterilized soil in the second exp.

The rate of multiplication of A. avenae, in the second exp., was slow both in sterilized and unsterilized soil and also in the roots in sterilized soil. Fig. 34, however, shows that the rate of multiplication in roots in sterilized soil was significantly higher in the nematode + fungus inoculated than nematode inoculated pots (P = 0·01 in R. solani and = 0·001 in C. coccodes). No marked difference of effect was observed in unsterilized soil. Detailed observations with analysis of variance are given in the Appendix XIX (a), (b), (c) and (d).

Incidence of disease: In the first exp. though disease incidence caused by the fungi was less in the fungus + nematode inoculated than in the fungus inoculated plants but was significant (P = 0·05) only with C. coccodes (Fig. 35). The detailed observations and analysis of variance are given in the Appendix XX.

Fig. 36 shows that in the second exp., in sterilized soil, the incidence of diseases was decreased significantly in the fungus + nematode inoculated than fungus inoculated plants (P = 0·05 in R. solani and = 0·01 in C. coccodes). No marked effect was, however, seen in unsterilized soil. The details of observations and analysis of variance are given in the Appendix XXI (a) and (b).

Survival of the fungi in the presence of A. avenae: Seedlings emerged after 25 to 30 days of sowing and observations on disease incidence were made up to 50 days after sowing. In R. solani

NUMBER OF APHELENCHUS AVENAE EXTRACTED FROM SOIL AND ROOTS OF TOMATO PLANTS IN THE PRESENCE OF RHIZOCTONIA SOLANAI & COLLETOTRICHUM COCCODES
(FIRST EXPERIMENT)

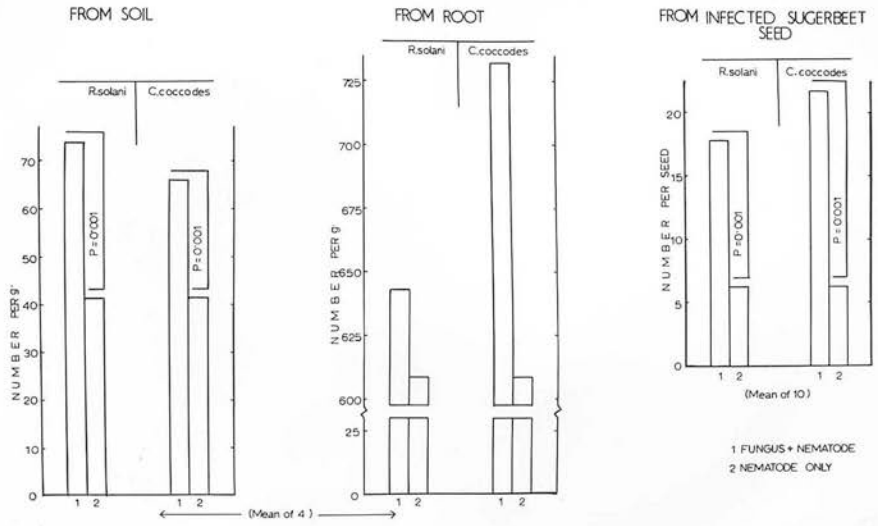


Fig. 33.

EFFECT OF APHELENCHUS AVENAE ON THE INCIDENCE OF DISEASES CAUSED BY RHIZOCTONIA SOLANI & COLLETOTRICHUM COCCODES ON TOMATO ROOTS
(FIRST EXPERIMENT)

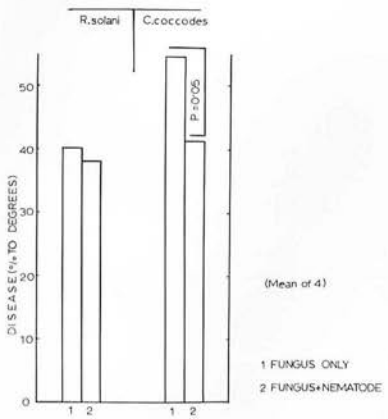


Fig. 35.

NUMBER OF APHELENCHUS AVENAE EXTACTED FROM SOIL AND ROOTS OF TOMATO PLANTS IN THE PRESENCE OF RHIZOCTONIA SOLANI & COLLETOTRICHUM COCCODES
(SECOND EXPERIMENT)

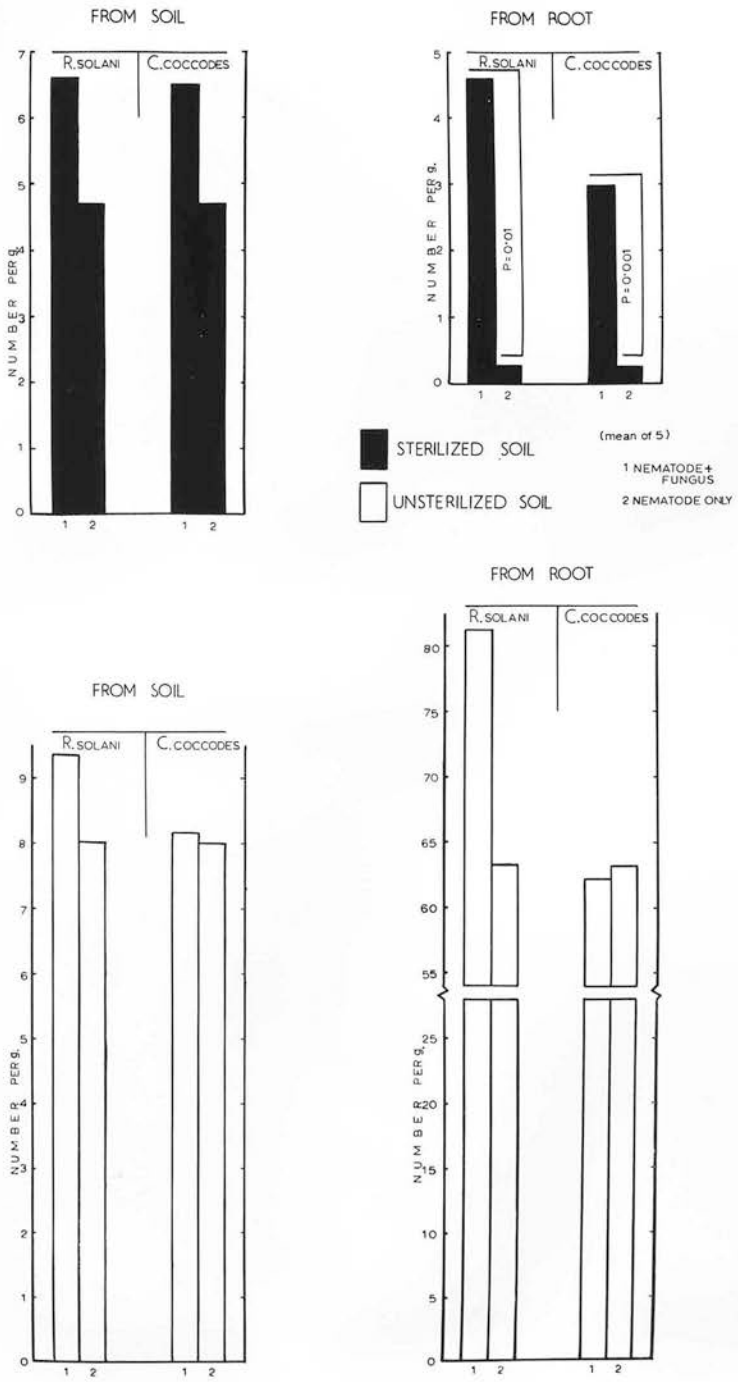


Fig. 34.

EFFECT OF APHELENCHUS AVENAE ON THE INCIDENCE OF DISEASES CAUSED BY RHIZOCTONIA SOLANI & COLLETOTRICHUM COCCODS ON TOMATO ROOTS
(SECOND EXPERIMENT)

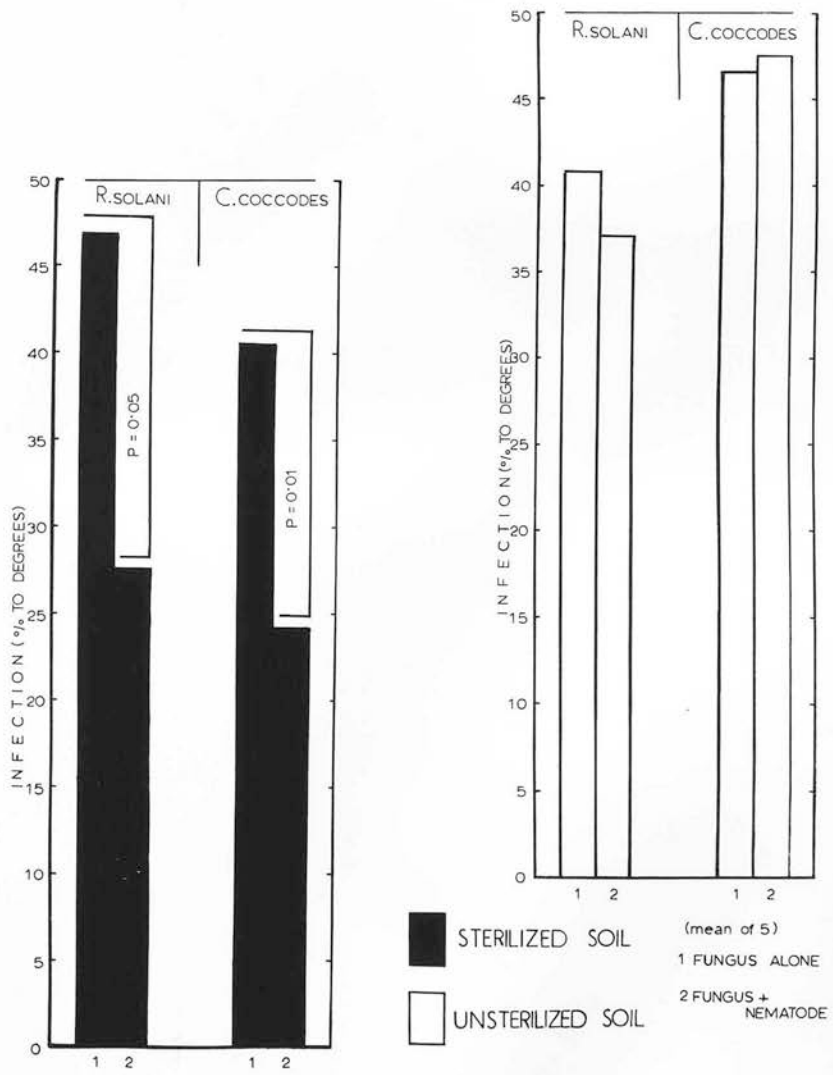


Fig. 36.

inoculated pots, some of the seedlings were killed by the fungus causing damping-off, some seedlings succumbed still earlier as the radicle and plumule emerged. C. coccodes attacked the seedlings on the collar region or occasionally even earlier on the radicle or plumule. A large number of seeds, particularly in C. coccodes inoculated pots did not germinate. The dead seedlings and seeds with incipient radicle and/or plumule on incubation on P.D.A. yielded colonies of the respective fungus.

Figs. 37 and 38 show that loss of seedlings in the nematode + fungus inoculated pots was less than fungus inoculated ones. The following table gives the percentage of kill and percentage of germination of seedlings in different treatments of fungus and nematode inoculations.

Table: Percentage of germination of tomato seedlings and percentage of kill due to R. solani and C. coccodes in the presence of A. avenae (mean of 4 pots each containing 20 seeds).

	R.solani	R.solani + A.avenae	C.coccodes	C.coccodes + A.avenae
% of germination	76·25(15·25)	80·00(16·00)	30·00(6·00)	60·00(12·00)
% of kill	50·80(7·75)	28·12(4·50)	45·83(2·75)	22·92(2·75)

Number in the bracket denotes the average number of seeds germinated and seedlings killed per pot, respectively.

Fig. 37.

Fig. 38.

Germination of seeds and death of seedlings of tomato in pots of the first exp. inoculated with A. avenae (N), R. solani (R) and C. coccodes (C).

DISCUSSION

In the first exp. with R. solani with the exception of dry weight of shoot, plant growth improved to a greater extent in the nematode + fungus than the fungus infected pots but the difference was not significant. In the second exp., however, the differences in all the growth measurements with C. coccodes and in the height with R. solani were significant. This may be explained by the fact that in the first exp. John Innes compost which was used as potting mixture was imperfectly sterilized and hence there was a possibility of some interactions due to other organisms. This is confirmed in the second exp. with unsterilized soil where no significant result was obtained in any observation. In addition, another factor may be the use of fewer nematodes in each inoculation compared with previous workers, e.g. Rhoades and Linford (1959) obtained increased weight of corn infected with Pythium arrhenomanes by adding 125,000 of A. avenae per 6 in. pot and Barker (1964) found increased growth of bean infected with R. solani by adding 100,000 of the same nematode per 5 in crock. No adverse effect of A. avenae was noticed on the growth of plants which is in agreement with the findings of Mankau and Mankau (1963), Southerland (1967) and Southerland and Fortin (1968).

Incidence of C. coccodes was checked significantly in the presence of A. avenae in both the experiments, this was also the case with R. solani in the second exp. This is in accord with the findings of Klink and Barker (1968) who obtained a decrease

in the incidence of Fusarium oxysporum f pisi on peas and that of R. solani on beans; and also of Barker (1964) in reducing the intensity of R. solani on beans, with the addition of A. avenae.

Extraction of greater number of nematodes in the first than the second exp. may be explained by the fact that, firstly, sugar-beet seeds used as inoculum medium in the first exp. increased the organic matter content of the soil and thus was responsible for supporting more worms since the nematodes received food from two sources - fungus and organic matter. This appears to be substantiated by the fact that the number of worms extracted from the sugarbeet seeds in the nematode + fungus treatment were significantly higher than where nematodes only were inoculated. Secondly, the plants in the second exp. were harvested earlier than the first.

Linford et al. (1938) found that the addition of organic matter is accompanied by an increase in the free-living, saprozoic, mycophagous and predaceous nematodes and A. avenae was found to be a dominant species. Fluctuation in the number of A. avenae according to different organic additives was recorded by Mankau (1962). Goodey (1935) and Thorne and Price (1935) recorded it to be a saprozoic nematode.

The number of A. avenae extracted from soil in the nematode + fungus treatment was significantly higher than the nematode inoculation in the first exp. but not in the second. This may be explained by the fact that in the second exp. after eating the fungus in the soil the nematode population decreased as the base of fungal inoculum was maize meal and sand which provided the soil

with practically no organic matter and therefore, the difference in their number between the nematode + fungus and nematode inoculated pots narrowed down. In the same exp. virtually no nematode entered the root in the nematode inoculated plants and so, the difference in their number between this treatment and nematode + fungus inoculation became significant as more nematodes were present in the latter treatment to feed on the fungi on root.

From the germination performance of seeds and the occurrence of disease, it appears that A. avenae can reduce the inoculum of both R. solani and C. coccodes in soil. Klink (1968) and Klink and Barker (1968) showed that A. avenae destroyed the fungal inoculum of F. solani f. phaseoli, F. solani f. pisi, R. solani and sclerotia of R. solani and a Sclerotium sp.

Attack of C. coccodes on tomato seedlings was unexpected as it is supposed to be a pathogen of old plants but laboratory test showed conclusively that this may be pathogenic on germinating seedlings.

GENERAL DISCUSSION AND CONCLUSIONS

In the associations between *Heterodera rostochiensis* and (1) *Rhizoctonia solani* & (2) *Colletotrichum coccodes* maximum growth reduction of tomato plants takes place if the nematode can enter into the roots earlier than the fungi. Incidence of disease and production of cysts are also increased in this treatment. The condition is, however, entirely reversed if the fungi can gain entrance first. In such cases the fungi hinder the development of *H. rostochiensis* as a result of which the adverse effect of potato cyst eelworm on plant growth is reduced. The fungi have been observed to have an adverse effect on the hatching of larvae from the cysts but not on their ability to penetrate the roots.

It has been observed from the histological studies that the giant cells formed by the nematodes appear to be attractive to the fungi for colonization. Rosindole reaction has shown the presence of indole derivative/s in the bodies of the nematodes which may be injected into the giant cells during the process of feeding and account for a causal factor of fungus attraction. On the other hand, in the tissues ramified by the fungus hyphae giant cells either cannot be formed or if the formation is initiated to some extent, further development is arrested. However, in the same root piece normal giant cells have been observed in the tissues not invaded by the fungus mycelium. Therefore, fewer cysts developed in the treatment where the fungus was followed by the nematode than where the nematode was followed by the fungus inoculation.

The effects of simultaneous inoculation lie between these

two treatments i.e. though the growth of the plants is decreased but is less than where the nematode is followed by the fungus inoculation and more than where the fungus is followed by the nematode. Similar is the case with the development of diseases by the fungi and production of cysts by the nematode. The reason appears to be that due to constant association of the fungus the action of H. rostochiensis cannot be as intense as where the nematode is followed by the fungus but is more favourable than where the fungus is followed by the nematode.

Though linear growth of R. solani has been found to be maximum at 27°C on P.D.A. and 25°C in soil (highest temperatures used) the rate of growth is decreased fairly quickly with time. The rate of growth in soil at 10°C shows a linear line whereas at 15° and 25°C quadratic curves. The rate of decrease is, however, significantly more at 25°C than at 15°C which is evident from the quadratic contrast. Many workers observed lower temperature for pathogenicity of R. solani than the optimum for its development in culture (Richards, 1923 and Walker, 1928). Besides, Leach (1947) has pointed out that damping-off due to R. solani depends not so much on the rate of growth either of the fungus or host but on the ratio of the emergence rate of host (Kotowski's coefficient of emergence) over the growth rate of the fungus which is inversely proportionate to the severity. It has also been found that R. solani can survive more efficiently at lower temperature than higher which may account for a factor responsible for its greater

attack in Scotland than in the south of England. Besides, a lower temperature for pathogenicity which again depends on the growth of the host plant may be another factor.

Variability in the rate of growth and cultural characters are noticed in different isolates of R. solani from potatoes and tomato. Here also, the pattern of growth rate is almost the same: best growth is obtained either at 20° or 27°C which is decreased fairly quickly with time.

Further, it seems that R. solani can secrete some substance which may stimulate the growth of tomato plants to some extent, especially at higher temperatures. In the experiment in Chapter I though no appreciable adverse effect of R. solani on the growth of plants has been noticed but in Chapter IV in sterilized soil the growth has been reduced considerably where the temperature of the glasshouse was lower (11·0° to 27·7°C, av. 20·0°C at day time and 4·4° to 15·0°C, av. 11·2°C at night) than the previous experiment. A close relation between temperature and pathogenicity of R. solani has been mentioned by Garrett (1956) who stated that this fungus can cause serious damage to roots of young cereals but with the advent of higher temperature the crop "grows away" from the disease.

The rate of multiplication of A. avenae is greater in R. solani culture than that of C. coccodes but no significant difference in any of the morphometric values of the nematode is observed in them. Greater number of eggs laid per female in R. solani than C. coccodes seems to be a factor for this

differential multiplication rate.

Significant control of R. solani and C. coccodes has been obtained with A. avenae in sterilized soil but not in unsterilized soil. All the previous workers who obtained control of root diseases used a very high number of nematodes. Considering these two facts i.e. presence of a very high number of worms and non-interference from other soil microorganisms, though the use of A. avenae seems to have limitation as a biological control agent against fungus diseases but its large multiplication in the presence of high organic matter in soil as has been occurred in the first experiment of the Chapter IV opens a new line of thinking to amend soil with organic matter along with A. avenae in further attempt to use it as a tool in controlling fungus diseases. From these observations, however, A. avenae can be considered a factor for occasional variable occurrence of R. solani and C. coccodes in glasshouse.

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APPENDICES

Statistical symbols used:

r	=	Correlation coefficient
S.S.	=	Sum of squares
M.S.	=	Mean square
D.F.	=	Degrees of freedom
F ratio ***	=	Significant at 0·1% level
" **	=	Significant at 1% level
" *	=	Significant at 5% level
C.D.	=	Critical difference
N.S.	=	Not significant
$\bar{m}$	=	mean

APPENDIX I.

Hatching of larvae of H. rostochiensis from cysts after different treatments with chemicals

Treatment	77°F						Room temperature (60° to 77°F)					
	Number in watch glass*			Total	Average per watch glass	Mortality ratio ($\frac{\text{Total}}{\text{Dead}}$)	Number in watch glass*			Total	Average per watch glass	Mortality ratio ($\frac{\text{Total}}{\text{Dead}}$)
	1	2	3				1	2	3			
0·5%$CuSO_4$ - 1 hour	309/108	630/100	622/100	1561/308	520·3/102·7	5·068	268/30	688/32	496/27	1452/89	484·0/29·7	16·315
0·5%$CuSO_4$ - 4 hours	92/22	265/40	362/71	719/133	239·7/44·3	5·406	1557/64	705/102	1198/91	3460/257	1153·0/85·7	13·463
0·5%$CuSO_4$ -14 hours	549/100	110/9	203/59	862/168	287·3/56	5·131	527/51	1015/70	1642/92	3184/213	1061·3/71·0	14·948
0·5%$CuSO_4$ -24 hours	163/60	385/66	528/81	1076/207	358·7/69·0	5·198	1080/78	472/63	1618/80	3170/221	1056·7/73·7	14·344
0·1%$CuSO_4$ - 8 days	8/3	13/5	2/2	23/10	7·7/3·3	2·300	33/12	53/17	34/14	120/43	40·0/14·3	2·791
0·01%$HgCl_3$ - 1½ min.	6/6	2/2	0/0	8/8	2·7/2·7	1·000	186/46	22/4	20/5	228/55	76·0/18·3	4·145
Control	358/98	298/80	503/14	1159/192	386·3/64·0	6·036	1864/57	802/72	1065/70	3731/199	1243·7/66·3	18·749

Number of the numerator denotes the number of total hatched out worms and that of denominator dead worms.

* Number in each watch glass is the total of 10 cysts.

Appendix II (a)

Correlation between the observed infection of the fungus on tomato roots and the corresponding number of colonies on P.D.A. plates.

With R. solani

x	y
5	3
4	2
7	8
3	2
3	3
4	2
5	3
7	5
6	4
6	3
50	35

$r = 0 \cdot 7699$

$y = -1 \cdot 25 + 0 \cdot 95x$

x = Observed infected root pieces per plate

y = Number of colonies corresponding to the infected root pieces.

Regression analysis:

Item	D.F.	S.S.	M.S.	F ratio
Linear	1	18·05	18·05	11·5988***
Error	8	12·45	1·5562	
Total	9	30·50		

Appendix II (b)

Correlation between the observed infection of the fungus on tomato roots and the corresponding number of colonies of P.D.A. plates

With C. coccodes

x	y
4	4
3	3
4	3
5	5
6	5
6	8
5	7
3	4
7	8
7	9
50	59

$r = 0{\cdot}8733$

$y = -0{\cdot}9 + 1{\cdot}3x$

x = Observed infected root pieces per plate

y = Number of colonies corresponding to the infected root pieces

Regression analysis:

Item	D.F.	S.S.	M.S.	F ratio
Linear	1	33·80	33·8000	25·5094***
Error	8	10·60	1·3250	
Total	9	44·4		

Appendix III (a)

Dry weight of shoot of tomato plant in different treatments of inoculation of *H. rostochiensis* and *R. solani* in house No. 1. (in g.)

	Control (1)	F (2)	F→N (3)	N→F (4)	N+F (5)	N+F+F (6)	N (7)
	1·21	1·60	1·73	0·29	0·37	0·54	0·40
	1·79	1·45	1·05	0·20	0·59	0·76	0·26
	1·59	1·48	1·45	0·22	0·46	0·72	0·20
	1·50	1·81	1·57	0·48	0·41	0·35	0·26
	0·85	1·40	1·34	0·66	0·56	0·63	0·21
	6·94	7·74	7·14	1·85	2·39	3·00	1·33
mean	1·388	1·548	1·428	0·370	4·78	0·600	0·266

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	6	9·4645	1·5774	37·8273 ***
Error	28	1·1676	0·0417	
Total	34	10·6321		

C.D. (P = 0·001) = 0·474

C.D. (P = 0·01) = 0·356

C.D. (P = 0·05) = 0·264

Abbreviations of the Appendices from III to V.

F = Fungus alone
F→N = Fungus first, then nematode
N→F = Nematode first, then fungus
N + F = Nematode and fungus combined **simultaneously**
N + F + F = Nematode and both the fungi combined **simultaneously**
N = Nematode alone.

Appendix III (b)

Dry weight of shoot of tomato plant in different treatments of inoculation of H. rostochiensis and R. solani in house No. 2. (in g.)

	Control (1)	F (2)	F→N (3)	N→F (4)	N + F (5)	N + F + F (6)	N (7)
	2·45	2·81	3·06	1·43	0·79	2·08	0·60
	1·83	2·02	2·26	0·45	2·03	1·02	0·93
	1·89	3·30	2·84	0·40	1·47	1·63	0·59
	1·83	2·39	2·09	1·13	1·70	1·84	0·61
	2·29	2·25	1·83	1·10	1·97	1·90	0·99
	10·29	12·77	12·08	4·51	7·96	8·47	3·72
$\bar{m}$	2·058	2·554	2·416	0·902	1·592	1·694	0·744

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	6	14·6603	2·4434	13·4326 ***
Error	28	5·0941	0·1819	
Total	34	19·7544		

C.D. (P = 0·001) = 0·988

C.D. (P = 0·01) = 0·743

C.D. (P = 0·05) = 0·551

Appendix III(c)

Dry weight of shoot of tomato plant in different treatments of inoculation of H. rostochiensis and C. coccodes in house No.1 (in g.)

	Control (1)	F (2)	F→N (3)	N→F (4)	N + F (5)	N + F + F(6)	N (7)
	1·21	0·65	1·22	0·21	0·32	0·54	0·40
	1·79	0·64	0·77	0·26	0·37	0·76	0·26
	1·59	0·63	1·28	0·29	0·48	0·72	0·20
	1·50	1·46	0·94	0·28	0·32	0·35	0·26
	0·85	1·22	0·80	0·20	0·61	0·63	0·21
	6·94	4·60	5·01	1·24	2·10	3·00	1·33
$\bar{m}$	1·388	0·920	1·002	0·248	0·420	0·600	0·266

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	6	5·5286	0·9214	17·000 ***
Error	28	1·5170	0·0542	
Total	34	7·0456		

C.D. (P = 0·001) = 0·540

C.D. (P = 0·01) = 0·406

C.D. (P = 0·05) = 0·301

Appendix III (d)

Dry weight of shoot of tomato plant in different treatments of inoculation of H. rostochiensis and C. coccodes in house No. 2 (in g.)

	Control (1)	F (2)	F→N (3)	N→F (4)	N + F (5)	N + F + F (6)	N (7)
	2·45	1·72	1·68	0·65	1·41	2·08	0·60
	1·83	1·61	1·99	0·70	0·73	1·02	0·93
	1·89	2·00	2·05	1·02	0·68	1·63	0·59
	1·83	1·69	1·59	0·60	1·05	1·84	0·61
	2·29	1·90	1·66	0·91	1·01	1·90	0·99
	10·29	8·92	8·97	3·88	4·88	8·47	3·72
$\bar{m}$	2·058	1·784	1·794	0·776	0·976	1·694	0·744

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	6	9·1075	1·5179	22·1268 ***
Error	28	1·9213	0·0686	
Total	34	11·0288		

C.D. (P = 0·001) = 0·610

C.D. (P = 0·01) = 0·459

C.D. (P = 0·05) = 0·340

Appendix IV (a)

Fresh weight of root of tomato plant in different treatments of inoculation of H. rostochiensis and R. solani in house No.1 (in g.)

	Control (1)	F (2)	F→N (3)	N→F (4)	N + F (5)	N + F + F (6)	N (7)
	2·01	2·32	2·90	0·36	1·01	1·36	0·83
	2·54	2·56	2·12	0·27	1·57	1·75	0·47
	2·26	2·21	2·30	0·38	1·07	1·84	0·36
	2·05	2·85	2·71	0·45	0·97	0·75	0·48
	1·87	2·09	2·13	0·87	1·73	1·41	0·35
	10·73	12·03	12·16	2·33	6·35	7·11	2·49
$\bar{m}$	2·146	2·406	2·432	0·466	1·270	1·422	0·498

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	6	35·465	5·911	58·5247 ***
Error	28	2·828	0·101	
Total	34	38·293		

C.D. (P = 0·001) = 0·738

C.D. (P = 0·01) = 0·555

C.D. (P = 0·05) = 0·412

Appendix IV (b)

Fresh weight of root of tomato plant in different treatments of inoculation of H. rostochiensis and R. solani in house No.2 (in g.)

	Control (1)	F (2)	F→N (3)	N→F (4)	N + F (5)	N + F + F(6)	N (7)
	4·12	3·15	4·04	3·09	1·66	3·92	0·95
	3·50	2·38	3·26	0·78	3·54	1·97	1·47
	2·22	4·70	3·52	0·65	2·87	2·53	0·77
	2·49	3·08	3·14	2·01	2·97	3·25	0·83
	2·36	3·19	2·77	1·56	3·03	3·24	1·57
	14·69	16·50	16·73	8·09	14·04	14·91	5·59
$\bar{m}$	2·938	3·300	3·346	1·618	2·808	2·982	1·118

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	6	22·5471	3·7578	6·9039 ***
Error	28	15·2409	0·5443	
Total	34	37·7880		

C.D. (P = 0·001) = 1·712

C.D. (P = 0·01) = 1·287

C.D. (P = 0·05) = 0·954

Appendix IV (c)

Fresh weight of root of tomato plant in different treatments of inoculation of H. rostochiensis and C. coccodes in house No.1 (in g.)

	Control (1)	F (2)	F→N (3)	N→F (4)	N + F (5)	N + F + F (6)	N (7)
	2·01	1·34	2·65	0·33	0·56	1·36	0·83
	2·54	2·26	1·64	0·48	0·57	1·75	0·47
	2·26	1·35	2·25	0·51	0·80	1·84	0·36
	2·05	2·47	2·22	0·64	0·49	0·75	0·48
	1·87	2·27	1·92	0·32	0·90	1·41	0·35
	10·73	9·69	10·68	2·26	3·32	7·11	2·49
$\bar{m}$	2·146	1·938	2·136	0·452	0·664	1·422	0·498

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	6	17·9990	2·9998	26·222 ***
Error	28	3·2038	0·1144	
Total	34	21·2028		

C.D. (P = 0·001) = 0·786

C.D. (P = 0·01) = 0·591

C.D. (P = 0·05) = 438

Appendix IV (d)

Fresh weight of root of tomato plant in different treatments of inoculation of H. rostochiensis and C. coccodes in house No.2 (in g.)

	Control (1)	F (2)	F→N (3)	N→F (4)	N + F (5)	N + F + F (6)	N (7)
	4·12	3·17	2·65	1·01	2·52	3·92	0·95
	3·50	2·17	2·98	2·20	1·13	1·97	1·47
	2·22	3·33	3·54	1·96	0·93	2·53	0·77
	2·49	2·43	2·76	1·16	1·94	3·25	0·83
	2·36	2·60	3·22	1·51	1·71	3·24	1·57
	14·69	13·70	15·15	6·84	8·23	14·91	5·59
$\bar{m}$	2·938	2·740	3·030	1·368	1·646	2·982	1·118

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	6	21·4054	3·5675	10·7444 ***
Error	28	9·2637	0·3308	
Total	34	30·6691		

C.D. (P = 0·001) = 1·337

C.D. (P = 0·01) = 1·006

C.D. (P = 0·05) = 0·745

Appendix V (a)

Number of cysts of *H. rostochiensis* and disease incidence caused by *R. solani* in different treatments of fungus and nematode inoculation in house No.1 (cysts per g. of root; disease incidence in degrees).

N (1)		F (2)		F⟶N (3)		N⟶F (4)		N + F (5)		N + F + F (6)	
Cysts	Disease	Cysts	Disease	Cysts	Disease	Cysts	Disease	Cysts	Disease	Cysts	Disease
179	-	-	24·96	11	30·14	313	68·54	116	59·06	22	-
280	-	-	26·20	22	34·26	160	62·70	63	44·30	14	-
246	-	-	21·19	11	25·31	227	60·35	128	49·18	17	-
242	-	-	23·20	6	34·22	188	64·48	55	33·92	33	-
367	-	-	25·38	8	42·34	187	33·32	59	66·32	28	-
1314			120·93	58	166·27	1075	289·39	421	252·78	114	
$\overline{m}$ 262·8			24·186	11·6	33·254	215·0	57·878	84·2	50·556	22·8	

Appendix V (a) (contd.)

Analysis of variance for cysts:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	4	247471·040	61867·760	23·8356 ***
Error	20	51912·000	2595·600	
Total	24	299383·040		

C.D. (P = 0·001) = 124·047 C.D. (P = 0·01) = 91·666 C.D. (P = 0·05) = 67·211

Analysis of variance for disease:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	3590·2856	1196·7619	11·8898 ***
Error	16	1601·4657	100·6541	
Total	19	5191·7513		

C.D. (P = 0·001) = 25·475 C.D. (P = 0·01) = 18·534 C.D. (P = 0·05) = 13·451

Appendix V (b)

Number of cysts of H. rostochiensis and disease incidence caused by R. solani in different treatments of fungus and nematode inoculation in house No.2 (cysts per g. of root; disease incidence in degrees)

N (1)		F (2)		F⟶N (3)		N⟶F (4)		N + F (5)		N + F + F (6)	
Cysts	Disease	Cysts	Disease	Cysts	Disease	Cysts	Disease	Cysts	Disease	Cysts	Disease
144	-	-	26·36	8	23·44	145	43·48	56	43·42	12	-
103	-	-	24·40	10	40·28	131	77·38	43	39·08	22	-
132	-	-	22·00	11	26·84	129	66·64	21	30·35	18	-
191	-	-	25·80	7	37·62	98	43·90	36	33·56	14	-
137	-	-	27·51	10	26·60	55	46·46	16	33·62	22	-
707			126·07	46	154·78	558	277·86	172	180·03	88	-
$\overline{m}$ 141·4			25·214	9·2	30·956	111·6	55·572	34·4	36·006	17·6	

Appendix V (b) (Contd.)

Analysis of variance for cysts:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	4	71609·76	17902·44	35·1455 ***
Error	20	10187·60	509·38	
Total	24	81797·36		

C.D. (P = 0·001) = 54·939. C.D. (P = 0·01) = 40·598. C.D. (P = 0·05) = 29·767.

Analysis of variance for disease:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	2606·6552	868·8851	10·5862 ***
Error	16	1313·2365	82·0773	
Total	19	3919·8917		

C.D. (P = 0·001) = 23·006. C.D. (P = 0·01 = 16·737. C.D. (P = 0·05) = 12·148.

Appendix V (c)

Number of cysts of *H. rostochiensis* and disease incidence caused by *C. coccodes* in different treatments of fungus and nematode inoculation in house No.1 (cysts per g. of root; disease incidence in degrees)

N (1)		F (2)		F → N (3)		N → F (4)		N + F (5)		N + F + F (6)	
Cysts	Disease	Cysts	Disease	Cysts	Disease	Cysts	Disease	Cysts	Disease	Cysts	Disease
179	-	-	48·15	3	28·76	170	69·14	46	34·54	22	-
280	-	-	28·28	5	51·28	179	62·63	74	73·83	14	-
246	-	-	35·58	11	28·00	162	48·52	109	68·54	17	-
242	-	-	33·38	7	36·18	213	71·78	103	40·70	33	-
367	-	-	35·64	8	26·67	248	70·42	36	27·70	28	-
1314			181·03	34	170·89	972	321·49	368	244·31	114	
$\overline{m}$ 262·8			36·206	6·8	34·178	194·4	64·298	73·6	48·862	22·8	

Appendix V (c) (contd.)

Analysis of variance for cysts:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	4	250143·04	62535·760	41·6394 ***
Error	20	30036·80	1501·840	
Total	24	280179·84		

C.D. (P = 0·001) = 94·363. C.D. (P = 0·01) = 69·731. C.D. (P = 0·05) = 51·128.

Analysis of variance for disease:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	2893·1900	964·3966	5·4853 **
Error	16	2813·0425	175·8151	
Total	19	5706·2325		

C.D. (P = 0·01) = 24·504. C.D. (P = 0·05) = 17·778.

Appendix V (d)

Number of cysts of *H. rostochiensis* and disease incidence caused by *C. coccodes* in different treatments of fungus and nematode inoculation in House No.2 (cysts per g. of root; disease incidence in degrees)

N (1)		F (2)		F → N (3)		N → F (4)		N + F (5)		N + F + F (6)	
Cysts	Disease	Cysts	Disease	Cysts	Disease	Cysts	Disease	Cysts	Disease	Cysts	Disease
144	-	-	36·54	10	31·84	114	64·04	69	36·06	12	-
103	-	-	37·68	7	31·72	98	45·18	47	26·81	22	-
132	-	-	31·36	11	38·45	100	78·50	61	42·05	18	-
191	-	-	31·06	5	34·16	133	90·00	20	27·30	14	-
137	-	-	27·58	11	43·66	153	52·44	21	36·42	22	-
707			164·22	44	179·83	598	330·16	218	168·64	88	
$\overline{m}$ 141·4			32·844	8·8	35·966	119·8	66·032	43·6	33·728	17·6	

Appendix V (d) (Contd.)

Analysis of variance for cysts:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	4	71609·76	17902·44	35·1455 ***
Error	20	10187·60	509·38	
Total	24	81797·36		

C.D. (P = 0·001) = 49·7805. C.D. (P = 0·01) = 36·7858. C.D. (P = 0·05) = 26·9719.

Analysis of variance for disease:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	3830·6162	1276·8721	12·0291 ***
Error	16	1698·3752	106·1484	
Total	19	5528·9914		

C.D. (P = 0·001) = 26·162. C.D. (P = 0·01) = 19·033. C.D. (P = 0·05) = 13·814.

Appendix VI.

Effect of R. solani and C. coccodes exudate on the hatching of H. rostochiensis larvae from cysts (per 6 cysts).

With R. solani

Root diffusate + water	Fungus exudate + root diffusate	Fungus exudate + water
219	186	22
395	135	110
97	115	12
200	76	88
271	30	40
1182	542	272
$\bar{m}$ 236·4	108·4	54·4

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	87373·33	43686·665	7·6357 **
Error	12	68655·60	5721·300	
Total	14	156028·93		

C.D. (P = 0·01) = 146·1512.

C.D. (P = 0·05) = 104·2434.

Appendix VI (Contd.)

With C. coccodes.

Root diffusate + water	Fungus exudate + root diffusate	Fungus exudate + water
219	20	0
395	44	4
97	6	32
200	35	18
271	30	29
1182	135	83
$\bar{m}$ 236·4	27·0	16·6

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	153780·93	76890·465	18·7956 ***
Error	12	49090·40	4090·866	
Total	14	202871·33		

C.D. (P = 0·001) = 174·6631

C.D. (P = 0·01) = 123·5747

C.D. (P = 0·05) = 88·1405

Appendix VII

Invasion of tomato root by H. rostochiensis larvae in the presence of R. solani and C. coccodes (larvae per g. of root)

With R. solani

Control at 15·5°C	Control at 26·6°C	Fungus first, then nematode at 15·5°C	Fungus first, then nematode at 26·6°C
243	111	292	188
218	93	253	190
397	288	161	248
206	268	299	250
163	252	211	213
1227	1012	1216	1089
$\bar{m}$ 245·4	202·4	243·2	217·8

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	6453·2	2151·0666	0·4121 (N.S.)
Error	16	83512·0	5219·5000	
Total	19	89965·2		

Appendix VII (Contd.)

With C. coccodes

Control at 15·5°C	Control at 26·6°C	Fungus first, then nematode at 15·5°C	Fungus first, then nematode at 26·6°C
243	111	268	249
218	93	157	95
397	288	244	258
206	268	161	295
163	252	198	84
1227	1012	1028	981
$\bar{m}$ 245·4	202·4	205·6	196·2

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	7488·4	2496·1333	0·3459 (N.S.)
Error	16	115470·4	7216·9000	
Total	19			

Appendix VIII

Growth rate of *R. solani* at different temperatures on P.D.A. plates in different dates (in mm. per hour)

No. of Plate	After 2 days			After 3 days			After 4 days			After 5 days		
	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C
1	0·66	0·37	0·04	0·75	0·54	0·33	0·79	0·67	0·42	0·75	0·71	0·42
2	0·71	0·33	0·08	0·75	0·58	0·33	0·83	0·75	0·46	0·71	0·79	0·46
3	0·50	0·37	0·04	0·54	0·54	0·37	0·58	0·67	0·42	0·71	0·75	0·50
4	0·58	0·37	0·04	0·67	0·54	0·33	0·83	0·67	0·37	0·66	0·62	0·50
	2·45	1·44	0·20	2·71	2·20	1·36	3·03	2·76	1·67	2·83	2·87	1·88
$\bar{m}$	0·6125	0·3600	0·0500	0·6775	0·5500	0·3400	0·7575	0·6900	0·4175	0·7075	0·7175	0·4700

Analysis of variance after 2 days:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·6350	0·3175	102·419 ***
Error	9	0·0279	0·0031	
Total	11	0·6629		

C.D. (P = 0·001) = 0·1864.

Appendix VIII (contd.)

Analysis of variance after 3 days:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·2323	0·11615	32·773 ***
Error	9	0·0319	0·00354	
Total	11	0·2642		

C.D. (P=0·001) = 0·2008 C.D. (P=0·01) = 0·1365 C.D. (P=0·05) = 0·0950

Analysis of variance after 4 days:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·2592	0·12960	22·461 ***
Error	9	0·0520	0·00577	
Total	11	0·3112		

C.D. (P=0·001) = 0·2534 C.D. (P=0·01) = 0·1722 C.D. (P=0·05) = 0·1199

Analysis of variance after 5 days:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·1570	0·07850	28·956 ***
Error	9	0·0244	0·00271	
Total	11	0·1814		

C.D. (P=0·001) = 0·1769 C.D. (P=0·01) = 0·1225 C.D. (P=0·05) = 0·0837

Appendix VIII (Contd.)

Mean growth rate over days

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·288001	0·144001	64·257 ***
Error	9	0·020166	0·002241	

27°C	20°C	13°C	
0·6888	0·5794	0·3194	(All different)

C.D. (P = 0·05) = 0·0758

C.D. (P = 0·01) = 0·1088

C.D. (P = 0·001) = 0·1601

Appendix VIII (Contd.)

Linear contrast over days of the change in growth rate

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·111983	0·055991	22·816 ***
Error	9	0·022084	0·002454	
Total	11	0·134066		

27°C	20°C	13°C
0·0816	0·2711	0·2991

C.D. (P=0·05) = 0·0793 C.D. (P=0·01) = 0·1139 C.D. (P=0·001) = 0·1676

Quadratic contrast over days of the change in growth rate

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·007629	0·003815	1·592 (N.S.)
Error	9	0·021562	0·002396	
Total	11	0·029192		

27°C	20°C	13°C
-0·0575	-0·0813	-0·1187

Appendix IX

Growth rate of different isolates of R. solani at different temperatures in different dates on P.D.A. plates (in mm. per hour)

After 2 days

No. of Plate	Isolate																				
	P1			P2			P3			P4			P5			P6			T		
	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C
1	0·44	0·33	0·08	0·50	0·50	0·00	0·71	0·71	0·42	0·44	0·56	0·21	0·33	0·44	0·19	0·48	0·52	0·10	0·54	0·46	0·23
2	0·39	0·35	0·14	0·64	0·50	0·04	0·77	0·75	0·44	0·44	0·62	0·29	0·42	0·35	0·17	0·48	0·54	0·04	0·54	0·46	0·33
3	0·42	0·44	0·04	0·50	0·47	0·04	0·77	0·69	0·29	0·54	0·46	0·21	0·46	0·46	0·17	0·50	0·52	0·02	0·54	0·58	0·14
4	0·47	0·23	0·04	0·47	0·33	0·02	0·64	0·64	0·19	0·42	0·56	0·23	0·39	0·31	0·14	0·56	0·48	0·04	0·71	0·37	0·25
	$\bar{m}$ = 0·4300	$\bar{m}$=0·3375	$\bar{m}$=0·0750	$\bar{m}$=0·5275	$\bar{m}$=0·4500	$\bar{m}$=0·0250	$\bar{m}$=0·7075	$\bar{m}$=0·6975	$\bar{m}$=0·3350	$\bar{m}$=0·4600	$\bar{m}$=0·5500	$\bar{m}$=0·2350	$\bar{m}$=0·4000	$\bar{m}$=0·3900	$\bar{m}$=0·1675	$\bar{m}$=0·5050	$\bar{m}$=0·5150	$\bar{m}$=0·0500	$\bar{m}$=0·5825	$\bar{m}$=0·4675	$\bar{m}$=0·2375

		I							
		P1	P2	P3	P4	P5	P6	T	
Temp	27°C	1·72	2·11	2·83	1·84	1·60	2·02	2·33	14·45
	20°C	1·35	1·80	2·79	2·20	1·56	2·06	1·87	13·63
	13°C	0·30	0·10	1·34	0·94	0·67	0·20	0·95	4·50
		3·37	4·01	6·96	4·98	3·83	4·28	5·15	32·58

I = Isolate
Temp = Temperature

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio	
I	6	0·7126	0·1188	29·7000 ***	C.D. (P = 0·001) = 0·1555)
Temp	2	2·1789	1·0894	272·3500 ***	C.D. (P = 0·01) = 0·1196) I x Temp
I x Temp	12	0·1983	0·0165	4·1250 ***	C.D. (P = 0·05) = 0·0899)
Error	63	0·2510	0·0040		
Total	83	3·3408			

Appendix IX (Contd.)

After 3 days

No. of Plates	Isolate																				
	P1			P2			P3			P4			P5			P6			T		
	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C
1	0·50	0·62	0·42	0·75	0·71	0·21	0·83	0·83	0·46	0·42	0·58	0·19	0·56	0·64	0·23	0·67	0·62	0·17	0·60	0·81	0·29
2	0·66	0·77	0·42	0·85	0·75	0·29	0·87	0·73	0·50	0·42	0·60	0·23	0·58	0·50	0·19	0·64	0·62	0·17	0·83	0·85	0·33
3	0·39	0·58	0·23	0·89	0·75	0·27	0·81	0·87	0·50	0·52	0·56	0·23	0·52	0·71	0·29	0·62	0·60	0·17	0·81	0·73	0·42
4	0·56	0·46	0·37	0·89	0·58	0·27	0·96	0·77	0·44	0·52	0·60	0·21	0·52	0·79	0·25	0·69	0·60	0·19	0·81	0·70	0·48
	$\bar{m}$ = 0·5275	$\bar{m}$ = 0·6075	$\bar{m}$ = 0·3600	$\bar{m}$ = 0·8400	$\bar{m}$ = 0·6975	$\bar{m}$ = 0·2600	$\bar{m}$ = 0·8675	$\bar{m}$ = 0·8000	$\bar{m}$ = 0·4750	$\bar{m}$ = 0·4700	$\bar{m}$ = 0·5850	$\bar{m}$ = 0·2150	$\bar{m}$ = 0·5450	$\bar{m}$ = 0·6600	$\bar{m}$ = 0·2400	$\bar{m}$ = 0·6550	$\bar{m}$ = 0·6100	$\bar{m}$ = 0·1750	$\bar{m}$ = 0·7625	$\bar{m}$ = 0·7475	$\bar{m}$ = 0·3800

		I						
		P1	P2	P3	P4	P5	P6	T
Temp	27°C	2·11	3·38	3·47	1·88	2·18	2·62	3·05	18·69
	20°C	2·43	2·79	3·20	2·34	2·64	2·44	2·99	18·83
	13°C	1·44	1·04	1·90	0·86	0·96	0·70	1·52	8·42
		5·98	7·21	8·57	5·08	5·78	5·76	7·56	45·94

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio	
I	6	0·7697	0·1283	26·1837 ***	C.D. (P = 0·001) = 0·1693)
Temp	2	2·5460	1·2730	259·7959 ***	C.D. (P = 0·01) = 0·1302) I x Temp
I x Temp	12	0·2758	0·0230	4·6939 ***	C.D. (P = 0·05) = 0·0979)
Error	63	0·3087	0·0049		
Total	83	3·9002			

Appendix IX (contd.)

After 4 days

No. of Plates	Isolate																			
	P1			P2			P3			P4			P5			P6			T		
	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C
1	0·37	0·60	0·33	0·77	0·96	0·44	0·75	0·77	0·42	0·73	0·77	0·33	0·54	0·67	0·27	0·69	0·67	0·33	0·71	0·77	0·42
2	0·69	0·73	0·35	0·79	0·94	0·42	0·77	0·85	0·46	0·60	0·79	0·33	0·52	0·75	0·27	0·71	0·64	0·39	0·67	0·77	0·37
3	0·60	0·56	0·33	0·81	0·89	0·33	0·83	0·71	0·44	0·69	0·67	0·42	0·52	0·64	0·21	0·60	0·67	0·37	0·73	0·73	0·42
4	0·47	0·46	0·37	0·77	0·62	0·39	0·50	0·75	0·37	0·71	0·77	0·33	0·48	0·39	0·21	0·69	0·71	0·37	0·79	0·71	0·39
	$\bar{m}$ = 0·5325	$\bar{m}$ = 0·5875	$\bar{m}$ = 0·3450	$\bar{m}$ = 0·7850	$\bar{m}$ = 0·8525	$\bar{m}$ = 0·3950	$\bar{m}$ = 0·7125	$\bar{m}$ = 0·7700	$\bar{m}$ = 0·4225	$\bar{m}$ = 0·6825	$\bar{m}$ = 0·7750	$\bar{m}$ = 0·3550	$\bar{m}$ = 0·5150	$\bar{m}$ = 0·6125	$\bar{m}$ = 0·2400	$\bar{m}$ = 0·6725	$\bar{m}$ = 0·6725	$\bar{m}$ = 0·3650	$\bar{m}$ = 0·7250	$\bar{m}$ = 0·7450	$\bar{m}$ = 0·4000

	I							
	P1	P2	P3	P4	P5	P6	T	
27°C	2·13	3·14	2·85	2·73	2·06	2·69	2·90	18·50
Temp 20°C	2·35	3·41	3·08	3·10	2·45	2·69	2·98	20·06
13°C	1·38	1·58	1·69	1·42	0·96	1·46	1·60	10·09
	5·86	8·13	7·62	7·25	5·47	6·84	7·48	48·65

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio	
I	6	0·4669	0·0778	13·1864 ***	C.D. (P = 0·001) = 0·1866)
Temp	2	2·0543	1·0271	174·0847 ***	C.D. (P = 0·01) = 0·1435) I x Temp
I x temp	12	0·0807	0·0067	1·1356 (N.S.)	C.D. (P = 0·05) = 0·1079)
Error	63	0·3705	0·0059		
Total	83	2·9724			

Appendix IX (Contd.)

After 5 days

No. of Plates	Isolate																	
	P1			P2			P4			P5			P6			T		
	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C	27°C	20°C	13°C
1	0·37	0·39	0·27	0·46	0·71	0·12	0·75	0·69	0·48	0·52	0·52	0·39	0·73	0·69	0·37	0·62	0·67	0·37
2	0·56	0·37	0·27	0·39	0·73	0·14	0·83	0·73	0·44	0·52	0·66	0·35	0·62	0·69	0·48	0·58	0·75	0·33
3	0·60	0·44	0·33	0·60	0·42	0·29	0·75	0·73	0·48	0·48	0·54	0·35	0·64	0·64	0·37	0·56	0·71	0·37
4	0·46	0·39	0·37	0·60	0·73	0·42	0·75	0·75	0·39	0·48	0·62	0·33	0·67	0·75	0·27	0·64	0·71	0·33
	$\bar{m}$ = 0·4975	$\bar{m}$ = 0·3975	$\bar{m}$ = 0·3100	$\bar{m}$ = 0·5125	$\bar{m}$ = 0·6475	$\bar{m}$ = 0·2425	$\bar{m}$ = 0·7700	$\bar{m}$ = 0·7250	$\bar{m}$ = 0·4475	$\bar{m}$ = 0·5000	$\bar{m}$ = 0·5850	$\bar{m}$ = 0·3550	$\bar{m}$ = 0·6650	$\bar{m}$ = 0·6925	$\bar{m}$ = 0·3725	$\bar{m}$ = 0·6000	$\bar{m}$ = 0·7100	$\bar{m}$ = 0·3450

		I					
		P1	P2	P4	P5	P6	T
Temp	27°C	1·99	2·05	3·08	2·00	2·66	2·40	14·18
	20°C	1·59	2·59	2·90	2·34	2·77	2·84	15·03
	13°C	1·24	0·97	1·79	1·42	1·49	1·38	8·29
		4·82	5·61	7·77	5·76	6·92	6·62	37·50

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio	
I	5	0·4659	0·0932	17·2592 ***	C.D. (P = 0·001) = 0·1813)
Temp	2	1·1228	0·5614	103·9629 ***	C.D. (P = 0·01) = 0·1390) I x Temp
I x Temp	10	0·1720	0·0172	3·1852 **	C.D. (P = 0·05) = 0·1043)
Error	54	0·2903	0·0054		
Total	71	2·0510			

Appendix IX (Contd.)

Mean growth rate over days

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
I	6	0·425513	0·070919	45·461 ***
Temp.	2	2·024957	1·012478	649·024 ***
I x Temp.	12	0·095816	0·007985	5·118 ***
Error	63	0·098263	0·001560	
Total	83	2·644549		

I x Temp. table

	27°C	20°C	13°C	Mean
P1	0·4969	0·4825	0·2725	0·4173
P2	0·6675	0·6619	0·2306	0·5200
P3	0·7675	0·7559	0·4108	0·6447
P4	0·5956	0·6588	0·3125	0·5223
P5	0·4900	0·5619	0·2506	0·4342
P6	0·6244	0·6225	0·2406	0·4958
T	0·6675	0·6738	0·3419	0·5610
Mean	0·6156	0·6310	0·2942	0·5136

C.D. (P = 0·05) (I = 0·0322
(Temp. = 0·0211
(I x Temp. = 0·0558

Appendix IX (Contd.)

Linear contrast over days in the change in growth rate

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
I	6	0·037431	0·006239	5·4632 ***
Temp	2	0·022995	0·011497	10·0674 ***
IxTemp	12	0·035656	0·002971	2·6016 **
Error	63	0·071915	0·001142	
Total	83	0·167998		

I x Temp. table

	27°C	20°C	13°C	Mean
P1	0·0208	0·0160	0·0690	0·0353
P2	-0·0105	0·0748	0·0788	0·0477
P3	-0·0050	0·0363	0·0438	0·0250
P4	0·1143	0·0715	0·0775	0·0878
P5	0·0270	0·0537	0·0563	0·0457
P6	0·0498	0·0595	0·1158	0·0750
T	0·0015	0·0700	0·0358	0·0358
Mean	0·0283	0·0545	0·0681	0·0503

C.D. (P = 0·05)

(I = 0·0276
(Temp. = 0·0181
(I x Temp. = 0·0478

Appendix IX (Contd.)

Quadratic contrast over days in the change in growth rate

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
I	6	0·145852	0·024309	8·6294 ***
Temp	2	0·011095	0·005547	1·9691 (N.S.)
I x Temp	12	0·046253	0·003854	1·3681 (N.S.)
Error	63	0·077442	0·002817	
Total	83	0·380642		

I x Temp. table

	27°C	20°C	13°C	Mean
P1	-0·0331	-0·1150	-0·0800	-0·0760
P2	-0·1475	-0·1131	-0·0969	-0·1192
P3	-0·1500	-0·0663	-0·0963	-0·1042
P4	0·0194	-0·0212	0·0287	0·0090
P5	-0·0400	-0·0744	0·0106	-0·0346
P6	-0·0394	-0·0187	-0·0294	-0·0292
T	-0·0762	-0·0850	-0·0481	-0·0698
Mean	-0·0667	-0·0705	-0·0445	-0·0606

C.D. (P = 0·05)

(I = 0·0433
(
(Temp. = 0·0284
(
(I x Temp. = 0·0750

Appendix X.

Growth rate of R. solani at different temperatures in soil in vitro (in mm. per hour)

At 25°C

x	y	x	y	x	y
2	0·31	4	0·64	8	0·87
2	0·29	4	0·62	8	0·89
2	0·27	6	0·69	8	1·02
2	0·25	6	0·73	8	0·98
2	0·27	6	0·64	10	0·64
2	0·31	6	0·64	10	0·85
4	0·62	6	0·71	10	0·62
4	0·64	6	0·69	10	0·62
4	0·66	8	0·81	10	0·75
4	0·62	8	0·83	10	0·77

x = Days

y = Growth rate

$$y = -0·1517 + 0·2523x - 0·0164x^2$$

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	4	1·2083	0·3021	81·6486 ***
Linear)	1	0·7482	0·7482	202·2162 ***
Quadratic)	1	0·3601	0·3601	97·3243 ***
Residual)	2	0·1000	0·0500	13·5135 ***
Error	25	0·0917	0·0037	

Table X (contd.)

At 15°C

x	y	x	y	x	y
2	0·04	6	0·46	10	0·52
2	0·02	6	0·48	10	0·48
2	0·04	6	0·44	12	0·50
2	0·04	6	0·46	12	0·46
2	0·04	8	0·54	12	0·42
2	0·02	8	0·50	12	0·46
4	0·06	8	0·50	12	0·89
4	0·06	8	0·50	12	0·50
4	0·10	8	0·46	14	0·64
4	0·10	8	0·66	14	0·44
4	0·10	10	0·39	14	1·08
4	0·08	10	0·46	14	0·42
6	0·35	10	0·75	14	1·02
6	0·39	10	0·48	14	0·33

$$y = -0{\cdot}2250 + 0{\cdot}1222\ x\ -0{\cdot}0045x^2$$

Analysis of Variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	6	2·0917	0·3486	15·425 ***
Linear)	1	1·7507	1·7507	77·465 ***
Quadratic)	1	0·1596	0·1596	7·062 *
Residual)	4	0·1814	0·0453	2·004 (N.S.)
Error	35	0·7897	0·0226	

Appendix X (contd.)

At 10°C

x	y	x	y	x	y
2	0·04	6	0·23	10	0·25
2	0·02	6	0·12	10	0·25
2	0·02	6	0·12	12	0·31
2	0·02	6	0·12	12	0·27
2	0·02	8	0·23	12	0·25
2	0·02	8	0·31	12	0·25
4	0·04	8	0·31	12	0·19
4	0·06	8	0·23	12	0·25
4	0·02	8	0·31	14	0·29
4	0·04	8	0·27	14	0·33
4	0·04	10	0·27	14	0·31
4	0·02	10	0·23	14	0·31
6	0·25	10	0·25	14	0·33
6	0·19	10	0·27	14	0·33

$$y = -0·0090 + 0·0249x$$

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Linear	1	0·4168	0·4168	143·7241 ***
Error	40	0·1195	0·0029	
Total	41	0·5363		

Appendix X (contd.)

Linear contrast over days in the change in growth rate (up to 10 days)

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·013295	0·006648	8·645 **
Error	15	0·011537	0·000769	
Total	17	0·024832		

25°C	15°C	10°C
0·1117	0·1021	0·0498

C.D.(P=0·05) = 0·0341 C.D.(P=0·01) = 0·0472 C.D.(P=0·001)=0·0652

Quadratic contrast over days in the change in growth rate (up to 10 days)

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·011319	0·005659	32·901 ***
Error	15	0·002580	0·000172	
Total	17	0·013898		

25°C	15°C	10°C
-0·0655	-0·0178	-0·0081

C.D.(P=0·05) = 0·0161 C.D.(P=0·01) = 0·0223 C.D.(P=0·001) = 0·0308

Appendix XI (a)

Growth rate of R. solani at different temperature treatments of 25°C (in mm. per hour).

After 2 days

1	3	4
0·31	0·04	0·08
0·29	0·08	0·00
0·27	0·04	0·08
0·25	0·04	0·08
0·27	0·08	0·08
0·31	0·04	0·08
1·70	0·32	0·40
$\bar{m}$ 0·2833	0·0533	0·0666

1 - Continuous 25°C

2 - First 6 days at 10°C then at 25°C.

3 - 10°C with 6 hours exposure at 25°C.

4 - 10°C with 3 hours exposure at 25°C.

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·1999	0·0999	142·714***
Error	15	0·0106	0·0007	
Total	17			

C.D. (P = 0·001) = 0·0610

C.D. (P = 0·01) = 0·0442

C.D. (P = 0·05) = 0·0319

Appendix XI (a) (Contd.)

	After 4 days		
	1	3	4
	0·62	0·58	0·42
	0·64	0·50	0·42
	0·66	0·33	0·33
	0·62	0·50	0·66
	0·64	0·58	0·66
	0·62	0·50	0·50
	3·80	2·99	2·99
$\overline{m}$	0·6333	0·4983	0·4983

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·0728	0·0364	4·044 *
Error	15	0·1360	0·0090	
Total	17	0·2088		

C.D. (P = 0·05) = 0·1065

Appendix XI (a) (Contd.)

After 6 days

	1	3	4
	0·69	0·66	0·50
	0·73	0·50	0·33
	0·64	0·42	0·50
	0·64	0·50	0·50
	0·71	0·50	0·50
	0·69	0·66	0·33
	4·10	3·24	2·66
$\bar{m}$	0·6833	0·5400	0·4433

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·1749	0·0874	14·0967 ***
Error	15	0·0934	0·0062	
Total	17	0·2683		

C.D. (P = 0·001) = 0·1832

C.D. (P = 0·01) = 0·1326

C.D. (P = 0·05) = 0·0958

Appendix XI (a) (Contd.)

After 8 days

	1	2	3	4
	0·81	0·58	0·58	0·66
	0·83	0·62	0·50	0·33
	0·87	0·71	0·50	0·33
	0·89	0·71	0·42	0·50
	1·02	0·58	0·66	0·50
	0·98	0·71	0·50	0·50
	5·40	3·91	3·16	2·82
$\overline{m}$	0·9000	0·6516	0·5266	0·4700

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	0·6566	0·2188	26·0476 ***
Error	20	0·1685	0·0084	
Total	23	0·8251		

C.D. (P = 0·001) = 0·2040

C.D. (P = 0·01) = 0·1507

C.D. (P = 0·05) = 0·1105

Appendix XI (a) (Contd.)

After 10 days

	1	2	3	4
	0·64	0·71	0·62	0·50
	0·85	0·77	0·58	0·50
	0·62	1·06	0·58	0·33
	0·62	0·75	0·66	0·50
	0·75	0·71	0·41	0·50
	0·77	0·77	0·45	0·33
	4·25	4·77	3·30	2·66
$\overline{m}$	0·7083	0·7950	0·5500	0·4433

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	0·4467	0·1489	13·5363 ***
Error	20	0·2209	0·0110	
Total	23	0·6676		

C.D. (P = 0·001) = 0·2310

C.D. (P = 0·01) = 0·1707

C.D. (P = 0·05) = 0·1251

Appendix XI (a) (Contd.)

After 12 days

2	3	4
1·23	0·75	0·50
0·73	0·58	0·50
1·06	0·66	0·33
1·16	0·50	0·50
0·98	0·62	0·66
1·19	0·62	0·50
6·35	3·73	2·99
$\bar{m}$ 1·0583	0·6216	0·4983

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	1·0390	0·5195	30·0173 ***
Error	15	0·2601	0·0173	
Total	17	1·2991		

C.D. (P = 0·001) = 0·3095

C.D. (P = 0·01) = 0·2239

C.D. (P = 0·05) = 0·1619

Appendix XI (a) (Contd.)

After 14 days

	2	3	4
	0·64	0·50	0·33
	0·69	0·54	0·50
	0·52	0·58	0·50
	0·52	0·54	0·50
	0·64	0·58	0·50
	0·62	0·58	0·50
	3·63	3·32	2·83
$\bar{m}$	0·6050	0·5533	0·4716

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·0541	0·0270	7·7142 **
Error	15	0·0539	0·0035	
Total	17	0·1080		

C.D. (P = 0·01) = 0·1001

C.D. (P = 0·05) = 0·0724

Appendix XI (b)

Growth rate of R. solani at different temperature treatments of 10°C (in mm. per hour)

After 2 days

	1	2	3	4
	0·04	0·04	0·04	0·02
	0·02	0·02	0·08	0·03
	0·02	0·04	0·04	0·05
	0·02	0·02	0·04	0·03
	0·02	0·04	0·08	0·05
	0·02	0·02	0·04	0·03
	0·14	0·18	0·32	0·21
$\bar{m}$	0·0233	0·0300	0·0522	0·0350

1- Continuous 10°C

2- First 6 days at 10°C, then at 25°C

3- 10°C with 6 hours exposure at 25°C

4- 10°C with 3 hours exposure at 25°C

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	0·0028	0·0009	4·500 **
Error	20	0·0040	0·0002	
Total	23			

C.D. (P = 0·01) = 0·0227

C.D. (P = 0·05) = 0·0166

Appendix XI (b) (Contd.)

After 4 days

	1	2	3	4
	0·04	0·06	0·14	0·08
	0·06	0·06	0·05	0·06
	0·02	0·08	0·11	0·07
	0·04	0·04	0·05	0·16
	0·04	0·04	0·08	0·19
	0·02	0·02	0·11	0·12
	0·22	0·30	0·54	0·68
$\bar{m}$	0·0366	0·0500	0·0900	0·1133

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	0·0225	0·0075	6·8182 **
Error	20	0·0239	0·0011	
Total	23	0·0464		

C.D. (P = 0·01) = 0·0540

C.D.(P = 0·05) = 0·0396

Appendix XI (b) (Contd.)

After 6 days

	1	2	3	4
	0·25	0·27	0·25	0·19
	0·19	0·31	0·22	0·28
	0·23	0·12	0·27	0·21
	0·12	0·14	0·27	0·21
	0·12	0·29	0·11	0·26
	0·12	0·21	0·25	0·28
	1·03	1·34	1·37	1·43
$\bar{m}$	0·1716	0·2233	0·2283	0·2383

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	0·0177	0·0059	1·5946 (N.S.)
Error	20	0·0744	0·0037	
Total	23	0·0921		

Appendix XI (b) (Contd.)

After 8 days

	1	3	4
	0·23	0·19	0·33
	0·31	0·27	0·38
	0·31	0·30	0·31
	0·23	0·30	0·31
	0·31	0·30	0·38
	0·27	0·30	0·33
	1·66	1·66	2·04
$\overline{m}$	0·2766	0·2766	0·3400

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·0160	0·0080	5·3333 *
Error	15	0·0228	0·0015	
Total	17	0·0388		

C.D.(P = 0·05) = 0·0490

Appendix XI (b) (Contd.)

After 10 days

	1	3	4
	0·27	0·29	0·26
	0·23	0·25	0·31
	0·25	0·30	0·28
	0·27	0·38	0·14
	0·25	0·47	0·36
	0·25	0·37	0·33
	1·52	2·06	1·68
$\bar{m}$	0·2533	0·3433	0·2800

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·0256	0·0128	3·0476 (N.S.)
Error	15	0·0626	0·0042	
Total	17	0·0882		

Appendix XI (b) (Contd.)

After 12 days

	1	3	4
	0·31	0·25	0·28
	0·27	0·22	0·19
	0·25	0·28	0·28
	0·25	0·33	0·26
	0·19	0·26	0·26
	0·25	0·24	0·24
	1·52	1·58	1·51
$\bar{m}$	0·2533	0·2633	0·2516

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·0004	0·0002	0·1428 (N.S.)
Error	15	0·0207	0·0014	
Total	17	0·0211		

Appendix XI (b) (Contd.)

After 14 days

	1	3	4
	0·29	0·30	0·28
	0·33	0·29	0·36
	0·31	0·25	0·26
	0·31	0·32	0·28
	0·33	0·30	0·26
	0·33	0·22	0·26
	1·90	1·68	1·70
$\bar{m}$	0·3166	0·2800	0·2833

Analysis of variance:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·0048	0·0024	2·1818 (N.S.)
Error	15	0·0160	0·0011	
Total	17	0·0208		

Appendix XII

Rate of multiplication of *A. avenae* in cultures of *R. solani* and *C. coccodes* (from single female)

No. of plate	*R. solani*		*C. coccodes*	
	after 13 days	after 21 days	after 13 days	after 21 days
1	163 x 10	1657 x 10	87 x 10	179 x 10
2	207 x 10	2242 x 10	77 x 10	166 x 10
3	109 x 10	3074 x 10	52 x 10	161 x 10
4	187 x 10	2212 x 10	75 x 10	185 x 10
	666 x 10	9185 x 10	291 x 10	691 x 10
$\bar{m}$	166·5 x 10	2296·25 x 10	72·75 x 10	172·75 x 10

Analysis of variance:

After 13 days

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	17578·13	17578·13	17·474 **
Error	6	6035·75	1005·96	
Total	7	23613·88		

After 21 days

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	9018504·4	9018504·4	52·845 ***
Error	6	1023949·6	170658·3	
Total	7	10042454·0		

Appendix XIII

Morphometrics of A. avenae in the cultures of R. solani and C. coccodes.

No. of observation	R. solani ♀ (1)									C. coccodes ♀ (2)									C. coccodes ♂ (3)					
	Length (μ)	a	b	b_1*	c	v (%)	Stylet length (μ)	Length of egg (μ)	Breadth of egg (μ)	Length (μ)	a	b	b_1*	c	v (%)	Stylet Length (μ)	Length of egg (μ)	Breadth of egg (μ)	Length (μ)	a	b	b_1*	c	Stylet Length (μ)
1	708	21·6	6·7	7·7	28·0	78·2	15·6	66·3	21·4	730	25·0	5·3	7·4	31·7	76·0	15·6	70·2	23·4	745	27·6	5·6	7·6	25·7	15·6
2	745	21·2	6·4	9·1	27·3	78·4	19·5	64·3	23·4	730	25·0	6·8	7·6	31·7	76·0	13·7	64·3	23·4	701	22·6	6·1	7·2	22·6	15·6
3	686	21·3	7·7	7·8	24·8	77·7	15·6	64·3	21·4	701	18·0	6·0	8·5	30·5	77·0	17·5	64·3	23·4	759	28·1	5·5	7·8	38·0	17·5
4	686	21·3	5·4	7·3	27·1	78·7	19·5	74·1	21·4	701	24·2	6·2	7·4	28·0	76·8	17·5	74·1	25·3	745	27·6	6·1	7·6	33·9	17·5
5	759	19·5	5·8	8·6	30·0	79·8	19·5	72·2	23·4	701	18·0	5·8	8·5	28·4	77·1	15·6	66·3	25·3	745	27·6	6·0	7·6	33·9	19·5
6	672	20·9	6·4	7·5	24·6	78·2	21·4	68·2	23·4	701	22·6	5·5	7·8	28·4	77·0	13·7	68·2	27·3	730	27·0	6·3	7·8	29·2	19·5
7	759	21·6	6·2	8·6	30·0	78·9	15·6	66·3	23·4	555	18·0	6·1	7·5	24·1	79·0	19·5	70·2	31·2	813	26·4	6·7	10·5	33·9	19·5
8	759	20·5	6·0	8·5	27·8	78·9	15·6	78·0	23·4	788	17·5	5·2	8·4	29·2	78·0	15·6	66·3	25·3	803	25·7	6·1	8·4	25·7	15·6
9	752	21·4	6·0	8·0	29·8	79·6	14·6	70·2	27·3	803	17·8	6·4	7·6	32·1	76·0	15·6	60·4	23·4	730	26·7	5·5	7·2	25·0	15·6
10	759	21·6	5·2	8·3	30·0	78·9	19·5	85·8	33·1	708	16·5	6·7	7·2	26·2	76·8	15·6	66·3	31·2	700	25·7	5·4	7·5	25·7	19·5
11	672	20·9	6·0	7·7	28·7	80·0	15·6	60·1	29·2	723	23·2	5·1	8·1	26·5	77·4	17·5	74·1	31·2						
12	774	19·8	5·6	8·3	28·3	77·3	15·6	73·0	29·2	905	19·3	4·7	8·9	27·3	77·9	17·5	70·2	31·2						
13	774	18·0	5·5	8·6	30·6	79·2	13·7	73·0	43·8	759	21·6	5·8	8·6	27·8	78·0	17·5	74·1	73·4						
14	715	20·4	5·4	8·0	30·5	79·6	13·7	60·1	36·5	686	22·0	5·8	7·6	27·1	76·6	19·5	64·3	23·4						
15	796	20·4	4·9	8·5	29·1	77·0	15·6	73·0	36·5	774	24·8	5·9	7·6	28·3	78·3	15·6	62·4	26·3						
16	715	17·5	5·6	8·3	26·2	77·6	15·6	69·3	40·1	679	21·7	6·8	7·6	26·8	77·4	19·5	62·4	19·5						
17	759	18·5	6·7	8·1	30·0	78·9	17·5	73·0	36·5	788	22·5	5·8	8·8	28·9	76·0	19·5	70·2	27·3						
18	905	25·8	6·8	8·7	31·6	75·8	15·6	73·0	43·8	759	22·9	5·6	7·8	27·8	78·0	17·5	74·1	23·4						
19	978	20·1	7·4	8·1	31·2	76·1	17·5	66·3	23·4	679	21·7	5·4	7·6	26·8	76·3	17·5	78·0	23·4						
20	1022	18·7	6·5	7·9	31·1	77·1	15·6	68·2	23·4	657	21·0	5·5	7·5	28·1	75·5	15·6	68·2	25·3						
21								79·9	23·4								72·2	23·4						
22								69·3	23·4								78·0	23·4						
$\bar{m}$	793·85	20·54	6·11	8·31	29·04	78·29	16·62	70·36	28·67	726·35	21·16	5·82	8·00	28·28	76·63	16·85	69·03	25·47	747·10	26·5	5·93	7·92	29·36	17·54

* b_1 = quotient of body length and distance from head to the base of median oesophageal bulb - after Goodey and Hooper (1965).

Appendix XIII (contd.)

Analysis of variance of length:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	46914·4	23457·2	2·650 (N.S.)
Error	47	416014·1	8851·3	
Total	49	462928·5		

Analysis of variance of 'a':

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	259·22	129·612	29·564 ***
Error	47	206·09	4·384	
Total	49	465·31		

C.D. between 1 and 2 = 2·322 (P = 0·001)
= 1·329 (P = 0·05)

C.D. between 1 and 3) = 2·850 (P = 0·001)
and 2 and 3)

Analysis of variance of 'b':

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	0·851	0·4255	1·1268 (N.S.)
Error	47	17·751	0·3776	
Total	49	18·602		

Analysis of variance of 'b_1':

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	1·403	0·7015	1·3092 (N.S.)
Error	47	25·184	0·5358	
Total	49	26·587		

Appendix XIII (Contd.)

Analysis of variance of 'c':

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	9·68	4·84	0·971 (N.S.)
Error	47	234·30	4·98	
Total	49	243·98		

Analysis of variance of 'v':

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	27·56	27·56	0·784 (N.S.)
Error	38	1334·78	35·12	
Total	39	1362·34		

Analysis of variance of stylet length:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	2	5·704	2·8520	0·7391 (N.S.)
Error	47	181·366	3·8588	
Total	49	187·070		

Analysis of variance of length of egg:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	19·37	19·37	0·6112 (N.S.)
Error	42	1331·22	31·69	
Total	43	1350·59		

Analysis of variance of breadth of egg:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	112·63	112·63	2·139 (N.S.)
Error	42	2211·24	52·64	
Total	43	2323·87		

Appendix XIV

Capacity of laying the multiple eggs inside the uterus of the females and vivipary in A. avenae.

(Gravid obese worms kept in the solid watch glasses at 25°C)

No. of observation	Eggs and larvae laid (after days)						Total no. of eggs and larvae laid
	1	2	3	4	5	6	
1	8 E 2 L	- -	- -	- -	- -	- -	8 E 2(+1*) L
2	15 E 2 L	8 E -	- -	3 E -	2 E -	1 E -	29 E 2 L
3	9 E -	3 E 1 L	1 E -	1 E -	1 E -	- -	15 E 1 L
4	11 E 2 L	6 E -	2 E 1 L	- -	- -	- -	19 E 3 L
5	1 E 3 L	died					1 E 3 L
6	1 E 3 L	died					1 E 3 L
7	- 3 L	1 E -	died				1 E 3 L
8	- 1 L	died					- 1 L
9	1 E 1 L	- -	died				1 E 1 L
10	- 1 L	died					- 1 L

* larva came out after 17 days.

E = Egg/s

L = Larva/e

Appendix XV

Hatching time, egg laying period and number of eggs laid per female of *A. avenae* in cultures of *R. solani* and *C. coccodes*.

No. of Observation	*R. solani*				*C. coccodes*			
	Hatching (after days)	Starting of egg laying (after days of hatch)	No. of eggs laid (per ♀)	Egg laying period (days)	Hatching (after days)	Starting of egg laying (after days of hatch)	No. of eggs laid (per ♀)	Egg laying period (days)
1	3	5	97	9	2	5	47	8
2	2	6	39	5	2	5	43	12
3	2	6	23	10	2	5	4	1
4	2	6	82	8	no hatching	-	-	-
5	2	6	17	3	2	5	55	12
6	2	6	75	11	no hatching	-	-	-
7	2	6	26	5	3	*	-	-
8	3	5	33	7	2	5	18	8
9	3	5	70	12	2	*	-	-
10	no hatching	-	-	-	2	4	94	16
11	2	5	52	5	2	5	39	10
12	2	*	-	-	3	5	13	5
13	2	5	111	7	2	6	20	5
14	2	*	-	-	2	6	18	8
15	2	5	33	8	2	5	39	5
16	2	5	130	17	2	*	-	-
17	2	5	48	6	no hatching	-	-	-
18	no hatching	-	-	-	2	5	79	9
19	2	*	-	-	3	5	12	3
20	2	5	69	8	2	5	25	8
21	2	5	173	11	2	*	-	-
22	3	5	154	19	2	6	13	5
23	no hatching	*	-	-	2	6	4	1
24	2	5	180	16	2	*	-	-
m	1·92	4·00	58·83	6·96	1·87	3·46	21·79	4·83

* Larva developed into normal female but did not lay eggs.

Appendix XV (Contd.)

Analysis of variance of number of eggs laid per ♀:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	16465·020	16465·020	8·4337 **
Error	46	89805·293	1952·288	
Total	47	106270·313		

Analysis of variance of egg laying period per ♀ :

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	54·187	54·187	1·990 (N.S.)
Error	46	1252·293	27·223	
Total	47	1306·480		

Appendix XVI (a).

Effect of Aphelenchus avenae on the growth of tomato plants inoculated with Rhizoctonia solani

(First experiment)

No. of observation	Control			Fungus only			Fungus + Nematode			Nematode only		
	Height of shoot (cm.)	Dry wt. of shoot (g.)	Dry wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Dry wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Dry wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Dry wt. of root (g.)
1	148	33·6	1·81	130	34·3	1·53	135	33·0	2·88	128	31·1	1·52
2	147	29·1	2·13	108	23·2	1·50	148	34·1	1·88	107	23·0	1·20
3	112	28·5	1·85	122	27·5	1·76	128	28·3	1·59	142	32·4	2·66
4	119	25·2	2·61	117	25·2	1·54	122	22·5	1·61	132	25·6	2·36
	526	116·4	8·40	477	110·2	6·33	533	117·9	7·96	509	112·1	7·74
$\bar{m}$	131·50	29·100	2·100	119·25	27·550	1·582	133·25	29·475	1·990	127·25	28·025	1·935

Analysis of variance of the height of Shoot:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	467·19	158·730	0·817 (N.S.)
Error	12	2329·25	194·104	
Total	15	10408·94		

Analysis of variance of the dry weight of Shoot:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	9·732	3·233	0·1560 (N.S.)
Error	12	249·406	20·783	
Total	15	259·138		

Analysis of variance of the dry weight of root:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	0·6005	0·2002	0·8069 (N.S.)
Error	12	2·9778	0·2481	
Total	15	3·5783		

Appendix XVI (b).

Effect of Aphelenchus avenae on the growth of tomato plants inoculated with Colletotrichum coccodes

(First experiment)

No. of observation	Control			Fungus only			Fungus + Nematode			Nematode only		
	Height of shoot (cm.)	Dry wt. of shoot (g.)	Dry wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Dry wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Dry wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Dry wt. of root (g.)
1	148	33·6	1·81	93	22·0	1·91	157	31·2	2·16	128	31·1	1·52
2	147	29·1	2·13	142	33·6	1·47	133	19·8	2·50	107	23·0	1·20
3	112	28·5	1·85	109	20·0	1·62	119	24·4	1·58	142	32·4	2·66
4	119	25·2	2·61	104	15·2	1·40	114	21·0	1·04	132	25·6	2·36
	526	116·4	8·40	448	90·8	6·40	523	96·4	7·28	509	112·1	7·74
$\bar{m}$	131·50	29·100	2·100	112·00	22·700	1·600	130·75	24·100	1·820	127·25	28·025	1·935

Analysis of variance of the height of shoot:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	995·25	331·75	0·960 (N.S.)
Error	12	4146·50	345·50	
Total	15	5141·75		

Analysis of variance of the dry weight of shoot:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	112·837	37·612	1·264 (N.S.)
Error	12	356·988	29·749	
Total	15	469·825		

Analysis of variance of the dry weight of root:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	0·5295	0·1765	0·6571 (N.S.)
Error	12	3·2237	0·2686	
Total	15	3·7532		

Appendix XVII (a).

Effect of Aphelenchus avenae on the growth of tomato plants inoculated with Rhizoctonia solani in sterilized soil (Second experiment)

No. of observation	Control			Fungus only			Fungus + Nematode			Nematode only		
	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)
1	77	3·62	1·96	68	3·26	2·44	71	3·70	1·99	68	2·61	2·19
2	66	3·01	2·02	70	3·46	2·09	69	3·60	1·71	72	3·22	1·55
3	68	3·18	1·98	58	2·51	1·67	78	3·21	2·08	74	4·07	2·62
4	74	2·81	1·72	70	3·23	1·77	75	4·04	2·06	78	3·37	2·26
5	67	3·12	1·86	51	1·73	1·14	75	2·98	2·65	79	3·49	1·81
	352	15·74	9·54	317	14·19	9·11	368	17·53	10·49	371	16·76	10·43
$\bar{m}$	70·0	3·148	1·908	63·4	2·838	1·822	73·60	3·506	2·098	74·2	3·352	2·086

Analysis of variance of the height of shoot:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	368·4	122·800	3·805 *
Error	16	516·4	32·275	
Total	19	884·8		

C.D. (P = 0·05) = 7·632

Analysis of variance of the dry weight of shoot:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	1·2500	0·4166	1·5834 (N.S.)
Error	16	4·2106	0·2631	
Total	19	5·4606		

Analysis of variance of the fresh weight of root:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	0·2753	0·0721	0·5337 (N.S.)
Error	16	2·1624	0·1351	
Total	19	2·4377		

Appendix XVII (b).

Effect of Aphelenchus avenae on the growth of tomato plants inoculated with Colletotrichum coccodes in sterilized soil.

(Second experiment)

No. of observation	Control			Fungus only			Fungus + Nematode			Nematode only		
	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)
1	77	3·62	1·96	65	2·22	1·62	71	3·88	2·37	68	2·61	2·19
2	66	3·01	2·02	70	3·24	1·94	78	3·61	1·99	72	3·22	1·55
3	68	3·18	1·98	60	1·60	1·37	80	3·32	2·29	74	4·07	2·62
4	74	2·81	1·72	70	2·30	1·99	72	3·71	1·94	78	3·37	2·26
5	67	3·12	1·86	55	1·56	0·98	80	3·34	2·29	79	3·49	1·81
	352	15·74	9·54	320	10·92	7·90	381	17·86	10·88	371	16·76	10·43
$\bar{m}$	70·0	3·148	1·908	64·0	2·184	1·580	76·2	3·572	2·176	74·2	3·352	2·086

Analysis of variance of the height of shoot:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	432·4	144·133	5·480 **
Error	16	420·8	26·300	
Total	19	1045·2		

C.D. (P = 0·01) = 9·464

C.D. (P = 0·05) = 6·869

Analysis of variance of the dry weight of shoot:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	5·6122	1·8707	8·4228 **
Error	16	3·5539	0·2221	
Total	19	9·1661		

C.D. (P = 0·01) = 0·870

C.D. (P = 0·05) = 0·632

Analysis of variance of the fresh weight of root:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	1·0768	0·3589	3·578 *
Error	16	1·6051	0·1003	
Total	19	2·6819		

C.D. (P = 0·05) = 0·424

Appendix XVII (c)

Effect of Aphelenchus avenae on the growth of tomato plants inoculated with Rhizoctonia solani in unsterilized soil.
(Second experiment)

No. of observation	Control			Fungus only			Fungus + Nematode			Nematode only		
	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)
1	60	2·70	1·51	60	2·03	1·52	54	1·66	1·10	60	1·94	1·42
2	55	2·24	1·12	65	1·70	1·20	70	2·80	2·52	50	1·44	1·07
3	59	2·58	1·68	60	1·84	1·49	61	2·23	2·13	56	2·37	1·79
4	62	2·06	2·02	69	3·16	2·15	68	2·63	2·41	53	1·95	1·58
5	61	2·49	1·90	61	3·38	2·32	58	1·92	1·41	66	2·23	1·92
	297	12·07	8·23	315	12·11	8·68	311	10·74	9·57	285	9·93	7·78
$\bar{m}$	59·4	2·414	1·646	63·0	2·422	1·736	62·2	2·148	1·914	57·0	1·986	1·556

Analysis of variance of the height of shoot:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	112·8	37·6000	0·479 (N.S.)
Error	16	297·0	18·7875	
Total	19	409·8		

Analysis of variance of the dry weight of shoot:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	0·6818	0·2273	0·735 (N.S.)
Error	16	4·9476	0·3092	
Total	19	5·6294		

Analysis of variance of the fresh weight of root:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	0·3504	0·1168	0·545 (N.S.)
Error	16	3·4266	0·2142	
Total	19	3·7770		

Appendix XVII (d).

Effect of Aphelenchus avenae on the growth of tomato plants inoculated with Colletotrichum coccodes in unsterilized soil.

(Second experiment)

No. of observation	Control			Fungus only			Fungus + Nematode			Nematode only		
	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)	Height of shoot (cm.)	Dry wt. of shoot (g.)	Fresh wt. of root (g.)
1	60	2·70	1·51	62	1·82	1·31	54	1·72	1·28	60	1·94	1·42
2	55	2·24	1·12	55	2·50	1·81	61	2·14	1·79	50	1·44	1·07
3	59	2·58	1·68	53	1·78	0·98	60	2·35	2·02	56	2·37	1·79
4	62	2·06	2·02	65	2·54	2·09	55	1·59	1·02	53	1·95	1·58
5	61	2·49	1·90	58	2·48	1·75	58	2·22	1·92	66	2·23	1·92
	297	12·07	8·23	293	11·12	7·95	288	10·02	8·02	285	9·93	7·78
$\bar{m}$	59·4	2·414	1·646	58·6	2·224	1·590	57·6	2·004	1·604	57·0	1·986	1·556

Analysis of variance of the height of shoot:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	19·95	5·6500	0·479 (N.S.)
Error	16	188·60	11·7875	
Total	19	205·55		

Analysis of variance of the dry weight of shoot:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	0·6160	0·2053	1·807 (N.S.)
Error	16	1·8180	0·1136	
Total	19	2·4340		

Analysis of variance of the fresh weight of root:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	3	0·0209	0·0070	0·045 (N.S.)
Error	16	2·4924	0·1558	
Total	19			

Appendix XVIII (a)

Number of *Aphelenchus avenae* extracted from soil and roots of tomato in the presence of *Rhizoctonia solani*.

(First experiment)

No. of observation	Fungus + Nematode			Nematode only		
	per g. of soil	per g. of root	per sugar beet seed	per g. of soil	per g. of root	per sugar beet seed
1	79·9	319	18	37·7	485	6
2	76·7	729	12	40·9	464	5
3	69·1	920	25	36·2	748	6
4	70·6	604	20	49·8	740	10
5			20			5
6			12			4
7			18			1
8			16			6
9			21			13
10			15			10
	296·1	2572	177	164·6	2437	63
$\bar{m}$	74·025	643·00	17·7	41·150	609·25	6·3

Analysis of variance:

From soil:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	2161·531	2161·531	69·489 ***
Error	6	186·638	31·106	
TOTAL	7	2348·169		

From root:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	2278·13	2278·13	0·052 (N.S.)
Error	6	263504·75	43917·46	
TOTAL	7	265782·88		

From Sugarbeet seed:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	649·8	649·800	24·008 ***
Error	18	487·2	27·066	
TOTAL	19	1137·0		

Appendix XVIII (b)

Number of *Aphelenchus avenae* extracted from soil and roots of tomato in the presence of *Colletotrichum coccodes*.
(First experiment)

No. of observation	Fungus + Nematode			Nematode only		
	per g. of soil	per g. of root	per sugar beet seed	per g. of soil	per g. of root	per sugar beet seed
1	57·8	544	17	37·7	485	6
2	70·2	711	20	40·9	464	5
3	66·6	872	14	36·2	748	6
4	69·6	805	21	49·8	740	10
5			25			5
6			30			4
7			24			1
8			24			6
9			23			13
10			19			10
	264·2	2932	217	164·6	2437	63
$\bar{m}$	66·050	733·00	21·7	41·150	609·25	6·3

Analysis of variance:

From soil:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	1240·020	1240·020	35·517 ***
Error	6	209·480	34·913	
Total	7	1449·500		

From root:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	30628·13	30628·13	1·375 (N.S.)
Error	6	133592·75	22265·46	
Total	7	164220·88		

From sugarbeet seed:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	1185·8	1185·8	41·031 ***
Error	18	502·2	28·9	
Total	19	1706·0		

Appendix XIX (a).

Number of Aphelenchus avenae extracted from soil and roots of tomato in the presence of Rhizoctonia solani in sterilized soil (second experiment)

No. of observation.	Fungus + Nematode		Nematode only	
	Per g. of soil	per g. of root	per g. of soil	per g. of root.
1	4·65	6·2	7·25	1·4
2	6·75	6·8	3·14	0
3	8·24	5·8	4·20	0
4	8·60	1·4	3·31	0
5	4·75	2·8	5·55	0
	32·99	23·0	23·45	1·4
$\bar{m}$	6·598	4·60	4·690	0·28

Analysis of variance:

From soil:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	9·101	9·101	2·825 (N.S.)
Error	8	25·778	3·222	
TOTAL	9	34·879		

From root:

Item	D.F.	S.S.	M.S.	F. ratio
Treatment	1	46·656	46·656	15·625**
Error	8	23·888	2·986	
TOTAL	9	70·544		

Appendix XIX (b)

Number of *Aphelenchus avenae* extracted from soil and roots of tomato plants in the presence of *Colletotrichum coccodes* in sterilized soil. (Second experiment)

No. of observation.	Fungus + Nematode		Nematode only	
	per g. of soil	per g. of root	per g. of soil	per g. of root
1	5·10	1·6	7·25	1·4
2	5·22	3·2	3·14	0
3	9·20	3·0	4·20	0
4	6·75	4·2	3·31	0
5	6·28	3·0	5·55	0
	32·55	15·0	23·45	1·4
$\bar{m}$	6·510	3·00	4·690	0·28

Analysis of variance:

From soil:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	8·280	8·280	2·900 (N.S.)
Error	8	22·840	2·855	
TOTAL	9	31·120		

From root:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	18·496	18·496	29·546***
Error	8	5·008	0·626	
TOTAL	9	23·504		

Appendix XIX (c)

Number of *Aphelenchus avenae* extracted from soil and roots of tomato in the presence of *Rhizoctonia solani* in unsterilized soil.

(Second experiment)

No. of observation.	Fungus + Nematode		Nematode only	
	per g. of soil	per g. of root	per g. of soil	per g. of root
1	6·94	67·9	7·12	118·4
2	14·64	120·5	5·70	35·2
3	8·62	90·1	10·09	12·0
4	10·21	78·8	10·20	95·0
5	6·30	49·4	7·17	31·2
	46·71	406·7	40·28	291·8
$\bar{m}$	9·342	81·34	8·056	58·38

Analysis of variance:

From soil:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	4·1344	4·1344	0·548 (N.S.)
Error	8	60·3127	7·5391	
TOTAL	9	64·4471		

From root:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	1317·904	1317·904	0·942 (N.S.)
Error	8	11195·420	1399·427	
TOTAL	9	12513·324		

Appendix XIX (d)

Number of Aphelenchus avenae extracted from soil and roots of tomato plants in the presence of Colletotrichum coccodes in unsterilized soil. (Second experiment)

No. of observation.	Fungus + Nematode		Nematode only	
	per g. of soil	per g. of root	per g. of soil	per g. of root
1	7·08	67·8	7·12	118·4
2	7·44	30·9	5·70	35·2
3	11·28	93·4	10·09	12·0
4	8·77	43·3	10·20	95·0
5	6·24	51·3	7·17	31·2
	40·81	286·7	40·28	291·8
$\bar{m}$	8·162	57·34	8·056	58·38

Analysis of variance:

From soil:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	0·0280	0·0280	0·007 (N.S.)
Error	8	31·4235	3·9279	
TOTAL	9	31·4515		

From root:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	2·704	2·704	0·002 (N.S.)
Error	8	10720·340	1340·042	
TOTAL	9	10723·044		

Appendix XX.

Effect of Aphelenchus avenae on the incidence of diseases caused by Rhizoctonia solani and Colletotrichum coccodes on tomato root (in degrees). (First experiment)

No. of observation.	R. solani.	R. solani + A. avenae	C. coccodes	C. coccodes + A. avenae.
1	41·70	28·88	50·68	36·72
2	40·40	42·88	64·62	42·70
3	32·84	45·18	49·42	40·52
4	45·70	38·40	53·28	45·60
	160·64	152·34	218·00	165·54
$\overline{m}$	40·160	38·085	54·500	41·385

Analysis of variance:

With R. solani:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	8·6113	8·6113	0·1671 (N.S.)
Error	6	309·0965	51·5161	
TOTAL	7	317·7078		

With C. coccodes:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	344·0065	344·0065	11·0787 *
Error	6	186·3075	31·0512	
TOTAL	7	530·3140		

Appendix XXI (a)

Effect of *Aphelenchus avenae* on the incidence of diseases caused by *Rhizoctonia solani* and *Colletotrichum coccodes* on tomato root in sterilized soil (in degrees).
(second experiment)

No. of observation.	R. solani	R. solani + A. avenae	C. coccodes	C. coccodes + A. avenae
1	54·66	28·49	47·90	23·04
2	31·60	39·44	34·40	24·61
3	59·59	29·06	42·52	20·78
4	39·68	18·80	43·42	34·40
5	48·76	22·24	38·96	18·80
	234·28	138·03	203·20	121·63
$\bar{m}$	46·856	27·606	40·640	24·326

Analysis of variance:

With R. solani:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	926·4061	926·4061	9·752 *
Error	8	759·9472	94·9938	
Total	9	1686·3533		

With C. coccodes:

Item	D.F.	S.S.	M.S.	F ratio
Treatment	1	665·3664	665·3664	18·909 **
Error	8	281·5012	35·1876	
Total	9	946·8676		

Appendix XXI (b)

Effect of *Aphelenchus avenae* on the incidence of diseases caused by *Rhizoctonia solani* and *Colletotrichum coccodes* on tomato root in unsterilized soil (in degrees) (second experiment)

No. of observation	*R. solani*	*R. solani* + *A. avenae*	*C. coccodes*	*C. coccodes* + *A. avenae*
1	51·90	43·24	45·65	39·26
2	28·00	28·76	68·09	47·90
3	44·30	43·36	25·28	50·80
4	46·88	36·90	43·78	44·10
5	32·78	33·80	40·52	54·66
	203·86	186·06	233·32	236·72
$\overline{m}$	40·772	37·212	46·664	47·344

Analysis of variance: not given as the differences between the treatments with both the fungi are very small.