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INTERRELATIONSHIPS BETWEEN *HETERODERA ROSTOCHIENSIS* AND SOIL FUNGI ON TOMATO (*)

by

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Rhizoctonia solani Kühn [= *Corticium solani* (Prill. *et* Del.) Bourd. *et* Ganz.] and *Colletotrichum coccodes* (Wallr.) Hughes [= *C. atramentarium* (Berk. *et* Br.) Taubenh.] were considered to be contributing factors of 'potato sickness' in the United Kingdom (Cheal, 1929; Edwards, 1929; Triffitt, 1931). Miles (1930) while considering *R. solani* to be a contributory factor disregarded *C. coccodes* to have any role. However, Millard *et al.* (1932) did not support this view. Dunn and Hughes (1964) did not find any synergistic effect between the potato cyst eelworm (*Heterodera rostochiensis* Woll.) (PCE) and *C. coccodes*. Greater reduction in the growth of potato and tomato in the presence of both *R. solani* and PCE than PCE alone was observed by Grainger and Clark (1963) and Dunn and Hughes (1964), respectively.

Graham (1966) noted that predominance of soil fungi adversely affected the production of cysts of PCE. James (1966 and 1968) and Ketudat (1968 and 1969) made similar observations and also found a suppressing effect of the fungi on the hatching of juveniles. The grey sterile fungus was found to inhibit the females from forming giant cells (Roy, 1968). The studies presented here attempted to identify the effects of the interaction between fungi and nematode.

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MATERIALS AND METHODS

Inoculations, assessment of nematode reproduction and disease incidence.

Both *R. solani* and *C. coccodes* were isolated from tomato roots and maintained on potato-carrot agar. The cultures for inoculation were prepared by growing on sterile sugarbeet seeds (Dunn and Hughes, 1967) for 4 weeks at 25 °C. Cysts were sterilized in 0.5% $CuSO_4$ for 24 h before putting them in a mixture of sterile root diffusate and water for hatching.

John Innes potting mixture was sterilized at 71 °C for 1.25 h. Tomato seeds, cv. Alisa Craig, were surface sterilized with $HgCl_2$ and sown in a wooden box containing sterilized soil. After one month, the seedlings were transplanted to a second box and 20 days later reported in 9 cm diameter plastic pots each containing 375 g sterilized soil. The following inoculations were made: 1 - Control; 2 - Fungus alone; 3 - Fungus first, then nematode; 4 - Nematode first, then fungus; 5 - Nematode and fungus combined simultaneously; 6 - Nematode and both the fungi combined simultaneously; 7 - Nematode alone.

The experiment was conducted in two houses simultaneously. In house No. 1, temperature of soil varied from 21 to 27 °C (average 22 °C) in the day and 11.6 to 15.8 °C (average 14 °C) at night. In house No. 2 with underground heating, temperature varied from 25 to 31.4 °C (average 26 °C) in the day and 17.7 to 23.9 °C (average 22.2 °C) at night.

The first inoculation with fungus or nematode, or both, were made on 12 July and in treatments with a second inoculation this was 18 days later. Rate of fungus inoculum was 3% of soil and that of PCE 18 larvae per g of soil. Five weeks after the last inoculation, the plants were uprooted and the roots washed by a strong jet of water on a sieve. Although most of the cysts were dislodged from the roots, a few still remained attached which were stained in lactophenol with 0.05% acid fuchsin and counted with a stereoscopic microscope. Cysts which remained in the soil were extracted by a Fenwick can. Total number of cysts was computed by adding the number from the soil, from the roots and those dislodged on the sieve and then expressed per g of root.

Disease incidence was estimated by means of a « disease recording tray ». This consisted of a tray ' A ' scribed with lines 1 cm apart,

a cover ' B ' and a moveable piece ' C ' to fix the cover on the tray (Fig. 1). The whole root or a portion of it was placed on the tray in a thin layer of water and the cover was put on it. Then ' C ' was moved along the length of the tray supported by the long stripes on the side walls and disease incidence measured by counting the diseased root pieces in 1 cm bands under a stereoscopic microscope.

Five replications were kept for each of the treatments of this as well as of the subsequent experiments. The treatments with *R. solani* were analysed seperately from that with *C. coccodes.*

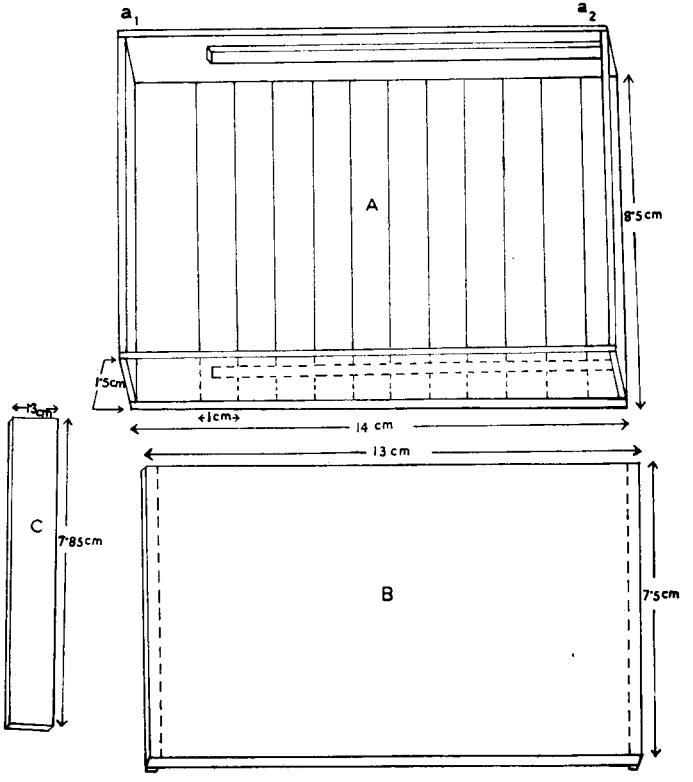

Fig. 1 - ' Disease recording tray ' to assess the percentage of disease on root.

Histological studies.

Root pieces fixed in FAA were dehydrated through grades of tertiary butyl alcohol and ethanol mixtures and embedded in paraffin

wax of 55 °C m. pt (Johansen, 1940). The tissues were sectioned at 12 μm thick with a microtome and stained in 0.5% safranin and 0.5% Fast Green (Jensen, 1962).

To perform the Rosindole reaction to detect indole derivative/s (Glenner, 1957), root tissues 18 days after nematode inoculation were fixed in calcium acetate (commercial formalin — 10 ml, water — 90 ml and calcium acetate — 2 g) for 6 h and then wax embedded tissues were sectioned at 9 μm. After removing the paraffin, the slides were placed into absolute ethanol and treated for 3 min in a solution containing 1 g p-dimethylaminobenzoaldehyde, 5 ml 60% perchloric acid, 1 ml concentrated HCl and 34 ml glacial acetic acid and then for 1 min in a solution of 5 ml concentrated HCl and 35 ml glacial acetic acid which was poured into a Choplin jar previously layered with 500 mg $NaNO_2$. The slides were brought into xylene through grades of glacial acetic acid and xylene mixtures and mounted in cellulose tridecanoate (cellulose caprate).

Hatching of juveniles in presence of fungus exudates.

The fungi were grown in potato dextrose broth for 21 days and *R. solani* was filtered through a sterilized Zeiss filter. As *C. coccodes* produced some mucilagenous substance which clogged the filter sheet, the liquid was simply pipetted out. The following treatments were made:

1 - 1 ml sterile water +1 ml sterile root diffusate (control); 2 - 1 ml *R. solani* exudate + 1 ml sterile water; 3 - 1 ml *R. solani* exudate + 1 ml sterile root diffusate; 4 - 1 ml *C. coccodes* exudate + 1 ml sterile water; 5 - 1 ml *C. coccodes* exudate + 1 ml sterile root diffusate.

Cysts of more or less uniform size (passing through 30 mesh but not 40) after surface sterilization were kept on sterile filter papers in sterile Petri dishes at 21 ± 1 °C. The filter papers were soaked with 1 ml sterile water + 1 ml *R. solani* exudate for the treatments 2 and 3, 1 ml sterile water + 1 ml *C. coccodes* exudate for 4 and 5 and 2 ml sterile water for the control.

After six days, 6 cysts were transferred into a bijou bottle containing the fungus exudate and root diffusate according to the treatments and incubated at 21 ± 1 °C. Counts of juvenile nematodes were made after 8 days.

Invasion of root by juveniles in the presence of fungi.

Tomato seedlings, about 5 weeks old, were planted out in 6.5 cm diameter plastic pots each containing 150 g John Innes compost. The pots were inoculated on the same day with the fungi and 18 days later with PCE juveniles. Inoculation with PCE alone served as control.

The experiment was carried out at two temperatures, 15.5 and 26.6° C, in water baths. Two 200 watt reflector lamps were hung over the water baths to provide 12 h day length. After 15 days, the plants were harvested and the roots stained with 0.05% acid fuchsin in lactophenol and comminuted in a homogenizer.

RESULTS

Growth of plants, reproduction of eelworm and diseases incidence.

Fig. 2 shows that inoculation with *R. solani* alone and before inoculation with PCE did not retard the growth of either shoot or root in either of the houses. Growth of shoot was checked signifi-

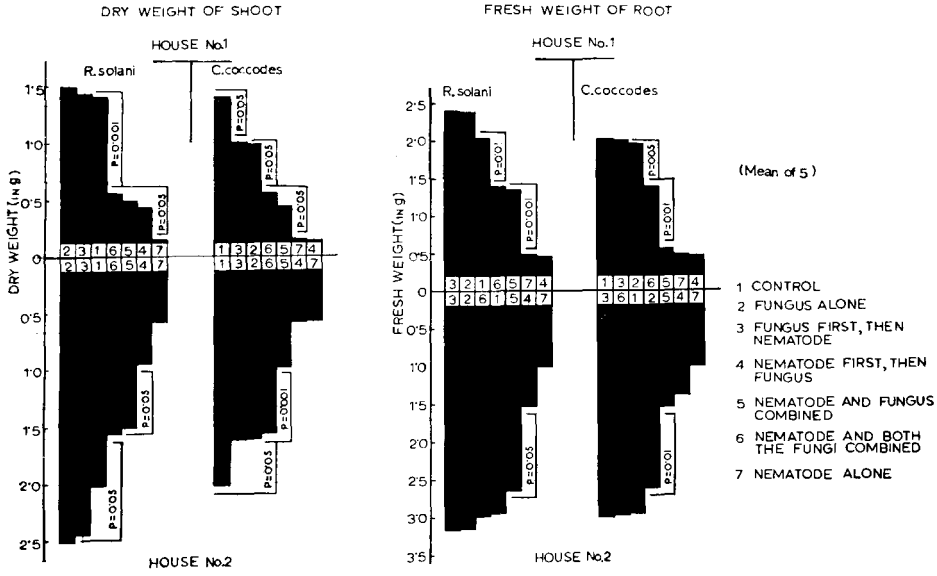

Fig. 2 - Weight of shoot and root of tomato plants in different treatments of inoculations of *H. rostochiensis, R. solani* and *C. coccodes.*

cantly in other treatments in both the houses, with growth inhibition most severe in the treatments where nematodes were inoculated alone or before the fungus.

With *C. coccodes*, growth of shoot was checked by all the fungus and nematode combinations in house 1. The maximum retardation occurred in the plants inoculated with nematode only or with nematode followed by fungus but with no significant difference between these treatments (4 and 7). Similar results were obtained in house 2 but with no significant differences among treatments 1, 2 and 3. There was a greater reduction in plant growth in the treatments with nematodes alone or nematode followed by fungus than in treatments fungus alone or fungus followed by nematode inoculation. Growth check in the treatments of simultaneous inoculation lay intermediate.

Fig. 3 shows that with *R. solani*, in both the houses, production of cysts on roots inoculated with the nematodes alone or when

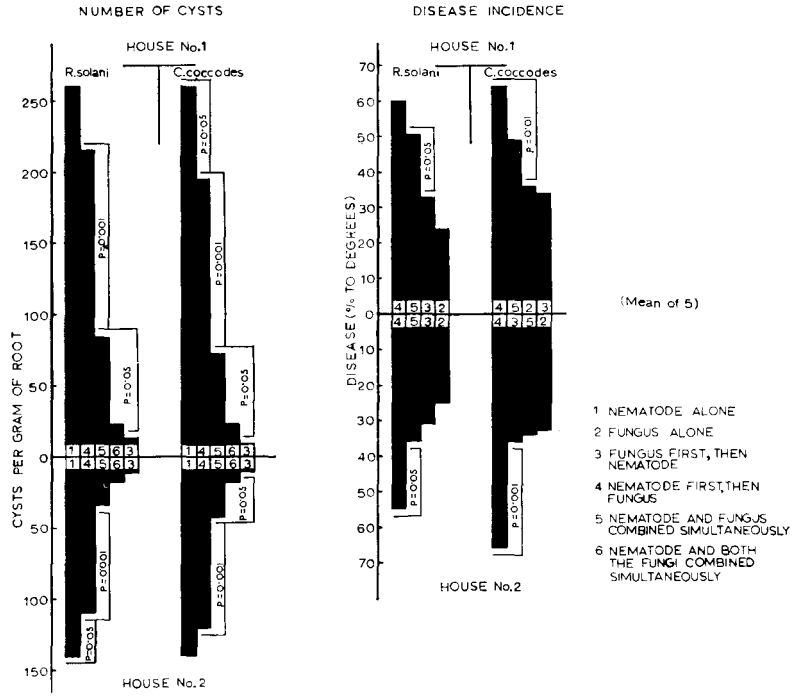

Fig. 3 - Number of cysts of *H. rostochiensis* and disease incidences of *R. solani* and *C. coccodes* in different treatments of fungus and nematode inoculations.

inoculated before the fungus was significantly greater than in other treatments. Also, the number of cysts was least when fungus inoculation preceded nematode inoculation.

With *C. coccodes*, the results were similar to those obtained with *R. solani* in that the number of cysts was significantly higher in treatment with nematodes followed by fungus inoculation than in treatment with fungus followed by nematode inoculation (Fig. 3).

Disease incidence (% converted to angles) was greatest when *R. solani* inoculation followed nematode inoculation, with significant difference between this and other treatments in house 2 but not in house 1 (Fig. 3). With *C. coccodes*, similar results were obtained but without significant differences among the treatments 2, 3 and 5.

Histological observations.

In the treatments where fungi preceded nematodes, giant cells failed to develop or, occasionally, deformed ones occurred (Fig. 4).

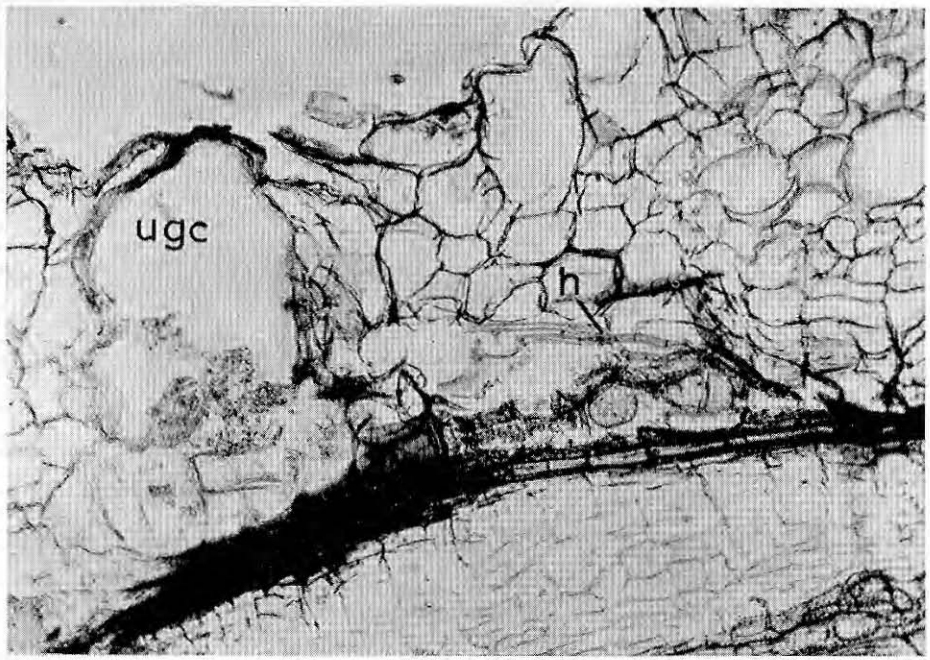

Fig. 4 - Longitudinal section of *R. solani* infected tomato root showing undeveloped giant cells (ugc) and hyphae (h) of the fungus in the treatment fungus followed by nematode.

Where the fungus was followed by nematode inoculation but in tissues which were not invaded by mycelium, giant cells could be formed. When the nematodes entered first and had stimulated formation of giant cells (Figs. 5 and 6) the fungal mycelia developed much profusely in the giant cells than the surrounding healthy tissues. Rosindole test gave positive reaction in the bodies of the invading larvae.

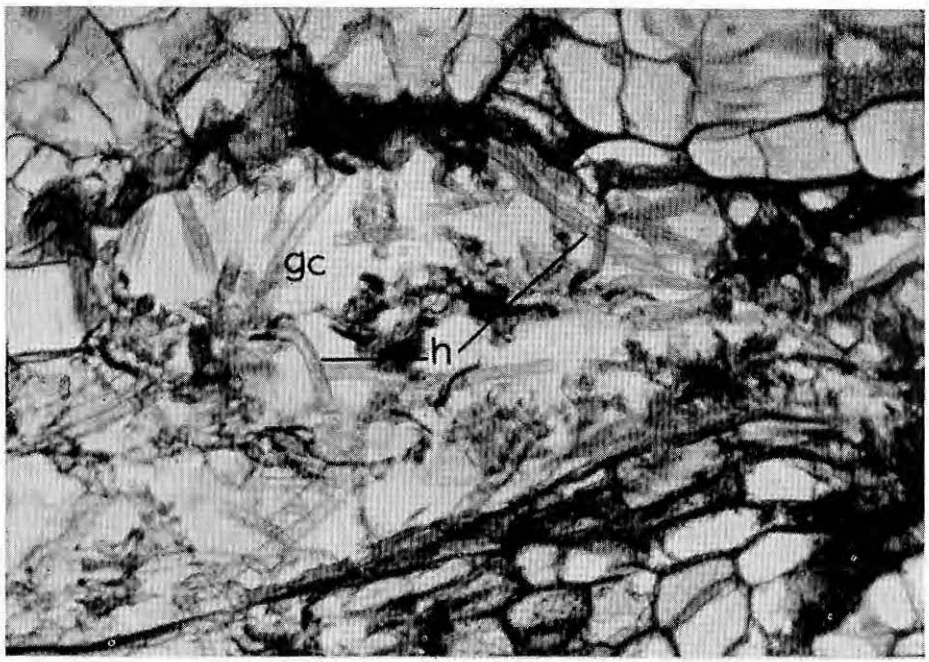

Fig. 5 - Long. sec. of giant cells (gc) showing profuse colonization by *R. solani* hyphae in the treatment nematode followed by fungus - nearby cells are uninvaded.

Hatching of larvae and invasion of roots.

A mean of 236 juveniles hatched from cysts immersed in water with root diffusate compared with 54 in *R. solani* exudate and water or 17 in *C. coccodes* exudate and water. Root diffusate increased hatching to 108 juveniles per cyst in the presence of *R. solani* exudate and to 27 with *C. coccodes* exudate.

No differences were found in the rate of invasion of roots by

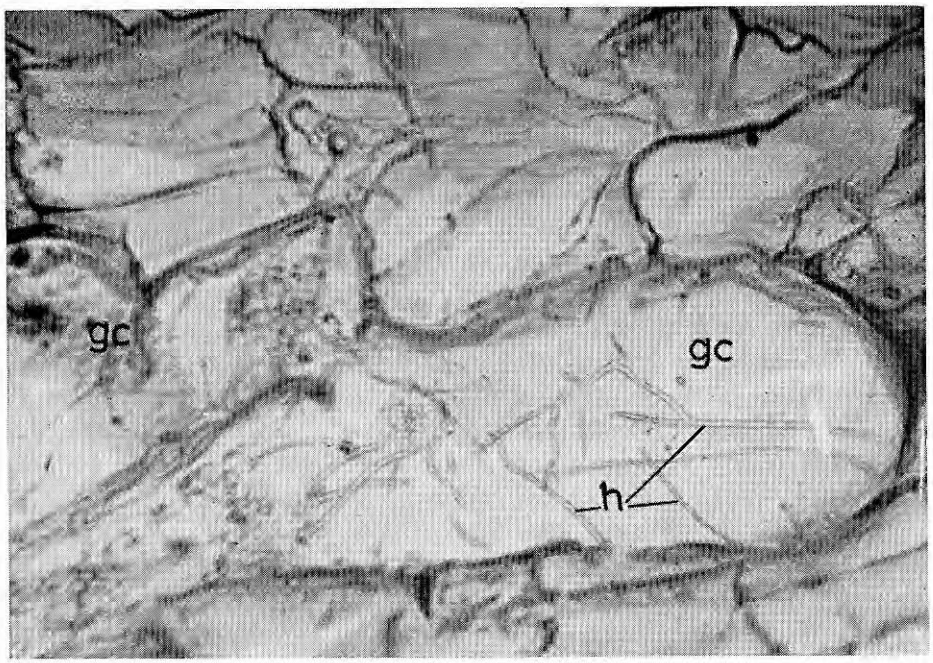

Fig. 6 - Long. sec. of giant cells showing colonization by *C coccodes* hyphae in the treatment nematode followed by fungus - nearby cells are free.

PCE juveniles in the presence of either fungus species compared with the control. At 15.5° C, 206 and 243 juveniles entered per g of root inoculated with *C. coccodes* and *R. solani*, respectively and at 26.6° C, 196 and 218, respectively.

DISCUSSION

Both *R. solani* and *C. coccodes* appear to act adversely on the hatching of PCE and production of new cysts. James (1966) recorded similar inhibitory effects by the grey sterile fungus and Ketudat (1968) by *Verticillium albo-atrum* Reinke and Berth. and *R. solani*. The fungi, on the other hand, do not affect penetration of roots by the juveniles.

In the present studies and those of other workers (Miles, 1930; Millard *et al.*, 1932; Goffart, 1938) no differences were found in growth reduction of plants inoculated with nematodes and fungi or

nematodes alone. On the contrary, Dunn and Hughes (1964) and Ketudat (1968) obtained greater reduction of growth of tomato plants in the presence of both *R. solani* and PCE than PCE alone. These differences in experimental results can probably be explained by the use of juveniles instead of cysts for inoculations in the present study, as a result of which all the nematodes after entering the soil attacked roots simultaneously causing severe damage. When cysts are added, larvae hatch out gradually over a period of time and, therefore, concentration of nematodes gradually increases; the plant can resist this initial attack to some extent. Also 9 cm pots were used instead of the 25 cm ones used by the other workers thus providing greater concentration of nematodes in the root zone. Therefore, the damage due to nematode attack alone caused such a severe reduction in growth that the difference between this treatment and the combined inoculation of fungus and nematode became small.

The lack of adverse effect of *R. solani* on the growth of tomato is in agreement with Cheal (1929), Edwards (1929) and Miles (1930) on potato. Garrett (1956) called this fungus a primitive parasite which constitutes a part of the normal microbiological environment for the roots of higher plants but in the course of evolution the root system had developed resistance to its attack.

Although *R. solani* is capable of infecting plant tissues by means of infection hyphae independent of any sort of injury, Polychronopoulos *et al.* (1969) observed that it penetrated sugarbeet roots wounded by *Heterodera schachtii* Schmidt without forming any infection structures. Both *R. solani* and *C. coccodes* have been observed to enter through natural growth cracks or other types of injuries. Therefore, beside biochemical changes, mechanical injury caused by the invading juveniles appears to contribute to increased fungal attack on roots in the treatment where nematodes were followed by fungus inoculation. Slootweg (1956) noted that *Cylindrocarpon radicicola* Wr. could infect roots of lily of the valley only when some injury had occurred and, therefore, suggested that the injuries caused by *Pratylenchus* or *Hoplolaimus* sp. provided infection courts for the fungus to enter. Tissues surrounding a wound tend to suberize but in case of nematode injury this (suberization) together with other changes which may otherwise give the plant some sort of protection against microorganisms may not occur (Du Charme, 1959; Christie, 1960).

Reduction of plant growth is accompanied by an increase in the number of cysts and incidence of disease. Increased disease incidence on roots inoculated first with the nematodes and then with the fungus can be explained in two ways. Firstly, mechanical injuries caused by the juveniles facilitate entry of the fungi; secondly, giant cells produced by the nematodes are colonized by the fungi in preference to normal cells. Greater colonization by *Phytophthora parasitica* var. *nicotianae* (Breda de Haan) Tucker of giant cells caused by *Meloidogyne incognita* Chitwood than of normal cells and by *R. solani* of giant cells caused by *H. schachtii* has been reported by Powell and Nusbaum (1960) and Polychronopoulos *et al.* (1969), respectively. Wilt of tobacco caused by *Fusarium oxysporum* var. *nicotianae* (Johnson) Snyd. and Hans. was more severe when its inoculation was preceded by root knot inoculations (Porter and Powell, 1967) and giant cells (by *M. incognita*) produced in both wilt resistant and susceptible varieties were colonized vigorously (Melendez and Powell, 1967).

Presence of indole material has been demonstrated in the galled tissues as well as in the root-knot nematodes themselves by Yu and Viglierchio (1964) and in the juveniles of *H. schachtii* by Johnson and Viglierchio (1969). In the present studies, indole material has been found in PCE which is likely to be injected into the giant cells during feeding and may be a factor contributing to increased fungus attack in them.

Normally the attack of *R. solani* is not observed deeper than the cortex but in giant cells the mycelium reaches up to the stele and may subsequently spread laterally. In the tissues ramified by mycelia of either *R. solani* or *C. coccodes*, giant cells either cannot be formed or become deformed as has been observed in case of the grey sterile fungus (Roy, 1968). 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 the fungus inoculation precedes nematode is that in the former two cases PCE gets more change to attack healthy roots and, therefore, the fungi could penetrate deep into the tissues through the giant cells causing extensive damage and decay.

Temperature differences between the two houses account for the greater number of cysts produced in house 1, and consequent poor plant growth compared with house 2. In house 1 the temperature

was more suitable for development of PCE (Fenwick, 1951) whereas that in house 2 was more congenial for growth of the plants (Bewley, 1951) enabling them to resist the attack of nematodes to some extent.

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SUMMARY

In investigations into the interrelationships between *Heterodera rostochiensis* Woll. and *Rhizoctonia solani* Kühn and *Colletotrichum coccodes* (Wallr.) Hughes on tomato (cv. Alisa Craig) it was observed that the plants suffered greater growth inhibition when *H. rostochiensis* attacked before the fungi than when the fungi preceded the nematode. Incidence of disease due to the fungi and production of cysts of the eelworm were also greater in the former treatment than the latter. The fungi depressed emergence of juveniles from cysts but did not affect entry of the larvae into roots. The fungi retarded the formation of giant cells by *H. rostochiensis* and, therefore, development of cysts decreased. On the other hand, if the nematodes could produce giant cells before fungus invasion these were favoured by the fungi for colonization. The juveniles of *H. rostochiensis* were found to possess some indole derivative/s in their bodies and its significance for increased colonization of the giant cells by the fungi has been discussed.

RIASSUNTO

Relazioni tra Heterodera rostochiensis *e funghi del terreno su Pomodoro.*

Studi sulle relazioni intercorrenti tra *Heterodera rostochiensis* Woll. e *Rhizoctonia solani* Kühn e *Colletotrichum coccodes* (Wallr.) Hughes su Pomodoro « Alisa Craig » indicano che le piante hanno sofferto riduzioni della crescita maggiori quando il nematode è stato inoculato prima dei funghi che viceversa. Anche l'incidenza della malattia e la produzione di cisti da parte del nematode sono state maggiori nel caso che gli attacchi di *H. rostochiensis* hanno preceduto quello dei funghi. I funghi hanno depresso l'emergenza di stadi giovanili del nematode dalle cisti, ma non la penetrazione degli stessi nelle radici. Tuttavia, essi hanno ritardato la formazione delle cellule giganti e quindi ridotto la produzione di cisti. Al contrario, la colonizzazione dei funghi è stata favorita quando i nematodi hanno provocato la formazione di cellule giganti. La presenza di derivati indolici nel corpo dei nematodi potrebbe essere la causa dell'aumentata colonizzazione da parte dei funghi sulle cellule giganti.

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