Interaction Between *Rhizoctonia solani* and *Meloidogyne hapla* on Radish in Gnotobiotic Culture

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ABSTRACT

There have been relatively few attempts to study the interaction between root-infecting fungi and nematodes in gnotobiotic cultures in agar medium. In this study of interaction between *Rhizoctonia solani* and *Meloidogyne hapla* on radish (*Raphanus sativus*) in gnotobiotic culture in petri-plates, it was observed that prior infection of roots by nematode favoured the colonizing capability of the fungus. Galls were preferred by the fungus and mycelium accumulated over them. Vigorous mycelial growth and abundant sclerotial formation was observed on galls. Extensive necrosis of galls due to penetration of fungal hyphae resulted and roots became obliterated and whole galled tissue was colonized. Non-galled portions did not show sclerotial formation, but more hyphal growth and penetration was observed in contrast to the roots inoculated wth fungus alone.

This indicates that the apparent physiological changes, especially, in the galled regions due to nematode infection pre-disposed the roots for invasion and rapid colonization by the fungus.

INTRODUCTION

Soil-borne fungi are known to interact with nematodes and enhance the necrosis and decay of roots (1). It has been proposed that root-knot nematodes in their early stage of infection and gall formation when galls remain smooth do not favour the invasion of galls by root-or-soil-inhabiting fungi. Later several infection courts are formed by cracking of galls. This facilitates the entry of root-invading fungi which eventually cause necrosis and root-decay (2, 12).

Root-knot nematodes have been reported to predispose tobacco plants to severe damage by minor tobacco pathogens such as *Pythium ultimum* and *Rhizoctonia solani* (1, 8). Melendez and Powell (7) observed the necessity of prior invasion of roots of tobacco by *Meloidogyne incognita* for the necrosis caused by *P. ultimum*. Melendez and Powell (8) found no appreciable fungal invasion of nematode-free roots but galled regions of the roots were readily invaded and rapidly colonized by *P. ultimum*. Root-

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knot nematode infection of tobacco is also known to enhance the invading and colonizing capabilities of *R. solani* (1). Even some non-aggressive secondary root-invaders and other soil-borne fungi have been found involved in necrosis and decay of roots infected with root-knot nematodes (11). Golden and Van Gundy (4) observed that the galled roots of tomato and okra infected with *M. incognita* were highly susceptible to *R. solani*. Fungal sclerotia were formed only on nematode-gall tissue.

In recent years, gnotobiotic culturing of roots have provided a useful tool for the study of different aspects of nematode-host relationships. This is a suitable technique to understand the mechanism of interaction of various pathogens inhabiting cultivated soil (13). *R. solani* is an ubiquitous soil-inhabiting fungus infecting a variety of cruciferous vegetables. Its involvement in certain disease complexes with some plant parasitic nematodes is natural (5, 6). Therefore, its possible interaction with *Meloidogyne hapla* on radish was studied in gnotobiotic agar culture.

MATERIALS AND METHODS

A weakly pathogenic isolate of *Rhizoctonia solani* Kuhn from crucifers maintained on potato-dextrose-agar in pure from and pure population of *Meloidogyne hapla* Chitwood, 1949 maintained on tomato in greenhouse, were selected for the study. For surface sterilization, seeds of radish (*Raphanus sativus* L.) were first stirred in 1% streptomycin sulphate for 30 minutes and washed repeatedly with sterilized distilled water. Then seeds were stirred in 0.2% Mercuric chloride for 15 minutes and were again repeatedly washed with sterilized distilled water. These surface sterilized seeds were plated on water agar (0.3%) and incubated for germination. After two days some seeds with emerging radicles were transferred to fresh water agar plates and were allowed to grow in an incubator. After five days, tops of the seedlings were incised from hypocotyl region and one seedling was placed in each of the 40 half-divided petri-plates with cut ends in one half containing Dropkin and Boone nutrient medium (3) and the root ends in the other half containing water agar (0.5%). Both ends were gently appressed at surface of the media. Plates were kept in four different sets as given below:

Set A. Plates without any nematode and fungus inoculation (check)
Set B. Plates for nematode inoculation only.
Set C. Plates for fungus inoculation only.
Set D. Plates for both the nematode and the fungus inoculation.

Roots of plants in each petri-plate of the sets that were to receive nematode were inoculated with 50 second stage larvae of *M. hapla*. Before inoculation, larvae were surface sterilized by the method described by Muller (9). Plates inoculated with *M. hapla* were observed for the formation of galls two weeks following inoculation. Wherever galls were recognizable in the set D, a semi-circular plug of *R. solani* grown on PDA was placed in the division having roots with water agar and plates were incubated. Plates of set C were also similarly inoculated with *R. solani*. All these operations were done at the Clean Bench (Ceag Schirp Reinraumtechnik, 4711 Bork/Westf. Deutschland) under aseptic conditions.

A week after inoculation, mycelial growth pattern and sclerotial formation of *R. solani* on the galled and non-galled regions of the roots inoculated with *M. hapla* and

on roots inoculated with the fungus alone were examined. Galled and non-galled regions of the roots from set D and root-portions from sets A and C and galled regions from set B were collected and fixed for sectioning. Some galls in set D were left and collected after 2 weeks of fungal inoculation for sectioning. Materials were dehydrated and embedded in monomere solution kept in gelatin capsules. Capsules were sectioned by rotary microtome and sections were stained with Toluidin Blue. Sections were mounted in Eukitt and examined microscopically.

RESULTS AND DISCUSSION

Galls were evident on the root tips one week after nematode inoculation. When root tips were excised and stained in lactophenol cotton blue, larvae in different stages were observed. Recognisable galls were formed 2 weeks after inoculation (Fig. 1). Roots became stunted and less luxuriant in comparison to the non-inoculated roots. After one week, sparse mycelial growth on the roots in the plates inoculated with *R. solani* alone was observed. Roots did not exhibit any apparent necrosis but were poorly growing. Myclelium lacked monilioid cells and no sclerotia had developed.

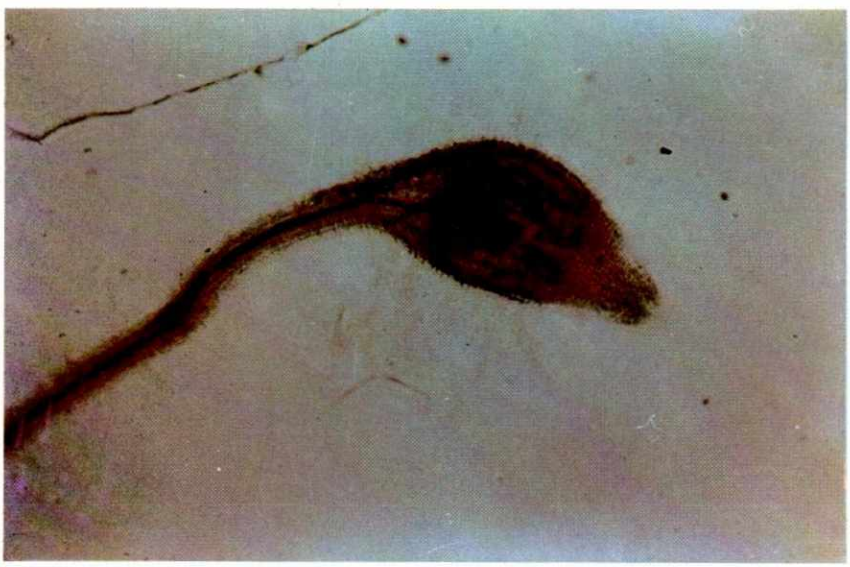

Fig. 1. A developing gall, caused by *Meloidogyne hapla* at a root tip of radish.

One week following fungal inoculation and three weeks after nematode inoculation, mycelial growth on galls was luxuriant with numerous sclerotia in various stages of their development. Mycelium and sclerotia were firmly attached to gall surfaces and entire gall surfaces were covered with monilioid cells (Fig. 2). Mycelial growth on the non-galled region was much less in comparison to those on galled region and there was no sclerotial formation (Fig. 2). These observations are similar to those of Golden and Van Gundy (4) who observed formation of black sclerotia by *R. solani* on the surface of cellophane membranes directly opposite to the galls induced by

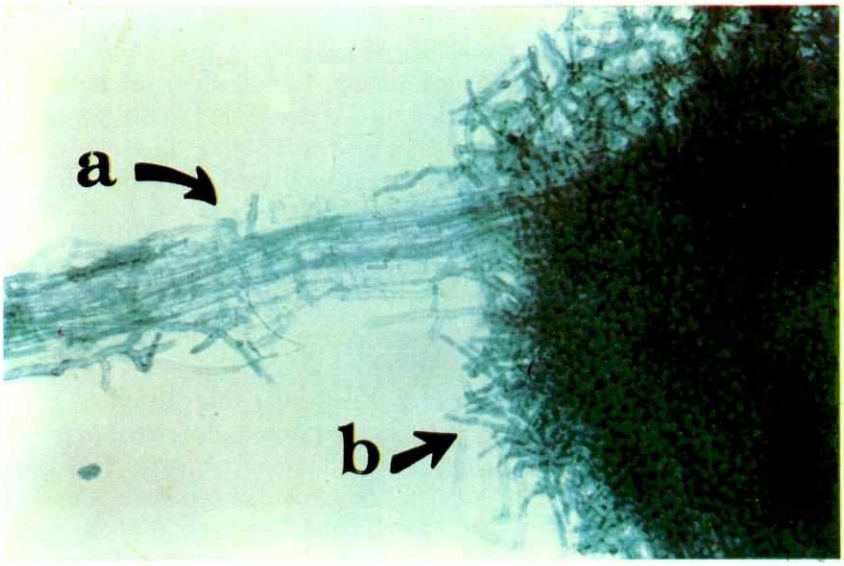

Fig. 2. Colonization of galled and non-galled areas. a. Part of the root adjacent to the gall showing sparse mycelial growth. b. Part of the gall heavily colonized with luxuriant mycelial growth and abundant sclerotial formation at the surface.

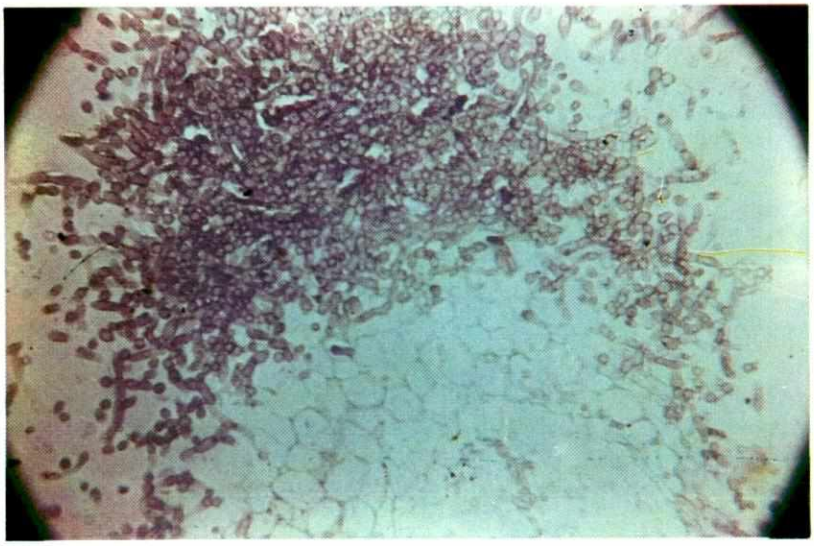

Fig. 3. A section of a gall with initial colonization by *Rhizoctonia solani* with evident monilioid cells mostly confined to cortical zone.

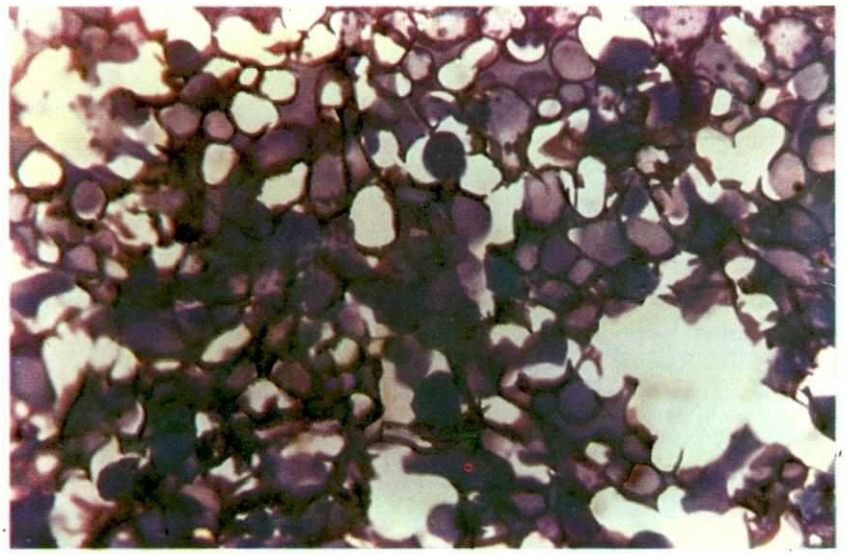

Fig. 4. A section of completely colonized gall showing monilioid cells and sections of sclerotia formed in decayed and obliterated gall.

M. incognita while non-galled portions of the nematode infected and the control treatment were free of sclerotia. They hypothesized that the leakage of nutrient from the root was presumably responsible for attracting the fungus to the galls and for initiating sclerotial formation. In the present study, accumulation of mycelium and abundant sclerotial formation on galled tissue, apparently, indicate some biochemical changes and release of nutrient favouring mycelial growth and scelerotial formation features. This supports the hypothesis of Golden and Van Gundy (4).

Histology of non-infected roots (Set A) showed a normal differentiation of simple and complex tissues in the roots growing on water agar. Sections of galls induced by *M. hapla* demonstrated the presence of females with giant cells in the vicinity of their neck region. Cortical cells proliferated to form the galls. Sections of roots inoculated with *R. solani* alone showed the presence of hyphae without any sclerotial formation. Necrosis of the roots was not apparent even after 3 weeks of inoculation. Sections of galls after one week of fungus inoculation showed that mycelium had traversed the cortical portion of galls and was approaching the stelar region. Giant cells were found to be colonized by the hyphae, and sclerotia were present on the gall surfaces. Initiation of sclerotial development in the cortical region was observed (Fig. 3), but the histology of galls after two weeks of *R. solani* inoculation showed complete colonization of cortical and stelar tissues. Root tissues were obliterated at several places. Monilioid cells and sclerotia in different stages of development were present throughout the root tissues with no evidence of *M. hapla* females or associated giant cells (Fig. 1, 4). Similar observations have been reported by Batten and Powell (1) and Golden and Van Gundy (4). Extensive *R. solani* colonization of *M. incognita* infected roots were observed by Batten and Powell (1). Vigorous hyphae encircled the nematode and were concentrated within the locus of nematode infection. Golden and Van Gundy (4) also observed the destruction of giant cells due to *R. solani* colonization.

The fungus was observed in the surrounding xylem elements in the roots of okra and tomato.

The growth pattern of *R. solani* on roots, in the present study, revealed its preference for galls. Non-galled regions of the roots were also invaded but not as well. Although this study demonstrated an interaction between two organisms on radish in gnotobiotic culture, it may not be obligatory in nature within a complex biotic community of the soil ecosystem. Nevertheless, this study has successfully demonstrated the possibility of such interaction in nature. According to Zuckerman (13), the results of even a few gnotobiotic experiments involving plant nematode-fungus interactions have confirmed the role of nematodes in enhancing the severity of other plant diseases as analysed by Powell (10). The present study in gnotobiotic system also supports this hypothesis.

ACKNOWLEDGEMENTS

The senior author is thankful to Dr. B. Weischer, Director Institut fur Nematologie, Munster (West Germany) for laboratory facilities and to the German Academic Exchange Service (DAAD), West Germany and the University Grants Commission, India for financial support during the study stay of the senior author under Indo-German Young Scientists Exchange Program.

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التأثير المشترك لفطر عفن الجذور (*Rhizoctonia solani*) ، وديدان تعقد الجذور (*Meloidogyne hapla*) على نباتات الفجل في بيئة معقمة

د. محمد واجد خان د. ج. مولر

المستخلص

تعتبر المحاولات السابقة لدراسة التأثير المشترك للفطريات التي تصيب الجذور ، والنيماتودا في بيئات آجار معقمة غير كافية نوعاً ما .

في هذه الدراسة التي أجريت لبحث التأثير المشترك لفطر عفن الجذور (*R. solani*) وديدان تعقد الجذور (*M. hapla*) على نبات الفجل تحت ظروف صناعية معقمة في أطباق بتري ، لوحظ أن إصابة الجذور بالنيماتودا .

أولاً : زادت من قدرة الفطر على إحداث العدوى ، كما أن العقد الناتجة عن الإصابة بالنيماتودا كان لها الأفضلية من قبل الفطر حيث أن الغزل الفطري تراكم حولها وكان النمو الفطري وتكوين أمشجة صلبة (*Sclerotia*) أغزر على هذه العقد .

وكان من نتيجة عملية الإختراق من قبل الفطر غزو أنسجة العقد كلية وتحللها بشكل ملحوظ ، كما بدأت الجذور في التلاشي تماماً ، وبينما لم تتكون أمشجة صلبة على الأجزاء غير المتعقدة فإن نمو الخيوط الفطرية كان أوضح بالمقارنة إلى الجذور التي لم تصب إلا بالفطر وحده . وهكذا يتبين أن حدوث تغييرات فسيولوجية خصوصاً في منطقة العقد نتيجة الإصابة بالنيماتودا جعل الفطر أقدر على عملية الإختراق والإنتشار السريع داخل الجذور .