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STUDIES ON THE INTERACTIONS OF *MELOIDOGYNE INCOGNITA*, *RADOPHOLUS SIMILIS* AND *FUSARIUM SOLANI* ON BLACK PEPPER (*PIPER NIGRUM* L.)

Promotor : dr.ir. A.F. van der Wal
hoogleraar in de nematologie
Co-promotor: dr.ir. P.W.F. de Waard
senior agronomist
specialized in black pepper

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STELLINGEN (THEOREMS)

1. *Radopholus similis* is the primary causal agent of yellow disease of black pepper.

   This thesis.

2. Yellowing of leaves of black pepper not associated with a stiff droop has other causes than *R. similis*, e.g. *Meloidogyne incognita* in combination with *Fusarium solani*.

   This thesis.

3. The symptom expression of yellow disease of black pepper can be modified by varying abiotic factors such as soil moisture and minerals.

   This thesis.

4. The control of plant diseases is like a continuous war between the grower and pathogens. To be successful, well planned strategies are needed, e.g. the control of yellow disease of black pepper should not be directed solely towards controling *R. similis*.

5. Despite the fact that *F. solani* is able to reduce the populations of *R. similis* and *M. incognita*, this fungus can not be used as a biological control agent.

   This thesis.

6. The first European currency was not ECU, but "peppercorn". It was used to pay taxes, tolls, rents and dowries already in the Middle Ages.

   Purseglove et al., 1981. Spices. Vol. 1, p. 10-29.

7. The value of black pepper as a remedial agent for spleen diseases, stomachaches, dysentery, itches, rheumatism, and impotence is under estimated in Western Europa.

   Kercher, J., 1989. Aroma therapie voor iedereen.

8. The increase interest of Dutch people in Indonesia as expressed in the increasing number of tourists to the "Gordel van smaragd" is not supported by an adequate Dutch effort to establish economic links.

9. The first challenge after having training abroad is how to minimize the "reverse culture shock".

Ika Mustika
Wageningen, 23 November 1990

CONTENTS

Preface

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1 General introduction

Black pepper[1] (*Piper nigrum* L.) is one of the oldest cultivated crops in Indonesia. Probably this plant came originally from Western Ghats, in the State of Kerala, India, when many Hindu colonists came to Indonesia, as early as 100 BC, and introduced this plant to Java (Purseglove et al., 1981; de Waard, 1986).

In the 16th century, it was grown only on a small scale. However, in the 18th century it became widely cultivated.
Before the 1950's Indonesia produced almost 80% of the world production of pepper. In 1984 the world production of pepper approximated 110,000 tonnes. Indonesia's share was only 25,000 tons or 22.7%, while Brazil, India and Malaysia produced as much as 33,000, 30,000 and 17,000 tons respectively (de Waard, 1986). Other countries including Malagasy, Sri Lanka, China and Kampuchea produced a total of 9800 tons.

Pepper is mainly used as a spicy and flavouring ingredient for the food industry (de Waard, 1986), as a constituent of several medicines (Kercher, 1989), and it has also insecticidal properties (Su & Hovart, 1981).
Pepper is now the fifth agricultural commodity in Indonesia preceded only by rubber, tea, oil palm and coffee. During 1979-1984, the average export value of pepper from Indonesia amounted to US $ 36.3 million per year (calculated from de Waard, 1986) while in 1984 alone, it amounted to US $ 87.9 million (de Waard, 1986).

In Indonesia this crop is mainly cultivated in the Lampung and South Sumatra provinces, particularly on the island of Bangka. These two areas produce almost 90% of the pepper in

[1] "Black pepper" in this thesis refers to the plant only.

this country. Other areas, where pepper is grown, are the provinces of Bengkulu, Aceh, West Sumatra, West, East and South Kalimantan, and South Sulawesi. However, the production of black pepper in Indonesia, is threatened by two main diseases, namely "foot rot" and "yellow disease". No effective control against these diseases has yet been found.

Foot rot disease, or " quick wilt disease", was reported by Muller in 1936. It damaged pepper crops in Indonesia (Lampung, South Sumatra and Java). *Phytophthora palmivora* var. *piperis* Muller was identified as the cause of this disease (Holliday & Mowat, 1963; Nambiar & Sarma, 1977). Later, other workers identified the fungus as *P. capsici* or *P. palmivora* MF-4 (Kasim, 1978; Tsao, et al., 1985a; 1985b). In 1967 the fungus destroyed pepper crops in Lampung, India and Sarawak, up to as much as 40%, 30% and 20% respectively (Anonymous, 1967; Nambiar & Sarma, 1977). Recently, foot rot disease was also reported to occur in Bangka. In Brazil a disease with similar symptoms was found, but the causal organism was identified as *Fusarium solani* f.sp. *piperi* Albuquerque, 1961 (Alconero et al., 1972). This fungus has also been consistently isolated from pepper plants affected by nematodes in Bangka (Bridge, 1978). In this study no further attention is paid to foot rot disease.

Yellow disease, or "slow wilt disease", was first reported by Van der Vecht in 1932 on the island of Bangka. This disease had destroyed up to 90% of the pepper crops in Bangka (Thorne, 1961). The same or similar symptoms were also found on pepper in Thailand (Sher et al., 1969; Bridge, 1978), and in India (Venkitesan & Setty, 1977; Nambiar & Sarma, 1977; Ramana et al., 1987).

Wahid (1976) and De Waard (1979) suggested that yellow disease was primarily caused by the lack of fertilizer, and nematodes were considered to be secondary pathogens. Mean-

while, Bridge (1978) isolated consistently the nematodes *Radopholus similis*, and *Meloidogyne incognita*, and the fungi *Fusarium oxysporum* and *Fusarium solani* from diseased roots. Based on these findings, he suggested that the yellow disease complex, rather than being caused by a single pathogen, a nematode or a fungus, was caused by the interaction of nematodes and fungi. In his opinion, *R. similis* was the major causal agent which could predispose the plants to secondary pathogens like *Fusarium* spp. Dropkin (1980) suggested that nematodes often initiate the pathological process in which other organisms, such as bacteria or fungi follow, and, in combination with the nematodes, inflict damage. Soil characteristics such as texture, moisture content (Dropkin, 1980) and pH (Wallace, 1970) affect crop diseases induced by nematodes. Soil moisture has also a direct influence on the capability of nematodes to cause disease symptoms (Dropkin, 1980). Data of the effect of *R. similis*, *M. incognita* and *F. solani* on pepper, particularly in Indonesia, are still scarce.

Nematodes associated with black pepper

Nematodes have been known to infect and cause damage to black pepper. Zimmerman (1901, cit. Winoto, 1972) was the first researcher who presented evidence that root-knot nematodes (*Meloidogyne* spp.) infected black pepper plantings in Java, Sarawak and Johore. Later, Van der Vecht (1950) reported that *R. similis* also infected black pepper, and causing yellow disease in Bangka. Sher et al. (1969) found a number of nematodes associated with black pepper in Thailand viz. *M. javanica, M. incognita, Tylenchulus semipenetrans, Rotylenchulus reniformis, Helicotylenchus erythrinae, Pratylenchus coffeae, Tylenchorhychus spp., Hemicriconemoides* spp., *Hoplolaimus seinhorsti, R. similis and Xiphinema* spp.. Bridge (1978) isolated consistently the following nematodes from black pepper in Bangka viz. *R. similis, M. incognita, Macrophostonia ornata, Xiphinema in-*

signe, Pratylenchus coffeae, Aphelenchus spp., *Ditylenchus* spp., and *Tylenchus* spp. Sundararaju et al. (1979) have listed as many as 35 species and genera of plant parasitic nematodes associated with black pepper. More recently, Mohandas et al. (1985) found that *Trophotylenchulus piperis* parasitized the roots of black pepper in Kerala, India.

Damaging effects of nematodes on black pepper have been studied by several workers. Winoto (1969) showed that *M. incognita* and *M. javanica* could both significantly reduce black pepper growth. Venkitesan and Setty (1978) studied the pathogenicity of *R. similis*, and found a significant growth reduction of black pepper. Other plant parasitic nematodes such as *M. arenaria, Hoplolaimus seinhorsti* and *Xiphinema ifacolum* also appeared to affect the growth of black pepper adversely (Lamberti & Ekanayake, 1983). Of these nematodes, *R. similis* and *M. incognita*, are often predominant in infecting black pepper roots. These two nematode species have been observed in increasing frequency in black pepper plantings in Indonesia (Mustika, 1978; Bridge, 1978; s'Jacob, personal communication) and in India (Nambiar & Sarma, 1977; Ramana & Mohandas, 1987; Ramana et al., 1987). Therefore these two species were selected for the experiments reported here.

Apart from black pepper, *R. similis* attacks many crops. This nematode is reported to reproduce on more than 250 plant species (O'Bannon, 1977). It has long been considered as the cause of yellow disease of black pepper, and "toppling disease" of banana (Van der Vecht, 1950; Christie, 1959; Thorne, 1961; Blake, 1961, 1966).

R. similis (Cobb, 1893) Thorne, 1949 was first recorded from banana roots in Fiji, and appeared to be widespread in tropical and subtropical regions (O'Bannon, 1977). *R. similis* is a migratory endoparasite, known as "burrowing nematode" (Cohn, 1972), This nematode penetrates the roots of the host, and migrates, destroying the cortex tissues, and de-

velops inside the roots from the eggs deposited along the path of migration. The juveniles hatched from these eggs continue to destroy tissues, and extensive lesions develop in the cortex and stele, frequently resulting in destruction of infested roots (Dropkin, 1980). Plants infected by *R. similis* usually display poor growth, yellow discoloration, wilting, and they eventually die (Williams & Siddiqi, 1973).

M. incognita (Kofoid & White, 1919) Chitwood, 1949 is a sedentary endoparasitic nematode, causing swellings of the roots. The nematode appears to be widespread on black pepper (Holliday & Mowat, 1963; Winoto, 1972; Sharma & Loof, 1974; Nambiar & Sarma 1977; Kueh & Teo, 1978; Kueh, 1979; Ichinohe, 1980).
Heavily infested roots by *Meloidogyne* spp. are deformed and vascular elements are disrupted. As a result, translocation of water and nutrients is impeded. The growth is retarded. Under dry conditions wilting may occur, and also symptoms of nutrient deficiencies may develop (Taylor & Sasser, 1978). The nematode may alter nutrient flow patterns in plant tissues, reducing root growth, which may contribute to lower plant yield (Hussey, 1985). On tomato, infection by *Meloidogyne* spp. is frequently associated with chlorosis, stunting, and aggravation of nutrient deficiency symptoms (McClure, 1977). Similar symptoms were also reported on black pepper infected by *Meloidogyne* spp. in Sarawak (Winoto, 1972; Kueh, 1979).

Nematode-fungus-plant interaction

Plants are usually attacked by several pathogens at the same time. The effects of multiple infection by different pathogens can either be greater than, smaller than or similar to the sum of effects attributed by each pathogen (Waller & Bridge, 1984). However, most pathogens interact synergistically on plants.

Synergistic interactions of *M. incognita* have been reported with *Phytophtora parasitica* var. *nicotianae* (Powell & Nusbaum, 1960), with *Rhizoctonia solani* on tobacco (Baten & Powell, 1971); with *F. oxysporum* f. sp. *vasinfectum* on cotton (Garber et al., 1979; with *F. oxysporum* f. sp. *lycopersici* on tomato (Morrel & Bloom, 1981); and with *F. oxysporum* Schlecht f. sp. *niveum* on summer-squash (Caperton et al., 1986).
Synergistic interaction of *R. similis* and fungi was also reported, with *F. oxyporum* f. sp. *cubense* on banana (Blake, 1961, 1966) and with *Fusarium* spp. on grapefruit seedlings (Feder & Feldmesser, 1961).

Waller and Bridge (1984) also recorded several synergistic interactions of nematodes and *Fusarium* spp. on some tropical crops: e.g. *M. incognita, Belonolaimus gracilis* and *Rotylenchulus similis* and *Helicotylenchus multicinctus* with *F. oxysporum* f. sp. *cubense* on banana; *Tylenchulus semipenetrans* with *F. oxysporum* f. sp. *lycopersici* on tomato.

Many researchers have reported that infection by nematodes or fungi caused mineral alterations in crops. Oteifa (1952) found that infection by *M. incognita* in lima bean (*Phaseolus lunatus* L.), decreased the total amount of N, P, K, Ca and Mg. McClure (1977) suggested that *M. incognita* is a "metabolic sink". Plants infected by this nematode frequently show chlorosis and nutrient deficiency symptoms. Ibrahim et al. (1982) found that infection by *F. oxysporum* f. sp. *vasinvectum* induced a significant reduction in K, Mn, Zn and Cu in cotton.

R. similis was found to reduce the concentrations of N, P and K in leaves of citrus (Feldman et al., 1961). The same phenomenon may also occur in the case of yellow disease on black pepper. De Waard (1979) reported that leave contents of N, P, K, Ca and Mg in yellow leaves of diseased plants were lower than those in healthy leaves of apparently

healthy plants. He also found that the root system was invaded by nematodes when yellow disease occurred. Recent field observations on the relationship between yellow disease and nematode populations, indicated that leaf contents of N, K and Ca in diseased plants were significantly lower than those of healthy plants. Root and soil populations of *R. similis* in diseased plants were significantly higher than in healthy plants, whereas the root and soil populations of *M. incognita* in affected plants tended to be higher than in healthy plants. There were no significant differences in soil N, P, K, Ca and Mg among plots with either diseased or healthy plants (Mustika, 1986, unpublished).

Considering the economic importance of black pepper for some 200,000 smallholders in Indonesia, strategies to control the yellow disease are needed, and hence actual knowledge of its cause is required to form the basis for these strategies. In this thesis, the role of two species of nematodes, *M. incognita* and *R. similis*, is studied in combination with the fungus *F. solani* under conditions of nonlimiting mineral nutrients. The experiments were conducted at the Department of Nematology, Wageningen Agricultural University (WAU), The Netherlands, from April 1987 to July 1989.

In the course of this investigation two *in vitro* studies and five pot experiments were carried out. Pot experiments were both conducted in the greenhouse and in a climate chamber. The first *in vitro* study was set up to test the influence of temperature on the multiplication of *R. similis* on carrot discs (Chapter 3). Two experiments were done in the greenhouse to evaluate the effect of *M. incognita* (Chapter 2) and *R. similis* (Chapter 4), on the growth and development on black pepper, in the presence of *F. solani*. The fourth pot experiment dealt with the interactions between *M. incognita*, *R. similis*, and *F. solani* (Chapter 5). *In vitro* study on the effect of a culture filtrate of *F. solani* on hatching of

M. incognita and *R. similis* was also carried out (Chapter 6). In order to check for tolerance to those three pathogens, the response of four cultivars of black pepper to infection was studied (Chapter 7). The last pot experiment was carried out to study the interactions of soil moisture, *R. similis*, *M. incognita* and *F. solani*, on leaf nutrient contents of black pepper (Chapter 8).

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2 Interactions of *Meloidogyne incognita* and *Fusarium solani* on black pepper (*Piper nigrum* L.)

Ika Mustika

ABSTRACT

Single node cuttings of black pepper (*Piper nigrum* L.) cv. Kuching were inoculated with *Meloidogyne incognita* alone and in combination with *Fusarium solani*. Two combinations of inoculation of *M. incognita* and *F. solani* were examined, i.e. simultaneous inoculation and *M. incognita* inoculated two weeks before *F. solani*. Soil moisture was at field capacity, and mineral nutrition was not limiting. Plants inoculated with *M. incognita* and *F. solani* either alone or in combination, did not provoke symptoms of yellow disease. Wilting and dying of plants occurred earlier when *M. incognita* was inoculated together with *F. solani*, compared to *F. solani* alone.
The population of *M. incognita* was reduced when *F. solani* was also present.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) were reported to infect black pepper (*Piper nigrum* L.) by Zimmerman in 1901 in Java, Indonesia (cit. Holliday & Mowat, 1963). Black pepper plantings in Sarawak were found to be infested with *Meloidogyne javanica* and *M. incognita* (Holliday & Mowat, 1963; Winoto, 1972), in India with *M. incognita* (Koshy & Sundararaju, 1979), in Bangka, Indonesia, with *M. incognita* (Bridge, 1978; s'Jacob, personal communication), in Brazil by *M. incognita* (Sharma & Loof, 1974; Ichinohe, 1980), and in Sri Lanka with *M. incognita* and *M. are-*

naria (Lamberti & Ekanayake, 1983). *M. incognita* and *M. javanica* were reported to cause growth reduction, leaf yellowing and decline on black pepper (Winoto, 1972; Kueh & Teo, 1978; Kueh, 1979).

Apart from nematodes, Bridge (1978) isolated consistently several fungi such as *Fusarium solani* and *F. oxysporum* from black pepper roots with yellow disease symptoms. *F. solani* has been recognized as the cause of decay of the underground stem and roots of black pepper in Brazil (Albuquerque cit. Alconero et al., 1972). The role of this fungus in association with *M. incognita* on black pepper in Indonesia is hardly documented. The aim of this study was to determine the effect of *M. incognita* alone or together with *F. solani*, to evaluate the role of *M. incognita* in predisposing black pepper to an attack of *F. solani*, and to observe whether or not these combinations of the pathogens could induce "yellow disease" symptoms.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse which was set to reach a maximum air temperature of 28 °C during the day, and a minimum night temperature of 23 °C, with relative humidity fluctuating from 50% to 80%. The soil temperature fluctuated between 19 °C, and 25 °C. The day length was at least 12 hours, using suplementary light.

M. incognita, isolated from black pepper roots in Indonesia, was cultured on tomato roots of c.v. Moneymaker. To prepare the inoculum of *M. incognita*, heavily infested tomato roots were chopped and incubated in a mistifier for one week. The resulting suspensions were collected and used as inoculum. Before inoculation, the nematodes were surface sterilized with a solution of 0.02% aqueous ethoxyethylmercury chloride and 0.1% streptomycin sulphate, using a micropore filter. After surface sterilization, the nematode suspensions were

diluted with sterile distilled water. Each plant was inoculated by placing 10 ml of this suspension containing 1250 second stage juveniles on the soil surface around the stem, at a distance of approximately 5 cm. Inoculum levels used in this study were based on the average number of *M. incognita* in 1 liter of soil taken from diseased plants (Bridge, 1978).

A culture of *F. solani*, isolated from black pepper roots, was grown on potato dextrose agar (PDA) in Petri dishes and incubated for six days at 30 °C. To prepare the inoculum of *F. solani*, three discs of this culture (3 mm in diameter) were transferred to 500 ml flasks containing 100 ml of potato dextrose broth (PDB) as described by Latin and Snell (1986). The flasks were put on a shaker operating at 96 rpm, while the temperature was maintained at 30 °C. After five days the contents of the flasks were mixed and filtered through two layers of filter paper. The filtrate was diluted with sterile distilled water to obtain inoculum of 10^5 microconidia per ml. Each plant was inoculated with 10 ml of inoculum as mentioned above. The inoculum levels used were according to Deverall (1981).

Three-months-old rooted pepper cuttings of cv. Kuching were transplanted into clay pots, one plant per pot, filled with 1250 g of autoclaved sandy soil. The composition of the soil is presented in Annex 1. Before planting, 3 g of Dolocal per pot were added (for composition see Annex 2). To maintain the pF value at approximately field capacity during the experiment, plants were watered every day with 100 ml of tap water. Nutrient solution (composition Annex 2) was applied weekly, at a rate of 100 ml per pot to reach a nonlimiting nutrient level.

Five treatments were tested and arranged in a randomized block design with five replicates. Each replicate consisted of five plants. The treatments were:

1) Uninoculated control (C).
2) Inoculated with *M. incognita* (Mi).
3) Inoculated with *F. solani* (Fs).
4) Inoculated with *M. incognita* and *F. solani* simultaneously (Mi+Fs).
5) First inoculated with *M. incognita*, after two weeks followed by an inoculation with *F. solani* (Mi)+Fs.

The experiment was terminated four months after nematode inoculation, and the following characteristics were measured:

1. Initial and final plant height, the length of internodes, leaf area[1], fresh and dry weight of shoot and roots.
2. Period between nematode inoculation and the first yellow leaf symptoms, percentage of wilted and dead plants, and type of droop.
3. Number of galls, final number of nematodes in soil and roots.
4. Histopathological changes in the roots.

The nematodes from soil and roots were extracted by means of the Oostenbrink elutriator and a centrifugation method respectively (s'Jacob & Van Bezooijen, 1984). Roots from all treatments were selected one month after nematode inoculation and prepared for histological studies. Specimens were fixed in FP 4:1, dehydrated in ethyl alcohol, embedded in paraffin, sectioned by using a microtome set up 10 µm, and stained with Cason's stain (s'Jacob & Van Bezooijen, 1984). Fresh unsectioned roots were stained with the modified acid-fuchsin method (Byrd et al., 1983).

RESULTS

Effects of *M. incognita* alone or in combination with *F. solani* on the growth of black pepper are shown in Table 1 and

[1] Measured by using a leaf area meter (Daiichi Borki Shokai Co., Tokyo, Japan).

2. In all treatments, there was a reduction of plant growth. In comparison with control plants, *M. incognita* or *F. solani* alone significantly reduced plant height, leaf area, and fresh and dry weights of shoots and roots. In addition, *F. solani* alone significantly reduced the number of nodes and leaves. The differences between the effects of *M. incognita* and *F. solani* alone or in combination on plant height, number of nodes, length of nodes and number of leaves were not significant.

In general, however, the strongest growth reduction was observed when plants were inoculated with *M. incognita* two weeks before *F. solani*.

Table 1 Effects of *M. incognita* and *F. solani* on plant height, number and length of nodes and number of leaves of black pepper.

Treatments *)	Plant height (cm) initial	final	Number of nodes	Length of nodes (cm)	Number of leaves
C	21.8 a	45.7 b	12.0 b	4.5 a	21.2 b
Mi	21.5 a	37.6 a	9.9 ab	4.1 a	15.8 ab
Fs	20.0 a	34.7 a	9.2 a	4.2 a	11.7 a
(Mi+Fs)	20.7 a	40.4 b	9.4 a	4.1 a	13.4 a
(Mi)+Fs	20.1 a	35.1 a	8.7 a	4.0 a	11.8 a

Numbers followed by the same letters in each column are not significantly different at 5% level (LSD-test).
*) See page 16.

Table 2 Effects of *M. incognita* and *F. solani* on leaf area, shoot and root weight of black pepper.

Treatment *)	Leaf area (cm^2)	Shoot weight (g) fresh	dry	root weight (g) fresh	dry
C	341 c	30.7 b	7.9 b	2.9 b	1.2 b
Mi	188 ab	19.9 a	5.1 a	3.2 b	1.3 b
Fs	231 b	16.5 a	5.4 a	1.5 a	0.4 a
(Mi+Fs)	198 ab	16.9 a	5.0 a	2.0 a	0.3 a
(Mi)+Fs	129 a	14.5 a	4.2 a	1.3 a	0.3 a

Numbers followed by the same letters in each column are not significantly different at 5% level (LSD-test).
*) See page 16.

The number of nematodes and galls tended to be reduced by about 50% when plants were inoculated with both *M. incognita* and *F. solani* as compared to that caused by *M. incognita* alone (Table 3).

Table 3 Effects of *M. incognita* and *F. solani* on number of galls, nematode numbers and disease symptoms on black pepper.

Treatment *)	Number of galls	Nematode populations			Period (days)		% plants wilt & dead
		soil	roots	total	wilt	dead	
C	-	-	-	-	-	-	-
Mi	77 (1.85) a	3937 (3.41)a	3831 (3.49)a	7768 (3.80)a	-	-	-
Fs	-	-	-	-	63 b	74 b	23
(Mi+Fs)	54 (1.57) a	904 (2.95)a	3636 (3.53)a	4139 (3.60)a	50 a	61ab	30
(Mi)+Fs	38 (1.51) a	1325 (2.96)a	1849 (3.18)a	3174 (3.14)a	51 a	59 a	36

Numbers followed by the same letters in each column are not significantly different at 5% level (LSD-test). Number in parentheses are averages of log x transformed original data.
*) See page 16.

Observations on the period between fungus inoculation and the first appearance of disease symptoms are presented also in Table 3. No wilted or dead plants were observed in the control or in plants inoculated with *M. incognita* alone. Infection by *F. solani* or in combination with *M. incognita* caused plants to wilt and eventually to die (Fig. 1). Wilting was due to the destruction of the underground stem. Wilting symptoms caused by *F. solani* occurred after 63 days, and death after 74 days. Infection with *M. incognita* and *F. solani* simultaneously, caused plants to wilt after 50 days, and to die after 61 days. Infection with *M. incognita* two weeks before *F. solani*, caused wilting within about the same period as in the case of simultaneous inoculation.

The percentage of plants wilted and dead by the end of the experiment, amounted to 30 and 36% respectively when plants were inoculated with *M. incognita* and *F. solani*, whereas those inoculated with *F. solani* alone, 23% showed wilting and death.

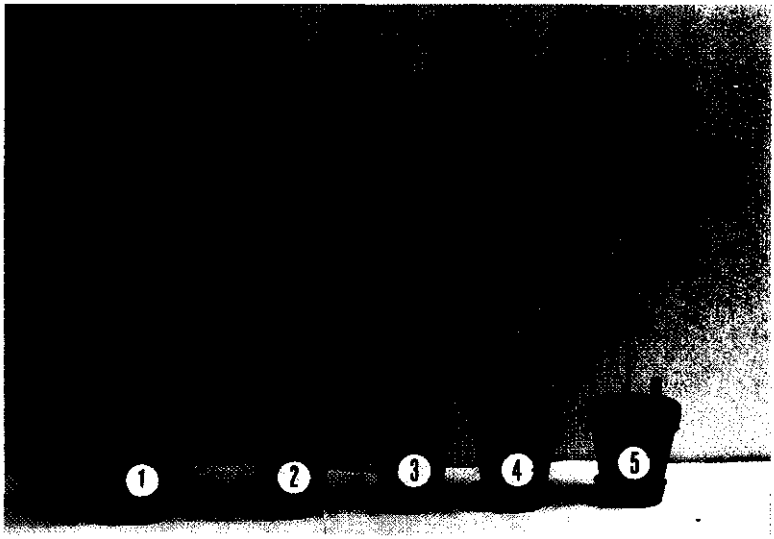

Figure 1 Symptom development of black pepper four months after inoculation. 1. Uninoculated plant; 2. Inoculated with *M. incognita*; 3. Inoculated with *F. solani*; 4. Inoculated with *M. incognita* and *F. solani* simultaneously; 5. Inoculated with *M. incognita* two weeks before *F. solani*.

Histological studies showed that *M. incognita* occupied the stelar portion of the roots. Two weeks after inoculation, the fourth stage juveniles of *M. incognita* were found to feed on the giant cells (Fig. 2A). One month after inoculation the giant cells occupied almost the entire part of the stele, and vascular system was damaged (Fig. 2B and Fig. 3B). In roots infected with *M. incognita* alone, the giant cells were well developed as compared with those infected with *M. incognita* and *F. solani* in combination. Two weeks after the inoculation with both combinations of *M. incognita* and *F. solani*, the giant cells were found to be colonized by fungal hyphae (Fig. 3A). One month after inoculation with both pathogens, the giant cells coalesced, and the stelar portion was completely destroyed (Fig. 3B). The xylem vessels of plants infected with *F. solani* were blocked with "gum like substances".

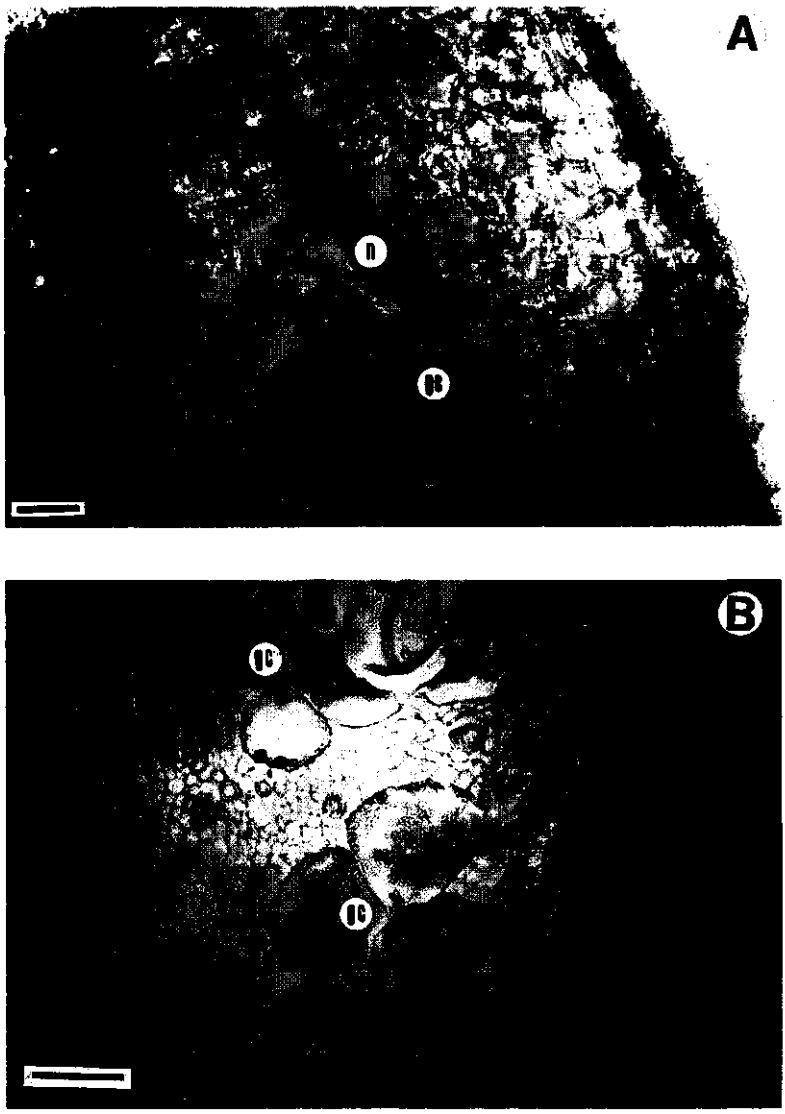

Figure 2 A. A fourth stage juvenile of *M. incognita* feeding on the giant cells (gc) two weeks after inoculation.
B. The giant cells (gc) in xylem vessels of black pepper roots induced by *M. incognita*, one month after inoculation (bar A = 100 µm; B = 250 µm).

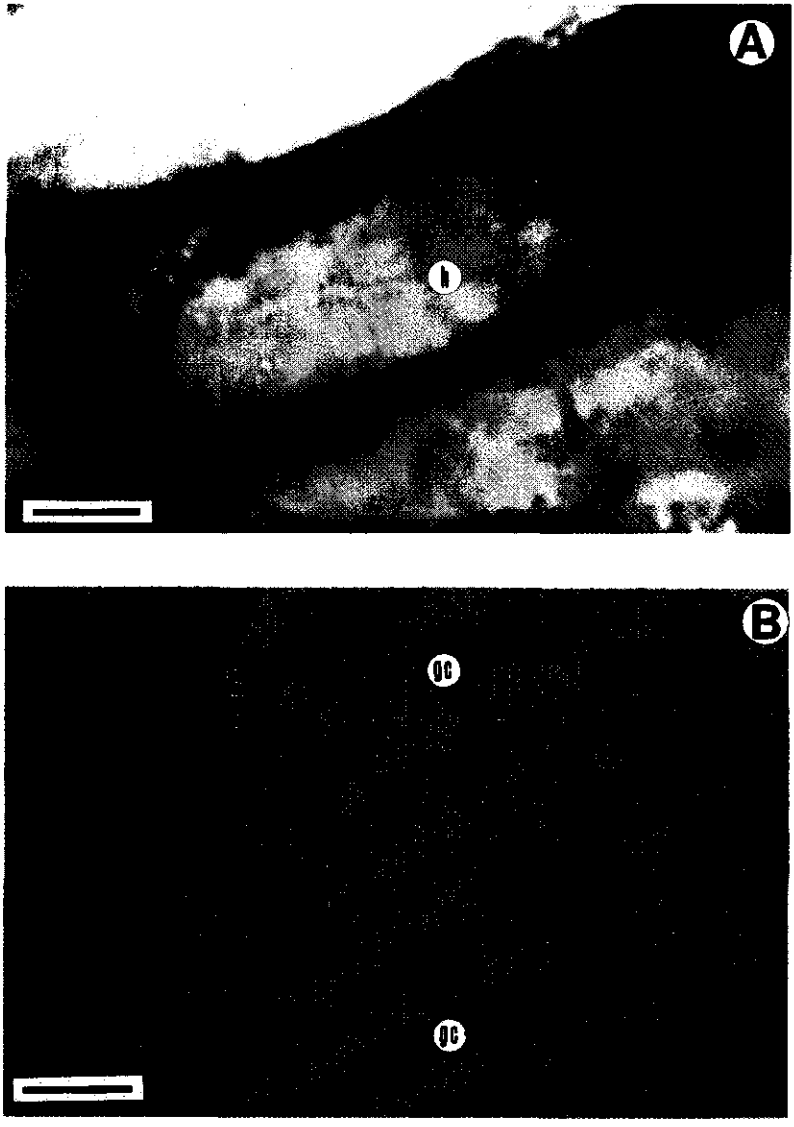

Figure 3 A. Giant cells (gc) in roots infected with *M. incognita* and *F. solani* showing the hyphae of *F. solani* (h).
B. Giant cells collapse in roots infected with *M. incognita* and *F. solani* one month after inoculation (bar A = 25 µm; B = 250 µm).

DISCUSSION

In this experiment, *M. incognita* alone or in combination with *F. solani* did not provoke the stiff droop and yellow leaves associated with "yellow disease". In contrast to *F. solani*, *M. incognita* alone did not cause plant wilt or death. But when this nematode was present together with *F. solani* inoculated either simultaneously or two weeks earlier, many plants wilted and died. Infection with *M. incognita* two weeks prior to *F. solani*, however, caused plant wilting and death earlier than when the two pathogens were inoculated simultaneously. Porter and Powell (1967) reported that wilting of tobacco plants was more serious when *M. incognita*, was present two or four weeks before *F. oxysporum*.
The present study has shown that *M. incognita* and *F. solani* alone were able to reduce leaf area and shoot weight considerably. The greatest reduction was observed when *F. solani* was inoculated two weeks after *M. incognita*. A similar effect was also reported on chickpea infected with *M. incognita* in combination with *F. oxysporum* f. sp. *ciceri* or *F. solani* (Mani & Sethi, 1987).

In the histological studies it was found that hyphae of *F. solani* colonized the giant cells. Melendez and Powell (1967) had made similar observations on tobacco infected with *M. incognita* and *F. oxysporum* f. sp. *nicotianae*. They found that the giant cells and xylem elements were heavily infected both in *Fusarium* resistant and susceptible plants. The giant cells which were extensively colonized, became apparently devoid of contents soon after invasion by the fungus. Fattah and Webster (1983) found that the structure of giant cells changed prior to fungal entry. Fungal secretions were translocated and killed the cells. According to Dropkin (1976) normal giant cells have a dense cytoplasm, an absence of vacuoles, many large polyploid nuclei, and thick walls

which are conditions for active metabolism in the cells. The giant cells surrounding the anterior portion of the nematode supply food to the nematode. Colonization by the fungus which may kill the giant cells, causes the nematodes to starve to death. This phenomenon has probably occurred in our experiment, where the number of *M. incognita* in soil and roots tended to decrease in the presence of *F. solani*.

Occlusion of the xylem vessels by "gum like substances" in roots infected with *Fusarium* in other crops has also been reported (Beckman & Halmos, 1962; Pennypacker & Nelson, 1972; Baayen & Elgersma, 1981). These substances are composed of various amounts of polysaccharides, among which are pectin and probably hemicellulose, together with oxidized polyphenols all of which are considered to be normal components of the substances. Their formation is a general phenomenon, and represents a factor in the response of plants to vascular infections (Baayen & Elgersma, 1985).

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3 Influence of temperature on the multiplication of *Radopholus similis* on carrot discs

Ika Mustika

ABSTRACT

The multiplication of *Radopholus similis* was studied on carrot disc cultures at different temperatures. Over a 35 days period at 20 °C, 25 °C, 27 °C and 30 °C the nematode multiplied as much as 11, 337, 439 and 56 times respectively. All stages of *R. similis* developed at temperatures ranging from 20 °C to 30 °C during 35 days of incubation. The nematode was able to complete at least one life cycle at the temperatures tested in this experiment. The optimum temperature for reproduction was 27 °C.

INTRODUCTION

Radopholus similis (Cobb) Thorne can be reared on excised okra root tissues (Feder, 1958) grapefruit, okra and alfalfa root callus tissues (Myers et al., 1965), carrot discs (O' Bannon & Taylor, 1968), citrus callus (Inserra & O'Bannon, 1974, banana fruit callus (Brown & Vessey, 1985), and carrot callus tissue (Reise et al., 1987).

A 200 times multiplication of *R. similis* collected from banana roots, was obtained after an incubation of 30 days at 27 °C on excised okra root tissue (Feder, 1958). On carrot discs, the multiplication of *R. similis*, after incubation for 45 days, ranged from 1 to 11 times, and after 75 days, it ranged from 46 to 399 times (Tarté et al., 1981). On banana fruit callus, the population of this nematode increa-

sed about 6 times when incubated for 30 days at temperatures ranging from 24 °C to 29 °C (Brown & Vessey, 1985). On carrot callus tissues incubated at 28 °C for 60 days, the population increased only 1.6 times (Reise et al., 1987). These data could indicate that at present carrot discs appeared to be a good, simple, and reliable medium for rearing *R. similis*. Effects of temperature on reproduction of *R. similis* on carrot discs, so far, have not been investigated.
Since large numbers of pure *R. similis* were needed in our experiments, a study was made on the multiplication of *R. similis* on carrot discs at four different temperatures.

MATERIALS AND METHODS

R. similis originally obtained from black pepper (*Piper nigrum* L.) roots was first reared on carrot discs. To prepare carrot discs, fresh carrots with attached leaves were freed of attached soil by using running water, a brush and soap. Carrots were then peeled and sliced transversely (15 mm in thickness, and 15-20 mm in diameter). After slicing, the carrot discs were surface sterilized in 10% Chlorine solution for 30 minutes and subsequently washed with sterile distilled water, and flamed, in a laminar flow hood under aseptic conditions. With a 3 mm cork borer, a 3 mm deep cavity, (approx. 3 mm in diameter and thickness), was made in the stele of carrot discs.
A single carrot disc was transferred into a sterile glass jar, 6 cm in diameter and 3.5 cm in height.

Nematodes were surface sterilized in a mixture of 0.02% aqueous ethoxyethylmercury chloride and 0.1% streptomycin sulphate for a period of 30 minutes, and washed with sterile distilled water. The nematodes were then transferred with a sterile handling needle into a drop of sterile distilled water which was already placed in the cavity of the carrot

discs. The culture jars were sealed with tape, and incubated at 25 °C for eight weeks. The populations of *R. similis* obtained from this culture were used as inoculum for the experiment.

Sixty carrot discs were prepared and inoculated with 20 females per disc. Incubation was done at 20 °C, 25 °C, 27 °C and 30 °C, each with 15 discs. Thirtyfive days after inoculation, carrot discs were blended in a microblender for 30 seconds to separate nematodes from the plant tissue. The suspensions obtained were washed through a 100 µm sieve to remove debris, and the nematodes were collected on a 10 µm sieve. From the suspension obtained the various stages of *R. similis* (eggs, juveniles, males and females), were counted separately. Data obtained were subjected to analysis of variance and compared using LSD tests.

RESULTS AND DISCUSSION

Unfortunately, 35 days after inoculation 8 out of the 60 carrot discs were contaminated. The numbers of uncontaminated discs at 20 °C, 25 °C, 27 °C and 30 °C were 13, 14, 13, and 12 respectively. The results of statistical analysis based on the numbers of *R. similis* recovered from uncontaminated discs are presented in Table 1.

Significantly higher numbers were found at 25 °C and 27 °C viz. 6730 and 8773 respectively, compared to only 214 at 20 °C, and 1120 at 30 °C. However, there was no significant difference in the multiplication factor (Pf/Pi) at 25 °C and 27 °C. At 25 °C and 27 °C, *R. similis* multiplied as much as 337 and 439 times, while at 20 °C and 30 °C it multiplied 11 and 56 times respectively. It is evident that the optimum temperature for reproduction of *R. similis* in this experiment was 27 °C.

Table 1 Effect of temperature on the population of *R. similis* 35 days after inoculation.

R. similis	Temperature (°C)			
	20	25	27	30
Eggs	6	3172	4484	509
	(1.05) a	(3.34) c	(3.33) c	(2.62) b
Juveniles	148	2996	3580	498
	(2.12) a	(3.30) c	(3.17) bc	(2.61) ab
Females	48	428	519	83
	(1.51) a	(2.55) b	(2.42) b	(1.71) a
Males	12	133	190	30
	(1.0) a	(1.9) c	(2.1) c	(1.4) b
E+J+F+M	214	6730	8773	1120
	(2.27) a	(3.68) c	(3.69) c	(2.97) b
Pf/Pi	11	337	439	56
	(0.97) a	(2.37) c	(2.40) c	(1.67) b
F:M:J:E	4:1:12:1	3:1:23:24	3:1:19:24	3:1:17:17

Numbers followed by the same letters in each row are not significantly different at 5% level (LSD-test).
Numbers in parentheses are averages of log x transformed original data.
E = eggs; J = juveniles; M = males; F = females. Pf/Pi = ratio of various stages (Pi = initial population = 20 females; Pf = final population).

Since the inoculum consisted of females only, the presence of all stages including males, and newly formed females of *R. similis*, indicated that at temperatures ranging between 20 °C to 30 °C during the 35 days of the incubation, this nematode was able to complete at least one life cycle. Loos (1962) found that the length of life cycle of *R. similis* collected from banana plants, reared on *Tephrosia candida* roots at temperatures fluctuating between 24 °C to 32 °C, was completed in 20-25 days. On citrus the life cycle of this nematode was completed in 18-20 days at temperatures ranging between 24 °C to 27 °C (DuCharme & Price, 1966).
In this experiment, the cultures were kept up to 35 days after inoculation. The population obtained in this experiment seemed to be a mixture between the first and the second generation. Except at 20 °C, eggs and juveniles were predominant.
The male-female ratios at 20 °C, ,25 °C, 27 °C and 30 °C were 1:4; 1:3; 1:3 and 1:3 respectively.

Tarté et al. (1981) found that there were differences in male-female ratios between four banana isolates of *R. similis* reared on carrot discs at 23 °C. At 45 days after inoculation an isolate with the lowest population increase of only a 1.40 fold had a male-female ratio 1:4, whereas in another isolate with a population increase of 11.27 fold, this ratio was 1:9. The differences in results between Tarté's work and our study are probably due to differences in nematode isolates, in temperature and time of observation used in both experiments.

We proved that the most favourable temperature for reproduction of the *R. similis* isolate of black pepper on carrot discs was 27 °C. Large numbers of this nematode in pure populations can be easily obtained with this culture method in a limited time under controlled conditions.

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4 Interactions of *Radopholus similis* with *Fusarium solani* on black pepper (*Piper nigrum* L.)

Ika Mustika

ABSTRACT

The effects of *Radopholus similis* and *Fusarium solani* alone or in combination have been studied on black pepper cv. Kalluvalli. *R. similis* alone significantly reduced plant height, number of nodes, length of nodes, number of leaves, leaf area, shoot and root weight. *F. solani* also caused such reductions, but to a lesser extent than did *R. similis*. Both combinations of *R. similis* and *F. solani* caused the same symptoms. Inoculation of *R. similis* two weeks before the fungus had no additional effect. *R. similis* alone caused growth reduction and yellow leaves with a stiff droop, but the damage was more obvious when *R. similis* acted together with *F. solani*. The females of *R. similis* penetrated the roots one day after inoculation, and deposited the eggs inside the roots within five days. Two weeks after inoculation, females and juveniles of *R. similis* were found to colonize the roots, and distinct necrotic tissues were observed. One month after inoculation, *R. similis* had destroyed vascular tissues. Obstruction of xylem vessels with "gum like substances" was also found in response to infection by *R. similis*.

INTRODUCTION

The burrowing nematode *Radopholus similis* is an important root parasite on many crops in tropical and subtropical areas (O'Bannon, 1977). Since 1932 this nematode has been

suspected of causing "yellow disease" on black pepper (*Piper nigrum* L.) on Bangka, Indonesia (Van der Vecht, 1950).
Jensen (1972) also thought that *R. similis* was the only important nematode which was responsible for the yellow disease of black pepper. The same or a similar disease was also reported on black pepper plantings in Thailand (Sher et al., 1961; Bridge, 1978), and in India (Venkitesan & Setty, 1977; Nambiar & Sarma, 1977; Ramana et al., 1987).
F. solani has frequently been isolated from roots of black pepper (Alconero et al., 1972; Nambiar & Sarma, 1977; Bridge, 1978).
Nematode-fungus disease complexes have been reported in many crops (Melendez & Powell, 1967; Carter, 1975; Garber et al., 1979; Fattah & Webster, 1983; Caperton et al., 1986). However, there is still insufficient information on the interaction between *R. similis* and *F. solani* on black pepper, especially in Indonesia. The effect of *R. similis* and *F. solani* on growth and symptom development, and on histological changes induced by these parasites on black pepper are reported here. In addition, mutual effects of *R. similis* and *F. solani* were studied.

MATERIALS AND METHODS

R. similis isolated from black pepper roots was cultured on carrot discs as described in Chapter 3. The nematodes were surface sterilized for 30 minutes in a mixture of 0.02% aqueous ethoxyethylmercury chloride and 0.1% streptomycin sulphate. The inoculum used was obtained by chopping six-week-old carrot discs incubated in a mistifier for one week. Each plant was inoculated by pipetting 100 ml of a suspension containing 1000 nematodes consisting of juveniles, males and females, around the stem, approximately 5 cm away, on the soil surface.
F. solani was prepared and each plant was inoculated as

described in Chapter 2. Three-month-old cuttings of black pepper cv. Kalluvalli were transplanted in clay pots, filled with 1 kg of autoclaved soil and planted with one cutting per pot. For soil composition see Annex 1. Before planting 3 g of Dolocal per pot were added (for the composition see Annex 2). Soil pH before and after the experiment was 4.9 and 5.8 respectively. To keep soil moisture near to field capacity, plants were watered daily with 100 ml of tap water. Plants were weekly fertilized with a 100 ml nutrient solution per pot, to ensure nonlimiting nutrient levels.

Five treatments were applied, viz.:
1) Control (Uninoculated plants) (C).
2) Inoculation with *R. similis* (Rs).
3) Inoculation with *F. solani* (Fs).
4) Inoculation with *R. similis* and *F. solani* simultaneously (Rs+Fs).
5) Inoculation with *R. similis* two weeks before *F. solani*. (Rs)+Fs.

Each treatment was replicated five times in a randomized block design with five plants per treatment. The whole experiment was performed in the greenhouse with an air temperature ranging between 19 °C and 25 °C, a soil temperature of 17 °C to 23 °C, and a relative humidity of 70% to 80%.
To reduce evaporation each pot was covered with a sheet of black polyethylene.

The experiment was terminated four months after nematode inoculation, and the following characteristics were recorded:
1. Plant height, length and number of nodes, number of leaves, leaf area, fresh and dry, shoot and root weight.
2. Leaf discoloration and leaf drooping.

3. Days between nematode inoculation, appearance of the first symptoms and the root decay index.
4. Nematode populations in soil, roots and in the stem base.

Leaf areas were measured by passing all the leaves through a leaf area meter. Dry weight of the shoots and roots were obtained by drying the material at 80 °C for 48 h. Root decay indexation was done similar to the method used by Palmer and Mc Donald (1974) as follows; 0 = no decay; 1 = slight decay (25%); 2 = moderate decay (26%-50%); 3 = heavy decay (51%-75%); 4 = more than 75% roots was decayed and dead plants.
Nematodes from root and stem base were extracted separately with the centrifugal flotation method. Nematodes from soil were extracted with the Oostenbrink elutriator (s'Jacob & Van Bezooijen, 1984). To check the presence of *F. solani*, root segments were taken from ten plants of each treatment, rinsed in sterile water, surface sterilized in CaOCl 5%, plated onto water agar (WA) and incubated at 25 °C. Plates were observed for the presence of *F. solani* for up to 15 days.

One month after nematode inoculation, some roots from all treatments were taken, and prepared for histological observations. The roots were killed and fixed in FAA, serially dehydrated in butyl alcohol, and finally embedded in paraffin. Transverse and longitudinal sections of 10 µm were prepared, stained with safranine and fast green (Johansen, 1940). Some fresh pieces of root were also stained with lactophenol cotton blue 0.1%.
In addition, one-month old rooted cuttings were transferred into plastic tubes containing 100 ml of the same soil as used in the above experiment. The plants were kept in a climate chamber at a constant temperature of 28 °C and relative humidity of 80%, exposed to a day length of 14 h using 360 Watt TLD. One week after transplanting, the plants were inoculated with 50 females of *R. similis*. One plant was

daily removed to observe the root invasion by *R. similis*. The observations were made by staining unsectioned roots with acid-fuchsin (Byrd et al., 1983).

RESULTS

Plant growth

Almost all values of the plant growth characteristics were reduced significantly in the presence of both parasites either alone or in combination (Table 1 and Table 2).
At the end of the experiment, *R. similis* alone reduced plant height by 26%, number of nodes by 31%, number of leaves by 39%, leaf area by 54%, dry shoot weight by 33%, and dry root weight by 27%. *F. solani* alone reduced these plant growth characteristics to a lesser extent (Table 1, 2, and 3).

Effects of *R. similis* and *F. solani* on plant height, alone or in combination, are shown in Table 1 and Fig. 1. Both *R. similis* and *F. solani* alone started to reduce plant height significantly from two months after nematode inoculation onwards, whereas combined inoculation of the two pathogens began to reduce plant height already one month after inoculation. Four months after inoculation *F. solani* alone had hardly any effect on plant height. When *R. similis* was present, simultaneously or two weeks prior to the fungus, plant height was reduced by 29% as compared to control plants.

Nematode populations

The effects of the interaction between *R. similis* and *F. solani* on the nematode population are presented in Table 4 and Fig. 2. The numbers of *R. similis* in the soil and in the roots were significantly higher when the plants were inoculated with *R. similis* alone, as compared with those of

combined inoculation with *F. solani*. *R. similis* was also found in the stem base, but in lower numbers compared with those in the roots. However, there were no significant differences in nematode numbers in the stem base, either in plants inoculated with *R. similis* alone or in combination with *F. solani*.

Total numbers of *R. similis* when inoculated alone were 2 or 3 times higher than those found in combinations with *F. solani*. The time of fungus inoculation, simultaneously or two weeks after the nematode, had no significant effect on the reduction of nematode numbers. There was only a tendency for numbers of *R. similis* to be lower in plants inoculated with nematode two weeks prior to the fungus.

The multiplication factor (Pf/Pi) of *R. similis* when inoculated alone was 13. This was significantly higher than the multiplication factor of 4 and 6, when this nematode was inoculated simultaneously or two weeks prior to the fungus. There was no significant difference in multiplication of *R. similis* when this nematode was present together with *F. solani* (Table 4).

Table 1 Effect of inoculation on plant height during experiment (in cm).

treatment *)	months after inoculation					final reduction **)
	(0)	(1)	(2)	(3)	(4)	
C	21.0 a	32.4 c	46.5 d	60.4 c	77.5 b	-
Rs	20.4 a	29.7 bc	36.9 b	44.1 a	57.3 a	26
Fs	21.0 a	31.0 c	41.9 c	53.5 b	72.1 b	7
(Rs+Fs)	20.5 a	26.9 b	34.0 b	43.6 a	55.0 a	29
(Rs)+Fs	20.8 a	24.2 a	29.7 a	42.0 a	54.7 a	29

Numbers followed by the same letters in each column are not significantly different at 5% level (LSD test).

*) See page 34.

**) Percent reduction of the height from control in column 4.

Table 2 Effects of inoculation on number of nodes (NN), length of nodes (LN), number of leaves (NL) and leaf area (LA in cm^2).

Treatments*)	NN	%**)	LN	%**)	NL	%**)	LA	%**)
C	15.6 c	-	7.5 c	-	20.1 c	-	1093 b	-
Rs	10.8 a	31	5.9 a	21	12.3 a	39	505 a	54
Fs	13.0 b	17	6.6 b	12	16.0 ab	20	932 b	15
(Rs+Fs)	10.8 a	31	5.8 a	23	12.0 a	40	520 a	52
(Rs)+Fs	10.7 a	31	6.0 a	20	10.7 a	47	456 a	58

Numbers followed by the same letters in each column are not significantly different at 5% level (LSD test).
*) See page 34.
**) Percent reduction from control.

Table 3 Effects of inoculation on shoot and root weights (g).

Treatments*)	shoot weights fresh	%**)	dry	% **)	root weights fresh	%**)	dry	% **)
C	46.6 c	-	15.0 d	-	8.1 c	-	2.1 b	-
Rs	22.6 a	52	7.4 a	50	3.1 ab	62	0.8 a	62
Fs	38.6 b	17	10.7 c	29	5.4 b	33	1.1 a	48
(Rs+Fs)	25.5 a	45	8.0 b	47	4.0 ab	51	1.1 a	48
(R)+Fs	18.7 a	60	5.4 a	64	2.6 a	68	0.8 a	62

Numbers followed by the same letters in each column are not significantly different at 5% level (LSD test).
*) See page 34.
**) Percent reduction from control.

Table 4 Effects of inoculation on nematode population in roots, soil and stem base of black pepper.

Treatments*)	roots	soil	stem base	total	multiplication factor (Pf/Pi)
Rs	11399 (4.02) b	984 (3.89) b	390 (2.49) a	12773 (4.07) b	13 b -
(Rs+Fs)	4437 (3.51) a	984 (2.91) b	304 (2.35) a	5725 (3.66) a	6 a -
(Rs)+Fs	3449 (3.51) a	422 (2.58) a	180 (2.26) a	4051 (3.54) a	4 a -

Numbers followed by the same letters in each column are not significantly different at 5% level (LSD test). Numbers in parentheses of log x transformed original data; Pi = initial population (1000 nematodes per pot); Pf = final population.
*) See page 34.

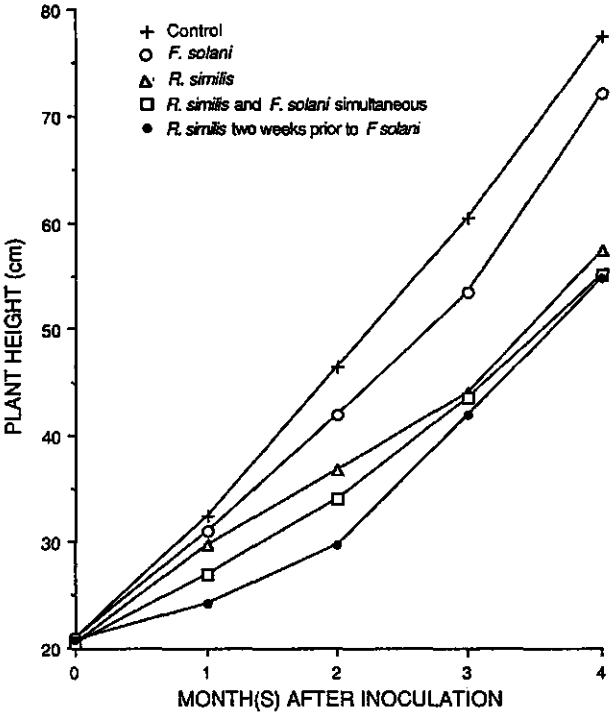

Figure 1 Effect of *R. similis* and *F. solani* on plant height of black pepper.

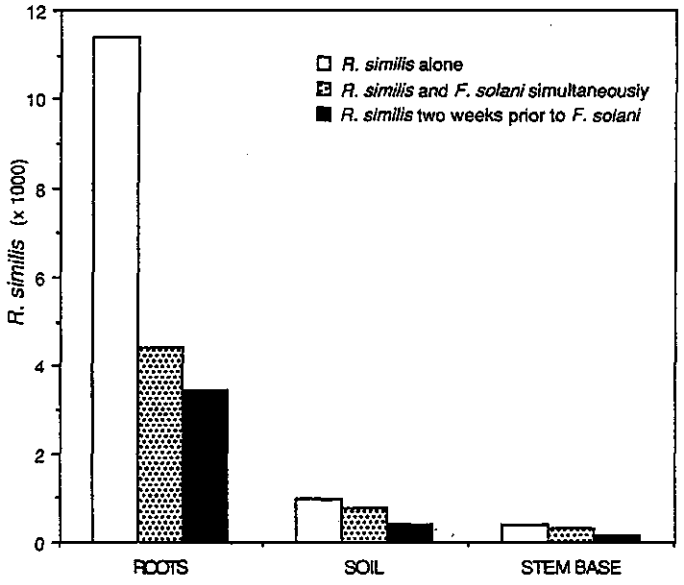

Figure 2 Population density of *R. similis* in roots, soil and stem base of black pepper four months after inoculation.

Development of symptoms

Plants inoculated with *R. similis* alone or in combination with *F. solani* showed yellow leaves with a stiff droop (Fig. 3), and root decay. More root decay occurred in the presence of both *R. similis* and *F. solani*. When *R. similis* was inoculated two weeks before *F. solani*, the root decay index increased significantly as compared to infection by *R. similis* alone. There were no significant differences in percent of yellowing and stiff droop, both in plants inoculated with *R. similis* alone or in combination with *F. solani* (Table 5).

The period between nematode inoculation and the first development of the discoloration of the leaves, was not significantly different between the treatments, and ranged from 44 to 47 days in average (Table 5). There were no yellow leaves

Figure 3 Disease symptoms of black pepper four months after inoculation. 1. Uninoculated plant; 2. Inoculated with *R. similis*; 3. Inoculated with *F. solani*; 4. Inoculated with *R. similis* and *F. solani* simultaneously; 5. Inoculated with *R. similis* two weeks before *F. solani*.

Table 5 Effects of inoculation on disease incidence on black pepper.

Treatments *)	Root decay	Yellowing plants (%) **)	Period between nematode inoculation and the first symptom (days)	Fungus isolation from root segments (%)
C	0.0 a	0.0 (0.0) a	-	0
Rs	2.8 b	28.0 (31.6) b	47 a	0
Fs	0.8 a	0.0 (0.0) a	-	30
(Rs+Fs)	3.4 bc	32.0 (34.2) b	44 a	60
(Rs)+Fs	3.8 c	32.0 (36.5) b	44 a	80

Numbers followed by the same letters in each column are not significantly different at 5% level (LSD test). Means in parentheses are averages arc sin $\sqrt{(\text{percent}/100)}$ transformations of original data.
*) See page 34.
**) % plants with yellow leaves and stiff droop.

with stiff droop or root decay observed either in control plants or in those inoculated with *F. solani* alone.
Isolation of *F. solani* from root segments revealed that the fungus was present in roots infected with both *F. solani* alone and in combination with *R. similis*. The percentage of recovery of *F. solani* from root pieces infected with *R. similis* and *F. solani* ranged from 60% to 80%, whereas in roots infected with *F. solani* alone it was only 30%.

Histological studies

One day after inoculation, females of *R. similis* were found in the tips of feeder roots. No root discoloration was observed, but cell destruction due to nematodes was noticeable (Fig. 4A). Three days after inoculation, nematodes were seen to move from the tips of the feeder roots to the larger roots (Fig. 4B). Within five days female nematodes were found to deposit several eggs inside those larger roots (Fig. 5A). On day 14, females and juveniles of *R. similis* were observed to colonize the roots (Fig. 5B). The newly hatched juveniles moved directly through the root cortex.

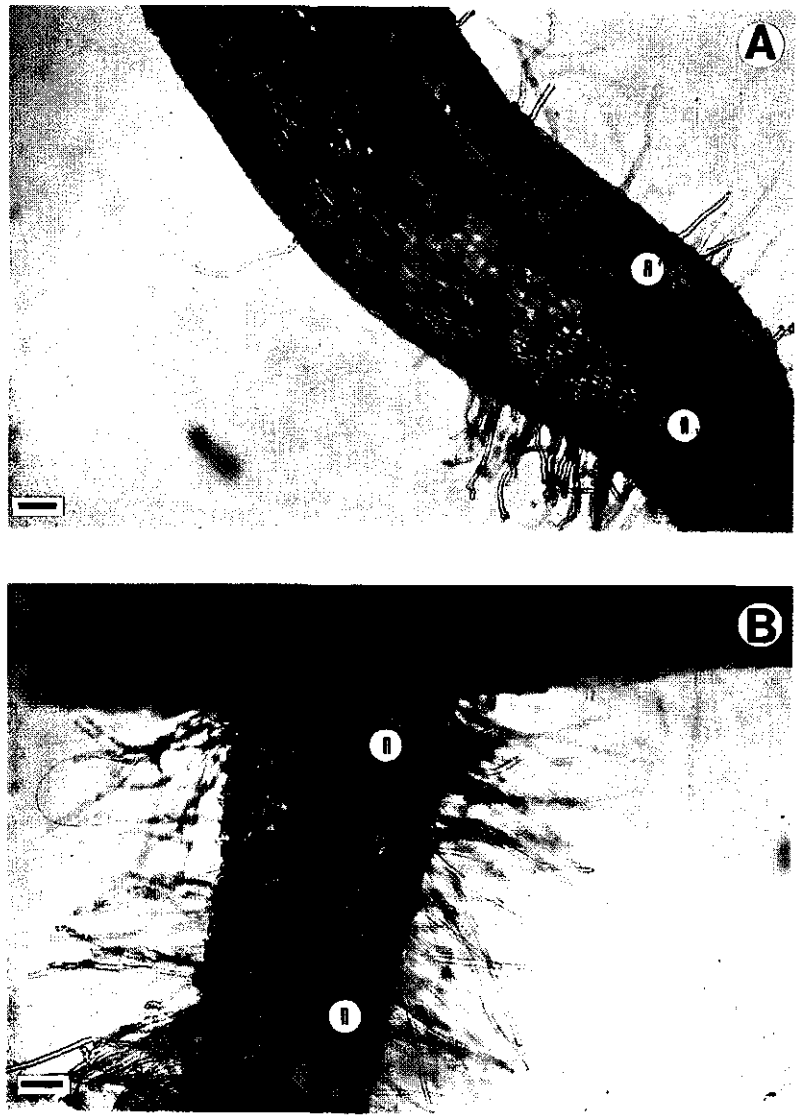

Figure 4 A. Two females of *R. similis* embedded in the root tips of black pepper one day after inoculation. Bar = 75 µm. B. *R. similis* moving from the feeder root to a secondary root at three days after inoculation. Bar = 75 µm.

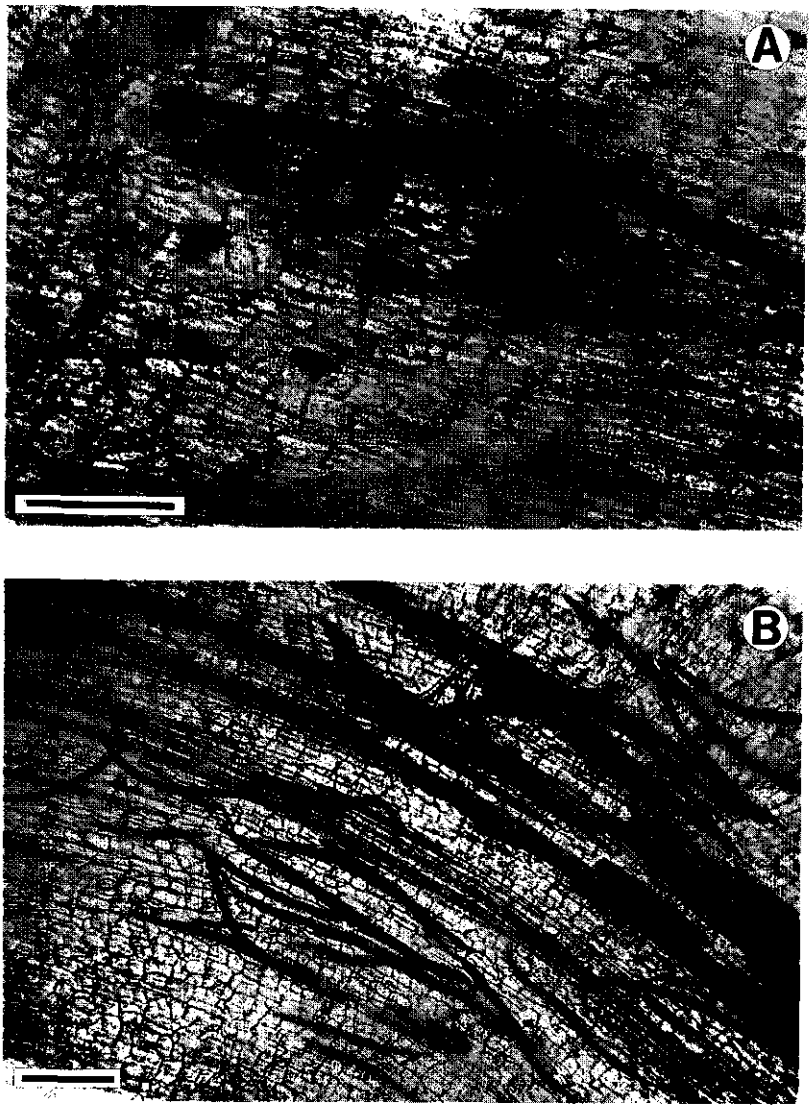

Figure 5 A. Female of *R. similis* depositing several eggs inside the root five days after inoculation (Bar = 200 µm). B. Females and juveniles of *R. similis* colonized the root of black pepper 14 days after inoculation, showing necrotic areas surrounding the nematodes starting to develop (Bar = 300 µm).

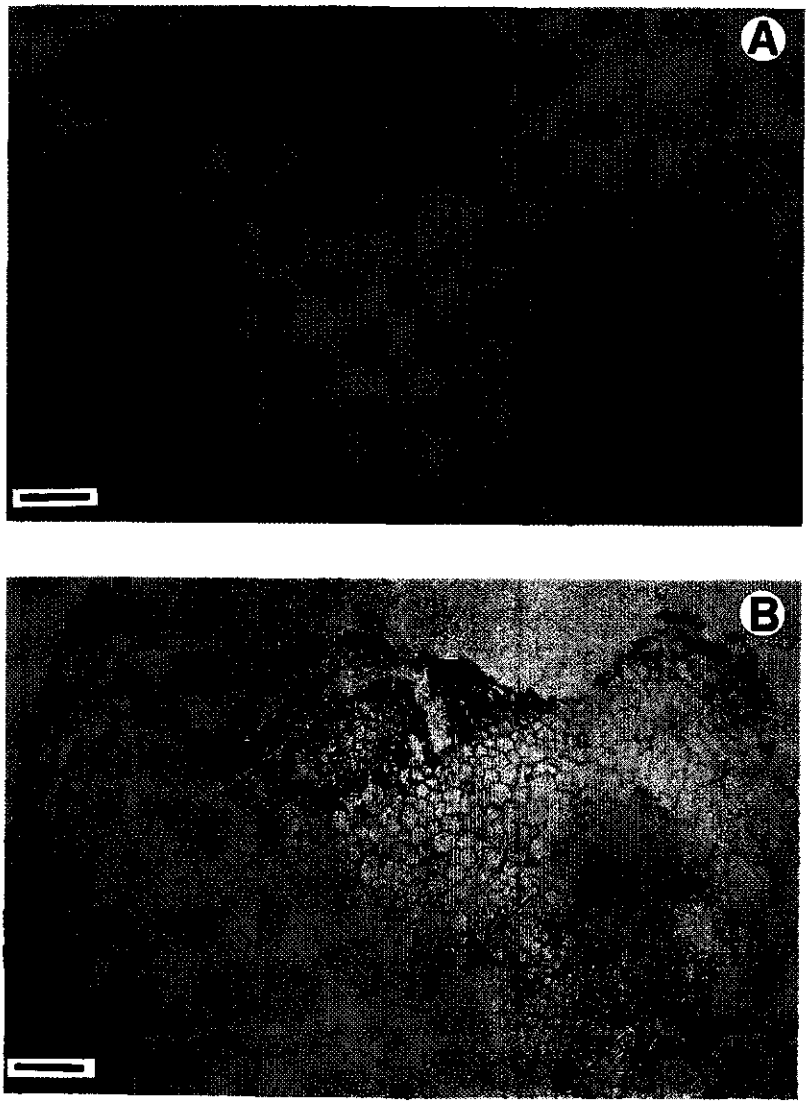

Figure 6 A. Transverse section of non infested root. B. An infected root with *R. similis* showing blocked xylem vessels with "gum like substances" (arrows). Bar = 250 µm.

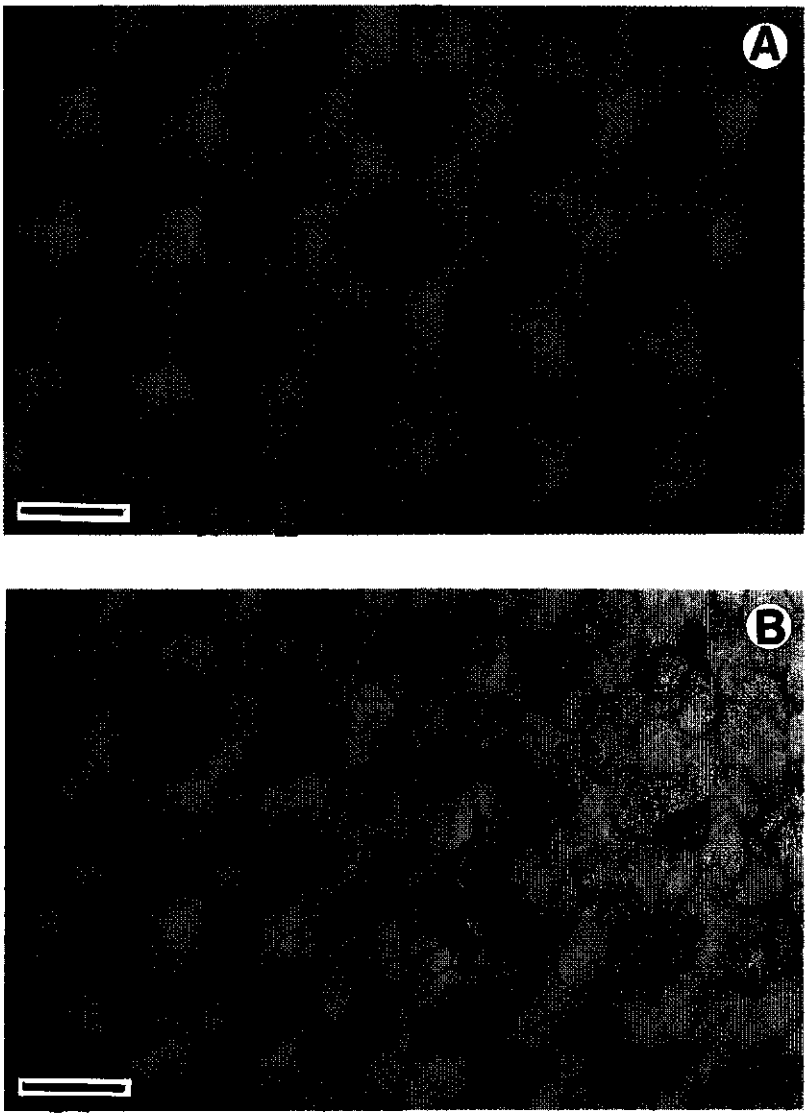

Figure 7 A. Transverse section of a root infected with *R. similis* (enlargement of Fig. 6B), showing necrotic cortex tissues and blocking of the xylem vessels (arrows). Bar = 500 µm.
B. Extensive necrosis in roots infected with *R. similis* and *F. solani*. Bar = 500 µm.

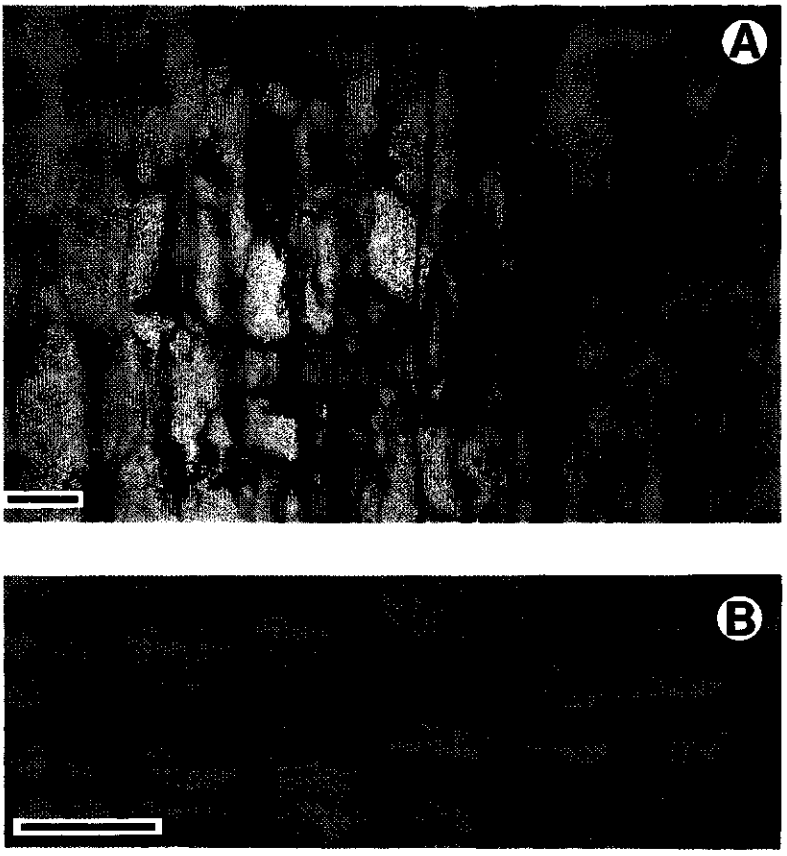

Figure 8 A and B. Longitudinal section of the root infected with *F. similis,* showing nematodes inside a cell, and surrounded by cells showing a distinct discoloration. (Bar A = 100 µm; B = 150 µm).

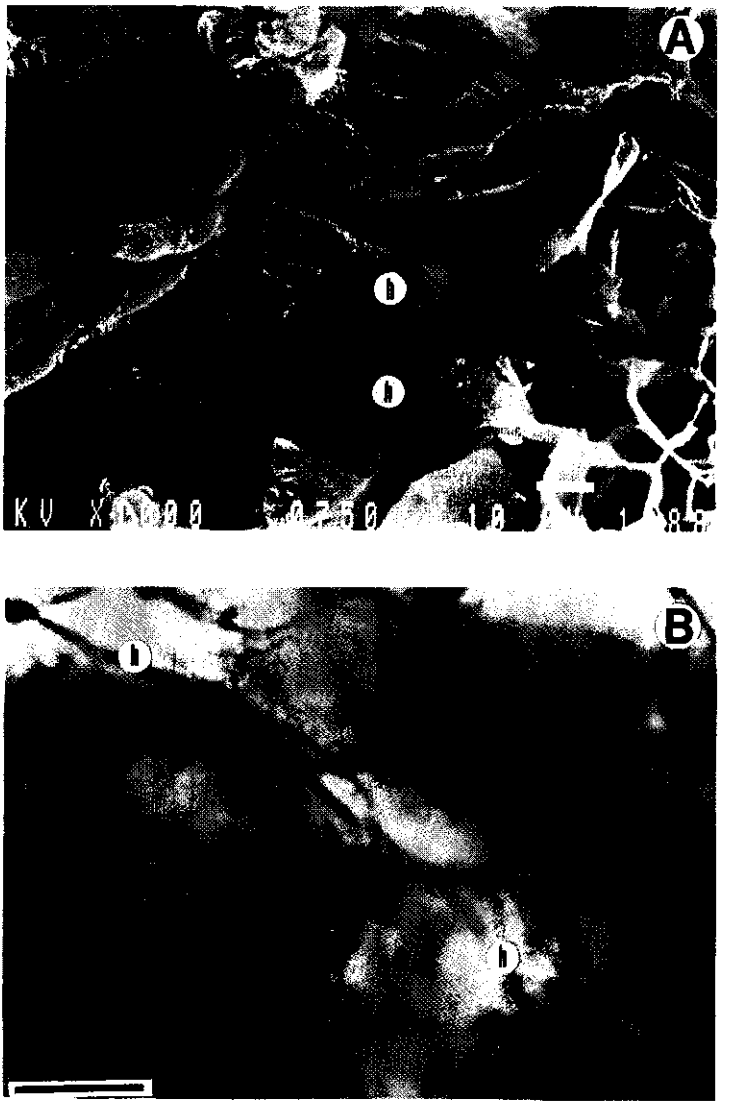

Figure 9 A. SEM micrograph showing the hyphae of *F. solani* (h) penetrating intracellular root tissues of black pepper (bar = 10 µm). B. Transverse section through the cortex, showing colonization of necrotic tissue by the hyphae of *F. solani* (h), the cell walls are destroyed (bar = 50 µm).

Root discoloration and cell destruction due to nematode feeding and movement were clearly observed two weeks after inoculation.
Four weeks after inoculation, *R. similis* induced an extensive necrosis in the cortex and in the vascular tissues. The xylem vessels were obstructed by "gum like substances" (Fig. 6B, and 7A). Plants inoculated with *R. similis* and *F. solani* showed also extensive necrosis of the vascular tissues (Fig. 7B). Here, xylem vessels were also obstructed with "gum like substances". In roots inoculated with both *R. similis* and *F. solani* obstruction of the xylem vessels, however, was more pronounced than in roots inoculated with either pathogen alone.
Nematodes were found surrounded by necrotic tissues in the cortex (Fig. 8A, 8B). SEM observations on roots infected with *F. solani* showed that the hyphae of this fungus penetrated the intracellular spaces in root tissues (Fig. 9A). In plants inoculated with both *R. similis* and *F. solani*, the hyphae were observed to be dispersed in the necrotic tissue (Fig. 9B).

DISCUSSION

Most of the observations showed an interaction between *R. similis* and *F. solani*, since plant growth was clearly retarded as compared with effects of inoculation with the pathogens alone. *R. similis* alone caused root decay and yellowing of the leaves with stiff drooping. *F. solani*, however, did not induce these typical symptoms. This indicates that *R. similis* alone can cause the symptoms typical for yellow disease, as described by Van der Vecht (1950).

Mountain & McKeen (1962) found that *Pratylenchus* was the dominant pathogen involved in the interaction with the *Verticillium* wilt fungus on cotton, tomato, eggplant and pep-

per (*Capsicum* spp.). Either pathogen was capable of causing disease, but damage was more obvious when both pathogens were present. Blake (1961, 1966) working with *R. similis*, *F. solani* and *Rhizoctonia solani* on banana, concluded that *R. similis* was the primary pathogen of root rot, increasing the invasive potential of weak fungal parasites. This phenomenon may also have occurred on black pepper infected with *R. similis* and *F. solani* together. Much lower numbers of *R. similis* in roots, soil and stem base were found when *F. solani* was also present. It appeared that *F. solani* had an antagonistic effect on the reproduction of this species. Antagonistic effects of *F. solani* on nematodes have been reported by several workers (Melendez & Powell, 1967; Fattah & Webster, 1981; Sakhuja & Sethy, 1986). When *R. similis* was inoculated two weeks before *F. solani*, the nematode multiplication was more reduced than when nematode and fungus were inoculated simultaneously. On the other hand, when *R. similis* and *F. solani* occurred together, *F. solani* could be recovered from 60 to 80% of the plants, much more than when *F. solani* was inoculated alone (Table 5).

The highest recovery of 80% was found when *R. similis* was inoculated two weeks prior to *F. solani*. The higher reduction of *R. similis* inside the roots as compared to simultaneous inoculation, indicated that *F. solani* grew faster in roots already infected with *R. similis*. This process might explain the tendency that the greatest damage occurred in plants infected with *R. similis* followed by *F. solani*.

A few weeks after *R. similis* invaded the roots, extensive cavities were formed. The stele was not invaded, but the cavities often distorted the vascular cells. This type of distortion was similar to those described by DuCharme (1959) in citrus and by Blake (1966) in banana infected with *R. similis*.

The present study shows that infection by *R. similis* alone caused blocking of the xylem vessels with "gum like substances". Distortion of the vessels was more distinct when

F. solani penetrated the roots already infected by *R. similis*. Blake (1966) has reported similar effects in banana infected with *R. similis* and *F. oxysporum*.

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5 Interactions of *Meloidogyne incognita*, *Radopholus similis*, and *Fusarium solani* on black pepper (*Piper nigrum* L.)

Ika Mustika and J.J. s'Jacob

ABSTRACT

Rooted cuttings of black pepper (*Piper nigrum* L.) cv. Kalluvalli were inoculated with *Meloidogyne incognita*, *Radopholus similis* and *Fusarium solani* alone and in several combinations. Compared with uninoculated plants, *M. incognita*, *R. similis* and *F. solani* alone significantly reduced plant height, fresh and dry shoot weight, and leaf area. *R. similis* alone caused greater plant growth reduction than either *M. incognita* or *F. solani*. No significant interactions were observed between the three pathogens in growth reduction. However, the poorest growth and the most distinct development of yellow leaves and stiff droop tended to occur when all three pathogens acted together. The populations of both *R. similis* and *M. incognita* were suppressed when coinhabiting black pepper roots. *R. similis* tended to be more dominant than *M. incognita*. Suppression of both nematode species was more pronounced when *F. solani* was present.

INTRODUCTION

Concomitant infestations of fields with different species of parasitic nematodes, as well as with other pathogens, are common. *Meloidogyne incognita* and *Radopholus similis* are two predominant nematodes on black pepper, and it is suspected that they play a role in the occurrence of "yellow disease" and "slow wilt disease" in Indonesia and India (Mustika, 1978;

Bridge, 1978; Nambiar & Sarma, 1977; Ramana et al., 1987). Data on the interactions between nematodes and fungi in disease complexes, as well as interaction between nematode populations, have been summarized (Pitcher, 1965; Eisenback, 1985, Eisenback & Griffin, 1987). Usually, such interactions are antagonistic to at least one of the individual species (Eisenback, 1985; Eisenback & Griffin, 1987).

Previous experiments showed that *M. incognita* and *R. similis* were suppressed by the presence of *F. solani*. Both nematode species, however, acted with *F. solani* synergistically with respect to symptom development. Since *M. incognita* and *R. similis* are frequently occurring in black pepper plantings concomitantly with *F. solani*, interactions between these three pathogens are possible.

This study was focused on two aspects: 1). The interaction between *M. incognita* and *R. similis* in the presence of *F. solani*; 2). The effect of various combinations of the three pathogens on growth and symptom development of black pepper.

MATERIALS AND METHODS

Inocula of *M. incognita*, *R. similis* and *F. solani* were prepared as described in Chapter 2 and Chapter 3. One month-old cuttings of black pepper cv. Kalluvalli were transplanted into clay pots, one plant per pot, filled with 750 g of autoclaved sandy loam soil mixed with perlite (2:1 v/v). The composition of the soil is presented in Annex 1. Before planting 3 g of Dolocal per pot were added (for the composition see Annex 2).
The following treatments were given:

A. Uninoculated control (C).
B. *M. incognita* (Mi).
C. *R. similis* (Rs).
D. *F. solani* (Fs)
E. *M. incognita* and *R. similis* ((Mi + Rs).
F. *M. incognita* and *F. solani* (Mi + Fs).
G. *R. similis* and *F. solani* (Rs + Fs).
H. *M. incognita, R. similis* and *F. solani* (Mi + Rs + Fs).

Each treatment was replicated four times in a completely randomized design with five plants per treatment. Inoculum densities used were 1000 nematodes and 10^6 microconidia of *F. solani* per pot. The nematodes were inoculated two weeks prior to the fungus. Plants were placed in a climate chamber under 14 h photoperiod with a constant air temperature set at 28 °C, a relative humidity of 75%-80%, and a soil temperature ranging from 23 °C to 25 °C. A combination of SON/T 400 W high pressure sodium lamps and HPI/T 400 W high pressure mercury iodine lamps was used.

To maintain the soil moisture at approximately field capacity, plants were watered every day with 50 ml tap water per pot. A 100 ml of nutrient solution was applied weekly to ensure nonlimiting nutrient levels (Annex 1).

Plant height was measured monthly. Four months after nematode inoculation, three plants from each treatment were uprooted and the shoots were cut at soil surface and fresh and dry matter were recorded. Leaf area per plant was measured by passing the leaves through a leaf area meter. Roots were washed free of adhering soil, and carefully dried with filter paper to remove excess water, weighed, and thereafter dried for 48 h at 80 °C. To assess the nematode density, a 200 g soil sample taken from the two remaining plants of each treatment was extracted with the Oostenbrink

elutriator as described by s'Jacob and Van Bezooijen (1984). Roots were blended, incubated in a mystifier with water at 27 °C for seven days. The nematode populations were counted. Data obtained were statistically analyzed.

RESULTS

Host response

Effects of pathogens on plant growth during the four months of the experiments are presented in Table 1 and Fig. 1. Compared with the control, each pathogen alone had a significant effect on plant height, fresh and dry shoot weight, and leaf area. When the three pathogens were inoculated together, the damage tended to increase. When *R. similis* acted alone, or was present in combination with *F. solani*, final plant height, shoot and root weight, as well as leaf area were significantly reduced. *R. similis* caused more damage than *M. incognita* or *F. solani* alone. Growth suppression by all three pathogens together was usually greater than that caused by each pathogen alone.

Table 1 The effects of *M. incognita*, *R. similis* and *F. solani* on the growth of black pepper four months after inoculation.

Treatments *)	Final height (cm)	Shoot weight (g) fresh	Shoot weight (g) dry	Root weight (g) fresh	Root weight (g) dry	Leaf area (cm^2)
C	79.8 d	65.9 d	14.7 d	13.4 b	2.7 b	995 d
Mi	61.2 c	48.1 c	10.5 c	14.9 b	2.7 b	772 c
Rs	38.2 ab	13.7 a	3.3 a	4.0 a	1.2 a	261 a
Fs	48.3 b	30.7 b	6.2 b	11.8 b	2.3 b	510 b
Mi+Rs	39.3 ab	17.1 a	4.2 ab	6.0 a	1.2 a	291 a
Mi+Fs	41.4 ab	20.3 a	4.6 ab	4.5 a	1.0 a	279 a
Rs+Fs	42.2 ab	17.5 a	4.1 a	4.1 a	1.1 a	243 a
Mi+Rs+Fs	35.4 a	13.3 a	3.3 a	4.5 a	0.8 a	295 a

Numbers followed by the same letters in each column are not significantly different at 5% according to LSD test.
* See page 55.

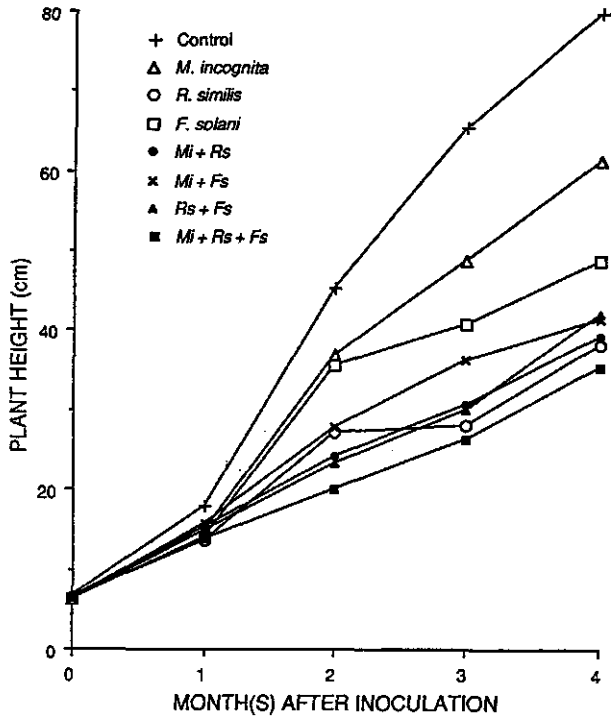

Figure 1 Effects of *M. incognita*, *R. similis*, and *F. solani* alone or in combination on plant height.

Table 2 The effect of *M. incognita*, *R. similis* and *F. solani* on symptom development four months after inoculation.

Treat-ments*)	root decay index	% of plants with symptoms**)
C	0.00 a	0 (0.0) a
Mi	0.75 b	0 (0.0) a
Rs	1.88 c	30 (32.9) b
Fs	0.88 b	0 (0.0) a
Mi+Rs	2.75 de	50 (45.0) bc
Mi+Fs	1.63 c	0 (0.0) a
Rs+Fs	3.00 e	50 (45.0) bc
Mi+Rs+Fs	4.13 f	65 (54.2) c

Numbers followed by the same letters in each column are not significantly different at 5% according to LSD test.
Figures in parentheses are averages arc $\sqrt{\sin x}$ transformations of original data.
*) See page 55.
**) % plants showing yellow leaves and stiff droop.

Differences between any pair of the two pathogens in causing damage, however, were not significant. Plants infested with *R. similis* alone showed yellow leaves and stiff drooping. When relating the root decay index to percentage of plants with yellow leaves and stiff droop, it is evident that increasing root decay indices in all combinations with *R. similis* resulted in higher percentages of affected plants. *M. incognita* alone or in combination with *F. solani* did not cause yellow leaves and stiff droop.

Nematode numbers

Numbers of *M. incognita* and *R. similis* in the absence and in the presence of *F. solani* are presented in Table 3 and 4, and summarized in Fig. 2. The numbers of *M. incognita* when present alone were significantly higher than in the presence of *R. similis* or *F. solani*, i.e. 23630, 954, and 5201 respectively (Table 3 and Fig. 2). Compared with *M. incognita* alone, the populations of *M. incognita* in combinations were reduced by 78% and 96% respectively. The greatest reduction in the population of *M. incognita* occurred when this nematode was present together with *R. similis* and *F. solani*.

Table 3 Numbers of *M. incognita* (second stage juveniles) alone or in combination with *R. similis* and *F. solani* in roots and soil.

Treatments*)	roots	soil	total	reduction **)
Mi	16585	7045	23630	-
	(4.22) c	(3.81) b	(4.37) c	-
Mi+Rs	132	822	954	96
	(2.07) a	(2.77) a	(2.87) ab	-
Mi+Fs	2327	2874	5201	78
	(3.10) b	(2.98) ab	(3.38) b	-
Mi+Rs+Fs	253	286	539	98
	(2.23) a	(2.42) a	(2.69) a	-

Numbers followed by the same letters in each column are not significantly different at 5% according to LSD test. Means in parentheses are averages of log x transformation pf original data.
*) See page 55.
**) % reduction compared to Mi alone.

Table 4 Numbers of *R. similis* alone or in combination with *M. incognita* and *F. solani* in roots and soil.

Treat-ments*)	roots	soil	total	reduction **)
Rs	1811	698	2509	-
	(3.25) b	(2.67) a	(3.39) b	-
Mi+Rs	715	445	1160	54
	(2.78) a	(2.52) a	(3.07) a	-
Rs+Fs	1056	333	1389	45
	(3.01) ab	(2.46) a	(3.13) ab	-
Rs+Mi+Fs	526	378	904	64
	(2.58) a	(2.52) a	(2.86) a	-

Numbers followed by the same letters in each column are not significantly different at 5% according to LSD test. Numbers in parentheses are averages of log transformed original data.
*) See page 55.
**) Reduction, % from *R. similis* alone.

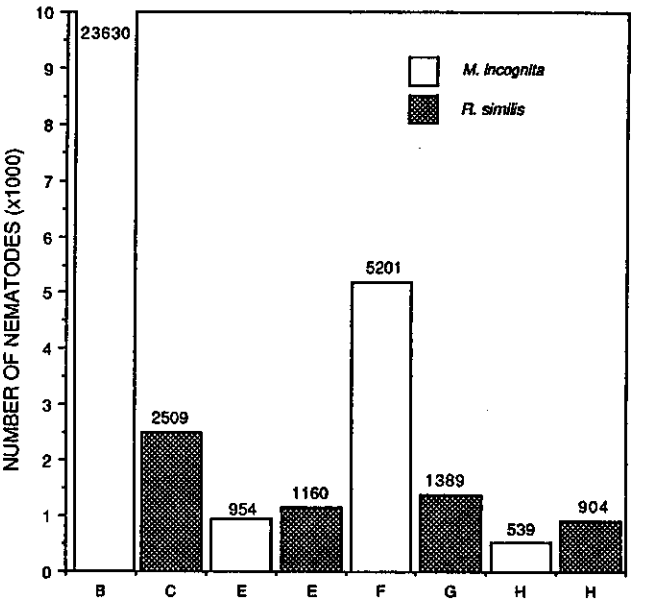

Figure 2 Total numbers of *M. incognita* and *R. similis* alone or in combination with *F. solani*.
B = inoculated with *M. incognita*; C = inoculated with *R. similis*; E = inoculated with *M. incognita* and *R. similis*; F = inoculated with *M. incognita* and *F. solani*; G = inoculated with *R. similis* and *F. solani*; H = inoculated with *M. incognita*, *R. similis* and *F. solani*.

The number of *R. similis* when present alone was 2509. It was significantly higher than in combination with *M. incognita* or with *F. solani*, i.e. 1160 and 1389 respectively (Table 3, Fig. 2). The lowest number of *R. similis* was 904 or only 36% of the treatment with *R. similis* alone, were obtained when this nematode was present together with *M. incognita* and *F. solani*. Although both nematode species were suppressed by *F. solani* when the three pathogens were present together, the number of *R. similis* tended to be higher than that of *M. incognita*.

DISCUSSION

The results indicated that the numbers of both *M. incognita* and *R. similis* were suppressed when they coinhabited the roots of black pepper. This result was similar to those obtained by other workers dealing with different nematode species and crops.

For example, *Pratylenchus penetrans* and *M. incognita* mutually inhibited reproduction when they coinhabited tomato roots (Estores & Chen, 1972). Herman et al. (1988) found that reproduction of *M. incognita* and *P. brachyurus* on soybean was mutually suppressed.

R. similis is a migratory endoparasite and *M. incognita* is a sedentary endoparasite. Migratory endoparasitic nematodes move through the root tissue and generally disturb feeding of already present sedentary endoparasitic nematodes (Eisenback, 1985; Eisenback & Griffin, 1987). Moreover, migratory endoparasitic nematodes often penetrate the roots earlier and suppress penetration by sedentary species. On black pepper, *R. similis* probably penetrated the roots earlier than *M. incognita*.

The populations of *M. incognita* and *R. similis* in black pepper appeared to be suppressed not only by mutual antago-

nistic reaction between both nematodes species, but also by an antagonistic interaction between nematodes and *F. solani*. Estores and Chen (1972) suggested the existence of substances produced either by *M. incognita* or by the infected host. These compounds caused suppression of the penetration by *P. penetrans*. Their studies might support the results described in Chapters 2 and 4.

R. similis alone and in all combinations with *M. incognita* and *F. solani*, provoked a clear yellow discoloration of the leaves consistently associated with stiff droop, root decay and occassionally dying of the plants.
M. incognita and *F. solani* alone caused some root decay, resulting in significant growth reduction. However, the typical yellow disease symptoms, viz. yellowing of the leaves and stiff droop, were exclusively found in the treatments with *R. similis*, either alone or in combination with *M. incognita* and/or *F. solani*. *M. incognita* together with *F. solani* also caused yellow leaves, but always without a stiff droop.

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6 Effect of a culture filtrate of *Fusarium solani* on hatching of *Meloidogyne incognita* and *Radopholus similis* at two different temperatures

Ika Mustika

ABSTRACT

The effect of a culture filtrate of *Fusarium solani* on hatching of *Meloidogyne incognita* and *Radopholus similis* at 20 °C and 25 °C was compared with hatching of both species in water at these temperatures. After an incubation of 15 days in a culture filtrate of *F. solani*, the hatch of *M. incognita* was inhibited by 92% at 20 °C and 84% at 25 °C, as compared with the hatch in water at these temperatures. The hatch of *R. similis* in this culture filtrate was inhibited only by 7% at 20 °C and 5% at 25 °C. *M. incognita* and *R. similis* hatched best in water at a temperature of 25 °C.

INTRODUCTION

The studies reported in Chapters 2, 4 and 5 have showed that populations of *Meloidogyne incognita* and *Radopholus similis* in black pepper decreased in the presence of *Fusarium solani*. Mani & Sethi (1984a) found that the presence of *F. oxysporum* f. sp. *ciceri* and *F. solani* inhibited egg hatch of *M. incognita*. This inhibitory effect was due to fungal toxins and residues of sugar and salts present in the culture filtrate (Mani & Sethi, 1984b).
Temperature influences many nematode activities such as hatching (Dao, 1970).

The objectives of this study were:

1) to evaluate whether the culture filtrate of a *F. solani* isolate of black pepper has an adverse effect on hatching of *M. incognita* and *R. similis*.
2) to determine whether an effect of culture filtrate is influenced by temperature.

MATERIALS AND METHODS

Two experiments were carried out to investigate the effect of a culture filtrate of *F. solani* on *M. incognita* and *R. similis*.
The temperatures selected were similar to the soil temperature used in previous pot experiments (Chapters 2, 4 and 5).

Experiment 1

F. solani isolated from roots of black pepper was cultured on PDA (Potato Dextrose Agar) in 10 cm Petri dishes and incubated at 25 °C. The *Fusarium* filtrate was prepared by transferring three discs of a culture (approx. 3 mm in diameter) into 250 ml flasks containing 100 ml of PDB (Potato Dextrose Broth). The flasks were placed on a shaker operating at 96 rpm and maintained at 30 °C. After five days the culture was filtered through three layers of filter paper to remove mycelium and conidia. The filtrate was then diluted with an equal volume of sterile distilled water according to Parmeter & Hood (1961).
To obtain inoculum of *M. incognita*, roots of black pepper infected with this nematode were collected and washed in running water.
Well developed root galls were excised and egg masses were collected and surface sterilized by placing them for 30 minutes in a Petri dish containing a mixture of 0.02% aqueous ethoxyethylmercury chloride and 0.1% streptomycin sulphate. Thereafter, they were transferred into a watch glass filled

with sterile distilled water. A single surface sterilized egg mass containing about 1000 eggs was then transferred into a Petri dish which had been filled with 4 ml of the autoclaved culture filtrate of *F. solani*, or into 4 ml of sterile distilled water as a control. The Petri dishes were incubated at 20 °C and 25 °C for three to 15 days. The treatments were replicated ten times. Juveniles and remaining eggs were counted after 3, 6, 9, 12 and 15 days. Percentages of hatch were calculated, dividing the number of juveniles by the sum of juveniles and remaining eggs, and then multiplied by 100.

Experiment 2

R. similis was obtained from two-month-old carrot disc cultures which had been prepared as described in Chapter 3. They were passed through a series of 90, 45, 20 and 10 µm sieves to separate eggs from females, males, juveniles, and debris. Surface sterilized eggs were collected in a small glass beaker filled with sterile distilled water, and used as inoculum. Two drops of this inoculum containing 900 to 1280 eggs of *R. similis*, were pipetted into Petri dishes (5 cm in diameter) containing 4 ml of *Fusarium* culture filtrate or sterile distilled water as in Experiment 1. The Petri dishes were incubated at 20 °C and 25 °C for 3 to 15 days. The percentages of hatch were assessed as in Experiment 1.

RESULTS

Eggs of both *M. incognita and R. similis* hatched faster and better in water than in *Fusarium* culture filtrate.

Experiment 1: *M. incognita*

In water, the hatching of *M. incognita* was rapid for 9 to

12 days, and then slowed down (Table 1, Fig. 1).
After 15 days, hatching had reached 80.4% at 20 °C, and 95.5% at 25 °C.

In *Fusarium* filtrate, hardly any hatching was found after 3 days.
After 15 days, hatching was still low at both temperatures in average 8%. The temperature effect was not significant.

Table 1 Hatching rate of *M. incognita* in water and culture filtrate of *F. solani* (FCF) at 20 °C and 25 °C (in percent).

Hatching media	T °C	days of incubation				
		3	6	9	12	15
		%	%	%	%	%
Water	20	8.8 c	31.7 b	44.7 b	68.8 b	80.4 b
Water	25	23.3 d	55.5 c	78.9 c	87.1 c	95.5 b
FCF	20	0.0 a	0.5 a	1.0 a	2.6 a	3.9 a
FCF	25	4.0 a	6.3 a	7.9 a	9.4 a	12.0 a

Numbers followed by the same letters in each column are not significantly different at 5% level (LSD test).

Experiment 2: ***R. similis***
In water, hatching of *R. similis* was rapid during the first 6 days, and then decreased (Table 2, Fig. 2).
The temperature effect was already significant after 3 days, and remained so during the 15 days of incubation. By this time approx. 80% hatching occurred.

In *Fusarium* filtrate, hatching finally ranged from 73.6% at 20 °C, to 76.1% at 25 °C. The temperature effect appeared to be significant during the first 6 days, and diminished in the following days.
Fusarium filtrate had only a minor inhibitory effect on the hatching of *R. similis*, whereas it seriously affected the hatching of *M. incognita*.

Table 2 Hatching rate of *R. similis* in water and culture filtrate of *F. solani* (FCF) at 20 and 25 °C (in percent).

Hatching media	T °C	days of incubation				
		3	6	9	12	15
		%	%	%	%	%
Water	20	17.8 a	58.2 ab	65.6 a	69.7 ab	77.4 b
Water	25	27.0 b	66.7 c	70.5 b	72.7 b	81.4 c
FCF	20	16.7 a	54.6 a	62.5 a	66.4 a	73.6 a
FCF	25	25.4 b	59.6 b	63.0 a	65.9 a	76.1 b

Numbers followed by the same letters in each column are not significantly different at 5% level (LSD test).

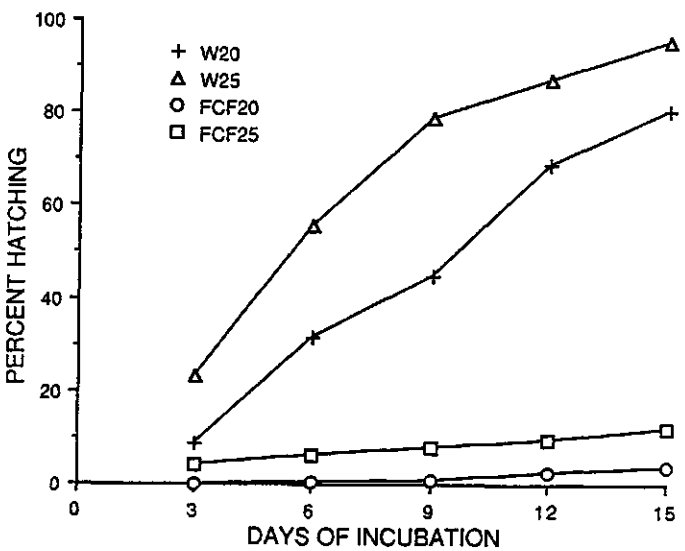

Figure 1 Hatching rate of *M. incognita* in water (W) and culture filtrate of *F. solani* (FCF) at 20 °C and 25 °C).

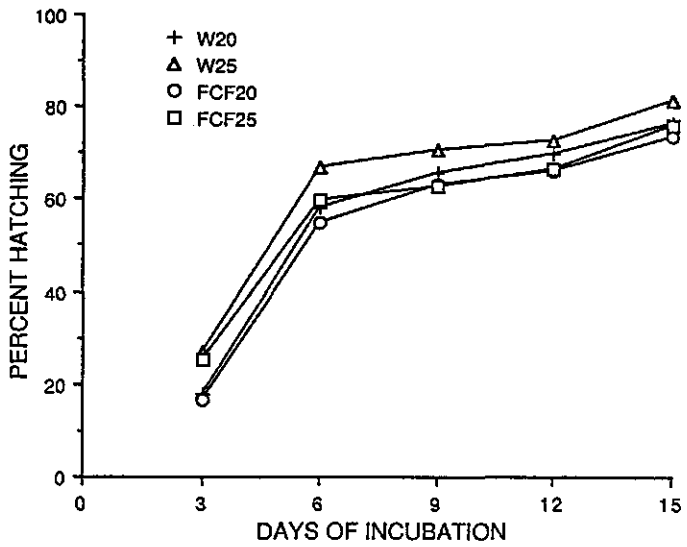

Figure 2 Hatching rate of *R. similis* in water (W) and culture filtrate of *F. solani* (FCF) at 20 °C and 25 °C.

DISCUSSION

These experiments revealed that the culture filtrate of *F. solani* inhibited the hatching of *M. incognita*, but had little effect on the hatching of *R. similis*. In previous pot experiments (Chapter 5), the populations of *M. incognita* and *R. similis* were reduced by 96% and 45% respectively in the presence of *F. solani*. The results of the present studies show that at 20 °C and 25 °C, the culture filtrate of *F. solani* reduced the hatch of *M. incognita* by 92% and 84% respectively (Table 1). The reduction of *M. incognita* by *F. solani* in pot experiments was possibly due to the inhibition of hatching. Apart from this, other mechanisms such as abortion of the giant cells may also occur, as reported by Fattah and Webster (1981).

In contrast, the culture filtrate of *F. solani* reduced the hatch of *R. similis* by only a few percent whereas in the pot experiments, the numbers of *R. similis* were reduced by 45% (Chapter 5). This difference indicates that the reduction of *R. similis* in the pot experiment was hardly due to the inhibition of hatching, and merely to an other mechanism. *R. similis* is an obligate parasite, and needs healthy root tissue to feed on. After the roots were colonized by *F. solani*, they decayed rapidly. *R. similis* might have been unable to survive in decaying roots, and might have moved in to other parts of the roots, or died, when no healthy roots were available.

It appeared that temperature had some influence on the inhibitory effect of the filtrate of *F. solani* upon *M. incognita* and *R. similis*. The highest rate of hatching was observed in water at 25 °C. A similar result was obtained by Dao (1970) with an isolate of *M. incognita* from Venezuela. At a higher and lower temperature the rate of hatching decreased considerably.

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7 Response of four black pepper cultivars to infection by *Radopholus similis*, *Meloidogyne incognita*, and *Fusarium solani*

Ika Mustika

ABSTRACT

The infection of four cultivars of black pepper (*Piper nigrum* L.) cv. Kalluvalli, Kuching, Cunuk & Jambi with *Radopholus similis* and *Meloidogyne incognita* alone or in combination with *Fusarium solani* was investigated. Absolute Growth Rate (AGR), Specific Leaf Area (SLA), and Leaf Area Ratio (LAR) were recorded. All four cultivars were susceptible to those pathogens. Of these four cultivars, cv. Kuching appeared to have tolerance to *M. incognita*.

INTRODUCTION

Nematodes induce alterations in plants, often resulting in reduced growth, lower yield, and quality loss (Lovey & Bird, 1973; Bird & Lovey, 1975; Evans, 1982; Melakeberhan et al., 1987; Nagesh & Dhawan, 1988).

The growth of the plants can be analyzed by measuring dry matter production, and can be expressed in terms of Absolute Growth Rate (AGR), Specific Leaf Area (SLA), and Leaf Area Ratio (LAR) (Hunt, 1982; Elliott & Bird, 1985; Nagesh & Dhawan, 1988; den Toom, 1988).

Nagesh and Dhawan (1988) found that *Heterodera avenae* reduced photosynthetic efficiency of wheat. Differences in growth rate between navy bean cultivars, associated with infection of *Pratylenchus penetrans*, were also reported (Elliott & Bird, 1985). Such information is not available for black pepper. Therefore an attempt was made to study a set growth

characteristics of four cultivars of black pepper and their response to infection by *Meloidogyne incognita* and *Radopholus similis* alone or in combination with *Fusarium solani*.

MATERIALS AND METHODS

Rooted cuttings of black pepper cv. Kalluvalli, Kuching, Cunuk and Jambi, one plant per pot, were grown in clay pots filled with 500 g of a mixture of autoclaved sandy loam soil (composition Annex 1) and perlite (2 : 1 v/v). The pots were placed in a growth chamber maintained under the conditions described in Chapter 5. Before planting, 3 g of Dolocal per pot were added (composition Annex 2).
Two weeks after planting the plants were inoculated with:
1) *R. similis* (Rs).
2) *M. incognita* (Mi)
3) *R. similis* + *M. incognita* + *F. solani* ((Rs+Mi+Fs).
Uninoculated plants (C) served as control.

The initial populations of *R. similis* and *M. incognita* were 500 nematodes per pot equivalent to 1 nematode per g of soil mixture, whereas the initial population of *F. solani* was set at 10^6 microconidia per pot. Nematodes and fungal inoculum were prepared as described in Chapters 4 and 5.

Plants were arranged in a randomized complete block design with three sets of six plants per treatment. Plants were watered daily with 50 ml of tap water per pot to keep soil moisture near to field capacity. In addition, every week the plants received 50 ml of a nutrient solution (composition Annex 2).

Thirty and sixty days after inoculation, the shoots of the plants were cut at soil surface. Leaf area, fresh and dry weight of the leaves, and fresh and dry weight of the shoots

were measured. Dry weight was determined after the plant material was dried at 80 °C for 48 h.

R. similis and *M. incognita* populations recovered from soil and root samples, were determined as described in Chapter 5. The Absolute Growth Rate (AGR)[1] was calculated according to Nagesh & Dhawan (1988) as follows:

$$AGR = (W2 - W1)/t2-t1$$

W1 and W2 represent the dry weight of the shoots 30 days (t1) and 60 days (t2) after inoculation.

Specific Leaf Area (SLA)[2], and Leaf Area Ratio (LAR)[3] were calculated according to Hunt (1982).

RESULTS

Growth characteristics

The results of the growth characteristics AGR, LAR and SLA are presented in Table 1.

Absolute Growth Rate (AGR)

In the control plants, the highest AGR was 0.83 gd^{-1} for cv. Kalluvalli, followed by 0.74 gd^{-1} for cv. Kuching, 0.38 gd^{-1} for cv. Jambi, and 0.34 gd^{-1} for cv. Cunuk. Both *R. similis* and *M. incognita* alone, or a combination of these two species with *F. solani* reduced the AGR in general.

R. similis alone reduced the AGR of Kalluvalli, Kuching,

[1] AGR (Absolute Growth Rate) = Fresh weight increase of the plant per day (gd^{-1}).

[2] SLA (Specific Leaf Area) = Total leaf area/total dry weight of leaves (cm^2g^{-1}).

[3] LAR (Leaf Area Ratio) = Total leaf area/total dry weight of the plant (cm^2g^{-1}).

Table 1 Effects of *R. similis*, and *M. incognita* alone, and both in the presence of *F. solani*, on the growth characteristics of four black pepper cultivars.

Cultivars	Treatment	AGR (gd^{-1})	LAR (cm^2g^{-1})	SLA (cm^2g^{-1})
Ka	C	0.83 g	106 ef	186 d
	Rs	0.15 bcd	87 abcd	147 ab
	Mi	0.48 f	86 abcd	156 ab
	Rs+Mi+Fs	0.34 e	85 abc	148 ab
Ku	C	0.74 g	112 f	173 cd
	Rs	0.20 d	78 a	177 d
	Mi	0.69 g	109 ef	180 d
	Rs+Mi+Fs	0.31 e	96 bcde	179 cd
Ja	C	0.38 ef	110 ef	174 cd
	Rs	0.03 a	82 ab	146 ab
	Mi	0.12 abcd	100 cdef	147 ab
	Rs+Mi+Fs	0.03 a	84 ab	172 cd
Cu	C	0.34 e	113 f	173 cd
	Rs	0.05 ab	80 a	128 ab
	Mi	0.16 cd	102 def	142 ab
	Rs+Mi+Fs	0.05 ab	85 ab	140 ab
Cultivars (C)		**	NS	*
Inoculation (I)		**	*	*
C x I		**	NS	NS

Numbers followed by the same letters in each column are not significantly different (P = 0.05) according to LSD test.
* significantly different (P = 0.05)
** significantly different (P = 0.01)
Ka = Kalluvalli
Ku = Kuching
Ja = Jambi
Cu = Cunuk
NS = non significant.

Jambi and Cunuk by 82, 73, 92 and 85% respectively as compared with each control. *M. incognita* alone did not significantly affect the AGR of cv. Kuching, and when *R. similis* and *F. solani* were involved, the AGR was reduced by 58%.
In cv. Kalluvalli, Jambi and Cunuk, the reduction of AGR by *M. incognita* alone was 42, 68 and 53% respectively. When *R. similis* and *F. solani* were also present, the reductions were 59, 92 and 85% respectively.

Leaf Area Ratio (LAR)

The LAR of uninoculated plants were not significantly different between the four cultivars. *R. similis* infection caused reduction in LAR of cv. Kalluvalli, Kuching, Jambi and Cunuk by 18, 30, 25 and 29% respectively. *M. incognita* caused a relative low and not significant reduction of LAR in the cv's Kuching, Jambi and Cunuk of 3, 9 and 10% respectively, whereas the LAR of cv. Kalluvalli was reduced by 19%. Combined infection with *R. similis*, *M. incognita* and *F. solani* caused a reduction of 20, 14, 23 and 25% for the cv's. Kalluvalli, Kuching, Jambi and Cunuk respectively.

Specific Leaf Area (SLA)

Infection by *R. similis*, or *M. incognita* alone or by *R. similis*, *M. incognita* and *F. solani* together did not affect the SLA of cv. Kuching. In cv. Kalluvalli, *R. similis* alone reduced the SLA by 21%, and by 16% to 26% in cv's. Jambi and Cunuk. *M. incognita* alone reduced the SLA of Kalluvalli, Jambi, and Cunuk by 16, 16 and 18% respectively.

Infection by *R. similis*, *M. incognita* and *F. solani*, reduced the SLA less than did the infection by *R. similis* or *M. incognita* alone in the cv's. Kalluvalli, Jambi and Cunuk.

Nematode populations

Nematode populations in the four cultivars tested are presented in Table 2 and Fig. 1.

Radopholus similis

Thirty days after inoculation, the number of *R. similis* in cv. Cunuk was 2 to 6 times higher as compared with those in any of the other cultivars. The number of *R. similis* in cv. Kalluvalli, Kuching, and Jambi were not significantly different, both in the presence and in the absence of *F. solani* (Table 2, Fig. 2).

Table 2 Total numbers of *M. incognita* and *R. similis* in four black pepper cultivars, 30 and 60 days after inoculation.

Cultivars	Treatments *)	*M. incognita*		*R. similis*	
		30	60	30	60
Ka	Mi	323 (2.49)c	913 (2.96)b	-	-
	Rs	-	-	776 (2.88)a	4326 (3.63)bc
	Mi+Rs+Fs	473 (2.66)c	873 (2.93)b	833 (2.88)a	1400 (3.15)a
Ku	Mi	43 (1.59)a	1566 (3.19)b	-	-
	Rs	-	-	903 (2.95)a	2350 (3.36)abc
	Mi+Rs+Fs	136 (2.09)bc	346 (2.49)a	843 (2.90)a	1566 (3.20)abc
Ja	Mi	40 (1.10)a	360 (2.46)a	-	-
	Rs	-	-	927 (2.96)a	1023 (3.01)ab
	Mi+Rs+Fs	147 (2.16)bc	213 (2.33)a	933 (2.91)a	1176 (3.05)a
Cu	Mi	190 (2.25)bc	1387 (3.13)b	-	-
	Rs	-	-	4530 (3.66)b	5333 (3.73)c
	Mi+Rs+Fs	100 (2.00)bc	373 (2.56)a	1706 (3.23)ab	1090 (3.17)a
Cultivars		+	++	++	++
Treatments		+	++	+	++
Interaction		NS	NS	NS	+

* See page 72 for treatments code.
Numbers followed by the same letters in each column are not significantly different at 5% level.
Numbers in parentheses are averages of log x transformed original data.
\+ = significant difference (P = 0.05)
++ = significant difference (P = 0.01)
NS = not significant
Ka = Kalluvalli; Ku = Kuching; Ja = Jambi; Cu = Cunuk
Initial populations Mi and Rs = 500 specimens/pot.

In the absence of *M. incognita* and *F. solani*, number of *R. similis* in cv. Kalluvalli, Kuching, and Jambi increased by 1.6; 1.8 and 1.9 times that of the initial population, whereas in cv. Cunuk numbers increased by 9 times. In the presence of *F. solani* and *M. incognita*, the number of *R. similis* was not reduced significantly, except in cv. Cunuk at 60 days after inoculation.

The population increase of *R. similis* from day 30 to day 60, was in average 2.25 times in Kalluvalli and Jambi. In cv. Cunuk, where the population of *R. similis* at day 30 was al-

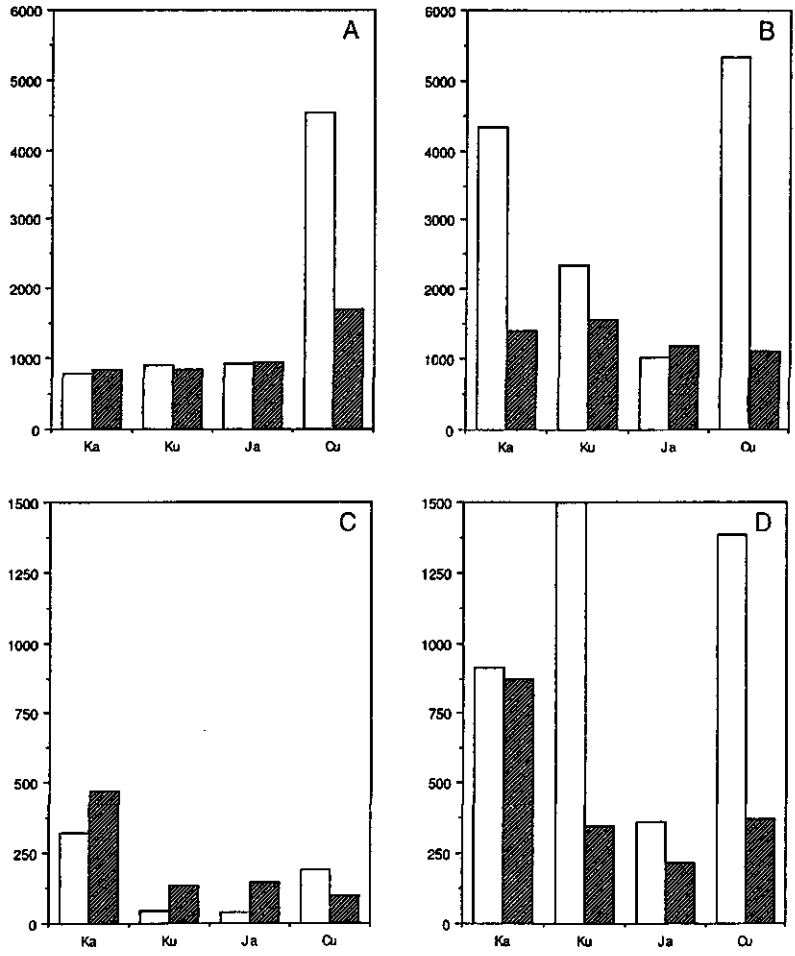

Figure 1 Numbers of *R. similis* and *M. incognita* in four cultivars of black pepper.
A. *R. similis* 30 days after inoculation.
B. *R. similis* 60 days after inoculation.
C. *M. incognita* 30 days after inoculation.
D. *M. incognita* 60 days after inoculation.
Ka = Kalluvalli; Ku = Kuching; Ja = Jambi; Cu = Cunuk.
(▭) = inoculated with *M. incognita* or *R. similis*
(▨) = inoculated with *M. incognita*, *R. similis* and *F. solani*.

ready much higher than in the other three cultivars, there was no further increase.

Meloidogyne incognita

In contrast to *R. similis*, thirty days after inoculation the populations of *M. incognita* in all four cultivars were lower than the initial population. Sixty days after inoculation, the numbers of *M. incognita* in the absence of *R. similis* and *F. solani* increased in cv's. Kalluvalli, Kuching and Cunuk in average by 2.56 times of that the initial population. In cv. Jambi, the number of *M. incognita* increased but to only 72% of the initial population.

DISCUSSION

The data obtained showed that the AGR of the four cultivars tested was significantly reduced by *R. similis* and *M. incognita* alone, or by a combination of these two species with *F. solani*, exept for *M. incognita* on cv. Kuching.

In cv. Kalluvalli and Kuching, the reduction of AGR by *R. similis*, *M. incognita* and *F. solani* together, was less than with *R. similis* alone (Table 1). This may be due to a competition between *R. similis* and *M. incognita* when they coinhabit black pepper roots. *R. similis* appeared to inflict more damage to all four cultivars than *M. incognita* at the same inoculum density.

With regard to infection by *M. incognita* cv. Kuching showed no significant difference in reduction of the AGR compared with the control (Table 1). The number of *M. incognita* in this cultivar, tended even to be higher than in the other cultivars. This implies that cv. Kuching seems to be the most tolerant amongst these four cultivars to *M. incognita*. No differences in tolerance to *R. similis* were found between

the cultivars.

The AGR, LAR and SLA of cv. Kuching inoculated with *R. similis*, *M. incognita* and *F. solani* tended to be greater than those of cv. Jambi and Cunuk. This result may explain why in field conditions such as on Bangka, where those three pathogens are present in association, the Kuching cultivar produced a higher yield than did Jambi and Cunuk (Nuryani, 1984). In Sarawak, where *M. incognita* and *M. javanica* were commonly found (Winoto, 1972; Kueh & Teo, 1978; Kueh, 1979) cv. Kuching was also reported to give the highest yield amongst six other cultivars viz. Jambi, Belantung, Kalluvalli, Balamcotta, Uthirincotta and Cheriakanikadan (Purseglove et al., 1981).

With regard to the SLA, which is a measure for relative leaf thickness, infection by *R. similis* and *M. incognita* alone, or by a combination of these two species with *F. solani*, had no effect on cv. Kuching. In cv. Kalluvalli and Cunuk, it was reduced significantly. In cv. Jambi the significant reduction in SLA was only observed in plants infected with *R. similis* and *M. incognita* alone. This indicates that in cv. Kalluvalli, Jambi and Cunuk, infection with those three pathogens caused the leaves to become thicker than in uninfected plants.

These data support the expression of symptoms occurring in the field with other cultivars. In the early stages of "yellow disease", leaves usually show a stiff droop and exhibit pale yellow discoloration, followed by an inwards curving as drought intensifies. The curving leaves are thicker than the healthy looking leaves. This is in agreement with the data on SLA obtained in this experiment.

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8 Effects of *Radopholus similis*, *Meloidogyne incognita* and *Fusarium solani* on leaf nutrient content and development of leaf symptoms in black pepper (*Piper nigrum* L.) at two soil moisture levels

Ika Mustika

ABSTRACT

Four-week-old rooted cuttings of black pepper (*Piper nigrum* L.) cv. Kalluvalli were grown at two soil moisture levels, one at approximately field capacity, and one at 60% of field capacity. They were inoculated with *Meloidogyne incognita*, *Radopholus similis* and *Fusarium solani* alone or in various combinations. Five weeks after the nematode inoculation Absolute Growth Rate, Leaf Area Ratio, Specific Leaf Area, leaf nutrient content for N, P, K, Ca and Mg, and the nematode populations were determined. In general, all plant growth characteristics of inoculated plants were reduced significantly as compared to uninoculated plants at both moisture levels. *R. similis* and *M. incognita* multiplied better in the moist than in the dry regime.

The average numbers of *R. similis* and *M. incognita* recovered in the dry regime were 19% and 66% respectively as compared to those in moist conditions. Leaf N concentration was significantly increased in plants grown in dry conditions when infected with *R. similis* or *M. incognita* alone, or in combination. Leaf P was not affected by the pathogens, whereas leaf K was reduced significantly in plants grown in the moist regime when inoculated with *R. similis* alone, in combination with *M. incognita*, or with a combination of *M. incognita* and *F. solani*. Leaf K in plants grown in dry conditions was not affected. Infection with all three pathogens, alone or in combination, increased significantly leaf

Ca level at both moisture levels. Leaf Mg was significantly increased by the pathogens, mainly in plants grown in the dry regime. *R. similis* alone caused yellow leaves and stiff droop. *M. incognita* together with *F. solani* could also cause yellow leaves but without stiff droop, and to a lesser extent. Dry condition enhanced the development of yellow leaves and the stiff droop.

INTRODUCTION

Soil moisture is one of the environmental factors influencing both nematode populations and plant growth. Nematodes need sufficient soil moisture for movement, feeding or invasion and reproduction. Sufficient soil moisture is also needed by the plants for good growth. Soil moisture may affect many physiological processes in plants and thereby may influence nematode populations. On the other hand, nematodes may also affect photosynthesis and plant growth, for instance by influencing the host's nutrient content, the translocation of photosynthetic products (Bird & Loveys, 1975) or the transpiration (Evans, 1982). For normal growth, an adequate supply, uptake and balanced distribution of nutrients within a plant is required. When nematodes infect plants the nutrient status may change and tends to alter the physiology of the host (Melakeberhan, et al., 1987).

There is ample information in the literature on the effect of nematode infection on plant nutrient status. It was suggested that the effect of nematode infection on the uptake of nutrients and distribution within the plant varies with nematode species and host.
Chitwood et al. (1952) found that leaves of peach seedlings infected with *Meloidogyne incognita* and *M. javanica* contained more K than uninfected plants. Nasr et al. (1980) showed that leaves of bitter almond and peach infected by *M. java-*

nica or *M. incognita* had greater concentrations of K, Ca, Mn and Cu. The leaf concentration of Fe was significantly decreased, whereas those of Mg and Zn were unaffected.

Radopholus similis was found to reduce the concentrations of N, P and K in leaves of citrus (Feldman et al., 1961). In rough lemon seedlings, *R. citrophillus* had no effect on leaf concentrations of K, Mg, and Zn, but reduced leaf levels of P, Ca, Fe and Mn (Smith & Kaplan, 1988).

In the case of "yellow disease" (the "geelziekte" according to Van der Vecht, 1950) of black pepper, which is suspected to be associated with *R. similis*, *M. incognita* and *Fusarium solani* (Bridge, 1978), de Waard (1979) showed that the concentrations of N, P, K, Ca and Mg in yellow leaves from diseased plants, were lower than those of healthy leaves from apparently healthy vines. Indications that water stress may be associated with disease incidence was also noted (de Waard, 1986).
Detailed information on the probable role of *R. similis*, *M. incognita* and *F. solani* on leaf nutrient concentrations of black pepper, is not available. The objective of the research reported in this Chapter, was to investigate the effect of *R. similis*, *M. incognita* and *F. solani* on the growth rate, symptom development, nematode populations, and the leaf nutrient content of black pepper grown at two soil moisture levels.

MATERIAL AND METHODS

Sandy loam soil, collected from the field was used in this study. The soil composition is presented in Annex 1. To remove course debris and large soil particles, the soil was passed through a sieve with a mesh of 5 mm. Thereafter the soil was autoclaved. The autoclaved soil was put in closed

plastic bags, and kept for one month in the greenhouse before use in the experiment. The pF characteristic of the treated soil is presented in Fig. 1.

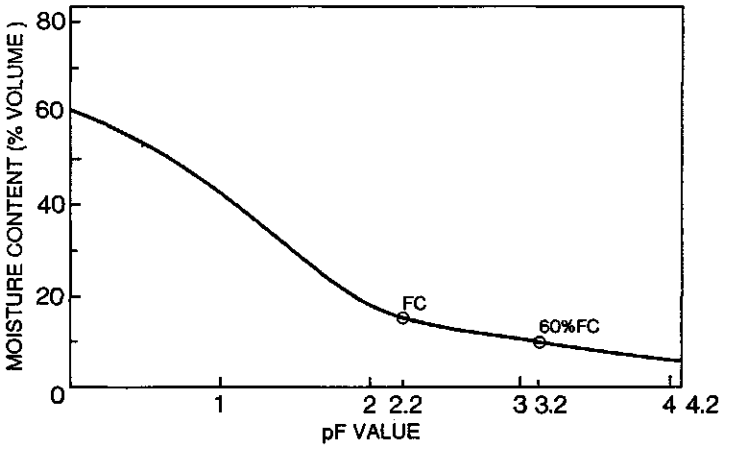

Figure 1 Moisture characteristic of sandy loam soil.
FC = Field capacity; Bulk density = 1.14 g cm^{-3}.

At the start of the experiment, 10 samples of 50 g of autoclaved soil were taken from the plastic bags for the measurement of the initial soil moisture content. This was determined by measuring the weight loss of the samples after drying in an oven at 110 °C for 24 h.

The pots were filled with one kg of soil. A single node, one-month-old rooted cutting of black pepper cv. Kalluvalli was weighed and planted in each pot. Before use in the experiment, the weight of each pot was also recorded. In order to reduce evaporation a sheet of black polyethylene was used to cover the soil surface.

The plants were placed in a climate chamber, under 14 h photoperiod with a constant air temperature of 28 °C, a relative humidity of 75% to 80%, and a soil temperature of 23 °C to 25 °C. A combination of SON/T 400 W lamps and HPI/T 400 W lamps was used.
The soil moisture was kept at approximately field capacity for one week by periodic watering and weighing each pot including soil, plant, plastic cover, label and bamboo support.
Two soil moisture levels, one at field capacity, and one at 60% of field capacity, were compared in this investigation. Field capacity was taken at pF = 2.2, derived from the pF curve (van Reeuwijk, 1986). Soil moisture content at field capacity and at 60% of field capacity (pF = 3.2) was 16% and 10% by volume respectively (Fig. 1). The plants were then divided into two groups of 144 plants each. To maintain soil moisture contents during the experiment, the pots were weighed and watered regularly, depending upon growth stage of the plants, and upon soil moisture levels.
The following treatments were applied, at both moisture levels.

1) Uninoculated control.
2) *M. incognita* (Mi).
3) *R. similis* (Rs).
4) *F. solani* (Fs).
5) Mi + Rs.
6) Mi + Fs.
7) Rs + Fs.
8) Mi + Rs + Fs.

Each treatment was replicated three times with six plants per replicate and arranged in a randomized complete block design. Initial inoculum was approximately 1000 nematodes and/or 10^6 microconidia of *F. solani* per pot. The nematodes were inoculated two weeks prior to the fungus. The inocula

of nematodes and fungus were prepared as described in Chapter 5.

Five weeks after inoculation, two plants from each treatment in both moisture blocks, were uprooted. Roots were washed. Roots and shoots including leaves, were weighed separately. The roots were then blended and incubated in a mistifier for seven days using water at a temperature of 27 °C, to separate nematodes from roots. Leaf area per plant was measured by passing the leaves through a leaf area meter.
All leaves from each plant, and the other parts of the shoot were then dried separately at 85 °C for 48 hours and weighed.

For nutrient analysis, mature and immature leaves without petiole were collected separately from two other plants in each treatment. Before drying, the leaves were cleaned with moist cotton using distilled water, and dried at 70 °C overnight (de Waard, 1969). Dry leaves of each plant were packed separately and sent[1] for analysis of N, P, K, Ca and Mg.

Plant growth measurements were made in terms Absolute Growth Rate (AGR)[2], Leaf Area Ratio (LAR)[3] and Specific Leaf Area (SLA)[4] (Hunt, 1982; Elliot & Bird, 1985). For the AGR, fresh weight of the plants at planting, and 7 weeks after planting were recorded, and further calculated as follows:

$$AGR = (W7 - WO)/(t2-t1) \quad (gw^{-1})$$

[1] To Bedrijfslaboratorium voor Grond- en Gewasonderzoek, Oosterbeek.
[2] AGR (Absolute Growth Rate) = fresh weight increase of the plant per week (gw^{-1}).
[3] LAR (Leaf Area Ratio) = Total leaf area/total dry weight of the plant (cm^2g^{-1}).
[4] SLA (Specific Leaf Area) = Total leaf area/total dry weight of leaves (cm^2g^{-1}).

W0 = weight at planting
W7 = weight at 7 weeks after planting.

The data were subjected to analysis of variance.

RESULTS

Nematode populations

The number of nematodes recovered from soil and roots is presented in Table 1, and Fig. 2 and 3. In general there were more nematodes in the moist regime than in dry regime.

Radopholus similis

Almost 4000 of *R. similis* were found in the moist regime, whereas only 495 in dry conditions. This difference was significant. When *R. similis* was present together with *M. incognita*, 3401 of *R. similis* were found. The increase was 3.4 times the initial population, whereas in the presence of *F. solani*, 3702 *R. similis* were found. It was an increase by 3.7 times as compared to the initial inoculum. In the moist regime, the number of *R. similis* when present alone, increased by 3.9 times that of the initial inoculum. When *R. similis* was present together with *F. solani* and *M. incognita*, the population increased to only 1602, or 1.6 times the initial inoculum, significantly less than in the three previous cases.

In the dry regime, the numbers of *R. similis* either alone or in combination with *M. incognita*, or *F. solani* were of a similar magnitude, but differed significantly from those in the moist conditions. In average, only 19% of the numbers *R. similis* found in the moist regime, were recovered under dry conditions. No effect of *F. solani* was found on multiplication of *R. similis* in dry regime, however, it was present in the moist.

Table 1 Total numbers of *M. incognita* and *R. similis* recovered five weeks after nematodes inoculation (data represent the sum of nematodes recovered from soil and roots).

Treatment *)	*M. incognita*		*R. similis*	
A. FC				
Mi	1516	(3.17) c	-	-
Rs	-	-	3944	(3.60) c
Mi + Rs	619	(2.69) ab	3401	(3.60) c
Mi + Fs	920	(2.95) bc	-	-
Rs + Fs	-	-	3702	(3.57) c
Mi + Rs + Fs	612	(2.78) abc	1602	(3.29) b
Total	3667		12649	
B. 60% FC				
Mi	1099	(2.89) bc	-	-
Rs	-	-	495	(2.69) a
Mi + Rs	256	(2.33) a	605	(2.27) a
Mi + Fs	539	(2.51) ab	-	-
Rs + Fs	-	-	577	(2.71) a
Mi + Rs + Fs	510	(2.67) ab	721	(2.85) a
Total	2404		2398	

*) See page 86.
Numbers followed by the same letter in each column are not significantly different at 5% level (LSD test). Figures in paratheses are averages log transformations of original data. FC = field capacity.

Meloidogyne incognita

A population increase of *M. incognita* from 1000 to 1517 was observed only in the moist regime, when *M. incognita* was present alone. In the presence of *R. similis*, the number of *M. incognita* was reduced by 38% to 619, as compared to the initial inoculum.

When *M. incognita* and *F. solani* were present together, the numbers of *M. incognita* were reduced by 8% to 920. The lowest number of 612 *M. incognita* was found in the presence of *R. similis* and *F. solani*, and amounted to a reduction of 39% from the initial inoculum.

In the dry regime, *M. incognita* could hardly reproduce. When present alone, the number of *M. incognita* was 1099, only a 10% increase over the initial inoculum. The number of *M. incognita* in the presence of *R. similis*, was reduced by 74% to

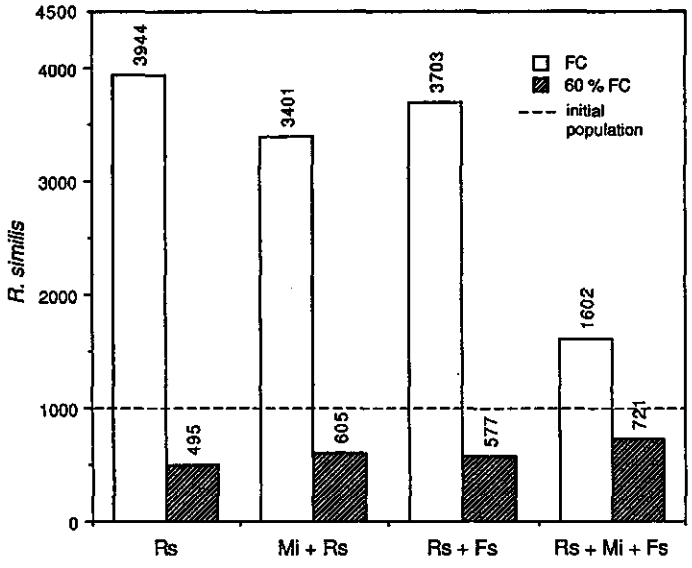

Figure 2 The population of *R. similis* in soil and roots in two soil moisture regimes, five weeks after inoculation. Rs = inoculated with *R. similis*; Mi+Rs = inoculated with *M. incognita* and *R. similis*; Rs+Fs = inoculated with *R. similis* and *F. solani*; Rs+Mi+Fs = inoculated with *R. similis*, *M. incognita* and *F. solani*; FC = field capacity.

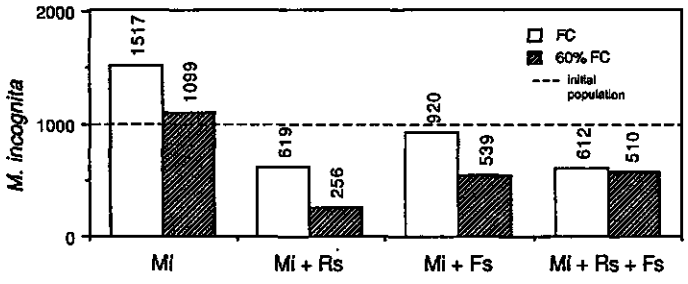

Figure 3 The population of *M. incognita* in soil and roots in two soil moisture regimes, five weeks after inoculation. Mi = inoculated with *M. incognita*; Mi+Rs = inoculated with *M. incognita* and *R. similis*; Mi+Fs = inoculated with *M. incognita*, *R. similis* and *F. solani*; FC = field capacity.

256, whereas in the presence of *F. solani*, it was reduced by 46% to 539, as compared to the initial inoculum.
When both *R. similis* and *F. solani* were involved, the number of *M. incognita* was 510 or 49% of the initial inoculum.
In average, only 66% of the numbers *M. incognita* found in the moist regime were recovered under dry conditions.

Plant growth

The results of plant growth analysis five weeks after inoculation are shown in Table 3. In general, both in the moist and in dry regimes, the growth of uninoculated control plants was better than that of inoculated plants.

The AGR, LAR and SLA were significantly affected by the pathogens. Only AGR showed a soil moisture effect. All three characteristics showed a significant interaction for moisture and pathogens.

Absolute Growth Rate (AGR)

In the dry regime the AGR of control plants reduced by 34% as compared to the moist treatment. When plants were infected by the respective pathogens alone or in combinations, reduction of AGR rose from 40% for *M. incognita*, to 84% for *M. incognita* together with *R. similis* in the moist regime, and from 58% for *M. incognita* to 90% for *M. incognita* together with *F. solani* in the dry regime. The data tend to show the trend that *R. similis* caused more reduction of AGR alone or in combination with the other pathogens, irrespective of moisture regime (Table 4, and Fig. 4).

Table 3 Effects of *M. incognita*, *R. similis* and *F. solani* on AGR, LAR, SLA, development of yellow leaves with stiff droop, and leaf concentration of N, P. K, Ca and Mg on black pepper five weeks after inoculation.

Treatment	AGR (gw^{-1})	LAR (cm^2g^{-1})	SLA (cm^2g^{-1})	% yellow^{+++}	N	P	K	Ca	Mg
A. FC									
Uninoculated	4.01 f	82.93 h	191 h	0	4.24 bc	0.48 a	2.11 cd	1:38 a	0.43 ab
M. incognita	2.42 e	58.71 ef	153 fg	0	4.54 cd	0.43 a	1.97 abc	1.61 bc	0.42 ab
R. similis	0.95 bc	58.14 ef	147 defg	0	4.22 b	0.33 a	1.84 a	1.80 c	0.45 abc
F. solani	1.72 d	61.20 fg	145 defg	0	4.32 cd	0.36 a	1.82 a	2.20 d	0.51 abc
Mi + Rs	0.63 ab	50.65 ef	168 g	0	3.82 a	0.40 a	1.85 a	1.67 bc	0.41 a
Mi + Fs	1.38 cd	46.44 de	150 efg	0	4.12 b	0.36 a	1.79 a	1.74 c	0.48 abc
Rs + Fs	1.14 bcd	17.25 a	115 abc	0	4.14 b	0.37 a	2.01 abc	1.65 bc	0.42 ab
Mi + Rs + Fs	1.06 bc	24.82 a	100 a	0	3.98 ab	0.45 a	1.85 a	2.12 c	0.96 g
B. 60% FC									
Uninoculated	2.64 e	73.22 gh	173 gh	0	4.14 b	0.36 a	2.08 bcd	1.46 ab	0.39 a
M. incognita	1.10 bc	45.05 d	120 abcd	0	5.02 e	0.39 a	2.12 cd	2.55 f	0.73 ef
R. similis	0.51 ab	38.36 cd	123 abcde	17	5.19 e	0.36 a	2.15 cd	2.24 de	0.58 bcd
F. solani	0.84 abc	47.24 de	128 bcdef	0	5.03 e	0.43 a	2.25 d	2.59 f	0.76 f
Mi + Rs	0.56 ab	34.49 cd	140 cdef	17	5.23 e	0.44 a	2.26 d	2.57 f	0.76 f
Mi + Fs	0.27 a	40.85 d	134 bcdef	6*	4.14 b	0.40 a	1.95 abc	2.44 ef	0.60 cde
Rs + Fs	0.28 a	34.66 cd	112 ab	12	4.79 d	0.33 a	1.98 abc	2.91 g	0.71 ef
Mi + Rs + Fs	0.95 bc	33.39 bc	113 abc	17	4.90 e	0.37 a	2.08 bcd	2.53 f	0.65 def
Inoculation (I)	++	++	+		NS	NS	NS	+	+
Soil moisture (S)	++	NS	NS		++	NS	++	+	++
I X S	++	+	+		+	NS	++	++	++

Numbers followed by the same letter in each column are not significantly different (P = 0.05) according to LSD test. +) = significantly different (P = 0.05); ++) = significantly different (P = 0.01); +++) = % plants with yellow leaves and stiff droop; NS = not significant; FC = field capacity; * = yellow leaves without stiff droop.

Leaf Area Ratio (LAR)

The LAR value of uninoculated plants in the moist and dry regimes shows no significant difference, but LAR values were significantly reduced by *M. incognita*, *R. similis*, and *F. solani* alone, or in combination (Table 3).
In the moist regime, the reduction of LAR varied from 26% to 79% for plants infected with *F. solani* alone, and for a combination of *R. similis* and *F. solani* respectively, as compared to uninoculated plants (Table 5).

Table 4 The reduction of AGR (%) by *M. incognita*, *R. similis* and *F. solani* alone or in combination. *)

Treatment**)	FC	60% FC
C	0	0
Mi	40	58
Rs	76	81
Fs	57	68
Mi+Rs	84	79
Mi+Fs	66	90
Rs+Fs	72	89
Mi+Rs+Fs	74	64

*) Compared to uninoculated plants.
**) See page 86.
FC = field capacity.

Table 5 The reduction of LAR by *M. incognita*, *R. similis* and or *F. solani* five weeks after inoculation.*)

Treatment**)	FC	60% FC
C	0	0
Mi	29	38
Rs	30	48
Fs	26	35
Mi+Rs	39	53
Mi+Fs	44	44
Rs+Fs	79	53
Mi+Rs+Fs	70	54

*) Compared to uninoculated plants.
**) See page 86.
FC = field capacity.

In the dry regime, reduction of LAR varied from 35% in plants infected with *F. solani*, to 54% in plants infected with *M. incognita*, *R. similis*, and *F. solani* together, as compared to uninoculated plants.

Specific Leaf Area (SLA)

The SLA values of the uninoculated plants grown in the moist and dry regimes were not significantly different. The SLA was significantly reduced by *M. incognita*, *R. similis* and *F. solani* either alone or in combination, as compared to the respective controls (Table 3).

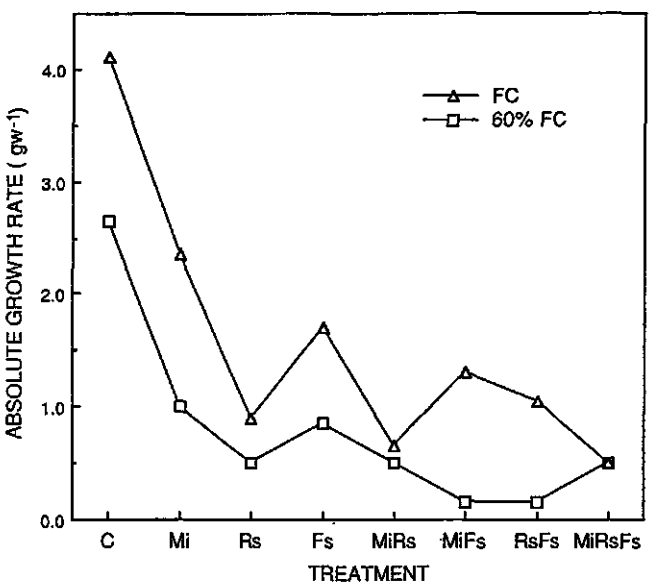

Figure 4 The Absolute Growth Rate (AGR) of black pepper five weeks after inoculation. C = uninoculated plants; Mi = inoculated with *M. incognita*; Rs = inoculated with *R. similis*; Fs = inoculated with *F. solani*; MiRs = inoculated with *R. similis* and *M. incognita*; RsFs = inoculated with *R. similis* and *F. solani*; MiFs = inoculated with *M. incognita* and *F. solani*; MiRsFs = inoculated with *M. incognita*, *R. similis* and *F. solani*. FC = Field capacity.

In the moist regime, the reduction of SLA varied from 20% in plants infected with *M. incognita*, to 48% in plants infected with *M. incognita*, *R. similis*, and *F. solani* together.

In the dry regime, *M. incognita*, *R. similis*, and *F. solani* alone or in combination reduced the SLA significantly, with a similar trend as observed for the reduction in the moist regime. This reduction of SLA varied from 19% in plants infected with a combination of *M. incognita*, and *R. similis*, to 35% in plants infected with a combination of *R. similis* and *F. solani*, or with a combination of the three pathogens as compared to inoculated plants (Table 6).

Table 6 The reduction of SLA (%) caused by *M. incognita*, *R. similis* and *F. solani* alone or in combination five weeks after inoculation.*).

Treatment**)	FC	60% FC
C	0	0
Mi	20	31
Rs	23	29
Fs	24	26
Mi+Rs	12	19
Mi+Fs	21	23
Rs+Fs	40	35
Mi+Rs+Fs	48	35

*) Compared to uninoculated plants.
**) See page 86.
FC = field capacity.

Symptom development

At five weeks after inoculation, wilting symptoms did not occur yet. Plants grown in the dry regime, started to develop yellow leaves with a stiff droop, and defoliation, whereas the appearence of plants grown in the moist regime remained apparently healthy (Table 3).
In the dry regime, plants inoculated with *R. similis* alone,

or in combination with *M. incognita* and/or *F. solani* appeared associated with the yellow leaves with a stiff droop, and started to defoliate. Plants inoculated with a combination of *M. incognita* and *F. solani* also started to show these symptoms, but without a stiff droop, and less frequently than in those inoculated with *R. similis*.
R. similis alone, or with *M. incognita*, and in combination with *M. incognita* and *F. solani*, caused 17% yellow leaves associated with a stiff droop. The combination of *R. similis* and *F. solani* showed only 12%.
Inoculation with *M. incognita* and *F. solani* caused 6% yellow leaves, but without stiff droop.
Uninoculated plants, and inoculated plants with *M. incognita* or *F. solani* alone, did not show any of these symptoms.
In the present study, the first yellow leaves appeared five weeks after inoculation, in dry regime, whereas these symptoms appeared about seven weeks after inoculation in the study of Chapters 4 and 5.

Leaf nutrients concentration

Nitrogen

There was no significant difference in level of N between controls. In the moist regime *M. incognita*, *R. similis* and *F. solani* alone increased leaf N but not significantly different with that in uninfected plants. Only in plants infected with a combination of *M. incognita* and *R. similis*, leaf N dropped and showed a significant reduction of 10% as compared to leaves of uninoculated plants (Table 3).
In the dry regime, infection by *M. incognita*, *R. similis* and *F. solani* alone increased leaf N levels significantly. Plants infected with a combination of *M. incognita* and *F. solani* did not show any change in leaf N.

Phosphate

There was no significant change in leaf P concentration either in the moist or in the dry regime.

Potassium

There was no significant difference in leaf K between controls. In the moist regime, leaf K concentration in all inoculated plants was significantly lower than that in the uninoculated. The decrease of leaf K ranged from 7% in plants inoculated with *M. incognita*, to 15% in plants inoculated with a combination of *M. incognita* and *F. solani* (Table 7).

In the dry regime, no significant change in leaf K was observed.

Table 7 Percent increase (+), and decrease (-) of leaf nutrient concentration on black pepper infected with *M. incognita*, *R. similis* and *F. solani* alone, or in combination, five weeks after inoculation. *)

Treatments **)	N		K		Ca		Mg	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Uninoculated	0	0	0	0	0	0	0	0
M. incognita	+ 7	+21	- 7	+2	+17	+75	- 2	+87
R. similis	0	+25	-13	+3	+30	+53	+ 5	+49
F. solani	+ 2	+21	-14	+8	+59	+77	+19	+95
Mi + Rs	-10	+26	-12	+9	+21	+76	- 5	+95
Mi + Fs	- 3	0	-15	-6	+26	+67	+12	+54
Rs + Fs	- 2	+16	- 5	-5	+20	+99	- 2	+82
Mi + Rs + Fs	- 6	+18	-12	0	+54	+73	+123	+67

*) Compared to uninoculated plants.
**) See page 86.
(1) field capacity.
(2) 60% field capacity.

Calcium

Between controls there was no significant difference of Ca levels. In plants grown in the moist regime, leaf Ca concentration was significantly higher for all treatments.

M. incognita, *R. similis* and *F. solani* alone increased leaf Ca by 17%, 30%, and 59% respectively as compared with uninoculated plants, whereas *M. incognita*, *R. similis* and *F. solani* together increased the Ca level by 54% (Table 7).

In the dry regime the increase of leaf Ca of inoculated plants was significantly higher than that of uninoculated plants. Plants infected with a combination of *R. similis* and *F. solani* had a leaf Ca concentration of 2.91%, almost twice that of uninfected plants. It should also be noted that in dry conditions the average increase of leaf Ca in association with either pathogen is much higher than in moist conditions.

Magnesium

Between controls there was no significant difference in leaf Mg concentrations. In the moist regime, a significant increase of leaf Mg was observed only in plants infected with *M. incognita*, *R. similis* and *F. solani* together. In this treatment the level of Mg was 0.96%, or more than twice the value in leaves of uninoculated plants.

In the dry regime, infection with *M. incognita*, *R. similis* or *F. solani* alone, increased leaf Mg concentration by 87%, 49%, and 95% respectively as compared to uninoculated plants. Inoculation with a combination of *M. incognita* with *R. similis*, or *M. incognita* with *F. solani* and *R. similis* with *F. solani*, increased leaf Mg by 95%, 54%, and 82% respectively. Infection with a combination of *M. incognita*, *R. similis* and *F. solani* caused leaf Mg to increase by 67%. It should be noted that for dry conditions the average level of leaf Mg showed also a much higher increase as compared to the levels for the moist conditions.

DISCUSSION

Nematode populations

The results show that the multiplication of *R. similis* was affected by soil moisture. The populations of *R. similis* in soil and roots increased when the plants were grown in soil at field capacity. Under dry conditions, the nematode numbers in soil and roots decreased. Probably the penetration of *R. similis* into the roots was less in the dry than in the moist regime. The number of nematodes in the roots in the moist regime were much higher than in the dry regime. A similar result was obtained on citrus infected with *R. similis* (O'Bannon & Tomerlin, 1971). Relationships between soil moisture and various nematode activities have consistently shown that field capacity provides the best conditions for movement and penetration (Wallace, 1971).
The multiplication of *M. incognita* was much lower than that of *R. similis*. This may be due to the fact that during the five weeks experiment, the live cycle of *M. incognita* was not completed yet.

In the moist regime, the average numbers of *R. similis* and *M. incognita* recovered from roots and soil were 3163 and 917, whereas in the dry regime it was only 599 and 601 respectively.
These data indicate that *R. similis* was more affected by drought than *M. incognita*. Another indication that *R. similis* is more sensitive to drought was found by Sossama and Koshy (1986), who showed that *R. similis* could survive for a maximum period of 14 months in moist soil, and only 3 months in dry soil.

Plant growth rate

The AGR and LAR of plants grown in the dry regime were significantly lower than those in the moist regime.
R. similis, *M. incognita* and *F. solani* alone or in combination are capable of reducing the growth of black pepper significantly both in the moist and dry regimes.
Reduction in AGR, LAR, and SLA due to nematode infection, has also been reported for other plants. Den Toom (1988) showed that the growth rate of *Lolium perenne* in terms of RGR, LAR, LWR and SLA, infected with *Tylenchorhynchus dubium* was reduced considerably when plants were grown under low soil moisture conditions. According to Nagesh and Dhawan (1988), decrease in growth rates of wheat infected with *H. avenae* were due to photosynthetic inefficiency and nutrient stress.
Apparently the effects of *R. similis*, *M. incognita* and *F. solani* on the growth of black pepper was more severe in the dry soil than in the moist soil, despite the fact that the numbers of *R. similis* and *M. incognita* were higher in plants grown in the moist than in the dry regime. Only in the dry regime yellow leaves and stiff droop were found in this study. Symptoms of "yellow disease" in the field support these findings. In the field, symptoms became more severe when soils were drier. In moist soil, it is probably water and nutrient availability that increases tolerance of black pepper to infection by *M. incognita*, *R. similis* and *F. solani*.

There was an interaction between the nematodes and/or the fungus with soil moisture on growth reduction. In the dry regime, the AGR of uninoculated plants was reduced by 34%, as compared to that in uninoculated plants grown in the moist regime. This implies that generally, the reduction of AGR was more severe when plants were grown in the dry regime, and were infected by *M. incognita*, *R. similis* and *F. solani* alone or in combination.

A similar trend was observed on LAR and SLA. Since the LAR is the ratio of leaf area to the total plant weight, and the SLA is the relative thickness of the leaves, the data showed that plants infected with *R. similis*, *M. incognita* and *F. solani* expanded their leaves much less than uninfected plants. Similar results were also obtained in Chapter 7.

Plant nutrient content

The infection of plants by *R. similis*, *M. incognita* and *F. solani* alone or in combination could alter leaf nutrient concentrations.

Under the moist regime, only infection by the combination of *M. incognita* and *R. similis* reduced leaf N concentration. In contrast, under the dry regime, the pathogens alone or in combination all increased leaf N concentration. Leaf Ca was found to increase in the presence of *R. similis*, *M. incognita* and *F. solani*, in plants grown at both moisture levels. More increase of leaf Ca, however, occurred in plants grown in the dry regime.

Leaf Mg was also found to increase after infection by *M. incognita*. *R. similis* and *F. solani* either alone or in combination. In the moist regime, however, a significant increase of leaf Mg was observed only in plants infected with a combination of *M. incognita*, *R. similis* and *F. solani*. Whereas in the dry regime, the increase of leaf Mg occurred in plants infected either with *M. incognita*, *R. similis*, and *F. solani* alone, or in combination.

The increase of leaf N, Ca and Mg may be due to the reduced size of leaves associated with reduced growth due to infection by nematodes and/or fungus.

Symptom development

Under field conditions, typical symptoms of yellow disease are relatively thick yellow leaves with a stiff droop, and noticeable defoliation around the vines. These symptoms occur both in young and old vines (Van der Vecht, 1950). Usually the disease starts in scattered patches, which gradually increase in size. As the disease advances, the affected area is surrounded by the diseased plants, in various stages of discoloration.

Leaf discoloration usually starts at the bottom of the vines, and spreads to the top, although in many cases symptoms may occur throughout the vines. As the disease advances, the number of leaves on the infected plants becomes less due to defoliation. Finally it causes the death of the vines. Mustika (1978) found that the root system was infected by *R. similis* and *M. incognita*.

In this experiment, the first yellow leaves were observed five weeks after inoculation in plants grown in the dry regime, when infected with *R. similis* alone or in combination with *M. incognita* and/or with *F. solani*. A stiff droop was observed, and plants started to defoliate. At this time no yellow leaves were observed in plants grown in the moist regime. This may be due to the availability of adequate water in the moist regime that might mask the appearance of yellow leaves. In Chapter 4, under moist conditions, the first yellow leaves and stiff droop were observed after 6 to 7 weeks. The percentage of plants with yellow leaves and a stiff droop observed in the present study was lower than that observed in the previous studies (Chapters 4 and 5). In the previous studies, observations were made up to four months after inoculation, as compared to only five weeks in the present study. These symptoms might also have developed in plants grown in the moist regime, if the observations were continued for a longer period. It appears that yellow leaves and stiff droop were only ob-

served when *R. similis* was present. *M. incognita* together with *F. solani* could also cause yellow leaves, but without the stiff droop. The results indicated that black pepper is more affected by *R. similis* than by *M. incognita*. *R. similis* might cause more root damage and thus more growth inhibition than *M. incognita*.

Plants in the dry regime, inoculated with *R. similis* alone, or in combination with *M. incognita* and/or *F. solani*, exhibited yellow leaves and a stiff droop. The leaf symptoms produced in this study are similar to the symptoms observed in the field, when *R. similis*, *M. incognita* and *F. solani* are present in soil and roots. The results showed that these pathogens did not affect leaf P levels, and increased levels of N, Ca and Mg irrespective of the moisture regime. In the moist regime, leaf K was reduced by the pathogens, whereas under dry conditions nutrient levels tended to remain unchanged. When compared to the normal levels for cv. Kuching found by De Waard (1969), the current nutrients in the leaves recorded for cv. Kalluvalli appeared present in adequate quantities.

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GENERAL DISCUSSION

One of the serious diseases of black pepper (*Piper nigrum* L.) in Indonesia, especially on the island of Bangka is "yellow disease". This disease was first reported in 1932, under the name "geelziekte" (Van der Vecht, 1950). He suspected the burrowing nematode *Radopholus similis* (Cobb) Thorne to be the cause of the disease. Other workers have reported that "yellow disease" was caused by infection of *R. similis* and *Fusarium solani* (Bridge, 1978), or by the lack of nutrients in the soil (de Waard, 1979). The work presented in this thesis was to discover possible biological causes in development of the disease under conditions of adequate mineral nutrition.

The results of these studies showed that *R. similis* alone was capable to reduce plant growth and to cause yellow leaves in association with a stiff droop. When *R. similis* was present together with *Meloidogyne incognita* and/or *F. solani*, reduction of plant growth and development of yellow leaves and the stiff droop tended to be more severe than in plants inoculated with *R. similis* alone.

The combination of these symptoms is typical for vines which have been affected by "yellow disease". The biological causal agents also account for the consistent observation that "yellow disease" spreads in a circular fashion from infected patches.

M. incognita inoculated in combination with *F. solani*, was also able to reduce plant growth considerably and to cause development of yellow leaves, but only in association with a flaccid wilting of leaves. These symptoms are of a different nature, and less intensive than those caused by *R. similis*.

Infection with *M. incognita*, *R. similis* and a combination of *M. incognita*, *R. similis* and *F. solani* reduced the growth of black pepper. Growth reduction varied amongst the cultivars tested. In cv. Kuching growth reduction by *M. incognita*

alone was much less than in cv. Kalluvalli, Jambi and Cunuk. Growth reduction caused by a combination of *M. incognita*, *R. similis* and *F. solani* was also less in cv. Kuching than in cv. Kalluvalli, Jambi and Cunuk. This result indicated that cv. Kuching was more tolerant to *M. incognita*. This tolerance remained manifest, when *R. similis* and *F. solani* were also present. There was no difference in tolerance to *R. similis* alone for any of the cultivars examined.

These results were supported by data of a field experiment in Bangka, where *M. incognita*, *R. similis* and *F. solani* usually occur in association. In the field cv. Kuching had the highest yield among several other cultivars including Jambi and Cunuk (Nuryani, 1984). This confirms that cv. Kuching may be considered to be the more tolerant cultivar even to combined effects of *M. incognita*, *R. similis* and *F. solani*.

Chemical analysis of leaf samples of black pepper vines displaying symptoms of "yellow disease" in the field, showed that yellow leaves of diseased vines were deficient in N, P, K, Ca and Mg as compared to leaves of apparently healthy vines (de Waard, 1979). A possible relation between leaf nutrient concentrations and nematode populations was not established.

Recently, Mustika (unpublished, 1986) found a relation between nematode populations and leaf nutrient content of black pepper. In diseased plants leaf concentrations of N, K and Ca were lower than in healthy ones, while root and soil populations of *R. similis* and *M. incognita* were much higher for diseased plants than for apparently healthy vines.

In general, leaf nutrient concentrations of N, P, K, Ca and Mg obtained in the controls from the pot experiment with cv. Kalluvalli described in Chapter 8, were higher as compared to those found by De Waard (1969) for cv. Kuching, and constantly well above normal levels. This is probably due to the difference in cultivars and to a difference in sampling

procedures. In our experiments, all mature and immature leaves were used for analysis, while only selected leaves were used by De Waard (1979). According to De Waard (1969), the younger leaves contained more P, Ca and Mg, but less N than the older leaves. Bearing in mind that cv. Kalluvalli was used, the established above normal concentrations of the five elements suggest that the levels of mineral nutrients in the leaves were nonlimiting during the experiment, and could not be a cause of yellowing of leaves.

The results of the experiment also indicated that *M. incognita*, *R. similis* and *F. solani* alone or in combination could alter leaf nutrient concentrations especially by raising N, Ca and Mg when plants were grown in the dry soil. Dry soil conditions also enhanced the yellowing of leaves, and development of the stiff droop despite the fact that numbers of *M. incognita* and *R. similis* were higher in the moist than in the dry soil. The increase of leaf concentrations of N, Ca and Mg obtained in this study may be due to the reduced size of leaves associated with the reduction of growth by nematodes and/or fungi.

It appeared that in pots with sufficient water and mineral nutrients, *R. similis*, *M. incognita* and *F. solani*, were able to cause yellow leaves with a stiff droop, the typical symptoms of "yellow disease". Drought enhanced the development of these symptoms. This effect may be reinforced by lack of nutrients.

The symptom expression of "yellow disease" in the field supports this finding. In the field, more severe symptoms were observed when the soil became drier. In moist soil, water availability increases tolerance of black pepper to *M. incognita*, *R. similis* and *F. solani* probably due to more active roots and better uptake of water and nutrients. The lower number of nematodes under the dry conditions was apparently more damaging than the higher number under wet conditions.

De Waard (1979) found that in Bangka the symptoms of "yellow

disease" appeared earlier in plots without mulch, than in plots with mulch. In plots with mulch, soil moisture was maintained at pF values close to field capacity, whereas in plots without mulch, pF values tended to rise towards wilting point. Wahid (1976) found that mulch was able to reduce nematode populations and the incidence of "yellow disease". Mulch created a crumb structure of the soil, stabilized the soil temperature to an even 25 °C at day and night, tended to provide more K, created a condition favourable for root development, and adequate opportunity for uptake of minerals (de Waard, 1979). These findings agree with those of Ichinohe (1980).

A similar disease was reported from India under the name "slow wilt disease" (Nambiar & Sarma, 1977; Ramana et al., 1987). The cause of this disease was considered to be *M. incognita*, *R. similis* and *Fusarium* spp., and water stress (Nambiar & Sarma, 1977).

From Brazil, similar disease symptoms were reported but *R. similis* was not found (Sharma & Loof, 1974; Ichinohe, 1976). Interactions between *M. incognita* and *F. solani* f. sp. *piperis* were suspected to be the cause of this disease. Yellow leaves did occur always, but the stiff droop was consistently absent.

In Sarawak, yellowing of black pepper plants was also reported; but *M. incognita* and/or *M. javanica* were considered to cause these disorders (Kueh & Teo, 1978, Kueh, 1979). The apparent tolerance of cv. Kuching to *M. incognita* provides a possible explanation why this cultivar is almost uniformly and succesfully cultivated in Sarawak, without widespread damage due to *Meloidogyne* spp. *R. similis* has not been reported from Sarawak so far.

In conclusion, *R. similis* alone can induce the yellow leaves and the stiff droop on black pepper under conditions of adequate leaf nutrient levels.

These symptoms are enhanced by additional infections of *M. incognita* and/or *F. solani*, by drought, and by poor mineral nu-

trition.

The name "yellow disease" of black pepper ("geelziekte") described by Van der Vecht in 1932, should be exclusively reserved for the effect of *R. similis* on black pepper alone or in association with other biological or non biological agents.

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SUMMARY

This study on the interactions between various cultivars of the black pepper plant (*Piper nigrum* L.) and three of its pathogens, *Meloidogyne incognita* (Kofoid & White), *Radopholus similis* (Cobb), Thorne and *Fusarium solani* f. sp. *piperi* Albuquerque was initiated to search for a biological cause of "yellow disease" of black pepper.
This disease was described already in 1932 by Van der Vecht as "geelziekte" and its symptoms on the aerial parts of the pepper plants were yellowing of the leaves, always associated with a stiff droop. Its cause was suspected to be the nematode *R. similis*. Later, the data in the literature were inconsistent. Some authors thought a number of organisms to cause this disease, others suspected mineral deficiencies to induce the above mentioned symptoms.

In the field, the disease is spreading from a few infected plants and gradually the patches with diseased plants become larger. This points towards a biological cause of this disease. Based on literature and field observations the three pathogens mentioned above were selected for this study.
Some additional studies were necessary particularly with respect to *R. similis*.

A method to rear *R. similis* under sterile conditions is described. It appeared that in 35 days an over 400 times multiplication of this nematode is possible when reared on sterile carrot discs at about 27 °C. An inoculum of good vitality and without contamination was obtained (Chapter 3). *R. similis* was already found in the roots of pepper plants 24 hours after inoculation. The nematode laid eggs inside the roots within five days. One month after the inoculation a clear destruction of tissues in the roots was found, and "gumlike" substances appeared to obstruct xylem vessels

(Chapter 4).
Only *R. similis* appeared to induce the typical symptoms of yellow disease. This was more pronounced, when *M. incognita* and/or *F. solani* were also present.
Infected plants were seriously reduced in growth when infected with *R. similis*, much more than when infected with either *M. incognita* or *F. solani* (Chapters 4, 5 and 8).

When both *R. similis* and *M. incognita* were present in the roots, the populations of both species developed less well than when they were inoculated separately. An even further reduction in nematode numbers was obtained when *F. solani* was inoculated also (Chapters 5 and 8). One of the causes might have been the inhibition of hatching of juveniles of *M. incognita*. A culture filtrate of *F. solani* appeared to inhibit this hatch of *M. incognita* (Chapter 6).

M. incognita appeared not to induce the typical symptoms of yellow disease. When this nematode was inoculated, alone or in combination with *F. solani*, flaccid wilting and yellowing of the leaves was found, but not the stiff droop without loss of turgor, typical for yellow disease.
When the plants were inoculated with *M. incognita* two weeks prior to the inoculation with *F. solani*, wilting and early death of the plants was observed. *F. solani* alone did cause wilting, but much less than in plants, also inoculated with *M. incognita*. The typical symptoms of yellow disease were not induced by *F. solani* (Chapter 5).

Four cultivars of black pepper, viz. Kalluvalli, Kuching, Jambi & Cunuk, were tested on their response to all three pathogens. All cultivars appeared susceptible for these pathogens, but cv. Kuching was hardly affected by *M. incognita*. It appeared to possess tolerance to this nematode (Chapter 7).
The soil moisture level appeared not only to influence the

growth of the pepper plants, and nutrient levels in the leaves, but also to affect the yellowing of the leaves.
Plants grown at 60% of field capacity showed less growth, compared to those grown at field capacity. Yellowing of the leaves was observed earlier under the drier conditions. At both soil moisture levels, there were sufficient minerals in the leaves. The positive effect of "mulch" on the growth of pepper can be understood since it maintains soil moisture at high levels. Yellowing could not have been the result of mineral deficiency.

In the discussion the results found in this study were further compared to observations made in various parts of the world.
The clear result in this study, that only *R. similis* induced the symptoms typical for yellow disease, also at nonlimiting mineral levels in the leaves, agrees with the findings in the field on Bangka. The patches and the gradual increase in size of these patches is consistent with the activities of *R. similis* in the soil. The symptoms tended to be more severe under conditions of drought and low mineral levels. In our studies similar results were obtained. *R. similis* appears the prime cause of yellow disease of pepper, while other biological or non biological factors aggravate the symptom expression.

The apparent tolerance of cv. Kuching to *M. incognita* gives a possible explanation for the wide spread and successful growth of this cultivar in Sarawak, where *R. similis* was not reported so far.

The yellowing of leaves found in pepper in Brazil, where *R. similis* also is not reported to occur, can now be attributed to an interaction of *M. incognita* and *F. solani*. Both pathogens are known to occur in pepper plantings in these regions. Field observations in Brazilian pepper plantings

suggest a less severe yellowing than on Bangka. This is in agreement with the results found in our studies.
The apparent differences in symptoms induced by various pathogens justify a distinction between yellowing of leaves associated with flaccid wilting, and yellowing of leaves associated with a stiff droop.
The cause of the yellowing of pepper leaves in Brazil is different from the one on Bangka. It is not correct to call a disease of pepper with symptoms involving yellowing of leaves and flaccid wilting the "yellow disease of pepper". This term, "yellow disease" or "geelziekte", first used by Van der Vecht in 1932, should be exclusively reserved for the disease caused by *R. similis*.

SAMENVATTING

In dit proefschrift wordt een onderzoek beschreven naar de interacties tussen *Meloidogyne incognita* (Kofoid & White), *Radopholus similis* (Cobb) Thorne en *Fusarium solani* f.sp. *piperi* Albuquerque op de zwarte peper (*Piper nigrum* L.). Doel van het onderzoek, dat bij de vakgroep Nematologie van de Landbouwuniversiteit te Wageningen werd uitgevoerd, was meer inzicht te krijgen in de oorzaak van de "geelziekte" (yellow disease) bij zwarte peper. Deze ziekte is reeds in 1932 beschreven door Van der Vecht, die vermoedde, dat de nematode *R. similis* de oorzaak was. De gegevens in de literatuur m.b.t. deze ziekte waren tegenstrijdig. Nu eens werd een mineraal tekort als oorzaak geopperd, dan weer werd een biologische oorzaak verondersteld. De hier beschreven proeven waren vooral gericht op de studie van een biologische oorzaak van de ziekte. De omstandigheden waren zo gekozen dat mineralen voldoende beschikbaar waren voor de plant.

De typische bovengrondse symptomen van geelziekte zijn gele bladeren, die naar beneden buigen en naar de twijgen toe neigen, zonder een spoor van verlies van turgor. In een vroeg stadium van de ziekte in het veld zijn slechts enkele planten aangetast. De aantasting breidt zich met deze planten als middelpunt geleidelijk uit in de aanplant. Dit verspreidingspatroon doet een biologische oorzaak vermoeden. Op grond van literatuurgegevens en veldervaring is de keuze voor de studie van de bovengenoemde nematoden en de schimmel gemaakt.

Enige aanvullende studies waren nodig voor onderzoek, speciaal m.b.t. *R. similis*.

Een in vitro studie bracht aan het licht dat de optimale vermenigvuldiging van 400 maal in 35 dagen plaats vond bij 27 °C (Hoofdstuk 2). *R. similis* werd al 24 uur na inoculatie in de wortels aangetroffen. Het aaltje legde eieren in de wortel binnen vijf dagen. Een maand na de inoculatie was een

destructie van het weefsel in de wortels duidelijk te zien, terwijl een 'gomachtige' substantie xyleem vaten verstopte (Hoofdstuk 4).

Alleen *R. similis* bleek in staat de eerder genoemde typische geelziekte symptomen te induceren, en wel in sterkere mate, als ook *M. incognita* en/of *F. solani* aanwezig waren. Daarnaast werden aangetaste planten ernstig geremd in de groei door *R. similis*, veel meer dan het geval was bij aantastingen door *M. incognita* en *F. solani* (Hoofstuk 4, 5 en 8).

Wanneer *R. similis* en *M. incognita* beide aanwezig waren, bleken de populaties van deze soorten zich minder goed te ontwikkelen dan bij afzonderlijke inoculatie. Een verdere reductie van de populatie ontwikkeling van beide soorten werd bewerkstelligd door toevoeging van *F. solani* aan de bodem (Hoofdstuk 5 en 8). Een van de oorzaken van dit verschijnsel zou kunnen zijn een blokkering van het uitkomen van de eieren door aanwezigheid van *F. solani*. Een cultuurfiltraat van *F. solani* bleek een dergelijk effect te hebben op de eieren van *M. incognita* (Hoofdstuk 6).

M. incognita alleen bleek geen symptomen van geelziekte te induceren. Wanneer dit aaltje werd geïnoculeerd, al dan niet samen met *F. solani*, werden wel verwelking en bladvergeling gevonden, maar niet het naar beneden buigen van het blad en naar de twijg toe neigen met behoud van turgor.
Werden de planten met *M. incognita* geïnoculeerd twee weken voor de inoculatie met *F. solani*, dan was verwelking van de plant en een vervroegd afsterven, vergeleken met de controle planten, het gevolg. *F. solani* alleen veroorzaakte wel verwelking, maar veel minder dan het geval was wanneer ook *M. incognita* aanwezig was. De typische geelziekte symptomen werden ook niet door *F. solani* geïnduceerd (Hoofdstuk 3).

Vier cultivars van zwarte peper, te weten Kalluvalli, Kuching, Jambi en Cunuk, werden onderzocht op hun reactie op

aantastingen door de drie hierboven genoemde pathogenen.
Alle vier cultivars bleken vatbaar voor deze pathogenen, maar een duidelijk verschil in tolerantie werd ook vastgesteld. Kuching bleek minder beschadigd te zijn door *M. incognita* dan Kalluvalli, Jambi en Cunuk (Hoofdstuk 7).

De hoeveelheid vocht in de bodem bleek niet alleen en duidelijk effect te hebben op de groei van de peper planten en de hoeveelheid nutriënten in het blad, maar ook het geel worden van de bladeren te beïnvloeden.
Planten die opgekweekt waren in grond met een vochtigheid van ca. 60% van de veldcapaciteit vertoonden een verminderde groei, vergeleken met die, opgekweekt bij veldcapaciteit. Ook werd bladvergeling onder drogere omstandigheden eerder waargenomen dan bij meer vocht in de bodem. Zowel bij veldcapaciteit als bij 60% ervan bleken zoveel mineralen in de bladeren aanwezig te zijn, dat de vergeling niet aan mineralen deficiëntie kon worden toegeschreven.
Het gunstige effect van "mulch" op de groei van de peper plant, o.a. door minder bladvergeling kan begrepen worden tegen de achtergrond van de hier gevonden verschillen in symptomen bij de twee vochttrappen.

In de discussie werden verder de gevonden resultaten vergeleken met onderzoeksresultaten uit het veld op diverse plaatsen in de wereld.
De hier gevonden resultaten, namelijk dat *R. similis* alleen en in ernstige mate symptomen van de geelziekte kan veroorzaken, ook bij een ruim voldoende aanwezigheid van mineralen in het blad, doet veel van de bevindingen in Bangka verklaren. Op dit eiland verspreidt de ziekte zich vanuit enkele aangetaste planten. Plekken met aangetaste planten worden geleidelijk groter, en de hevigheid van de symptomen wordt versterkt door droogte en slechte minerale voeding van de planten. Dit sluit volledig aan bij hetgeen nu is gevonden, namelijk dat de nematode *R. similis* geelziekte veroorzaakt,

waarbij andere biotische en abiotische factoren het effect van dit aaltje versterken.

De gevonden tolerantie van Kuching voor *M. incognita* geeft mogelijk de verklaring voor de uitgebreide en succesvolle verbouw van deze cultivar in Sarawak, waar, voor zover bekend, *R. similis* niet voorkomt.

De gevonden vergelingsziekte symptomen van peper (*Piper nigrum*) in Brazilië, waar, voor zover bekend, *R. similis* niet voorkomt, kan toegeschreven worden aan de interactie van *M. incognita* en *F. solani*, die daar beide voorkomen. Ook wijzen de veldwaarnemingen in Brazilië erop, dat de vergeling daar minder hevig optreedt dan b.v. op Bangka. Ook dit sluit aan bij de in dit proefschrift beschreven resultaten. De oorzaak van de vergelingsziekte van zwarte peper in Brazilië is niet dezelfde als die op Bangka. Het is onjuist deze vergeling, gepaard gaande met verwelking, te beschrijven als "de geelziekte" van peper.
De term "geelziekte" ofwel "yellow disease" dient uitsluitend gebruikt te worden voor de geelziekte van de peper, veroorzaakt door *R. similis* en beschreven door Van der Vecht in 1932.

RINGKASAN

Penelitian mengenai interaksi antara *Meloidogyne incognita* (Kofoid & White), *Radopholus similis* (Cobb), Thorne dan *Fusarium solani* f. sp. *piperi* Albuquerque pada tanaman lada (*Piper nigrum* L.) telah dilakukan dengan menggunakan beberapa kultivar lada.
Tujuan penelitian ini adalah untuk menegaskan kembali mengenai terjadinya penyakit kuning pada tanaman lada yang sekarang ini di dalam pustaka terdapat ketidak pastian mengenai penyebabnya.
Penyakit kuning pada tanaman lada ditemukan oleh Van der Vecht pada tahun 1932 dengan nama "geelziekte". Gejala yang nampak pada bagian tanaman di atas permukaan tanah adalah daun menguning, helaian daun kaku dan menekuk. Penyakit tersebut diduga disebabkan oleh *R. similis*. Tetapi beberapa peneliti berpendapat bahwa penyebabnya adalah bermacam-macam organisma, atau kekurangan unsur hara.

Dilihat dari penyebaran penyakit di lapangan, diduga bahwa penyebab penyakit tersebut adalah faktor biologi.
Berdasarkan pustaka dan pengamatan lapangan, ketiga patogen tersebut di atas, dipilih sebagai bahan dalam penelitian ini. Beberapa penelitian tambahan, terutama mengenai *R. similis* perlu dilaksanakan.

Hasil penelitian menunjukkan bahwa pada potongan wortel steril, populasi *R. similis* dapat meningkat sampai lebih dari 400 kali, selama 35 hari pada 27 °C (Bab 2). *R. similis* masuk ke dalam akar lada pada 24 jam setelah inokulasi, dan meletakkan telurnya di dalam akar pada 5 hari setelah inokulasi. Satu bulan setelah inokulasi nematoda tersebut menyebabkan kerusakkan pada jaringan pembuluh, dan pembuluh xylem tersumbat oleh "cairan seperti getah" (Bab 4).
Hanya *R. similis* yang terbukti dapat menyebabkan gejala khas

penyakit kuning. Gejala ini nampak lebih parah apabila *M. incognita* dan *F. solani* terdapat bersama-sama *R. similis*. Tanaman yang diinokulasi dengan *R. similis*, pertumbuhannya lebih terhambat dibandingkan dengan yang diinokulasi dengan *M. incognita* atau *F. solani* (Bab 4, 5, dan 8).

Apabila *R. similis* dan *M. ncognita* terdapat bersama-sama pada akar lada, kedua jenis nematoda tersebut saling menghambat, sehingga populasinya menurun. Populasi kedua jenis nematoda tersebut semakin berkurang apabila *F. solani* juga terdapat bersama-sama (Bab 5, dan 8). Hasil penelitian menunjukkan bahwa filtrat *F. solani* dapat menekan penetasan telur-telur *M. incognita* dan *R. similis* (Bab 6).

M. incognita terbukti tidak menyebabkan gejala khas penyakit kuning. Apabila nematoda ini diinokulasikan bersama-sama dengan *F. solani*, tanaman layu dan daun menguning, tetapi tidak menunjukkan gejala khas penyakit kuning seperti daun kaku dan menekuk. Apabila tanaman diinokulasi dengan *M. incognita* dua minggu sebelum inokulasi dengan *F. solani*, gejala layu terjadi lebih awal. *F. solani* sendiri dapat menyebabkan tanaman layu, tetapi tidak separah yang disebabkan oleh serangan bersama *M. incognita* dan *F. solani*. Cendawan ini tidak menyebabkan gejala khas penyakit kuning (Bab 5).

Untuk mengetahui tanggap kultivar lada terhadap serangan *M. incognita*, *R. similis* dan *F. solani*, dilakukan penelitian dengan menggunakan 4 kultivar lada yaitu Kalluvalli, Kuching, Jambi dan Cunuk. Hasil penelitian ini menunjukkan bahwa keempat kultivar tersebut rentan terhadap *M. incognita*, *R. similis*, dan terhadap gabungan kedua jenis nematoda tersebut dengan *F. solani*. Kultivar Kuching tampak lebih toleran terhadap *M. incognita*, dibandingkan dengan Kalluvalli, Jambi dan Cunuk (Bab 7).

Kelembaban tanah mempengaruhi pertumbuhan tanaman lada, dan persenan hara pada daun. Pada 60% kapasitas lapang, pertumbuhan tanaman lebih terhambat dibandingkan dengan pertumbuhan tanaman lada pada kapasitas lapang. Gejala kuning terjadi lebih awal pada tanaman yang ditanam pada tanah dengan kadar air 60% kapasitas lapang. Baik pada kapasitas lapang maupun pada 60% kapasitas lapang, ternyata persenan hara daun cukup. Oleh karena itu pengaruh baik mulsa terhadap pertumbuhan tanaman lada, dapat dijelaskan antara lain adalah karena mulsa dapat menciptakan kadar air tanah yang cukup tinggi. Kekurangan hara bukanlah penyebab gejala kuning pada "penyakit kuning" tanaman lada.

Hasil penelitian ini dapat dibandingkan dengan penelitian-penelitian lain yang dilakukan di berbagai tempat di dunia. Hasil yang nyata dari penelitian ini, bahwa *R. similis* dapat menyebabkan gejala khas penyakit kuning, juga pada tingkat hara yang tidak terbatas pada daun, sesuai dengan keadaan di daerah Bangka. Gejala penyakit kuning cenderung lebih banyak terjadi pada keadaan kekeringan dan rendahnya tingkat hara. Hasil yang serupa didapatkan pada penelitian ini. Oleh karena itu dapat disimpulkan bahwa *R. similis* adalah penyebab utama penyakit kuning lada, sedangkan faktor biologi dan non-biologi lain menguatkan gejala ini.

Di Sarawak, kultivar Kuching berhasil diusahakan secara luas, walaupun dilaporkan adanya *M. incognita* dan *M. javanica*. Hal ini disebabkan karena kultivar Kuching adalah toleran terhadap *M. incognita*. Selain itu di Sarawak tidak dilaporkan adanya *R. similis*. Gejala kuning dilaporkan juga pada tanaman lada di Brazil, tetapi tidak dijumpai adanya *R. similis*. Diduga bahwa gejala kuning pada tanaman lada di daerah Brazil disebabkan oleh interaksi antara *M. incognita* dan *F. solani*. Hal ini sesuai dengan hasil penelitian yang telah diuraikan di atas.

Perbedaan yang nampak pada gejala penyakit yang ditimbulkan oleh *R. similis, M. incognita, F. solani* adalah daun menguning, helaian daun kaku, dan menekuk, tetapi tidak layu.
Gejala kuning pada tanaman lada di Brazil berbeda dengan yang terdapat di Bangka. Gejala kuning disertai layu pada tanaman lada tidak bisa dikategorikan sebagai "penyakit kuning lada". Istilah "penyakit kuning" atau "geelziekte" yang pertama kali digunakan oleh Van der Vecht pada tahun 1932, adalah khusus untuk penyakit yang disebabkan oleh *R. similis*.

Curriculum vitae

Ika Mustika was born in Rangkasbitung on December 31, 1943, where he also attended highschool. He entered the Faculty of Agriculture, Padjaran University in Bandung in 1963, and received his Ir-degree in 1972. From 1981 to 1984 he continued his studies at the Faculty of Graduate Studies, Gadjah Mada University, Yogyakarta (MSc).

From 1974 till 1984 he worked as a plant pathologist at the Research Institute for Spices and Medicinal Crops, Bangka. In 1984 he started to work as a plant nematologist in the main office of the same institute in Bogor. From 1986 till the end of 1990 he was granted a leave of absence to fulfil the requirements for the degree of "doctor" in de Landbouw- en milieuwetenschappen at the Wageningen Agricultural University, The Netherlands, in the Department of Nematology.

He is (co-)author of the following papers:

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ANNEX 1

Physical and chemical characteristics of natural soil used in the experiments.

Characteristics of soil	
1. Physical	
a. 0-2 µ (clay)	5.3%
b. 2-16 µ (silt)	4.4%
c. 16-50 µ (silt)	4.1%
d. 50-105 µ (sand)	6.6%
e. 105-150 µ (sand)	12.2%
f. 150-210 µ (sand)	18.2%
g. 210-2000 µ (sand)	49.2%
2. Classification	sandy loam soil
3. pH KCL	4.3
pH water	4.9
4. Mineral composition	
a. N water	16 (mg N/100 g)
b. N total	0.13 (mg N/100 g)
c. P	105 (mg P205/100 g)
d. K	13 (mg K20/100 g)
e. Ca	0
f. Mg	73 (mg MgO/kg)
g. Fe	0.92 (mg Fe203/100 g)

ANNEX 2

Fertilizer applications on black pepper over period of four months in pot experiments*).

Time of application (week)	Fertilizers (g/100 ml water)			
	Kristallon	Fe Chelaat	MNSO4	Kieserite
1	0.50	-	-	0.50
2	0.50	0.125	-	-
3	0.50	-	-	-
4	0.50	0.125	0.25	-
5	0.50	-	-	0.50
6	0.50	0.125	-	-
7	0.50	-	-	-
8	0.50	0.125	0.25	-
9	1.00	-	-	1.00
10	1.00	0.250	-	-
11	1.00	-	-	-
12	1.00	0.250	0.25	-
13	1.00	-	-	1.00
14	1.00	0.250	-	-
15	1.00	-	-	-
16	1.00	0.250	0.50	-

Kristallon (19% N; 6% P205; 20% K20; 4% MgO).
Fe Chelaat, Sequestrene 330 Fe (FeDTPA). Kieserite (MgSO4.2H20) 26%.

* Before planting 3 g of Dolocal/pot were added. The composition of Dolocal was Ca = 32-34%; Mg = 3.0-3.2%; Mn = 70-1000 ppm; Cu = 1-10 ppm; B = 150-2000 ppm (De Waard, 1979).