Institut für Pflanzenkrankheiten
der
Rheinischen Friedrich-Wilhelms-Universität Bonn

Biological control of plant parasitic nematodes with antagonistic bacteria on different host plants

Inaugural-Dissertation

zur

Erlangung des Grades

Doktor der Agrarwissenschaften
(Dr.agr.)

der

Hohen Landwirtschaftlichen Fakultät

der
Rheinischen Friedrich-Wilhelms-Universität

zu Bonn

vorgelegt im Januar 2002

von
Magdy Mahdy

aus
Shebin El-kom/ Egypt

Referent: Prof. Dr. R. A. Sikora

Korreferent: Prof. Dr. A. Skowronek

Tag der mündlichen Prüfung:

Gedruckt bei:

Biological control of plant parasitic nematodes with antagonistic bacteria on different host plants

Root-knot nematodes, *Meloidogyne* spp., are recognized as the most economically important genus of plant parasitic nematodes worldwide. The nematode causes severe damage and yield loss to a large number of cultivated plants and especially on vegetable crops in the tropics and subtropics. In the investigations conducted in this study, the potential control of plant parasitic nematodes using different antagonistic bacteria was studied with particular reference to the control of species of *Meloidogyne* on tomato.

The results obtained from the research are presented in four chapters. The first chapter gives a: comprehensive introduction to the problems associated with root-knot nematode attack, outlines the major control methods being used and and gives an insite into integrated pest management now as well as the potential use of biological control in future integrated strategies for nematode management.

In the second chapter the results of experiments on the biological control activity of the plant health promoting rhizobacterium *Bacillus cereus* strain S18 for biocontrol of the three major species of *Meloidogyne* are discussed. The results showed that *B. cereus* introduced either as a soil drench or as a root dip, reduced the number of galls and egg masses of *M. incognita* on tomato significantly. *B. cereus* applied 10 days before nematode inoculation caused significant reductions in root galling and number of galls. No differences, however, were detected between the different application times in the number of egg masses produced. It was also shown that *B. cereus* does not control all three major species of *Meloidogyne* to the same degree. *B. cereus* had little to no biological control activity toward *M. arenaria,* but gave significant control of *M. incognita* and *M. javanica*. Furthermore, the results demonstrated that *B. cereus* is an effective biological control agent of *M. incognita* on a broad spectrum of host plants.

In the third chapter the plant health promoting rhizobacterium *Rhizobium etli* strain G12 was tested for control of different genera of plant parasitic nematodes on a broad spectrum of crops. *R. etli* exhibited strong biocontrol activity towards *M. incognita* on different host plants. The reduction rates varied however between crops. Results showed that *R. etli* had the ability to control three economically important species of *Meloidogyne*. The highest reduction was against *M. incognita* and *M. javanica*, whereas it had little effect on *M. arenaria*. It was also demonstrated that increasing inoculum densities of *R. etli* caused increased reductions in nematode infection. Reduction in the number of galls occurred at 10^{10} cfu/ml whereas reductions in egg mass number were detected at 10^{6} to 10^{10} cfu/ml. *R. etli* also caused significant reductions in sugar beet cyst nematode, *Heterodera schachtii* infection. *R. etli* reduced significantly the number of cysts/plant and number of eggs and juveniles/plant. The bacteria, however, did not affect the number of eggs and juveniles/cyst. The experimental data also showed that *R. etli* can reduce infection of the cyst nematode *Globodera pallida* on potato, but had no activity towards the migratory endoparasitic root-lesion nematode *Pratylenchus zeae* on maize.

In the fourth chapter the spore-forming endoparasitic bacterium *Pasteuria penetrans* (Pp3) was used as a biocontrol agent for the biological control of *M. javanica* on tomato. The results revealed abiotic factors affect attachment. Culture filtrates of the two antagonistic rhizobacteria *B. cereus* S18 and *R. etli* G12 reduced attachment of Pp3 spores to the cuticle of *M. javanica* juveniles at 100 strength and dilutions of 10 percent of the original fermentation broth. The experiments also demonstrated that percolates of chicken manure compost treated soil had a strong negative effect on the attachment of Pp3 spores to *M. javanica* juveniles. In greenhouse tests *P. penetrans* multiplied quickly and within 6 months and gave good biological control of *M. javanica* when initially introduce into the planting soil during seedling production. The seedlings with *P. penetrans* in the potting soil were then grown for two additional tomato cropping cycles in a sand substrate previously infested with root-knot nematodes, at temperatures above 25 C. The overall results of these studies demonstrated the importance of three different bacterial antagonists for root-knot nematode control and supplied new information on how to improve activity of the bioloigcal control agents as well as ideas on their use in integrated management under field conditions.

Biologische Bekämpfung pflanzenparasitärer Nematoden mit antagonistischen Bakterien an verschiedenen Wirtspflanzen

Wurzelgallennematoden der Gattung *Meloidogyne* zählen weltweit zu den wirtschaftlich wichtigsten pflanzenparasitären Nematoden. Nematoden dieser Gattung verursachen hohe Schäden und Ertragsausfälle an den meisten landwirtschaftlichen Kulturpflanzen, vor allem aber an Gemüse in tropischen/subtropischen Regionen. In der vorliegenden Arbeit wurden Möglichkeiten zur biologischen Bekämpfung von *Meloidogyne* mit antagonistischen Bakterien an verschiedenen Kulturpflanzen untersucht.

Die Ergebnisse der Arbeit wurden in vier Kapiteln dargestellt. Im ersten Kapitel wurde eine kritische Darstellung der schädigende Wirkung von Wurzelgallennematoden und deren Bekämpfungsmöglichkeiten vorgenommen, sowie die Bedeutung eines integrierten Pflanzenschutzes dargestellt und mögliche Strategien zum Einsatz biologischer Bekämpfungsverfahren für die Zukunft entwickelt.

Im zweiten Kapitel sind die Ergebnisse zur biologischen Bekämpfung der drei wichtigsten *Meloidogyne*-Arten mit dem pflanzengesundheitsfördernden Bakterium *Bacillus cereus* S18 dargestellt. Die Ergebnisse zeigen, dass eine Gieß- oder Tauchbehandlung von Tomaten mit *B. cereus* S18 zu einer signifikanten Reduzierung der Anzahl Gallen und Eiermassen von *Meloidogyne* spp. führt. Eine besonders gute Wirkung wurde erzielt, wenn *B. cereus* S18 10 Tage vor den Nematoden appliziert wurde. Keine Unterschiede zeigten sich im Applikationszeitpunkt auf die Anzahl gebildeter Eiermassen. Die antagonistische Wirkung von *B. cereus* S18 war nicht gegen alle drei *Meloidogyne*–Arten gleich gut ausgeprägt. Im Gegensatz zu *M. incognita* und *M. javanica*, zeigte *B. cereus* S18 nur eine geringe Wirkung gegen *M. arenaria*. Weiterhin zeigten die Ergebnisse eine gute Bekämpfung von *M. incognita* mit *B. cereus* S18 an einem breiten Spektrum von Wirtspflanzen.

Im dritten Kapitel wurde die antagonistische Wirkung des Rhizsophärebakteriums *Rhizobium etli* Isolat G12 gegen verschiedene Nematodenarten an unterschiedlichen Wirtspflanzen untersucht. *R. etli* G12 zeigt eine hohe Wirksamkeit gegen *M. incognita* an verschiedenen Wirtspflanzen, wobei die Wirkung in Abhängigkeit der Wirtspflanze variierte. *R. etli* G12 zeigte eine sehr gute Wirkung gegen *M. incognita* und *M. javanica* und eine geringer Wirkung gegen *M. arenaria*. Mit Erhöhung der Inokulumdichte von *R. etli* G12 bis auf 10^{10} cfu/m war eine Steigerung der Wirksamkeit verbunden. *R. etli* G12 zeigte weiterhin eine gute Wirkung gegen *Heterodera schachtii* an Zuckerrübe. Die Anzahl Zysten/Pflanze und Anzahl Eier + Larven/Pflanze war signifikant reduziert. *R. etli* G12 hatte jedoch keine Wirkung auf die Anzahl Eier + Larven pro Zyste. An Kartoffeln führte *R. etli* G12 zu einer Reduzierung des Befalls mit *Globodera pallida*. Demgegenüber zeigte das Bakterium keine Wirkung gegen den wandernden Endoparasiten *Pratylenchus zeae* an Mais.

Im vierten Kapitel wurde der obligate Endoparasit *Pasteuria penetrans* (Pp3) zur biologischen Bekämpfung von *M. javanica* an Tomate eingesetzt. Die Ergebnisse zeigten, dass die Anhaftung der *P. penetrans*-Sporen an die Nematodenlarven durch abiotische Faktoren beeinflusst wird. Eine Behandlung der *P. penetrans*-Sporen mit Kulturfiltraten der beiden antagonistischen Bakterien *B. cereus* S18 und *R. etli* G12 führte zu einer verringerten Anhaftung. Auch das Perkolat eines mit 5 % und 20 % Hühnermistkompost behandelten Bodens führte zu einer Verringerung der Sporenanhaftung an *M. javanica*. In Gewächshausversuchen mit Tomaten konnte sich *P. penetrans* gut vermehren. Bei Behandlung der Sämlinge mit *P. penetrans* war nach 6 Monaten eine gute Bekämpfung von *M. javanica* gegeben.

Die Ergebnisse der vorliegenden Arbeit zeigen die Bedeutung von drei verschiedenen bakteriellen Antagonisten für die Bekämpfung von Wurzelgallennematoden an Gemüse. Weiterhin wurden Strategien entwickelt, wie eine Verbesserung der Wirksamkeit zu erzielen ist und wie sich die biologischen Bekämpfungsverfahren in ein integriertes Pflanzenschutzverfahren integrieren lassen, um eine nachhaltige Bekämpfung der Nematoden im Freiland zu erzielen.

I- Table of Contents

Chapter I. General Introduction

I- Table of Contents

Chapter II. Effect of the plant health-promoting rhizobacterium *Bacillus cereus* strain S18 on root-knot nematodes, *Meloidogyne* spp. on different host plants

I- Table of Contents

Chapter III. Antagonistic activity of the rhizobacterium *Rhizobium etli* strain G12 towards different plant parasitic nematodes on different host plants

Table of Contents

Chapter I: General Introduction

The soil around plant roots that forms the rhizosphere is a dynamic, complex zone. All plant parasitic nematodes are obligate parasities and must enter this zone to reach their host and cause damage (Kerry and Hominick, 2000). Root-knot nematodes, *Meloidogyne* spp., recognized as among the most economically important and complex group of plant parasitic nematodes, cause damage and high yield losses on most cultivated plants throughout the world especially in developing countries (Sasser, 1979b; Sasser and Carter, 1985; Sasser and Freckmann, 1987; Netscher and Sikora, 1990).

Fifty one species have been described for the genus *Meloidogyne* by Jepson (1987) and four species are of high economic importance to vegetable production, *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*. The most dominant species worldwide was shown to be *M. incognita* in 53% of all field samples followed by *M. javanica* 30% and *M. arenaria* and *M. hapla* 8% (Johnson and Fassuliotis, 1984).

M. incognita (Kofoid and White, 1919) Chitwood 1949, *M. javanica* (Treub, 1885) Chitwood 1949 and *M. arenaria* (Neal, 1889) Chitwood 1949, are cosmopolitan in distribution being found in most of the warmer regions of the world. The optimum temperature for *M. hapla* is 5°C lower than the other three species. *M. incognita, M. javanica* and *M. arenaria* occur in tropical areas with an average temperature of 25°C, whereas *M. hapla* is found at high altitudes in the tropics (Netscher and Sikora, 1990).

Although nematode problems occur in all areas of the world where crops are grown, Mai (1985) revealed that the most evident damage occurs in warm areas because:

* higher temperatures and longer growing seasons result in more generations per year resulting in higher nematode populations and more crop damage.
* the greater number of susceptible crops per year in warm areas results in higher nematode build-up.
* some of the more damaging species such as *M. incognita*, occur in warmer areas and
* more severe diseases complexes occur in warmer areas.

1. Life cycle of *Meloidogyne* spp.

The life cycle of root-knot nematodes, *Meloidogyne* spp. is shown in figure and is briefly described below:

Adult females embedded in host roots produce eggs which may be free in the soil or together in a gelatinous matrix which may still adhere to old root tissue segments of the plant host. The nematode develops to the second stage juvenile in the egg. The infective second stage juvenile then hatches and moves into the soil to search for the host root. When a suitable host root is reached the juveniles invade near the root tip. They penetrate the cortex until the juvenile makes contact with the vascular cylinder. Here it forms giant cells upon which the developing nematode feeds. The juvenile grows slightly in length and much in width. As development increases the juveniles become flask shaped and undergo three further moults (Taylor and Sasser, 1978). After the last moult either a true male, which appears as a long filiform nematode inside the cuticle of the fourth larval stage is produced or an adult female evolves which is pyriform in shape. The females secrete a gelatinous matrix into which they lay up to 500 eggs (Tyler1933). Most juveniles develop into females and only under adverse conditions are high numbers of males observed.

2. Abiotic factors and development

There are many abiotic factors affecting the development and distribution of *Meloidogyne* spp. such as temperature, soil moisture, soil texture and soil pH. The optimum temperature for the different *Meloidogyne* species can vary greatly, but for tropical species like *M. incognita* it ranges from 25 to 30 °C. Thomason and Lear (1961) suggested that the nematode reproduced well on tomato at 20, 25 and 30°C. However *M. incognita* and *M. javanica* reproduced even at 35°C, although very few galls and egg masses appeared above 32°C. Walker (1960) also found that the juveniles of *M. arenaria* have greater tolerance to heat than the three other species.

Figure 1: Life cycle of root-knot nematodes, *Meloidogyne* spp. (**A**) embryo developed within eggs; (**B**) second stage juvenile within egg; (**C**) hatched second stage (**D**)developed second stage; (**E**) third stage for female(♀) and male (♂); (**F**) fourth stage female and male and (**G**) mature female (periform) and male (vermiform) (Taylor and Sasser, 1978).

Soil type and soil pH have been shown to influence nematode distribution (Taylor *et al*., 1982). Soil type may also influence the types of crops grown, thereby affecting nematode distribution, population build-up and damage intensity. Migration of nematode juveniles decreased with increasing clay content more than 30% (Prot and Van Gundy, 1981). In sandy soils the juveniles were able to move over distances of up to 75 cm in 9 days horizontally and vertically (Prot, 1977).

Soil pH from 4-8 affects root-knot nematode survive and reproduction greatly (Ferris and Van Gundy, 1979). *M. javanica* emergence *in vitro* was greatest between 6.4 and 7.0 and inhibited below pH 5.2 (Wallace, 1966). However, many tropical soils are very acid with pH levels of 4.5 and this does not prevent high density build-up of *Meloidogyne* populations (Netscher and Sikora, 1990).

3. Root-knot nematode population development

The number of *Meloidogyne* generations per year varies according to species. Usually there are multiple generations, but in some species there is only one, e.g. *M. nassi* which attacks cereals under temperate climatic conditions (De Guiran and Ritter, 1979). The rate of development increases for most species with temperature up to 28°C. The minimum time required for the life cycle of *M. incognita* was 87 days at 16°C and 25 days at 27°C (De Guiran and Ritter, 1979). The mean number of generations of tropical species of *Meloidogyne* range between 7-10 generations. *M. arenaria* for example has 9 generations per year and the duration of the life cycle can vary from 18 days in summer to 54 days in winter as reported by Scotto la Massese (1961).

4. Root-knot nematode races

There are four dominant species of *Meloidogyne*, that cause severe damage to crops especially vegetable crops (Netscher and Sikora, 1990) and these species are *M. incognita, M. javanica M. arenaria* and *M. hapla*. The differential host test that depends on the reaction of specific hosts to infection is considered one of the most important methods for identifying races of

these four major species (Sasser, 1954). The use of host differentials allows determination of the four main species and many important races of *Meloidogyne*. Taylor and Sasser (1978) added tobacco with resistance to many *M. incognita* populations and discovered physiological races within *Meloidogyne* species (Table 1). Four races of *M. incognita* have now been recorded Taylor *et al*., (1982). Race 1 is the commonest race and does not reproduce on cotton nor tobacco which are the marker hosts for this race.

Table 1: Identification of the most common species and races of *Meloidogyne* using the differential host test (Sasser and Carter, 1985; Hartman and Sasser 1985).

Nematode species and races	**Cotton cv. Deltapine**	**Tobbaco cv. NC95**	**Pepper cv. California Wonder**	**Watermelon cv. Charleston Gray**	**Peanut cv. Florunner**	**Tomato cv. Rutgers**
M. incognita						
Race 1	-	-	+	+	-	+
Race 2	-	+	+	+	-	+
Race 3	+	-	+	+	-	+
Race 4	+	+	+	+	-	+
M. arenaria						
Race 1	-	+	+	+	+	+
Race 2	-	+	+	+	-	+
M. javanica	-	+	-	+	-	+
M. hapla	-	+	+	-	+	+

(+) indicates a susceptible host **(-)** indicates a resistant host.

Sasser (1972); Taylor and Sasser (1978) and Eisenbeack *et al*., (1981) separated *M. arenaria* into two races, race 1 reproduces on peanut, but race 2 does not. Host races of *M. arenaria* are distributed throughout the world and are morphologically indistinguishable (Sasser, 1979a; Osman *et al*., 1985). Race 2 of *M. arenaria* is the commoner race (Taylor *et al*., 1982).

5. Root-knot damage

The presence of galls on the root system is the most evident diagnostic symptom caused by root-knot nematodes. Root-knot nematodes limit vegetable production worldwide and cause high losses especially in vegetable crops (Netscher and Sikora, 1990). Most vegetable crops have been recorded as a host for at least one of the most frequently occurring species of root-knot nematodes, *M. incognita, M. javanica* and *M. arenaria*.

The losses in vegetable crops ranged from 17-20% on eggplant, 18-33% on melon and 24-38% on tomato as reported by Netscher and Sikora (1990). It is very difficult to grow important vegetables such as tomato in tropical or semi-tropical soil infested with root-knot nematodes, particularly *M. incognia*. Root-knot nematodes cause serious economic losses also to tuber crops like carrot and potato by direct damage to the plant through reduced tuber quality Brodie *et al*., (1993).

Another important problem is the involvement of root-knot in disease complexes. On vegetables for example complexes of *M. incognita* with the wilt fungi *Fusarium oxysporum* on tomato cause plant death (Jenkins and Coursen, 1957). Root-knot also interacts with *Fusarium oxysporum f. sp. conglutinans* on cabbage (Fassuliotis and Rau, 1969); *Fusarium oxysporum f. sp. lycopersici* on okra (Khan and Saxena, 1969); *Sclerotium rolfsii* on eggplant (Goswami *et al.*, 1970) and with *Rhizoctonia solani* on okra and tomato (Golden and Van Gundy, 1975).

The second most important root-knot species, *M. javanica*, also interacts with *Fusarium* and reduces growth of tomato (Bergeson *et al*., 1970). It also increased *Verticillium* wilt of tomato (Valdez, 1978). *M. arenaria* has been reported to interact with *F. oxysporum* on watermelon (Sumner and Johnson, 1972).

Bacterial canker caused by *Corynebacterium michiganense* (Moura *et al*., 1975) and bacterial wilt caused by *Ralstonia solanacearum* (Valdez, 1978) was increased when root-knot nematode is present.

6- Integrated Pest Management (IPM)

Integrated pest management is a socially acceptable, environmentally responsible and economically practical method of controlling pest populations. IPM incorporates a variety of cultural, biological and chemical methods to efficiently manage pest populations, while lowering dependence on chemical means of control.

Development of IPM can be divided into seven components: biological monitoring, environmental monitoring, the decision maker, decision support systems, the decision, procedure implementation and the system (Bird, 1987). Many different control methods have been used in the past to reduce root-knot nematode damage.

The use of the following control methods varies with crop, country, economics, availability and environmental laws.

6.1 Physical Methods

Different methods of physical control are used to control root-knot nematodes in IPM.

6.1.1 Heating: Heat is the physical factor most widely used in nematode population reduction (Bird, 1987). This method is considered to be a very effective method to control plant parasitic nematodes. Heat treatment is more effective in moist rather than dry soil, due to the increased thermal conductivity and metabolic activity of target organisms (Barker, 1962). Two types of heating are used:

6.1.1.1 Steam sterilization of nurseries and greenhouse soil is done by heating to 82-93°C at a 15 cm depth for 30 minutes. This temperature is sufficient to kill the nematode and gives better results against root-knot nematodes than those obtainable with applications of systemic nematicides which do not kill root-knot (Lamberti *et al*., 1976).

6.1.1.2 Soil solarization is a rather recently developed technique, which has shown promise for the control of several soil-borne pathogens (Katan *et al*., 1976; Katan, 1987). Solarization also is a unique method of mulching that integrates pest control, soil and water conservation and increased growth response of crops (Stapleton and DeVay, 1986). The efficacy of soil solarization is based on the sensitivity of nematodes to relatively high temperature > 40-50°C. Soil solarization with clear plastic has been attempted as a means of raising soil temperatures to these lethal levels to control soil-borne diseases (Katan, 1981). This technique however, is only adaptable to regions, for example the Middle East, where sufficient solar energy is available for long periods of time. Solarization also has been shown to have a potential in the subtropical climate of Florida where it reduced root-knot, *Verticillium* wilt and weeds (Overman and Jones, 1986).

In some solarization experiments control of these nematodes was inconsistent (Greco *et al*., 1985; Barbercheck and Van Broembsen, 1986), in other investigations excellent control was achieved by soil solarization under greenhouse conditions (Cenis, 1984; Cartia *et al*., 1988; 1989).

Soil solarization reduced significantly important genera of phytoparasitic nematodes: *Meloidogyne, Heterodera, Globodera, Pratylenchus, Rotylenchulus, Rotylenchus, Tylenchulus,, Tylenchorhynchus, Xiphinema, Belonolaimus, Criconemella, Ditylenchus, Criconemella* and *Dolichodorus* (Davis and Sorensen, 1976; Siti *et al*., 1982; Stapleton and DeVay, 1983; Porter and Merriman, 1983; Greco *et al*., 1985; McSorley and Parrado, 1986; Heald and Robinson, 1987; Stapleton, 1990).

However, phytoparasitic nematodes are more resistant to the effects of solarization than most other plant pests and pathogens. Also, populations of root-knot nematodes must be effectively controlled to a greater soil depth than other targeted organisms. Although soil depths to which phytoparasitic nematodes were controlled by solarization varied, only a few reports examined effects below 30 cm (Stapleton and DeVay, 1983; Stapleton *et al*., 1987). Porter and Merriman (1983) demonstrated significant reductions of *Meloidogyne javanica, Tylenchulus semipenetrans* and *Criconemella xenoplax* to a 26 cm depth in microplot in Victoria.

Heald and Robinson (1987) showed that *Rotylenchulus reniformis* was effectively controlled only to a 15 cm depth in Texas. Stapleton and DeVay (1983) however reported more than 95% reduction of *Meloidogyne hapla* juveniles and males at 90 cm soil depth in Australia.

Abu-Gharbieh *et al*., (1987) found that hot season solarization with black polyethylene film, which was then perforated and left in place as a cool season mulch for vegetable crops, was an effective and economic practice for root-knot control (Fig. 2).

6.1.2 Flooding: Thames and Stoner (1953) reported effective root-knot nematode control on two vegetable crops produced after flooding previous rice fields for three months. Sikora (1989) also observed less severe root-knot nematode damage in Philippine vegetable cropping systems based on paddy rice-vegetable rotations when flooding was maintained for at least 4 months than in rotations without paddy rice. IFAS (1989) recommend alternating flooding with drying during the summer vegetable season in Florida for root-knot nematode control.

6.2 Rotations

Because root-knot nematodes are obligate parasites on plants, freshly hatched juveniles must find suitable hosts to parasite and reproduce. When rotations are used to control *Meloidogyne* species, such rotations must be designed with susceptible hosts and with non-hosts or resistant hosts to *Meloidogyne*. Because the most widespread and pathogenic species of root-knot nematodes are polyphagous and can have hunderds of host plants it is very difficult to design rotation schemes which are effective in controlling the nematode and that are at the same time economical (Lamberti, 1979b).

Lamberti (1979b) mentioned that *M. nassi* which has non-host plants among the cereals and other species of root-knot with few hosts may be easily controlled by crop rotation. Crops sequences he tested in a three year rotational system in soil infested with *M. incognita* in Italy, led to slight increases in yields of tomato when grown in the winter season .

A number of rotations exist in the tropics, especially in Asia, which are predominantly composed of cruciferous crops moderately resistant or tolerant to *Meloidogyne* spp., together with a small number of highly susceptible crops. Rotations of this design can be effectively used to reduce *Meloidogyne* densities (Page, 1979; Sikora *et al*., 1988)

However, Netscher and Sikora (1990) stated that the root-knot nematodes are extremely polyphagous, therefore relatively few non-host plants are available for *Meloidogyne* control through crop rotation in most countries or in intensive greenhouse production cropping systems.

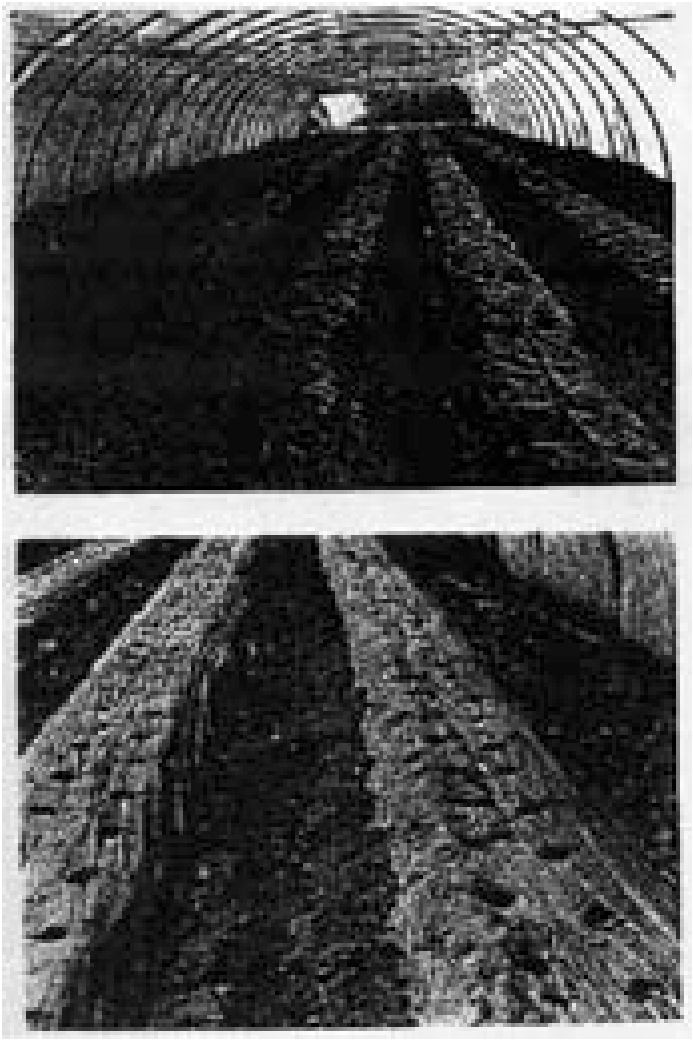


Figure 2: Soil solarization by using black polyethylene (above photo) and then planting the transplants into the plastic holes after 6-8 weeks of the solarization (below photo) (Abu-Gharbieh, 1988)

6.3 Chemical Control

During the last few decades, root-knot nematode control has been based on the use of chemical pesticides applied to the soil or the plant (Duponnois *et al*., 2001). There are two types of nematicides used in control of root-knot nematodes, fumigants and non-fumigants. Soil fumigation is the most common measure used to achieve economical control in agricultural land (Lamberti, 1979a). The polyphagous nature of the more important species of root-knot nematodes means that the use of chemicals is likely to be the main method of control.

Minton and Baujard (1990) also reported that chemicals are the major means of controlling nematodes including *M. arenaria*, *M. hapla*, *Pratylenchus brachyurus*, *Belonolaimus longicaudatus* and other nematodes in peanut.

Fumigant nematicides are used to treat soil as drenches, root dips, foliar applications or seed treatments. They can be formulated as gases, volatile liquids, gels, flowables, spray concentrates or granules (Bird, 1987). The gaseous nature of fumigants makes them extremely useful as nematicides. When fumigants are released in the soil, they volatilize and move effectively through soil pore spaces and into soil moisture films containing nematodes. In most cases they are broad spectrum contact nematicides effective against nematode eggs and juveniles and adults (Bird, 1987; Netscher and Sikora, 1990). The fumigants are phytotoxic (Bird, 1987) and must be applied weeks before planting.

Fumigants are generally more effective in controlling root-knot nematodes and in increasing crop yield than the non-fumigant nematicides, because fumigants have a broader spectrum of activity, controlling soil insects, fungal diseases and weeds as well as other plant parasitic nematodes as reported by Netscher and Sikora (1990).

Lamberti (1979b) and Johnson (1985) reported that the fumigant nematicides were highly effective in control of *Meloidogyne* in vegetables. In the past the most commonly used fumigant nematicides were: methyl bromide, chloropicrin, ethylene dibromide, 1,2-dibromo-3-chloropropan and metham sodium. The efficacy of these fumigants is affected by abiotic factors such as soil texture, moisture, temperature and organic matter (Bird, 1987).

Methyl bromide is generally very effective against most plant parasitic nematodes (Abdalla and Lear, 1975). Excellent control of all root-knot nematode species has been obtained with methyl bromide, which penetrates soil and intact root fragments readily (Khatoom, 1981; Noling, 1989; Abou-Jawadah *et al*., 2000; Oka *et al*., 2000). This highly effective fumigant has been removed from the market due to side-effects on the atmosphere and good alternatives have not yet been found for the tropics and subtropics.

The fumigant nematicide metham sodium was effective in controlling root-knot nematodes and soil fungi when applied through drip irrigation (Roberts, 1988). On the other hand Lamberti (1979b) found that metham sodium does not give good results against root-knot nematodes even when applied in very high dosages. It is also not as effective at higher soil temperature. The fumigant ethylene dibromide gives very efficient control to root-knot nematodes (Lamberti, 1979b). It is not as effective against other pest problems like weeds and fungi.

Hodges and Lear (1973) found that root-knot nematodes are killed by short exposure to 1,2-dibromo-3-chloropropan (DBCP). DBCP was frequently used to control nematodes in established orchards, vine-yards or rose plantings (Raski and Schmitt, 1964; Lownsbery *et al*., 1968; Johnson *et al*., 1969). It is no longer on the market.

The non-fumigant granular or liquid nematicide compounds are water soluble (Netscher and Sikora, 1990). The nematicides are not effective against nematode eggs and in most cases do not kill the juveniles or adults at the recommended concentrations used as reported by Netscher and Sikora (1990).

These granular or liquid non-fumigant systemic nematicides are effective in delaying infection for some weeks. Non-fumigant systemic nematicides are non-phytotoxic and they can be applied effectively by surface and drip irrigation (Overman, 1974; Johnson, 1985; IFAS, 1989). However, they are not as effective as fumigants in increasing yield because they do not have broad spectrum activity (Netscher and Sikora, 1990).

The most common non-fumigant systemic nematicides used to control nematode were: oxamyl, fenamiphos, carbofuran and aldicarb (Lamberti, 1979b). The systemic action of aldicarb inhibit root invasion by *M. incognita* juveniles under greenhouse conditions and inhibits egg hatch and juvenile migration and host invasion (Hough and Thomason, 1975; Vovlas and Lamberti, 1976). Vovlas and Lamberti (1976) reported that carbofuran and oxamyl prevented root invasion of tomato by *M. incognita* and was very effective against root-knot nematodes for 12 and 25 days. Phenamiphos is a systemic non-fumigant sold in a granular and a liquid formulation. Bunt (1975) and Roca *et al*., (1975) revealed that the granular formulation is more effective than the liquid against root-knot nematodes, because of its longer persistence.

6.4 Resistance

Resistance can be defined as the character, or characters of a plant that inhibit nematode reproduction (Taylor and Sasser, 1978). The use of resistant cultivars is a preferred, important, economical and environmentally safe method to managing root-knot nematode damage (Netscher and Mauboussin, 1973; Roberts, 1992; 1995; Trudgill, 1995).

However, Netscher and Sikora (1990) mentioned that resistant cultivars of crops susceptible to one species of *Meloidogyne* do not necessarily protect the crop against all other species of *Meloidogyne*. The evaluation of resistance to various species showed that some crops were resistant to two or more species whereas others carried resistance to only one species (Marks *et al*., 1968; Southards and Priest, 1973). For example, tomato with the Mi gene was found to carry resistance to *M. incognita* and *M. javanica*, but not *M. arenaria*. Resistance is also

known in common bean to *M. incognita*, sweet potato to *M. incognita* and *M. javanica* and pepper to *M. incognita*, *M. arenaria* and *M. javanica*, but not *M. hapla* as reported by Fassuliotis (1985).

Hendy *et al*., (1985) reported the presence of five dominant genes which when present in one genotype protect against *M. incognita, M. javanica* and *M. arenaria*. Also root-knot populations which were capable of attacking resistant cultivars have been detected (Sikora *et al*., 1973; Netscher, 1977; Prot, 1984; Fargette, 1987; Berthou *et al*., 1989).

6.5 Soil Amendments

Reductions in plant parasitic nematode populations in response to applications of organic amendments has been reported (Muller and Gooch, 1982). The incorporation of organic material into the soil has been shown repeatedly to reduce root-knot nematode densities (Stirling, 1991). He suggested that the mechanism of action may be due to: improvement of soil structure and aggregation resulting in increased aertion and water-holding capacity; to improvement in plant nutrition; to the release of toxic by-products to nematodes of microbial basis or to the enhancement of the growth of organisms able to compete with or destroy nematodes.

Baby and Manibhushanrao (1993) found that organic amendments were associated with suppression of nematode populations through stimulation of antagonistic soil microflora during their decomposition.

A number of organic amendments have been used to manage root-knot nematodes. Their use is associated with reduced infection, or survival of nematodes and increased numbers of microbial antagonists of nematodes (Linford *et al.*, 1938; Watson, 1945; Mankau and Minteer, 1962; Mankau, 1968; Sitaramaiah and Singh, 1978).

Plant based soil amendments such as castor, sesame, sorghum-sudangrass, velvet bean and zinnia incorporated into soil or applied as mulch were chosen to control nematodes under

greenhouse conditions, because they have been previously used as organic amendments to suppress root-knot nematodes when used in crop rotation (Watson, 1922; 1936; 1945; Watson and Goff, 1937; Lear, 1959; Mankau and Minteer, 1962; Mankau, 1968; Mian and Rodrigeuz-Kabana, 1982b; Hung, 1984; Rodrigeuz-Kabana *et al*., 1988; Rich *et al*., 1989; McSorley and Gallaher, 1993; McSorley *et al.*, 1994).

The use of nitrogenous organic matter as a soil amendment is a successful strategy for the management of *Meloidogyne* spp. and other plant parasitic nematodes in vegetables and other root-knot susceptible crops (Mian and Rodrigeuz-Kabana, 1982a; Rodrigeuz-Kabana *et al.*, 1990).

Oil cakes, sawdust, urea and bagasse also have been used in managing root-knot nematodes with some success (Singh and Sitaramaiah, 1966; 1967; Sikora *et al*., 1973). Culbreath *et al*., (1985) suggested that when chitin was combined with waste products from the paper industry, root-knot nematodes were reduced.

6.6 Biological Control

Biological control of soil-borne pathogens by introduced microorganisms has been studied for over 65 years (Cook and Barker, 1983; Barker, 1987), but during most of that time it has not been considered commercially feasible. Biocontrol of nematodes was first studied by Duddington (1951).

The development of biological control agents is also considered an effective alternative for nematode control on vegetables (Van Gundy, 1985; Kerry, 1987; Sikora, 1992). Biological control is considered to encompass control that results from the action of soil microorganisms and the soil microfauna and is mediated through mechanisms such as parasitism, predation, competition and antibiosis (Stirling, 1991).

There are three major types of organisms that are antagonistic to nematodes. Each group differs in its mode of action (a) predators are organisms which actively seek out nematodes

and then consume them, (b) parasites are organisms which grow within their host and obtain their nutrition from the host and if they are capable of causing disease in the host they are known as pathogens, (c) the third group of antagonists influence nematode abundance through mechanisms other than predation and parasitism (Stirling, 1991).

Sikora (1992) suggested the term antagonistic potential for all parasites, predators, pathogens, competitors and other organisms in soil that work together to repel, inhibit, or kill plant parasitic nematodes. Antagonists most likely to be receptive to management for the biological control of nematodes are: predacious or trapping fungi; endoparasitic fungi; fungal pathogen/parasites of females, endomycorrhizal and mutualistic fungi; plant-health promoting rhizobacteria and obligate bacterial parasites.

6.6.1 Fungi: A number of different fungi were studied as biocontrol agents against plant parasitic nematodes especially root-knot nematodes.

6.6.1.1 Nematode-trapping fungi

These fungi known as predatory fungi, consist of a sparse mycelium which has been modified to form organs capable of capturing nematodes (Stirling, 1991).

Duddington (1962); Barron (1977) and Gray (1987; 1988) described six different types of traps that have been found in the nematode trapping fungi (Fig. 3). A few species of hyphomycetes and two genera in zygomycetes (*Stylopage and Cystopage*) capture nematodes by means of an adhesive that is produced directly on their hyphae. Another trapping structure is the adhesive branch which is produced by a few species of nematode-trapping fungi such as *Monacrosporium cionopagum*.

Some predacious fungi produce erect branches of one to three cells on the hyphae (Fig. 3f) and these may anastomose to form single loops or two-dimensional networks (Fig. 3e). A thin film of adhesive material is secreted over the entire surface of each branch. Adhesive network traps are a further development from adhesive branches. Further loops are produced on this

loop or on the parent hyphae, until a complex three-dimensional, adhesive-covered network of anastomosed loops exists (Fig. 3b). The adhesive nets are the most common type of trap found in the nematode-trapping fungi and they are found in almost all soils. *Arthrobotrys oligospora* the most frequently found predatory fungus in soil, uses this trapping mechanism. The fourth type of adhesive trapping device is the adhesive knob (Fig. 3c,d). Adhesive knobs are one of the most common trapping mechanisms among the hyphomycetes and are also found in basidiomycetes. Non-constricting rings are the most infrequent trapping device in the nematophagous fungi (Fig. 3g). Constricting rings are formed in a similar manner to non-constricting rings, but they are attached to the hyphae by a shorter stalk (Fig. 3h,i) and close when a nematode passes through the ring.

6.6.1.2 Female and egg parasitic fungi and pathogens

This group of fungi parasitize nematode females and/or eggs.

The zoosporic pathogens of females are closely related to the zoosporic species which attack vermiform nematodes. *Catenaria auxiliaries* is a widespread fungus in *Heterodera schachtii* in Europe (Tribe, 1977a,b) and has been found in *H. glycines* and *H. avenae* (Crump *et al*., 1983; Stirling and Kerry, 1983). *Nematophthora gynophila* was found in *H. avenae* infested soils (Kerry and Crump, 1980).

In addition to the zoosporic fungi, a wide range of other fungi have been found in association with nematode females, cysts and eggs. These fungi are facultative parasites such as, *Verticillium* spp., one of the most important pathogens of root-knot and cyst nematodes. Most species in this genus have been recorded as occurring in cysts and/or eggs of *Globodera*, *Heterodera* and *Meloidogyne* (Morgan-Jones and Rodriguez-Kabana, 1988). *V. chlamydosporium* is the most widely studied species. These fungi form branched mycelial networks which when in close contact with the egg shell, penetrated the egg wall and destroy its contents (Lysek, 1978; Morgan-Jones *et al.*, 1983; Lysek and Krajci, 1987; Lopez-Llorca and Duncan, 1988). Morgan-Jones *et al.*, (1983) and Meyer *et al*., (1990) suggested that *V. chlamydosporium* itself might produce a toxin that affects egg hatch.

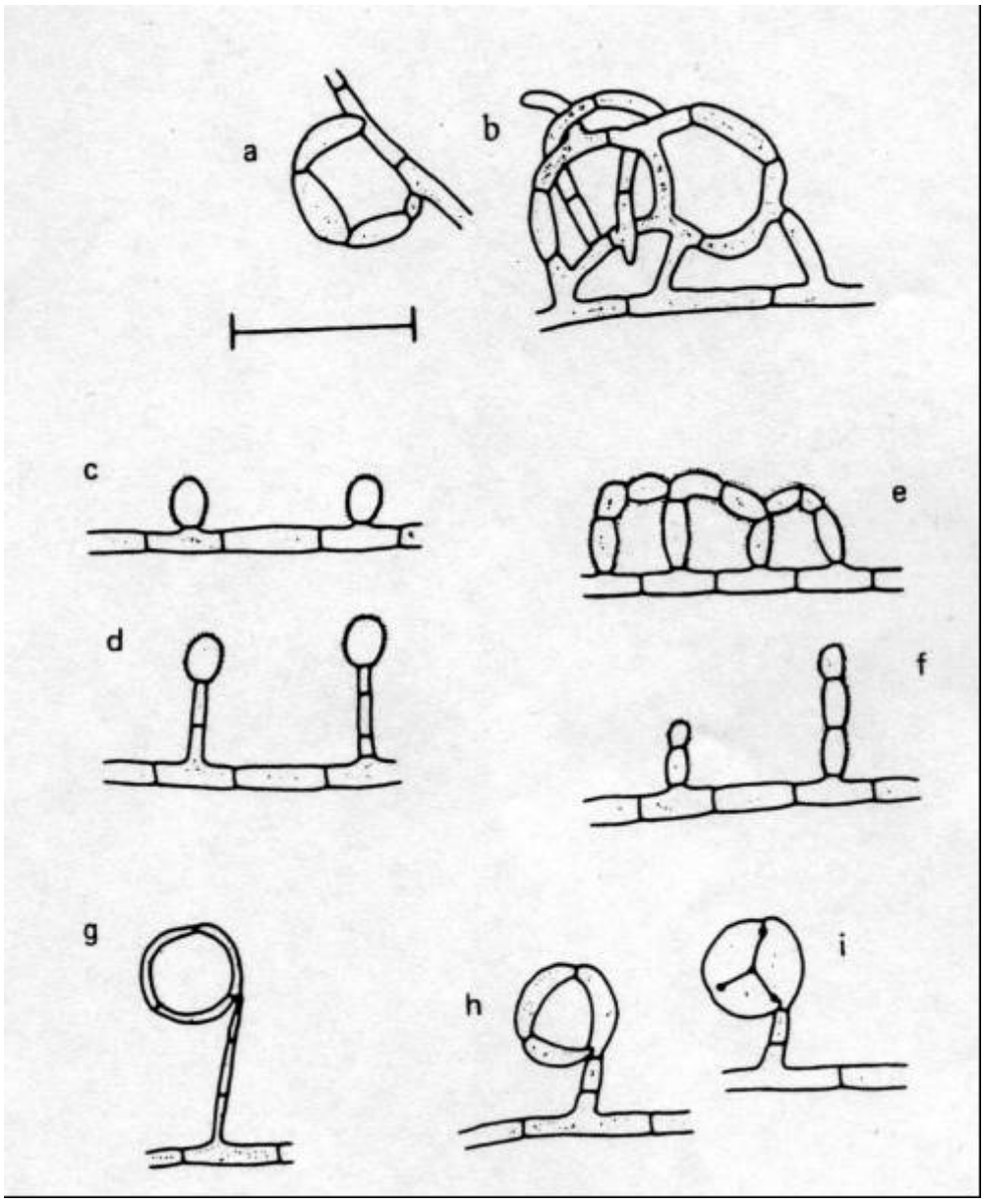


Figure 3: Trapping organs of predatory nematophagous fungi: (a) adhesive nets simple and; (b) complex; (c) adhesive spore sessile and; (d) stalked, (e) simple two-dimensional adhesive networks; (f) adhesive knobs and branches; (g) non-constricting rings, (h) constricting rings, open and; (i) closed (Gray, 1988).

Paecilomyces has been one of the principal genera in biological control in recent years. Lysek (1976) was the first to observed it in association with nematode eggs. Jatala *et al*., (1979) found it parasitizing eggs of *M. incognita* and in 1985 and 1986 Jatala revealed that *P. lilacinus* showed promise as a biological control agent against several nematode species. This fungus penetrates the nematode eggs directly by individual hyphae (Morgan-Jones *et al*., 1984).

Dactylella oviparasitica was the first parasite found in *Meloidogyne* eggs (Stirling and Mankau, 1978a,b). This fungus produces appressoria on the egg surface and then penetrates the eggs through these specialized structures and through enzymatic penetration especially by chitinase production (Stirling and Mankau, 1979).

6.6.1.3 Mycorrhizal fungi

The mycorrhizas are symbiotic associations between plant roots and certain species of fungi. In practical agricultural terms, mycorrhizas are traditionally ectomycorrhizas and vesicular-arbuscular mycorrhizas as reported by Ikram (1990) (Fig. 4).

The ectomycorrhizas grows around the root surface and are easily seen with the naked eye while endomycorrhizas grow inside roots. The ectomycorrhizas are common to many temperate forest tree species and this group is characterized by a sheath of visibly-swollen fungal tissue (mantle) which encloses the ultimate rootlets of the root system, together with intercellular infection of the epidermis and cortex (Fig. 4a). Some species of ectomycorrhizas have been shown to reduce ectoparasitic nematode damage of forest tree roots, but are not effective against root-knot.

Endomycorrhizae are the most spectacular and widespread type of mycorrhizas present in nearly all major agronomic crops (vegetables, fruit crops, flowers, forest tree and plantation crops). The invading hyphae of endomycorrhizae grow to the root surface, forms an appressorium and penetrates into the cortex. Hyphal growth in root cortical cells are both inter-and intracellular where they form the two diagonstic structures, vesicles and arbuscules

(Fig. 4b). Many species do not form vesicles, therefore, the new name arbuscular mycorrhizal fungi.

A plant with a well-established symbiont density is stronger because it has:

a) increased resistance to nematode parasities and root pathogens

b) increased tolerance to drought, salt and other abiotic stress factors

c) improved phosphate uptake

d) less root injury following transplanting

The colonization of plants with endomycorrhizal fungi apart from providing plants with nutrients also has a depressive effect on root-knot nematodes. The obligate symbiotic endomycorrhizal fungi protect their host against root-knot nematode attack by competition as reported by Sikora (1978) and Hussey and Roncadori (1982). Sikora (1978) found that penetration and development of *M. incognita* in tomato was significantly reduced by the endomycorrhizal fungus, *Glomus mosseae* under glasshouse conditions. Because these fungi occur commonly together with plant parasitic nematodes in the roots or rhizosphere of the same plants, they interact with both host plant and nematodes (Stirling, 1991).

Smith (1987) explained possible hypotheses for the beneficial effects of endomycorrhizal fungi on plants parasitized by nematodes. He postulated that the symbiotic mycorrhizae:

(1) reduce or alter root exudates consequently affecting egg hatch or nematode attraction

(2) retard nematode development or reproduction within root tissue

(3) parasitize female nematodes and their eggs.

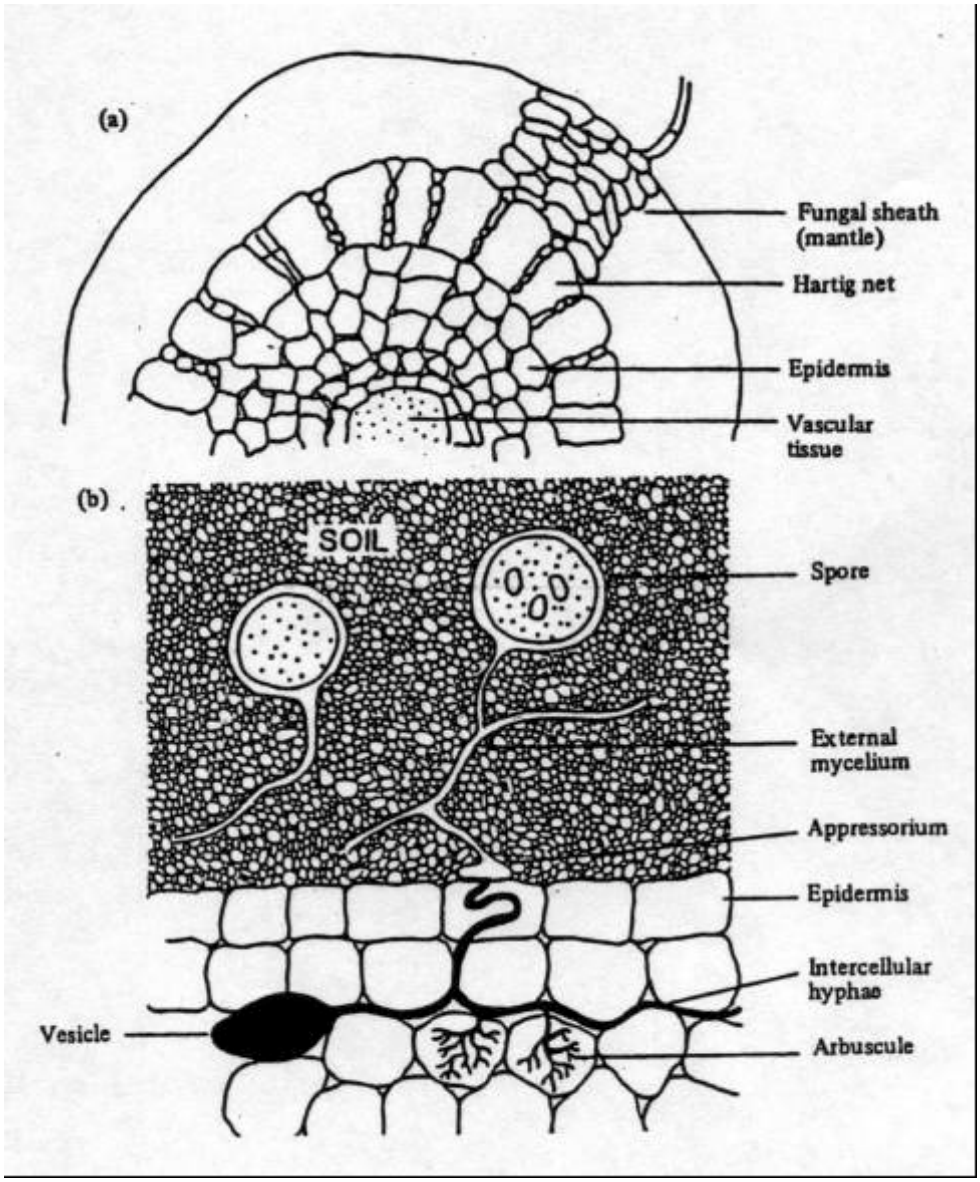


Figure 4: Diagrammatic representation (a) ecto- and (b) endomycorrhizae (Ikram, 1990)

6.6.1.4 Endophytic fungi

Another group of fungi that has been recently used as a biocontrol agent against plant parasitic nematodes especially root-knot nematodes, are endophytic fungi (Hallmann *et al*., 1997). These saprophytic fungi colonize healthy plant tissue without causing symptoms. When the colonization is successful it leads to protection of the plant against biotic and/or abiotic stress, these fungi are called mutualistic endophytic fungi (Carroll, 1990).

Mousa and Hague (1988) found that in the soybean disease complex between *Fusarium oxysporum f. sp. glycines* and *M. incognita* active colonization of the giant cells by the fungus resulted in reduced development of juveniles and an increased proportion of males.

Hallmann and Sikora (1993) evaluated 200 isolates of endophytic fungi, representing different genera, isolated from tomato roots. Forty isolates were screened for their ability to control *M. incognita* in pot experiments. Hallmann and Sikora (1994, 1995) found a reduction in gall formation by *M. incognita* between 52 and 75% after application of four endophytic strains of the fungus *Fusarium oxysporum*. They also found that *M. incognita* attraction and penetration of tomato seedlings was significantly reduced following treatment with the culture filtrate of *Fusarium oxysporum*.

6.6.2 Bacteria

6.6.2.1 Endophytic bacteria

Endophytic bacteria have recently been found internally in the root tissue and in the rhizosphere where they persist in most plant species and plant tissues. They have been found in fruits and vegetables and in both stems and roots without doing harm to the plant (McInory and Kloepper, 1995; Hallmann *et al*., 1997, 1999; Hallmann, 2001).

Recent research has demonstrated that bacterial endophytes can improve plant growth and reduce disease symptoms caused by several plant pathogens such as *Fusarium oxysporum f.*

sp. vasinfectum on cotton (Chen *et al*., 1995), *Verticillium albo-atrum* and *Rhizoctonia solani* on potato and on cotton (Kloepper *et al*., 1992; Pleban *et al*., 1995).

Hallmann *et al*., (1995) found some evidence that endophytic bacteria may contribute to control of plant parasitic nematodes. They evaluated 7 isolates of endophytic bacteria isolated from cucumber and cotton roots against the root-knot nematode, *M. incognita* and they found a significant reduction of 50% in the number of galls on cucumber.

Munif *et al*., (2000) screened the endophytic bacteria isolated from tomato roots towards *M. incognita* on tomato under greenhouse conditions. They showed antagonistic properties in the screening of 21 out of 181 endophytic bacteria towards *M. incognita*.

6.6.2.2 Rhizobacteria

Another strategy used for the biological control of nematodes is based on the introduction of bacteria colonizing the rhizosphere of the host plant or so called rhizobacteria.

These microorganisms that can grow in the rhizosphere also provide front line defence for roots against pathogen attack and are considered ideal for use as biocontrol agent (Weller, 1988). Rhizosphere bacteria have the ability to colonize plant roots (Schroth and Hancock, 1982) and they also have positive effects on plant growth. They have been named plant growth promoting rhizobacteria (PGPR) by Kloepper *et al.*, (1991) or plant health promoting rhizobacteria (PHPR) by Sikora (1988).

Application of these rhizobacteria to sugar beet seed and potato seed pieces caused significant decreases in early root infection of the sugar beet cyst nematode *Heterodera schachtii* and potato cyst nematode *Globodera pallida* (Racke and Sikora, 1985; Oostendorp and Sikora, 1986).

Several rhizosphere bacteria with antagonistic activity against plant parasitic nematodes have been identified. Their potential as biocontrol agents is consider great.

Zavaleta-Meija and Van Gundy (1982) found that rhizobacteria have biocontrol activity towards root-knot nematode and they showed that more than 12% of the rhizobacteria tested reduced the number of galls of *M. incognita* on cucumber and tomato. Sikora (1992) reported that 7-10% of the rhizosphere bacteria isolated from potato, sugar beet or tomato root systems have antagonistic activity against cyst and root-knot nematodes. Sikora and Hoffmann-Hergarten (1993) revealed that PHPR influence the intimate relationship between the plant parasitic nematode and its host by regulation of nematode behaviour during the early root penetration phase of parasitism which is extremly important for crop yield.

Sikora (1988) found that *Bacillus subtilis* was effective in controlling *M. incognita* on cotton and sugar beet, *M. arenaria* on peanut and *Rotylenchulus reniformis* on cotton. Strains of *Pseudomonas chitinolytica* also were shown to reduce *M. javanica* on tomato as reported by Spiegel *et al*., (1991). Smith (1994) reported that *Bacillus* sp. strain 23a reduced *M. javanica* densities on tomato and *Pseudomonas fluorescens* strain Pf1 reduced the number of galls and egg masses of *M. incognita* on tomato roots (Santhi and Sivakumar, 1995). *B. cereus* strain S18 also decreased *M. incognita* on tomato as reported by Keuken (1996).

6.6.2.3 Obligate bacterial parasite, *Pasteuria penetrans*

The name *P. penetrans* (Thorne) Sayre and Starr is given to a group of spore forming bacteria which are parasitic to a number of important plant parasitic nematodes (Birchfield and Antonpoulos, 1976; Starr and Sayre, 1988). It is one of the most efficient natural enemies of root-knot nematodes (Mankau, 1975; Stirling, 1984; Gowen and Ahmed, 1990). The bacteria have been reported from nearly 200 nematode species from a wide range of environments (Spull, 1981; Sayre and Starr, 1985; Sturhan, 1985), but its occurrence and abundance seems to be variable. This variability is thought to be due to several factors, including differences in the specificity of isolates of *P. penetrans* to populations and species of *Meloidogyne* (Stirling, 1985; Channer and Gowen, 1992; Davies and Danks, 1993).

P. penetrans is a very common parasite of *Meloidogyne* and is often observed attached to nematode juveniles. The spore form can resist drought, exposure to fumigant nematicides (Mankau and Prasad, 1972) and extreme temperature (Chen and Dickson, 1998).

The inability to produce mass cultures of *P. penetrans* in quantities sufficient for lange scale use is the major factor limiting practical work with these bacteria (Birchfield and Antonpoulos, 1976; Gowen and Ahmed, 1990). Stirling and Wachtel (1980) were able to produce large numbers of spores by inoculating tomato with infected *Meloidogyne* juveniles. Dried tomato roots were then milled into a powder containing *Pasteuria* spores.

The populations of *P. penetrans* which parasitize *Meloidogyne* not only prevent nematode reproduction, but also reduce infectivity of spore-encumbered juveniles. A reduction in infectivity may be evident when nematodes have as few as 15 spores attached (Davies *et al*., 1988). Juveniles are prevented from invading roots when large enough numbers of spores are present in soil (Stirling, 1984; Stirling *et al*., 1990).

7- Integrated Pest Management (IPM) In The Future

The different control methods available for use against plant parasitic nematodes, especially root-knot nematodes have been outlined above. However, there are problems associated with the use of some of these methods. Problems can be related to costs, availability, positioning in the cropping system, extension needs or lack of farmer acceptance.

During the last few decades, nematode control has been based on the use of chemical pesticides applied to soil or the plant. New efforts are being made to develop management strategies that do not rely on nematicides, or are aimed at reducing the use of pesticides materials (Rodriguez-Kabana and Morgan-Jones, 1987). These pesticides are very expensive (Duponnois *et al*., 2001) and toxic to humans and the environment especially when used in an inappropriate manner by farmers.

Using resistant cultivars and/or rotations with non-host crops are effective for root-knot nematode control. However, the wide host range of species of *Meloidogyne* (Jepson, 1987) make them difficult to control by rotation. Resistant cultivars are only available for a few crops. Their use is also limited by the occurrence of virulent races and species mixtures able to breakdown the resistance (Triantaphyllou and Sasser, 1960; Roberts, 1992).

Triantaphyllou and Sasser (1960) and Netscher (1977) found that resistance breaking races were also selected from single egg mass populations of *M. incognita* and *M. javanica* under laboratory conditions. Resistance breakdown due to high soil temperatures also has been observed with the Mi gene in tomato (Berthou *et al*., 1989). Dropkin (1969) showed that the resistant tomato cultivar Nematex was highly resistant at 28°C to *M. incognita*, whereas it was susceptible at 32°C.

Solarization is limited to only a few countries where sun light intensity and temperature are sufficient e.g North Africa and Middle East and where farmers can afford the plastic mulch.

New effective IPM strategies to control root-knot nematodes on vegetables and on other crops must be developed using a mixture of the following technologies:

(1) Use of nematode free transplants.
(2) Planting green manure crops as non hosts in rotation.
(3) Using trap crops or toxic plants like, Tagetes or Crotolaria
(4) Adding organic soil amendments and amendments which contain chitinous materials
(5) Rotating resistant and susceptible cultivars to prevent resistant breaking races.
(6) Selective use of nematicides by professionals.
(7) Incorporation of flooding and steam sterilization of soil where economical.
(8) Solarization with plastic mulches where solar radiation permits.
(9) Increased use of biofumigation following incorporation of organic matter into soil coupled with solarization.
(10) Selecting good biocontrol agents to reduce infection.

Using biological control agents in IPM must be advanced particularly for vegetable production. IPM plus biocontrol is more environmentally safe and economical than some pesticides. The loss of methyl bromide fumigation warrents even more research efforts to develop IPM in vegetable production.

Enhancement or application of biocontrol agents to IPM must be promoted and researched. Biocontrol agents need to be studied like: female and egg parasites fungi *V. chlamydosporum* (Kerry *et al*., 1984); mycorrhizal fungi *Glomus* spp (Sikora, 1978); mutualistic fungal (Hallmann *et al*., 1997) and bacterial endophytes (Munif *et al*., 2000); plant-health promoting rhizobacteria (Becker *et al*., 1988; Racke, 1988; Oostendorp and Sikora 1989, 1990) and the endospore forming bacterium *P. penetrans* (Mankau, 1975; Stirling and Wachtel, 1980; Davies *et al*., 1988; Gowen and Ahmed, 1990; Dickson *et al*., 1994) . All these agents are considered potential tools in future IPM to suppress root-knot nematodes and must be researched in more detail.

A promosing group are the plant-health promoting rhizobacteria that are effective in reducing early root infection damage (Sikora, 1988; Oostendorp and Sikora, 1989) especially of root-knot nematodes (Becker *et al*., 1988; Hoffmann-Hergarten *et al*., 1998). Rhizobacteria *Bacillus cereus* strain S18 and *Rhizobium etli* strain G12 have been shown to be successful towards root-knot nematodes and must be considered prime candidates for inclusion in IPM strategies.

Important characteristics of these bacteria are:
(1) they present in the rhizosphere and also may grow endophytically in the plant tissue
(2) both rhizobacteria will control *M. incognita* on different host plants
(3) they can be easily produced in industrial fermentors
(4) they are very easy to applied as a soil drench, seed treatment or root dipping
(5) economically low levels of inoculum for targeted application make them attractive

Another very important group is the obligate parasite *Pasteuria penetrans*. These bacteria can be applied economically as a spore suspension to the transplants as a root powder and can be passively spread to produce natural suppressive soils.

Important characteristics of this bacterium are:
(1) Safty to users and environment
(2) Mass production is very easy on root-knot infested living plants
(3) Resistant against extreme biotic and abiotic factors
(4) Spores can be stored many years without lossing viability
(5) Very easy to apply as a spore suspension or as a root powder to seedlings

The objectives of this work were
(1)- Evaluate the effect of the plant health-promoting rhizobacterium *Bacillus cereus* strain S18 on root-knot nematodes, *Meloidogyne* spp. on different host plants

(2)- Evaluate the antagonistic activity of the rhizobacterium, *Rhizobium etli* strain G12 towards different genera and species of plant parasitic nematodes especially root-knot on different host plants.

(3)- Evaluate the attachment of the obligate endoparasitic bacteria *Pasteuria penetrans* towards different populations of root-knot nematodes.

(4)- To develop a strategy for incorporating these biocontrol agents into IPM programs for reducing the impact of root-knot on vegetables.

8- References

Abdalla, N., and B. Lear. 1975. Lethal dosages of methyl bromide for four plant-parasitic nematodes and the effect of soil temperature on its nematicidal activity. Plant Disease Reporter 59:224-228.

Abu-Gharbieh, W. I. 1988. Root-knot nematodes, *Meloidogyne* spp., in Jordan: Biology and Control. Pp. 1-68, eds. University of Jordan, Amman.

Abu-Gharbieh, W. I., H. M. Saleh, and H. A. Abu-Blan. 1987. Effect of solarization using different thicknesses of black plastic tarping on soil-borne pathogens. Workshop, Advances in agriculture through plastic house technology. Amman, Jordan:25.

Abou-Jawdah, Y., K. Melki, S. L. Hafez, H. Sobh, Y. El-Masri, and P. Sundararj. 2000. Alternatives to methyl bromide for root-knot nematode management on cucumber in Lebanon. Nematropica 30:41-45.

Baby, U. I., and K. Manibhushanrao. 1993. Control of rice sheath blight through the integration of fungal antagonists and organic amendments. Tropical Agriculture (Trinidad) 70:224-244.

Barbercheck, M. E., and S. L. Von Broembsen. 1986. Effects of soil solarization on plant parasitic nematodes and *Phytophthora cinnamomi* in South Africa. Plant Disease 70:945.

Barker, K. F. 1962. Principles of disinfestations of heat-treated soil and planting material. Journal of Austeralian Inst. Agicultural Science 28:118.

Barker, K. F. 1987. Evolving concepts of biological control of plant pathogens. Annual Review of Phytopathology 25:67-85.

Barron, G. L. 1977. The nematode destroying fungi. Canadian Biological Publications Ltd, Guelph.

Becker, J. O., E. Zavaleta-Mejia, S. F. Colbert, M. N. Schroth, A. Weinhold, J. G. Hancock, and S. D. Van Gundy. 1988. Effects of rhizobacteria on root-knot nematodes and gall formation. Phytopathology 78: 1466-1469.

Bergeson, G. B., S. D. Van Gundy, and I. J. Thomason. 1970. Effect of *Meloidogyne javanica* on rhizosphere microflora and *Fusarium* wilt of tomato. Phytopathology 69:1245-1249.

Berthou, F., A. Ba-Diallo, and G. De Guiran. 1989. Caracterisation chez les nematodes *Meloidogyne* Goeldi (Tylenchida) de types virulents viv a vis du gene Mi de la tomate dans deux zones maraicheres au Senegal. Agronomie 9 :877-885.

Birchfield, W., and A. A. Antonpoulos. 1976. Scanning electron microscope observations of *Duboscquia penetrans* parasitizing root-knot larvae. Journal of Nematology 8:272-283.

Bird, G. W. 1987. Role of nematology in integrated pest management programs. Pp. 114-130 *in* J. A. Veech, and D. W. Dickson, eds. Vistas on nematology. Hyatlsville, Maryland.

Brodie, B. B., K. Evans, and J. Franco. 1993. Nematode parasites of potatoes. Pp. 87-132 *in* K. Evans, D. L. Trudgill, and J. M. Webster, eds. Plant parasitic nematodes in temperate agriculture. CAB International, Wallingford, Oxon, UK.

Bunt, J. A. 1975. Effect and mode of action of some systemic nematicides. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent, Belgium 75:1-127.

Carroll, G. C. 1990. Fungal endophytes in vascular plants: Mycological research opportunities in Japan. Transactions Mycological Society Japan 31:103-116.

Cartia, G., N. Greco, and G. Cirvilleri. 1988. Solarizzaztione e bromuro di metile nella difesa dai parassiti del pomodoro in ambiente protetto. Proceedings Giornate Fitopatologiche, Lecce, 16-20 May, Italy 1:437-488.

Cartia, G., N. Greco, and T. Cipriano. 1989. Effect of solarization and fumigants on soil-borne pathogens of pepper in greenhouse. Acta Horticulture 255:111.

Cenis, J. L. 1984. Control of the nematode *Meloidogyne javanica* by soil solarization.Pp. 132 *in* Proceedings of the sixth Congress Union Phytopathological Mediterranea, 1-6 October, Cairo, Egypt.

Channer, A. G., and S. R. Gowen. 1992. Selection for increased host resistance and increased pathogen specificity in the *Meloidogyne-Pasteuria penetrans* interaction. Fundamental and Applied Nematology 15:331-339.

Chen, C., E. M. Bauske, G. Musson, R. Rodriguez-Kabana, and J. W. Kloepper. 1995. Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. Biological Control 5:83-91.

Chen, Z. X., and D. W. Dickson. 1998. Review of *Pasteuria penetrans*: biology, ecology, and biological control potential. Journal of Nematology 30:313-340.

Chitwood, B. G. 1949. Root-knot nematodes. Proceedings of Helminthological Society of Washington 16:90-104.

Cook, R. J., and K. F. Barker. 1983. The nature and practice of biological control of plant pathogens. American Phytopathological Society, St. Paul.

Crump, D. H., R. M. Sayre, and L. D. Young. 1983. Occurrence of nematophagous fungi in cyst nematode populations. Plant Disease 67:63-64.

Culbreath, A. K., R. Rodriguez-Kabana, and J. Morgan-Jones. 1985. The use of hemicellulosic waste matter for reduction of the phytotoxic effect of chitin and control of root-knot nematodes. Nematropica 15:49-75.

Davies, K. G., B. R. Kerry, and C. A. Flynn. 1988. Observations on the pathogencity of *Pasteuria penetrans*, a parasite of root-knot nematodes. Annals of Applied Biology 112:491-501.

Davies, K. G., and C. Danks. 1993. Interspecific differences in the nematode surface coat between *Meloidogyne incognita* and *M. arenaria* related to the adhesion of the bacterium *Pasteruria penetrans*. Parasitology 105:475-480.

Davis, J. R., and L. H. Sorensen. 1976. Influence of soil solarization at moderate temperatures on potato genotypes with differeing resistance to *Verticillium dahliae*. Phytopatholoy 76:1021.

Dickson, D. W., M. Oostendorp, R. Giblin-Davis, and D. J. Mitchell. 1994. Control of plant-parasitic nematodes by biological antagonists. Pp. 575-601 *in* D. Rosen, F. D. Bennett, and J. L. Capinera, eds. Pest management in the subtropics. Biological control-a Florida Perspective. Andover, UK: Intercept.

Dropkin, V. 1969. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: Reversal by temperature. Phytopathology 59:1632-1637.

Duddington, C. L. 1951. Two new predacious hyphomycetes. Transactions of the British Mycological Society 34:598-603.

Duddington, C. L. 1962. Predacious fungi and the control of eelworms. Pp. 151-200 *in* C. L. Duddington, and Carthy, J. D., eds. Viewpoints in biology, Vol. I, Butterworths, London.

Duponnois, R., J. L. Chotte, and S. Sall. 2001. The effect of organic amendments on the interactions between a nematophagous fungus *Arthrobotrys oligospora* and the root-knot nematode, *Meloidogyne mayaguensis* parasitizing tomato plants. Biological Fertlization Soils 34:1-6.

Eisenback, J. D., H. Hirschmann, J. N. Sasser, and A. C. Triantaphyllou. 1981. A guide to the four most common species of root-knot nematodes, *Meloidogyne* species with pictorial keys. Pp. 47 *in* The Department of Plant Pathology and Gentics, North Carolina State University and USAID, Raleigh, NC, USA.

Fargette, M. 1987. Use of the esterase phenotype in taxonomy of the genus *Meloidogyne*. 2. Esterase phenotypes in West African populations and their characterization. Revue de Nematologie 10:45-55.

Fassuliotis, G. 1985. The role of the nematologist in the development of resistant cultivars. Pp. 233-240 *in* J. N. Sasser, and C. C. Carter, eds. An advanced treatise on *Meloidogyne*, Vol. I Biology and Control, Raleigh: North Carolina State University Graphics.

Fassuliotis, G., and G. J. Rau. 1969. The relationship of *Meloidogyne incognita acrita* to the incidence of cabbage yellows. Journal of Nematology 1:219-222.

Ferris, H., and S. D. Van Gundy. 1979. *Meloidogyne* ecology and host interrelationships. Pp. 205-230 *in* Lamberti, F., and Taylor, C. E., eds. Root-knot nematodes, *Meloidogyne* species: systematics, biology and control. Academic Press, London.

Golden, J. K., and S. D. Van Gundy. 1975. A disease complex of okra and tomato involving the nematode, *Meloidogyne incognita* and the soil-inhabiting fungus, *Rhizoctonia solani*. Phytopathology 65:265-273.

Goswami, B. K., D. V. Singh, C. L. Sethi, and J. N. Gupta. 1970. Studies on association of root-knot nematodes, *Meloidogyne incognita* (Kofoid and White) Chitwood and *Sclerotium rolfsii* Sacc. In brinjal (*Solanum melongena* L.). Indian Phytopathology 23:587-589.

Gowen, S. R., and R. Ahmed. 1990. *Pasteuria penetrans* for control of pathogenic nematodes. Aspects of Applied Biology 24:25-32.

Gray, N. F. 1987. Nematophagous fungi with particular reference to their ecology. Biological Reviews 62:245-304.

Gray, N. F. 1988. Fungi attacking vermiform nematodes. Pp. 3-38 *in* Poinar, G. O., and Jansson, H. B., eds. Diseases of nematodes, Vol. II, CRC Press Inc., Boca Raton.

Greco, N., A. Brandonisio, and F. Elia. 1985. Control of *Ditylenchus dipsaci*, *Heterodera carotae* and *Meloidogyne javanica* by solarization. Nematologia Mediterranea 13:191.

Guiran, G. De., and M. Ritter. 1979. Life cycle of *Meloidogyne* species and factors influencing their development. Pp. 173-191 *in* Lamberti, F., and Taylor, C. E., eds. Root-knot nematodes, *Meloidogyne* species: systematics, biology and control. Academic Press, London.

Hallmann, J. 2001. Plant interactions with endophytic bacteria. Pp. 87-119 *in* M. J. Jeger, and N. J. Spence, eds. Biotic interactions in plant-pathogen interactions. CAB International, Wallingford, UK.

Hallmann, J., A. Quadt-Hallmann, W. F. Mahaffee, and J. W. Kloepper. 1997. Bacterial endophytes in agricultural crops. Canadian Journal of Microbiology 43:895-914.

Hallmann, J., J. W. Kloepper, R. Rodriguez-Kabana, and R. A. Sikora. 1995. Endophytic rhizobacteria as antagonists of *Melodogyne incognita* on cucumber. Phytopathology 85:1136.

Hallmann, J., and R. A. Sikora. 1993. Fungal endophytes and their potential for biological control of root-knot nematodes. Proceedings of 6th International Congress of Plant Pathology, Montreal, Canada.

Hallmann, J., and R. A. Sikora. 1994. Influence of *Fusarium oxysporium*, a mutualistic fungal endophyte on *Meloidogyne incognita* infection of tomato. Journal of Plant Diseases and Protection 101:475-481.

Hallmann, J., and R. A. Sikora. 1995. Occurrence of plant parasitic nematodes and non-pathogenic species of *Fusarium* in tomato plants in Kenya and their role as mutualistic synergists for biological control of root-knot nematodes. International Journal of Pest Management 40:321-325.

Hallmann, J., R. Rodriguez-Kabana, and J. W. Kloepper. 1999. Chitin-mediated changes in bacterial communities of the soil, rhizosphere and within roots of cotton in relation to nematode control. Soil Biology and Biochemistry 31:551-560.

Hartman, K. M., and J. N. Sasser. 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. Pp. 69-78 *in* Barker, K. R., C. C. Carter, and J. N. Sasser, eds. An advanced treatise on *Meloidogyne*. Vol. II Methodology, Raleigh:North Carolina State University Graphics.

Heald, C. M., and A. F. Robinson. 1987. Effects of soil solarization on *Rotylenchulus reniformis* in the lower Rio Grande Valley of Texas. Journal of Nematology 19:93.

Hendy, H., E. Pochard, and A. Dalmasso. 1985. Transmission heriditaire de deux nouvelles sources de resistance aux nematodes du genre *Meloidogyne* chez le piment *Capsicum annum* L. comptes rendus de l'Academie d'Agriculture:817-822.

Hodges, L. R., and B. Lear. 1973. Effect of time of irrigation on the distribution of 1,2-Dibromo-3-chloropropane in soil after shallow injection. Pesticides Science 4:795-799.

Hoffmann-Hergarten, S., M. K. Gulati, and R. A. Sikora. 1998. Yield response and biological control of *Meloidogyne incognita* on lettuce and tomato with rhizobacteria. Journal of Plant Diseases and Protection 105:349-358.

Hough, A., and I. J. Thomason. 1975. Effect of aldicarb on the behaviour of *Heterodera schachtii* and *Meloidogyne javanica*. Journal of Nematology 7:221-229.

Huang, S. P. 1984. Cropping effects of marigolds, corn and okra on population levels of *Meloidogyne javanica* and on carrot yields. Journal of Nematology 16:396-398.

Hussey, R. S., and R. W. Roncadori. 1982. Vesicular-arbuscular mycorrhizae may limit nematode activity and improve plant growth. Plant Disease 66:9-14.

IFAS, Institute of Food and Agricultural Sciences. 1989. Florida nematode control guide. Pp. 138 *in* Entomology and Nematology Department and Cooperative Extension Service, University of Florida, Gainesville, USA.

Ikram, A. 1990. Beneficial soil microbes and crop productivity. The Planter 66:640-648.

Jatala, P. 1985. Biological control of nematodes. Pp. 303-308 *in* Sasser, J. N. and C. C. Carter, eds. An advanced treatise in *Meloidogyne*, Vol. I. Biology and control, Raleigh: North Carolina State University Graphics.

Jatala, P. 1986. Biological control of plant parasitic nematodes. Annual Review of Phytopathology 24:453-489.

Jatala, P., R. Kaltenbach, and M. Bocangel. 1979. Biological control of *Meloidogyne incognita acrita* and *Globodera pallida* on potatoes. Journal of Nematology 11:303.

Jenkins**,** W. R., and B. W. Coursen. 1957. The effect of root-knot nematodes, *Meloidogyne incognita acrita* and *M. hapla* on *Fusarium* wilt of tomatoes. Plant Disease Reporter 41:182-186.

Jepson, S. B. 1987. Identification of root-knot nematodes *Meloidogyne* spp. Pp. 265, eds. CAB International, Wallingford, Oxon, UK.

Johnson, A. W. 1985. The role of nematicides in nematode management. Pp. 249-267 *in* J. N. Sasser, and C. C. Carter, eds. An advanced treatise in *Meloidogyne*, Vol. I Biology and Control, Raleigh: North Carolina State University Graphics.

Johnson, A. W., and G. Fassuliotis. 1984. Nematode parasites of vegetable crops. Pp. 323-372 *in* W. R. Nickle, eds. Plant and insect nematodes. Marcel Dekker Inc., New York and Basel.

Johnson, D. E., B. Lear, S. T. Miyagawa, and R. H. Sciaroni. 1969. Multiple application of 1,2-Dibromo-3-chloropropane for control of nematodes in established rose plantings. Plant Disease Reporter 53:34-37.

Katan, J. 1981. Solar heating (solarization) of soil for control of soil-borne pests. Phytopathology 19:211-236.

Katan, J. 1987. Soil solarization. Pp. 77-105 *in* J. Wiley and Sons, eds. Innovative approaches to plant disease control, Ilan Chet, New York, USA.

Katan, J., A. Greenberger, H. Alon, and A. Grinstein. 1976. Solar heating by polyethylene mulching for the control of diseases caused by soil-borne pathogens. Phytopathology 66:683-688.

Kerry, B. R. 1987. Biological control. Pp. 233-263 *in* Brown, R. H., and Kerry, B. R., eds. Principles and practice of nematode control in crops. Academic Press, New York, USA.

Kerry, B. R., and D. H. Crump. 1980. Two fungi parasitic on females of cyst nematodes *Heterodera* spp. Transactions of the British Mycological Society 74:119-125.

Kerry, B. R., A. Simon, and A. D. Rovira. 1984. Observations on the introduction of *Verticillium chlamydosporuim* and other parasitic fungi into soil for control of the cereal cyst-nematode *Heterodera avenae*. Annals of Applied Biology 105:509-516.

Kerry, B. R., and W. B. Hominick. 2000. Biological control. *In* Biology of Nematodes, eds. Dl Lee. Reading, UK: Harwood Academic. In Press.

Keuken, O. 1996. Interaktionen zwischen dem Rhizosphärebakterium *Bacillus cereus*, dem Wurzelgallennematoden *Meloidogyne incognita* und tomate. Ph.D. disseratation, Bonn University, Bonn, Germany.

Khan, A. M., and S. K. Saxena. 1969. Relationship of root-knot to wilt of okra caused by *Fusarium oxysporium var. lycopersici*. Pp. 9 *in* All India Nematology Symposium, New Delhi.

Khatoom, M. 1981. Effect of methyl bromide soil fumigation and black plastic mulching on cucumber grown under plastic tunnels. M. Sc Thesis, Faculty of Agriculture, University of Jordan.

Kloepper, J. W., G. Wei, and S. Tuzun. 1992. Rhizosphere population dynamics and internal colonization of cucumber by plant growth-promoting rhizobacteria which induce systemic resistance to *Colletotricum orbiculare*. Pp. 185-191 *in* E. S. tjamos, eds. Biological control of plant diseases. Plenum Press, New York.

Kloepper, J. W., R. Rodriguez-Kabana, J. A. McInory, and D. J. Collins. 1991. Analysis of populations and physiological characterization of microorganisms in rhizosphere of plants with antagonistic properties to phytopathogenic nematodes. Plant and Soil 136:95-102.

Kofoid, C. A., and W. A. White. 1919. A new nematode infection in man. Journal of American Medicin Association 72:567-569.

Lamberti, F. 1979a. Economic importance of *Meloidogyne* spp. in subtropical and Mediterranean climates. Pp. 341-357 *in* Lamberti, F., and Taylor, C. E., eds. Root-knot nematodes, *Meloidogyne* species: systematics, biology and control. Academic Press, London.

Lamberti, F. 1979b. Chemical and cultural control. Pp. 403-423 *in* Lamberti, F., and Taylor, C. E., eds. Root-knot nematodes, *Meloidogyne* species: systematics, biology and control. Academic Press, London.

Lamberti**,** F., D. Dandria, N. Vovlas, and J. Aquuilina. 1976. Prove di lotta contro i nematodi galligeni del pomodoro da mensa in serra a Malta. Colture Protette 5:27-30.

Lear, B. 1959. Application of castor pomace and cropping of castor beans to soil to reduce nematode populations. Plant Disease Reporter 63:459-460.

Linford, M. B., F. Yap, and J. M. Oliveira. 1938. Reductions of soil populations of the root-knot nematode during decomposition of the organic matter. Soil Science 45:127-141.

Lopez-Llorca, L. V., and G. H. Duncan. 1988. A study of fungal endoparasitism of the cereal cyst nematode *Heterodera avenae* by scanning electron microscopy. Canadian Journal of Microbiology 34:613-619.

Lownsbery, B. F., J. T. Mitchell, W. H. Hart, F. M. Charles, M. H. Gerdts, and A. S. Greathead. 1968. Responses to post-planting and pre-planting soil fumigation in California peach, walnut and prune orchards. Plant Disease Reporter 52:890-894.

Lysek, H. 1976. Autodehelminthization of soil in lowland deciduous forests. Universitatis Palackianae Olomucensis Facultatis Medicae 41:73-106.

Lysek, H. 1978. A scanning electron microscope study of the effect of an ovicidal fungus on the eggs of *Ascaris lumbricoides*. Parasitology 77:139-141.

Lysek, H., and D. Krajci. 1987. Penetration of ovicidal fungus *Verticillium chlamydosporium* through the *Ascaris lumbricoides* egg shells. Folia Parasitologia 34:57-60.

Mai, W. F. 1985. Plant-parasitic nematodes: Their threat to agriculture. Pp. 11-18 *in* K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise in *Meloidogyne*, Vol. II Methodology, Raleigh: North Carolina State University Graphics.

Mankau, R. 1968. Reduction of root-knot disease with organic amendment under semifield conditions. Plant Disease Reporter 52:315-319.

Mankau, R. 1975. *Bacillus penetrans* n. comb. causing a virulent disease of plant-parasitic nematodes. Journal of Invertebrate Pathology 26:333-339.

Mankau, R., and N. Prasad. 1972. Possibilities and problems in the use of sporozoan endoparasite for biological control of plant parasitic nematodes. Nematropica 2:7-8.

Mankau, R., and R. J. Minteer. 1962. Reduction of soil population of citrus nematode by the addition of organic matter. Plant Disease Reporter 46:375-378.

Marks, C. F., I. J. Thomason, and C. E. Castro. 1968. Dynamics of the permeation of nematodes by water, nematicides and other substances. Experimental Parasitology 22:321-337.

McInory, J. A., and J. W. Kloepper. 1995. Survey of indeginous bacterial endophytes from cotton and sweet corn. Plant and Soil 173:337-342.

McSorley, R., D. W. Dickson, J. A. De Brito, T. E. Hewlett, and J. J. Frederick. 1994. Effects of tropical rotation crops on *Meloidogyne arenaria* population densities and vegetable yields in microplots. Journal of Nematology 26:175-181.

McSorley, R., and J. L. Parrado. 1986. Application of soil solarization to Rockdale soils in a subtropical environment. Nematropica 16:125.

McSorley, R., and R. N. Gallaher. 1993. Population dynamics of plant parasitic nematodes on cover crops of corn and sorghum. Journal of Nematology 25:446-453.

Meyer, S. L. F., R. N. Huettel, and R. M. Sayre. 1990. Isolation of fungi from *Heterodera glycines* and in vitro bioassays for their antagonism to eggs. Journal of Nematology 22:532-537.

Mian, I. H., and R. Rodriguez-Kabana. 1982a. Soil amendments with oil cakes and chicken litter for control of *Meloidogyne arenaria*. Nematropica 12:205-220.

Mian, I. H., and R. Rodriguez-Kabana. 1982b. Survey of the nematicidal properties of some organic materials available in Alabama as amendments to soil for control of *Meloidogyne arenaria*. Nematropica 12:235-246.

Minton, N. A., and P. Baujard. 1990. Nematode parasites of peanut. Pp. 285-320 *in* M. Luc, R. A. Sikora, and J. Bridge, eds. Plant parasitic nematodes in subtropical and tropical agriculture, CAB International, Wallingford, Oxon, UK.

Morgan-Jones, G., J. F. White, and R. Rodriguez-Kabana. 1983. Phytonematode pathology:ultrastructural studies. I. Parasitism of *Meloidogyne arenaria* eggs by *Verticillium chlamydosporium*. Nematropica 13:245-260.

Morgan-Jones, G., J. F. White, and R. Rodriguez-Kabana. 1984. Phytonematode pathology:ultrastructural studies. I. Parasitism of *Meloidogyne arenaria* eggs and larvae by *Paecilomyces lilacinus*. Nematropica 14:57-71.

Morgan-Jones, G., and R. Rodriguez-Kabana. 1988. Fungi colonizing cysts and eggs. Pp. 39-58 *in* Poinar, G. O., and Jansson, H. B., eds. Diseases of nematodes, Vol. II, CRC Press Inc., Boca Raton.

Moura, R. M. De., E. Echandi, and N. T. Powell. 1975. Interaction of *Corynebacterium michiganense* and *Meloidogyne incognita* on tomato. Phytopathology 65:1332-1335.

Mousa, E. M., and N. G. M. Hague. 1988. Influence of *Fusarium oxysporium* f. sp. *glycines* on the invasion and development of *Meloidogyne incognita* on soybean. Reveu de Nematologie 11:437-439.

Muller, R., and P. S. Gooch. 1982. Organic amendments in nematode control: An examination of the literature. Nematropica 12:319-326.

Munif, A., J. Hallmann, and R. A. Sikora. 2000. Evaluation of the biocontrol activity of endophytic bacteria from tomato against *Meloidogyne incognita*. Mededelingen Faculteit Landbouwkundige, Universiteit Gent 65: 471-480.

Neal, J. C. 1889. The root-knot disease of the peach, orange and other plants in Florida, due to the work of the *Anguillula*. Bulletin of U. S Bur. Ent. 20:1-31.

Netscher**,** C. 1977. Observations and preliminary studies on the occurrence of resistance breaking biotypes of *Meloidogyne* spp. on tomato. Cahiers O. R. S. T. O. M. Serie Biologie 11:173-178.

Netscher, C., and J. C. Mauboussin. 1973. Resultats d'un essai concernant l'efficacite comparee d'une variete resistance et de certains nematicides contre *Meloidogyne javanica*. Cahiers O. R. S. T. O. M. Serie Biologie 21:97-102.

Netscher, C., and R. A. Sikora. 1990. Nematode parasites of vegetables. Pp. 237-283 *in* M. Luc, R. A. Sikora, and J. Bridge, eds. Plant parasitic nematodes in subtropical and tropical agriculture, CAB International, Wallingford, Oxon, UK.

Noling, J. 1989. Nematode management in vegetable crops. Florida Nematode Control Guide.

Oka, Y., S. Nacar, E. Putievsky, U. Ravid, Z. Yaniv, and Y. Spiegel. 2000. Nematicidal activity of essential oils and their components against the root-knot nematode. Phytopathology 90:710-715.

Oostendorp, M., and R. A. Sikora. 1986. Utilization of antagonistic rhizobacteria as a seed treatment for the biological control of *Heterodera schachtii* in sugar beet. Revue de Nematologie 9:304 (Abstr.).

Oostendorp, M., and R. A. Sikora. 1989. Seed treatment with antagonistic rhizobacteria for the suppression of *Heterodera schachtii* early root infection of sugar beet. Revue de Nematologie 12:77-83.

Oostendorp, M., and R. A. Sikora. 1990. In vitro interrelationships between rhizosphere bacteria and *Heterodera schachtii.* Revue de Nematologie 13:269-274.

Osman, H. A., D. W. Dickson, and G. C. Jr. Smart. 1985. Morphological comparisons of host races 1 and 2 of *Meloidogyne arenaria* from Florida. Journal of Nematology 17:279-285.

Overman, A. J. 1974. Nematicides in linear drip irrigation for full-bed mulch of tomato. Proceedings of the Soil and Crop Science Society of Florida 34:197-200.

Overman, A. J., and J. P. Jones. 1986. Soil solarization, reaction and fumigation effects on double-cropped tomato under full-bed mulch. Proceedings Florida State Horticultural Society 99:315-318.

Page, S. L. J. 1979. An assesment of the importance and control of plant parasitic nematodes of vegetable crops in Bangladesh. Pp. 36 *in* O. D. M. Report of visit to Bangladesh. Ascot, Berks., UK. Ministry of Overseas Development.

Pleban, S., F. Ingel, and I. Chet. 1995. Control of *Rhizoctonia solani* and *Sclerotium rolfsii* in the greenhouse using endophytic *Bacillus* spp. Europa Journal of Plant Pathology 101:665-672.

Porter, I. J., and P. R. Merriman. 1983. Effect of solarization of soil on nematode and fungal pathogens at two sites in Victoria. Soil Biology and Biochemistry 15:39.

Prot, J. C. 1977. Amplitude et cinetique des migrations de nematode *Meloidogyne javanica* sous l'influence d'un plant de tomate. Cahiers O. R. S. T. O. M. Serie Biologie 11:157-166.

Prot, J. C. 1984. A naturally occurring resistance breaking biotype of *Meloidogyne incognita* on tomato : Reproduction and pathogenicity on tomato cultivars Roma and Rossol. Revue de Nematologie 7: 23-28.

Prot, J. C., and S. D. Van Gundy. 1981. Effect of soil texture and the clay component on migration of *Meloidogyne incognita* second stage juveniles. Journal of Nematology 13:213-219.

Racke, J. 1988. Untersuchungen zur biologischen Bekämpfung von *Globodera pallida* (Stone) an Kartoffeln durch Pflanzgutbehandlung mit antagonistisch wirkenden Rhizobakterien. Ph.D. dissertation, Universität Bonn, Bonn, Germany.

Racke, J., and R. A. Sikora. 1985. Einfluss von Rhizosphärenbakterin auf *Rhizoctonia solani* und den Befall der kartoffelsorte Hansa mit *Globodera pallida*. Biotechnologische Vorträge zur Resistenzselektion, Vorträge für Pflanzenzüchtung Statusseminar Grünbach, 9: 21-28.

Raski, D. J., and R. V. Schmitt. 1964. Grapevine responses to chemical control of nematodes. Am. J. En. Vitic. 15:199-203.

Rich, J. R., G. S. Rahi, C. H. Opperman, and E. L. Davis. 1989. Influence of the castor bean (*Ricinus communis*) lectin (ricin) on motility of *Meloidogyne incognita*. Nematropica 19:90-103.

Roberts, P. A. 1988. Effects of metam-sodium applied by drip irrigation on root-knot nematodes, *Pythium ultimum* and *Fusarium* sp. in soil and on carrot and tomato roots. Plant Disease 72:213-217.

Roberts, P. A. 1992. Current status of the availability, development and use of host plant resistance to nematodes. Journal of Nematology 24:213-227.

Roberts, P. A. 1995. Conceptual and practical aspects of variability in root-knot nematodes related to host plantresistance. Annual Review of Phytopathology 33:199-221.

Roca, F., F. Lamberti, and A. Siniscalco. 1975. Studi sulla persistenza di alcuni nematocidi granulari nella lotta contro i nematodi galligeni, *Meloidogyne* spp. Proceedings Giornate Fitopatologiche:265-269.

Rodriguez-Kabana, R., D. Boube, and R. W. Young. 1990. Chitinous materials from blue crab for control of root-knot nematode. II. Effect of soybean meal. Nematropica 20:153-168.

Rodriguez-Kabana, R., and G. Morgan-Jones. 1987. Biological control of nematodes: Soil amendments and microbial antagonists. Plant and Soil 100:237-247.

Rodriguez-Kabana, R., P. S. King, D. G. Robertson, and C. F. Weaver. 1988. Potential of crops uncommon to Alabama for management of root-knot and soybean cyst nematodes. Annals of Applied Nematology 2:116-120.

Santhi, A., and V. Sivakumar. 1995. Biocontrol potential of *Pseudomonas fluorescens* (Migula) against root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, 1949 on tomato. Journal of Biological Control 9:113-115.

Sasser, J. N. 1954. Identification and host-parasite relationships of certain root-knot nematodes, *Meloidogyne* spp. Pp. 31 *in* Bulletin of the Maryland Agricultural Experiment Station A-77.

Sasser, J. N. 1972. Physiological variations in the genus *Meloidogyne* as determined by differential hosts. OEPP/EPPO Bulletin 6:41-48.

Sasser, J. N. 1979a. Pathogenicity and host ranges in *Meloidogyne* species. Pp. 256-268 *in* Lamberti, F., and Taylor, C. E., eds. Root-knot nematodes, *Meloidogyne* species: systematics, biology and control. Academic Press, London.

Sasser, J. N. 1979b. Economic importance of *Meloidogyne* in tropical countries. Pp. 359-374 *in* Lamberti, F., and Taylor, C. E., eds. Root-knot nematodes, *Meloidogyne* species: systematics, biology and control. Academic Press, London.

Sasser, J. N., and C. C. Carter. 1985. Overview of the international *Meloidogyne* project 1975-1984. Pp. 19-24 *in* K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise in *Meloidogyne*, Vol. II Methodology, Raleigh: North Carolina State University Graphics.

Sasser, J. N., and D. W. Freckmann. 1987. A world perspective on nematology: The role of the society. Pp. 7-14 *in* J. A. Veech, and D. W. Dickson, eds. Vistas on nematology. Hyatlsville, Maryland, USA.

Sayre, R. M., and M. P. Starr. 1985. *Pasteuria penetrans* (ex Thorne, 1940) nom. Rev., comb. n., sp. n., a mycelial and endospore-forming bacterium parasitic in plant-parasitic nematodes. Proceedings of the Helminthological Society of Washington 52:149-165.

Scotto la Massese, C. 1961. In: Les Nematodes. C. N. R. A. Versailles, 16-17 November.

Schroth, M. N., and J. G. Hancock. 1982. disease-suppressive soil and root colonizing bacteria. Science 216:1376-1381.

Sikora, R. A. 1978. Einfluß der endotrophen Mykorrhiza *Glomus mosseae* auf das Wirt-Parasit-Verhältnis von *Meloidogyne incognita* an Tomaten. Journal of Plant Disease and Protection 85:197-202.

Sikora, R. A. 1988. Interrelationship between plant health-promoting rhizobacteria, plant parasitic nematodes and soil microorganisms. Mededelingen Faculteit Landbouwkundige Rijksuniversiteit Gent 53: 867-878.

Sikora, R. A. 1989. Importance of plant parasitic and entomopathogenic nematodes to vegetable production in the Philippines. Pp. 37 *in* GTZ Report, Eschborn.

Sikora, R. A. 1992. Management of the antagonistic potential in agricultural ecosystems for the biological of plant parasitic nematodes. Annual Review of Phytopathology 30:245-270.

Sikora, R. A., K. Sitaramaiah, and R. S. Singh.1973. Reaction of root-knot nematode resistant tomato cultivars to *Meloidogyne javanica* in India. Plant Disease Reporter 57:141-143.

Sikora, R. A., P. Reckhaus, and I. adamou. 1988. Presence, distribution and importance of plant parasitic nematodes in irrigated agricultural crops in Niger. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent, Belgium 53:821-834.

Sikora, R. A., R. S. Singh, and K. Sitaramaiah. 1973. Control of root-knot through organic and inorganic soil amendments.3. Effect of rice husk and sugarcane bagasse. Haryana Journal of Horticultural Science 2:123-127.

Sikora, R. A., and S. Hoffmann-Hergarten. 1993. Biological control of plant parasitic nematodes with plant-health promoting rhizobacteria. Pp. 166-172 *in* Lumsden, R. D., and J. L. Vaughn, eds. Pest Management: Biologically Based Technologies. American Chemical Society, Washington, DC.

Singh, R. S., and K. Sitaramaiah. 1966. Incidence of root-knot of okra and tomatoes in oil-cake amended soil. Plant Disease Reporter 50:668-672.

Singh, R. S., and K. Sitaramaiah. 1967. Effect of decomposing green leaves, sawdust and urea on the incidence of root-knot in okra and tomato. Indian Phytopathology 20:349-355.

Sitaramaiah, K., and R. S. Singh. 1978. Effect of organic amendments on phenolic content of soil and plant and response of *Meloidogyne javanica* and its host to release compounds. Plant and Soil 50:671-679.

Siti, E., E. Cohn, J. Katan, and M. Mordechai. 1982. Control of *Ditylenchus dipsaci* in garlic by bulb and soil treatments. Phytoparasitica 10:93.

Smith, G. S. 1987. Interactions of nematodes with mycorrhizal fungi. Pp. 292-300 *in* J. A. Veech, and D. W. Dickson, eds. Vistas on nematology. Hyatlsville, Maryland.

Smith, L. J. 1994. The potential of rhizobacteria as biological control agents of root-knot nematode. Thesis, University of Queenland, Australia.

Southards, C. J., and M. F. Priest. 1973. Variation in pathogenicity of seventeen isolates of *Meloidogyne incognita*. Journal of Nematology 5:63.67.

Spiegel, Y., E. Cohn, S. Galper, E. Sharon, and I. Chet. 1991. Evaluation of a newly isolated bacterium, *Pseudomonas chitinolytica* sp. nov., for controlling the root-knot nematode *Meloidogyne javanica*. Biocontrol Science and Technology 1: 115-125.

Spull, V. W. 1981. *Bacillus penetrans* in South African plant-parasitic nematodes. Nematologica 27:244-245.

Stapleton, J. J. 1990. Feasiability of sprayable polymer mulches for soil solarization and soil sealing applications. Phytopathology 80:892.

Stapleton, J. J., and J. E. DeVay. 1983. Response of phytoparasitic and free-living nematodes to soil solarization and 1,3-dichloropropene in California. Phytopathology 73:1429-1983.

Stapleton, J. J., and J. E. DeVay. 1986. Soil solarization: a non-chemical approach for management of plant pathogens and pests. Crop Protection 5:190.

Stapleton, J. J., B. Lear, and J. E. De Vay. 1987. Effect of combining soil solarization with certain nematicides on target and nontarget organisms and plant growth. Annals and Applied of Nematology 1:107.

Starr, M. P., and R. M. Sayre. 1988. *Pasteuria thornei* sp. nov. and *Pasteuria penetrans* sensu stricto emend., mycelial and endospore-forming bacterium parasitic, respectively, on plant-parasitic nematodes of the genera *Pratylenchus* and *Meloidogyne*. Annals de l'Institut Pasteur, Microbiologie 139:11-31.

Stirling, G. R. 1984. Biological control of *Meloidogyne javanica* with *Bacillus penetrans*. Phytopathology 74:55-60.

Stirling, G. R. 1985. Host specificity of *Pasteuria penetrans* within the genus *Meloidogyne*. Nematologica 31:203-209.

Stirling, G. R. 1991. Antagonists of nematodes. Pp. 50-98 *in* G. R. Stirling, eds. Biological control of plant parasitic nematodes. CAB International, Wallingford, Oxoid, UK.

Stirling, G. R., and B. R. Kerry. 1983. Antagonists of the cereal cyst nematode *Heterodera avenae* Woll. In Australian soils. Australian Journal of Experimental Agriculture and Animal Husbandry 23:318-324.

Stirling, G. R., and M. F. Wachtel. 1980. Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. Nematologica 26:308-312.

Stirling, G. R., and R. Mankau. 1978a. *Dactylella oviparasitica*, a new fungal parasite of *Meloidogyne* eggs. Mycologia 70:774-783.

Stirling, G. R., and R. Mankau. 1978b. Parasitism of *Meloidogyne* eggs by a new fungal parasite. Journal of Nematology 10:236-240.

Stirling, G. R., and R. Mankau. 1979. Mode of parasitism of *Meloidogyne* and other nematode eggs by *Dactylella oviparasitica.* Journal of Nematology 11:282-288.

Stirling, G. R., R. D. Sharma, and J. Perry. 1990. Attachment of *Pasteuria penetrans* spores to *Meloidogyne javanica* and its effects on infectivity of the nematode. Nematologica 36:246-252.

Sturhan, D. 1985. Untersuchungen über Verbreitung und wirte des Nematodenparasiten *Bacillus penetrans*. Mitteleilungen aus der Biologischen Bundesanstalt für Land und Forstwirtschaft, Berlin 226.

Sumner, D. R., and A. W. Johnson. 1972. The effect of nematodes and crop sequence on *Fusarium* wilt of watermelon. Phytopathology 62:791 (Abstr.).

Taylor, A. L., and J. N. Sasser. 1978. Biology, identification and control of root-knot nematodes, *Meloidogyne* spp. Pp. 111 *in* Cooperative Publication Department of Plant Pathology, North Carolina State University and U. S. Agency International Development, Washington D. C., North Carolina State University Graphics, USA.

Taylor, A. L., J. N. Sasser, and L. A. Nelson. 1982. Relationship of climate and soil characteristics to geographical distribution of *Meloidogyne* species in agricultural soils. Pp. 65 *in* Cooperative Publication Department of Plant Pathology, North Carolina State University and U. S. Agency International Development, Washington D. C., North Carolina State University Graphics, USA.

Thames, W. H., and W. N. Stoner. 1953. A preliminary trial of lowland culture rice in rotation with vegetable crops as a means of reducing root-knot nematode infestations in the Everglades. Plant Disease Reporter 37:187-192.

Thomason, I. J., and B. Lear. 1961. Rate of production of *Meloidogyne* spp. as influenced by soil temperature. Phytopathology 51:520-524.

Triantaphyllou, A. C., and J. N. Sasser. 1960. Variation in perineal patterns and host specifity of *Meloidogyne incognita*. Phytopathology 50:724-735.

Tribe, H. T. 1977a. Pathology of cyst nematodes. Biological Reviews 52:477-507.

Tribe, H. T. 1977b. A parasite of white cysts of *Heterodera*; *Catenaria auxiliaris*. Transactions of the British Mycological Society 69:367-376.

Treub, M. 1885. Onderzoekingen over Sereh-Ziek Suikkeriet gedaan in s'Lands Plantentuin te Buitenzorg. Medad. PlTuin., Batavia:1-39.

Trudigill, D. L. 1995. Seminar: host and plant temperature effects on nematode development rates and nematode ecology. Nematologica 41:398-404.

Tyler, J. 1933. Reproduction without males in aseptic root cultures of the root-knot nematode. Hilgardia 7: 373-388.

Valdez, R. B. 1978. Nematodes attacking tomato and their control. Pp. 136-152 *in* First International Symposium on Tropical Tomato, AVRDC Publications 78-59.

Van Gundy, S. D. 1985. Biological control of nematodes: status and prospect in agricultural IPM systems. Pp. 467-477 *in* Biological Control in Agricultural IPM Systems. Academic Press, London.

Vovlas, N., and F. Lamberti. 1976. Studies on the systemic action of some chemicals in the control of root-knot nematodes. Nematologie Mediterranea 4:111-113.

Walker, J. T. 1960. The effect of hot water at different temperatures on larvae of various species of *Meloidogyne*. Phytopathology 50:658(Abstr.).

Wallace, H. R. 1966. Factors influencing the infectivity of plant parasitic nematodes. Proceedings of the Royal Society, B, 164:592-614.

Watson, J. R. 1922. Bunch velvet bean to control root-knot. Bulletin 163. University of Florida Agricultural Experiment Station, Gainesville, FL, USA.

Watson, J. R. 1936. Plants susceptible to root-knot field and truck crop. Bulletin 483. University of Florida Agricultural Experiment Station, Gainesville, FL, USA.

Watson, J. R. 1945. Mulches to control root-knot. Proceedings of the Florida Academy of Science 7:151-153.

Watson, J. R., and C. C. Goff. 1937. Control of root-knot in Florida. Bulletin 311. University of Florida Agricultural Experiment Station, Gainesville, FL, USA.

Weller, D. M. 1988. Biological control of soil-borne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathology 26: 379-407.

Zavaleta-Meija, E., and S. D. Van Gundy. 1982. Effects of rhizobacteria on *Meloidogyne* infection. Journal of Nematology 14: 475A-475B.

1. General Introduction

Plant parasitic nematodes are important factors affecting crop growth and yield in all agricultural production zones (Webster, 1985). Root-knot nematodes, *Meloidogyne* spp. are the most important throughout the world, especially in the tropics and subtropics where they cause a high reduction in the yield of economic crops especially vegetables (Luc *et al.*, 1990). Severe attack by root-knot nematodes can prevent successful cultivation of many species of vegetables (Mai, 1985; Netscher and Sikora, 1990). Vegetable crops are important for balanced nutrition and also are very susceptible to soil-borne pathogens that leads to interactions with the root-knot nematodes and causes disease syndromes (Sikora and Carter, 1987).

Biological control activity of plant health-promoting rhizobacteria (PHPR) against plant parasitic nematodes has been demonstrated for the sedentary endoparasitic species of the root-knot nematodes, *Meloidogyne* spp. (Becker *et al*., 1988; Sikora, 1988; Spiegel *et al*., 1991; Hallmann *et al*., 1997).

PHPR are at present a promising non-chemical alternative for controlling a number of plant parasitic nematodes and diseases. PHPR also are important to crop management because in addition to the biological control of nematodes and diseases they often have the ability to improve plant growth and are not phytotoxic (Suslow and Schroth, 1982, Sikora, 1988, Hallmann *et al*., 1994).

The PHPR *Bacillus cereus* strain S18 is a well studied biocontrol agent against plant parasitic nematodes in particular cyst and root-knot species (Oostendorp and Sikora, 1989; Racke and Sikora, 1992; Sikora and Hoffmann-Hergarten, 1993). Insunza *et al*., (2000) also reported that *B. cereus* was associated with nematode biocontrol and plant growth promotion under greenhouse conditions. They found that nematicidal activity towards nematodes in naturally infested field soil reduced nematode densities by 50-100%.

2. General Materials and Methods

2.1 Host Plants

Three different host plants susceptible to root-knot nematodes, *Meloidogyne* spp. were tested:
A) Tomato (*Lycopersicon esculentum* cv. Hellfrucht Frühstamm)
B) Cucumber (*Cucumis sativus* cv. Vorgebirgs Trauben)
C) Pepper (*Capsicum annuum* cv. Yolo Wonder B)
Seedlings of each host plant were prepared as follows:
Tomato seedlings were first raised in seed beds in sand under greenhouse conditions. Two weeks old tomato seedlings were then transplanted (one plant/pot) into experimental plastic pots filled with 500 cm^3 of a soil/sand (1:2, v/v) mixture. The soil substrate was not sterilized. Cucumber and pepper seedlings were sown into the plastic pots (3 seeds/pot) filled with the same soil. After two weeks plants were thinned to one plant per pot.

2.2 *Bacillus cereus* strain S18

The rhizobacterium, *B. cereus* strain S18 originated from the Soil-Ecosystem Phytopathology and Nematology Lab., Plant Pathology Institute, Bonn University and was first isolated from unsterile dry tomato seeds cv. Rheinlands Rhum. The bacterium was initially identified as *Bacillus subtilis* and in 1996 was reidentified as *Bacillus cereus*. *B. cereus* was grown on tryptic soy agar (TSA) (Oxoid). Long term storage was carried out in tryptic soy broth (TSB) amended with 20% glycerol at –80°C. Bacterial inoculum was produced by fermentation in 100 ml of TSB in 250 ml flasks on a rotary shaker at 100 rpm for 24 hours at 28°C after transfer of starter inoculum from the TSA. The bacterial suspension was centrifugated at 8000 rpm for 10 minutes, the supernatant was discarded and the pellet was resuspended in a sterile ¼ strength Ringer-solution (Merck). Bacterial density was adjusted with a spectral photometer to an optical density of OD_{560nm}= 2.0 representing approximately 10^9 colony-forming units (cfu)/ml.

A soil drench method was used in all the experiments. The bacteria in Ringer-solution was inoculate in a 5 ml bacterial suspension (10^9 cfu/ml) per plant in three or four holes made around the plants to a depth of 4 cm. The controls were treated with 5 ml of Ringer-solution.

2.3 Root-knot nematodes, *Meloidogyne* spp.

All three root-knot species *M. incognita* race 3 (Kofoid and White 1919) Chitwood 1949, *M. javanica* (Treub, 1885) Chitwood 1949 and *M. arenaria* (Neal, 1889) Chitwood 1949 race 1 used in the tests were supplied by Dr. D. W. Dickson from the University of Florida, Gainesville, USA. The nematodes were multiplied on tomato plants grown in plastic pots filled with sterilized soil-sand mix (1:2, v/v) at 25°C under greenhouse conditions.

To obtain inoculum tomato roots infested with *M. incognita, M. javanica* or *M. arenaria* were removed from the pots and gently washed with tap water to remove the soil particles from the roots. Roots were cut in small pieces and then were macerated for two periods of 10 seconds each at high speed by using a Waring blender. This method released the highest number of nematodes from roots. The macerated root solution was then placed in a Duran bottle containing sodium hypochlorite (NaOCl). Water was added to adjust the final concentration of NaOCl to 1.05% as described by (Hussey and Barker, 1973). The solution in the Duran bottle was vigioursly shaken for 3 minutes to release the eggs from the egg matrix as NaOCl removes the gelatin matrix of egg masses. The solution was then poured through different size sieves to remove the root tissue. Eggs were collected on the 20 micrometer (µm) sieve and washed several times with tap water to remove residual NaOCl. Eggs were then transferred to a counting slide and counted.

Egg Masses Staining: Egg masses of *Meloidogyne* were stained by dipping the roots in 0.015% Phloxine B solution for 20 minutes as described by (Daykin and Hussey, 1985) and then washing the stained roots with tap water to remove the residual Phloxine B.

Gall Index Measuring: The gall index of *Meloidogyne* infested roots was measured as described by (Zeck, 1971) on a scale from 0-10.

2.4 Statistical Analysis

Data were analysed according to standard analysis of variance by a one way ANOVA with the software statgraphics (Statistical Graphics Crop., Rockville, MD). Variance homogeneity for all treatments was confirmed by the Bartlett test. The comparison between means was carried out either with Duncan's Multiple Range Test or by using T-Test at $P<0.05$ as given in the tables and/or figures.

3. Experimental Program

3.1 Effect of application method on biocontrol efficacy

3.1.1 Introduction

The study of methods of application of PHPR is important, because it can play a major role in the level of antagonistic activity of *B. cereus* S18 against root-knot nematodes. Several studies dealt with the subject of rhizobacteria against several plant parasitic nematodes. For example, when tomato, cucumber and clover were treated with rhizobacteria as soil drenches or root dip treatments they suppressed significantly the penetration of nematodes in the roots and reduced the root galling of the root-knot nematode, *M. incognita* under greenhouse conditions as reported by Zavaleta-Meija and Van Gundy (1982) and Becker *et al*., (1988). The same results were obtained by Sikora (1988) on cotton, tomato, peanut and sugar beet when treated with *Bacillus subtilis* to control *M. incognita*, *M. arenaria* and *Rotylenchulus reniformis* under greenhouse conditions. He revealed that *B. subtilis* reduced *M. incognita* 43% to 66% on cotton when applied as a powder and 38% to 62% when applied as a liquid seed dressing. Oka *et al*., (1993) found that when the tomato roots were dipped in the bacterial suspension of *B. cereus* it did not reduce the galling index or the number of juveniles (J_2) invading the roots. However, they also found that fewer juveniles invaded tomato roots when *B. cereus* was mixed with soil or when juveniles were pre-exposed to the bacteria suspended in the soil. Keuken (1996) reported that a soil drench of tomato plants with *B. cereus* S18

under greenhouse conditions led to significant increases in root length compared with the control. He also reported that a soil drench application of *B. cereus* S18 led to significant increases in plant growth e.g fresh shoot and root weight as well as root length in the absence of the nematode**.** Similar results were noted by Sadlers (1996) who confirmed that application of *B. cereus* led to high increases in tomato plant growth. He also noted that the increase in plant growth was not affect by *B. cereus* application method. He demonstrated that treatment by root dipping had no effect on plant growth.

Treatment of cucumber seedlings with the fluorescent *Pseudomonas* stains BS_{8651} and BS_{8661} as a soil drench or as a seed treatment reduced damping-off caused by *Pythium ultimum* under greenhouse conditions as reported by Vogt and Buchenauer (1997). They also found that when the fluorescent *Pseudomonas* strain BS_{8651} was applied as a soil drench or as a seed treatment it resulted in reductions of the foliar disease of cucumber seedlings caused by the powdery mildew fungus *Sphaerotheca fuliginea.* Similar results were obtained by Sikora *et al*., (1990a) who found that treatment of sugar beet seeds with fluorescent pseudomonad rhizosphere bacteria can protect sugar beet seedlings from damping-off caused by species of *Pythium*. Hoffmann-Hergarten *et al*., (1998) revealed that root penetration as well as gall formation of root-knot nematode, *M. incognita* was significantly reduced after bacterial application to the seed or as a soil drench.

This experiment was carried out to evaluate the best application method of the PHPR *B. cereus* S18 towards *M. incognita* on tomato plants.

3.1.2 Materials and Methods

Sixteen 3 week old tomato plants “Hellfrucht Frühstamm“ were treated by dipping their roots in bacterial suspensions of *B. cereus* S18 (10^9 cfu/ml) prepared as described before (see 2.2) for 30 minutes. Another sixteen plants were soil drenched and received 5 ml of the same bacterial suspension per plant by pipetting the solution into three or four holes in the soil around the shoot. Eight plants from each treatment were then inoculated with 2000 eggs of *M.*

incognita /plant by pipetting in three holes in the root zone three days after treating the plants with the bacterial suspensions. Each treatment was replicated 8 times and plants were arranged in a completely randomised design in the greenhouse at approximately 25°C with artificial light 12 hours/day. Plants were watered every day and fertilized weekly with 10 ml per plant of Poly Crescol (14+10+14, 2g/liter water).

Plants were harvested for evaluation of fresh shoot and root weight, root length, gall index, number of galls and egg masses, 8 weeks after nematode inoculation. Root length was measured by using the Comiar Root Length Scanner (Hawker De Havilland, AUS). Gall index was measured as described by Zeck (1971) on a scale from 0-10. Egg masses of *M. incognita* were stained and recorded as described before (see 2.3).

3.1.3 Results and Discussion

Using *B. cereus* S18 either as a soil drench or as a root dip reduced the gall index by 59% and 54% respectively compared to the bacteria untreated plants (Fig. 1). No significant differences in the level of *M. incognita* control between the two *B. cereus* S18 application methods was detected.

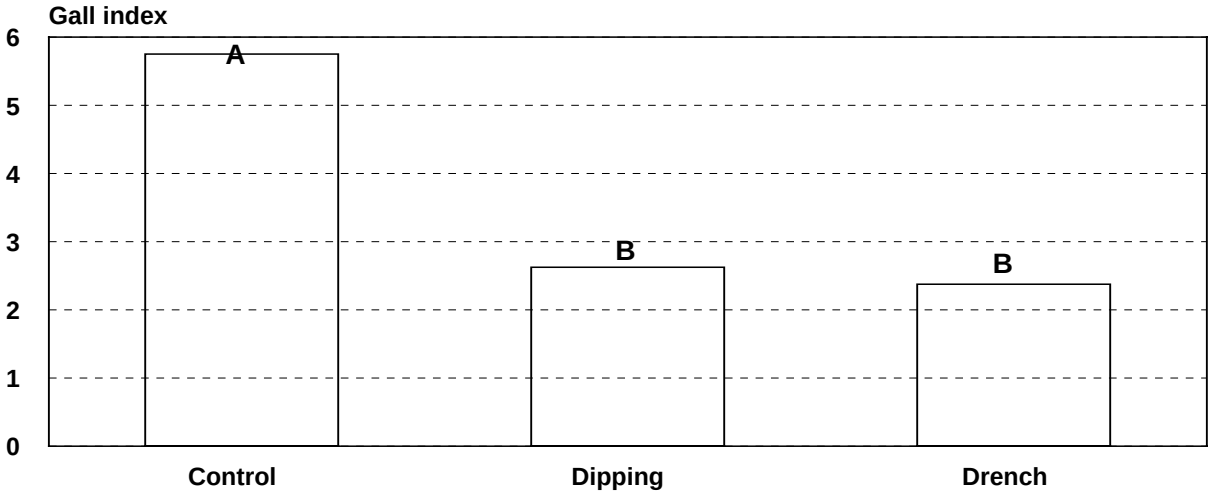


Figure 1: Effect of application method of *Bacillus cereus* strain S18 on the gall index of *Meloidogyne incognita* on tomato eight weeks after nematode inoculation. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P < 0.05$) n=8.

The number of galls (Fig. 2a) and egg masses (Fig. 2b) were reduced by 60% and 72% in the plants treated with *B. cereus* S18 as a soil drench, while the reduction was 60% and 75% in the bacteria plants treated by root dipping. No significant differences were found between the two application methods in the number of galls or egg masses of *M. incognita* compared with the plants treated with *M. incognita* alone.

These results agree with those of Oka *et al*., (1993) who found that fewer second stage juveniles invaded tomato roots when *B. cereus* was mixed with soil. The results differ, however, in that they found that dipping the roots in a suspension of the bacterium did not change juvenile infectivity. This is the opposite of the present findings.

The *B. cereus* S18 plants treated as a soil drench in the present study showed no significant differences in fresh shoot (Fig. 3a), root weight (Fig. 3b) or root length (Fig. 4).

However, root weight and length (Fig. 3b, 4) increased in the root dipping treatment when compared to the bacteria untreated plants.

Results also showed that fresh shoot and root weight as well as root length were significantly enhanced when the plants were treated with *B. cereus* S18 combined with *M. incognita* when compared with the treated plants with *M. incognita* alone. Fresh shoot and root weight increased 28 % and 69 % in the bacteria plants treated as a soil drench, while the rate of increase was 20 % and 59% when the plants were treated by root dipping.

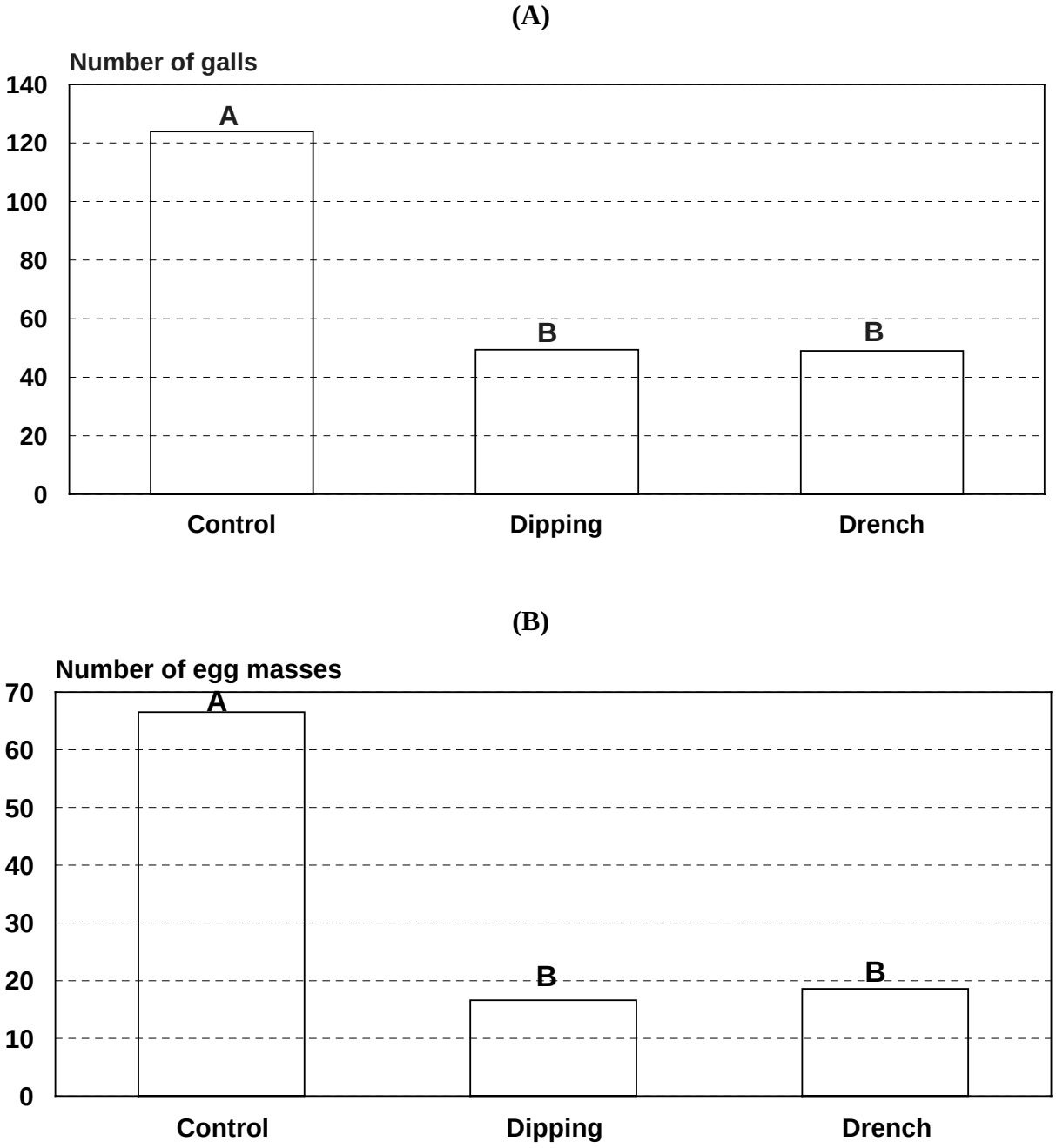
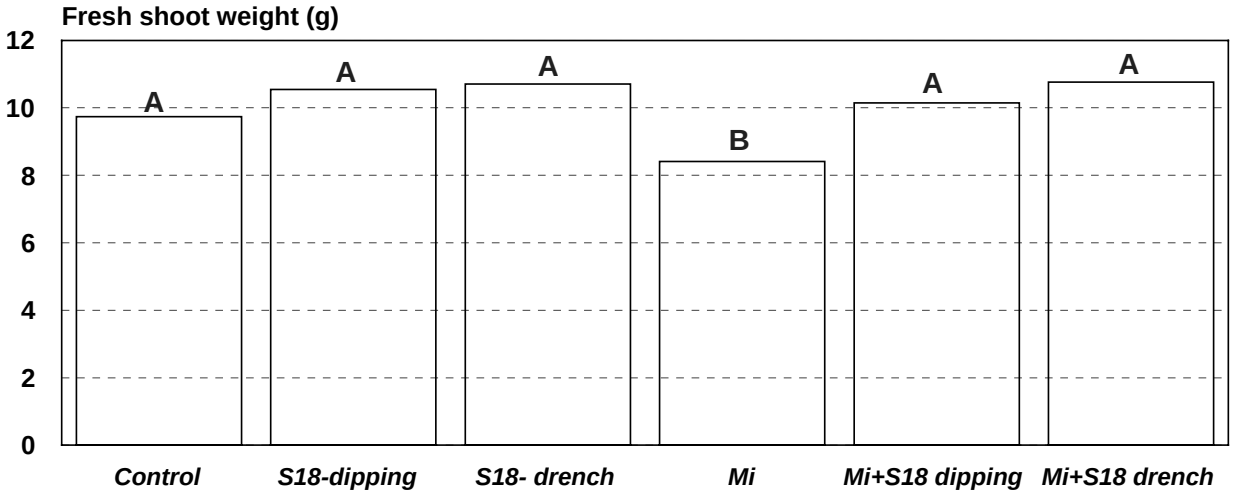


Figure 2: Effect of *Bacillus cereus* S18 application method on the number of galls (A) and egg masses (B) of *Meloidogyne incognita* on tomato eight weeks after nematode inoculation. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P< 0.05$) n=8.

(A)



(B)

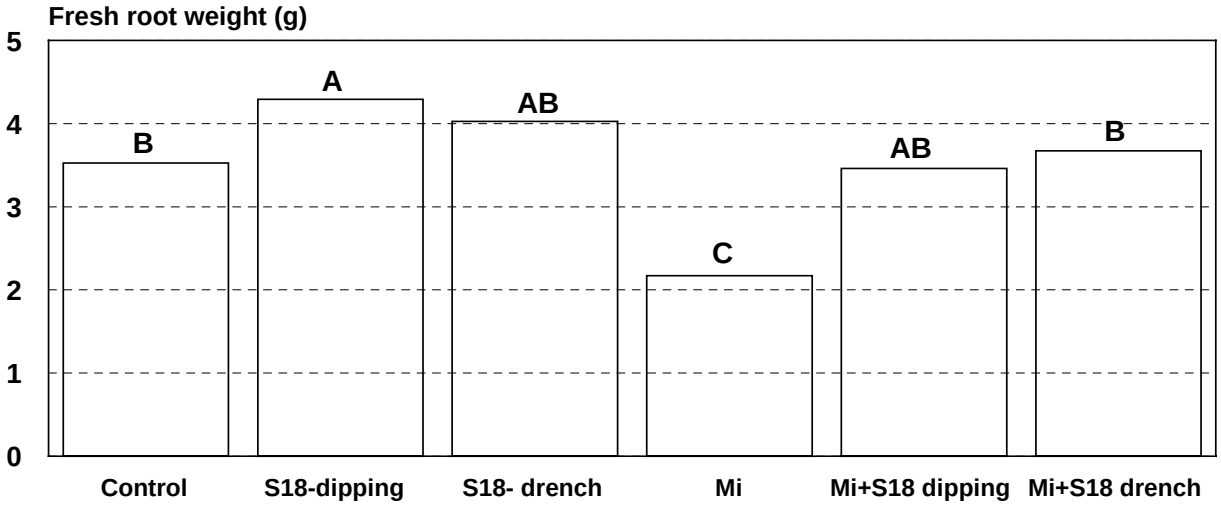


Figure 3: Effect of *Bacillus cereus* S18 application method on fresh shoot (A) and root weight (B) of tomato plants infected with *Meloidogyne incognita* (Mi) eight weeks after nematode inoculation. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P < 0.05$) n=8.

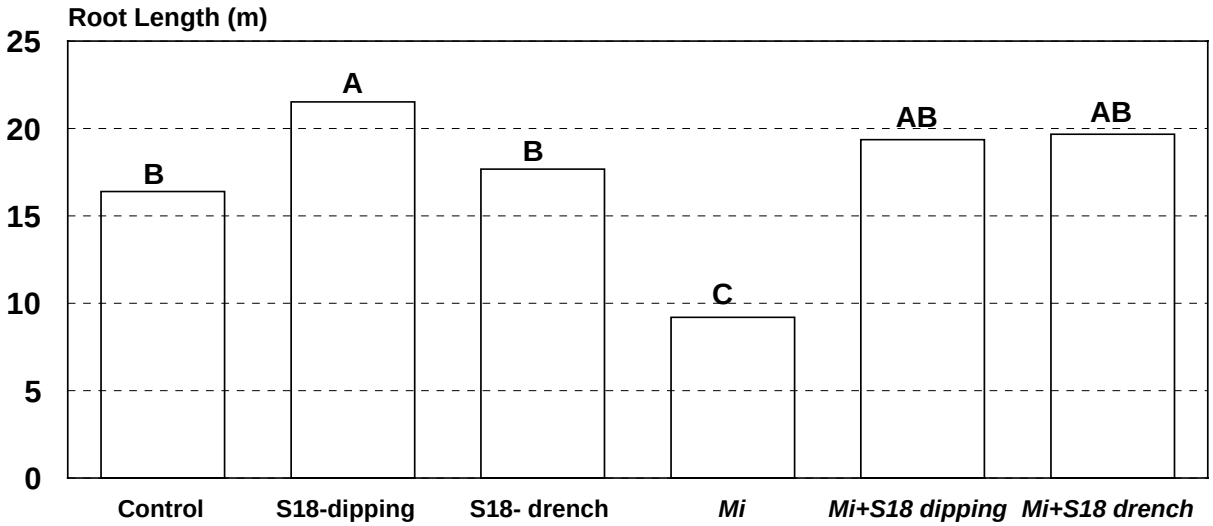


Figure 4: Effect of *Bacillus cereus* S18 application method on root length of tomato plants infected with *Meloidogyne incognita* (Mi) eight weeks after nematode inoculation. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P< 0.05$) n=8.

The *B. cereus* S18 plants treated with both application methods with *M. incognita* showed a twofold increase in root length when compared to the bacteria untreated plants with the nematode. Keuken (1996) reported that the application form of *B. cereus* S18 has a direct effect on the quality of rhizosphere or/and rhizoplane colonization and shoot and root growth. Treatment of potato tubers or the introduction of antagonistic fluorescent pseudomonads to soil has resulted in increases in crop yield as reported by Kloepper *et al*., (1980) and Geels and Schippers (1983). Similar results were obtained by Hoffmann-Hergarten *et al*., (1998) who found that treatment of tomato and lettuce with *B. cereus* S18 resulted in enhanced seedling biomass.

3.2 Effect of application time on biocontrol efficacy

3.2.1 Introduction

Time of application of the biocontrol agent is one of the most essential factors influencing the effectiveness of bacterial antagonists against many types of plant pathogens. This experiment was carried out to evaluate the best timing of PHPR *B. cereus* S18 application to control *M. incognita* on tomato.

3.2.2 Materials and Methods

In this experiment three application times were tested:

1- simultaneously with nematode inoculation

2- 10 days before nematode inoculation

3- 10 days after nematode inoculation

Three week old tomato plants cultivar "Hellfrucht Frühstamm" were inoculated with *B. cereus* S18 as a soil drench (see 2.2) by adding a 5 ml bacterial suspension (10^9 cfu/ml) per plant at the times mentioned above. Plants were inoculated with 2000 eggs of *M. incognita* per plant by pipetting in three holes around the roots. Ringer-solution treated plants as well as plants inoculated with *B. cereus* S18 alone served as controls. Each treatment was replicated 8 times in a completely randomised design under greenhouse conditions at 25°C with artificial light 12 hours/day.

Plants were watered daily and fertilized weekly with 10 ml per plant of Poly Crescol (14+10+14, 2g/liter water). Eight weeks after nematode inoculation the plants were removed and evaluated for fresh shoot and root weight, root length, gall index and total number of galls and egg masses.

3.2.3 Results and Discussion

Significant differences in gall index, total number of galls and egg masses between plants treated with *B. cereus* S18 at different times were observed. When *B. cereus* S18 was applied 10 days before *M. incognita* inoculation gall index decreased significantly when compared with the other application times (Fig. 1).

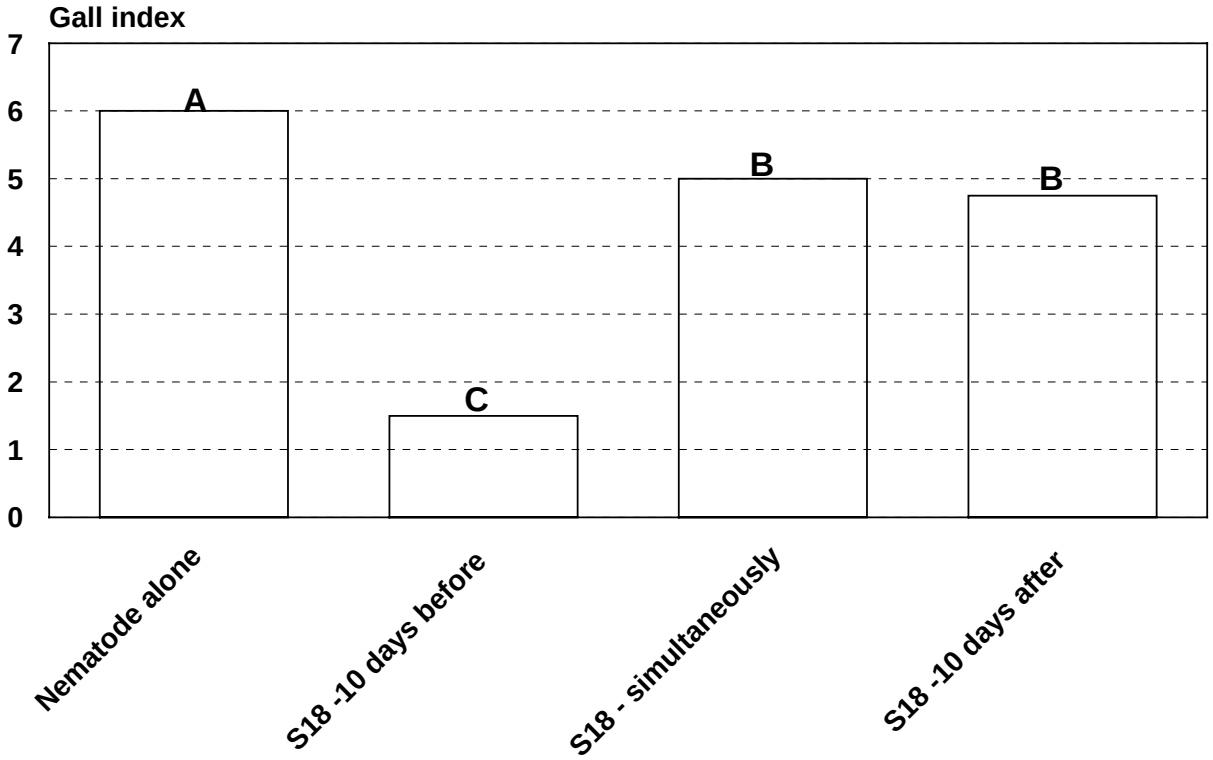


Figure 1: Effect of *Bacillus cereus* S18 application time on the galling of *Meloidogyne incognita* on tomato plants eight weeks after nematode inoculation. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P< 0.05$) n=8.

The highest reduction in the total number of galls also was obtained when *B. cereus* S18 was applied 10 days before nematode inoculation (Fig. 2a). Egg mass numbers were greatly reduced in all treatments over the control. However, there were no differences between the different times of application of *B. cereus* S18 against *M. incognita* when compared with the

plants treated with the nematode alone (Fig. 2b).

In the present study the introduction of *B. cereus* S18 ten days before nematode inoculation was the most effective application technique reducing significantly root galling, number of galls and number of egg masses of *M. incognita*. When *B. cereus* S18 was applied ten days before the nematode time was available for bacterial colonization of the rhizosphere or/and rhizoplane.

Bacteria colonization is important for nematode penetration reduction as reported by Sikora (1992). This 10 days period of time was sufficient for the rhizobacteria to establish and to possibly produce metabolites such as Zwittermicin A and Kansoamin that affect nematode juvenile infectivity (Silo-Suh *et al*., 1994, Milner *et al.*, 1996).

When *B. cereus* S18 was applied alone or combined with *M. incognita* it enhanced plant growth over the controls. The highest fresh shoot (Fig. 3a) and root weight (Fig. 3b) enhancement was obtained when *B. cereus* S18 was applied 10 days before or simultaneously with the nematode. The rate of increase was 26% and 17% over the nematode alone. *B. cereus* S18 alone also caused a slight increase in fresh shoot weight, but not root weight.

Results also showed no significant effect on fresh shoot or root weight when *B. cereus* S18 was applied 10 days after nematode inoculation when compared with the nematode alone.

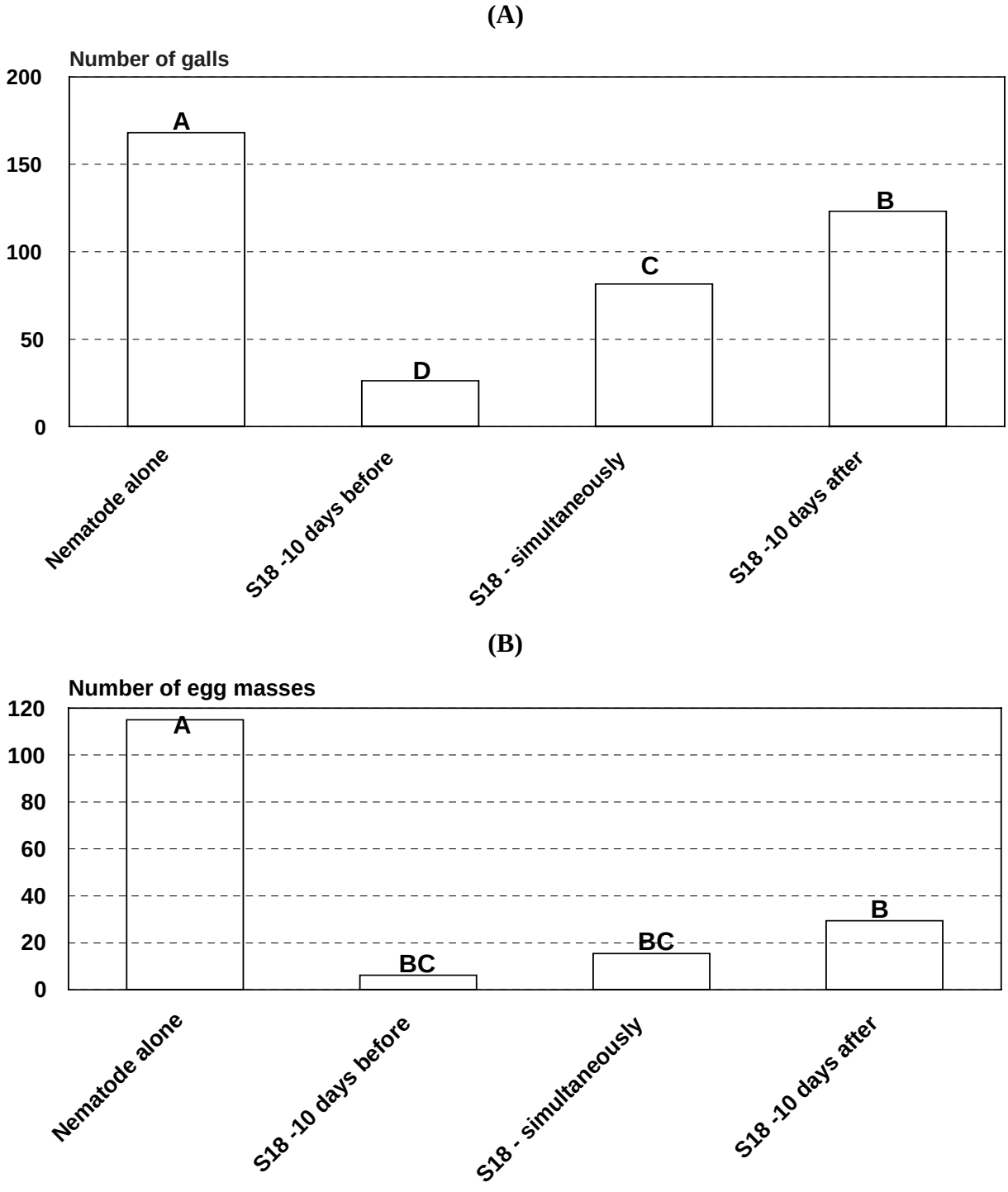


Figure 2: Effect of *Bacillus cereus* S18 application time on the number of galls (A) and egg masses (B) of *Meloidogyne incognita* on tomato eight weeks after nematode inoculation. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P < 0.05$) n=8.

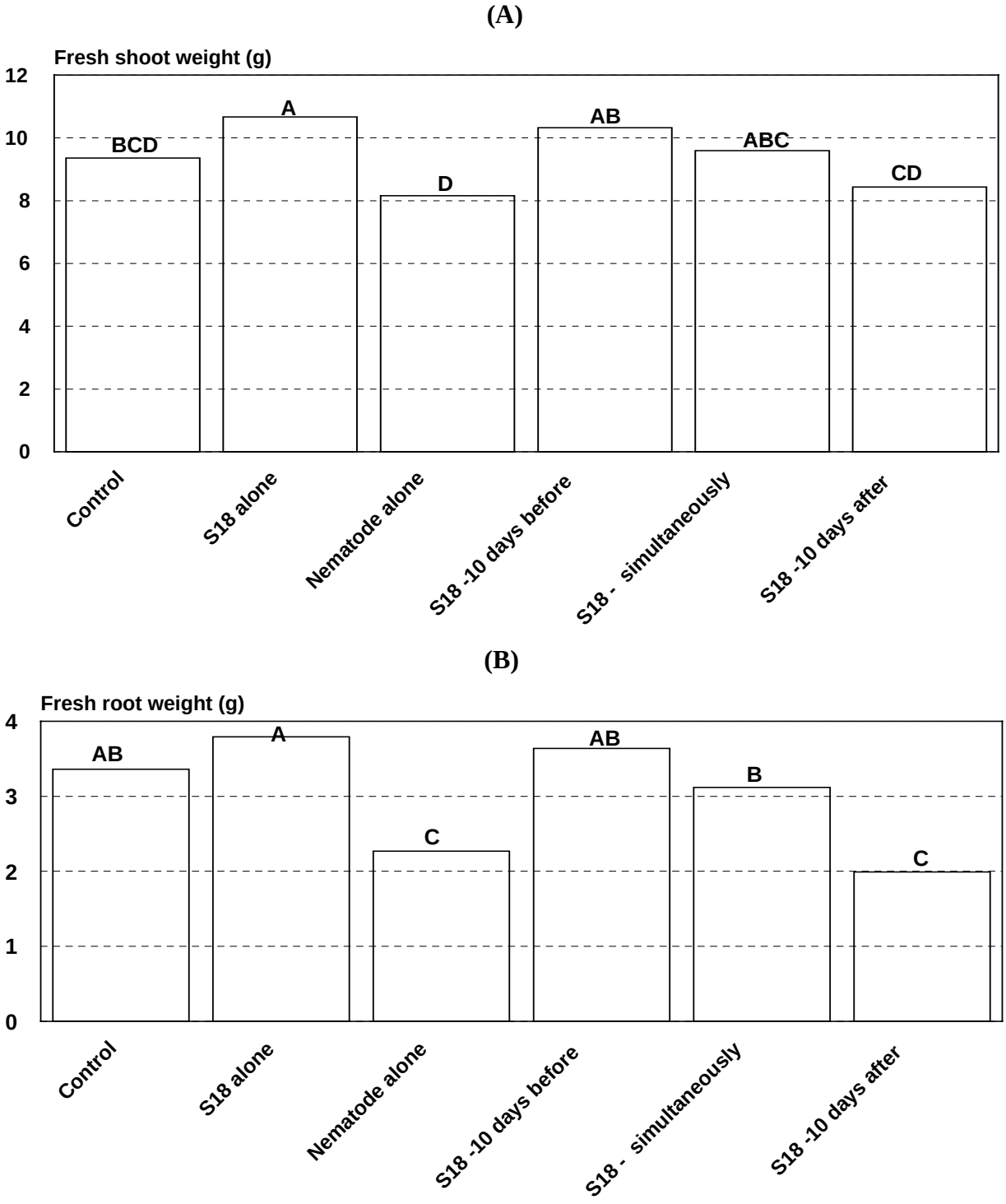


Figure 3: Effect of *Bacillus cereus* S18 application time on fresh shoot (A) and root weight (B) of tomato plants infected with *Meloidogyne incognita* eight weeks after nematode inoculation. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P < 0.05$) n=8.

The greatest increase in root length was obtained when *B. cereus* S18 was applied 10 days before nematode inoculation followed by simultaneous application (Fig. 4). A non-significant increase in root length was obtained when *B. cereus* S18 was applied 10 days after nematode inoculation when compared with the plants treated with the nematode alone. A slight root length enhancement was also obtained when the plants were treated with PHPR *B. cereus* S18 alone over the control.

Similar results were obtained by Keuken (1996) who found that the rhizobacterium *B. cereus* S18 enhanced the yield of tomato in the absence of the nematode under field conditions. Hoffmann-Hergarten *et al.,* (1998) also revealed that *B. cereus* S18 has positive effects on seedling development as it enhanced plant establishment at the seedling stage.

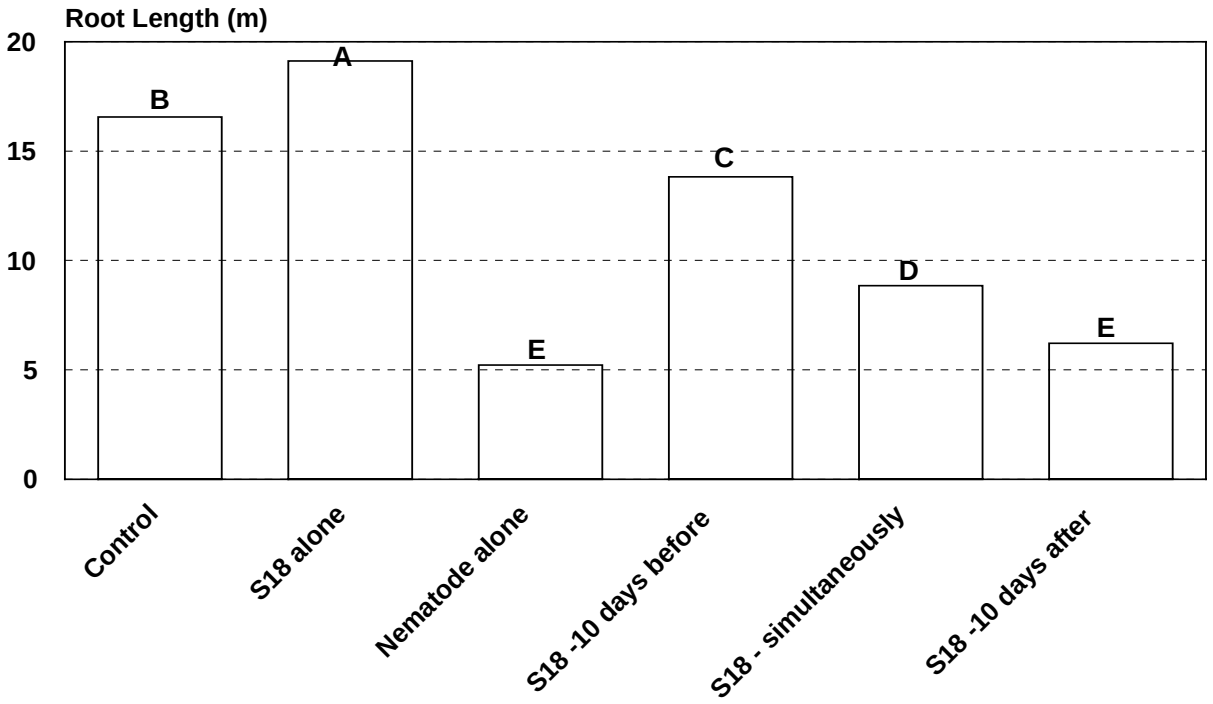


Figure 4: Effect of *Bacillus cereus* S18 application time on root length of tomato plants infected with *Meloidogyne incognita* eight weeks after nematode inoculation. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P< 0.05$) n=8.

3.3 Biological control potential towards different species of *Meloidogyne*

3.3.1 Introduction

In the past most studies on PHPR focused on one bacterium controlling one pest or pathogen. For example up to now *B. cereus* S18 was only used to control *M. incognita* on tomato (Keuken, 1996). Very little is known about the control potential of single PHPR strains against a broad spectrum of species within the same nematode genus. This experiment was performed to evaluate the biocontrol activity of *B. cereus* S18 towards different species of *Meloidogyne* on tomato.

3.3.2 Materials and Methods

Plants in this experiment were inoculated with three different species of *Meloidogyne* that were cultured and extracted as mentioned previously (see 2.3). Three week old tomato plants, cv. "Hellfrucht Frühstamm" were inoculated with 5 ml of a bacterial suspension (10^9 cfu/ml) per plant as a soil drench by pipetting the solution in three or four holes in the root zone. The controls received 5 ml Ringer-solution. Three days later the plants were inoculated with 2000 eggs/plant of *M. incognita, M. javanica* or *M. arenaria* by pipetting the juveniles in three holes made in the root zone. Non-bacterized plants as well as plants inoculated with *B. cereus* S18 alone served as controls. Each treatment was replicated 10 times in a completely randomised design in the greenhouse at 25°C with 12 hours/day of artificial light. Plants were watered daily and fertilized weekly with 10 ml per plant of Poly Crescol (14+10+14, 2g/liter water).

Eight weeks after nematode inoculation the experiment was terminated and shoot and root fresh weight, root length and total number of galls and egg masses were recorded.

3.3.3 Results and Discussion

The number of galls and egg masses of all 3 *Meloidogyne* species were reduced on plants treated with *B. cereus* S18. The rate of reduction however varied between the different species of *Meloidogyne*. A significant reduction of approximately 50% and 33% in the number of galls was observed for *M. incognita and M. javanica*, while it had no effect on the number of galls of *M. arenaria* when compared with the non-bacterized control (Fig. 1a). For *M. incognita*, *M. javanica* and *M. arenaria* the reduction in the number of egg masses was significant when compared with the non-bacterized control (Fig. 1b).

The results on the biological control of *B. cereus* S18 towards different species of *Meloidogyne* demonstrated that different levels of biological control activity are expressed according to the root-knot nematode species being tested.

The data demonstrated that some species of root-knot may not be controlled as effectively by this antagonistic bacterium as others. *B. cereus* S18 caused significant reductions in the number of galls and egg masses of *M. incognita* and *M. javanica*, but only reduced the number of egg masses of *M. arenaria*. A reduction in gall number usually indicates biocontrol activity during the infection process whereas a reduction in the number of egg masses indicates possible effects on nematode development after penetration or delayed penetration. Both mechanisms seem to apply for *B. cereus* S18, but to different degrees depending on species of *Meloidogyne*. More study of mechanisms of action are needed.

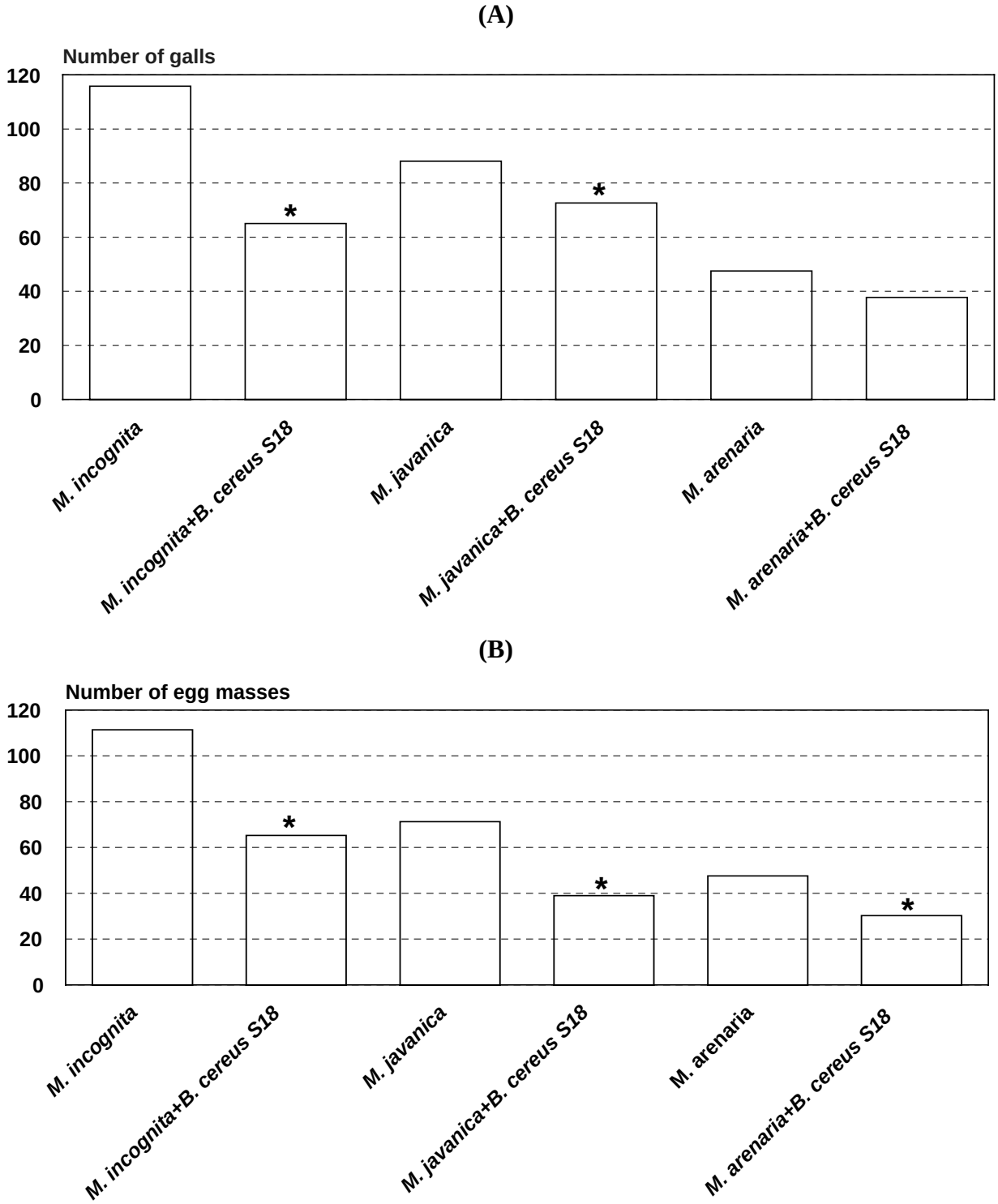
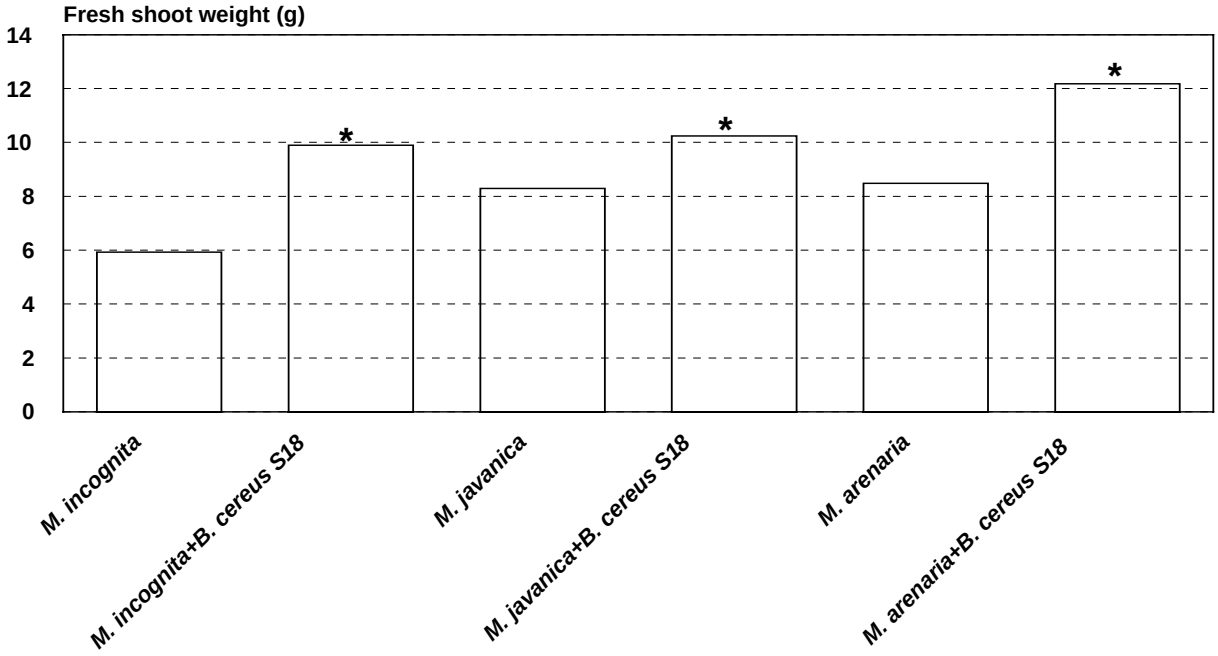


Figure 1: Effect of *Bacillus cereus* S18 on the number of galls (A) and egg masses (B) of *Meloidogyne incognita, M. javanica* or *M. arenaria* on tomato 8 weeks after nematode inoculation. Columns followed by * are significantly different compared with the bacteria untreated plants of the same nematode species according to T- Test ($P< 0.05$) n=10.

Plant shoot (Fig. 2a) and root weight (Fig. 2b) as well as root length (Fig. 3) were significantly greater in the nematode infested plants treated with *B. cereus* S18 when compared to nematode treated plants without *B. cereus* S18.

The fact that *B. cereus* S18 by its own only improves plant growth marginally indicates that the observed growth enhancement of bacterized and nematode-infested plants is probably mainly due to the biocontrol effect and not caused by plant growth promoting activity as reported for other rhizobacteria (Kloepper *et al*., 1989).

(A)



(B)

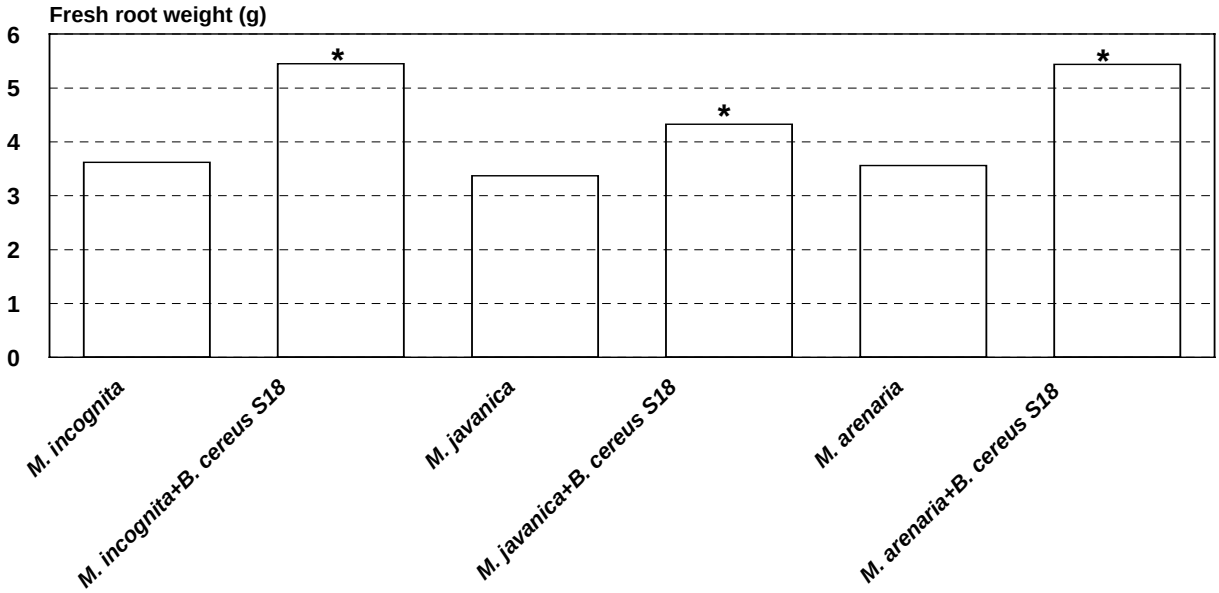


Figure 2: Effect of *Bacillus cereus* S18 on fresh shoot (A) and root weight (B) of tomato infected with *Meloidogyne incognita*, *M. javanica* or *M. arenaria* on tomato 8 weeks after nematode inoculation. Columns followed by * are significantly different compared with the nematode alone treated plants of the same nematode species according to T- Test ($P< 0.05$) n=10.

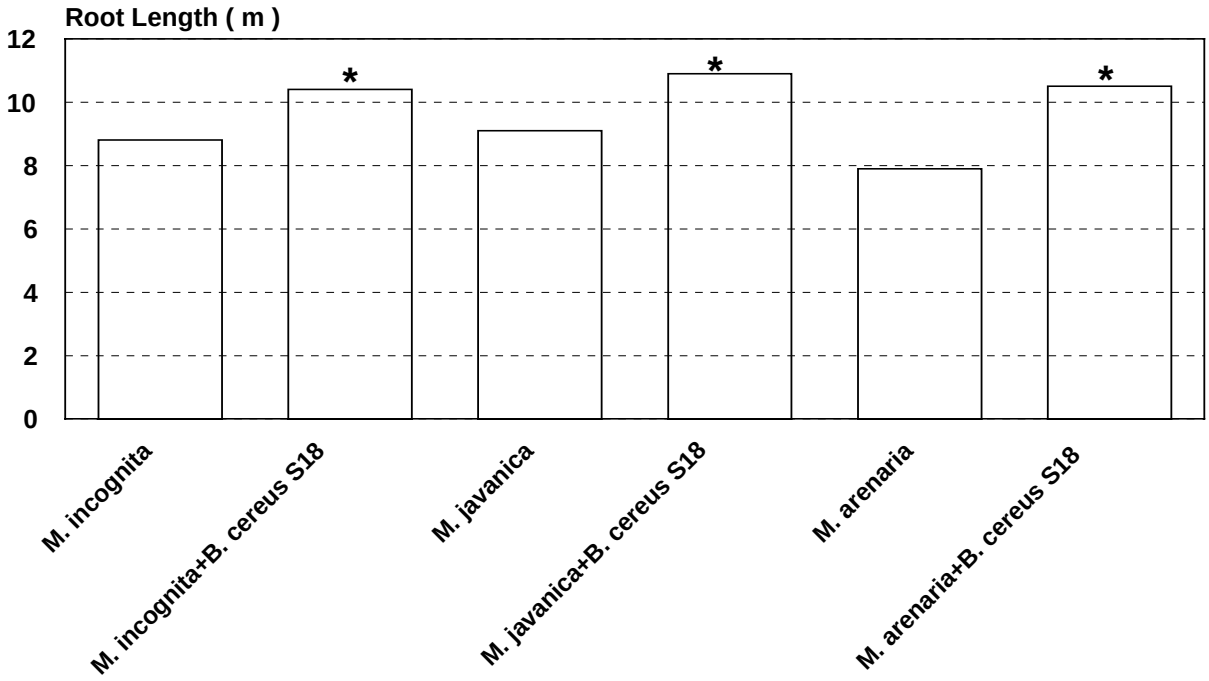


Figure 3: Effect of *Bacillus cereus* S18 on root length of tomato infected with *Meloidogyne incognita*, *M. javanica* and *M. arenaria* on tomato 8 weeks after nematode inoculation. Columns followed by * are significantly different compared with the nematode alone treated plants of the same nematode species according to T- Test ($P< 0.05$) n=10.

3.4 Biological control potential towards *M. incognita* on different host plants

3.4.1 Introduction

Few past studies dealt with the influence of the host plant on the biocontrol activity of PHPR towards the plant pathogens and parasites. Atkinson *et al*., (1975) and Azad *et al*., (1985) found that plant genotype influences the quantity and composition of the rhizosphere microflora, through differences in root exudates. Aström and Gerhardson (1988) and Aström (1991) also found that rhizobacteria isolates react differently on different plant species and to different genotypes within a species. Similar results were obtained by Sikora *et al*., (1990a, 1990b). They reported that rhizobacteria isolated from the rhizosphere had different levels of plant health-promoting activity towards *Pythium*. The level of biological control was influenced to various degree by microbial competitors, plant species, cultivar and environmental factors. Sikora (1992) suggested that the influence of plant species and cultivar on establishment and antagonistic activity of rhizobacteria must be defined to optimize nematode control with PHPR. This experiment was carried out to evaluate the influence of different host plants on the biocontrol activity of *B. cereus* S18 against *M. incognita*.

3.4.2 Materials and Methods

In this experiment three different host plants were used as mentioned before (see 2.1). Three week old plants were inoculated with 5 ml of a *B. cereus* S18 bacterial suspension (10^9 cfu/ml) per plant as a soil drench by pipetting in three holes around the roots. Two thousand *M. incognita* eggs were inoculated 3 days after bacteria inoculation by pipetting into three holes in the root zone. Plants were watered daily and fertilized weekly with 10 ml per plant of Poly Crescol (14+10+14, 2g/liter water). Each treatment was replicated 10 times in a completely randomised design in the greenhouse at 25°C with 12 hours per day of artificial light.

Sixty days after nematode inoculation shoot and root fresh weight, root length and number of galls and egg masses were measured.

3.4.3 Results and Discussion

Results revealed that when the three host plants were treated with *B. cereus* S18 a significant reduction in the percentage of galls and egg masses produced when compared with the bacteria untreated plants of the same crop species was obtained. The percentage reduction varied only slightly between the different host plants.

Cucumber showed the highest reductions in both the number of galls and egg masses followed by pepper and then tomato. The reduction in the number of galls on cucumber was (75%) followed by pepper (72%) and tomato (63%) (Fig. 1). The significant reduction in number of galls was similar on all crops. However, a higher reduction in egg masses was shown with cucumber (76%), followed by pepper (72%) and tomato (55%) (Fig. 1).

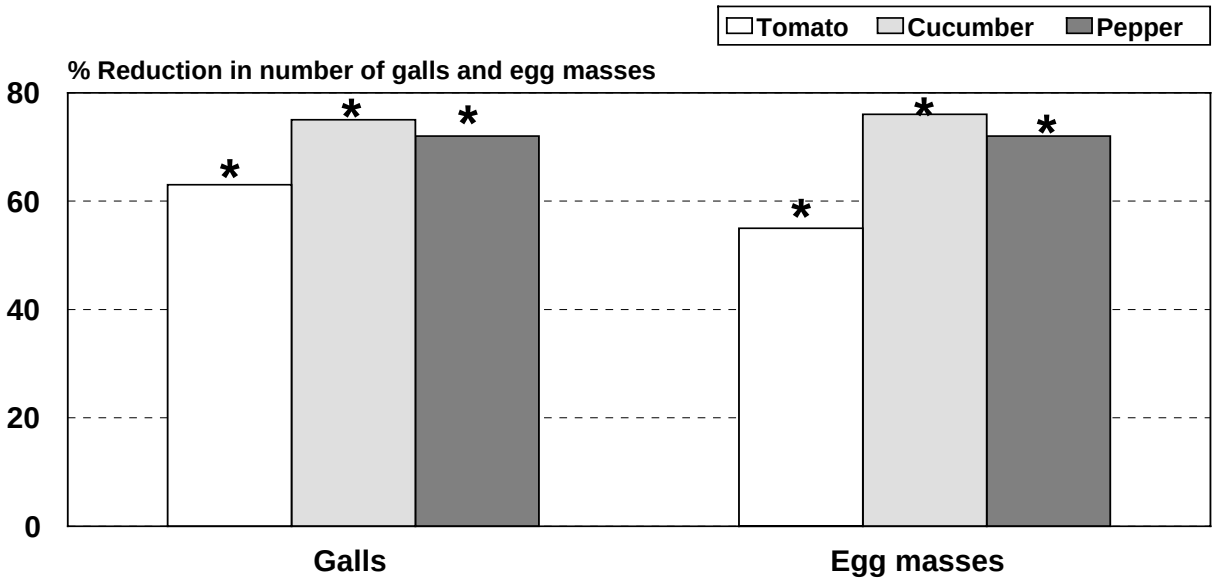


Figure 1: Effect of *Bacillus cereus* S18 on the number of galls and egg masses of *Meloidogyne incognita* on different host plants 8 weeks after nematode inoculation in percent of the control. Each plant reflects the results of a separate experiment. Columns under one plant species with * indicate significant differences when compared with the controls of the same crop plant treated with *Meloidogyne incognita* alone ($P<0.05$, $n=10$).

The results on the biological control of *B. cereus* S18 towards *M. incognita* on different host plants revealed that these bacteria reduced the number of galls and egg masses of *M. incognita* significantly on all tested host plants.

The rate of reduction varied only slightly between the different host plants. Somewhat similar results were obtained by Hackenberg and Sikora (1992). They revealed that cultivar influenced the level of biological control by *Agrobacterium radiobacter* towards potato cyst nematode, *Globodera pallida*.

The results demonstrate that *B. cereus* S18 has strong biocontrol activity against *M. incognita* on a broad spectrum of plant hosts. The reduction in the number of galls and egg masses indicates that *B. cereus* S18 probably affects nematode penetration.

Delayed juvenile penetration also slowed nematode development and egg laying in roots. *B. cereus* S18 may interfere with the host-finding process by receptor blockage on roots or by modifying root exudates of the host plant, thus hindering the attraction, hatching or penetration behaviour of nematodes. These types of mechanisms of action were suggested by others (Becker *et al*., 1988; Oostendorp and Sikora, 1990; Spiegel *et al*., 1991).

The results also showed that *B. cereus* S18 enhanced significantly plant growth of all tested crops when compared with the plants treated with *M. incognita* alone. The highest level of enhancement for fresh shoot weight were obtained on pepper (179%) followed by cucumber (58%) and tomato (36%) (Fig. 2). Significant enhancement of fresh root weight were detected on tomato (106%) followed by pepper (81%) and cucumber (53%) (Fig. 2).

Root length of all tested plants treated with *B. cereus* S18 were significantly increased when compared with the bacteria untreated plants (Fig. 3). The levels of increased root length were very high for pepper (144%) followed by tomato (70%) and cucumber (58%).

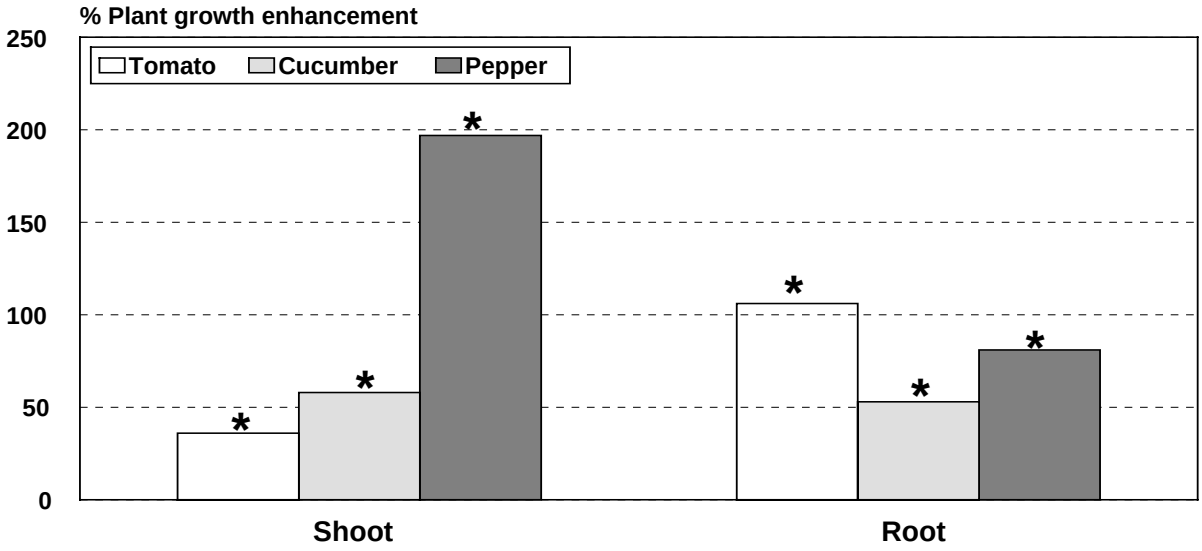


Figure 2: Effect of *Bacillus cereus* S18 on the shoot and root weight of different host plants infected with *Meloidogyne incognita* measured 8 weeks after nematode inoculation in percent of the control. Each plant reflects the results of a separate experiment. Columns under one species with * indicate significant differences when compared with the same crop plant treated with *Meloidogyne incognita* alone ($P<0.05$, $n=10$).

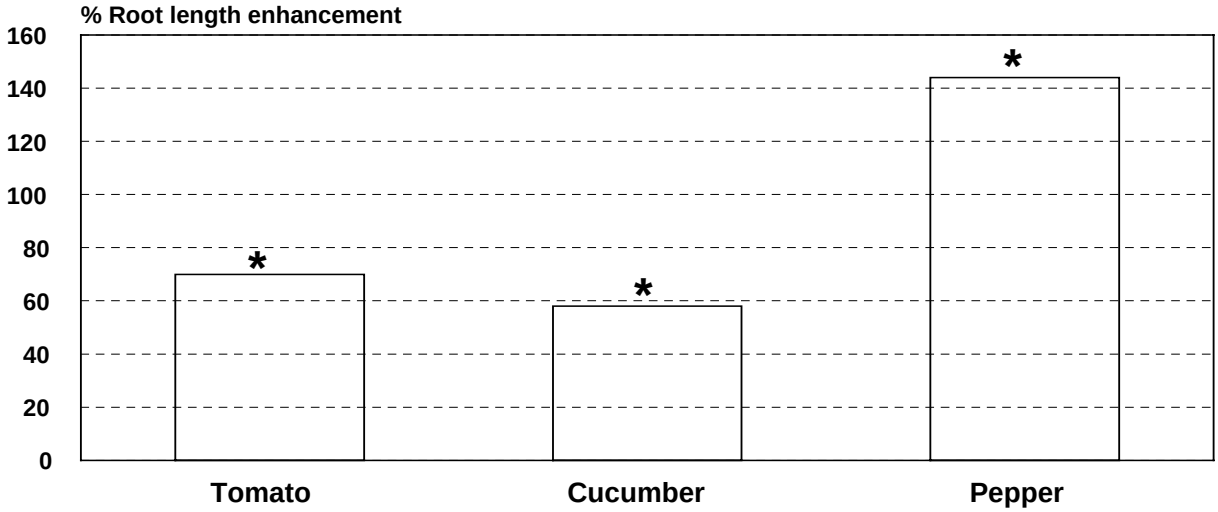


Figure 3: Effect of *Bacillus cereus* S18 on root length of different host plants infected with *Meloidogyne incognita* measured 8 weeks after nematode inoculation in percent of the control. Each column reflects the results of a separate experiment. Columns under one species with * indicate significant differences when compared with the same crop plant treated with *Meloidogyne incognita* alone ($P<0.05$, $n=10$).

3.5 General Conclusions

A) Results revealed that the form of application of *B. cereus* S18 to plants either as a soil drench or as a root dipping does not affect the biocontrol efficacy of *B. cereus* S18 towards root-knot nematode, *M. incognita*.

B) When the PHPR *B. cereus* S18 was applied 10 days before nematode inoculation it led to significant reductions in gall index and number of galls. Results showed no differences between the different application times of *B. cereus* S18 in the number of egg masses. Application of the bacteria after nematode penetration did not affect nematode development.

C) Results revealed that *B. cereus* S18 does not control all three different species of root-knot nematodes to the same level. *B. cereus* S18 had little to no biocontrol activity on *M. arenaria. B. cereus* S18 showed significant effects towards all aspects of *M. incognita* and *M. javanica*.

D) The results confirmed that *B. cereus* S18 is an effective biocontrol agent towards *M. incognita* on a broad spectrum of hosts plant. Results also demonstrated that all three crops treated with *B. cereus* S18 combined with *M. incognita* showed plant growth enhancement when compared with the bacteria untreated crops.

4. References

Aström, B. 1991. Intra-and interspecific variation in plant response to inoculation with deleterious rhizosphere pseudomonads. Journal of Phytopathology 131:184-192.

Aström, B., and B. Gerhardson. 1988. Differential reactions of wheat and pea genotypes to root inoculation with growth-affecting rhizosphere bacteria. Plant and Soil 109:263-269.

Atkinson, T. G., J. L. Jr. Neal, and R. I. Larson. 1975. Genetic control of the rhizosphere microflora of wheat. Pp. 216 *in* Biology and Control of Soil-Borne Plant Pathogens, eds. G. W. Bruehl, Pp. 116-122. St. Paul. American Phytopathological Society.

Azad, H. R., J. R. Davis, W. C. Schnathorst, and C. I. Kado. 1985. Relationship between rhizoplane and rhizosphere bacteria and *Verticillum* wilt resistance in potato. Archive of Microbiology 140:347-351.

Becker, J. O., E. Zavaleta-Mejia, S. F. Colberts, M. N. Schroth, A. R. Weinhold, J. G. Hancock, and S. D. Van Gundy. 1988. Effects of rhizobacteria on root-knot nematodes and gall formation. Phytopathology 78:1466-1469.

Chitwood, B. G. 1949. Root-knot nematodes. Proceedings of Helminthological Society of Washington 16:90-104.

Daykin, M. E., and R. S. Hussey. 1985. Staining and histopathological techniques in nematology. Pp. 39-48 *in* K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise in *Meloidogyne*, Vol. II Methodology, Raleigh: North Carolina State University Graphics.

Geels, P., and B. Schippers. 1983. Selection of antagonistic fluorescent *Pseudomonas* spp. and their root colonization and persistence following treatment of seed potatoes. Phytopathology 108:193-206.

Hackenberg, C., and R. A. Sikora. 1992. Influence of cultivar, temperature and soil moisture on the antagonistic potential of *Agrobacterium radiobacter* against *Globodera pallida*. Journal of Nematology 24:594.

Hallmann, J., R. Rodriguez-Kabana, and J. W. Kloepper. 1994. Nematode interactions with endophytic bacteria. International Workshop on Plant Growth-Promoting Rhizobacteria (3rd: 1994: Adelaide, S. Aust.). Improving plant productivity with rhizosphere bacteria.

Hallmann, J., R. Rodriguez-Kabana, and J. W. Kloepper. 1997. Nematode interactions with endophytic bacteria. Pp. 243-245 *in* A. Ogoshi, K. Kobayashi, Y. Homma, F. Kodama, N. Kondo, and S. Akino, eds. Plant growth-promoting rhizobacteria-present status and future prospects. Nakanishi printing, Sapporo.

Hoffmann-Hergarten, S., M. K. Gulati, and R. A. Sikora. 1998. Yield response and biological control of *Meloidogyne incognita* on lettuce and tomato with rhizobacteria. Journal of Plant Diseases and Protection 105:349-358.

Hoffmann-Hergarten, S., O. Keuken, M. K. Gulati, and R. A. Sikora. 1998. Yield response and biological control of the root-knot nematode, *Meloidogyne incognita* on tomato treated with *Bacillus cereus*. Journal of Plant Diseases and Protection 105:364-367.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 57:1025-1028.

Insunza, V., S. Alström, and B. Eriksson. 2000. Root-associated bacteria from nematicidal plants and their suppressive effects on trichodorid nematodes in potato. Proceedings of the Fifth International PGPR Workshop, Cordoba, Argentina.

Keuken, O. 1996. Interaktionen zwischen dem rhizosphärenbakterium *Bacillus cereus* dem wurzelgallennematoden *Meloidogyne incognita* und tomate. Ph.D. disseration, University of Bonn, Bonn, Germany.

Kloepper, J. W., M. N. Schroth, and T. D. Miller. 1980. Effects of rhizosphere colonization by plant development and yield. Phytopathology 70:1078-1092.

Kloepper, J. W., R. Lifshitz, and R. M. Zablotowich. 1989. Free-living bacterial inocula for enhancing crop productivity. Trends in Biotechnology 7:39-44.

Kofoid, C. A., and W. A. White. 1919. A new nematode infection in man. Journal of American Medicin Association 72:567-569.

Luc, M., R. A. Sikora, and J. Bridge. 1990. Plant parasitic nematodes in tropical and subtropical agriculture. Pp. 675, eds. CAB International, Wallingford, Oxon, UK.

Mai, W. F. 1985. Plant parasitic nematodes: their threat to agriculture. Pp. 11-17 *in* J. N. Sasser, and C. C. Carter, eds. An advanced treatise on *Meloidogyne*, Vol. I Biology and Control, Raleigh: North Carolina State University Graphics.

Milner, J. L., L. A. Silo-Suh, J. C. Lee, H. He, J. Clardy, and J. Handelsmann. 1996. Production of kansoamine by *Bacillus cereus* UW85. Applied and Environmental Microbiology 62:3061-3065.

Neal, J. C. 1889. The root-knot disease of the peach, orange and other plants in Florida, due to the work of the *Anguillula*. Bulltin of U. S Bur. Ent. 20:1-31.

Netscher, C., and R. A. Sikora. 1990. Nematode parasites of vegetables. Pp. 237-283 *in* M. Luc, R. A. Sikora, and J. Bridge, eds. Plant parasitic nematodes in tropical and subtropical agriculture. CAB International, Wallingford, Oxon, UK.

Oka, Y., J. Chet, and Y. Spiegel. 1993. Control of the root-knot nematode *Meloidogyne javanica* by *Bacillus cereus*. Biocontrol Science and Technology 3:115-126.

Oostendorp, M., and R. A. Sikora. 1989. Seed treatment with antagonistic rhizobacteria for suppression of *Heterodera schachtii* early root infection of sugar beet. Revue de Nematologie 12:77-83.

Oostendorp, M., and R. A. Sikora. 1990. In vitro interrelationships between rhizosphere bacteria and *Heterodera schachtii*. Revue de Nematologie 13:269-274.

Racke, J., and R. A. Sikora. 1992. Isolation, formulation and antagonistic activity of rhizobacteria toward the potato cyst nematode *Globodera pallida*. Soil Biology and Biochemistry 24:521-526.

Sadlers, H. M.1996. Wirkung eines selektierten rhizospärenbakterienstammes auf das wachstum und die pflanzengesundheit von tomate. Ph. D. disseration, Universität Bonn, Germany.

Sikora, R. A. 1988. Interrelationship between plant health-promoting rhizobacteria, plant parasitic nematodes and soil microorganisms. Mededelingen Faculteit Landbouwkundige Rijksuniversiteit Gent 53:867-878.

Sikora, R. A. 1992. Management of the antagonistic potential in agricultural ecosystems for the biological of plant parasitic nematodes. Annual Review of Phytopathology 30:245-270.

Sikora, R. A., F. Bodenstein, and R. Nicolay.1990a. Einfluß der Behandlung von Rübensaatgut mit Rhizosphärebakterien auf den Befall durch pilze der Gattung *Pythium*. I . Antagonistische Wirkung verschiedener Bakterienisolate gegenüber *Pythium* spp. Journal of Phytopathology 129:111-120.

Sikora, R. A., F. Bodenstein, and R. Nicolay.1990b. Einfluß der Behandlung von Rübensaatgut mit Rhizosphärebakterien auf den Befall durch pilze der Gattung *Pythium*. II . Untersuchungen zum Wirkungsmechanismus. Journal of Phytopathology 129:121-132.

Sikora, R. A., and S. Hoffmann-Hergarten. 1993. Biological control of plant parasitic nematodes with plant health-promoting rhizobacteria. Pest Management: Biologically based technologies. Proceedings of Beltsville Symposium XVIII, Agricultural Research Service, U.S. Department of Agriculture. Beltsville, Maryland.

Sikora, R. A., and W. W. Carter. 1987. Nematode interactions with fungal and bacterial plant pathogens – fact or fantasy. Pp. 307-312 *in* J. A. Veech, and D. W. Dickson, eds. Vistas on nematology. Hyatlsville, Maryland.

Silo-Suh, L. A., B. J. Lethbridge, S. J. Raffel, H. He, J. Clardy, and J. Handelsman. 1994. Biological activities of two fungistatic antibiotics produced by *Bacillus cereus* UW85. Applied and Environmental Microbiololgy 60:2023-2030.

Spiegel, Y., E. Cohn, S. Galper, E. Sharon, and I. Chet. 1991. Evaluation of a newly isolated bacterium, *Pseudomonas chitinolytica* sp. Nov., for controlling the root-knot nematode *Meloidogyne javanica*. Biocontrol Science and Technology 1:115-125.

Suslow, T. V., and M. N. Schroth. 1982. Rhizobacteria of sugar beet. Effects of seed application and root colonization on yield. Phytopathology 72:199-206.

Treub, M. 1885. Onderzoekingen over Sereh-Ziek Suikkeriet gedaan in s'Lands Plantentuin te Buitenzorg. Medad. PlTuin., Batavia:1-39.

Vogt, W., and H. Buchenauer. 1997. Enhancement of biological control by combination of antagonistic fluorescent *Pseudomonas* strain and resistance inducers against damping-off and powdery mildew in cucumber. Journal of Plant Diseases and Protection 104:272-280.

Webster, J. M. 1985. Interaction of *Meloidogyne* with fungi on crop plants. Pp. 183-192 in J. N. Sasser, and C. C. Carter, eds. An advanced treatise on *Meloidogyne,* Vol. I Biology and Control, Raleigh: North Carolina State University Graphics..

Zavaleta-Meija, E., and S. D. Van Gundy. 1982. Effects of rhizobacteria on *Meloidogyne* infection. Journal of Nematology 14:475A-475.

Zeck, W. M. 1971. A rating scheme for field evaluation of root-knot nematode infestations. Pflanzenschutznachrichten Bayer 24:141-144.

1. General Introduction

Crop loss caused by sedentary endoparasitic root-knot nematodes, *Meloidogyne* spp. are of major concern to agriculture (see chapter 2). However, other important sedentary plant parasitic nematodes, for example, the cyst nematodes, *Globodera* spp. and *Heterodera* spp. as well as root-lesion nematodes, *Pratylenchus* spp. also cause serious injury to crop plants.

Potato cyst nematodes *G. pallida* (Stone, 1973) Behren, 1975 and *G. rostochiensis* (Wollenweber, 1923) Behren, 1975 are major parasities of Solanaceae particularly potato, tomato and also egg plant. The sugar beet cyst nematode, *Heterodera schachtii* Schmidt, 1871 is another important cyst nematode. It has a wide host range on many plants especially Chenopodiaceae where it infects sugar beet, cabbages, canola, mustard and most other brassicas. The beet cyst nematode is present in most sugar beet growing areas and causes serious yield losses when sugar beet is grown intensively (Schmidt, 1992). All three cyst nematodes are widespread and are found in almost all important potato and sugar beet growing regions in the world (Whitehead, 1972).

The migratory endoparasitic root-lesion nematodes, *Pratylenchus* spp. are important plant parasites and are widely distributed worldwide on many crops. Species in this genus cause damage to the root cortex. *Pratylenchus zeae* Graham, 1951, for example, is considered a serious pest of maize, tobacco, cotton, sweet corn, sugarcane and rice (Fortuner, 1976). It is extremely difficult to control (Luc and Reversat, 1985) since nematicides are too expensive and resistance is not available in important hosts. *P. zeae* has been reported to cause up to 50% yield losses on maize (Hollis, 1962; Adeniji *et al*., 1979; Bridge, 1994).

Since the rhizosphere provides the first line of defence for roots against nematode attack, it is generally considered that rhizosphere bacteria are ideal biocontrol agents for use in controlling parasitic nematodes.

This chapter focus on one of the better studied antagonistic rhizobacteria and its activity against a broad spectrum of plant parasitic nematodes. The objectives of the following studies were to evaluate the biocontrol activity of *R. etli* strain G12 towards four different endoparasitic genera of plant parasitic nematodes. In addition, different host plants were studied to check for their effects on the antagonistic activity of *R. etli* G12.

2. General Materials and Methods

2.1 Host Plants

Different host plants susceptible to different plant parasitic nematodes were tested:

Tomato seedlings (*Lycopersicon esculentum* cv. Hellfrucht Frühstamm) were first raised in seed beds in sand under greenhouse conditions at 25°C. Two weeks old tomato seedlings were then transplanted (one plant/pot) into plastic pots filled with 500 cm^3 of a mixture of soil/sand (1:2, v/v). The substrate was not sterilized and was used for all the plants tested.

Cucumber (*Cucumis sativus* cv. Vorgebirgs Trauben) and pepper seedlings (*Capsicum annuum* cv. Yolo Wonder B) were sowed into the plastic pots (3 seeds/pot) filled with the same soil. After two weeks plants were thinned to one plant per pot.

Cotton (*Gossypium hirsutum* cv. Carolina Queen), soybean (*Glycin max* cv. Young Soybean) and maize (*Zea mays* cv. Hybridmais Liberal) were grown first by sowing 3 seeds directly in plastic pots filled with 500 cm^3 of the unsterilized soil-sand mixture under greenhouse conditions. Seedlings of approximately the same age of each host plant tested were thinned to one plant per pot 2 or 3 weeks after planting.

Seedlings of sugar beet (*Beta vulgaris* cv. Ariana) were raised in seed beds in sand under greenhouse conditions at 22°C. Three weeks old sugar beet seedlings were then transplanted, one plant/pot, into plastic pots filled with 500 cm^3 of the unsterilized soil-sand mixture.

Potato tubers were pregerminated at room temperature in the dark for two to three weeks. Sprouts approximately 2 cm in length with adjacent tuber tissue were cut and sowed two pieces/pot in plastic pots filled with the soil mixture. After two weeks the plants were thinned to one plant/pot.

2.2 *Rhizobium etli* strain G12

The rhizobacterium *R. etli* strain G12 was originally isolated from the rhizosphere of potatoes (Racke and Sikora 1992). The bacterium was initially identified as A*grobacterium radiobacter* and in 1998 was renamed *Rhizobium etli*. This rhizobacterium has been shown to reduce early root infection by *G. pallida* (Hasky-Günther *et al*., 1998) and the root-knot nematode, *M. incognita* (Hallmann, 2001). *R. etli* was grown on King's B agar (King *et al*., 1954) for 48 hours at 25°C. Storage of the bacteria for long periods was made in King's B nutrient solution (pH 5.8) amended with 20% glycerol at –80°C. Bacterial inoculum was produced by transferring one loop of bacteria from the agar into 100 ml flasks containing 50 ml of King's B nutrient solution for fermentation. The flasks were placed on a rotary shaker at 100 rpm and the bacterial suspension was incubated at 28°C for 24 hours. The bacterial suspension was then centrifugated at 8000 rpm for 10 minutes and the pelleted bacterial cells were resuspended in sterile ¼ strength Ringer-solution (Merck). The bacterial density of *R. etli* G12 was adjusted to optimal density OD_{560nm}= 2.0 which equalled approximately 10^{10} colony forming units cfu/ml.

The bacterial suspensions used were applied as a soil drench in all experiments. The bacteria in Ringer-solution was inoculate in 5 ml of the bacterial suspension (10^{10}cfu/ml) per plant by pipetting the solution in three or four holes made in the root zone to a depth 4 cm. The control was treated with 5 ml of Ringer-solution.

2.3 Plant Parasitic Nematodes

The three root-knot species of *Meloidogyne*, *M. incognita* race 3 (Kofoid and White, 1919) Chitwood 1949, *M. javanica* (Treub, 1885) Chitwood 1949 and *M. arenaria* (Neal, 1889) Chitwood 1949 race 1 used in this chapter were discussed in chapter 2 (see 2.3).

The potato cyst nematode, *Globodera pallida* was originally isolated from an infested potato field in Wegberg, district Heinsberg and multiplied on the potato cv. Hansa by sowing one germinated potato pieces/pot (16 cm in diameter) filled with *G. pallida* infected soil under greenhouse conditions (20°C). Pots were watered daily and fertilized every week with 10 ml/plant of Poly Crescol (14+10+14, 2g/liter water). After three months the watering was stopped, the potato tuber and the potato shoot were discarded. The soil was then added to a container and stored dry at 15°C in the dark.

The sugar beet cyst nematode, *Heterodera schachtii*, originated from a culture maintained on sugar beet cv. Ariana in plastic pots filled with a sterilized mixture of sand/soil (2:1, v/v) in a greenhouse at 22°C. Two weeks after transplanting of sugar beet seedlings into these pots, each plant was inoculated with 2000 *H. schachtii* juveniles. These pots were used as a stock culture for the experiments. Cysts were extracted 8 weeks after nematode inoculation.

Cysts of *Globodera* and *Heterodera* were extracted by a wet sieve technique (modified after Ayoub, 1980) using a 800µm and 250µm sieves. Cysts were separated from the organic material by washing the residue on 250µm sieve, containing the cysts and organic material, with $MgSo_4$-solution (1.28 g/ml, Merck) into 100 ml glass tubes containing additional $MgSo_4$-solution. The test tubes were left for 5 minutes during which the cysts float and the organic material sinks. The upper 1/3 of the solution which contains the cysts was poured through a 250µm sieve to collect the cysts. The cysts were then washed immediately with tap water to remove the Mg So_4-solution and to prevent damage to the eggs.

Eggs and juveniles were separated from the cyst wall in a tissue homogenizer by putting the extracted cysts with a little tap water into the homogenizer. The cysts were carefully squashed and then the homogenizer contents were poured through two combined 45µm and 25µm sieves to separate the eggs and juveniles from the cyst wall. Eggs and juveniles were collected on the bottom of the 25µm sieve and washed with tap water and then the number of eggs and juveniles/ml were counted under microscope.

The root-lesion nematode, *Pratylenchus zeae*, was cultured *in vitro* on Murashige medium (Sigma) using excised maize roots as a food source. The culture was supplied by Dr. John Bridge, CABI, UK. The medium was prepared as follow:

10 g Murashige medium (Sigma)

15 g Agar agar

1000 ml Dist. Water

The media was autoclaved for 20 minutes at 121°C. Media and was poured into Petri dishes when cooled down to 40°C. Maize seeds cv. Hybridmais Liberal were surface sterilized first by soaking in ethanol (95%) for 40 seconds and then in a sodium hypochlorite solution containing 2.5% active chlorine. After sterilization, the grains were washed 4-5 times with sterile water and placed on Murashige medium in Petri dishes and incubated at 24°C for 5-7 days in the dark. After 7 days the seed was severed from the roots and removed from Petri dishes. The remaining roots were then inoculated with a disc of old Murashige media having a high number of *P. zeae*. The newly inoculated nematode Petri dishes were incubated under dark conditions at 24°C. The nematodes were extracted after 12 weeks for experimental purpose on Oostenbrink dishes.

2.4 Statistical Analysis

Data were analysed according to standard analysis of variance by a one way ANOVA with the software statgraphics (Statistical Graphics Crop., Rockville, MD). Variance of homogeneity for all treatments was confirmed by the Bartlett test. The comparison between means was carried out either with Duncan's Multiple Range Test or by using the T-Test at $P<0.05$ as given in the tables and/or figures.

3. Experimental Program

3.1 Influence of plant species on the biological control activity of the antagonistic rhizobacterium *R. etli* G12 toward the root-knot nematode *M. incognita*

3.1.1 Introduction

Several rhizosphere bacteria with antagonistic activity against plant parasitic nematodes have been identified. Becker *et al*., (1988) reported that rhizosphere bacteria were effective against *M. incognita* and caused a reduction of root galling on tomato and cucumber in greenhouse tests. Sikora (1988) found that *Bacillus subtilis* was also effective in controlling *M. incognita* on cotton and sugar beet, *M. arenaria* on peanut and *Rotylenchulus reniformis* on cotton. Strains of *Pseudomonas chitinolytica* also were shown to reduce *M. javanica* on tomato as reported by Spiegel *et al*., (1991). Racke and Sikora (1992) found that the rhizobacteria *Agrobacterium radiobacter* and *Bacillus sphaericus* caused significant reduction in potato root infection by *Globodera pallida*. Smith (1994) reported that *Bacillus* sp. strain 23a reduced *M. javanica* densities on tomato. *B. cereus* strain S18 decreased *M. incognita* on tomato as reported by Keuken (1996). Hallmann (2001) reported that *Rhizobium etli* strain G12 has been shown to control species of *Meloidogyne* on tomato.

Little is known, however, about the influence of plant species on the antagonistic activity of a specific rhizosphere bacteria strain (Sikora, 1988; Keel *et al*., 1991). The aim of this work was to evaluate the influence of host plant species on the antagonistic activity of the rhizosphere bacterium *R. etli* strain G12 towards *M. incognita*.

3.1.2 Materials and Methods

In this experiment five different host plants were used. The tested crops were tomato cv. Hellfrucht Frühstamm, cucumber cv. Vorgebirgs Trauben, pepper cv. Yolo Wonder B, cotton cv. Carolina Queen and soybean cv. Young Soybean. Three week old plants were inoculated with 5 ml of *R. etli* G12 bacterial suspension (10^{10} cfu/ml) applied as a soil drench as

mentioned before (see 2.2). Controls were treated with 5 ml Ringer-solution. The soil substrate used was a non-sterilized soil-sand mixture (1:2, v/v). Three days after bacteria inoculation each plant was inoculated with *M. incognita* by pipetting 2000 eggs in three holes in the root zone as described in chapter 2 (see 2.3). Plants inoculated with *R. etli* G12 or *M. incognita* alone also served as controls. Each treatment was replicated 8 times and plants were arranged in a completely randomised design in the greenhouse at a mean temperature of 25°C. Plants were fertilized every week with 10 ml/plant of Poly Crescol (14+10+14, 2g/liter water). Each crop plant was tested separately because of the size of the overall experiment. Experiments were terminated 8 weeks after nematode inoculation. The following plant growth parameters were recorded: shoot and root fresh weight and root length. Root length was measured using a Comair Root Scanner (Hawker De Havilland, AUS). Gall index, number of galls and egg masses also were recorded. Gall index was measured as described by Zeck (1971) on a scale from 0-10. Egg masses of *M. incognita* were recorded by dipping the roots in 0.015% Phloxine B staining-solution for 20 minutes as described by Daykin and Hussey (1985) and then washing the stained roots with tap water to remove the residual Phloxine B.

3.1.3 Results and Discussion

The *R. etli* G12 treated vegetable plants (tomato, cucumber and pepper) showed a significant reduction in gall index when compared with the bacterial untreated plants of the same crop species (Fig. 1). A mean reduction in the gall index of approximately 45% over all three vegetable crops was measured. The bacteria, however, only reduced galling to a level of approximately 20% on the field crops cotton and soybean. The reduction in the galling index for all crops ranged from 17% for cotton to 50% for tomato.

Important was the fact that efficacy as measured by counting the actual number of galls per root system, did not show a strong influence of crop species on the level of biocontrol (Fig. 2a). Even though the level of control varied between the tested crops when efficacy was measured as a reduction in galling index. The reduction in the number of galls per root system was equally high for both the vegetable and the field crops with reductions on tomato of

(47%) followed by cotton (41%), pepper (39%), soybean (38%) and (34%) on cucumber (Fig. 2a).

When the differences in biocontrol efficacy on the different crops was compared using galls per gram root, however, there was an increase in biocontrol on pepper, cucumber and cotton over tomato and soybean (Fig. 2b).

Results also revealed that an even higher significant reduction in the number of galls per gram root was obtained on the vegetable crops pepper and cucumber on a per gram root basis (Fig. 2b). The significant reduction in the number of galls/gram root was (57%) for pepper; (54%) for cucumber and (53%) for cotton.

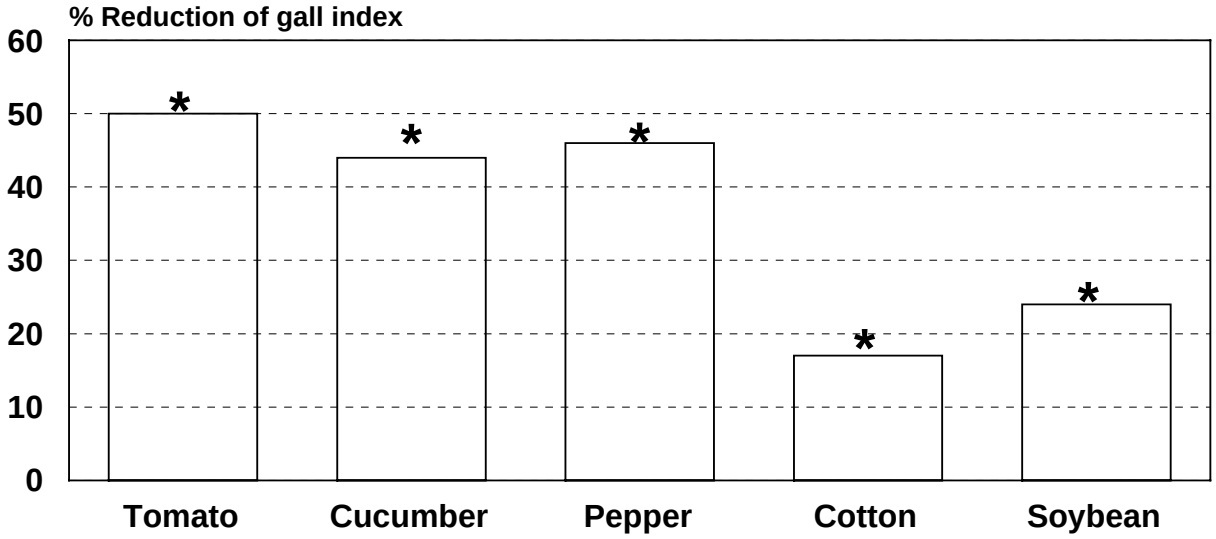


Figure 1: Effect of *Rhizobium etli* G12 on the gall index of *Meloidogyne incognita* on different host plants 8 weeks after nematode inoculation in percent of the control. Each plant reflect the results of a separate experiment. Columns under one species with * indicate significant differences when compared with the same crop plant treated with *Meloidogyne incognita* alone ($P<0.05$, n=8, T-Test).

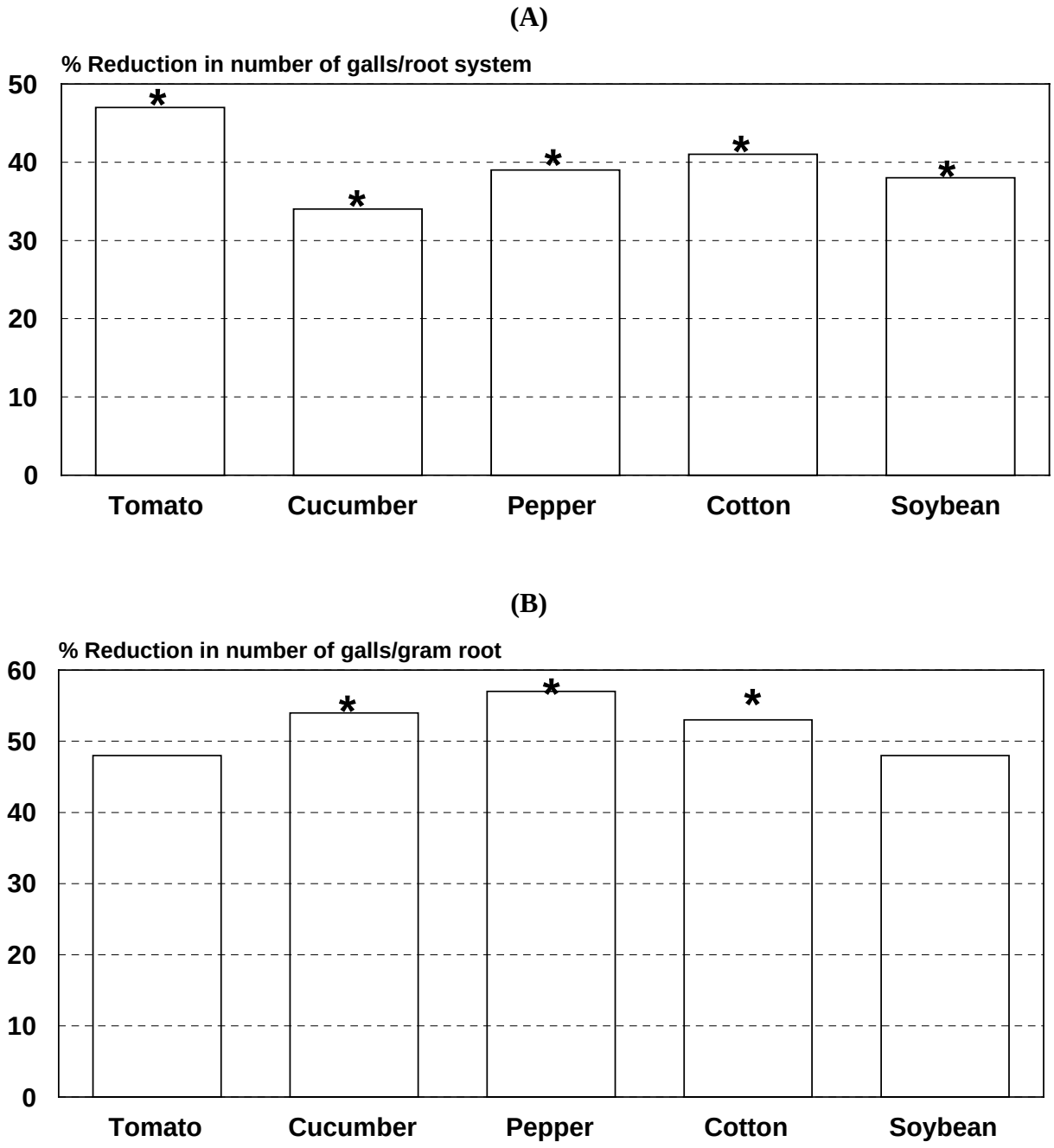


Figure 2: Effect of *Rhizobium etli* G12 on the number of galls per root system (A) and per gram root (B) of *Meloidogyne incognita* on different host plants 8 weeks after nematode inoculation in percent of the control. The columns for each plant reflect the results of a separate experiment. Columns under one species with * indicate significant differences when compared with the same crop plant treated with *Meloidogyne incognita* alone ($P<0.05$, $n=8$, T-Test).

Plant species did affect efficacy when the number of egg masses per root system was taken into consideration (Fig. 3a). Egg mass number is a measure of the level of nematode development and an indication that the adult egg laying stage has been reached. The reduction in number of egg masses/root system was extremely high for pepper (70%) and tomato (62%) and lower for cucumber (47%), cotton (47%) and lowest for soybean (37%) on a per root system basis (Fig. 3a).

The higher reduction in number of egg masses/gram root was shown with the same crops (80%) for pepper; (63%) for cucumber and (57%) for cotton (Fig. 3b).

The results, verified earlier findings that showed that *R etli* G12 has a direct effect on nematode root penetration leading to a reduction in the number of females developing in the root and therefore overall galling levels. Slight differences, especially those associated with high levels of variation as seen in these tests envolving plant species interactions with a biocontrol agent, require more exact criteria of measurement such as number of galls or number of females per gram root since some antagonistic agents can directly increase root growth.

A new finding was the detection of a significant reduction in the number of egg masses produced per root system as affected *R. etli* and by crop species. These results indicate that either (1) nematode penetration is delayed or (2) bacteria influences the speed of juvenile development to the adult stage after the nematode penetration the roots.

Results obtained also revealed that the rate of reduction in the number of galls and egg masses varied when the efficacy was counted per gram root compared with efficacy counted per root system. This variation in efficacy may be due to the growing rate of each root crop, as the roots of tomato, cucumber and soybean grow faster than the other crops.

The results demonstrated that a subjective root galling index is not exact enough for determining differences in efficacy of interactions between the different plant species.

Standard galling indexes are often used for nematicide studies where differences in galling between the control and the chemical treatment are often extremely high.

The results obtained indicated that:
(1) R. *etli* can significantly reduce root-knot on a wide range of host plants
(2) host plant species has an influence on the level of biocontrol activity of *R etli* G12, but not as strong as initally detected using galling indexes
(3) biocontrol of the nematode by this bacteria on field crops is slightly poorer than control on vegetable crops
(4) the bacteria reduces nematode infection on all crops over non-bacterial controls
(5) speed of penetration or rate of juvenile development after nematode penetration of the root tissue seems to be adversely affected by the bacteria.

The mode of action envolved in the biocontrol of root-knot nematode may be: (1) systemic induced resistance (2) alteration in root exudates of the crop plants that affect attraction and/or penetration of the nematode thereby delaying development and (3) bacterial production of toxic metabolites that affect nematode behaviour.

The broad effectivity of *R. etli* G12 on different plant species can probably be best explained by its known ability to induced resistance. Induced systemic resistance is defined as an enhancement of plant defense mechanisms in a broad spectrum of plants against several pathogens (Schönbeck *et al*., 1993). *R. etli* G12 induced resistance to nematodes was first described for cyst nematodes by Hasky-Günther *et al*., (1998) and studied in detail by Reitz *et al*. (2000, 2001).

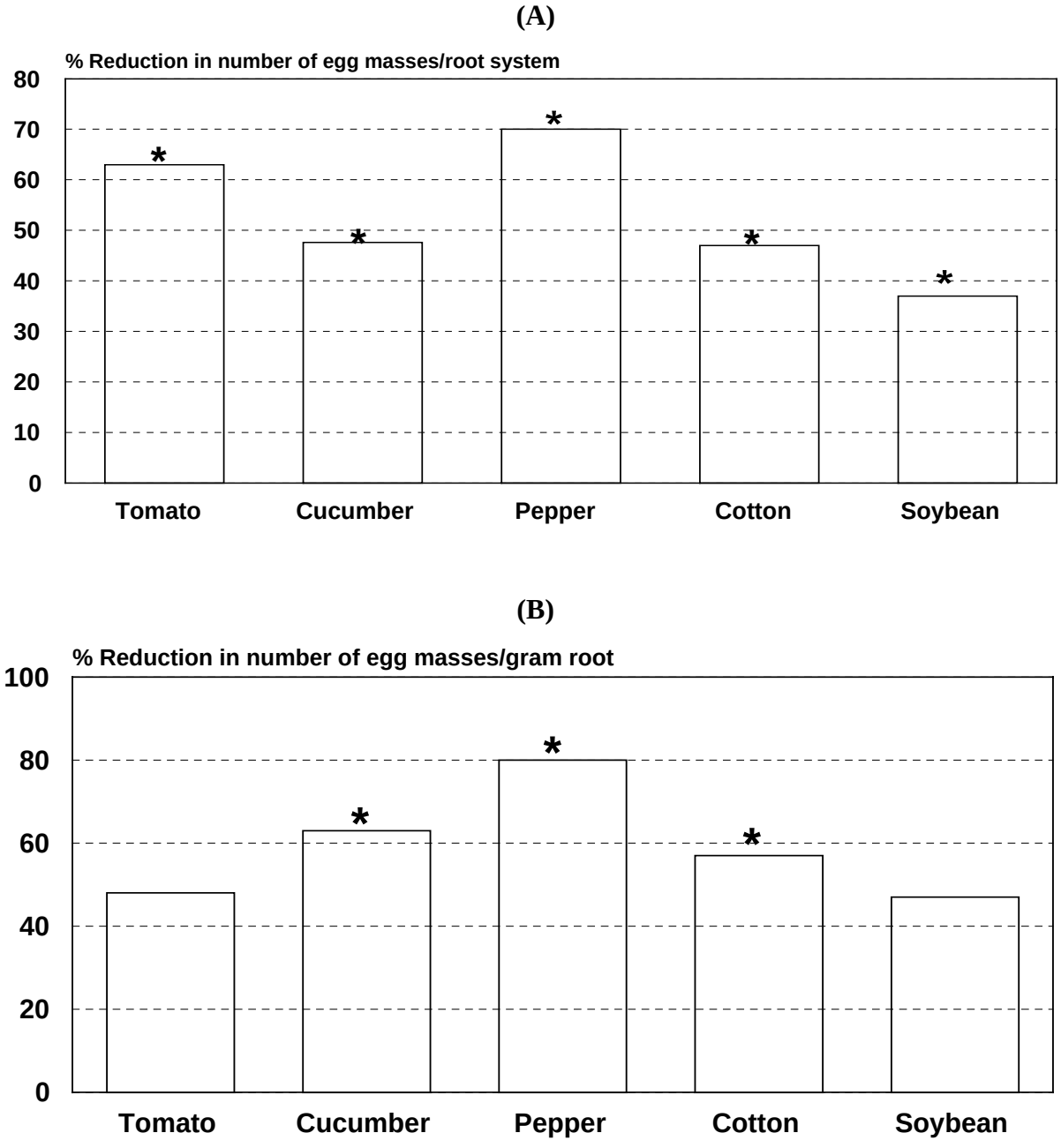


Figure 3: Effect of *Rhizobium etli* G12 on the number of egg masses per root system (A) and per gram root (B) of *Meloidogyne incognita* on different host plants 8 weeks after nematode inoculation in percent of the control. The columns for each plant reflect the results of a separate experiment. Columns under one species with * indicate significant differences when compared with the same crop plant treated with *Meloidogyne incognita* alone ($P<0.05$, n=8, T-Test).

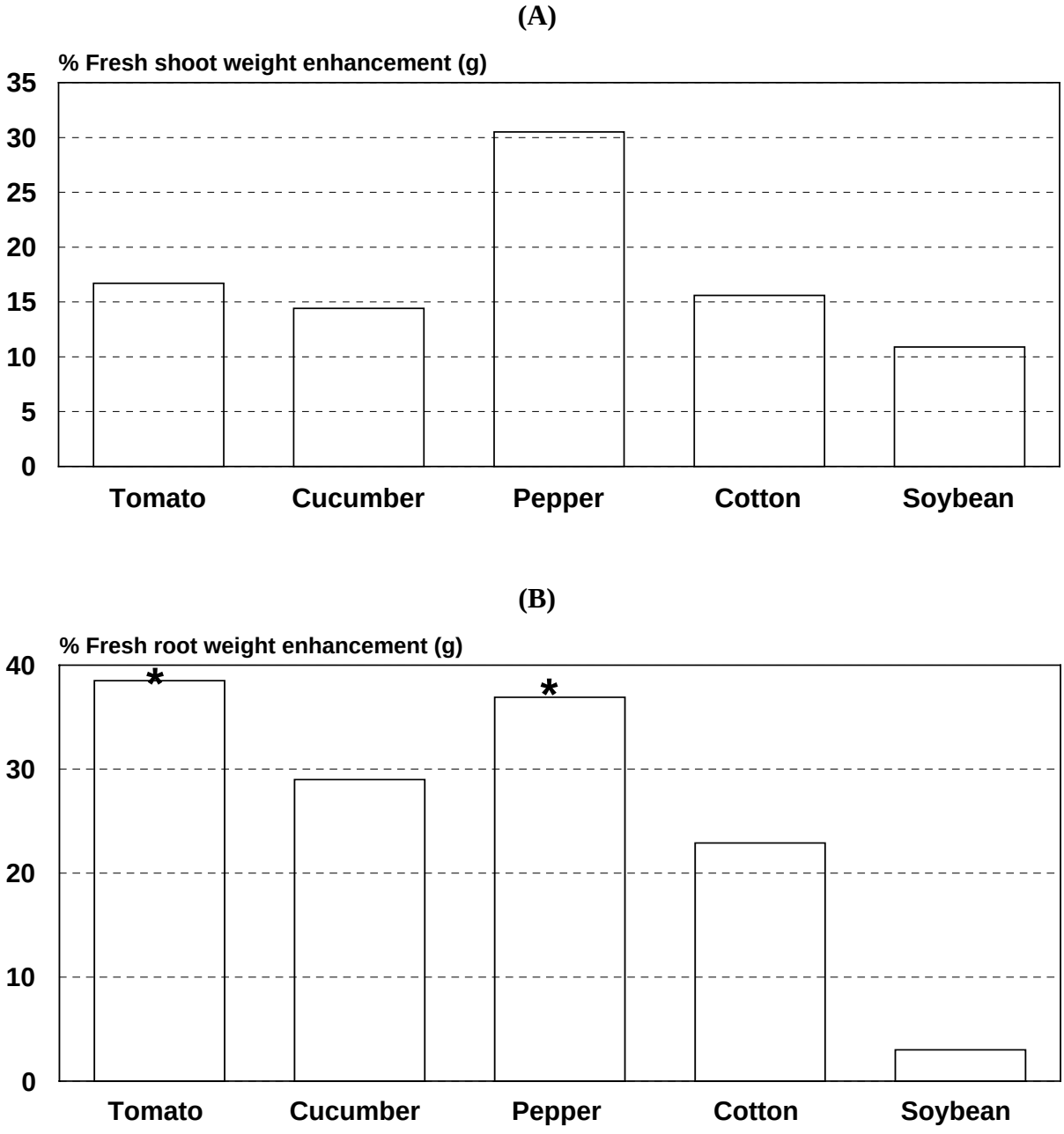


Figure 4: Effect of *Rhizobium etli* G12 on the shoot (A) and root (B) fresh weight of different host plants infected with *Meloidogyne incognita* measured 8 weeks after nematode inoculation in percent of the control. The columns for each plant reflect the results of a separate experiment. Columns under one species of plant with * indicate significant differences when compared with the same crop plant treated with *Meloidogyne incognita* alone ($P<0.05$, n=8, T-Test).

R. etli G12 biocontrol of root-knot led to increased plant growth of all crops tested, but the differences were not always significant when compared to the bacteria free controls. Data showed increases in shoot weight for pepper when treated with *R. etli* G12 compared with the bacteria untreated plants of the same crop (Fig. 4a). A non-significant stimulation of shoot fresh weight was observed for plants treated with *R. etli* G12 combined with *M. incognita* ranging from 11% for soybean to 31% for pepper (Fig. 4a).

Results also showed no significant increases in root weight for cucumber, cotton or soybean. Tomato and pepper showed significant enhancement in fresh root weight when treated with *R. etli* G12 compared with the control (Fig. 4b). The increase in root fresh weight was (39%) for tomato and (37%) for pepper.

There were no significant differences in root length between nematode infested plants treated and non-treated with *R. etli*, even though, root length increases ranged from 11% for cucumber to 24% for pepper (Fig. 5).

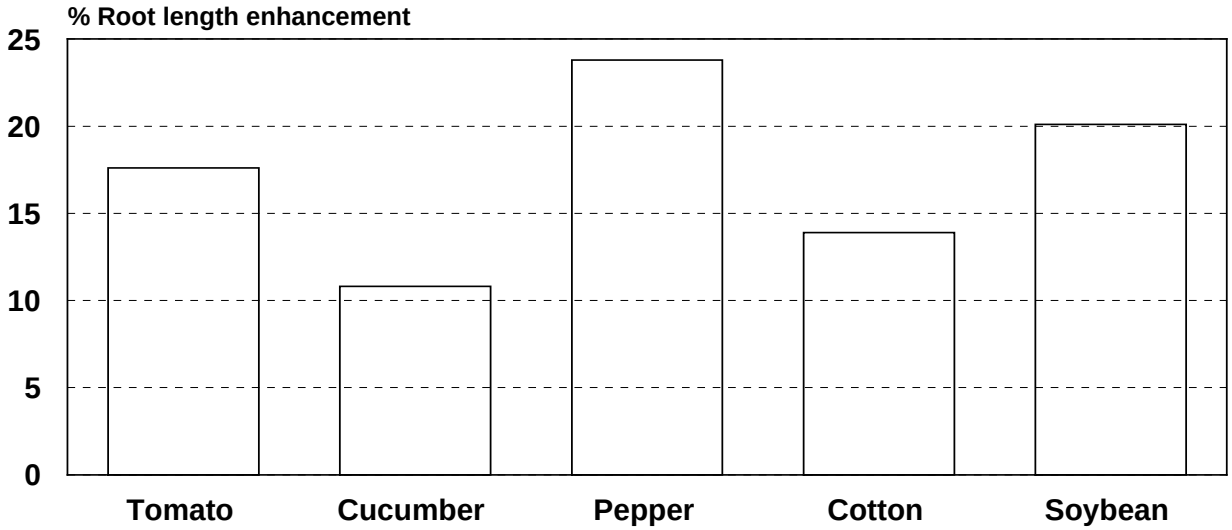


Figure 5: Effect of *Rhizobium etli* G12 on root length of different host plants infected with *Meloidogyne incognita* measured 8 weeks after nematode inoculation in percent of the control. The columns for each plant reflect the results of a separate experiment. The differences between treatments were not significant when compared with the same crop plant treated with *Meloidogyne incognita* alone ($P<0.05$, n=8, T-Test).

The results revealed that the significant increases in fresh root weight for tomato and pepper was the result of significant antagonistic activity of *R. etli* G12 against *M. incognita*.

3.2 Biocontrol activity of *R. etli* G12 towards different species of *Meloidogyne* on tomato

3.2.1 Introduction

Different studies have dealt with the role of rhizobacteria in controlling plant parasitic nematodes. All of these studies concentrated on the effects on a specific plant parasitic nematode such as: *M. incognita* (Becker *et al*., 1988, 1989, Kloepper *et al*., 1992); *M. hapla* (Honglin *et al*., 1995); *M. javanica* (Spiegel *et al*., 1991); *Criconemella xenoplax* (Kluepfel *et al*., 1993); *Heterodera glycines* (Kloepper *et al*., 1992); *H. schachtii* (Oostendorp and Sikora, 1989, 1990); *G. pallida* (Racke and Sikora, 1992); *M. incognita* (Keuken, 1996) and *G. rostochiensis* (Cronin *et al*., 1997). The control potential of one antagonistic rhizobacterium toward a broad spectrum of species within the same nematode genus has not been studied. The objective of this study was to evaluate the biocontrol activity of *R. etli* G12 towards different species of *Meloidogyne* on one host plant. This was important due to the fact that (1) tomato is infested by many species of root-knot world wide and (2) in some fields multiple species complexes can exist (Sasser and Freckman, 1987).

3.2.2 Materials and Methods

Egg extraction of the three different species of root-knot nematodes, *M. incognita, M. javanica* and *M. arenaria* was outlined in chapter 2 (see 2.3). Three week old tomato plants cv. Hellfrucht Frühstamm were inoculated with 5 ml of the bacterial suspension (10^{10} cfu/ml) per plant as a soil drench. Controls received 5 ml Ringer-solution. The soil substrate used was a non-sterilized mixture of soil-sand (1:2, v/v). Plants were inoculated after three days with 2000 eggs per plant of either *M. incognita, M. javanica* or *M. arenaria* by pipetting the solution in three holes in the root zone. Nontreated plants as well as plants treated with *R. etli* G12 or the nematodes alone served as controls. Each treatment was replicated 8 times within a completely randomised design under greenhouse conditions with a mean temperature of

25°C and 12 hours of artificial light per day.

Plants were watered every day and fertilized weekly with 10 ml/plant of Poly Crescol (14+10+14, 2g/liter water). The experiment was terminated 8 weeks after nematode inoculation and fresh shoot and root weight as well as root length, gall index, total number of galls and egg masses were determined.

3.2.3 Results and Discussion

All plants treated with *R. etli* G12 had a reduced degree of root galling for all three species of *Meloidogyne*. This reduction varied between the different species of *Meloidogyne*. A significant reduction in gall index was observed for all the tested three species *M. incognita, M. javanica* and *M. arenaria*. For *M. incognita* and *M. javanica* the reduction in gall index was approximately 50% and for *M. arenaria* it was 27% compared with the bacteria untreated plants (Fig. 1).

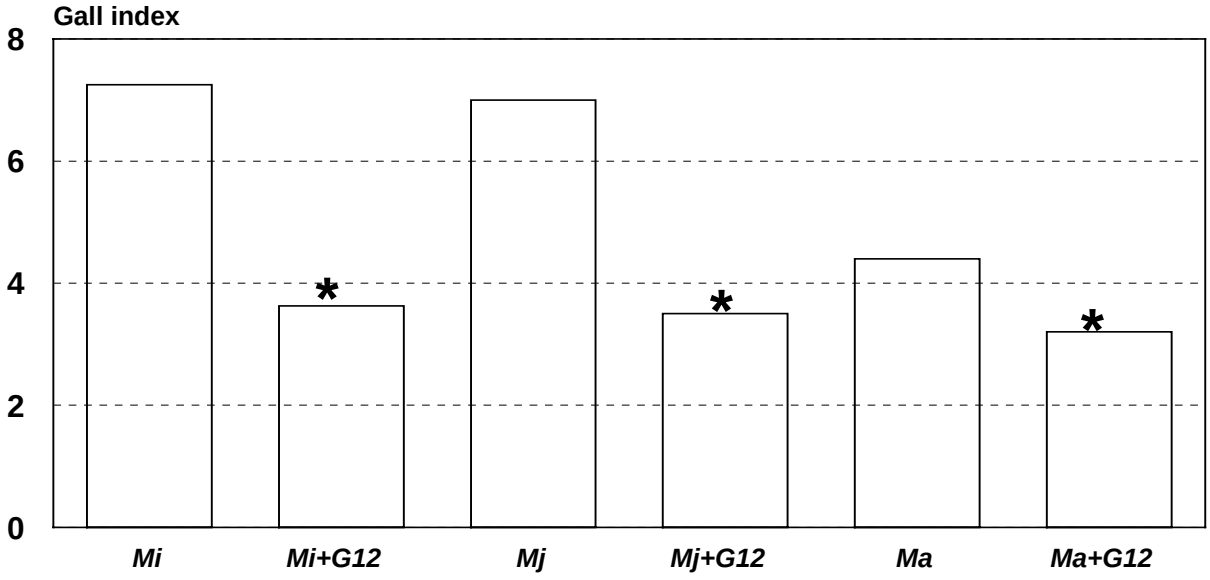


Figure 1: Effect of *Rhizobium etli* G12 applied as a drench on the gall index of *Meloidogyne incognita* (Mi), *M. javanica* (Mj) and *M. arenaria* (Ma) on tomato eight weeks after nematode inoculation. Columns followed by * are significantly different compared with the plants treated with the same nematode species alone according to T-Test ($P < 0.05$) n=8.

Significant reductions in the number of galls was detected for *M. incognita* (52%), *M. javanica* (50%). The non-significant reduction in number of galls was shown for *M. arenaria* when compared to the treated plants with nematode alone (Fig. 2a).

Results also showed significant reductions in the number of egg masses. The reduction was (64%) for *M. incognita* and for *M. javanica* (46%). *M. arenaria* showed a non-significant reduction in number of egg masses when compared to the bacteria untreated plants (Fig. 2b).

The results of this study demonstrated that *R. etli* G12 can reduce root-knot nematode infestation of multiple species on tomato plants. The level of biocontrol varied between the three species of *Meloidogyne*. The highest reduction in root galling, number of galls and egg masses was shown for *M. incognita* and *M. javanica*, with the lowest reduction seen for *M. arenaria*. These results also demonstrated that *R. etli* G12 reduced or delayed the penetration of both *M. incognita* and *M. javanica*. The antagonistic bacteria may also delay the process of development of the juveniles which succeeded in penetrating the roots as seen through the significant reduction in the number of egg masses.

R. etli G12 only had a minor effect toward *M. arenaria*. This may be based on a need for a different mode of action of bacterial antagonists in relation to different nematode species (Hoffmann-Hergarten *et al*., 1998). *M. arenaria* is known to have a different host range than *M.incognita* and *M. javanica*. The fact that *M. arenaria* does not infect horticultural crops as effectively as *M. incognita* and *M. javanica* may also be a reason for poorer biocontrol.

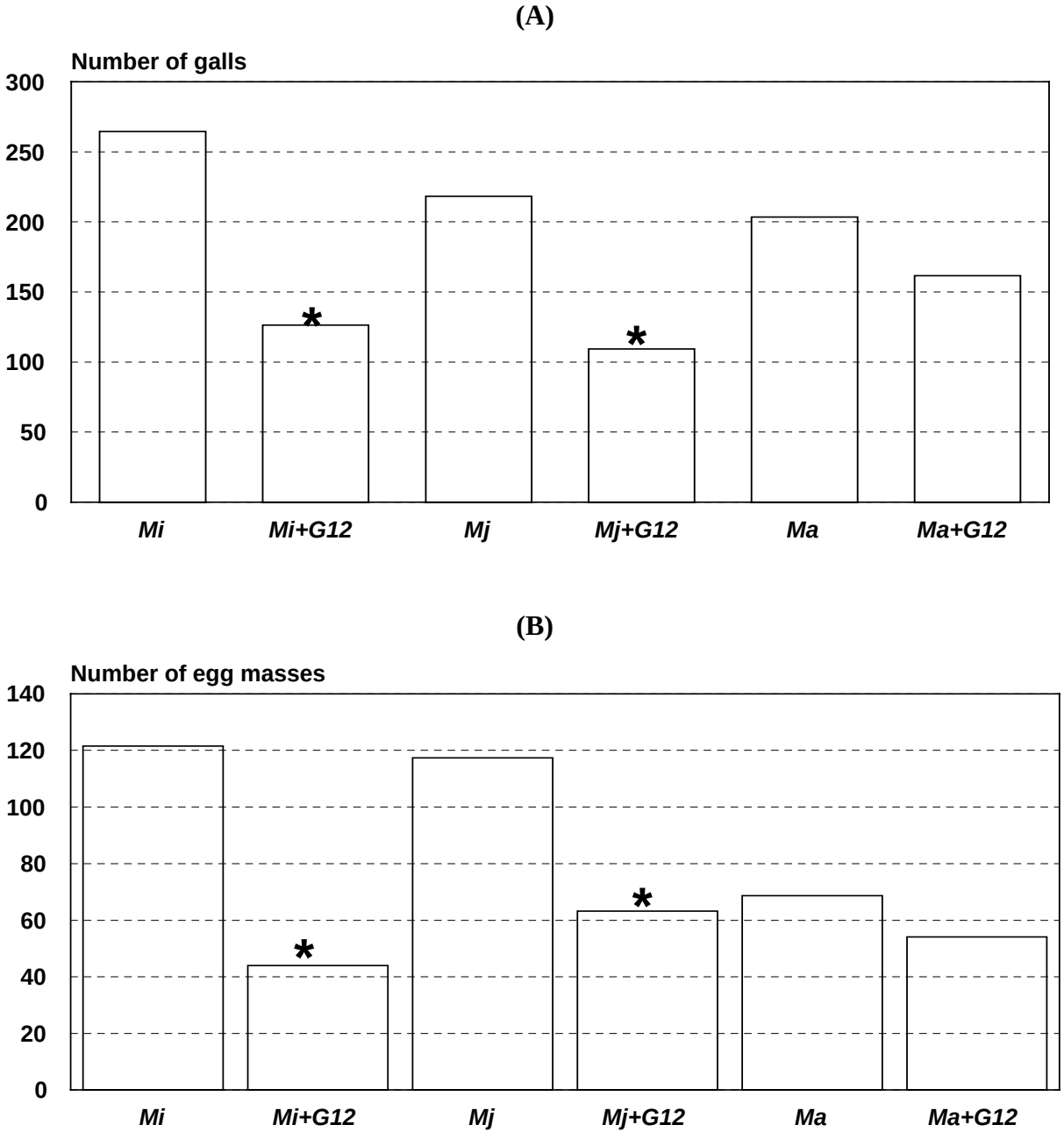


Figure 2: Effect of *Rhizobium etli* G12 on the number of galls (A) and egg masses (B) of *Meloidogyne incognita* (Mi), *M. javanica* (Mj) and *M. arenaria* (Ma) on tomato eight weeks after nematode inoculation. Columns followed by * are significantly different compared with the plants treated with the same nematode species alone according to T-Test ($P< 0.05$) n=8.

A significant increase in fresh shoot weight was only seen with the plants treated with *M. incognita* alone (Fig. 3a). The other two species of *Meloidogyne* treated plants combined with *R. etli* G12 showed no significant enhancement in shoot weight when compared with the plants treated with each species alone. Results also showed non-significant enhancement in shoot weight for the plants treated with *R. etli* G12 alone compared with the non-treated control (Data not shown).

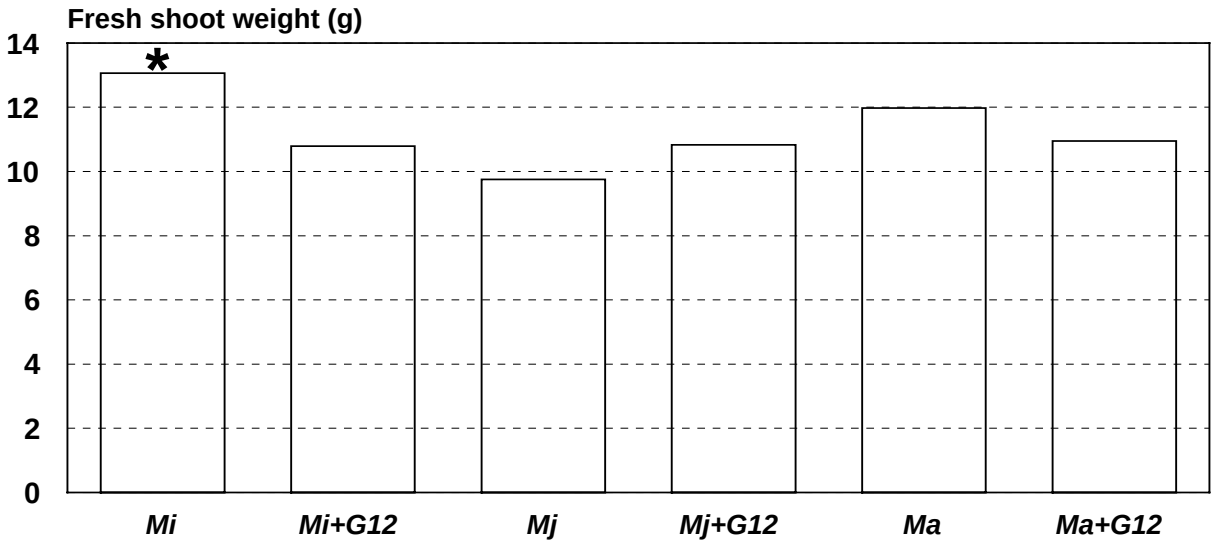
Results showed significant increases in fresh root weight for the plants treated with *R. etli* G12 combined with *M. incognita* when compared with the plants treated with *M. incognita* alone (Fig. 3b). No significant increases in root weight was detected for the others species. Results also showed that non-significant enhancement in root weight for the treated plants with *R. etli* G12 alone compared with the non-treated control (Data not shown).

Results showed significant increases in root length only for the plants treated with *R. etli* G12 combined with *M. incognita* when compared with the plants treated with *M. incognita* alone (Fig. 4).

In the treatments absolute control and tomato treated with only *R. etli* G12 there was no enhancement of root length (control, 13 cm and *R. etli*, 12.5 cm) (Data not shown).

The increase in root weight and length of *R. etli* G12 treated plants infested with the species of *M. incognita* indicates that *R. etli* G12 have ability to reduce nematode root injury by this species. This biocontrol leads to improved root weight and length. This means that enhancement refers to the antagonistic activity of *R. etli* G12 towards the different species of root-knot nematodes and not to direct plant growth promotion.

(A)



(B)

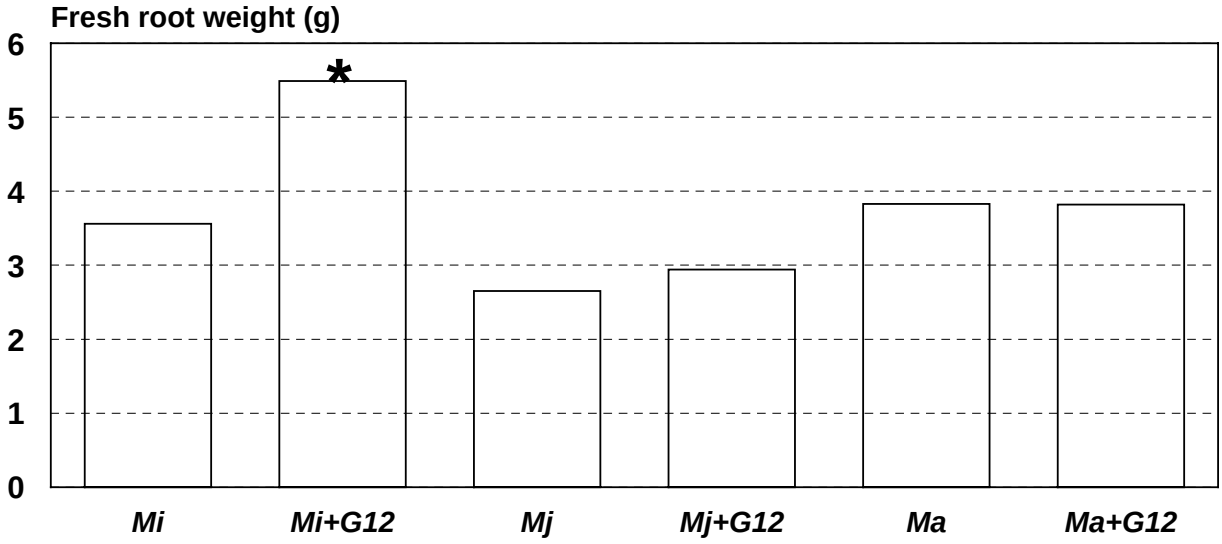


Figure 3: Effect of *Rhizobium etli* G12 on fresh shoot (A) and root (B) weight of tomato infected with *Meloidogyne incognita* (Mi), *M. javanica* (Mj) and *M. arenaria* (Ma) on tomato eight weeks after nematode inoculation. Columns followed by * are significantly different compared with the plants treated with the same nematode species alone according to T-Test ($P< 0.05$) n=8.

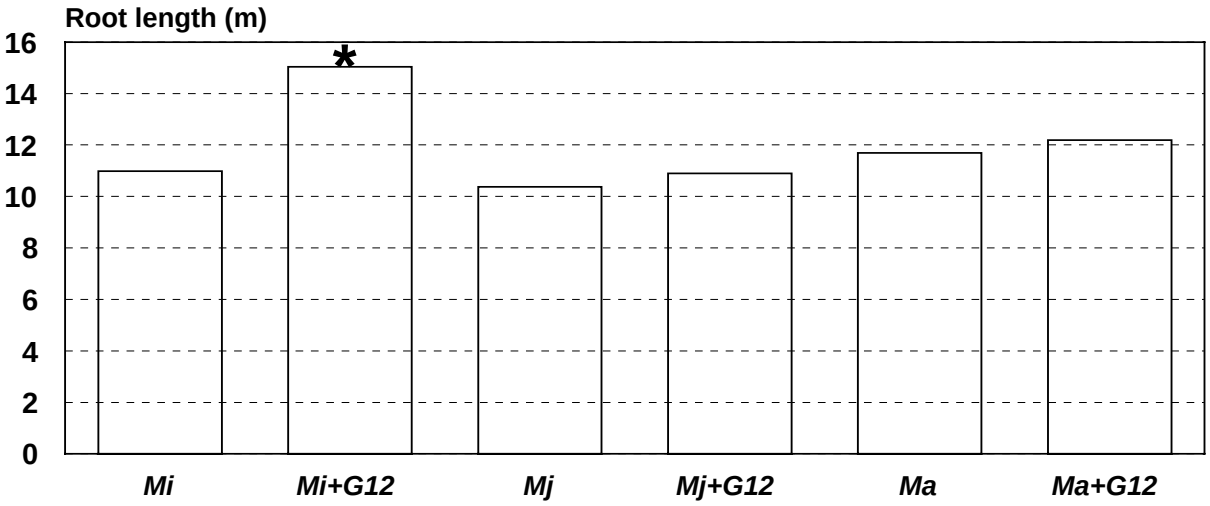


Figure 4: Effect of *Rhizobium etli* G12 on the root length of tomato infected with *Meloidogyne incognita* (Mi), *M. javanica* (Mj) and *M. arenaria* (Ma) on tomato eight weeks after nematode inoculation. Columns followed by * are significantly different compared with the plants treated with the same nematode species alone according to T-Test ($P< 0.05$) n=8.

3.3 Effect of *R. etli* G12 inoculum density on biocontrol activity toward *M. incognita* on tomato

3.3.1 Introduction

There are many different factors influencing the antagonistic activity of rhizobacteria towards plant pathogens and parasities. Some of them are biotic and others abiotic in nature. In addition, control can be influenced by fermentation systems and inoculum form. For example, different rhizobacteria inoculum densities have been shown to influence the antagonistic ability of the biocontrol agent *Bacillus subtilis* against different soil-borne potato diseases under greenhouse condition (Schmiedeknecht *et al*., 1998). Oostendorp and Sikora (1989) demonstrated that inoculum density was an important factor influencing the efficacy of *Pseudomonas fluorescens* toward *Heterodera schachtii*. Racke and Sikora (1992) revealed that the number of colony forming units (cfu) in the inoculum applied to the seed pieces of potato was important in determining the level of antagonistic activity toward *Globodera pallida*. The objective of this study was to determine the optimum *R. etli* G12 inoculum density for effective biocontrol of *M. incognita* on tomato.

3.3.2 Materials and Methods

A non-sterilized sand-soil mixture (2:1, v/v) was prepared and 500 cm^3 of soil added seperately to each experimental pot. The soil was first placed in plastic bags and each bag inoculated with 3000 *M. incognita* eggs. The bag was shaken vigorously to distribute the eggs homogeneously into the soil. The soil of each bag was then put into 500 cm^3 experimental plastic pots. The nematode untreated soil served as controls. Three week old tomato plants cv. Hellfrucht Frühstamm were dipped for 30 minutes in different bacterial suspensions of *R. etli* G12 produced as described before (see 2.2). The tested bacterial concentrations were: 10^4, 10^6, 10^8 and 10^{10} cfu/ml. The different bacterial suspensions were prepared in 500 ml glass beaker containing 500 ml sterilized ¼ strength Ringer-solution as follow:

a) 10^{10} cfu/ml

b) 10^8 cfu/ml: 5 ml of 10^{10} + 495 ml Ringer-solution

c) 10^6 cfu/ml: 5 ml of 10^8 + 495 ml Ringer-solution

d) 10^4 cfu/ml: 5 ml of 10^6 + 495 ml Ringer-solution

From each concentration two serial dilution replicates were prepared to calculate the actual number of cfu/ml. The nematode treated soil alone or the roots dipped in Ringer-solution served as controls. After dipping the tomato roots in the different bacterial suspensions each plant from each treatment was transplanted into pots. Each treatment was replicated 8 times in a completely randomised design under greenhouse conditions at approximately 25°C with 12 hours/day of artificial light. Plants were watered daily and fertilized every week with 10 ml per plant of Poly Crescol (14+10+14, 2g/liter water).

The experiment was terminated 8 weeks after nematode inoculation. The plants were removed and the roots were washed carefully with tap water. The fresh shoot and root weight, root length, gall index, total number of galls and egg masses was evaluated.

3.3.3 Results and Discussion

The results revealed that all the tested *R. etli* G12 densities reduced the degree of nematode galling. This reduction increased with increasing inoculum densities. A significant reduction in gall index was obtained with 10^8 and 10^{10} cfu/ml (32% and 40%) (Fig. 1).

Similar results were obtained when number of galls was counted. Plants treated with 10^8 and 10^{10} cfu/ml of *R. etli* G12 showed significant reductions in the number of galls (33% and 36%) respectively compared with the plants treated with *M. incognita* alone (Fig. 2a).

A significant reduction in number of egg masses was observed at densities 10^6, 10^8 and 10^{10} cfu/ml of *R. etli* compared with the nonbacterized plants (Fig. 2b).

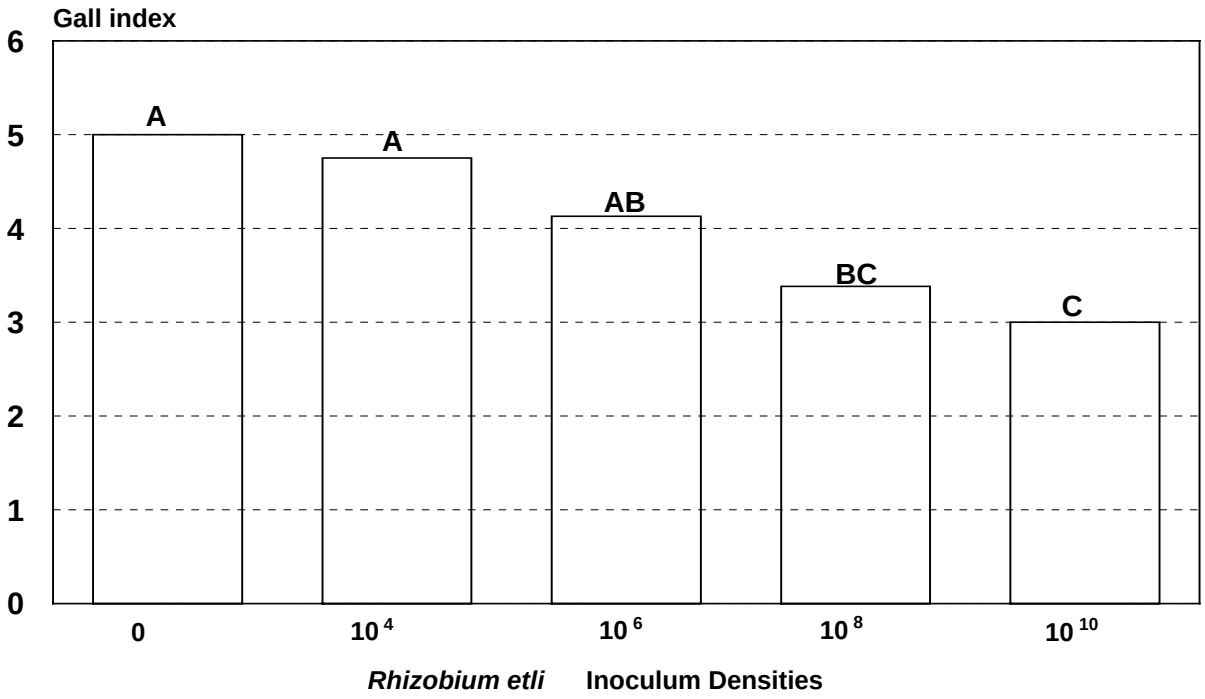


Figure 1: Effect of different inoculum densities of *Rhizobium etli* G12 applied as a drench on the gall index of *Meloidogyne incognita* on tomato 8 weeks after nematode inoculation. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P< 0.05$) n=8.

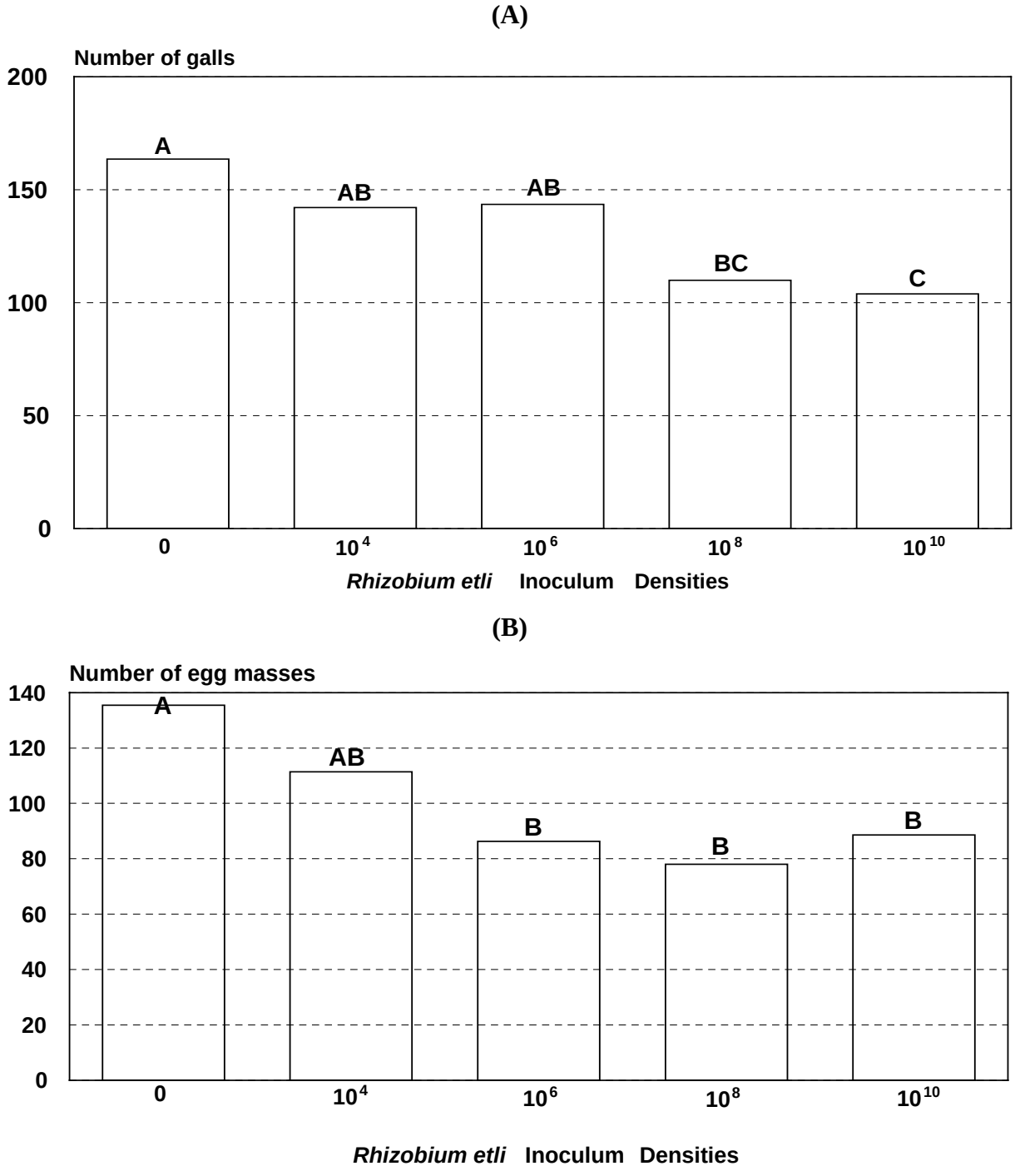
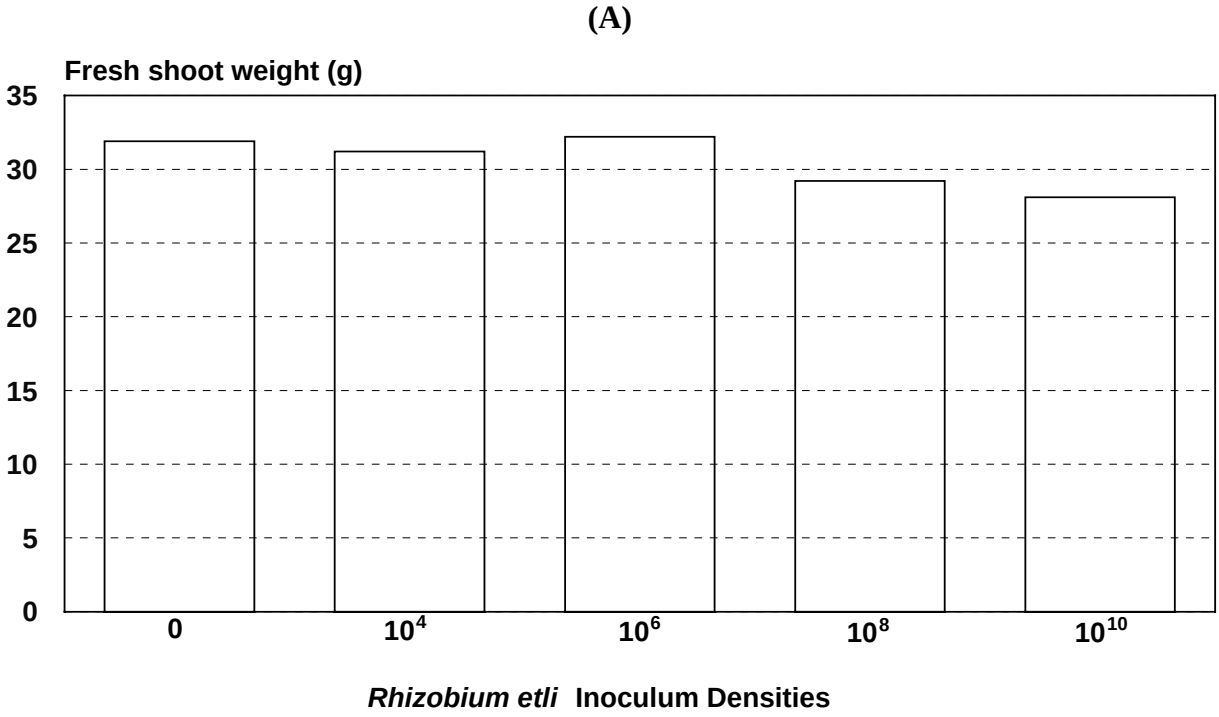


Figure 2: Effect of different inoculum densities of *Rhizobium etli* G12 applied as a drench on the number of galls (A) and egg masses (B) of *Meloidogyne incognita* on tomato 8 weeks after nematode inoculation. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P< 0.05$) n=8.

Results found significant reductions either in root galling and in the number of galls and egg masses of *M. incognita* observed at inoculum densities of 10^6 to 10^{10} cfu/ml of *R. etli* G12. These results are similar to those obtained by Oostendorp and Sikora (1989) who found that the antagonistic activity of *Pseudomonas fluorescens* against *H. schachtii* increased when the inoculum density was increased. When the bacterial inoculum density was high it led to less effective nematode penetration that could be due to lowered oxygen levels at the root or to the interspecific competition caused by nutritional deficiency effects (Trolldenier, 1979; Oostendorp and Sikora 1989).

Results showed that no significant differences between all the tested *R. etli* G12 inoculum densities combined with *M. incognita* in plant growth parameters either fresh shoot (Fig. 3a) and root (Fig. 3b)weight as well as root length (Fig. 4) compared with the treated plants with *M. incognita* alone. Plants treated with *R. etli* G12 alone were not used, because this bacteria alone had no effects on the plant growth enhancement in previous experiments (Data not shown).



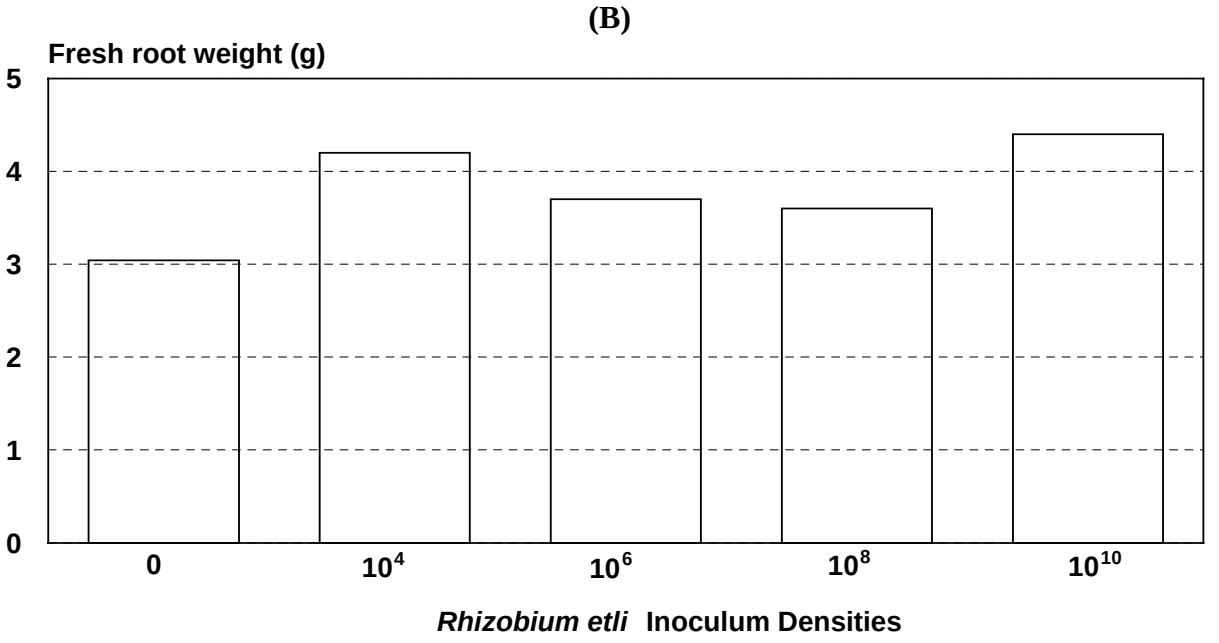


Figure 3: Effect of different inoculum densities of *Rhizobium etli* G12 on the fresh shoot (A) and root (B) weight of tomato infected with *Meloidogyne incognita* 8 weeks after nematode inoculation. The differences between treatment were not significant according to Duncan's Multiple Range Test ($P< 0.05$) n=8.

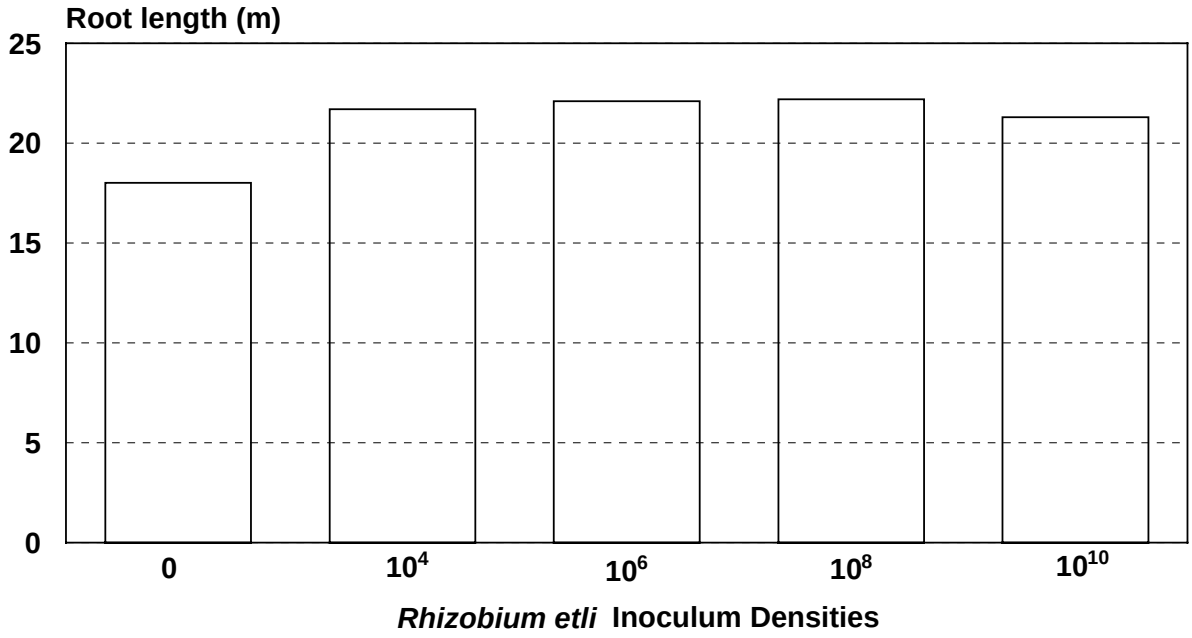


Figure 4: Effect of different inoculum densities of *Rhizobium etli* G12 on the root length of tomato infected with *Meloidogyne incognita* 8 weeks after nematode inoculation. The differences between treatment were not significant according to Duncan's Multiple Range Test ($P< 0.05$) n=8.

3.4 Biocontrol activity of *R. etli* G12 towards sugar beet cyst nematode, *H. schachtii*

3.4.1 Introduction

Oostendorp and Sikora (1989) showed that when sugar beet seeds were treated with rhizobacteria, the early root penetration of *H. schachtii* in sugar beet was reduced under greenhouse and field conditions. Neipp and Becker (1999) also found that rhizobacteria treated sugar beet reduced *H. schachtii* numbers in roots. This nematode can occur with *M. incognita* together and causes significant economic losses in sugar beet production in subtropic regions. The objective of this study was to evaluate the possible antagonistic effects of *R. etli* G12 towards *H. schachtii*.

3.4.2 Materials and Methods

Five millilitres of *R. etli* G12 bacterial suspension (10^{10}cfu/ml) were inoculated into the soil by pipetting it in three holes around the roots of three weeks old sugar beet plants cv. Ariana grown as described before (see 2.1). Three days later 1000 juveniles of *H. schachtii* were inoculated per plant by pipetting them into holes around the root zone. The soil substrate used was a non-sterilized soil-sand mixture (1:2, v/v). Plants receiving 5 ml Ringer-solution or treated with *R. etli* G12 or *H. schachtii* alone served as controls. Treatments were replicated 8 times in a completely randomised design under greenhouse conditions at 22°C with a 12 hours/day of artificial light. Plants were watered every day and fertilized every week with 10 ml per plant of Poly Crescol (14+10+14, 2g/liter water).

Eight weeks after nematode inoculation the plants were removed to determine fresh shoot and root weight, number of cysts per plant and number of eggs and juveniles per cyst and per plant.

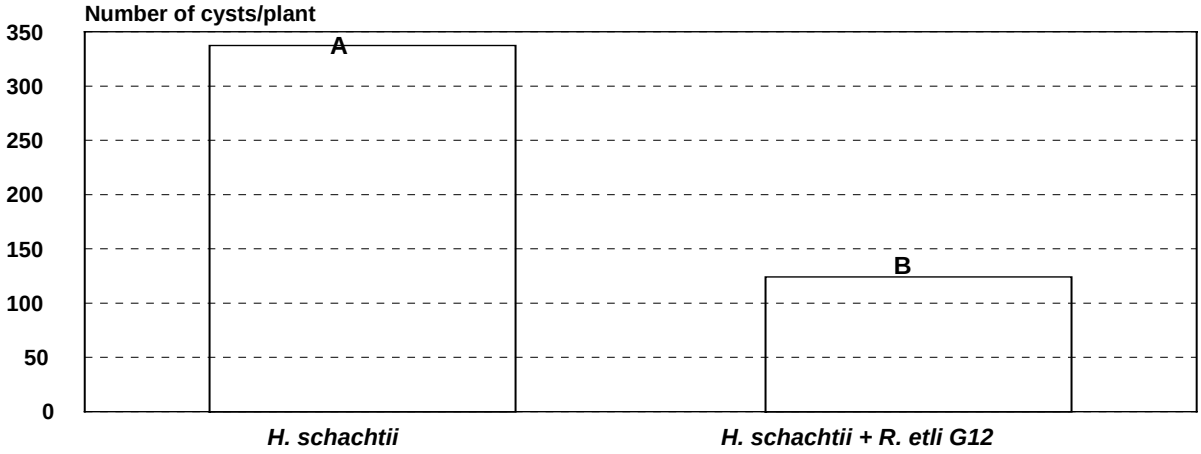
3.4.3 Results and Discussion

Results of this study showed a significant reduction in *H. schachtii* infection when plants were treated with *R. etli* G12 compared with the bacteria untreated plants. The number of cysts per plant was reduced significantly by 63% when *H. schachtii* infested plants were treated with *R. etli* G12 compared with the bacteria untreated plants (Fig. 1a). Results also showed that *R. etli* G12 reduced significantly the number of eggs and juveniles per plant by 65% (Fig. 1b). These results indicate that the rhizobacterium *R. etli* G12 may inhibit the early root penetration of *H. schachtii* into sugar beet. Similar results with other bacteria were obtained by Oostendorp and Sikora (1989). *R. etli* G12 is a gram-negative bacteria and it may have lectin binding structures. The reduction in nematode penetration may be related to the ability of this bacteria to bind to root surface lectins, thereby interacting with normal host recognition (Lotan *et al.*, 1975).

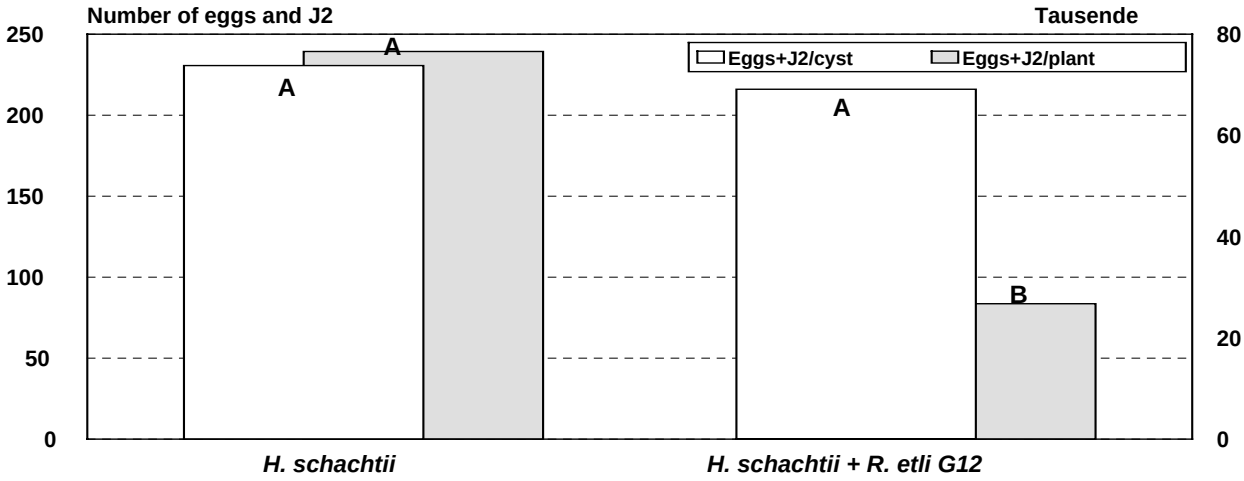
A non-significant reduction was detected for the number of eggs and juveniles per cyst compared with the plants treated with *H. schachtii* alone (Fig. 1b). The non-significant reduction in number of eggs and juveniles per cyst may indicate that *R. etli* G12 does not have the ability to affect the developmental process as shown by Kluepfel *et al*., (1993).

Results also revealed that *R. etli* G12 treated plants showed significant enhancement in both fresh shoot and root weight when compared with bacteria untreated plants (Fig. 1c). Data also showed no significant differences in fresh shoot and root weight in plants treated with *R. etli* G12 alone compared with the control (Data not shown). Although *R. etli* did not enhance root and shoot growth alone, the antagonistic activity of *R. etli* G12 toward *H. schachtii* did compensate for root loss due to *H. schachtii*.

(A)



(B)



(C)

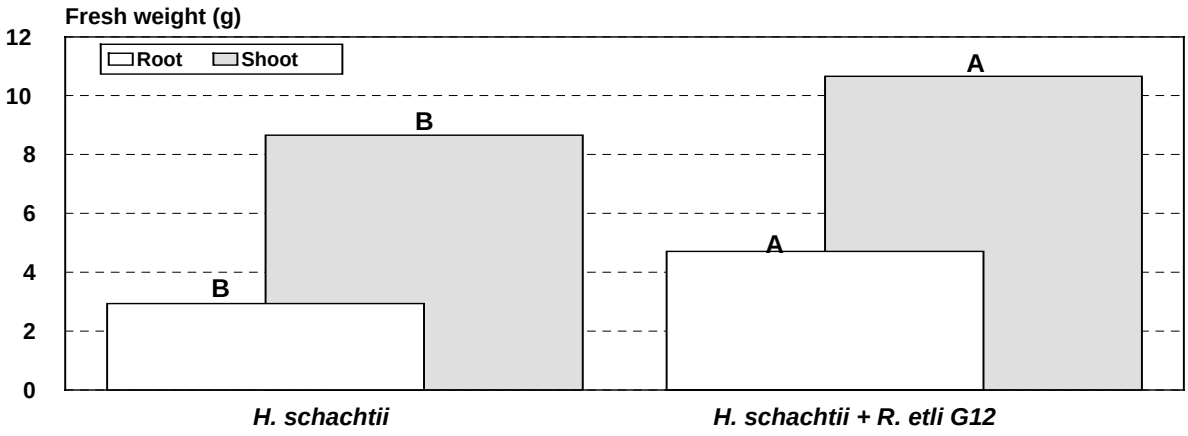


Figure 1: Effect of *Rhizobium etli* G12 on the number of *Heterodera schachtii* cysts/plant (A); number of eggs and juveniles/cyst and per plant (B) and on fresh shoot and root weight (C) of sugar beet. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P< 0.05$) n=8.

3.5 Biocontrol activity of *R. etli* G12 on potato cyst nematode, *G. pallida* reproduction

3.5.1 Introduction

A number of studies dealt the biological control of *G. pallida* on potato using rhizobacteria. Racke and Sikora (1985) found that when the potato tuber was treated with *R. etli* G12 it inhibited early root penetration of *G. pallida* in potato under greenhouse and field conditions. These bacteria were isolated from the potato rhizosphere. They also reduced the reproductive capability of *G. pallida* in one field trial. The objective of the following test was to re-confirmed the influence of *R. etli* G12 on the reproduction capacity of *G. pallida* under control conditions .

3.5.2 Materials and Methods

Three week old potato cv. Hansa grown as described before (see 2.1) were inoculated with a 5 ml *R. etli* G12 bacterial suspension (10^{10}cfu/ml) prepared as described before (see 2.2) and applied as a soil drench by pipetting into 3-4 holes around the root zone. Three days later the plants were inoculated with 1000 juveniles of *G. pallida* per plant by pipetting into 4 holes made around the root zone. The soil substrate was a soil-sand mix.(1:2, v/v). The soil used was a nonsterilized soil. Plants receiving 5 ml of Ringer-solution or treated with *R. etli* G12 or *G. pallida* alone served as controls. Each treatment was replicated 8 times in a completely randomised design under greenhouse conditions. Plants were watered daily and fertilized weekly with 10 ml per plant of Poly Crescol (14+10+14, 2g/liter water).

Plants were removed 8 weeks after nematode inoculation to evaluate the fresh shoot and root weight as well as number of cysts per plant, number of eggs and juveniles per cyst and per plant.

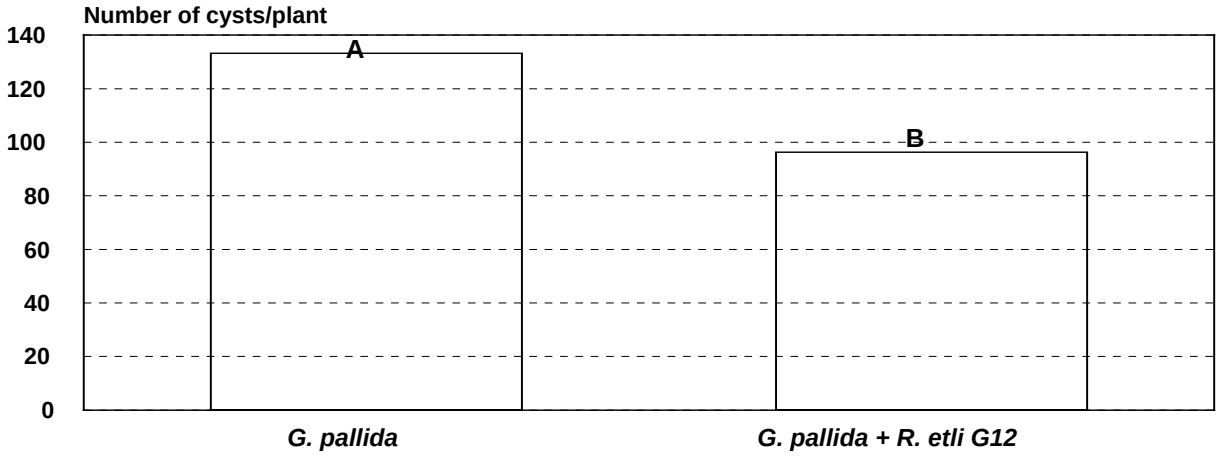
3.5.3 Results and Discussion

Results of this study revealed that all *R. etli* treated plants showed a significant reduction in nematode infection compared with the treated plants with *G. pallida* alone. Plants treated with *R. etli* G12 showed a significant reduction of 28% in the number of cysts per plant when compared with the plants treated with *G. pallida* alone (Fig. 1a). Results also showed that the number of eggs and juveniles per cyst and per plant were reduced significantly by 24 and 49% respectively for the plants treated with *R. etli* G12 combined with *G. pallida* compared with the *G. pallida* treated plants alone (Fig. 1b).

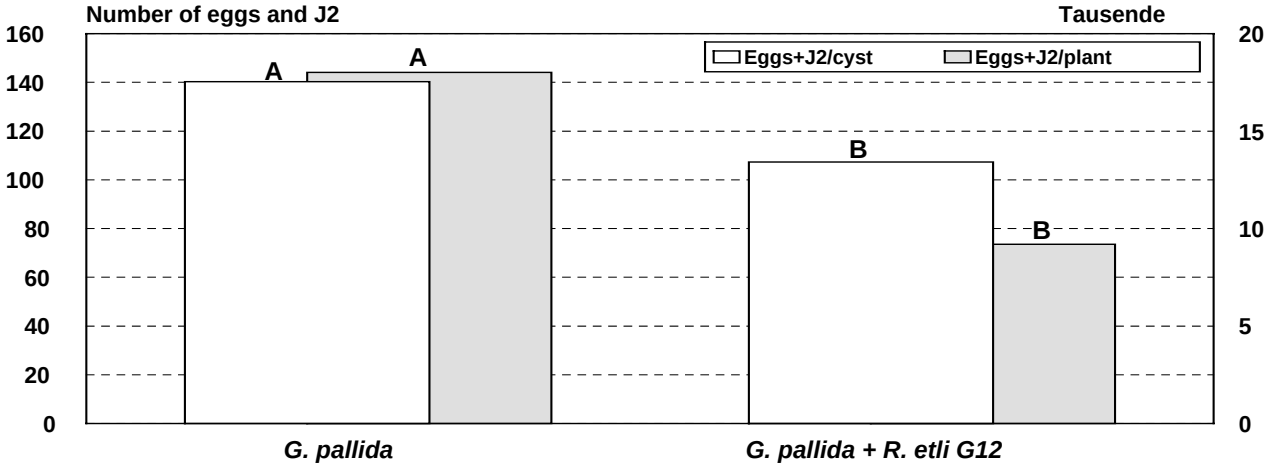
These results revealed that when potato plants were treated with the rhizobacterium *R. etli* G12, the number of cysts/plant, the number of eggs and juveniles per cyst and per plant were significantly reduced compared with the plants treated with *G. pallida* alone. Similar results were obtained by Racke and Sikora (1985; 1986). They found that when the rhizobacteria were applied to potato seed pieces it caused significant decreases in early root infection of the potato cyst nematode. Hackenberg and Sikora (1990) and Racke and Sikora (1992) confirmed that the antagonistic rhizobacterium *Agrobacterium radiobacter* (now *R. etli*) suppressed *G. pallida* early root penetration by 20 to 40% in greenhouse and field experiments. The reduction in *G. pallida* root penetration may be due to the heavily colonized root tips that may cause alteration of root exudates that affects nematode attraction to the root (Racke and Sikora 1992). Induced systemic resistance may also play a major role against *G. pallida* (Hallmann *et al*., 1998; Hasky-Günther *et al*.; 1998; Reitz *et al*., 2000)

Results also showed that there was no significant effect of *R. etli* G12 on plant shoot growth enhancement when compared with the plants treated with *G. pallida* alone. *R. etli* G12 treated plants combined with *G. pallida* showed non-significant enhancement in shoot weight (Fig. 1c). However plants treated with *R. etli* G12 combined with *G. pallida* showed significant enhancement in root weight compared with the plants treated with *G. pallida* alone (Fig. 1c). The significant enhancement in root weight for the bacteria treated plants combined with *G. pallida* may be due to colonization of *R. etli* G12 on or in the roots. This colonization may protect the root against nematode penetration and allows the roots to grow strongly.

(A)



(B)



(C)

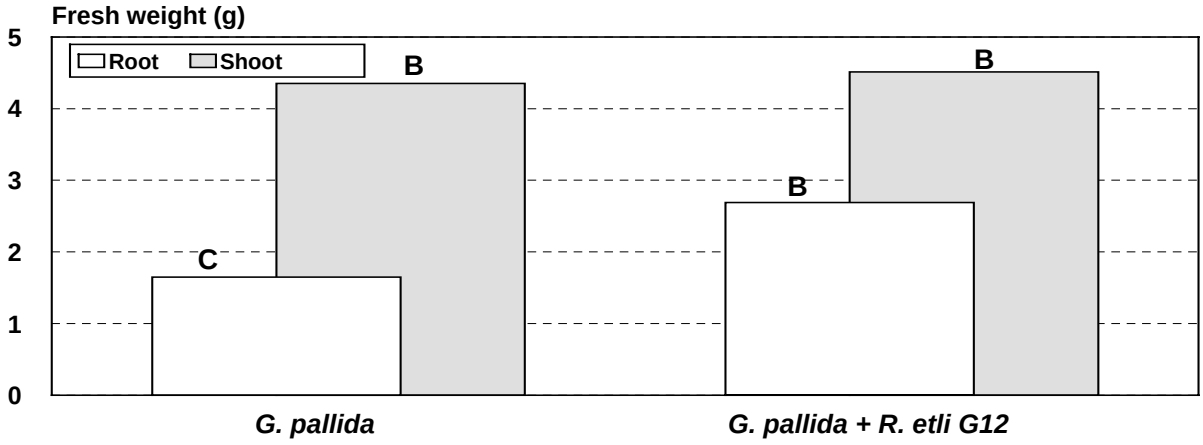


Figure 1: Effect of *Rhizobium etli* G12 on the number of *Globodera pallida* cysts/plant (A); number of eggs and juveniles/cyst and per plant (B) and on fresh shoot and root weight (C) of potato. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P < 0.05$) n=8.

3.6 Biocontrol activity of *R. etli* G12 towards root-lesion nematode, *P. zeae* on maize

3.6.1 Introduction

Several plant species are hosts for the migratory endoparasitic root-lesion nematode, *P. zeae* Graham. These nematodes cause high losses in important agricultural crops. Very little is known about the activity of rhizobacteria on migratory endoparasitic plant nematodes. Kluepfel *et al*., (1993) found that rhizobacteria seem to have the potential to reduce plant damage due to the ectoparasitic nematode *Criconemella xenoplax*. The objective of this study was to evaluate the ability of the rhizobacterium, *R. etli* G12 to control the root-lesion nematode, *P. zeae* on maize.

3.6.2 Materials and Methods

Three weeks old maize plants cv. Hybridmais Liberal grown as mentioned before (see 2.1) were inoculated with 5 ml of *R. etli* G12 bacterial suspension (10^{10}cfu/ml) as a soil drench. Three days after bacteria inoculation, 600 juveniles of *P. zeae* produced as described before (see 2.3) were pipetted into 3-4 holes around the roots. Plants treated only with Ringer-solution or inoculated with *R. etli* G12 or *P. zeae* plants served as controls. Treatments were replicated 8 times in a completely randomised design in the greenhouse at 25°C with a 12 hours/day of artificial light. Plants were watered daily and fertilized weekly with 10 ml per plant of Poly Crescol (14+10+14, 2g/liter water).

Fresh shoot and root weight as well as number of nematodes per root system were measured 8 weeks after nematode inoculation. The number of nematodes per root system were determined by dipping the roots of treated plants either with *P. zeae* alone or combined with *R. etli* G12 separately into glass tubes containing lactic acid/fuchsin acid (0.1%) staining solution. Roots were left for 30 minutes in the staining solution and then heated in a microwave for 10-20 seconds to stain the nematode inside the roots (Ferris, 1985). The roots were washed to remove the residual staining solution and then were macerated in water using an Ultra-Turrax (20,000 rpm). Stained nematodes were then counted using a binocular and a counting dish.

3.6.3 Results and Discussion

Results of this study revealed that plants treated with *R. etli* G12 showed non-significant reduction in the number of *P. zeae* nematodes per root system compared with the bacteria untreated plants (Fig. 1).

Results also showed that there was no significant difference in the number of nematode per gram root compared with the bacteria untreated plants (Fig. 1).

The results demonstrated that *R. etli* G12 does not have an antagonistic potential toward *P. zeae* on maize. The findings were not in agreement with those obtained by Kluepfel and McInnis (unpublished data) who found that some strains of rhizobacteria stimulated nematode population increase. These results infer that the mode of action of bacteria differed between nematodes of different genera of plant parasitic nematodes (Sikora and Hoffmann-Hergarten,1993; Hasky-Günther, 1996; Hoffmann-Hergarten *et al*., 1998).

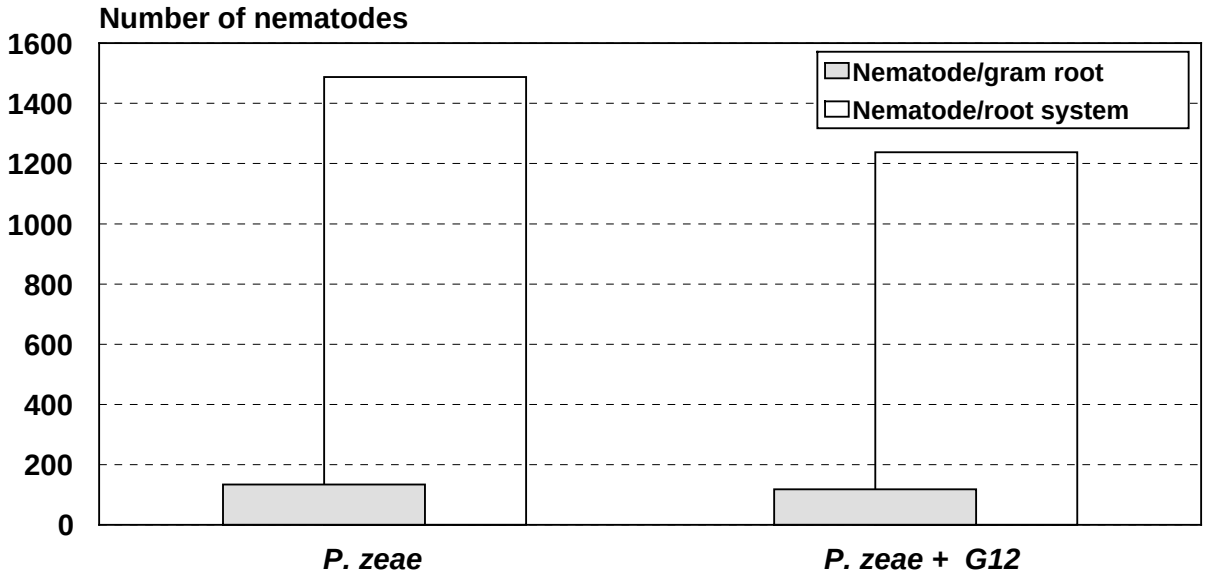


Figure 1: Effect of *Rhizobium etli* G12 on the number of *Pratylenchus zeae* on maize eight weeks after nematode inoculation. Results not significantly different according to Duncan's Multiple Range Test ($P < 0.05$) n=8.

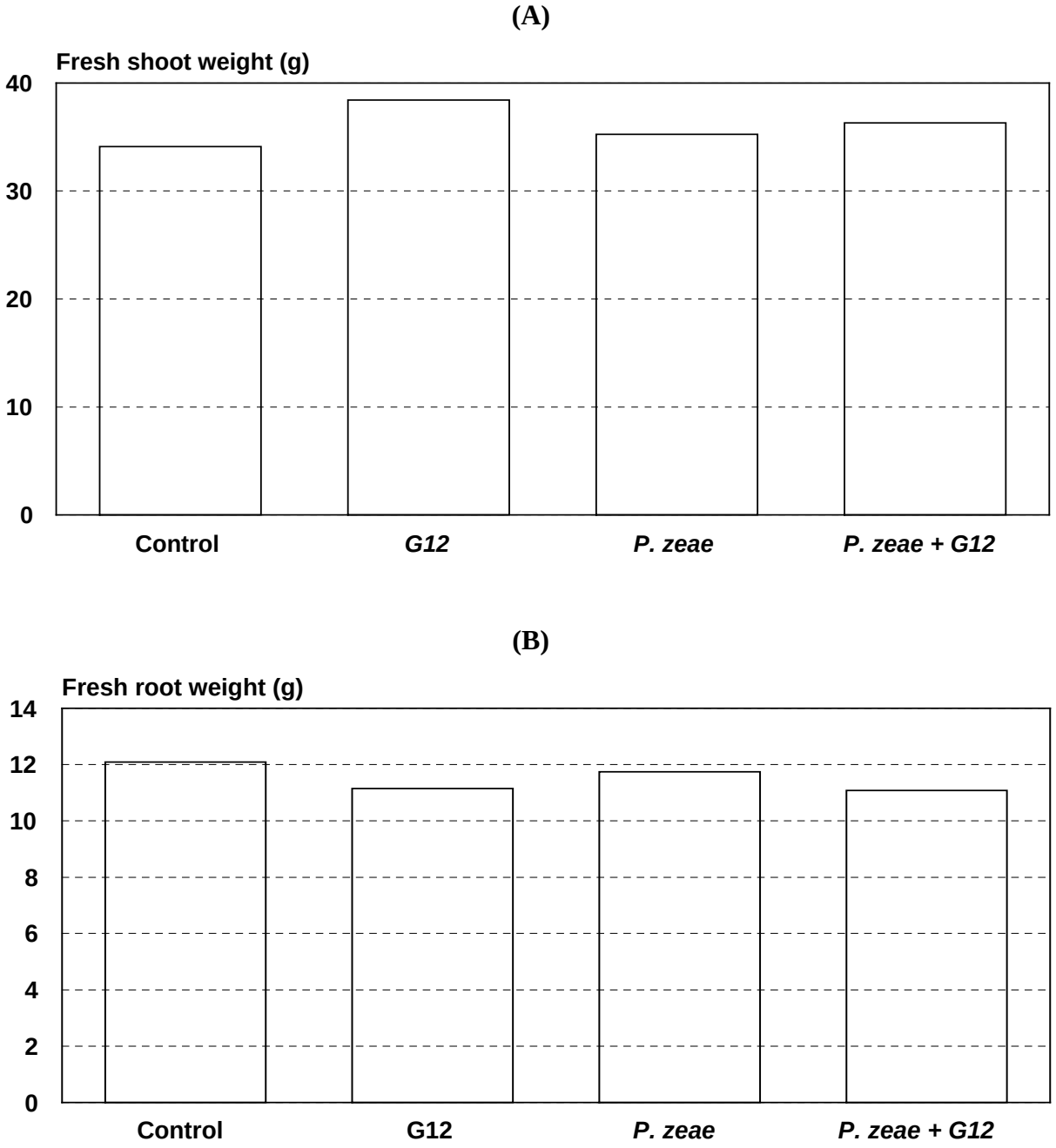


Figure 2: Effect of *Rhizobium etli* G12 on the fresh shoot (A) and root (B) weight of maize infected with *Pratylenchus zeae* eight weeks after nematode inoculation. Results were not significantly different according to Duncan's Multiple Range Test ($P < 0.05$) n=8.

R. etli treated plants showed no significant enhancement in either fresh shoot (Fig. 2a) or root (Fig. 2b) weight compared with non-bacterized plants. These results may show that maize roots are not supportive of *R. etli* G12 establishment which also resulted in the lack of antagonistic activity of *R. etli* G12 against *P. zeae*.

3.7 General Conclusions

A) *Rhizobium etli* strain G12 exhibited strong biocontrol activity towards the root-knot nematode, *Meloidogyne incognita* on different host plants. The reduction rates, however, varied between the different crops.

B) *R. etli* G12 had the ability to control three economically important species within the genus *Meloidogyne*. However, the reduction varied greatly between the different species. The highest reduction was against *M. incognita* and *M. javanica*, whereas *R. etli* had little effect on *M. arenaria.*

C) Increasing inoculum densities of *R. etli* G12 caused increased reductions in nematode infection. Biocontrol required a minimum density of 10^8 cfu/ml for root galling reduction whereas a reduction in number of galls occurred at 10^{10} cfu/ml. Significant reductions in egg masses was detected at bacterial densities of 10^6 to 10^{10} cfu/ml.

D) *R. etli* G12 treated plants caused significant reductions in sugar beet cyst nematode, *Heterodera schachtii* infection. *R. etli* reduced significantly the number of cysts/plant and the number of eggs and juveniles/plant. The bacteria did not affect the number of eggs and juveniles per cyst and therefore the reproduction activity of the individual female.

E) *R. etli* G12 reduced infection by the potato cyst nematode, *Globodera pallida*. The number of cysts/plant, number of eggs and juveniles per cyst and per plant were reduced significantly when plants were treated with *R. etli* G12 combined with *G. pallida*.

F) *R. etli* G12 had no effect towards the migratory endoparasitic root-lesion nematode, *Pratylenchus zeae* on maize.

4. References

Adeniji, M. O., E. Gichure, and J. Muigai. 1979. Distribution and crop association study of plant parasitic nematodes in Kenya. Pp. 98-131 *in* C. N. Karue, and D. M. Mukunya, eds. Second report of the university of Nairobi plant protection programme. Nairobi, Kenya.

Ayoub, S. M. 1980. Plant nematology-an agricultural training aid. Pp. 81, Sacramento, CA, USA, Nema Aid Publication.

Becker, J. O., E. Zavaleta-Mejia, S. F. Colbert, M. N. Schroth, A. Weinhold, J. G. Hancock, and S. D. Van Gundy. 1988. Effects of rhizobacteria on root-knot nematodes and gall formation. Phytopathology 78:1466-1469.

Becker, J. O., S. F. Colbert, M. N. Schroth, and S. D. Van Gundy. 1989. Field evaluation of a rhizobacterial strain for affecting root-knot nematodes. Biological and Cultural Tests for Control of Plant Diseases 4:31.

Behren, E. C. 1975. *Globodera* Skarbilovich, 1959, eine sebstandige Gattung in der Unterfamilie Heteroderinae Skarbilovich, 1947 (Nematoda: Heteroderinae). Pp. 12-26, *in* Vortragstagung 24 Aktuellen Problemen der Phytonematologie in Rostock.

Bridge, J. 1994. Priorities in plant pathology, a national and regional view. Pp. 22-24 *in* J. A. Sutherland, eds. Crop protection and the Kenyan smallholder farmer. National Agricultural Research Laboratories, Nairobi, Kenya.

Chitwood, B. G. 1949. Root-knot nematodes. Proceedings of Helminthological Society of Washington 16:90-104.

Cronin, D., Y. Moenne-Loccoz, A. Fenton, C. Dunne, D. Dowling, and F. O' Gara. 1997. Role of 2,4-diacetylphloroglucinol in the interactions of the biocontrol pseudomonad strain

F113 with the potato cyst nematode *Globodera rostochiensis*. Applied and Environmental Microbiology 63:1357-1361.

Daykin, M. E., and R. S. Hussey. 1985. Staining and histopathological techniques in nematology. Pp. 39-48 *in* K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise in *Meloidogyne*, Vol. II Methodology, Raleigh: North Carolina State University Graphics.

Ferris, J. M. 1985. Crop loss prediction and modelling for management decisions. Pp. 27-33 *in* B. N. Zuckermann, W. F. Mai, and M. B. Harrison, eds. Plant nematology-laboratory manual. University of Massachussetts, Agriculture Experimental Station, Amherst.

Fortuner, R. 1976. *Pratylenchus zeae*. C. I. H. Descriptions of plant-parasitic nematodes. Set 6, No. 77. Commonwealth Institute of Helminthology: St. Albans, England.

Graham, T. W. 1951. Nematode root rot of tobacco and other plants. South Carolina Agriculture Experimental Station Bulletin 390:25.

Hackenberg, C., and R. A. Sikora. 1990. Influence of abiotic and biotic factors on the interrelationship between plant health promoting rhizobacteria and *Globodera pallida* on potato. Nematologica 36:355-356.

Hallmann, J. 2001. Plant interactions with endophytic bacteria. In press *in* M. J. Jeger, and N. J. Spence, eds. Biotic interactions in plant-pathogen associations. CAB International, Wallingford, United Kingdom.

Hallmann, J., K. Hasky-Günther, S. Hoffmann-Hergarten, M. Reitz, and R. A. Sikora. 1998. Similarities and differences in the mode of action of two rhizosphere bacteria antagonistic to *Globodera pallida* on potato. IOBC Bulletin 21:41-43.

Hasky-Günther, K. 1996. Untersuchungen zum Wirkungsmechanismus der antagonistischen Rhizosphärebakterin *Agrobacterium radiobacter* isolate G12 und *Bacillus sphaericus* isolate B43 gegenüber dem kartoffelzystennematoden *Globodera pallida* an Kartoffel. Ph.D. disseratation, Bonn University, Bonn, Germany.

Hasky-Günther, K., S. Hoffmann-Hergarten, and R. A. Sikora. 1998. Resistance against the potato cyst nematode *Globodera pallida* systematically induced by the rhizobacteria *Agrobacterium radiobacter* G12 and *Bacillus sphaericus* B43. Fundamental and Applied Nematology 21:511-517.

Hoffmann-Hergarten, S., M. K. Gulati, and R. A. Sikora. 1998. Yield response and biological control of *Meloidogyne incognita* on lettuce and tomato with rhizobacteria. Journal of Plant Diseases and Protection 105:349-358.

Hollis, J. P. 1962. A survey of plant parasitic nematodes and their control in Kenya. F.A.O. Plant Protection Bulletin 10:97-106.

Honglin, T., W. Shouhua, and W. Xiuying. 1995. Screening of rhizobacteria antagonistic to plant-parasitic nematodes. Acta Agriculturae Universitatis Pekinensis 21:11-15.

Keel, C., B. Koller, and G. Defago. 1991. Plant growth-promoting rhizobacteria progress and prospects. Pp. 418, Proceeding of the Second International Workshop on Plant Growth-Promoting Rhizobacteria. Interlaken, Switzerland.

Keuken, O. 1996. Interaktionen zwischen dem Rhizosphärebakterium *Bacillus cereus*, dem Wurzelgallennematoden *Meloidogyne incognita* und tomate. Ph.D. disseratation, Bonn University, Bonn, Germany.

King, E. O., M. K. Warth, and D. E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. Journal of Laboratory and Clinical Medicine 44:301-307.

Kloepper, J. W., R. Rodriguez-Kabana, J. A. McInroy, and R. W. Young. 1992. Rhizosphere bacteria antagonistic to soybean cyst (*Heterodera glycines*) and root-knot (*Meloidogyne incognita*) nematodes: Identification by fatty acid analysis and frequency of biological control activity. Plant and Soil 139:75-84.

Kluepfel, D. A., T. M. McInnis, and E. I. Zehr. 1993. Involvement of root-colonizing bacteria in peach orchard soils suppressive of the nematode *Criconemella xenoplax*. Phytopathology 83:1240-1245.

Kofoid, C. A., and W. A. White. 1919. A new nematode infection in man. Journal of American Medicin Association 72:567-569.

Lotan, R., N. Sharon, and D. Mirelman. 1975. Interaction of wheat gram agglutinin with bacterial cells and cell wall polymers. European Journal of Biochemistry 55:257-262.

Luc, M., and G. Reversat. 1985. Possibilites et limites des solutions genetiques aux affections provoquees par les nematodes sur les cultures tropicales. Comptes Rendus des Seances de L' Academie d' Agriculture de France 71:781-791.

Neal, J. C. 1889. The root-knot disease of the peach, orange and other plants in Florida, due to the work of the *Anguillula*. Bulletin of U. S Bur. Ent. 20:1-31.

Neipp, P. W., and J. O. Becker. 1999. Evaluation of biocontrol activity of rhizobacteria from *Beta vulgaris* against *Heterodera schachtii*. Journal of Nematology 31:54-61.

Oostendorp, M., and R. A. Sikora. 1989. Seed treatment with antagonistic rhizobacteria for the suppression of *Heterodera schachtii* early root infection of sugar beet. Revue de Nematologie 12:77-83.

Oostendorp, M., and R. A. Sikora. 1990. In vitro interrelationships between rhizosphere bacteria and *Heterodera schachtii*. Revue de Nematologie 13:269-274.

Racke, J., and R. A. Sikora. 1985. Einfluss von Rhizosphärenbakterin auf *Rhizoctonia solani* und den Befall der kartoffelsorte Hansa mit *Globodera pallida*. Biotechnologische Vorträge zur Resistenzselektion, Vorträge für Pflanzenzüchtung Statusseminar Grünbach, 9: 21-28.

Racke, J., and R. A. Sikora. 1986. Influence of rhizobacteria on *Globodera pallida* early root infection and *Erwinia caratovora* tuber soft rot of potato. Revue de Nematologie 9:305.

Racke, J., and R. A. Sikora. 1992. Isolation, formulation and antagonistic activity of rhizobacteria toward the potato cyst nematode *Globodera pallida*. Soil Biology and Biochemistry 24:521-526.

Reitz, M., S. Hoffmann-Hergarten, J. Hallmann, and R. A. Sikora. 2001. Induction of systemic resistance in potato by rhizobacterium *Rhizobium etli* strain G12 is not associated with accumulation of pathogensis-related proteins and enhanced lignin biosynthesis. Journal of Plant Diseases and Protection 108:11-20.

Reitz, M., K. Rudolph, I. Schröder, S. Hoffmann-Hergarten, J. Hallmann, and R. A. Sikora. 2000. Lipopolysaccharides of *Rhizobium etli* strain G12 act as inducing agent of systemic resistance in potato towards infection by the cyst nematode *Globodera pallida*. Applied and Environmental Microbiology 66:3515-3518.

Sasser, J. N., and D. W. Freckmann. 1987. A world of perspective on nematology. The role of society. Pp. 7-14, *in* Veech, J. A., and D. W. Dickson, eds. Vistas on nematology. Hyattsville, Maryland, USA.

Schmidt, A. 1871. Über den Rüben-Nematoden (*Heterodera schachtii* A. S.). Zeitschriften Ver. Rübenzucker-Ind. Zollver. 21:1-119.

Schmidt, K. 1992. Zeitdiskrete modelle zur vorhersage der populationdynamik der rübenzystnematoden *Heterodera schachtii* (Schmidt) in abhängigkeit von der Fruchtfolge und des temperaturmusters. Ph.D. disseratation, Bonn University, Bonn, Germany.

Schmiedeknecht, G., H. Bochow, and H. Junge. 1998. Use of *Bacillus subtilis* as biocontrol agent. II. Biological control of potato disease. Journal of Plant Diseases and Protection 105:376-386.

Schönbeck, F., U. Steiner, and T. Kraska. 1993. Induced resistance: criteria, mechanisms, practical application and estimation. Journal of Plant Diseases and Protection 100:541-557.

Sikora, R. A. 1988. Interrelationships between plant health-promoting rhizobacteria, plant parasitic nematodes and soil microorganisms. Mededelingen Faculteit Landbouwkundige Rijksuniversiteit Gent 53:867-878.

Sikora, R. A., and S. Hoffmann-Hergarten. 1993. Biological control of plant parasitic nematodes with plant-health promoting rhizobacteria. Pp. 166-172, *in* Lumsden, R. D., and J. L. Vaughn, eds. Pest Management: Biologically Based Technologies. American Chemical Society, Washington, DC.

Smith, L. J. 1994. The potential of rhizobacteria as biological control agents of root-knot nematode. Thesis, University of Queenland, Australia.

Spiegel, Y., E. Cohn, S. Galper, E. Sharon, and I. Chet. 1991. Evaluation of a newly isolated bacterium, *Pseudomonas chitinolytica* sp. nov., for controlling the root-knot nematode *Meloidogyne javanica*. Biocontrol Science and Technology 1:115-125.

Stone, A. R. 1973. *Heterodera pallida* n. sp. (Nematoda:*Heterodera*) a second species of potato cyst nematode. Nematologica 18:591-606.

Treub, M. 1885. Onderzoekingen over Sereh-Ziek Suikkeriet gedaan in s'Lands Plantentuin te Buitenzorg. Medad. PlTuin., Batavia:1-39.

Trolldenier, G. 1979. Effects of mineral nutrition of plant and soil oxygen on rhizosphere organisms. Pp. 235-240 *in* Schippers and Gams, eds. Soil-Borne Plant Pathogens. Academic press, London.

Whitehead, A. G. 1972. Cyst nematode of potatoes and sugar-beet: biology and control. Span 15:114-117.

Wollenweber, H. W. 1923. Krankheiten und Beschädigungen der Kartoffel. Arb. Forschungsinstitut für Kartoffelbau 7:1-56.

Zeck, W. M. 1971. A rating scheme for field evaluation of root-knot nematode infestations. Pflanzenschutznachrichten Bayer 24:141-144.

1. General Introduction

Pasteuria penetrans (Thorne) Sayre and Starr is a gram positive bacterium and one of the most promising biological control agents of root-knot nematodes, *Meloidogyne* spp. This bacterium is a widespread obligate spore-forming parasite with a mycelial-like vegetative stage (Brown *et al*., 1985; Stirling 1991; Dickson *et al*., 1994; Chen *et al*., 1996). *P. penetrans* prevents *Meloidogyne* spp. reproduction (Sayre and Werign, 1977) and reduces the ability of juveniles to penetrate roots. The attachment of the endospores to the cuticle of the nematodes is the first step in the life cycle of the bacterium and is essential for its reproduction (Freitas *et al*., 1997). *Pasteuria* spp. have been associated with more than 200 different nematode species belonging to 100 nematode genera (Sayre and Starr, 1988; Sturhan, 1989). More recently *P. penetrans* has shown great potential in suppressing field populations of several plant parasitic nematodes throughout the world (Fulton, 1998), especially root-knot nematodes, *Meloidogyne* spp.(Dickson *et al*., 1994; Chen and Dickson, 1998).

Life cycle of *P. penetrans*

The non-motile endospores of *Pasteuria* attach to the cuticle of second stage juveniles when juveniles migrate through the soil. Nematodes with 10-15 attached spores are less capable of invading roots (Davies *et al*., 1988). Nematodes that carry fewer than 5 spores may escape parasitism because the spores become detached before or during root invasion (Ratnasoma *et al*., 1991) or because the spores fail to germinate. However, when the juveniles have an optimum number of 5-10 spores/juveniles of *P. penetrans* spores attached to their cuticle, the spores begin to germinate after the juveniles penetrate into roots. The germ tube from the spores penetrates the nematode cuticle and microcolonies are formed that proliferate through the body. These colonies develop into a vegetative spherical colony consisting of dichotomously branched, septate mycelium (Sayre and Starr, 1988). The cub-shaped sporangia develops to endospores that completely fill the nematode body (Fig. 1). Infected females of *Meloidogyne* spp. can contain up to 2.5 million non-motile endospores (Hewlett and Dickson, 1993) that are released into the soil environment upon degradation of the nematode carcass.

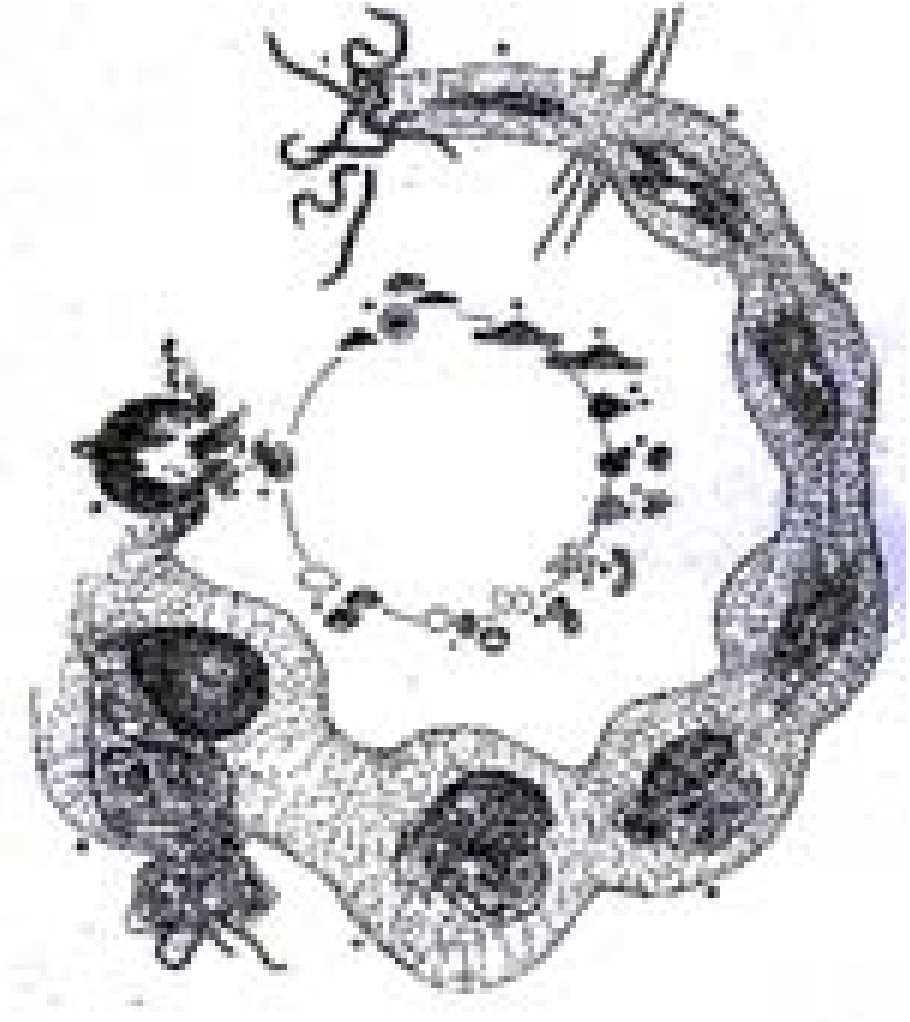


Figure 1: Life cycle of *Meloidogyne* spp. with and without *Pasteuria penetrans* (Fulton, 1998)

Outer cycle

1) juveniles penetrate root tip, **2**) migrating intercellularly in cortex, **3**) juveniles establishing feeding sites in the vascular system, *P. penetrans* endospores germinate, **4**) third-stage juveniles, **5**) fourth-stage juveniles, and **6,7**) young females, **8**) female infected with *P. penetrans* lays no eggs, whereas a healthy female forms an egg mass, and **9**) infected female body degrades and releases mature endospores into soil.

Inner cycle

The inner cycle illustrates the life cycle of *P. penetrans* and its various developmental stages. **a**) mature endospores, **b**) endospore attached to cuticle of *Meloidogyne*, **c**) germinating endospore, **d**) microcolonies formed, **e**) septations in rapidly growing thallus, **f**) dichotomously branched hyphae with elongated terminal cells, **g**) fragmented thalli separated from the thallus and visible forespore, **h**) cell wall separates forespore from parasporium of the egg-shaped sporangium, **i**) differentiation of spore core and perisporal fibers, **j**) mature endospores surrounded by exosporium and sporangium, and **k**) endospores released into soil.

2. General Materials and Methods

2.1 Root-knot nematodes, *Meloidogyne* spp.

The cultures of the different root-knot nematode species of *Meloidogyne* were discussed in chapter II (see 2.3). Eggs of *Meloidogyne* spp. were extracted from infected tomato roots by using 1.05% sodium hypochlorite according to the modified method of Hussey and Barker (1973) as described in chapter II (see 2.3). The eggs collected on a 20µm sieve were poured into a Duran bottle filled with 500 ml tap water and aerated continuously for 10 days at room temperature. The hatched juveniles were separated from the unhatched eggs using the method of Oostenbrink (1960) and the number of juveniles/ml was counted. Gall index was measured as described by Zeck (1971) on a scale from 0-10.

2.2 *Pasteuria penetrans*

2.2.1 *P. penetrans* Isolates

Seven *P. penetrans* isolates were evaluated for their ability to attach to *M. incognita* juveniles in the first experiment. The seven *P. penetrans* isolates tested were:

Country	Abbreviation	Country	Abbreviation
Barbados	Pp Bar	**South Africa**	Pp3
Great Britain	Pp GB	**Papua New Guinea**	Pp PNG
Malawi	Pp Mal	**Australia**	Pp1
Ivory Coast	Pp IvC		

These isolates were obtained from Prof. Dr. Simon R. Gowen, Department of Agriculture, Reading University, Early Gate Reading, RG6 2AT, United Kingdom.

2.2.2 *P. penetrans* Spore Suspension

Spore suspensions of the different *P. penetrans* isolates were prepared by adding 0.1 gram of *Pasteuria* root powder to a small amount of tap water in a pestle and morter. After mixing thoroughly the root debris was removed by pouring the suspension through a 25µm sieve (Stirling and Wachtel, 1980). The concentration of the spore suspension passing through the sieve was measured with a Fuchs Rosenthal slide.

2.2.3 *P. penetrans* Multiplication

The multiplication of *P. penetrans* was carried out in two phases:

In vitro: One thousand second stage juveniles of *Meloidogyne javanica* were added to 1 ml of a *P. penetrans* spore suspension adjusted to 10^5 spores/ml and 4ml distilled water in 5 cm diameter Petri dishes and incubated for 24 hours at a room temperature of approximately 20°C. After 24 hours the number of attached spores per juvenile was counted. Then the juveniles with attached spores were separated by pouring the suspension through a 20µm sieve to collect the juveniles with attached spores on the sieve. One thousand juveniles encumbered with Pp spores were then inoculated per plant by pipetting the solution around the roots of tomato cv. Hellfrucht Frühstamm in plastic pots filled with a non-sterilized sand-soil mixture (2:1, v/v). The tomatoes were maintained at 30°C with 16 hours (light) and 8 hours (dark) in a climatic chamber. After 750 degree days (approximately 40 days at the base temperature 10°C) the root systems were removed, washed and air-dried. The dried roots were then ground in a grinder until it became powder like. Alternatively, the root systems were soaked in tap water in glass beakers for 4 days or until the root softened. *P. penetrans* infected females were then removed with a twisser and collected in distilled water in small Petri dishes and stored in a refrigerator at 9°C. The *Pasteuria* root powder (Stirling and Wachtel, 1980) and the *Pasteuria* infected females served as a source of *P. penetrans* inoculum.

2.3 Statistical Analysis

Data were analysed according to standard analysis of variance by a one way ANOVA with the software statgraphics (Statistical Graphics Crop., Rockville, MD). Variance homogeneity for all treatments was confirmed by the Bartlett test. The comparison between means was carried out either with the Duncan's Multiple Range Test or by using the T-Test at $P<0.05$ as given in the tables and/or figures.

3. Experimental Program

3.1 Attachment rate of seven *P. penetrans* isolates at different spore concentrations to *M. incognita* juveniles using two passive exposure methods

3.1.1 Introduction

Hewlett and Dickson (1993) suggested that rapid endospore attachment to nematodes can be achieved using a centrifuge technique. The most common method of stimulating attachment of *P. penetrans* endospores to nematode juveniles, however, is by making a nematode-endospore water suspension that is left stationary or agitated at room temperature for 24 hours as reported by Hewlett and Serracin (1996).

P. penetrans have a high degree of specificity among populations of the parasite. For example, spores of populations from *M. incognita* and *Pratylenchus brachyurus* attached only to *Meloidogyne* spp. and *P. brachyurus* respectively and not to the range of other nematode tested (Dutky and Sayre, 1978). Davies *et al*., (1988) suggested that among *P. penetrans* isolates the greatest spore attachment occurred when spores were exposed to the species of *Meloidogyne* from which they were originally isolated. On the other hand tests by Stirling (1985) with *P. penetrans* isolates from *M. javanica* and *M. incognita* showed that spore attachment was not always related to the species of the recipient nematode. Stirling (1991) also found that even within isolates parasitic in the same nematode there is considerable variation in the attachment capacity of *P. penetrans* infecting nematodes in that genus. He also found that spores collected from root-knot nematode in one location do not always attach to and infect populations from other locations. Sharma and Davies, (1996) found that certain populations of *Pasteuria* can attach to a wide range of nematodes, even attach to nematodes from different genera, whereas other populations show a restricted host range (Stirling, 1985).

In some studies high attachment of *P. penetrans* spores to nematode juveniles was found with increasing spore concentrations (Sharma and Davies, 1996).

The objectives of the following investigations were to evaluate whether exposure method, *P. penetrans* isolate or spore concentration affects attachment of *P. penetrans* endospores to the cuticle of *M. incognita* juveniles.

3.1.2 Experimental Design

3.1.2.1 Test to determine optimum method to stimulate attachment

Materials and Methods

This test was carried out for the seven *P. penetrans* isolates. The *P. penetrans* spore suspensions of the seven isolates were prepared as described before (see 2.2.2). *M. incognita* juveniles were extracted as mentioned before (see 2.1). Approximately 1000 juveniles in 1 ml water were added to 10 glass centrifuge tubes. A 1 ml *P. penetrans* spore suspension (5.0 x 10^5 spores/ml) was then added to the tubes with 4 ml distilled water.

Two methods to stimulate attachment were compared for the seven *P. penetrans* isolates.

A) Centrifugation: the centrifuge tubes were incubated for 30 minutes at room temperature 20°C and then centrifugated at 3000 rpm (Hettich Universal II Centrifuge) for 10 minutes. After 10 minutes the number of spores attached to the nematode cuticle were counted on 50 juveniles of *M. incognita* randomly selected under a microscope (X400).

B) Incubation: the other centrifuge tubes were incubated for 24 hours at room temperature 20°C without centrifugation. After 24 hours the number of attached spores per juvenile were counted on 50 juveniles of *M. incognita* randomly selected under a microscope (X400).

Results and Discussion

The results revealed that 20% less spores attached to the juveniles with the centrifuge method than when incubated in water. There were, however, no statistically significant differences

between the two methods used to stimulate attachment efficacy of the seven tested isolates of *P. penetrans*. The mean number of attached spores ranged between 0.2-1.2 spore/juvenile and was very low (Fig. 1).

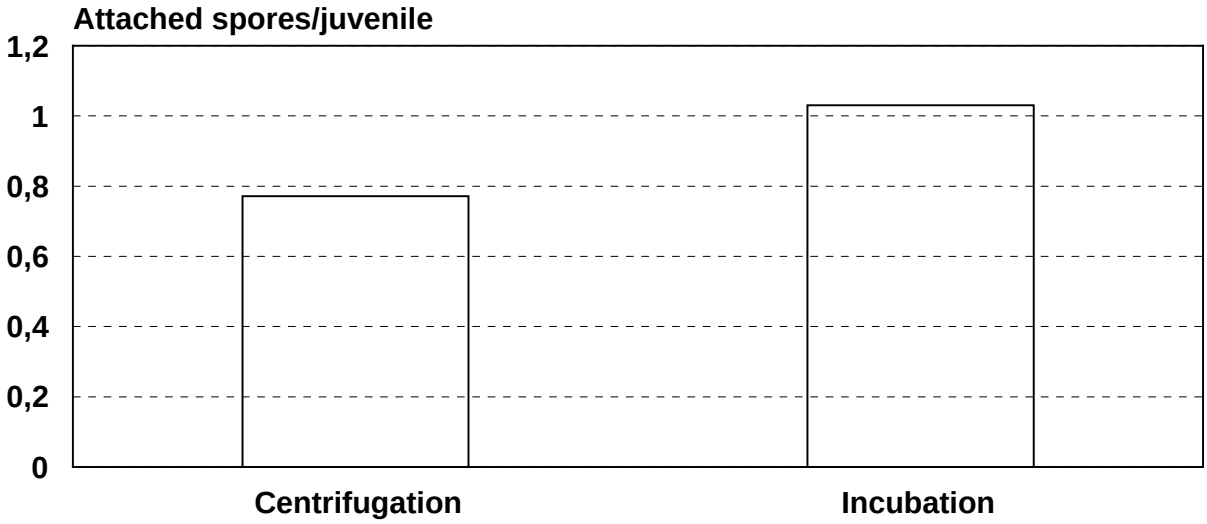


Figure 1: Effect of different exposure methods on the attachment of *Pasteuria penetrans* spores to *Meloidogyne incognita* juveniles. The differences between methods was not significant according to T-Test ($P<0.05$).

Although there were no significant differences between the two attachment methods, the centrifugation method was selected for all further tests as it is a rapid technique for attaching spores to the nematode. Hewlett and Dickson (1993) found that when using the centrifugation method to attach endospores to nematode juveniles most of the attached endospores were without sporangial walls. When the endospores descend on the nematode surface, the centrifugal force ruptures the sporangial wall and allows the endospore to contact the surface of the juvenile cuticle. This process also may result in the sloughing off of the sporangial wall and exosporium because these layers are not connected to the endospore and then the peripheral fibers conform with the juvenile cuticle to establish the attachment as described by Chen *et al*., (1997).

3.1.2.2 Influence of the origin of *P. penetrans* spores on attachment rate

Materials and Methods

The seven isolates of *P. penetrans* used in this study were: Barbados (Pp Bar), Great Britain (Pp GB), Malawi (Pp Mal), Ivory Coast (Pp IvC), South Africa (Pp3), Papua New Guinea (Pp PNG) and Australia (Pp1).

Spore suspensions of the different *P. penetrans* isolates were prepared as described before (see 2.2.2) and the centrifugation method was used in this test (see 3.1.2.1).

Results and Discussion

Results showed that three of the seven *P. penetrans* isolates attained a 50% higher and significantly different attachment rate to *M. incognita* juveniles. These three isolates originated from Great Britain, Ivory Coast and Papua New Guinea (Fig. 2). The other four isolates had a lower attachment rate. Pp Barbados isolate showed absolutely no attachment to *M. incognita* juveniles.

The isolates which attached to nematode juveniles had been originally isolated from the same *Meloidogyne* species. This effect is known, because isolates of *Pasteuria* are highly specific and the greatest attachment of spores occurred when they were exposed to the species of *Meloidogyne* from which they were originally isolated as suggested by Davies *et al*., (1988); Espanol *et al*., (1997) and Regina *et al*., (1999). Channer and Gowen (1992) also found that different populations of *M. incognita* varied in their susceptibility to spore attachment. In Fig. 3 a juvenile with a high level of spore attachment is seen.

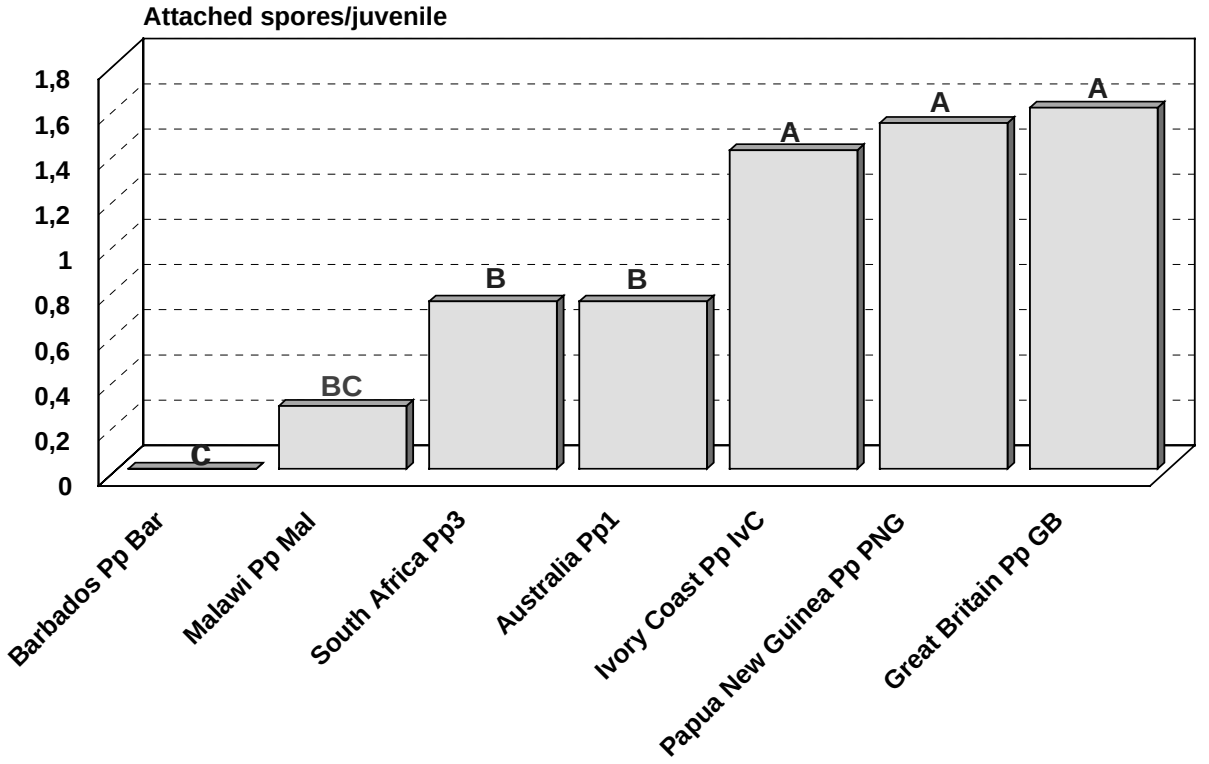


Figure 2: Attachment of different *Pasteuria penetrans* isolates to *Meloidogyne incognita* juveniles using the centrifuge method. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test (P< 0.05).

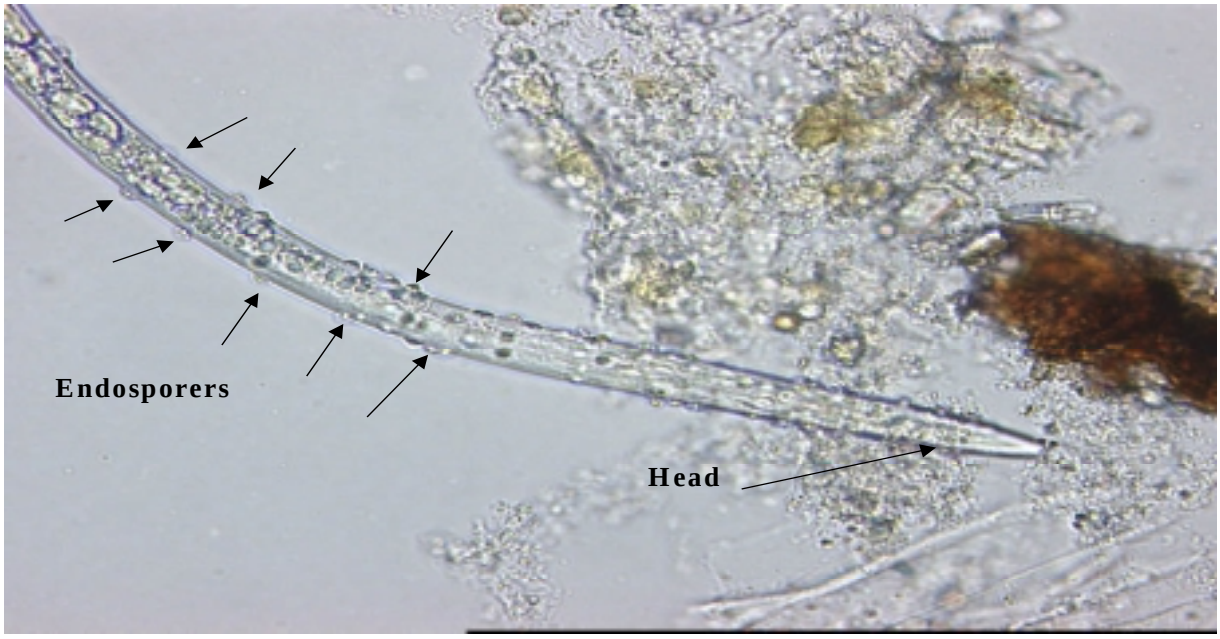


Figure 3: Second stage juvenile of *Meloidogyne javanica* with extensive spores of *Pasteuria penetrans* isolate Pp3 attached to cuticle.

3.1.2.3 Influence of spore concentration on rate of attachment

Materials and Methods

The spore suspensions of the seven *P. penetrans* isolates were prepared by using the method of Stirling and Wachtel (1980). The macerate of root material containing bacteria spores in water (see 2.2.2) was washed through a 25µm sieve into flasks and adjusted to final spore concentrations of (3.3×10^3; 1.67×10^4; 3.3×10^4; 5.0×10^4; 8.3×10^4; 1.67×10^5 and 5.0×10^5 spores/ml).

The attachment rate of the seven *P. penetrans* isolates was evaluated by counting the number of attached spores on 50 juveniles of *M. incognita* after centrifugation (see 3.1.2.1).

Results and Discussion

The results of this test confirmed that the attachment rate of the different isolates of *P. penetrans* to *M. incognita* juveniles increased with increasing concentrations of spores in the suspension from 3.3×10^3 to 5.0×10^5 spore/ml (Fig. 4). The highest rate of attachment was obtained with the isolates Pp Great Britain (Pp GB), Pp Papua New Guinea (Pp PNG), Pp Ivory Coast (Pp IvC) and Pp3 South Africa (Pp3) respectively with spore concentration of 8.3×10^4 to 1.67×10^5 spore/ml. Pp Mal had low levels of attachment. The *P. penetr*ans isolate from Barbados (Pp Bar) again showed no attachment at all spore concentrations.

In general the overall level of attachment was lower than expected in this test even though the level of attachment was similar to that obtained in the previous two studies.

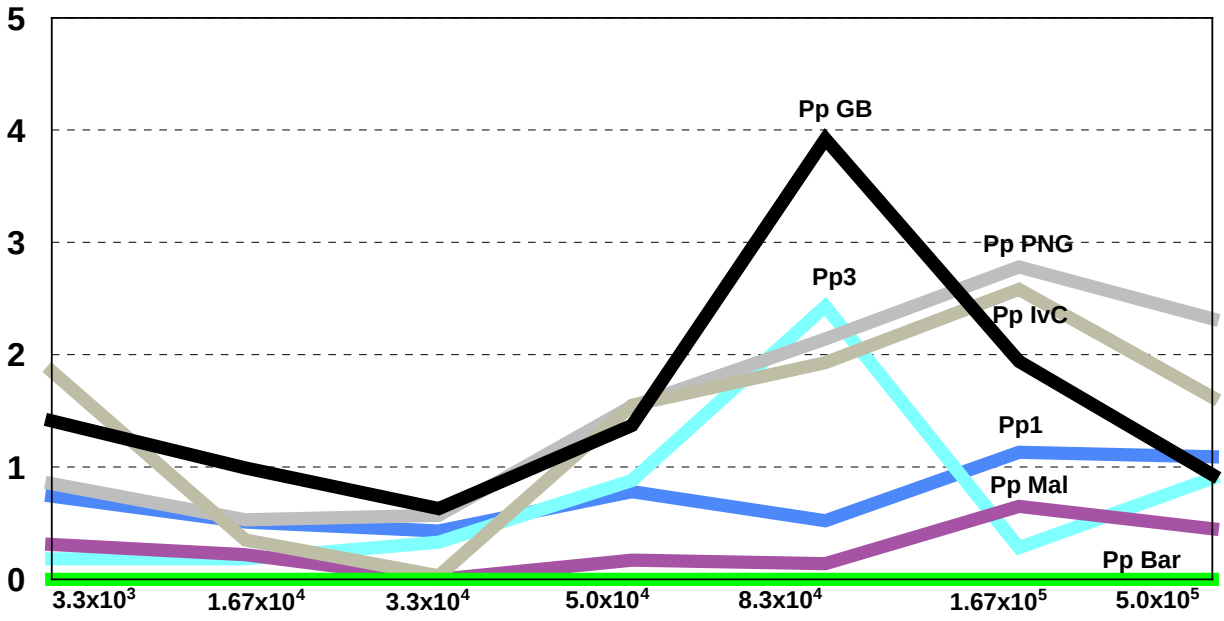


Figure 4: Effect of increasing spore concentrations of seven *Pasteuria penetrans* isolates on the rate of attachment to *Meloidogyne incognita* in spores per juvenile using the centrifugation technique.

These results agree with Davies *et al*., (1988), Stubbs (1998) and Sharma and Davies (1996) who found that the attachment of spores to each juvenile increased when the spore density was increased from 10^3 to 10^5 spore/ml. Davies *et al*., (1988) found that the number of spores that attached to the cuticles of juveniles of *M. incognita* was greatly affected by the concentration of spores in the suspension and few spores attached at concentrations below 10^3 spore/ml, whereas more spores attached as concentrations increased.

3.1.3 General Discussion

All tests showed low levels of attachment 0.2-1.2 spores/juvenile that was not expected when compared to an expected optimum attchment of between 5-10 spores/juvenile in the literature. The lack of attchment was even more confusing in that multiple spore types and different concentrations were used. Some earlier studies dealt the reasons for poor attachment. For example, Stirling (1981) revealed an increase in the number of spores attached as temperature

increased and this is probably the result of increased nematode mobility with temperature. However, temperature may also have affected the chemical interaction between spores and the nematode cuticle (Stirling *et al*., 1990). In the present tests the temperature was 20°C and this temperature was too low for good attachment when compared with the optimum temperature > 25°C for good attachment. Ahmed (1990) and Stirling *et al*., (1990) found that endospore attachment to juveniles increased with increasing temperature to ca. 30°C. The rate of endospore attachment at 27°C was approximately double that at 18°C (Stirling *et al*., 1990). The maximum number of *P. penetrans* endospores attaching to *Meloidogyne* juveniles was observed at 30°C (Ahmed, 1990; Hatz and Dickson, 1992; Orui, 1997). Hatz and Dickson (1992) found that low numbers of endospores attached per juvenile above 30°C. The low levels of attachment in the present study was clearly due to low temperatures at incubation.

Pembroke *et al*., (1998) confirmed that many factors affect results obtained during attachment studies such as: nematode density, spore density, maturity and age of spores, age of juveniles, temperature, time of exposure and pH. Stubbs (1998) reported that levels of attachment might vary even when using dishes of different size.

The attachment of *P. penetrans* spores to the nematode cuticle was not affected by pH between 4.5 and 8.5 (O'Brien, 1980). He found in other studies that optimum pH levels are between 4.5 and 5.5. Davies *et al*., (1988) observed that attachment was higher at pH 7 than at 4 or 9 in tap water, but lower at pH 7 than at pH 4 or 9 in distilled water. They also found that changes in pH and the use of tap water which was rich in salts compared with distilled water both affected attachment. However, Ahmed (1990) found that attachment was highest at pH 9 and decreased at low pH values. The previous tests were carried out in distilled water and the pH 7.3 might have had slight effects on attachment.

The variability of *P. penetrans* attachment to *Meloidogyne* species is thought to be due to several factors including differences in the specificity of isolates of *P. penetrans* to populations and species of *Meloidogyne* (Stirling, 1985; Channer and Gowen, 1992; Davies and Danks, 1993). Stirling (1985) reported differences between four isolates of *P. penetrans* in the ability of their spores to attach to juveniles from 15 single egg mass populations of

root-knot nematodes. Spores of *P. penetrans* from six populations of *Meloidogyne* only adhered to species of *Meloidogyne* and they adhered in greatest number to the species from which they had been originally isolated (Davies *et al*., 1988). Several studies suggest that host specificity is caused by differences in the amount and nature of surface proteins of the endospores (Davies *et al*., 1992; 1994). Variations in endospore attachments may be attributed to differences in the surface composition of nematode species, races and populations as well as the heterogeneity of the endospore surfaces (Chen and Dickson, 1998).

The length of time taken for second stage juveniles to become encumbered with spores in a water suspension of spores was dependent on juvenile age (Davies *et al*., 1991). They suggested also that the younger juveniles are more active and became encumbered with spores more rapidly than older ones or as the cuticle became less conducive for the adhesion of *Pasteuria* spores. They reported decreased spore attachment on older second stage juveniles of root-knot nematodes. The second stage juveniles used in the tests conducted in this study were less than 7 days old and therefore did not affect attachment greatly.

Pasteuria penetrans endospores can survive prolonged periods under dry conditions (Stirling and Wachtel, 1980; Oostendorp *et al*., 1990). Storage of *P. penetrans* spores for a long time does not affect the attachment viability. Spores were viable for a period of more than one year (Mani, 1988). Suspensions of spores can be stored frozen or in a desiccated root powder for long periods apparently without loss of binding ability to juveniles (Giannakou *et al*., 1997). Endospore suspensions either in distilled or tap water can be refrigerated (4°C) for several months. Endospore suspensions have been stored frozen and remained viable and able to attach to root-knot nematode juveniles (Hewlett and Serracin, 1996). The attachment of *P. penetrans* spores also was not affected by their age (Giannakou *et al*., 1997). They reported that a much higher proportion of females escaped infection when juveniles were treated with spores from 11 year old rather than from fresh root/spore preparations. The spores used in the present study were not more than one year old. Therefore poor attachment was not caused by spore aging

3.2 Effect of the nematode antagonistic rhizobacteria *B. cereus* S18 and *R. etli* G12 culture filtrates on the attachment of *P. penetrans* (Pp3) to *M. javanica* juveniles

3.2.1 Introduction

A number of biotic and abiotic factors can influence the attachment of *P. penetrans* spores to juveniles of species of *Meloidogyne*. Investigations have dealt with the effect of soil abiotic factors, for example, soil type, soil moisture (Stirling and Wachtel, 1980; Oostendorp *et al*., 1991) and soil temperature (Stirling, 1981; Hatz and Dickson, 1992) on *P. penetrans* spore attachment to plant parasitic nematodes.

However, little is known about the effect of biotic factors which also may influence the attachment of *P. penetrans* spores to nematode cuticles (Duponnois *et al*., 1997). Duponnois *et al*., (1999) showed that the soil microflora stimulated the attachment of *P. penetrans* spores on nematode juveniles and thereby reduced *M. incognita* juvenile penetration into tomato roots. They found that the rhizosphere bacteria *Enterobacter cloacae* and *Pseudomonas mendocina* stimulated plant growth, inhibited the reproduction of the root-knot nematode *M. incognita* and increased the attachment of the endospores of *P. penetrans* on the nematodes *in vitro*. They found also that *E. cloacae* increased the reproduction of *P. penetrans* in nematode infested plant roots. They reported also that both bacteria could modify the structure of spores. In particular, the sporangial wall and the exosporium could be changed exposing the parasporal fibers and allowing them to make better contact with the nematode cuticle.

The objective of the following experiment was to evaluate the effect of different concentrations of culture filtrates of the rhizobacteria *B. cereus* strain S18 and *R. etli* strain G12 on the attachment of *P. penetrans* spores to *M. javanica* juveniles *in vitro*.

3.2.2 Materials and Methods

The culture of the rhizobacteria *B. cereus* S18 was discussed in chapter II (see 2.2) and *R. etli* G12 in chapter III (see 2.2). The cell free culture filtrates of both rhizobacteria were prepared

by centrifugation of the tryptic soy broth and King's B nutrient solution used for fermentation at 8000 rpm for 10 minutes. The supernatants were poured through a sterile 0.2µm filter paper and collected in sterile 250 ml Erlenmeyer flasks. The culture filtrate concentrations used were 100, 50, 25 and 10%. *M. javanica* juveniles were extracted as described before (see 2.1). The *P. penetrans* isolate from South Africa (Pp3) was used in this study. The spore suspension of *P. penetrans* was prepared as described before (see 2.2.2) and number of spores was adjusted to 10^5 spores/ml. Four mililitres from each concentration of the cell free fermentation broth was added to 1 ml distilled water containing 1000 juveniles of *M. javanica* and 1 ml spore suspension (10^5 spore/ml) in Petri dishes (5 cm in diameter). Each treatment was replicated 3 times. Petri dishes with water served as controls. Petri dishes were incubated 24 hours at room temperature (approximately 20°C). After 24 hours the number of attached spores per juvenile were counted on 50 juveniles randomly selected under a microscope (X400).

3.2.3 Results and Discussion

Results of this study revealed that the highest rate of attachment of *P. penetrans* spores to *M. javanica* juveniles was obtained in the control treatment. A significant reduction in attachment was seen at all filtrate concentrations and for both *B. cereus* S18 and *R. etli* G12 (Fig. 1). The lowest level of attachment to *M. javanica* juveniles was attained with the 100% culture filtrate of both rhizobacteria when compared with the control. Ten fold dilutions of the culture filtrate still led to significant reductions in attachment but to a lesser degree.

These results showed for the first time that culture filtrates of rhizobacteria can reduce attachment of *P. penetrans* spores to nematode juveniles. The negative effect of the culture filtrates on the mobility of juveniles probably reduced the degree of juvenile contact with spores because the attachment process depends on the movement of juveniles (Stirling, 1984; Pembroke *et al*, 1998). For this reason at low concentrations of both culture filtrates higher levels of attachment were detected.

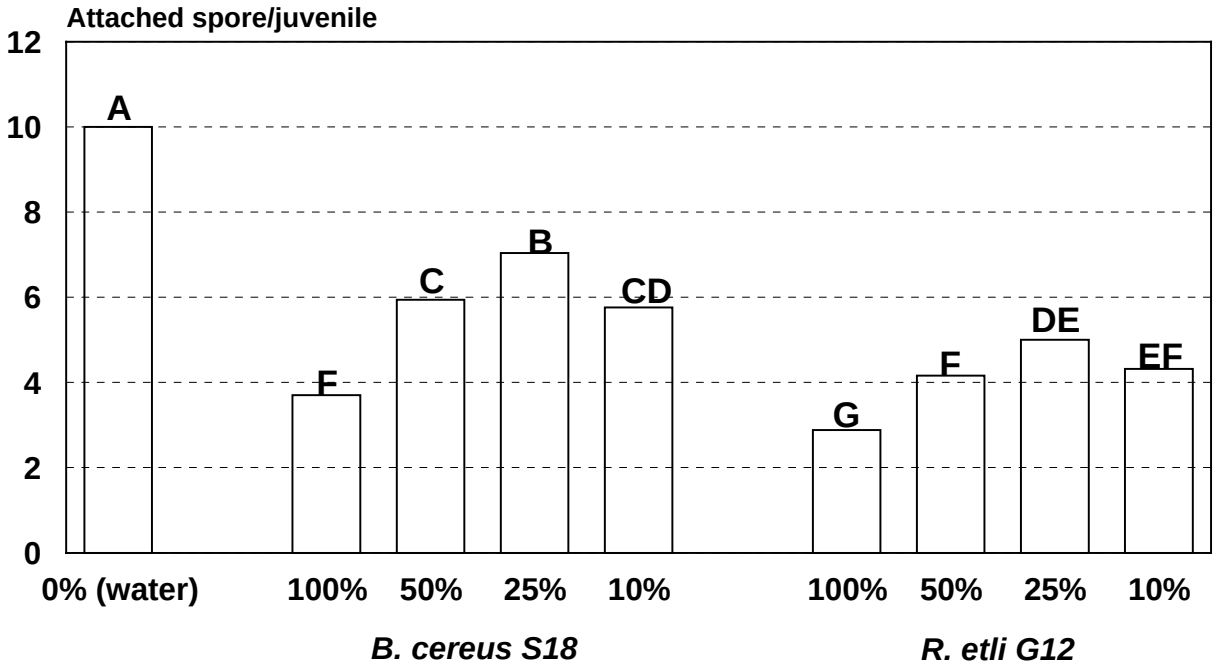


Figure 1: Effect of different concentrations of *Bacillus cereus* S18 and *Rhizobium etli* G12 culture filtrates on the *in vitro* attachment of *Pasteuria penetrans* spores to *Meloidogyne javanica* juveniles after 24 hours. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P< 0.05$).

These results do not agree with those obtained by Duponnois *et al.*, (1999) who found that rhizosphere bacteria increased attachment of *P. penetrans* spores to *M. incognita*. They found that the rhizosphere bacteria *Enterobacter cloacae* and *Pseudomonas mendocina* stimulated plant growth, inhibited the reproduction of the root-knot nematode *M. incognita* and increased the attachment of the endospores of *P. penetrans* on the nematodes. They also reported that both bacteria could modified the structure of spores and allowing them to make improved contact with the nematode cuticle.

The differences in results could be related to bacteria species. The bacteria used in the other studies may have stimulated nematode movement. Hasky-Günther (1996) showed higher numbers of *G. pallida* were attracted to the root surface when PHPR were present. It should be noted that concentrations of PHPR filtrates at this level will never occur in field applications for biocontrol.

3.3 Effect of chicken manure compost on the attachment of *P. penetrans* (Pp3) spores to *M. javanica* juveniles

3.3.1 Introduction

Adding organic amendments to soil for controlling plant diseases has received considerable attention. Organic amendments also have been used to manage plant-parasitic nematodes. The mechanism of action has been attributed to the improvement of soil structure and aggregation resulting in increased aeration and water-holding capacity to improvement in plant nutition to release of toxic products to nematodes or to the enhancement of antagonistic organisms able to compete with or destroy nematodes (Stirling, 1991). Studies demonstrated that pre-plant treatment with chicken manure 1kg/m2 reduced *Meloidogyne* population levels in the soil by 50%, over three tomato cropping periods (Anonymous, 1976). Chicken manure and oil cakes have been the most popular type of organic amendments used and have shown promise in reducing nematode populations (Muller and Gooch, 1982). Chicken manure is effective for control of *M. incognita* on several hosts plant (Derrico and Maio, 1980) and *Tylenchulus semipenetrans* on citrus (Mankau and Minteer, 1962).

Organic matter can influence also the spore attachment of *P. penetrans* to the nematode cuticle (Ratnasoma and Gowen, 1996). The attachment of the spores on the nematode cuticle are considered one of two important phases for *P. penetrans* reproduction (Sayre and Werign, 1977).

The objective of this study was to evaluate the effect of chicken manure compost on the attachment of Pp3 spores on *M. javanica* juveniles.

3.3.2 Materials and Methods

In this experiment a commercial dry chicken manure compost (Fehnland Naturdünger, Naturdünger Backhus GmbH, Bösel) was used to determine the effect of percolate of chicken

manure treated pots on *P. penetrans* attachment on the cuticle of *M. javanica* juveniles.

Chicken manure contents

Organic substance	45%
pH level	9.4%
Nitrogen (N)	1.8%
Phosphate (P2O5)	3.9%
Botassium Oxide (K2O)	3.2%
Calcium Oxide (CaO)	4.5%

Plastic pots (10 cm in diameter) were filled with 300 ml of a non-sterilized sand-soil mixture (1:1, v/v). The compost was mixed with the soil at two concentration 5 and 20% (v/v). Pots untreated with compost served as controls. Each treatment was replicated 6 times. Pots were left under greenhouse conditions at 25°C for one week. The pots were then flooded daily with 100 ml tap water 7 days in a row and the soil percolate from each treatment collected every day in a glass beaker and stored in a refrigerator at 4°C . The collected soil percolates were centrifugated and the three supernatants were used for the attachment test.

The attachment test was carried out in Petri dishes 5 cm in diameter. A suspension of 1000 freshly hatched juveniles of *M. javanica* (see 2.1) in 1 ml distilled water was added to a 1 ml Pp3 spore suspension (10^5 spores/ml). To this solution an additional 4 ml of the soil percolate was added from each treatment. The Petri dishes were incubated for 24 hours at room temperature approximately 20°C. The number of attached spores per juvenile were recorded on 20 nematode juveniles randomly chosen under a microscope (X400).

3.3.3 Results and Discussion

Results of this study showed that chicken manure percolate drastically reduced Pp spore attachment (Fig. 1). A significant reduction in attachment of *P. penetrans* spores to the cuticle of *M. javanica* juveniles occurred in the soil percolate from the compost 5 and 20% treatment when compared with the compost-free treated soil (Fig. 1). The results revealed that the soil percolate of chicken manure compost treated pots at either 5% or 20% caused a 80% reduction in attachment of *P. penetrans* spores to the nematode cuticle. Also, there were no

significant differences between the two compost concentrations used in attachment of *P. penetrans* spores to the nematode cuticle.

It is known that spore attachment is affected by pH (Davies *et al*., 1988; Ahmed, 1990; Orui, 1997). The highest attachment in their studies was at pH 9 and decreased at lower pH levels (Ahmed, 1990). The results obtained in the present study may be due to the pH of the chicken manure compost treated pots. The compost has a pH 9.4 and it probably drops greatly after mixed with soil at both concentrations compared with the compost untreated pots. Rodriguez-Kabana (1986) suggested that there is also evidence that some phytotoxic effects caused by organic amendments may be related to changes in soil pH (Walker, 1971; Brown, 1987).

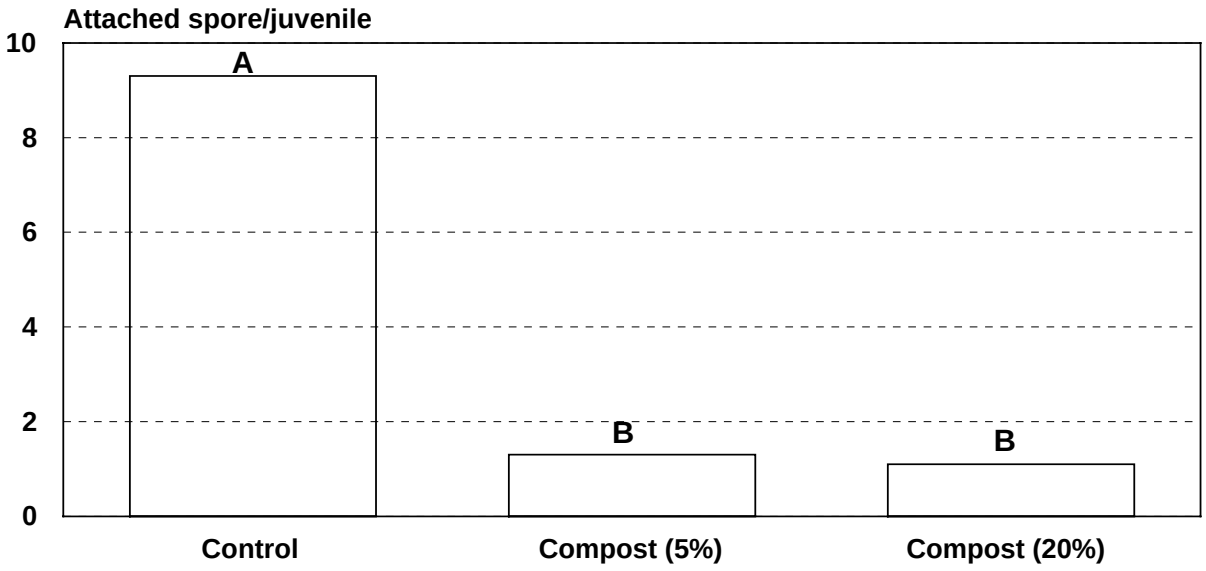


Figure 1: Effect of soil percolates from chicken manure compost treated soil on the attachment of *Pasteuria penetrans* spores to *Meloidogyne javanica* juveniles. Columns followed by different letters are significantly different from another according to Duncan's Multiple Range Test ($P< 0.05$).

3.4 Biological control of *M. javanica* with *P. penetrans* (Pp3) on tomato

3.4.1 Introduction

The endospore-forming bacteria *P. penetrans* is known as an obligate parasite of root-knot nematodes, *Meloidogyne* spp. It has been tested for control of *M. javanica* (Stirling, 1984) and *M. incognita* (Nishizawa, 1984; Sayre, 1984; Dickson *et al*., 1994). Studies with this bacteria confirmed that it has a high potential as a biological control agent (Mankau, 1975). Although attachment was obtained in previous experiments is not always correlated with good levels of control. This study aimed to evaluate the biological control of *P. penetrans* toward root-knot nematode, *M. javanica* on tomato over 6 months from the first inoculation and measure population development of *P. penetrans* over time under monoculture with a good root-knot host.

3.4.2 Materials and Methods

The experiment was carried out in a growth chamber at 30°C with 16/8 hours day/night of artificial light. A non-sterilized pure sand substrate was used. The sand of each pot (20 pots) was added to plastic bags separately and each bag inoculated with 10,000 eggs of *M. javanica* extracted as outlined before (see 2.1). Each bag was shaken vigorously to distribute the eggs homogeneously into the sand. The sand of each bag was then put into seperate plastic pots (14 cm in diameter).

Two treatments were used in this experiment:

A) *M. javanica* alone

B) *M. javanica* + *P. penetrans* (Pp3)

Three week old tomato plants cv. Hellfrucht Frühstamm were inoculated with a 10 ml Pp3 spore suspension (10^5 spore/ml) prepared as described before (see 2.2.2) in three holes in the root zone. Plants were then transplanted into pots in a growth chamber. Each treatment was replicated 10 times. The plants were watered daily and fertilized every week with 10 ml/plant of Poly Crescol (14+10+14, 2g/liter water). Plants were removed when 750 degree days (10°C base temperature) was reached approximately 2 months after nematode inoculation.

Roots were removed carefully to leave as much sand in the pots as possible. The roots were then washed with tap water and the gall index (Zeck, 1971) and number of galls determined.

The number of Pp3 infected females was evaluated. Females of *M. javanica* were collected by cutting the root system of each plant in 2 cm pieces and submerging the roots in a beaker full of tap water for 4 days at room temperature (20°C) until they became soft (Ratnasoma and Gowen, 1996). The roots were then washed through stacked 500µm and 250µm sieves to separate the females from the root debris.

The *Pasteuria* infected females (Fig. 4) were distinguished by their opaque dull creamy white to amber colour compared to white, glistening females (Mankau and Imbriani, 1975; Mankau and Prasad, 1977). All collected females were crushed under a cover slip (three females on a slide) in a drop water and the presence of *Pasteuria* spores verified (Fig. 3) using the microscope (X400).

After evaluation the chopped roots from each treatment were re-incorporated into the same pot. Three week old tomato plants were again transplanted into these pots but without adding additional nematode eggs or Pp3 spore suspensions.

After another 2 months the plants were again harvested and the roots examined as above. New tomato plants were again transplanted into the pots after incorporation of the roots. Then 6 months after the first inoculation the last tomato rotation was evaluated according to the gall index, number of galls and number of Pp3 infected females.

3.4.3 Results and Discussion

3.4.3.1 After two months

Results revealed that the root-knot nematode, *M. javanica* was not affected by *P. penetrans* (Pp3) after 2 months. Results showed no significant differences in gall index or number of galls compared with the Pp3 untreated plants (Fig. 1). The results also showed that the mean number of females infected with *P. penetrans* spores was very low after 2 months or 6.25% of the females (Fig. 2).

3.4.3.2 After four months

P. penetrans reduced the number of galls significantly compared with the *P. penetrans* untreated plants. The reduction in the number of galls was 53% (Fig. 1). The mean number of infected females increased from 6.25% after 2 months to 27.8% after 4 months (Fig. 2).

3.4.3.3 After six months

The results obtained after 6 months showed that biocontrol of *M. javanica* by *P. penetrans* increased further over the results obtained after 2 and 4 months. The number of galls was reduced significantly when compared with the plants treated with the nematode alone. The rate of reduction in number of galls was as high after 6 months as compared to 4 months (Fig. 1). The mean number of Pp3 infected females increased 6-fold to 37.9% when compared with the results obtained after 2 months (Fig. 2).

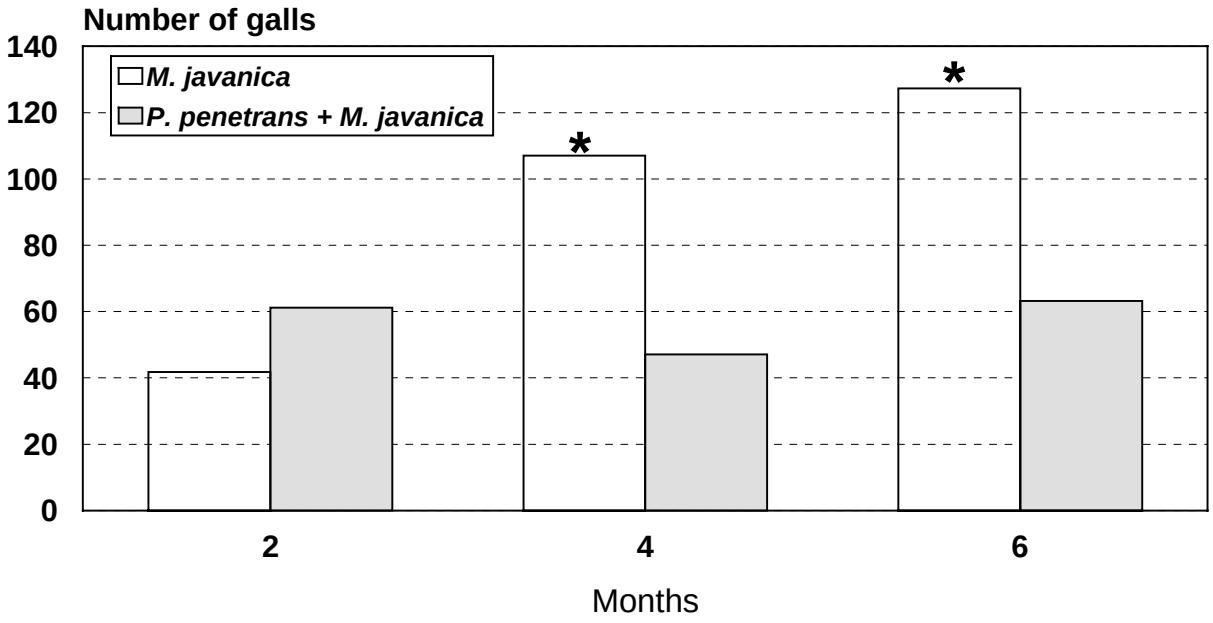


Figure 1: Effect of *Pasteuria penetrans* (Pp3) on the number of galls of *Meloidogyne javanica* on tomato after two, four and six months. Columns followed by * are significantly different when compared with the bacteria treated plants according to T-Test (P< 0.05) n=10.

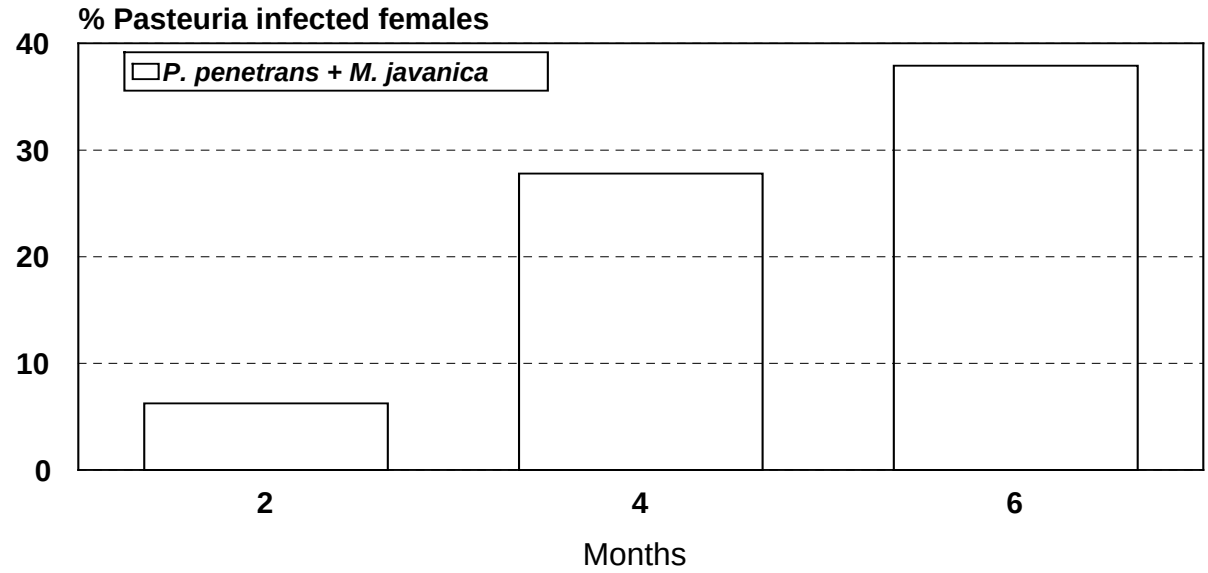


Figure 2: Biological control of *Meloidogyne javanica* with *Pasteuria penetrans* (Pp3) on tomato after two, four and six months measured as the number of Pp3 infected females in percent. The columns were not significantly different when compared with the bacteria treated plants according to T-Test (P< 0.05) n=10.

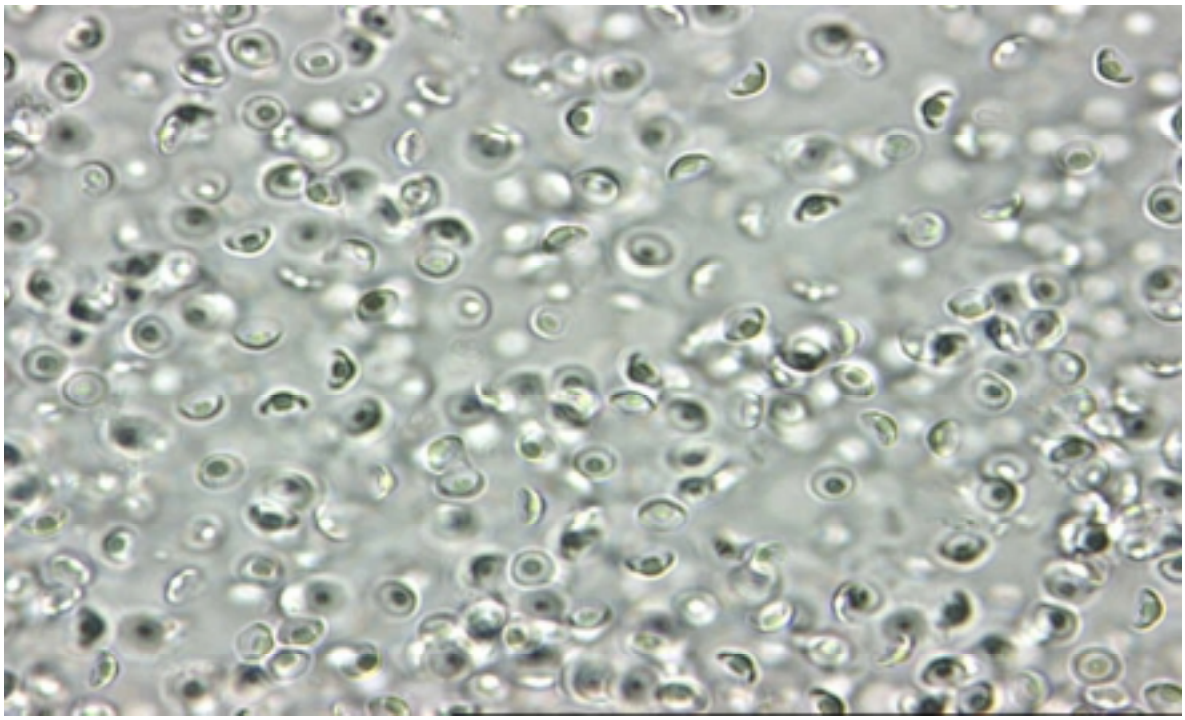


Figure 3: Endospores of *Pasteuria penetrans* isolate Pp3 originated from South Africa.

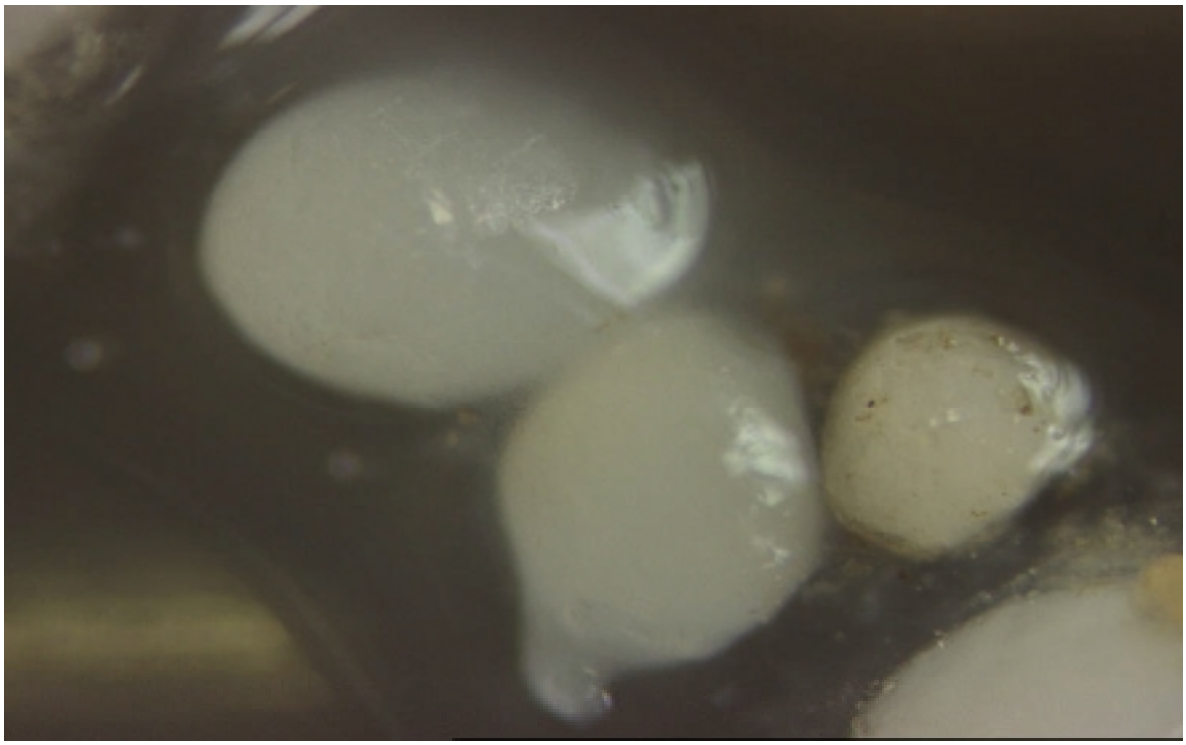


Figure 4: *Pasteuria penetrans* infected females of *Meloidogyne javanica* .

These results showed that the obligate endoparasitic bacteria *P. penetrans* when applied at 1.0 x 10^5 spore/ml to transplants quickly established in pure sand culture under tomato. Three life cycles of root-knot were needed in order to induce significant reduction in galling. These results suggested that the concentration of spores in soil increased quickly and led to an improvement in nematode control over a short period of time. This was accomplished by ensuring that the root systems containing spore filled cadavers of female root-knot nematodes are retained in the soil after crop harvested (Gowen and Tzortzakakis, 1994). Similar success in increasing Pp was also achieved by (Daudi *et al*., 1990) who re-incorporated the root systems. The use of pure sand in these tests eliminated the negative effects of introduced organic matter and subsequent effects of microbial metabolites in such material that were shown to negatively affect attachment (see 3.2 and 3.3). The use of higher temperatures 30°C verses 20°C also increased effectiveness by promoting attachment.

The results demonstrate that Pp when introduced on seedlings could be effective in vegetable production systems in short periods of time where near pure sand exists as in most of North Africa and West Asia.

3.5 General Concolusions

A) Results suggested that there are no significant differences between the two methods used to stimulate of *P. penetrans* spore attachment to root-knot juveniles either the centrifugation or the incubation method.

B) Attachment differed between different *P. penetrans* isolates and showed the high specificity of *P. penetrans* spores to distinct species of *Meloidogyne*.

C) As spores concentration of *P. penetrans* increased, rate of attachment increased.

D) Culture filtrates of two nematode antagonistic rhizobacteria *B. cereus* S18 and *R. etli* G12 reduced attachment of *P. penetrans* spores to the cuticle of *M. javanica* juveniles regardless of concentration.

E) The percolate of chicken manure compost treated soil had a drastic negative effect on the attachment of *P. penetrans* spores to *M. javanica* juveniles.

F) *P. penetrans* demonstrated quick build-up over 6 months and good biocontrol toward root-knot nematode, *M. javanica* when introduce to sand cultured tomato at high temperatures on seedlings.

G) Temperature below 25°C has a strong negative effect on attachment.

4. References

Ahmed, R. 1990. Studies on the efficacy of *Pasteuria penetrans* for the biological control of *Meloidogyne* species. Ph.D. dissertation, University of Reading, Reading, UK.

Anonymous. 1976. Pp. 479 *in* Philippines. International Rice Research Institute, Manila, Philippines, eds. Annual report for 1975.

Brown, R. H. 1987. Control strategies in low crops. Pp. 351-387 *in* R. H. Brown, and B. Kerry, eds. Principales and practice of nematode control in crops. Academic Press, Sydney, Australia.

Brown, S. M., J. L. Kepner, and G. C. Smart, Jr. 1985. Increased crop yields following application of *Bacillus penetrans* to field plots infested with *Meloidogyne incognita*. Soil Biology and Biochemistry 17:483-486.

Channer, A. G., and S. R. Gowen. 1992. Selection for increased host resistance and increased pathogen specificity in the *Meloidogyne-Pasteuria penetrans* interaction. Fundamental and Applied Nematology 15:331-339.

Chen, Z. X., and D. W. Dickson. 1998. Review of *Pasteuria penetrans*: Biology, ecology, and biological control potential. Journal of Nematology 30:313-340.

Chen, Z. X., D. W. Dickson, and T. E. Hewlett. 1996. Quantification of endospore concentrations of *Pasteuria penetrans* in tomato root material. Journal of Nematology 28:50-55.

Chen, Z. X., D. W. Dickson, L. G. Freitas, and J. F. Preston. 1997. Ultrastructure, morphology and sporogensis of *Pasteuria penetrans*. Phytopathology 87:273-283.

Daudi, A. T., A. G. Channer, R. Ahmed, and S. R. Gowen. 1990. *Pasteuria penetrans* as a biocontrol agent of *Meloidogyne javanica* in the field in Malawi and in microplots in Pakistan. Proceedings of Brighton Crop Protection Conferences, Pests and Diseases. Brighton, UK: British Crop Protection Council.

Davies, K. G., and C. Danks. 1993. Interspecific differences in the nematode surface coat between *Meloidogyne incognita* and *M. arenaria* related to the adhesion of the bacterium *Pasteuria penetrans*. Parasitology 105:475-480.

Davies, K. G., K. R. Kerry, and C. A. Flynn. 1988. Observations on the pathogenicity of *Pasteuria penetrans*, a parasite of root-knot nematodes. Annals of Applied Biology 112:491-501.

Davies, K. G., V. Laird, and B. R. Kerry. 1991. The motility, development and infection of *Meloidogyne incognita* encumbered with spores of the obligate hyperparasite *Pasteuria penetrans*. Revue de Nematologie 14:611-618.

Davies, K. G., M. Redden, and T. K. Pearson. 1994. Endospore heterogeneity in *Pasteuria penetrans* related to adhesion to plant-parasitic nematodes. Letters in Applied Microbiology 19:370-373.

Davies, K. G., M. P. Robinson, and V. Laird. 1992. Proteins involved in the attachment of a hyperparasite *Pasteuria penetrans* to its plant-parasitic nematode host, *Meloidogyne incognita*. Journal of Invertebrate Pathology 59:18-23.

Derrico, F. P., and F. D. Maio. 1980. Effect of some organic materials on root-knot nematodes on tomato in field preliminary experiments. Nematologia Mediterranea 8:107-111.

Dickson, D. W., M. Oostendorp, R. Giblin-Davis, and D. J. Mitchell. 1994. Control of plant-parasitic nematodes by biological antagonists. Pp. 575-601 *in* D. Rosen, F. D. Bennett,

and J. L. Capinera, eds. Pest management in the subtropics. Biological control-a Florida perspective. Andover, UK: Intercept.

Duponnois, R., C. Netscher, and T. Mateille. 1997. Effect of the rhizosphere microflora on *Pasteuria penetrans* parasitizing *Meloidogyne graminicola*. Nematologia Mediterranea 25:99-103.

Duponnois, R., M. BA. Amadou, and M. Thierry. 1999. Beneficial effects of *Enterobacter cloacae* and *Pseudomonas mendocina* for biocontrol of *Meloidogyne incognita* with the endospore-forming bacterium *Pasteuria penetrans*. Nematology 1:95-101.

Dutky, E. M., and R. M. Sayre. 1978. Some factors affecting infection of nematodes by the bacterial spore parasite *Bacillus penetrans*. Journal of Nematology 10:285 (Abstr.).

Espanol, M., S. Verdejo-Lucas, K. G. Davies, and B. R. Kerry. 1997. Compatibilty between *Pasteuria penetrans* isolates and *Meloidogyne* populations from Spain. Biocontrol Science and Technology 7:219-230.

Freitas, L. G., D. J. Mitchell, and D. W. Dickson. 1997. Temperature effects on attachment of *P. penetrans* endospores to *Meloidogyne arenaria* race 1. Journal of Nematology 29:547-555.

Fulton, E. W. 1998. Suppression of *Meloidogyne* spp. by *Pasteuria penetrans*. Ph.D. dissertation, University of Florida, USA.

Giannakou, I. O., B. Pembroke, S. R. Gowen, and K. G. Davies. 1997. Effects of long term storage and above normal temperatures on spore adhesion of *Pasteuria penetrans* and infection of the root-knot nematode *Meloidogyne javanica*. Nematologica 43:185-192.

Gowen, S. R., and E. Tzortzakakis. 1994. Biological control of *Meloidogyne* spp. with *Pasteuria penetrans*. Bulletin OEPP 24:495-500.

Hasky-Günther, K. 1996. Untersuchungen zum Wirkungsmechanismus der antagonistischen Rhizosphärebakterin *Agrobacterium radiobacter* isolate G12 und *Bacillus sphaericus* isolate B43 gegenüber dem kartoffelzystennematoden *Globodera pallida* an Kartoffel. Ph.D. disseratation, Bonn University, Bonn, Germany.

Hatz, B., and D. W. Dickson. 1992. Effect of temperature on attachment, development, and interactions of *Pasteuria penetrans* on *Meloidogyne arenaria*. Journal of Nematology 24:512-521.

Hewlett, T. E., and D. W. Dickson. 1993. A centrifugation method for attaching endospores of *Pasteuria* spp. to nematodes. Journal of Nematology 25:785-788.

Hewlett, T. E., and M. Serracin. 1996. *Pasteuria* spp. as biological control agent. Thinc 1996 Workshop Manual.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 57: 1025-1028.

Mani, A. 1988. Studies on the bacterial parasite *Pasteuria penetrans*: I. Spore viability after storage, II. Culture on citrus nematode *Tylenchulus semipenetrans*. International Nematology Network Newsletter 5:24-25.

Mankau, R. 1975. *Bacillus penetrans* n. comb. causing a virulent disease of plant-parasitic nematodes. Journal of Invertebrate Pathology 26:333-339.

Mankau, R., and J. L. Imbriani. 1975. The life cycle of an endoparasite in some tylenchid nematodes. Nematologica 21:89-94.

Mankau, R., and N. Prasad. 1977. Infectivity of *Bacillus penetrans* in plant parasitic nematodes. Journal of Nematology 9:40-45.

Mankau, R., and R. J. Minteer. 1962. Reduction of soil population of citrus nematode by the addition of organic matter. Plant Disease Reporter 46:375-378.

Muller, R., and P. S. Gooch. 1982. Organic amendments in nematode control: An examination of the literature. Nematropica 12:319-326.

Nishizawa, T. 1984. Effects of two isolates of *Bacillus penetrans* for control of root-knot and cyst nematodes. Pp. 160-161 *in* Proceedings of the First International Congress of Nematology, Guelph, Ontario, Canada. Bur. Nematology, Gainesville, Florida, USA.

O'Brien, P. C. 1980. Studies on parasitism of *Meloidogyne javanica* by *Bacillus penetrans*. Journal of Nematology 12:234.

Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. Pp. 85-102 *in* J. N. Sasser, and W. R. Jenkins, eds. Nematology. Raleigh, NC: North Carolina State University Press.

Oostendorp, M., D. W. Dickson, and D. J. Mitchell. 1990. Host range and ecology of isolates of *Pasteuria* spp. from the southeastern U.S.A. Journal of Nematology 22:525-531.

Oostendorp, M., D. W. Dickson, and D. J. Mitchell. 1991. Population development of *Pasteuria penetrans* on *Meloidogyne arenaria*. Journal of Nematology 23:58-64.

Orui, Y. 1997. Effect of spore sonication on attachment and host-attachment range of *P. penetrans* to the root-knot nematode. Applied Entomology and Zoology 32:101-107.

Pembroke, B., S. R. Gowen, and I. Giannakoue. 1998. Advanced of ideas for the use of *Pasteuria penetrans* for the biological control of root-knot nematodes, *Meloidogyne* spp.

Brighton Crop Protection Pests and Diseases 3:555-560.

Ratnsoma, H. A., and S. R. Gowen. 1996. Spore attachment of *Pasteuria penetrans* on juveniles of *Meloidogyne incognita* as affected by pH and organic matter. Nematologia Mediterranea 24:283-285.

Ratnsoma, H. A., S. R. Gowen, and N. G. M. Hague. 1991. Observations on the detachment of spores of *Pasteuria* from pre-parasitic second-stage juveniles of *Meloidogyne* spp. Nematologia Mediterranea 19:225-227.

Regina, M. D. G., R. Onivaldo, L. G. Freitas, and D. W. Dickson. 1999. Attachment of endospores of *Pasteuria penetrans* to males and juveniles of *Meloidogyne* spp. Nematology 1:267-271.

Rodriguez-Kabana, R. 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. Journal of Nematology 18:129-135.

Sayre, R. M. 1984. *Bacillus penetrans* a biocontrol agent of *Meloidogyne incognita* on cucumber. Pp. 116 *in* Proceedings of the First International Congress of Nematology, Guelph, Ontario, Canada. Bur. Nematology, Gainesville, Florida, USA.

Sayre, R. M., and M. P. Starr. 1988. Bacterial diseases and antagonisms of nematodes. Pp. 69-101 in G. O. Poinar, Jr., and H.-B. Jansson, eds. Diseases of nematodes. Boca Raton, FL: CRC Press.

Sayre, R. M., and W. P. Wergin. 1977. Bacterial parasite of a plant nematode: Morphology and ultrastructure. Journal of Bacteriology 129:1091-1101.

Sharma, S. B., and K. G. Davies. 1996. Characterization of *Pasteuria* isolated from *Heterodera cajani* using morphology, pathology, and serology of endospores. Systemic and Applied Microbiology 19:106-112.

Stirling, G. R. 1981. Effect of temperature on infection of *Meloidogyne javanica* by *Bacillus penetrans*. Nematologica 27:458-462.

Stirling, G. R. 1984. Biological control of *Meloidogyne javanica* with *Bacillus penetrans*. Phytopathology 74:55-60.

Stirling, G. R. 1985. Host specificity of *Pasteuria penetrans* within the genus *Meloidogyne*. Nematologica 31:203-209.

Stirling, G. R. 1991. Biological control of plant-parasitic nematodes: Progress, problems, and prospects. Wallingford, UK: CAB International.

Stirling, G. R., and M. F. Wachtel. 1980. Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. Nematologica 34:308-312.

Stirling, G. R., R. D. Sharma, and J. Perry. 1990. Attachment of *Pasteuria penetrans* spores to the root-knot nematode *Meloidogyne javanica* in soil and its effects on infectivity. Nematologica 36:246-252.

Sturhan, D. 1989. New host and geographical records of nematode-parasitic bacteria of the *Pasteuria penetrans* group. Nematologica 34:350-356.

Stubbs, V. 1998. The use of *Tagetes erecta* and *Pasteuria penetrans* within crop rotations for the control of *Meloidogyne* spp. in microplots in Zimbabwe. Ph.D. dissertation, University of Reading, Reading, UK.

Walker, J. T. 1971. Populations of *Pratylenchus penetrans* relative to decomposing nitrogenous soil amendments. Journal of Nematology 3:43-49.

Zeck, W. M. 1971. A rating scheme for field evaluation of root-knot nematode infestations. Pflanzenschutznachrichten Bayer 24: 141-144.

Summary and Future Strategies

Plant-parasitic nematodes especially root-knot nematodes, *Meloidogyne* spp. are consider the most economically important group worldwide. They attack a wide range of crops, especially vegetable crops, and cause severe damage and high yield losses. Root-knot nematodes are a major pest problem in crop production in most Mediterranean countries and especially in newly reclaimed desert areas e.g. in Egypt, Jordan and Morroco. Many books and publications have dealt with the different methods used to control of root-knot nematodes. The methods used include: cultural, physical, and chemical appraches. However, in most cases soil fumigation with nematicides is used. Nematologists now tend to improve integrated pest management (IPM) of phytonematodes using biological control by the introduction of nematode antagonistic microorganisms to the soil as a safe alternative.

Root-knot nematodes, *Meloidogyne* can be reduced to below economic levels without fumigation by using integrated pest management systems (IPM). This management system is socially acceptable, environmentally responsible and an economically practical method of controlling root-knot nematode populations. In IPM systems a variety of cultural, physical, resistant cultivars and biological control agents are incorporated into the production system (Fig. 1). The use of chemical control of nematodes is now limited, because nematicides are expensive, unsafe for humans and toxic to the environment. The loss of nematicides can be offset with biocontrol agents. Biologicals used in pest management of nematodes in integration such as crop rotation, resistant varieties, solarization and adding soil amendments to increase natural antagonists must be propagated at the grower level and with extension agencies.

There are many promising biocontrol agents against root-knot nematodes such as: vesicular-arbuscular mycorrhizal fungi (AMF), mutualistic fungal and bacterial endophytes, egg pathogenic fungi, obligate parasites and antagonistic plant health promoting rhizobacteria (PHPR).

PHPR used in the present study are very important, because they can be applied as a soil drench to seedlings or as a seed treatment in seed beds containing sterile soil or potting mixtures. The PHPR like *Bacillus cereus* S18 colonize the rhizosphere. Some PHPR can also colonize the plant tissue endophytically such as *Rhizobium etli* G12. The mode of action of PHPR leads to reduced nematode penetration into roots. They can also delay nematode development in roots and thereby reduce total population densities. The use of PHPR to reduce root-knot early root penetration will produce overall yield increase. However, PHPR must be used in combination with other IPM methods, because they do not give 100 percent control of the nematode. They only reduce early root penetration which leads to yield increase.

The results of the present studies also showed that management of the root-knot nematode, can be accomplished by the inoculation of seedlings with the obligate endospore-forming bacterium, *Pasteuria penetrans* as a spore suspension at high spore concentration (10^5 spores/ml) or by mixing the *Pasteuria* root powder into the seed beds. This will not protect the first growing crop in the field, but over time will lead to development of a suppressive soil. The *P. penetrans* isolate must be tested first to determine if it attaches well to root-knot populations existing in the fields.

After growing seedlings inoculated with PHPR and *Pasteuria penetrans* they can be transplanted to the field. The field soil can also be treated first with solarization before transplanting the seedlings to reduce root-knot population densities further and thereby supporting the biocontrol agents being used. The seedlings also can be planted in a planting hole containing compost which favors the buildup of naturally occurring antagonists and simultaneously promotes root growth. Solarization and organic matter reduce root-knot densities and add support to the biocontrol systems which normally do not work well at high root-knot threshold levels.

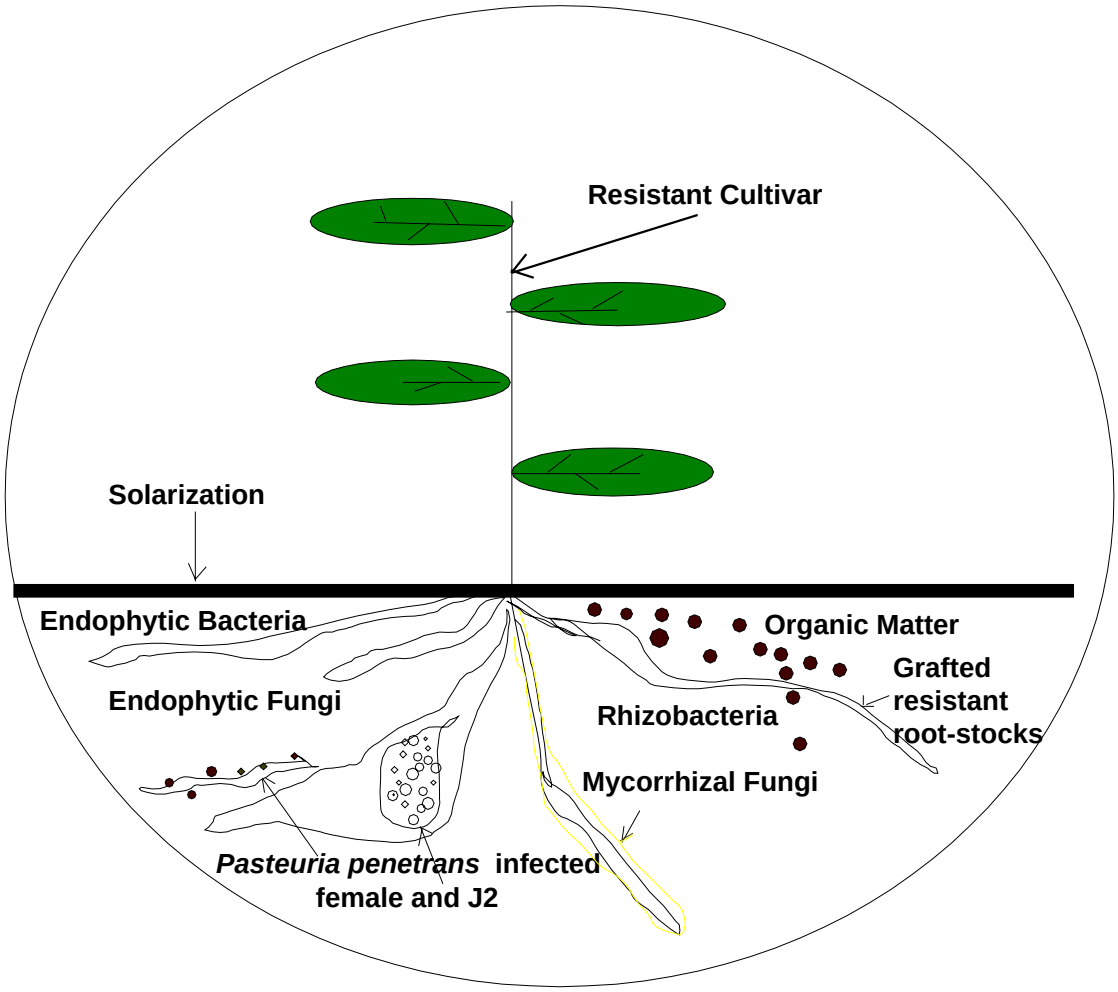


Figure 1: Diagrammatic representation of IPM methods that can be used to manage species of root-knot nematodes, *Meloidogyne*, on vegetable crops in tropical and Mediterranean climatic growing zones.

Below is a list containing acceptable IPM methods that can be combined as needed for root-knot control.

(1) Soil treatment before planting or between crops

* Soil drying
* Soil solarization in hot season
* Rotation with non-host crops

(2) Biological enhancement during seedling production

* Arbuscular mycorrhizal fungi
* Mutualistic fungal or bacterial endophytes
* *Pasteuria penetrans*
* Resistant cultivars

(3) Biological enhancement of seedlings prior to transplanting

* Plant health-promoting rhizobacteria e.g. *B. cereus* S18 and *R. etli* G12
* Grafting resistant/tolerant root stocks

(4) Stimulation of natural antagonistic potential

* Incorporation of organic composts e.g. chicken manure
* Planting and incorporation of green manure e.g. caster, sesame

(5) Management methods during crop growth

* Systemic nematicides
* Optimum plant fertilization and irrigation

(6) Control methods at harvest

* Trap cropping using hosts and degree day monitoring
* Antagonistic cropping with e.g. Tagetes or Crotolaria
* Removal or composting roots to kill nematodes (not with *P. penetrans*)
* Biofumigation with plant residues or green manure crops

In conclusion, the logical use of the methods listed above can lead to effective IPM of root-knot over time in the field. However research, both basic and applied is still needed to make such approaches available to the farmer under practical conditions. Government support for small scale industry may be needed in order to produce biocontrol agents on a large scale for market use by resource poor farmers. In addition, training of extension experts is required to teach them how to optimize IPM combined with biological control for root-knot nematodes mangement.

Acknowledgments

First of all, words will not help me to express my feelings towards my supervisor Prof. Dr. Richard A. Sikora. I would like to thank him for acceptance into his laboratory. I am proud to have been a member of his team. Many thanks for his encouragement, advice as well as moral and scientific support. He was truthfully a Doktorvater to me in the true sense of the word. I will never forget him and his support the rest of my life. Really thanks!

My special thanks go to Dr. Johannes Hallmann for his constant support and helpful and critical remarks in my experimental program. Many thanks also for his discussions and comments on my research work.

I would like to thank Prof. Dr. A. Skowronek for accepting the evaluation of my thesis.

To my BÖS-friends and colleagues from the section of Soil-Ecosystem Phytopathology and Nematology and all members of the Institute of Plant Pathology, I wish to thank you all for the great atmosphere in the laboratory, friendship and the nice memories.

I would like to thank Prof. Dr. Simon R. Gowen and Ms. Barbara, Department of Agriculture, Reading University, United Kingdom for supplying me with the *Pasteuria penetrans* isolates.

Many thanks to my lovely wife Suzan El-Mosallamy for maintaining a comfortable atmosphere and for her great and unending support during the long hard process of writing the thesis. Special also thanks to my family in Egypt.

Finally, I would like to thank the Egyptian Government and the Faculty of Agriculture, Department of Plant Pathology, Menofiya University, Shebin El-Kom, Egypt for the financial support during my stay in Germany.

Curriculum Vitae

Familiy Name	Mahdy
First Name	Magdy, El-Sayed
Date of Birth	05 February 1967
Place of Birth	Shebin El-Kom, Menofiya Governorate, Egypt
Nationality	Egyptian
Sex, Marital Status	Male, Married

Education

1973-1978	Primary School
1979-1981	Middle School
1982-1984	Secondary School
1985-1989	Faculty of Agriculture, Department of Plant Pathology, Menofiya University, Shebin El-Kom, Egypt Degree: B. Sc in Agricultural Science
1990-1994	Position: Demonstrator in Dept. of Plant Pathology, Fac. of Agir., Menofiya Univ., Shebin El-Kom, Egypt Degree: Magister in Agricultural Science (Plant Pathology)
1995-1997	Position: Assistant Lecturer in Fac. of Agir., Menofiya Univ., Shebin El-Kom, Egypt
Since 04/1997	Ph. D. Scholarship of the Egyptian Government in the Section of Soil-Ecosystem Phytopathology and Nematology , Institute of Plant Pathology, University of Bonn, Germany