INTEGRATED PEST MANAGEMENT OF *MELOIDOGYNE INCOGNITA* ON BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA*)

by

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

1.1 Bambara groundnut

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is a leguminous crop indigenous to tropical Africa especially West Africa where it is believed to have originated (Johnson, 1968; Kay, 1979). It is an annual herbaceous plant similar to the groundnut (*Arachis hypogaea L.)* in growth habit but botanically related to cowpea (*Vigna unguiculata* (L) Walp.*)* (Fig.1.1*)*. It forms its pods below the soil surface. The pods are harder and less fibrous than those of the groundnut. Mature pods have a wrinkled surface and usually contain one or more seeds which are harder than those of the groundnut. The crop is adaptable to various climatic conditions and has the ability to produce high yields under optimal conditions and at least some yield under drought stress (Azam-Ali, 1992). Although it generally prefers hot climate, it can tolerate cold conditions but requires three to four frost-free months. It is adapted to a wide range of soils but thrives well on loose sandy, well-drained loam soils with a pH between 5.0 and 6.5 (Johnson, 1968; Kay, 1979).

Coudert (1982) estimated world production of bambara at 330000 tons. About 50 % of this is from West Africa. Yields are generally low because farmers plant bambara groundnut after the main crop and usually when the rainy season is coming to an end because of the believe that it can tolerate conditions which are unsuitable for other crops (Linneman, 1988). In Botswana unreliable source of seed supply in addition to a lack of agronomic information concerning production of bambara groundnut contribute to poor yields (Harris &

Azam-Ali, 1992). However, a survey of small-scale farmers revealed that most farmers prefer bambara groundnut to common groundnut because of its tolerance to erratic rainfall, which makes it difficult to successfully grow groundnuts (Von Rudloff, unpublished report).

Small-scale farmers throughout sub-Saharan Africa cultivate bambara groundnut mainly on a subsistence farming basis. It ranks third in importance after groundnut and cowpea (Rachie & Silvestre, 1977). Farmers favour bambara groundnut for its nutritional value and versatility. Mature seeds are rich in protein and carbohydrates but low in lipids (Brough & Azam-Ali, 1992). Immature kernels are boiled in their green state either shelled or unshelled and provide an early source of nutritious food for the family or they are sold as a snack. Dry seeds are soaked and boiled to prepare various types of dishes.

The commercial value of bambara groundnut remains unexplored though canning of seeds has been reported from Ghana and Zimbabwe (Linneman, 1987). According to Linneman (1988), the crop is the main source of income for small-scale farmers in Nigeria where it attains higher prices than groundnut.

1.2 Root-knot nematodes (*Meloidogyne species*)

Root-knot nematodes are a major problem affecting crop production throughout the world. Yield losses due to root-knot nematodes are estimated at 5 % on worldwide basis (Taylor & Sasser, 1978). These losses occur mainly in small-scale farmer's fields where losses as high as 25 % are reported. Major species of root-knot nematodes such as *M. incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, and *M. arenarea* (Neal) Chitwood are

found within the tropics between 35 °S and 35 °N latitudes and more are common towards the equator while *M. hapla* (Chitwood), is a cool-temperate species. These species account for 95 % of the root-knot nematodes received by the International *Meloidogyne* Project (IMP) from agricultural soils (Sasser and Carter, 1985). The species were identified through the investigations of differences in their host preferences and morphological characteristics such as perineal patterns of females, stylets of females, heads and stylets of males, stylets of second-stage juveniles and tails of males and second-stage juveniles. Cytology and biochemistry were also used (Eisenback, Hirschman & Triantaphyllou, 1980; Eisenback, 1985; Jepson, 1983a & b; Taylor & Sasser, 1978; Triantaphyllou, 1985; Hussey, 1985). Because of the variations in responses of some species to different hosts, the North Carolina Differential Host Test was developed to further distinguish species into host specific races (Sasser & Triantaphyllou, 1977). On this basis, *M. incognita* was found to have four distinct host races and *M. arenarea* two, whereas *M. hapla* and *M. javanica* do not have host races. Although *M. incognita* is the most abundant root-knot nematode in the world, in South Africa it is second to *M. javanica* in distribution and host range (Kleynhans, Van den Berg, Swart, Marais & Buckley, 1996). *M. incognita* race 2 constitutes most of the South African population and has a wide host range while host race 4 is common in tobacco and cotton growing areas and host race 1 was reported only once on banana (Kleynhans et al., 1996).

1.3 Damage caused by *Meloidogyne species*

Root-knot nematode *M. incognita* race 2 attacks almost all cultivated crops in Botswana (Busang, 1983). Hosts include vegetables such as tomato (*Lycopersicon esculentum* Mill.), spinach (*Spinacia oleracea* L.), beetroot (*Beta vulgaris* L.), carrot (*Daucus carota* L.),

cabbage (*Brassica oleracea capitata* L.), pepper (*Capsicum frutescens* L.) and pea (*Pisium sativum* L.). An unidentified root-knot nematode species was found on pigweed (*Amaranthus thunbergii* Morq.), prostrate globe amaranth (*Gomphrena celoides* Matt.) and *Pavonia trocumbens* (Department of Agricultural Research, Botswana, unpublished). Serious damage caused by root-knot nematodes was observed on bambara groundnut in Sebele, Botswana (D. Wigglesworth, Grooms Cottage, Scarborough, Beaminister, Dorset, UK). Similar observations were made in the Botswana College of Agriculture fields (Phadima, 1994). Affected plants were stunted and chlorotic, had few or no pods and in severe cases plants died prematurely. Mwindilila (1994) observed root-knot nematodes and *Fusarium oxysporum* Schelcht. emend. Snyd. & Hans on bambara groundnut in Content Farm, Sebele. Root-knot nematodes were also reported on bambara groundnut in the former Rhodesia (Martin, 1959; Johnson, 1968), South Africa (Mc Donald & De Waele, 1989), Nigeria (Ogbuji, 1979) and Malawi (Hillocks, Stokes & Jones,1995). *M. javanica* was the most common species identified from infested fields although *M. incognita* was also encountered in Botswana and Nigeria. Although the disease was reported to be severe in all the areas it was found, no statistical data is available to support these findings.

1.4 Control of *Meloidogyne* species

Very little research has been conducted on pests and diseases of bambara groundnut although indications are that the crop is not free from pests and diseases as previously thought (Karikari, 1971; Linneman, 1990). It is therefore necessary to investigate appropriate control measures that will reduce yield losses reported under small-scale farmer production systems. Although chemical control through use of nematicides has proved to be

most effective, nematicides are generally expensive and small-scale farmers cannot afford them. Many nematicides are being removed from the market because of their hazardous effects on the environment. Available alternatives could be non-chemical methods that utilise resources that are easily accessible to the farmer and/or are part of his production practices, including amongst others the use of resistant/tolerant crop varieties, organic soil amendments, solarization and biological control. An integrated control strategy will provide a solution to the escalating pest and disease problem limiting crop production under small-scale farmers' situation.

1.4.1 Resistance/Tolerance

Resistance in plants to parasitic nematodes is defined as an active defence mechanism that inhibits, restricts, retards or alters nematode development (Dalmasso, Castagnone-Serero & Abad, 1992). Resistant varieties suppress reproduction and development of the nematode resulting in lower initial populations and consequently less potential damage for the next crops (Fassuliotis, 1987; Cook & Evans, 1987; Trudgill, 1991). Although initial infection occurs, most resistant cultivars have tolerance to injury and produce reasonable yields (Roberts, 1995). Hence resistance has been used on a number of crops to manage nematodes effectively (Cook & Evans, 1987; Fassuliotis, 1979; Evans & Trudgill, 1978; Trudgill, 1991; Roberts, 1995). With the growing concern about the hazardous effects of nematicides on the environment coupled with their expensiveness and unavailability, resistant varieties have proved to be a good alternative strategy for controlling plant-parasitic nematodes (Dalmasso et al., 1992). Some resistant varieties have an added advantage in that they are easy to use and provide resistant green manure. Unfortunately it has been observed that the efficiency of

resistant varieties is limited to a few species or pathotypes and ultimately the nematode may overcome the resistance (Trudgill, 1991).

Tolerance is a general adaptive phenomenon of plants faced with multiple environmental stresses and is therefore not a true mechanism of resistance (Canto-Saenz, 1985; Wallace, 1987; Trudgill, 1991). A tolerant variety is an efficient host of the nematode but it has the ability to endure attack without sustaining severe losses in yield (Evans & Trudgill, 1990). Wallace (1987) attributes this to some environmental conditions that influence tolerance. Although tolerance is a desirable characteristic of the plant, tolerant varieties have the potential to increase nematode population densities to extremely damaging levels (Dalmasso et al., 1992).

The mechanisms involved in resistance are complex and have been investigated by several researchers (Jatala & Russell, 1972; Rich, Keen & Thomason, 1977; Kaplan, Thomason & van Gundy, 1979; Kaplan, & Keen, 1980; Veech, 1981; Giebel, 1982; Trudgill, 1991). Two types of resistance, passive and active resistance were identified as similar to genetically operating mechanisms in plants (Giebel, 1982). Passive resistance is pre-infectional and is conditioned by anatomical, physiological and chemical barriers that may hinder invasion of the plant by the nematode. Mechanisms involved in passive resistance include production of toxins that kill the nematode and a lack of, or inadequacy of substances necessary for development and reproduction of a certain nematode species. For example, α terthienyl contained in *Tagetes* species is toxic to *Meloidogyne* and *Pratylenchus* species (Veech, 1981). Coumestrol from lima beans (*Phaseolus lunatus* L.) inhibits nematode activity in

resistant cultivars (Rich et. al., 1977) while resistance of soybean (*Glycine max* L.) can be attributed to the production of glyceolin-resistant soybean roots (Kaplan & Keen, 1980). A lack of hatching factor in certain resistant sweet potato (*Ipomoea batatas* L.) hybrids is associated with the inability of cyst nematode eggs to hatch (Jatala & Russell, 1972). Unlike passive resistance, active resistance is post-infectional and operates in host plants due to contact with parasites (Giebel, 1982). It is based on tissue hypersensitivity to nematode infection due to presence of the parasite.

Very little is known about the mechanisms responsible for tolerance. However, it is believed that host tolerance is related to the physiology of the whole plant, which depends on multiple genes interacting with crop conditions and the age of the plant (Dalmasso et. al., 1992). Spiegel, Cohn & Kafkafi (1982) showed that tomato plants grown in soil with a high nitrate level were tolerant to root-knot nematodes. Similarly, deficiencies in irrigation or in nutritive elements often affect the growth and yield of plants infected by nematodes (Evans & Haydock, 1990). Canto-Saenz & Brodie (1982) reported that plant age at time of inoculation with *M. incognita* did not affect host efficiency of potatoes (*Solanum tuberosum* L.). However, roots of plants inoculated when five and ten days old were significantly reduced.

Very little research has been done on resistance of bambara groundnuts to *Meloidogyne* species. Ogbuji (1979) evaluated seven bambara groundnut cultivars for resistance to *M. incognita* and *M. javanica* in Nigeria and reported that all cultivars were susceptible to both species. Pod production (yield) in infected plants was significantly reduced or in most cases totally absent. Ogbuji (1979) concluded that both resistance and tolerance were lacking in

the seven genotypes tested. In a related study in South Africa involving *M. javanica*, Mc Donald & De Waele (1989) reported absence of resistance in all fifteen bambara groundnut genotypes evaluated. Mc Donald & De Waele (1989) noticed a significant difference in yield amongst the genotypes and concluded that there is a possibility that tolerance exists in some bambara groundnut genotypes. Although so far no resistance has been reported on bambara groundnut, cowpea, a near relative of bambara groundnut is reported to be resistant to *M. incognita* (Fassuliotis, 1979; Sirohi & Dasgupta, 1993; Pandey et al., 1995). Thirty cowpea cultivars resistant to *M. incognita* were reported in the United States of America (Fassuliotis, 1979) and five in India (Pandey, Hasan, Bhaskar, Ahmad & Kohli, 1995). Sirohi & Dasgupta (1993) studied mechanisms of resistance in cowpea and concluded that the resistance present involves mRNA synthesis that directs the production of a set of proteins associated with expression of disease resistance. Groundnut, which is similar to bambara groundnut morphologically, is not attacked by *M. incognita* and is therefore resistant to this species (Taylor & Sasser, 1978; Ibrahim, Rezk & Ibrahim, 1991). However, the crop is reported to be highly susceptible to *M. javanica* (Taylor & Sasser, 1978).

1.4.2 Organic amendments

Soil organic amendment is an ancient agricultural practice involving incorporation of animal manure, composts, plant residues and other organic materials into the soil (Stirling, 1991; Bridge, 1996). It is practised by both small-scale and large-scale farmers alike with the aim of improving soil fertility and subsequently plant growth (Stirling, 1991). The practice also improves the physical characteristics of the soil and suppresses soil-borne pests and diseases (Chen, Hoitink, Schimittner & Tuoivinen, 1988; Stirling, 1991). The use of organic

amendments to suppress plant-parasitic nematodes has gained a lot of attention recently. Commonly used amendments are mainly bioproducts and wastes from agricultural and other activities (Bridge, 1996). Manure from poultry and cattle has been used as soil amendments and significantly reduced plant parasitic-nematodes on a number of crops (Main & Rodriguez-Kabana, 1982; Sivakumar & Vidhyasekaram, 1990; Chindo & Khan, 1990; Poswal & Akpa, 1991; Gonzalez & Canto-Saenz, 1993; Kaplan & Noe, 1993; Gamliel & Stapleton, 1993a & b; Riegel, Fernandez & Noe, 1996). Addition of chicken litter to the soil reduced *M. arenarea* population on squash (*Cucurbita pepo L.*) significantly and subsequently increased growth and yield (Rodriguez-Kabana, 1982). Control was directly proportional to the amount of litter added within the range of 0-5 % w/w litter to dry soil. Chindo & Khan (1990) reported a remarkable reduction of *M. incognita* population on tomato with a concomitant increase in growth and yield. The population, however, increased again at harvest in treatments that received less than 4 ton/ha. Chindo & Khan (1990) attributed the fact that there was no increase in *M. incognita* population in treatments with 4 and 8 ton/ha to the prolonged residual effect at these dosages that effectively controlled the nematode throughout the season. An observed decrease in population density and increase in growth and yield could be ascribed to a number of factors such as: increased nutrient availability to the plant, improvement in soil physical condition that enabled plants to efficiently utilise nutrients consequently minimising nematode damage, changes in biotic and abiotic environment of the plant that ultimately altered the host-parasite relationships, and release of some toxic substances during decomposition of poultry manure (Chindo & Khan, 1990). This confirms previous findings by other researchers (Wilkinson, 1976; Main & Rodriguez-Kabana, 1982). Chindo & Khan (1990) recommended 4 ton/ha as a compromise

level of poultry manure for both nematode and plant based on the fact that there was no significant difference in yield between plants amended with 4 ton/ha versus 8 ton/ha. Plants amended with 8 ton/ha tended to grow more vegetatively and flowered late, which could be contrary to what the farmer wanted. Riegel et al., (1996) in a study investigating the effects of chicken litter on *M. incognita* in cotton (*Gossypium hirsutum* L.) over a two-year period, recorded lower population density at the end of the season in litter-amended plots than in control plots. Plant growth and yield were enhanced by addition of litter and was attributed to high quantities of nutrients contained in chicken litter. Riegel et al. (1996) observed an increase in microbe densities in litter amended treatments than non-amended treatments. There was a rapid increase in bacterial numbers that lasted throughout the growing season after addition of the litter. *M. incognita* numbers were consequently lower in these plots at midseason and this, according to Riegel et al. (1996), could be the result of ammoniacal nitrogen released during decomposition of litter that decreased nematode numbers while other factors increased bacterial densities. Main & Rodriguez-Kabana (1982) concluded that addition of litter into the soil might have introduced new bacteria thus providing a source of food for existing and incoming organisms, hence stimulating the bacterial population. Riegel et al. (1996) isolated several bacteria and funji from litter and litter-amended soil, viz. *Arthobacter, Bacillus, Pseudomonas, Acremonium, Aspergillus, Eurotium, Paecilomyces, Petriella and Scopulariopsis.*

The effect of using cattle manure is not as widely researched as the use of poultry manure. Poswal & Akpa (1991) reported good control of *M. incognita* with cow dung in Nigeria.

M. incognita population on *Coleus forskohli* (Wild.) Briq. (Labiate) was effectively controlled with a combination of farmyard manure and *Paecilomyces lilacinus* (Thom) Samson (Sivakumar & Vidhyasekaram, 1990).

The mode of action of organic amendments is complex and involves several mechanisms (Stirling, 1991). These mechanisms include improvement of soil structure and fertility, release of nematoxic compounds and stimulation of predators and antagonistic microbes. A decrease in *M. incognita* population and an increase in tomato yield in soil amended with poultry manure was attributed to factors such as increased nutrient availability to the plant, improvement in soil physical condition, changes in biotic and abiotic environment of the plant, and release of nematoxic substances during decomposition of manure (Chindo & Khan, 1990). Riegel et al. (1996) suggested that the observed reduction in *M. incognita* population at midseason could have been due to increased bacterial numbers resulting from the addition of chicken litter. According to Riegel et al. (1996), chicken litter could have stimulated microbial activity and resulted in increased bacterial numbers that lasted throughout the growing season. The ammoniacal nitrogen released during decomposition could also have contributed to the low *M. incognita* population at midseason. The ammoniacal nitrogen decreased nematode numbers while other factors increased bacterial densities. Main & Rodriguez-Kabana (1982) suggested that addition of litter to soil might have introduced new bacteria thus providing a source of food for existing and incoming organisms, hence stimulating the bacterial population. Chicken litter contains a wide range of bacteria and fungi, some of which are toxic/antagonistic to nematodes and other microorganisms (Lovett, 1972; Jatala, 1986; Stirling, 1991; Siddiqui & Husain, 1991; Riegel,

et al.,1996). Lovett (1972) isolated toxigenic fungi such as *Aspergillus*, *Penicillium*, *Fusarium* and *Scopulariopsis* from litter and feeds. Riegel et al. (1996) isolated fungal and bacterial genera, which include *Acremonium*, *Aspergillus*, *Eurotium*, *Paecilomyces*, *Petriella*, *Scopulariopsis*, *Arthrobacter*, *Bacillus* and *Pseudomonas* from litter, and litter-amended soil. Some of these micro-organisms are reported to be antagonistic to nematodes (Jatala, 1986; Stirling, 1991, & Siddiqui and Husain, 1991).

1.4.3 Biofumigation

Biofumigation is the process of amending soil with organic matter that release toxic gases which reduce or eliminate soil-borne pests and pathogens. Organic matter from cruciferous crops is of interest for use in biofumigation since these crops are known to contain different types and concentrations of sulfur-containing glucosinolates in their tissues (Sang, Minchinton, Johnstone & Truscott, 1984). These glucosinolates break down upon decomposition to release volatiles such as allylisothiocyanate (AITC); methylisothiocyanates (MITC); butylisothiocyanates (BITC); phenylisothiocyanates (PITC); volatile aldehydes and sulfides (Lewis & Papavizas, 1971). Of these, MITC and AITC are the most volatile with the highest vapour pressure and have well-documented biocidal activity (Lewis & Papavizas, 1971). The ability of some B*rassica* species to release volatile biocidal compounds suggests that incorporation of green tissue of these plants, as "green manure" could be useful for the control of a wide range of soil-borne pathogens. MITC is an active ingredient of metham sodium, which is widely used as a soil fumigant against many soil-borne pathogens (Gamliel & Stapleton, 1997). Allylisothiocyanates were found in leaf extracts of various *Brassica* species and were effective in suppressing several fungal pathogens (Mayton, Olivier, Vaugh

& Loria, 1996). However, there was variability in the suppressive activity of volatile biocidal compounds and AITC concentration within and across *Brassica* species tested. Mayton et al. (1996) suggested that *Brassica* species with high concentrations of AITC might provide greater control of soil-borne pathogens than species with little volatile activity. For example, hemp (*B. juncea* L.) contained the highest concentration of AITC amongst *Brassica* species included in their study, and had the highest degree of suppressive activity. Mojtahedi, Santo, Wilson & Hang, (1993) reported that when green tissue of rapeseed (*B. napus* cv. Jupiter) was used as a soil amendment, it effectively reduced *M. chitwoodi* population densities at the zone of incorporation. The amendment provided protection from the nematode for up to six weeks. This contradicts earlier findings by Brown, Morra, McCaffrey, Auld & Williams (1991) that green materials releasing volatile AITC is unlikely to be effective in soil after four weeks. Mojtahedi et al. (1993) observed that the concentration of glucosinolates increased with the age of the plant. Two-month old rapeseed plants were more effective in controlling *M. chitwoodi* population (Mojtahedi, Santo, Hang & Wilson, 1991). Johnson, Golden, Auld & Summer (1992) reported that six-month old plants were less effective. The effectiveness of green manure amendment from rapeseed cv. Jupiter increased with increase in the dosage added to the soil. According to Mojtahedi et al. (1993), second-stage juveniles were more sensitive than eggs within egg masses. Most eggs did not yield infective juveniles when rapeseed cv. Jupiter was applied at 49 mg/g soil. The second-stage juvenile population density declined sharply with no nematode survival at 39 mg/g soil. Rapeseed was only effective in the amended zone (top 5 cm). McLeod , Somers & Gendy (1995) reported that rapeseed varieties Rangi, Humus and Arran reduced root-knot and citrus nematode population densities while non-*Brassica* crops included in the study failed.

McLeod & Da Silva (1994) observed that rapeseed var. Humus applied at 20 and 40 g/550 g soil was as effective as 8 mg of fenamiphos nematicide treatment.

Although biofumigation has potential as an alternative control to nematicides, it is likely to have some disadvantages such as the release of phytotoxic compounds and additional time before planting for amendment decomposition.

1.4.4 Solarization

Soil solarization is a non-chemical method of disease control accomplished by sealing the soil surface with a clear plastic tarpulin to trap solar radiation and accumulate heat (Chen & Katan, 1980; Chen et al., 1988; Pullman, De Vay & Garber, 1981 and Gamliel & Stapleton, 1997). In order for soil solarization to be more effective, it must be carried out during the hottest season of the year and the plastic tarpulins must be kept tight against the soil surface. The soil must be kept moist during the solarization period (Heald & Robinson, 1987). Under ideal conditions, soil temperatures can be raised to levels that are lethal to many soil-borne pests and pathogens (Gamliel & Stapleton, 1997). Solarization is reported to have controlled a wide range of pathogens including plant-parasitic nematodes such as *Meloidogyne, Heterodera, Pratylenchus and Paratylenchus* (Gamliel & Stapleton, 1997; Stapleton & De Vay, 1983). According to Stapleton & De Vay (1983), population densities of total plant-parasitic nematodes were reduced by 61-96 % compared with the control. In order to attain pathogen control down to 45-60 cm depth, solarization is usually conducted for a period of four weeks or longer (Gamliel & Stapleton, 1997). Significant nematode control was achieved at various depths under ideal soil solarization conditions.

Temperatures in excess of 50 °C were measured in the top few centimetres of the soil under these conditions (Katan, 1981, Stapleton & De Vay, 1983 and Heald & Robinson, 1987). Control of phytoparasitic nematodes was satisfactory near the soil surface and population density reduction decreased with increasing soil depths. Gamliel & Stapleton (1997) reported low soil temperatures in field soil during soil solarization compared with artificial heating methods. They also suggested that the effects on living and non-living soil components are likely to be less drastic especially for high temperature tolerant nematode species such as *Meloidogyne incognita.* Stapleton & De Vay (1983) concluded that the degree of reduction of phytoparasitic nematodes depend on several factors such as the degree of solar heating, crop and cropping history, nematode distribution in the soil and soil depth. Stapleton & De Vay (1983) suggested that a significant part of the nematicidal effect of soil solarization may be directly or indirectly due to maintaining a high soil moisture content for several weeks, changes in soil gas composition and/or accumulation of volatile compounds. Although there are changes in soil gas composition as well as accumulation of volatile compounds, no negative side effects such as phytotoxicity have been reported with soil solarization (Gamliel & Stapleton, 1997).

Soil solarization has advantages and disadvantages. The main disadvantage of soil solarization is the long duration of the treatment, its dependency on warm climate and the fact that it does not control all pathogens (Gamliel & Stapleton, 1995). Stapleton & De Vay (1983) reported partial control of *M. incognita* in solarized soil. It is therefore necessary to improve the efficacy of the technique to enable it to be used under a wider range of conditions and to shorten the duration of the treatment. There are several reports of

successful control when solarization was used in combination with other methods such as soil organic amendment, biofumigation and chemical control (Ramirez-Villapudua & Munnecke, 1987, 1988; Ristaino, Perry & Lumsden, 1991; Gamliel & Stapleton, 1993a & b, 1995).

1.4.5 Integrated pest management (IPM)

Integrated pest management (IPM) has been described as a system of management which considers the interactions amongst the whole range of organisms, whether beneficial, neutral or pests, with the long-term objective of increasing the level of pest suppression by means of natural as opposed to chemical means (Tait, 1987). According to Tait (1987), IPM is a holistic approach in which pest control by natural factors is the controlling influence on decision making. IPM seeks to reduce the use of pesticides, and to determine better ways of maintaining their effectiveness as pest control agents so as to alleviate environmental and health concerns. Hence, most IPM systems will involve minimum use of pesticides. It has also been stated that the purpose of IPM is to make crop production more efficient and to protect the environment from the misuse of pesticides (Hall, 1995). According to Hall (1995), this could be achieved by utilising all relevant pest control technologies in an integrated program. Hall (1995) suggested that such integration could consist of any of the following approaches:

1. Monitoring pest population or weather conditions and applying pesticides as required to control pests
2. Integration of many practices to control one pest or one kind of pest
3. Integration of all farming practices to provide protection against all pests within a crop, or on a farm, or over a wider geographic area.

The increase in food requirements for the increasing world population has made it necessary to investigate suitable pest control methods that will minimise damage to crops without compromising the safety of humans, livestock and the environment. IPM therefore holds promise as an alternative to hazardous chemicals. However, the implementation of IPM requires several kinds of integration in order for it to be successful. These include the integration of pesticides with other pest control techniques such as genetic resistance in plants, cultural practices, biological control, and biotechnology. It is also essential that technologies for the control of one group of pests must be integrated with those for the other pests (Hall, 1995). Most researchers involved with IPM have implemented these requirements (Stapleton & DeVay, 1983, Rammirez-Villapudua, 1987, 1988, Duncan, 1991).

1.5 Objectives

The study was conducted to evaluate different nematode control strategies with the aim of developing an IPM model for the control of *M. incognita* on bambara groundnut. Several experiments were conducted in the greenhouse and microplots to:

- Identify bambara groundnut genotypes with resistance/tolerance to *Meloidogyne incognita* race 2
- Determine the effectiveness of soil organic amendment as a control measure for *M. incognita* race 2 on bambara groundnut
- Determine the effectiveness of biofumigation on *M. incognita* race 2 on bambara groundnut
- Determine the effectiveness of biofumigation combined with soil solarization on root-knot nematodes on bambara groundnut.

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Fig. 1.1: Eight-week old *Vigna subterranea* plant grown in the greenhouse

CHAPTER 2

SCREENING OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA*) LANDRACES FOR RESISTANCE/TOLERANCE TO *MELOIDOGYNE INCOGNITA* RACE 2.

ABSTRACT

Greenhouse studies were conducted to evaluate fifty bambara groundnut (*Vigna subterranea* (L). Verdc.) landraces obtained from Botswana and South Africa for resistance/tolerance to *Meloidogyne incognita* race 2. Each landrace was inoculated with 5000 *M. incognita* eggs and evaluated for galls and egg masses eight weeks later. Host suitability was determined using Canto-Saenz's host suitability designations. None of the landraces was resistant to *M. incognita* race 2. However, landraces HVA 38-3, SB 4-4C, CLRDE and Swazi V4 showed slight tolerance to the nematode. The nematode reduced growth and yield of all landraces.

2.1 Introduction

The root-knot nematode, *M. incognita* is a serious problem in many crop-production areas throughout the world. The nematode affects all cultivated crops including bambara groundnut (*Vigna subterranea*). Although there is very little information regarding M. *incognita* on bambara groundnut, there are indications that the nematode can severely affect the crop and result in significant yield losses (Ogbuji, 1979). This is especially a problem in small-scale-farmer situations where the crop ranks third in importance after cowpea and

groundnut as a main source of food and income. Control of *M. incognita* on bambara groundnut in small-scale-farming systems can best be achieved by the use of resistant varieties. Ogbuji (1979) concluded that both resistance and tolerance are lacking in the Nigerian bambara groundnut genotypes screened against M. *incognita* and *M. javanica.* Mc Donald & De Waele (1989) made similar observations with *M. javanica* on bambara groundnut although they suggested that tolerance might exist in some genotypes. The objective of the present study was therefore to evaluate bambara groundnut landraces from Botswana and South Africa for resistance and/or tolerance to *M. incognita* race 2 which is the predominant host race in these two countries.

2.2 Materials and Methods

The study was conducted at two locations, the Botswana College of Agriculture (BCA) and the University of Pretoria (UP) experimental farm over a two-year period using germplasm from Botswana and South Africa (Fig 2.1).

Experiments at Botswana College of Agriculture

Two experiments were conducted at BCA between February and July 1996 in a greenhouse at temperatures maintained between 20 and 30 °C. The soil used in both experiments was a sandy loam (75 % sand, 5 % silt, 20 % clay and pH 6.0). The topsoil was mixed with river sand at a ratio of 2:1 and fumigated with methyl bromide prior to use. Fifteen bambara groundnut landraces were used in experiment 1 and five landraces in experiment 2 (Table 2.2a and 2.2b). Thirty-five centimeter diameter plastic pots were filled with soil and arranged on benches in a completely randomised design. To ensure optimum nitrogen

fixation seeds were treated with a cowpea group inoculant, *Bradyrhizobium* spp. (*Vigna*) obtained from Stimuplant (P.O. Box 2013, Swavelpoort, Pretoria 0036) before planting. For each landrace both *M. incognita*-inoculated and non-inoculated (control) plants were included using 5 replicated pots per treatment with three seeds planted in each pot. Pots were watered daily with tap water. Seedlings were thinned to one per pot six weeks after emergence and fertilised weekly with a solution of Multifeed P_{43}® fertiliser applied at 100 g /liter of water (Plaaskem (Pty) Ltd, P.O.Box 87005, Houghton, 2041).

Nematode inoculum was obtained from BCA from heavily galled spinach roots. Identification of the species and race were done using the North Carolina Differential Host Test (Sasser & Triantaphyllou, 1977) and was confirmed by Dr. K. Kleynhans (Agricultural Research Council, Plant Protection Research Institute, Pretoria) by means of morphological studies. Five thousand *M. incognita* race 2 eggs were extracted from heavily galled spinach roots using the NaOCl technique described by Hussey & Barker (1973). A 5 ml suspension containing 5000 eggs was applied to each plant. The suspension was pipetted into depressions made around the crown of each seedling. After inoculation, plants were allowed to grow for ten weeks to enable the nematodes to complete two life cycles and the crop to reach maturity. Plants were sprayed with cypermethrin applied at the rate of 150 ml/ha to control aphids. Powdery mildew was controlled with a foliar spray of triforine applied at the rate of 1 ml/liter of water.

The plants were harvested ten weeks after inoculation. Tops (shoots) were cut from each plant, the fresh mass determined and then dried to measure dry mass. Roots were gently

washed free of soil and their fresh weight determined. Each root sample was stained in 0.15 g/liter aqueous solution of Phloxine B (Hussey & Barker, 1973) for 15 minutes before being evaluated for galls and egg masses. Galls and egg masses were rated using a 0-5 root gall or egg mass index (Taylor & Sasser, 1978) where 0 = 0 galls or egg masses, 1 = 1 - 2, 2 = 3 - 10, 3 = 11 – 30, 4 = 31 -100 and 5 ≥ 100 galls or egg masses per root system. To obtain the final population (Pf), eggs were extracted from roots using the NaOCl technique of Hussey & Barker, (1973). The reproduction factor (R factor) of each landrace was calculated by dividing the final population with the initial population (Pi). Canto-Saenz (1985) host suitability designations were assigned to determine resistance or tolerance.

Experiments at the University of Pretoria

Five experiments were conducted in a greenhouse at UP between February 1996 and April 1997. Greenhouse temperatures between 20 and 30 °C were maintained throughout. The procedures followed in all experiments were the same as for experiments conducted at the BCA except where specified.

The soil used was a 2:1 mixture of topsoil and river sand with 80 % sand, 4 % silt, 14 % clay and a pH of 5.4. The soil was steam-pasteurised at 100 °C for 1 hour before being used. Artificial light was provided by means of 250 watt mercury vapour lights for 2 hours after sunset from April to end of July to increase day length since bambara groundnut is sensitive to photoperiod (Linneman, 1994). Most of the germplasm used in the experiments was obtained from South Africa. Germplasm used in experiment 6 was a mixture of germplasm

from Botswana and South Africa selected on the basis of their performance in previous experiments (Table 2.2c-g).

Harvesting of experiments 1 to 4 was done twelve to fourteen weeks after inoculation. Experiment 5 was harvested six weeks after inoculation. No yield data was collected for experiment 3.

All data were analysed by ANOVA and where necessary means were separated by Duncan's multiple-range test (SAS, BMDP Statistical Software, Los Angeles, CA). Gall and egg mass index values were ranked before they were analysed statistically. Where ranked gall index and egg mass index values were identical, only gall index values were presented.

2.3 Results

Expriments at Botswana College of Agriculture

All the landraces tested in experiment 1 did not differ significantly with regard to gall index and egg mass index. Landrace OM1 was significantly different from the other landraces in final population and R factor (Table 2.3a). *M. incognita* race 2 reduced plant growth (fresh weight of roots and dry mass of shoots) although there was no significant difference between landraces. Landraces Goo B differed significantly from Gab C, JB Pop 2, JB Pop 5, JB Pop 10, JB Pop 11 and Jac C with regard to reduction in yield (number of pods). However, there was no significant difference between landraces in the reduction of the dry mass of pods (Table 2.3b).

In experiment 2 some of the five landraces tested were significantly different from the other in gall and egg mass index. WS 52 had the highest gall and egg mass index and was significantly different from SB 4-4E and S10, which had the lowest indices. There were no significant differences between landraces in terms of the final population and R factor. All landraces were susceptible to the nematode (Table 2.3c). The highest reduction in dry mass of shoots was recorded for S13 and this was significantly different from S10 and SB 4-4E. S13 and WS 52 had the highest reduction in dry weight of pods compared with other landraces. There was no significant difference between landraces in terms of the reduction in fresh weight of roots and number of pods (Table 2.3d).

Experiments at the University of Pretoria.

All landraces tested in experiment 1 were susceptible to *M. incogita* race 2. Landrace K1 was significantly different from CLDRE in terms of the final population and R factor (Table 2.3e). SB 8-1 had the highest gall index value of 22.80 and was significantly different from V4 S1 and CLDRE with gall index values of 11.20 and 10.40 respectively. K1 had the highest egg mass index value of 22.90 and was significantly different from CLDRE with an egg mass index value of 7.90 (Table 2.3e). Swazi V4 and V4 S1 were significantly different from K1 in the reduction in dry weight of shoots (Table 2.3f). Swazi V4 was significantly different from SB-81 in terms of reduction in number of pods. There were no significant differences between landraces in the reduction of dry weight of pods and fresh weight of roots (Table 2.3f).

In experiment 2, Potgietersrus and Marabastad differed significantly from HVA 38-3 in terms of gall and egg mass indices. SB 4-4C was significantly different from Potgietersrus and Marabastad in terms of gall index but not in egg mass indices. SB 4-4C and HVA 38-3 did not differ significantly in both gall and egg mass index. No significant differences occurred between landraces in the final population and R factor values (Table 2.3g). The highest reduction in dry weight of shoots was recorded for M4 while Groblersdal and SB 4-4C had the lowest values. No significant differences occurred between landraces in terms of reduction in fresh weight of roots, number of pods and fresh weight of pods (Table 2.3h).

In experiment 3, there were no significant differences between the eight landraces in gall and egg mass index, final population and R factor values. All the landraces were susceptible to the nematode (Table 2.3i). Landraces did not differ significantly with regard to the reduction in growth (Table 2.3j).

The six landraces tested in experiment 4 did not differ significantly from each other in gall index, egg mass index, final population, and R factor values (Table 2.3k). Landraces differed significantly in the reduction in fresh weight of roots. There were no significant differences between landraces with regard to the reduction in dry mass of shoots and number of pods (Table 2.3*l*).

In experiment 5, no significant differences occurred between landraces with regard to the gall index, final population and R factor values. However, SB 20–2A differed significantly from Goo B in terms of egg mass index (Table 2.3m). The reduction in dry mass of shoots ranged from 5.26 to 2.32. The highest reduction occurred in S9 and this was significantly different

from SB 20-2A that had the lowest value. There were no significant differences between landraces in terms of the reduction in fresh weight of roots. SB 20-2A had the highest reduction in number of pods and was significantly different from S9, Goo B and DIPC whereas SB 20-2A, JB Pop 11 and CLDRE did not differ significantly (Table 2.3n).

2.4 Discussion

All landraces tested at BCA in experiment 1 were susceptible to *M. incognita* race 2 according to Canto-Saenz's (1985) host suitability designations. The R factors for all landraces were above 1.00 and gall indices above 2.00. Canto-Saenz's designations are based jointly on host efficiency (nematode reproduction on host) and damage to the plant (gall index) and is a better way of categorising resistance in plants than using gall index or egg mass index only. The same landraces are susceptible even when other evaluation methods are used (Taylor & Sasser, 1978; Hadisoeganda & Sasser, 1982). There were no significant differences between landraces in terms of the reduction in plant mass (dry weight of shoots and fresh weight of roots). This shows that all the fifteen landraces responded in a similar way to infection by *M. incognita* race 2. Landraces differed significantly in yield reduction (number of pods) due to infection. However, a significant reduction in dry mass of pods was only recorded in one experiment (Table 2.3d). This could be attributed to the fact that pod formation did not occur at the same time for all landraces. Some landraces formed pods late and at the time of harvest some of the pods were still immature. This is a common phenomenon in bambara groundnut especially when pod formation coincides with reduced day-length (Linneman, 1994) as it was the case in this experiment. Most of the landraces showing smaller reductions in yield due to *M. incognita* race 2 infection were the JB

populations obtained from the Department of Agricultural Research, Botswana. It is possible that these landraces are of similar origin with differences between them being due more to genetic drift in small isolated populations rather than any conscious selection (Wigglesworth, 1996). Interestingly, all landraces from BCA collection except one (Gab C) had greater yield reductions as a result of infection by *M. incognita* race 2. Again this may be an indication of a similarity in origin of these landraces since they were collected from farmers within the same region. It is possible that seed could have been bought from outside Botswana and that selection of larger light coloured seed for planting took place. This is a common practice by small-scale farmers in Botswana.

In experiment 2, the five landraces from the Potchefstroom collection showed significant differences in gall and egg mass indices. Coincidentally, the gall index and egg mass index values were identical and all landraces were susceptible according to Canto-Saenz's designations. The landraces were also significantly different in terms of the reductions in plant mass (dry weight of shoots) and yield (dry weight of pods). There was a positive correlation between gall index (plant damage) and the reduction in plant mass and yield due to infection by the nematode. Landraces with high gall indices showed greater reduction in growth, due to *M. incognita* infection than those with low gall indices. This shows that *M. incognita* had an effect on all five landraces tested in this experiment.

Of the landraces evaluated in experiment 1 at the University of Pretoria, CLDRE was a marginal case. Although it is susceptible according to Canto-Saenz's designations, it had a low R factor and a low gall index (1.10 and 2.24 respectively). V4 S1 and Swazi V4 showed

the lowest reduction in dry weight of shoots due to *M. incognita* infection. These three landraces were susceptible to M. *incognita* according to Canto-Saenz's interpretation but they could otherwise be considered tolerant because they were able to withstand the attack by the nematode and produce higher yields than the other landraces.

In experiment 2, HVA 38-3 performed better than others and had the lowest gall indices and R factor values. However, this landrace is susceptible because its gall index is greater than 2. Potgietersrus and Marabastad had high gall indices that correlated positively with the reduction in plant mass. All landraces tested in experiments 3 and 4 were susceptible and no significant differences occurred between landraces. Although there were reductions in plant mass as well as in yield, landraces did not differ significantly

The six landraces tested in experiment 5 did not differ from each other in gall index, final population and R factor values. Goo B had the lowest egg mass index and differed significantly from SB 20-2A. This verified results from earlier experiments where the same landrace reacted in a similar way when compared to others except that the gall index and egg mass index values were lower. This could be due to a number of factors including among others the time when the experiment started, different soils used at BCA and UP, and differences in greenhouse temperatures.

The results of this study confirm earlier reports that bambara groundnut is susceptible to *M. incognita* (Ogbuji.1979). Other reports of susceptibility of bambara groundnut to root-knot nematodes involved studies with *M. javanica*. Although (Mc Donald & De Waele, 1989)

suggested a possibility that tolerant bambara groundnut landraces may exist, their results were based on *M. javanica*. *M. incognita* race 2 reduced growth and yield of all landraces evaluated. No previous studies have been done involving *M. incognita* race 2 on bambara groundnut. The results of this study have therefore shown that this nematode is a serious problem on bambara groundnut, and that no significant resistance or tolerance exsist in the landraces screened. Consequently, other measures will have to be explored for control of this nematode on bambara groundnut.

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Table 2.2a: *Vigna subterranea* landraces used in Experiment 1 at Botswana College of Agriculture.

Landrace	Source
DIPC	Botswana College of Agriculture
OM 1	Botswana College of Agriculture
OM 6	Botswana College of Agriculture
Gab C	Botswana College of Agriculture
JB Pop 2	Department of Agricultural Research, Botswana
JB Pop 3	Department of Agricultural Research, Botswana
JB Pop 4	Department of Agricultural Research, Botswana
JB Pop 5	Department of Agricultural Research, Botswana
JB Pop 10	Department of Agricultural Research, Botswana
JB Pop 11	Department of Agricultural Research, Botswana
NTSR	National Seed Testing Centre, Zimbabwe
Ram R	Botswana College of Agriculture
Gac C	Botswana College of Agriculture
Jac C	Botswana College of Agriculture
Goo B	Botswana College of Agriculture

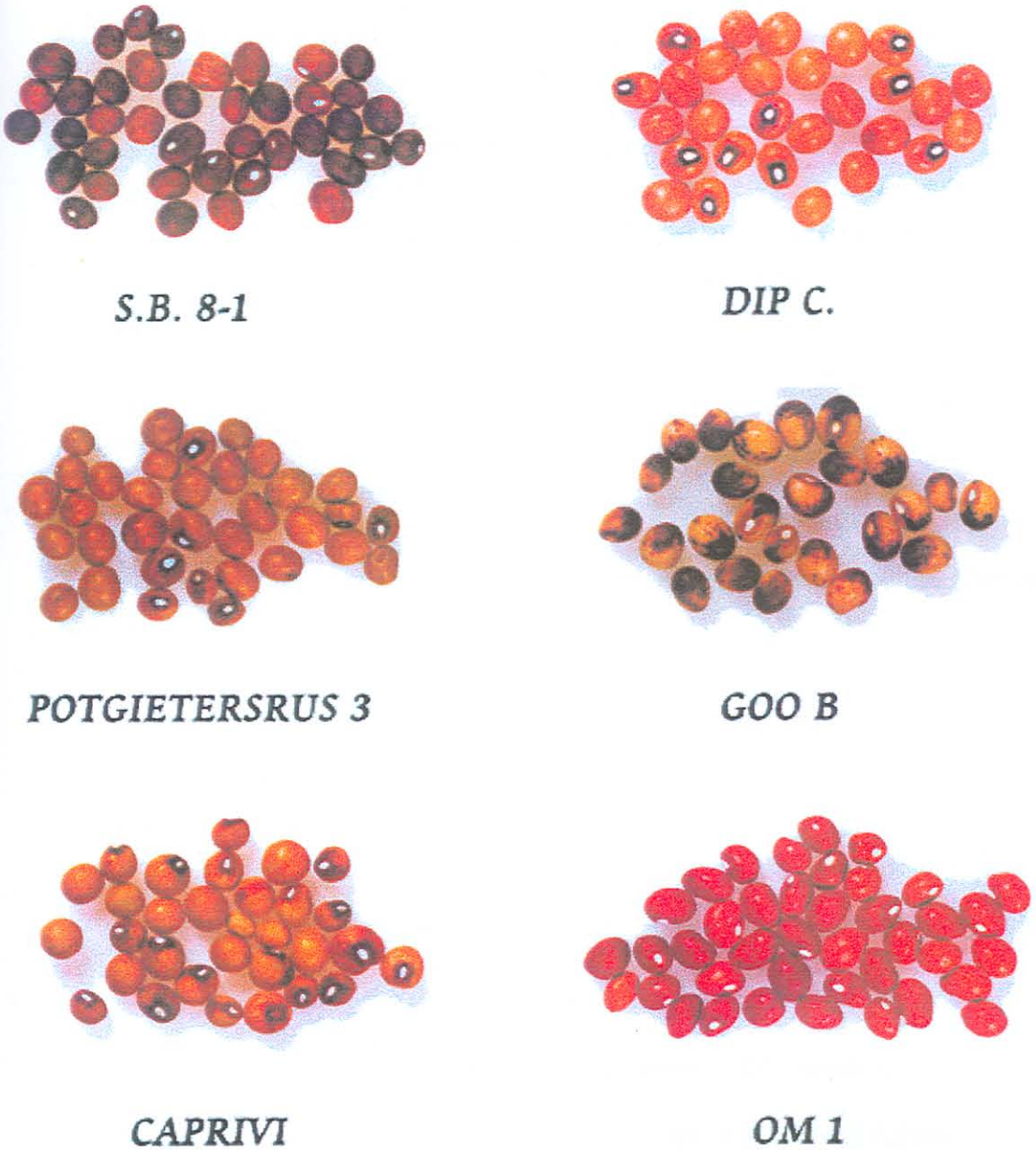

Fig 2.1: Bambara groundnut (*Vigna subterranea*) seeds – a selection of some examples of the landraces used in the present study.

Table 2.2b: *Vigna subterranea* landraces used in Experiment 2 at Botswana College of Agriculture.

Landrace	Source
SB 4-4E	Oil and Protein Seeds Centre, ARC*, Potchefstroom, South Africa
S13	Oil and Protein Seeds Centre, ARC, Potchefstroom, South Africa
S10	Oil and Protein Seeds Centre, ARC, Potchefstroom, South Africa
A57	Oil and Protein Seeds Centre, ARC, Potchefstroom, South Africa
WS 52	Oil and Protein Seeds Centre, ARC, Potchefstroom, South Africa

*Agricultural Research Council

Table 2.2c: *Vigna subrerranea* landraces used in experiment 1 at the University of Pretoria.

Landrace	Source
K1	Oil and Protein Seed Centre, ARC*, Potchefstroom
V4 S1	Plant Protection Research Institute, ARC, Pretoria, South Africa
CLDRE	Oil and Protein Seed Centre, ARC, South Africa
Swazi V4	Oil and Protein Seed Centre, ARC, South Africa
SB 8-1	Oil and Protein Seed Centre, ARC, South Africa
Caprivi	Oil and Protein Seed Centre, ARC, South Africa

*Agricultural Research Council

Table 2.2d: *Vigna subtrerranea* landraces used in experiment 2 at the University of Pretoria.

Landrace	Source
ETL-76469	Oil and Protein Seed Centre, ARC*, Potchefstroom
SB 4-4C	Oil and Protein Seed Centre, ARC, Potchefstroom
HVA 38-3	Oil and Protein Seed Centre, ARC, Potchefstroom
M4	Oil and Protein Seed Centre, ARC, Potchefstroom
Potgietersrus	Oil and Protein Seed Centre, ARC, Potchefstroom
Groblersdal	Oil and Protein Seed Centre, ARC, Potchefstroom
Marabastad	Oil ad Protein Seed Centre, ARC, Potchefstroom
A12	Oil and Protein Seed Centre, ARC, Potchefstroom

*Agricultural Research Council

Table 2.2e: *Vigna subterranea* landraces used in experiment 3 at the University of Pretoria.

Landrace	Source
Swazi V 4	Oil and Protein Seed Centre, ARC*, Potchefstroom
Caprivi Sel 1	Oil and Protein Seed Centre, ARC, Potchefstroom
MV 8817	Oil and Protein Seed Centre, ARC, Potchefstroom
ZB S2	Oil and ProtienSeed Centre, ARC, Potchefstroom
Sel van Potch. Mengel	Oil and Protein Seed Centre, ARC, Potchefstroom
WS 51	Oil and Protein Seed Centre, ARC, Potchefstroom
S9	Oil and Protein Seed Centre, ARC, Potchefstroom
SB 20-2 A	Oil and Protein Seed Centre, ARC, Potchefstroom

*Agricultural Research Council

Table 2.2f: *Vigna subterranea* landraces used in experiment 4 at the University of Pretoria.

Landrace	Source
ZB S1	Oil and Protein Seed Centre, ARC,* Potchefstroom, South Africa
MAD	Oil and Protein Seed Centre, ARC, Potchefstroom, South Africa
PGR 3	Oil and Protein Seed Centre, ARC, Potchefstroom, South Africa
Red Eye Ex.Zim	Oil and Protein Seed Centre, ARC, Potchefstroom, South Africa
V4 S4	Plant Protection Research Institute, ARC, Pretoria, South Africa
Gravelot	Plant Protection Research Institute, ARC, Pretoria, South Africa
Caprivi Sel 2	Plant Protection Research Institute, ARC, Pretoria, South Africa
WS 50	Plant Protection Research Institute, ARC, Pretoria, South Africa

*Agricultural Research Council

Table 2.2g: *Vigna subterranea* landraces used in experiment 5 at the University of Pretoria.

Landrace	Source
JB Pop 11	Department of Agricultural Research, Botswana
CLDRE	Oil and Protein Seed Centre, ARC*, Potchefstroom, South Africa
SB-20-2A	Oil and Protein Seed Centre, ARC, Potchefstroom, South Africa
S9	Oil and Protein Seed Centre, ARC, Potchefstroom, South Africa
Goo B	Botswana College of Agriculture
DIPC	Botswana College of Agriculture

*Agricultural Research Council

Table 2.3a: Reaction of *M. incognita* race 2 on fifteen *Vigna subterranea* landraces in experiment 1 at Botswana College of Agriculture.

Landrace	Ranked GI *	Final Population**	R factor	Host Suitability Designation ***
JB Pop 4	47.00a	19730b	3.94b	Susceptible
Jac C	47.00a	13482b	4.08b	Susceptible
JB Pop 5	47.00a	16810b	3.36b	Susceptible
DIPC	40.30a	21380b	4.30b	Susceptible
JB Pop 3	40.30a	21690b	4.42b	Susceptible
JB Pop 11	40.30a	10830b	2.18b	Susceptible
Gac C	40.30a	21600b	4.32b	Susceptible
Gab C	40.30a	22550b	4.52b	Susceptible
Goo B	38.50a	10850b	2.18b	Susceptible
Ram R	38.50a	16300b	3.28b	Susceptible
OM 1	32.10a	55520a	11.10a	Susceptible
OM 6	31.30a	11310b	2.24b	Susceptible
JB Pop 10	31.30a	17660b	3.50b	Susceptible
NTSR	30.60a	22950b	4.56b	Susceptible
JB Pop 2	24.60a	15110b	3.00b	Susceptible

Each value is the mean of 5 replicates. Means in the same column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test.

GI = Gall index, R factor = Final population ÷ Initial population. *Taylor & Sasser (1978), ** Number of eggs per 20 g roots, **Canto-Saenz (1985).

Table 2.3b: Effect of *M. incognita* race 2 on plant mass and yield of fifteen *Vigna subterranea* landraces in experiment 1 at Botswana College of Agriculture.

Landrace	Reduction in dry wt. of shoots (g)*	Reduction in fresh wt. of roots (g)*	Reduction in number of pods*	Reduction in dry wt. of pods (g)*
Ram R	6.84a	18.60a	22.75ab	5.13a
Goo B	4.48a	19.32a	53.00a	3.98a
OM 1	5.60a	8.46a	23.60ab	5.46a
NTSR	2.74a	22.50a	17.20ab	5.02a
Gac C	2.44a	16.38a	16.75ab	4.60a
OM 6	5.86a	11.20a	24.40ab	4.00a
Gab C	3.14a	18.02a	11.60b	2.94a
B Pop 4	4.58a	22.00a	11.80b	4.26a
JB Pop 10	2.46a	40.52a	8.00b	2.36a
Jac C	3.36a	28.26a	8.60b	3.02a
JB Pop 5	3.02a	22.60a	10.00b	3.38a
JB Pop 3	3.50a	12.68a	14.60ab	2.68a
DIPC	2.28a	20.98a	15.60ab	4.88a
JB Pop 2	2.90a	27.20a	12.00b	1.88a
JB Pop 11	2.88a	16.66a	11.60b	6.12a

Each value is the mean of 5 replicates. Means in each column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test.

*Non-inoculated minus inoculated.

Table 2.3c: Reaction of *M. incognita* race 2 on five *Vigna subterranea* landraces in experiment 2 at Botswana College of Agriculture.

Landrace	**Ranked GI ***	**Final Population ****	**R factor**	**Host Suitability Designation*****
WS 52	19.00a	16690a	3.34a	Susceptible
S13	14.20ab	25930a	5.18a	Susceptible
A57	14.20ab	33220a	6.66a	Susceptible
SB 4 - 4E	9.40b	26180a	5.22a	Susceptible
S10	8.20b	25640a	5.12a	Susceptible

Each value is the mean of 5 replicates. Means in each column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. GI = Gall index, R factor = Final population ÷ Initial population. * Taylor & Sasser (1978), ** Number of eggs per 20 g root, *** Canto-Saenz (1985).

Table 2.3d: Effect of *M. incognita* on plant mass and yield of five *Vigna subterranea* landraces in experiment 2 at Botswana College of Agriculture.

Landrace	Reduction in dry wt. of shoots (g)*	Reduction in fresh wt. of roots (g)*	Reduction in number of pods*	Reduction in dry wt. of pods (g)*
S13	1.94a	18.80a	7.44a	6.04a
S10	0.48b	18.60a	4.92a	4.88ab
SB 4 – 4E	0.58b	18.36a	10.76a	1.76b
A57	1.16ab	16.10a	5.28a	1.32b
WS 52	1.02ab	16.80a	3.98a	6.46a

Each value is the mean of 5 replicates. Means in each column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test. * Non-inoculated minus inoculated.

Table 2.3e: Reaction of six *Vigna subterranea* landraces to *M. incognita* race 2 in experiment 1 at the University of Pretoria.

Landrace	Ranked G1*	Ranked EI*	Final Population **	R factor	Host Suitability Designation ***
SB 8 – 1	22.80a	12.10ab	9320ab	1.86ab	Susceptible
Swazi V4	18.00ab	18.20ab	8360ab	1.66ab	Susceptible
K1	18.00ab	22.90a	14300a	2.84a	Susceptible
Caprivi	12.60ab	18.30ab	8020ab	1.62ab	Susceptible
V4 S1	11.20b	13.60ab	11160ab	2.24ab	Susceptible
CLDRE	10.40b	7.90b	5580b	1.10b	Susceptible

Each value is the mean of 5 replicates. Means in each column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. GI = Gall index, EI = Egg mass index, R factor = Final population ÷ Initial population. *Taylor & Sasser (1978), ** Number of eggs per 20 g roots, 8**Canto-Saenz (1985)

Table 2.3f: Effect of M. *incognita* race 2 on plant mass and yield of six *Vigna subterranea* landraces in experiment 1 at the University of Pretoria.

Landrace	Reduction in dry wt. of shoots (g)*	Reduction in fresh wt. of roots (g)*	Reduction in number of pods	Reduction in dry wt. of pods (g)*
K1	1.46a	11.80a	3.25ab	2.87a
SB 8 - 1	0.92ab	7.06a	1.50b	2.23a
Caprivi	0.74ab	6.76a	2.80ab	2.74a
CLDRE	0.98ab	5.42a	2.60ab	3.00a
Swazi V4	0.48b	4.86a	3.80a	3.46a
V4 S1	0.56b	4.82a	1.60ab	2.12a

Each value is the mean of 5 replicates. Means in each column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test.

*Non inoculated minus inoculated.

Table 2.3g: Reaction of *M. incognita* race 2 on eight V*igna subterranea* landraces in experiment 2 at the University of Pretoria.

Landrace	Ranked GI *	Ranked EI *	Final Population **	R factor	Host suitability Designation ***
Potgietersrus	29.50a	28.40a	9540a	1.92a	Susceptible
Marabastad	26.50a	27.00a	7246a	1.44a	Susceptible
M4	24.50ab	25.80a	9580a	1.90a	Susceptible
Groblersdal	23.50ab	21.80a	7820a	1.56a	Susceptible
ETL – 76469	22.30ab	23.00a	6760a	1.36a	Susceptible
A12	18.50ab	18.00a	8380a	1.66a	Susceptible
SB 4 - 4C	13.90bc	15.20ab	6600a	1.34a	Susceptible
HVA 38 – 3	5.30c	4.80b	2500a	0.50a	Susceptible

Each value is the mean of 5 replicates. Means in each column followed by the same letter do not differ significantly at P≤ 0.05 according to Duncan's multiple range test.

GI = Gall index, EI = Egg mass index. R factor = Final population ÷ Initial population.

*Taylor & Sasser (1978), ** Number of eggs per 20 g roots, *** Canto-Saenz (1985).

Table 2.3h: Effect of *M. incognita* race 2 on plant mass and yield of eight *Vigna subterranea* landraces in experiment 2 at the University of Pretoria.

Landrace	Reduction in dry wt. of shoots (g)*	Reduction in fresh wt. of roots (g)*	Reduction in number of pods*	Reduction in fresh wt. of pods (g)*
Potgietersrus	0.58ab	6.22a	1.00a	1.80a
M4	0.88a	5.08a	1.00a	1.80a
Marabastad	0.46ab	2.74a	3.00a	2.78a
HVA 38 – 3	0.48ab	2.50a	0.50a	1.40a
Groblersdal	0.36b	4.58a	0.00a	0.70a
A12	0.44ab	4.50a	2.40a	3.80a
ETL – 76469	0.46ab	4.18a	3.00a	2.20a
SB 4 - 4C	0.26b	3.24a	4.50a	3.05a

Each value is the mean of 5 replicates. Means in each column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. * Non-inoculated minus inoculated.

Table 2.3i: Reaction of *M. incognita* race 2 on eight *Vigna subterranea* landraces in experiment 3 at the University of Pretoria.

Landrace	Ranked GI*	Final Population **	R factor	Host Suitability Designation***
SB 20-2A	27.40a	5460a	1.10a	Susceptible
Swazi V5	25.50a	6080a	1.24a	Susceptible
ZB S2	22.40a	5840a	1.50a	Susceptible
MV 8817	19.30a	6980a	1.40a	Susceptible
S9	19.30a	2925a	0.60a	Susceptible
Caprivi Sel 1	19.30a	6260a	1.28a	Susceptible
Sel van Potch. Mengel	16.20a	6360a	1.28a	Susceptible
WS 51	14.60a	6040a	1.20a	Susceptible

Each value is the mean of 5 replicates. Means in each column followed by the same letter are not significantly different at P≤ 0.05 according to Duncan's multiple range test. G.I.= gall index, R factor = Final population ÷ Initial population.

*Taylor & Sasser (1978), ** Number of eggs per 20 g roots, *** Canto-Saenz (1985).

Table 2.3j: Effect of *M. incognita* race 2 on plant mass of eight *Vigna subterranea* landraces in experiment 3 at the University of Pretoria.

Landraces	Reduction in dry wt. of shoots (g)*	Reduction in fresh wt. of roots (g)*
ZB S2	2.02a	8.70a
MV 8817	2.00a	7.30a
S9	1.64a	7.68a
Caprivi Sel 1	1.26a	6.00a
Swazi V5	1.36a	8.70a
WS 51	1.70a	11.20a
SB 20 - 2A	1.26a	8.38a
Sel van Potch. Mengel	1.18a	5.70a

Each value is the mean of 5 replicates. Means in each column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test.

* Non-inoculated minus inoculated.

Table 2.3k: Reaction of *M. incognita* race 2 on six *Vigna subterranea* landraces in experiment 4 at the University of Pretoria.

Landrace	Ranked GI *	Final Population **	R factor	Host Suitability Designation ***
Red Eye Ex. Zim.	22.30a	9074a	1.83a	Susceptible
Gravelot	18.50a	9560a	1.92a	Susceptible
MAD	14.70a	7404a	1.48a	Susceptible
V4 S4	13.30a	6460a	1.30a	Susceptible
Caprivi Sel 2	12.50a	7240a	1.46a	Susceptible
PGR 3	11.70a	8120a	1.64a	Susceptible

Each value is the mean of 5 replicates. Means in each column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. GI = Gall index, R factor = Final population ÷ Initial population.

*Taylor * Sasser (1978), ** Number of eggs per 20 g roots, ***Canto-Saenz (1985).

Table 2.3*l*: Effect of *M. incognita* race 2 on plant mass and yield of six *Vigna subterranea* landraces in experiment 4 at the University of Pretoria.

Landrace	Reduction in dry wt. (g) of shoots*	Reduction in fresh wt. (g) of roots*	Reduction in number of pods
PGR 3	1.86a	12.30a	4.00a
Gravelot	1.68a	4.30b	4.67a
MAD	1.42a	6.38ab	3.00a
V4 S4	1.16a	4.20b	4.00a
Caprivi Sel 2	1.08a	6.04ab	1.50a
Red Eye Ex. Zim.	0.92a	4.80ab	1.67a

Each value is the mean of 5 replicates. Means in each column followed by the same letter do not differ significantly at P≤ 0.05 according to Duncan's multiple range test.

* Non-inoculated minus inoculated.

Table 2.3m: Reaction of *M. incognita* race 2 on six *Vigna subterranea* landraces in experiment 5 at the University of Pretoria.

Landrace	Ranked GI *	Ranked EI *	Final Population **	R factor	Host Susceptibility Designation ***
SB 20 - 2A	18.50a	22.10a	22800a	4.56a	Susceptible
CLDRE	18.50a	17.80ab	25140a	5.03a	Susceptible
S9	17.00a	16.20ab	19620a	3.92a	Susceptible
DIPC	17.00a	16.20ab	23200a	4.64a	Susceptible
JB Pop 11	14.00a	13.10ab	17660a	3.53a	Susceptible
Goo B	8.00a	7.60b	14950a	2.99a	Susceptible

Each value is the mean of replicates. Means in each column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. GI = Gall index, EI = Egg mass index, R factor = Final population ÷ Initial population.

* Taylor & Sasser (1978), ** Number of eggs per 20 g roots, ***Canto-Saenz (1985).

Table 2.3n: Effect of *M. incognita* race 2 on plant mass and yield of six *Vigna subterranea* landraces in experiment 5 at the University of Pretoria.

Landrace	Reduction in dry wt. of shoots (g)*	Reduction in fresh wt. ofroots (g)*	Reduction in number of pods*	Reduction in dry wt. of pods (g)*
	shoots (g)			
S9	5.26a	5.82a	6.00b	4.20a
DIPC	2.52ab	6.60a	8.60b	3.14a
SB 20 - 2A	2.32b	4.86a	21.60a	6.94a
JB Pop 11	3.12ab	6.22a	11.40ab	3.90a
CLDRE	4.34ab	7.14a	12.00ab	7.86a
Goo B	2.62ab	6.70a	4.8b	3.94a

Each value is the mean of 5 replicates. Means in each column followed by the same letter do not differ significantly at P≤ 0.05 according to Duncan's multiple range test. * Non-inoculated minus inoculated.

CHAPTER 3

CONTROL OF *MELOIDOGYNE INCOGNITA* RACE 2 ON BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA)* BY MEANS OF SOIL AMENDMENTS WITH POULTRY AND CATTLE MANURE.

ABSTRACT

The effects of soil amendment with cattle and poultry manure on bambara groundnut (*Vigna subterranea* (L.) Verdc) variety DIPC were evaluated in the greenhouse. Treatments consisted of soil amended with 0.2, 0.4 and 0.8 kg/m^2 of each manure. Control treatments consisted of non-amended soil and a treatment with commercial fertilizer, $(NH_4)_2SO_4$. A nematicide treatment with fenamiphos was added for comparison. All treatments were evaluated with and without nematodes. The nematode population decreased as the manure application rate was increased. Poultry manure at 0.8 kg/m^2 was as effective as fenamiphos in reducing the nematode population. However, phytotoxicity was observed on plants receiving these treatments. Shoot mass was increased by 132 %-270 % in plants treated with cattle manure at 0.4 kg/m^2 and 0.8 kg/m^2 compared to the untreated control. A decrease in weight was recorded with the 0.8 kg/m^2 poultry manure, fenamiphos and fertilizer treatments. Cattle manure at 0.4 kg/m^2 was less phytotoxic than poultry manure at the same rate. The fertilizer treatment and the untreated control did not differ significantly with regard to nematode reduction, plant growth and yield.

3.1 Introduction

Organic soil amendment is widely used by farmers throughout the world with the aim of improving soil fertility and plant growth. The practice has been found to improve the physical characteristics of the soil and to reduce soil-borne pests and diseases (Stirling, 1991). According to Bridge (1996), amendments improve the nutrient and water-holding capacity of the soil thereby improving plant growth and hence increasing tolerance to nematodes. Riegel, Fernandez, & Noe (1996) suggested that organic amendment stimulate microbial activity in the soil. Some of these microorganisms are antagonistic to nematodes. A wide range of materials including amongst others poultry and cattle manure help control nematodes (Bridge, 1987; Rodriguez-Kabana, 1986; Chindo & Khan, 1990; Poswal & Akpa, 1991).

Incorporation of chicken manure into nematode-infested soil is reported to have reduced *M. incognita* numbers by 80 % (Kaplan & Noe, 1993). Previously, chicken litter was shown to reduce *M. incognita* populations on tomato with a concomitant increase in growth (Chindo & Khan, 1990). Riegel et al. (1996) reported low population density of *M. incognita* on cotton grown in plots amended with chicken litter.

Very little work has been done involving the use of cattle manure as an amendment to control *M. incognita.* Poswal & Akpa (1991) reported successful control of *M. incognita* with cow dung in Nigeria. Apart from this study most of the work involving cattle manure is unpublished. In Botswana small-scale farmers incorporate cattle manure into the soil mainly to improve soil fertility. It has been observed that addition of cattle manure to the soil

reduces soil-borne pests and diseases including root-knot nematodes (unpublished observation).

Control of root-knot nematodes is best achieved through the use of nematicides. Nematicides are generally very expensive and their supply is erratic in rural areas. They are also hazardous to the environment as well as humans and livestock. It is therefore necessary to investigate non-chemical strategies like organic soil amendment using materials such as poultry and cattle manure as alternatives to nematicides.

The objectives of the study were therefore to investigate the efficacy of poultry and cattle manure on *M. incognita* race 2 and to determine the effective application dosage.

3.2 Materials and Methods

The experiment was conducted from January to April 1998 in the glasshouse at the University of Pretoria. The soil used in the experiment was a steam-pasteurized sandy loam soil (80 % sand, 4 % silt, 14 % clay and pH 6.0) prepared by mixing top soil with river sand at the ratio of 2:1. Twenty-five centimeter diameter plastic pots were each filled with 8 kg of soil and treated with a basal dressing of NPK (2:3:2) applied at the rate of 500 kg/ha.

The experiment consisted of 6 replicates of the following treatments:

- Soil amended with 0.2 kg/m^2 cattle manure
- Soil amended with 0.2 kg/m^2 poultry manure
- Soil amended with 0.4 kg/m^2 cattle manure

- Soil amended with 0.4 kg/m^2 poultry manure
- Soil amended with 0.8 kg/m^2 cattle manure
- Soil amended with 0.8 kg/m^2 poultry manure
- Untreated control
- Control supplemented with $(NH_4)_2SO_4$ applied at the rate of 80 kg N/ha to supply the equivalent amount of N as in 0.4 kg/m^2 poultry manure
- Fenamiphos applied at 2 liter/ ha included as a separate treatment for comparison.

Manure samples were ground into a fine powder, sifted to remove large particles, and subsequently added to the soil. All treatments except fenamiphos were applied separately to *M. incognita* race 2-inoculated and non-inoculated soil. Fenamiphos was applied to *M. incognita*-inoculated soil only. The amendments were thoroughly mixed with soil and watered to field capacity using a watering can. Chemical analysis of the two types of manure was done by the soil analysis laboratory, Department of Plant Production and Soil Science, University of Pretoria.

Inoculation was done immediately after application of amendments to the soil. Each treatment received 1 g of heavily galled tomato roots infected with a South African isolate of *M. incognita* race 2 (egg mass index = 5). Each egg mass contained an estimated 250 eggs. Roots were cut into 1-cm-long pieces, mixed with 5 ml of tap water, and incubated in petri dishes for 24 hours to allow eggs to hatch. The resulting inoculumn consisted of a mixture of eggs and juveniles. The inoculumn was placed in depressions in the center of each pot and

covered with soil. Amended treatments were set aside for a week to allow the manure to decompose before planting.

Planting was done one week after inoculation and the fenamiphos treatment was applied at this stage. Three seeds of bambara groundnut variety DIPC were planted per pot. Treatments were arranged on benches in the greenhouse in a completely randomized design and watered daily. Seedlings were thinned to one seedling per pot four weeks after planting. To assess the effects of treatments at midseason (four weeks after planting), one plant per pot was removed, shoots were cut and dried in an oven at 60 °C for three days and weighed. Roots were washed free of soil and stained in 0.15 g/liter aqueous solution of Phloxine B for 15 minutes and evaluated for galls and egg masses using a gall/egg mass rating scale of 0-5 as described by Taylor & Sasser (1978). Root mass was not determined at midseason.

Plants were harvested after ten weeks and evaluated following the same procedures used at midseason. In addition, roots were gently washed free of soil and their fresh mass determined. Roots were then evaluated for galls and egg masses as previously explained. Pods were counted and oven-dried at 60 °C for three days to determine their dry mass. Data were analyzed by ANOVA and the means separated by Duncan's multiple range test (SAS, BMDP Statistical Software, Los Angeles, CA). Gall and egg mass index data were ranked and means separated using Kruskal-Wallis tests for non-parametric data (Steel & Tourie, 1981). Because the gall and egg mass index values were the same, only the gall index data are presented.

3.3 Results

Results of the chemical analysis of manure samples are presented in Table 3.3a. According to this chemical analysis, poultry manure contained more nutrients than cattle manure. Phytotoxicity was observed in all plants that received the 0.8 kg/m^2 dosage of poultry manure and in plants treated with fenamiphos. These treatments had to be terminated because they died prematurely. No galls or egg masses formed on all plants grown in soil amended with 0.8 kg/m^2 poultry manure and in soil treated with fenamiphos. Phosphorus deficiency symptoms (dark leaves) were noticed in the control, fertilizer, cattle manure (0.2 kg/m^2 and 0.4 kg/m^2) and poultry manure (0.4 kg/m^2) treatments. Leaves were darker green in these plants than in other treatments. There was great variation in leaf growth among plants. Treatments that received a lower dosage of both poultry and cattle manure tended to have tiny, sharp pointed leaves while those receiving higher dosages had broader leaves.

The effects of the different amendments on *M. incognita* at midseason are presented in Table 3.3b. Treatments showed significant differences with regard to gall index, egg mass index and dry mass of shoots. The non-amended control had the highest gall and egg mass index compared to other treatments. No significant differences in gall and egg mass index occurred in plants receiving cattle manure applied at all three dosages, poultry manure applied at 0.2 kg/m^2 and 0.4 kg/m^2 and the control amended with $(NH_4)_2SO_2$.

Plants that received cattle manure at the rate of 0.4 kg/m^2 and 0.8 kg/m^2 had the highest increase in shoot weight compared to the non-amended control (147 % and 162 % respectively). The lowest increase in shoot weight (22 %) was recorded in plants grown in

soil treated with cattle manure at the rate of 0.2 kg/m^2. A decrease in weight was recorded in plants that received poultry manure at the rate of 0.8 kg/m^2, plants treated with fenamiphos, and in plants grown in soil amended with $(NH_4)_2SO_4$ (Table 3.3b).

At harvest, the gall indices of plants grown in the non-amended control soil were significantly different from those treated with fenamiphos and poultry manure applied at 0.4 kg/m^2. The gall index was higher in plants grown in non-amended soil than in any of the treatments. Other treatments were not significantly different from the non-amended control in gall indices (Table 3.3c).

Cattle manure increased the dry mass of shoots by 270 %, 132 % and 49 % when it was applied at rates of 0.8 kg/m^2, 0.4 kg/m^2 and 0.2 kg/m^2 respectively compared to the non-amended control. No significant differences occurred in the dry mass of shoots of plants from soil treated with poultry manure at the rate of 0.2 kg/m^2 and 0.4 kg/m^2 (Table 3.3c). A decrease in shoot mass was recorded in plants grown in soil treated with fenamiphos and in those from the control supplemented with $(NH_4)_2SO_4$. The dry mass of shoots of plants from $(NH_4)_2SO_4$-supplemented control was not significantly different from those from the non-amended non-supplemented control (Table 3.3c).

Treatment of soil with fenamiphos, and $(NH_4)_2SO_4$ reduced the fresh weight of bambara groundnut roots compared to the non-amended control. Root weight was increased by the addition of other treatments (Table 3.3 c). There was no significant increase in root weight

between poultry manure applied at the rate of 0.2 kg/m^2, and 0.4 kg/m^2, and cattle manure applied at all three rates.

Yield (number and dry mass of pods) was increased in plants treated with cattle manure applied at all three rates and poultry manure applied at the rate of 0.2 kg/m^2 and 0.4 kg/m^2 compared to the non-amended control. Treatment of soil with fenamiphos, and $(NH_4)_2SO_4$ resulted in a decrease in yield (Table 3.3c)

3.4 Discussion

The general decrease in galling and reproduction of *M. incognita* race 2 on plants grown in soil amended with poultry and cattle manure at midseason confirms earlier report that addition of manure to soil reduce population densities of plant-parasitic nematodes (Main & Rodriguez-Kabana, 1982). Effective control was achieved when poultry manure was applied at the rate of 4-10 ton/ha. In the present study, poultry manure applied at the rate of 0.8 kg/m^2 (8 ton/ha) was as effective as fenamiphos applied at 2 liter/ha. Phytotoxicity due to poultry manure only occurred at 0.8 kg/m^2 and not at the lower rates.

The phytotoxicity observed with fenamiphos-treated plants suggests that this nematicide is not suitable for use on bambara groundnut. The nematicide has not been tested on this crop before and is therefore not registered on bambara.

Plants from soil amended with cattle manure at 0.4 kg/m^2 and 0.8 kg/m^2 had the highest increase in shoot weight probably because of an increase in nutrients. Unlike poultry

manure, cattle manure was not phytotoxic when applied at high dosages. The general decrease in shoot weight observed in treatments receiving poultry manure at 0.8 kg/m^2, fenamiphos, and $(NH_4)_2S0_4$ can be attributed to phytotoxicity.

The gall index on plants grown in soil amended with poultry manure at 0.4 kg/m^2 was significantly lower than in other treatments except fenamiphos where no galls formed. Gall indices declined with increasing rates of amendments thus confirming findings by Chindo & Khan (1990). In soil amended with 0.4 kg/m^2 poultry manure, there was a sustained reduction in gall formation (reflected in the gall index) up to harvest. Poultry manure applied at the rate of 0.2 kg/m^2 was not different from the control. For soil amendment to be effective against root-knot nematodes, the application rate, which is lower than the threshold for phytotoxicity but still effective against the nematode, should be applied. In this study it is 0.4 kg/m^2 (4 ton/ha) and corresponds to previous findings by Chindo & Khan (1990).

Cattle manure applied at 0.4 kg/m^2 and 0.8 kg/m^2 was as effective as poultry manure at 0.4 kg/m^2 in reducing galling. This efficacy along with the fact that it was less phytotoxic gives cattle manure an advantage over poultry manure. When cattle manure was applied at 0.4 kg/m^2 and 0.8 kg/m^2, it had an added advantage in that it increased shoot weight of bambara groundnut plants by 132 % and 270 % respectively compared to 62 % for poultry manure at 0.4 kg/m^2. All the amendments caused an increase in root weight over the untreated control. Cattle manure applied at 0.4 kg/m^2 resulted in 134 % increase in yield (number of pods) compared to the non-amended control. The consistent increase in yield and plant growth makes this treatment viable for effective nematode management. A dosage of

0.8 kg/m^2 (8 ton/ha) is equally effective but this quantity will be uneconomic to use when 4 ton/ha could give the same results.

The $(NH_4)_2SO_4$ treatment was not different from the control with regard to nematode reduction, and growth and yield parameters. According to Sinclair (1975), for inorganic fertilizers to be effective as nematicides, the material must be applied in excess of those required for crop fertilization. The fertilizer in this treatment was applied at the rate equivalent to the amount of N in 0.4 kg/m^2 poultry manure. This produced similar results to poultry manure at the rate of 0.4 kg/m^2 in terms of gall indices thus supporting previous reports (Riegel et al., 1996). However, growth and yield decreased indicating phytotoxicity, probably due to the high rate of N provided.

The results obtained in this study suggest that the control achieved when using poultry and cattle manure could be a result of increased nutrient availability to the plant or production of toxicants fom decomposition of the manure (Chindo & Khan, 1990). Production of toxic products can further be supported by the phytotoxicity observed on plants receiving these amendments at high dosages. It is also possible that control could be due to changes in microbial activity in the soil as a result of addition of organic matter (Riegel et. al., 1996). Soil microorganisms were reported to have been stimulated by the addition of manure to the soil (Rodriguez-Kabana, 1986). According to Rodriguez-Kabana (1986), some of these microorganisms may be antagonistic or predators of the nematode.

It has been established in this study that control of *M. incognita* race 2 on bambara groundnut by means of soil organic amendment using cattle manure, rather than poultry manure, can be more advantageous because of the lower phytotoxicity associated with cattle manure. The increase in growth and yield were also more pronounced on plants receiving cattle manure than in those receiving poultry manure.

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Table 3.3a: Results of chemical analysis for poultry and cattle manure samples

Manure	N %	P %	K %	CA %	Mg %	Na %	SO4 %	Cu $mgkg^{-1}$	Fe $mgkg^{-1}$	Mn mg/kg^{-1}	Zn mg/kg^{-1}	C %
Poultry	3.50	1.90	2.25	7.44	1.42	0.30	11.25	44	2796	425	306	1.19
Cattle	2.15	1.60	1.85	2.56	1.29	0.70	1.93	56	1496	42	318	1.18

Table 3.3b: Effect of different organic amendments on galling and reproduction of *M. incognita* race 2 on *Vigna subterranea* at midseason.

Treatment	Ranked gall index	Dry wt. of shoots (g)	% Increase in shoot weight
Untreated control	275.50a	2.98c	-
$(NH_4)_2SO_4$	214.00ab	2.56cd	-14 %
Poultry manure (0.4 kg/m^2)	178.50ab	5.01b	68 %
Poultry manure (0.2 kg/m^2)	163.00ab	5.47b	84 %
Cattle manure (0.2 kg/m^2)	159.50ab	3.64c	22 %
Cattle manure (0.4 kg/m^2)	159.50ab	7.36a	147 %
Cattle manure (0.8 kg/m^2)	143.00ab	7.81a	162 %
Fenamiphos (2 liters/ha)	96.00c	1.63 de	-45 %
Poultry manure (0.8 kg/m^2)	96.00c	1.02e	-66 %

Each value is the mean of 6 replicates. Means in each column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. Ranked gall indices followed by the same letter do not differ significantly at $P \leq 0.05$ according to Kruskal-Wallis test for non-parametic data.

Table 3.3c: Effect of different organic amendments on galling caused by *M. incognita* race 2 and on growth and yield of *Vigna subterranea* at harvest

Treatment	Ranked gall index	Dry wt. of shoots (g)	Fresh wt. of roots (g)	Number of pods	Dry wt. of pods (g)
Untreated control	273.00a	3.62d -	33.14bc -	11.33bc -	0.16bc -
Poultry manure (0.2 kg/m^2)	210.00ab	5.90c (63 %)	56.12a (69 %)	15.33ab (35 %)	1.53a (856 %)
Cattle manure (0.2 kg/m^2)	183.00ab	5.40c (49 %)	56.61a (71 %)	16.50ab (46 %)	0.76abc (375 %)
$(NH_4)_2SO_4$)	158.50abc	2.53de (-30 %)	19.20c (-42%)	10.00bc (-12 %)	0.48bc (200 %)
Cattle manure (0.4 kg/m^2)	129.50abc	8.40a (132 %)	57.02a (72 %)	26.50a (134 %)	1.27ab (694 %)
C attle manure (0.8 kg/m^2)	103.00abc	13.40a (270 %)	59.55a (80 %)	16.83ab (49 %)	0.99abc (519 %)
Poultry manure (0.4 kg/m^2)	95.00bc	5.88c (63 %)	47.41ab (43 %)	18.50ab (63 %)	1.05ab (556 %)
Fenamiphos (2 liter/ha)	24.00c	1.93e (-47 %)	18.13c (-45 %)	2.50c (-78 %	0.16c (-)

Each value is the mean of 6 replicates. Means in each column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test. Ranked gall indices followed by the same letter do not differ significantly at $P \leq 0.05$ according to Kruskal-Wallis test for non-parametic data.

Figures in brackets represent % increase in growth and yield compared to control.

CHAPTER 4

CONTROL OF *MELOIDOGYNE INCOGNITA* RACE 2 ON BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA*) BY MEANS OF BIOFUMIGATION AND SOLARIZATION.

ABSTRACT

Experiments were conducted in the greenhouse and in microplots in the field to test the effects of biofumigation, and solarization individually and in combination on *M. incognita* race 2 on bambara groundnut (*Vigna subterranea* (L.) Verdc.). The greenhouse experiments evaluated biofumigation alone and consisted of soil amended with two *Brassica* species, B. *oleracea capitata* L. varieties Drumhead and Glory of Enkhuizen, and *B. napus* L. variety Forage Star. Other treatments included fenamiphos and an untreated control. The microplot experiments investigated the combined effect of biofumigation and solarization on *M. incognita*. Treatments comprised solarized soil amended with cabbage, unsolarized soil amended with cabbage, solarized non-amended soil, untreated control, and aldicarb. The greenhouse and microplot treatments were each inoculated with *M. incognita* race 2.

Biofumigation significantly reduced the *M. incognita* population during the first four weeks of incubation and control declined thereafter. Cabbage residues were effective when applied at higher rates (4 kg/m^2 and 6 kg/m^2). Phytotoxicity was observed with fenamiphos, Glory of

Enkhuizen, and forage rape treatments applied at 6 kg/m^2. Growth and yield increased in treatments with variety Drumhead at all application rates.

The microplot experiment revealed that combination of biofumigation and solarization resulted in better control of the nematode than each treatment alone. The control achieved with biofumigation combined with solarization was similar to that obtained with the aldicarb treatment.

4.1 Introduction

Control of soil-borne plant diseases by means of biofumigation is gaining much attention from researchers in different disciplines. The ability of some *Brassica* crops to release biocidal compounds suggests that incorporation of green tissues of such plants into the soil could be useful for the control of a wide range of soil-borne pathogens. A number of *Brassica* crops are reported to be effective in reducing populations of some nematode species (Lewis & Papavizas, 1971; Lazerri, Tacconi, & Palmieri, 1993: Motjahedi, Santo, Hang & Wilson, 1993; McLeod & Da Silva, 1994; Mayton, Olivier, Vaugh & Loria, 1996). This was attributed to the production of glucosinolates by the *Brassica* species (Lewis & Papavizas, 1971). According to Borek, Morra, Brown & McCaffrey (1994), glucosinolates release numerous compounds among them allylisothiocyanates (AITC) upon degradation. These are volatile compounds similar in toxicity to methylisothiocyanate (MITC), an active ingredient in fumigants such as metham sodium (Lewis & Papavizas, 1971).

The environmental and human health hazards posed by synthetic soil fumigants have greatly restricted their availability and use. As a result attempts are now being made to find suitable alternatives to synthetic fumigants. Biofumigation has therefore gained much attention as a possible alternative to most synthetic fumigants and the results obtained are promising. According to Motjahedi, et al. (1993), green tissue of rapeseed (*Brassica napus L.*) was successfully used to reduce *M. chitwoodi* populations in the soil. McLeod & Da Silva (1994) have also reported effective control of *M. incognita*, *M. javanica* and *Tylenchulus semipenetrans* with rapeseed in Australia. Motjahedhi, Sang, Hang & Wilson (1991) reported superior control of root-knot nematodes by green material of *Brassica* crops than other crops.

The usefulness of biofumigation may be limited by the low concentrations of toxic volatile compounds released during the breakdown of glucosinolates. Sang, Minchinton, Johnstone & Truscott (1984) reported that the nature and level of glucosinolates vary with different plant species. There is also a remarkable variation in the glucosinolate profile from tissues within one plant. Consequently, biofumigation may not always be effective unless it is complemented by additional control measures such as solarization.

Gamliel & Stapleton (1993) reported that solarized cabbage-amended soil induced the breakdown of more complex compounds and resulted in a wide range of volatile compounds such as alcohols, aldehydes, sulfides and isothiocyanates. Stapleton & Duncan (1998) observed that the level of biocidal activity in unsolarized cabbage-amended soil is often relatively weak and unpredictable compared to solarized cabbage-amended soil. *M.*

incognita galling on tomato was reduced by 95-100 % when cruciferous soil amendments were combined with a sublethal heating regime and by 38-100 % in amended soil without heating (Stapleton &Duncan, 1998).

The objectives of this study were to determine the effectiveness of different *Brassica* species as biofumigants for the control of *M. incognita* race 2 on bambara groundnut. The combined effect of biofumigation and solarization on *M. incognita* race 2 populations was also investigated.

4.2 Materials and Methods

Biofumigation experiment in the greenhouse

The experiment was conducted from September 1997 to April 1998 in the greenhouse with temperature maintained between 20 °C and 30 °C. The soil used was a steam-pasteurized sandy loam soil (80 % sand, 4 % silt, 14 % clay, and pH 6.0) prepared by mixing topsoil with river sand at the ratio of 2:1. The soil received a basal fertilizer dressing of NPK 2:3:2 applied at the rate of 500 kg/ha prior to planting. The volume of soil contained in a twenty-25 cm diameter pot was weighed and the amount of plant biomass required as amendment per pot calculated accordingly. Cabbage (*Brassica oleracea capitata* L.) varieties Drumhead and Glory of Enkhuizen, and forage rape (*Brassica napus* L.) variety Forage Star were grown in the field for three and a half months for use in this study. The plants were harvested and washed free of soil. The leaves and stems of each variety were chopped into small pieces and used as soil amendment.

The experiment comprised the following four treatments:

- Soil amended with cabbage varieties Drumhead and Glory of Enkhuizen applied at 2; 4 and 6 kg/m^2
- Soil amended with forage rape variety Forage Star applied at 2; 4 and 6 kg/m^2
- Fenamiphos applied at 2 liter/ha
- Non-amended control

Each treatment was replicated six times and was applied separately to *M. incognita* inoculated and uninoculated plants respectively. Quantities of 8 kg of soil were augmented with the various plant materials, put into clear plastic bags, and watered to field capacity. The bags were subsequently kept for four weeks in the greenhouse at a temperature of 20 to 30 °C.

Meloidogyne inoculum consisted of 10 g of heavily galled tomato roots (gall/egg mass index = 5) infested with a Botswana isolate of *M. incognita* race 2. Each egg mass had an estimated content of 250 eggs. Roots were washed free of soil and cut into small pieces, which were incubated in petri dishes for 12 hours to facilitate hatching of eggs. The resulting inoculum consisted of a mixture of eggs and juveniles. The inoculum was incorporated into the soil, the bags sealed tightly to prevent fumes from escaping, and incubated for four weeks at room temperature. During this period, bags were occasionally shaken to mix the contents. At the end of the incubation period, bag contents were transferred to 25 cm diameter plastic pots placed on benches in the greenhouse. The soil mixture was aerated for two weeks to expel volatile compounds. The fenamiphos treatment was applied at this stage. Three seeds of bambara groundnut variety DIPC were sown per pot and watered to maintain soil

moisture. After emergence, plants were fertilized weekly with a solution of Multifeed P_{43} ® applied at 100 g/liter (Plaaskem Pty Ltd, P.O. Box 87005, Houghton, 2041). After four weeks (midseason) plants were thinned to one seedling per pot and the removed seedlings were assessed for nematode damage. Roots were washed, stained with Phloxine B and evaluated for gall and egg mass incidence using a rating scale of 0-5 (Taylor & Sasser, 1978). Shoots were dried in an oven at 60 °C for three days and the dry mass determined.

Plants were harvested after eight weeks and assessed following the same procedures used at midseason. The fresh weight of roots was obtained prior to evaluating roots for galls and egg masses. Pods were counted, dried in an oven at 60 °C for six days, and weighed.

Biofumigation and solarization experiment in the field

Fifteen microplots each measuring 1.5 m x 1.5 m and 1 m deep were constructed in an orchard at the University of Pretoria experimental farm. The microplots consisted of holes in the ground, lined with pre-fabricated fiberglass walls. The bottoms of the microplots were open to facilitate drainage. These were filled with sandy loam soil (75 % sand, 5 % silt, 20 % clay and pH 6.0) prepared by mixing topsoil with coarse river sand at the ratio of 2:1. The soil was fumigated for six weeks using dazomet applied at the rate of 500 g/m^2. An aeration period of two weeks was allowed.

The experiment consisted of five treatments:

- Soil amended with cabbage at 4 kg/m^2
- Soil amended with cabbage at 4 kg/m^2 and solarized

- Non-amended solarized soil
- Untreated control
- Aldicarb applied at 5 g/m^2

The experimental design comprised a completely randomized design with 3 replicate micro plots per treatment. Each microplot contained sixteen plants (total of 48 plants per treatment).

Cabbage variety Drumhead was raised in the field for three and a half months for use as an amendment in this study. The crop was harvested, green tissue (heads, leaves and stems) were cut into small pieces, applied to the soil at the rate of 4 kg/m^2 and mixed thoroughly. The soil was watered to field capacity throughout the biofumigation period.

Each plot was inoculated before application of treatments with tomato roots heavily infested with a South African isolate of *M. incognita* race 2 with an egg mass index rating of 5 (Taylor & Sasser, 1978). Roots were chopped into small pieces and placed in each plot in 15 cm-deep holes in the previously marked spots where the plants would be planted. Each hole received 5 g of inoculum.

Solarization commenced in January 1999. Soil in the microplots was covered with 30 µm clear polyethylene sheets for four weeks coinciding with biofumigation. A digital data logger (1200 series Grant squirrel logger, UK) was used to measure soil temperature in four plots selected at random to measure soil temperature in solarized amended soil, unsolarized amended soil, solarized non-amended soil and the untreated control. Thermocouples were

inserted at 15 and 30 cm depths and calibrated to record temperature hourly. The temperature data was down loaded weekly at 12:00 noon. The polyethylene was removed after four weeks and the soil aerated for two weeks to expel residual fumes resulting from biofumigation.

Planting was done in March 1999. All plots received a basal dressing of NPK (2:3:2) applied at 500 kg/ha prior to planting. Aldicarb treatment was applied to the designated plots at 5 g/m^2. Three seeds of bambara groundnut (variety DIPC) were planted together at a depth of 15 cm where the inoculum was previously placed. There were two rows per plot with a spacing of 60 cm between rows and each row consisted of eight holes with a 15 cm spacing in between. The plants were watered by means of a watering can with each plot receiving twenty liters of water per day. Plants were thinned to one seedling per hole four weeks after emergence (leaving sixteen plants per plot). Ammonium nitrate was applied at the rate of 100 kg/ha. Aphids were controlled by means of cypermethrin applied as a foliar spray at the rate of 150 ml/ha.

Six plants per row were harvested eight weeks after planting and evaluated for galls and egg masses. Shoots were dried in an oven at 60 °C for three days and weighed. Roots were washed to remove soil and their fresh weight determined. Roots were subsequently placed in a 0.15 g/liter aqueous solution of Phloxine B for 15 minutes to stain egg masses. The incidence of root galls and egg masses was assessed according to a scale of 0-5 (Taylor & Sasser, 1978). Yield was measured by counting the number of pods per plant. Pods were dried in an oven at 60 °C for four to five days and weighed.

All data were analyzed statistically using ANOVA (SAS, BMDP Statistical Software Inc, Los Angeles, CA). Gall and egg-mass index data were ranked before analysis. Mean separation was done according to Duncan's multiple range test.

4.3 Results

Biofumigation experiment in the greenhouse

At the end of the incubation period the cabbage and forage rape residues in the soil were decomposing. Amended soil was wetter than the untreated control soil because of the sap released from disintegrating plant tissues. There was a pungent odor released from bags containing *Brassica* amendments. Germination of bambara groundnut seed was poor and slow in soil amended with Glory of Enkhuizen at all dosages, forage rape at 6 kg/m^2 and in fenamiphos treated soil. Plants grown in soil receiving these treatments showed phytotoxicity symptoms. These plants were stunted, with varying degrees of chlorosis and tiny puckered leaves Fig. 4. 1). Roots showed various degrees of discoloration and browning. In most cases, roots were very short and thin, had no rootlets and were disintegrated.

At midseason, there were more galls and egg masses on plants grown in non-amended soil (control) than in amended soil (Fig. 4.2). There was however no significant difference between the control and the *Brassica* amendments applied at the lower dosage (2 kg/m^2). Drumhead applied at 4 kg/m^2 was not significantly different from the control treatment. Gall and egg mass indices decreased with an increase in dosage (Fig. 4. 2).

Forage rape applied at 2 kg/m^2 and 4 kg/m^2 and Drumhead at 2 kg/m^2 differed significantly from other treatments in terms of the dry mass of shoots. The dry mass of shoots increased by 83 % and 88 % in treatments receiving forage rape at 2 kg/m^2 and 4 kg/m^2 respectively, 37 % in treatments receiving Drumhead at 2 kg/m^2, and 6 % in treatments receiving Glory of Enkhuizen at 2 kg/m^2 compared to the untreated control (Table 4.3a). A decrease in shoot mass was recorded for the other treatments. No significant differences were detected in the dry mass of shoots of plants grown in soil treated with Glory of Enkhuizen applied at 4 kg/m^2 and 6 kg/m^2. The dry mass of shoots of plants receiving forage rape applied at 2 kg/m^2 was not significantly different from those receiving 4 kg/m^2 but differed significantly from those treated with 6 kg/m^2. Plants grown in soil treated with fenamiphos differed significantly from those receiving forage rape at 2 kg/m^2 and 4 kg/m^2 in terms of shoot dry mass. These plants however did not significantly differ from plants in the other treatments (Fig. 4.3).

At harvest plants from the untreated control were significantly different from those receiving fenamiphos, forage rape, and Glory of Enkhuizen at 6 kg/m^2 in terms of gall and egg mass indices. The fenamiphos treatment decreased gall and egg mass incidence by 80 % compared with the untreated control. Very few galls (72 % decrease compared to the control) formed on plants grown in soil amended with forage rape, and glory of enkhuizen at 6 kg/m^2 (Table 4.3b, Fig. 4.4)

The fresh weight of roots of plants from the untreated control and forage rape treatment applied at 2 kg/m^2 differed significantly from those receiving other treatments except for Drumhead at 2 kg/m^2 and forage rape treatment at 4 kg/m^2. With the exception of forage

rape applied at 4 kg/m^2, application of amendments at 4 kg/m^2 and 6 kg/m^2 did not have any significant effect on the fresh weight of roots. The root mass decreased with an increase in application rate (Fig. 4.5).

The dry weight of shoots of plants from the untreated control and treatments with drumhead at 2 kg/m^2, and forage rape at 2 kg/m^2 and 4 kg/m^2 did not differ significantly but were significantly different from other treatments. There were no significant differences in dry weight of plants from treatments with fenamiphos and those receiving Drumhead at 4 kg/m^2, and forage rape at 2 kg/m^2 and 4 kg/m^2. The dry mass of shoots of plants from treatments with Drumhead applied at 4 kg/m^2 and 6 kg/m^2 were not significantly different from those treated with Glory of Enkhuizen at 2 kg/m^2. No significant differences in dry mass of shoots were noticed in plants receiving Glory of Enkhuizen at 2 kg/m^2 and 4 kg/m^2. These plants were also not significantly different from plants from treatments with Drumhead at 4 kg/m^2 and 6 kg/m^2, and forage rape at 6 kg/m^2 (Fig. 4.6).

The lowest yield (number of pods) was recorded in plants grown in soil amended with Glory of Enkhuizen at 6 kg/m^2 while the highest number was recorded in plants from forage rape at 2 kg/m^2. There was no significant difference in the number of pods from plants receiving forage rape at 2 kg/m^2 and 4 kg/m^2, the untreated control, and Drumhead at 2 kg/m^2. The number of pods from plants treated with fenamiphos differed significantly with those from forage rape treatments applied at 6 kg/m^2 (Fig. 4.7). The dry mass of pods followed the same trend as the number of pods (Fig. 4.8).

Biofumigation and solarization experiment

The results are presented in Tables 4.3a & 4.3b. Plants from unsolarized non-amended treatments had the highest gall and egg mass indices. Solarization alone decreased gall and egg mass indices by 22 % compared with the untreated control. Biofumigation without solarization decreased gall and egg mass indices by 41 % while biofumigation combined with solarization decreased gall and egg mass indices by 66 %. Treatment with aldicarb did not differ significantly from the biofumigation and solarization treatments (Table 4.3c).

Root growth was increased by 50 % in plots treated with aldicarb compared to the untreated control. Generally root growth was reduced by the other treatments. Treatments differed significantly from each other except the solarized and unsolarized amended treatments, which did not differ from each other (Table 4.3d). There was no significant difference between solarized non-amended treatment and unsolarized amended treatments.

The dry mass of shoots of plants from treatments with aldicarb, solarized amended and unsolarized amended treatments did not differ significantly. An increase of 54 % and 46 % in dry mass was recorded in plants from plots treated with aldicarb and those amended with cabbage residues combined with solarization respectively. There were no significant differences between plants from solarized non-amended and unsolarized non-amended treatments (Table 4.3 d). The mean maximum weekly soil temperature was 37.6 °C in solarized amended soil and 36.2 °C in solarized non-amended soil at 15 cm depth. Temperatures at 30-cm depth averaged 31.1 °C in solarized non-amended treatments and 32.3 °C in solarized amended plots. Weekly minimum temperatures averaged 25.6 °C and

27.7 °C at 15 cm in solarized non-amended plots and solarized amended plots respectively. Maximum weekly temperatures at 30 cm averaged 26.9 °C and 28.1 °C respectively in solarized non-amended and solarized amended treatments. Temperatures in unsolarized non-amended and unsolarized amended treatments followed the same trend as in solarized non-amended and solarized amended treatments at both 15 and 30 cm depths. Comparison of solarized amended and unsolarized amended treatments revealed great differences in mean weekly temperatures at both depths. The maximum weekly temperatures in these two treatments differed by an average of 5.8 and 3.9 °C while the weekly minimum temperatures differed by 4 and 2.7 °C at 15 and 30 cm respectively (Table 4.3e).

Weekly maximum temperatures in unsolarized non-amended and solarized non-amended treatments differed by an average of 5 °C and 2.9 °C and weekly minimum temperatures by an average of 2.6 ° C at 15 cm and 2.3 °C at 30 cm respectively (Table 4.3 f).

4.4 Discussion

Soil amendment with *Brassica* residues significantly reduced gall formation caused by *M. incognita* race 2 on bambara groundnut in this study. However, the measure of control declined towards harvest in all amendments at all dosages. This confirms an earlier report that brassica residues can only be effective as biofumigants for a period of six weeks (Motjahedi et. al., 1993). Other reports suggested that control of nematodes from green material releasing isothiocyanates is unlikely after four weeks (Brown, Morra, McCaffrey, Auld & Williams, 1991). The significant reduction in gall and egg mass indices at midseason suggests that the greatest reduction of nematode populations occurred during the first four

weeks when residues were incubated in the soil. Thereafter there was little or no activity by the biofumigants on successive generations of the nematode. Although there are no previously reported studies involving biofumigation against *M. incognita* on bambara groundnut, there are reports of successful control of this nematode on other crops using *Brassica* residues (McLeod & Da Silva, 1994; McLeod, Somers & Gendy, 1995). Although previous studies evaluated brassica crops other than the ones used in this study, the overall potential of *brassica* crops as biofumigants is confirmed. Residues were not very effective in reducing galling and reproduction of the nematode when they were applied at 2 kg/m^2 (20 ton/ha). Significant reductions in nematode population occurred when forage rape and Glory of Enkhuizen were applied at 4 kg/m^2 and 6 kg/m^2 (40 ton/ha and 60 ton/ha) and Drumhead at 6 kg/m^2 (60 ton/ha). Previously, several rapeseed varieties were reported to be superior to cabbages in reducing populations of soil-borne diseases including nematodes (Motjahedi et al., 1993; Mayton et al., 1996). Mayton et al. (1996) reported variations in isothiocyanate production between and within *Brassica* species. This could account for the differences in performance between the brassica crops tested in this study. Cabbage variety Glory of Enkhuizen seemed to be similar to forage rape in its performance. Some reports suggested that cabbage contains little or no isothiocyanates and that this could account for its inferior performance when compared with other *Brassica* (Lewis & Papavizas, 1971). However in this study it was difficult to accurately conclude that the high reduction of *M. incognita* race 2 population experienced when forage rape and Glory of Enkhuizen were applied at higher dosages was due to biofumigation alone. The phytotoxicity observed at the onset in plants grown in soil receiving these treatments could have contributed to the reduction in nematode population in one way or another. Generally *Brassica* amendments are effective when they

are applied at higher dosages. According to McLeod & Da Silva (1994), a dosage of 4 kg/m^2 (40 ton/ha) is appropriate to give significant control. Although attempts were made to reduce phytotoxicity by aerating the soil for two weeks before planting, this did not alleviate the problem in the present study. This was reflected in the poor germination of plants grown in soil amended with Glory of Enkhuizen at all dosages and forage rape at 4 kg/m^2 and 6 kg/m^2.

Addition of *Brassica* residues to the soil had a slight effect on plant growth at midseason compared to control. Forage rape at 2 kg/m^2 and 4 kg/m^2 increased dry mass of shoots by over 80 % while the two cabbage varieties were able to increase growth only when applied at 2 kg/m^2. This could possibly be ascribed to the nutrient effect as a result of addition of organic matter to the soil. Keinath (1996) observed increase in growth and yield of watermelon after incorporating cabbage residues into mulched soil.

At harvest, growth and yield were generally better in plants grown in soil amended with forage rape at 2 kg/ha and 4 kg/ha and Drumhead at 2 kg/m^2. The poor growth of plants grown in soil amended with Glory of Enkhuizen at all three dosages could be attributed to phytotoxicity. More severe phytotoxicity symptoms were exhibited on these plants than those receiving other treatments. The same applies to forage rape applied at 6 kg/m^2. Although forage rape at 4 kg/m^2 performed better than Drumhead at the same dosage, the later was less phytotoxic. Plants grown in soil treated with fenamiphos were expected to grow better than those receiving *Brassica* amendments but this was confounded by phytotoxicity. This is confirmed by results from pilot experiments in this study. It should be noted that fenamiphos is not registered for use on bambara groundnut.

Solarization and biofumigation individually were successful in reducing nematode populations under field conditions by 22 % and 41 % respectively. Notably, when biofumigation and solarization were combined, the nematode population was reduced by 66 % thus giving similar control as aldicarb. This confirms previous reports that the efficacy of solarization or biofumigation can be enhanced by combined application of the two methods (Gamliel & Stapleton, 1997). According to Gamliel & Stapleton (1997), pathogen control in solarized amended soil is attributed to a combination of thermal killing and biotoxic volatile compounds. Compounds such as allylisothiocyanate, phenylisothiocyanate and aldehydes are released in the vapour phase from solarized cabbage-amended soil and are reported to have biocidal activity (Gamliel & Stapleton, 1997). Although volatile compounds were not analyzed in this study, the pungent smell experienced from solarized cabbage-amended plots is an indication that volatile gases were liberated during decomposition. These compounds along with thermal heating could have contributed to the successful control achieved in this study. Stapleton & Duncan (1998) concluded that combination of soil amendment with solarization is a feasible option for the development and implementation of effective soil fumigation. The maximum average temperature in solarized amended treatments increased by 5.8 °C at 15 cm and 3.9 °C at 30 cm compared to unsolarized amended plots. Stapleton & De Vay (1983) reported that *M. incognita* can not be effectively controlled by solarization alone because it is resistant to high temperatures. Propagules of *M. incognita, Pythium ultimum* and *Sclerotium rolfsii* were previously killed by heating soil amended with different *Brassica* residues to a maximal temperature of 38 °C (Gamliel & Stapleton, 1995). The average maximum temperature at 15 cm in solarized

cabbage-amended soil in this study was 37.6 °C confirming previous findings by Gamliel & Stapleton (1995).

Combination of biofumigation with solarization was as good as using aldicarb in terms of increasing the dry mass of shoots of bambara groundnut in this study. Previously, increases in plant growth and yield of watermelon were reported following treatment with a combination of biofumigation and solarization (Katan, 1981; Keinath, 1996). This increase could be attributed to biological changes in the soil such as increases in microbes beneficial to plant growth (Katan, 1981). Keinath (1996) intimated that solarization improved the efficacy of organic amendments including biofumigation thus resulting in improvement in plant growth. Combination of biofumigation and solarization could therefore be a suitable alternative to the use of nematicides. The reduction in root growth in plants treated with a combination of biofumigation and solarization could be attributed to phytotoxicity. Although aldicarb has not been tested on bambara groundnut, it is registered on cowpea, a close relative of bambara groundnut.

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Fig. 4.1: Phytotoxicity symptoms on eight-week old *Vigna subterranea* treated with cabbage variety Glory of Enkhuizen at 6 kg/m^2.

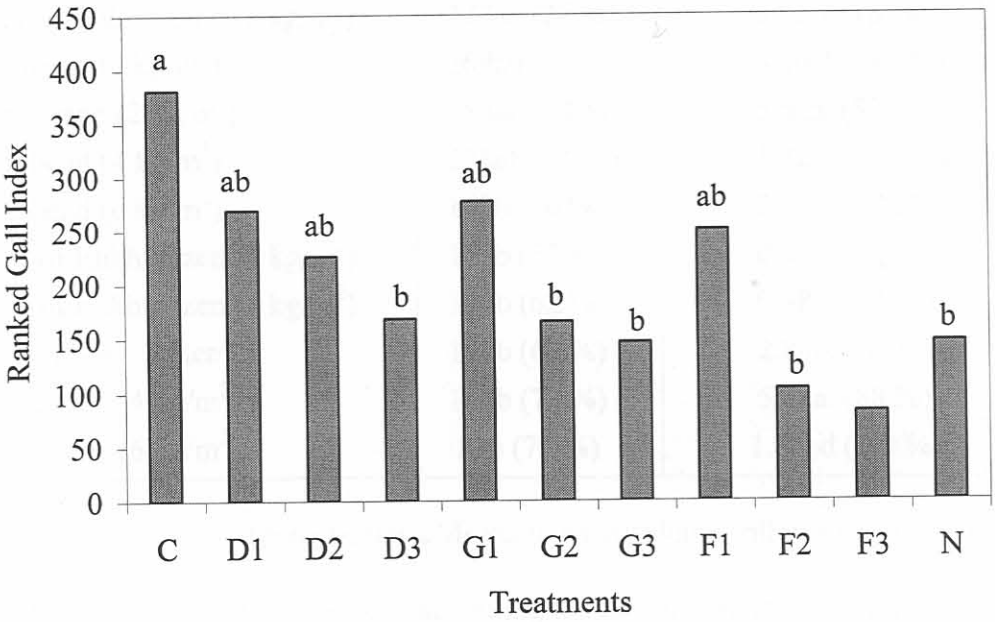

Fig. 4.2: Effect of soil amendment with different *Brassica* species and fenamiphos treatment on galling of *Vigna subterranea* infested with *M. incognita* race 2 at midseason. Bars followed by the same letter are not significantly different at $P \leq 0.05$ according to Kruskal-Wallis test. C = Control, D = Drumhead, G = Glory of Enkhuizen, F = Forage rape, N = Fenamiphos. 1 = 2 kg/m^2, 2 = 4 kg/m^2, 3 = 6 kg/m^2.

Table 4.3a: Effect of soil amendment with different *Brassica* spp. on galling of bambara groundnut roots and on plant growth.

Treatment	Ranked GI*	Dry wt. of shoots (g)
Control	381a -	3.07bc -
Glory of Enkhuiezen (2 kg/m^2)	277ab (27 %)	3.25bc (6 %)
Drumhead (2 kg/m^2)	269ab (30 %)	4.20ab (37 %)
Forage rape (2 kg/m^2)	250ab (34 %)	5.62a (83 %)
Drumhead (4 kg/m^2)	226ab (41 %)	1.32cd (-57 %)
Drumhead (6 kg/m^2)	168b (56 %)	2.10cd (-32 %)
Glory of Enkhuiezen (4 kg/m^2)	165b (57 %)	0.97d (-71 %)
Glory of Enkhuiezen (6 kg/m^2)	146b (62 %)	0.98d (-71 %)
Fenamiphos (2 liter/)	146b (62 %)	2.80bcd (-9 %)
Forage rape (4 kg/m^2)	103b (73 %)	5.78a (88 %)
Forage rape (6 kg/m^2)	82b (79 %)	1.83cd (-40 %)

Each value is the mean of 6 replicates. Means in each column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test. Ranked GI in a column followed by the same letter do not differ significantly according to the Kruskal-Wallis test. * GI = Gall index. Figures in brackets represent % decrease in gall index, and increase in dry wt. of shoots respectively, compared to the control.

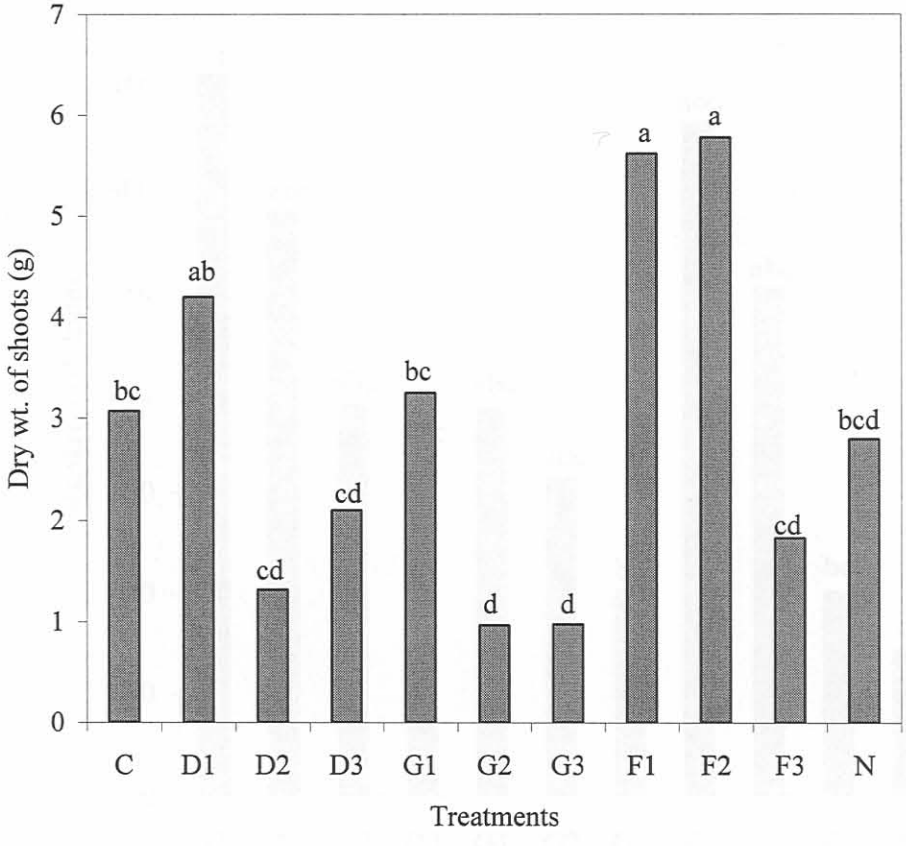

Fig. 4.3: Effect of soil amendment with different *Brassica* species on the dry wt. of shoots of *Vigna subterranea* grown for four weeks in soil infested with *M. incognita* race 2. Bars followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple rang test. C = Control, D = Drumhead, G = Glory of Enkhuizen, F = Forage rape, N = Fenamiphos. 1 = 2 kg/m^2, 2 = 4 kg/m^2, 3 6 kg/m^2.

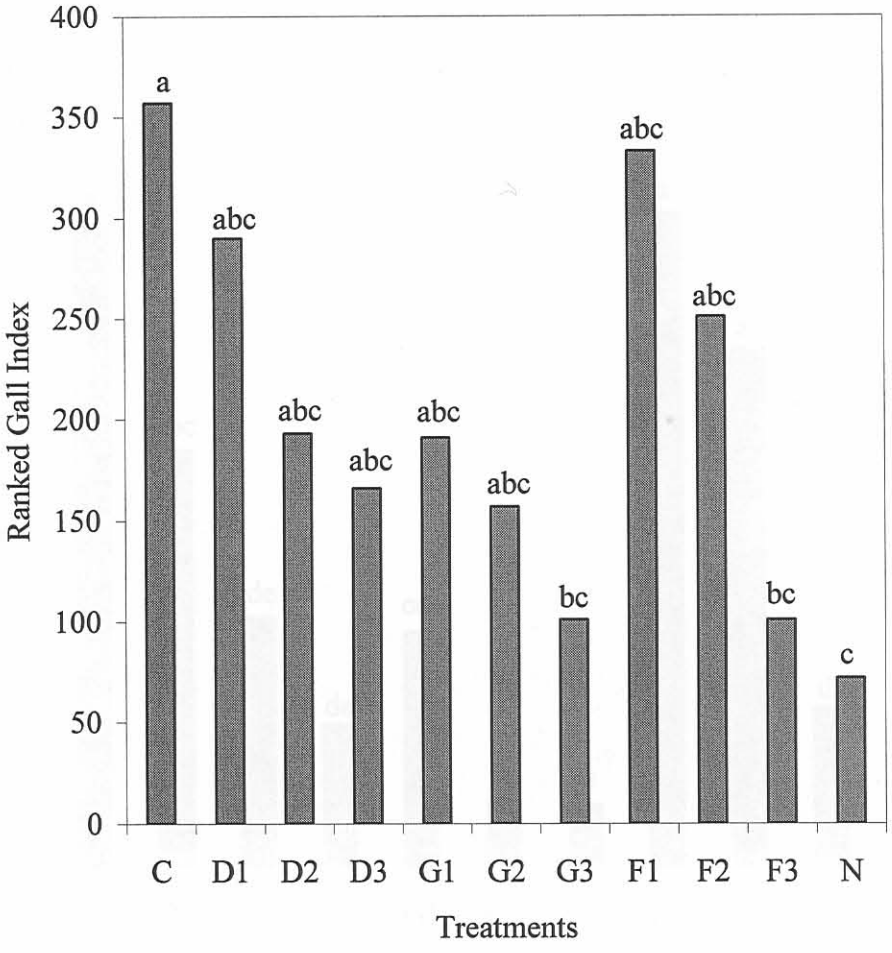

Fig. 4.4: Effect of soil amendment with different *Brassica* species on roots of eight-week old *Vigna subterranea* plants grown in the greenhouse in soil infested with *M. incognita* race 2. Bars followed by the same letter are not significantly different at $P \leq 0.05$ according to the Krukal-Wallis test. C = Control, D = Drumhead, G = Glory of Enkhuizen, F = Forage rape, N = Fenamiphos. 1 = 2 kg/m^2, 2 = 4 kg/m^2, 3 = 6 kg/m^2.

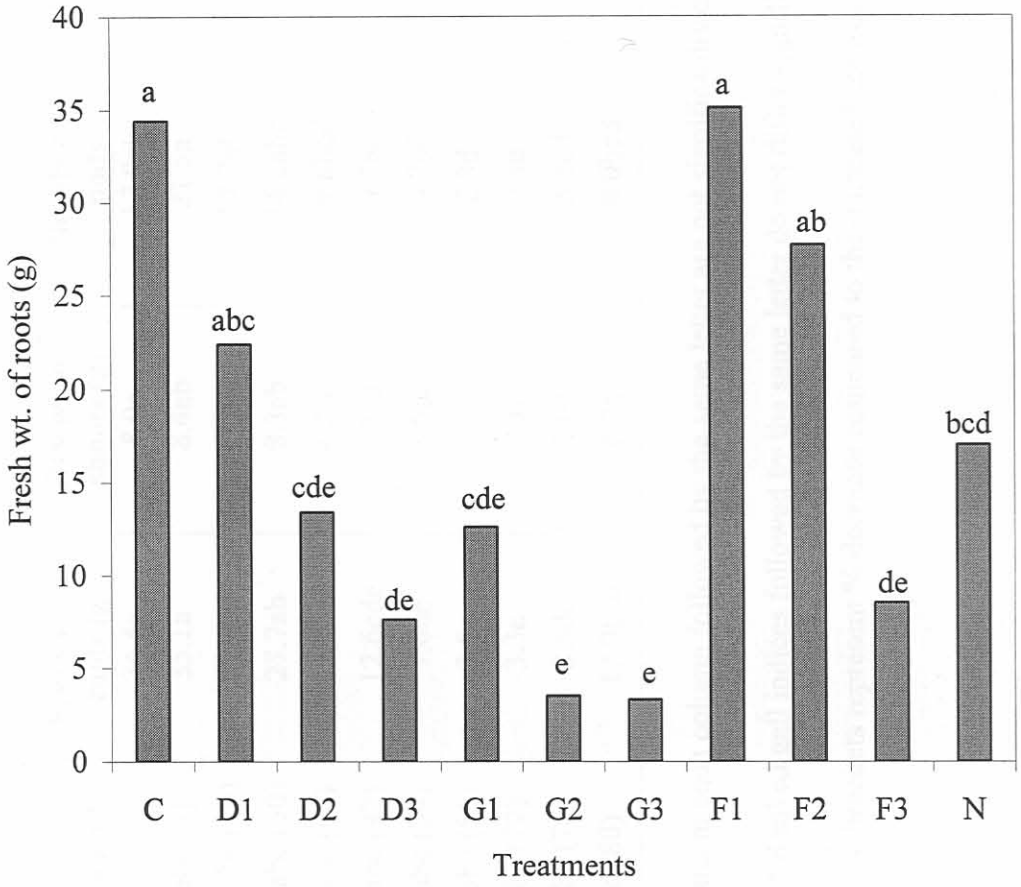

Fig. 4 5: Effect of soil amendment with different *Brassica* species on fresh wt. of roots of eight-week old *Vigna subterranea* plants grown in the greenhouse in soil infested with *M. incognita* race 2. Bars followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test. C = Control, D = Drumhead, G = Glory of Enkhuizen, F = Forage rape, N = Fenamiphos. 1 = 2 kg/m^2, 2 = 4 kg/m^2, 3 = 6 kg/m^2.

Table 4.3b: Effect of soil amendment with different *Brassica* species. on growth, yield, and galling of *Vigna subterranea* grown for eight weeks in soil infested with *M. incognita* race 2 in the greenhouse.

Treatments	Gall index*	Fresh wt. of roots (g)	Dry wt. of shoots (g)	Number of pods	Dry wt. of pods
Untreated control	357.0a -	34.4a	8.9a	17.0ab	4.6bc
Forage rape (2kg/m^2)	332.5abc (7)	35.1a	8.4ab	21.5a	9.7a
Drumhead (2kg/m^2)	290.0abc (19)	22.4abc	8.0ab	17.7ab	6.1ab
Forage rape (4kg/m^2)	251.0abc (30)	27.7ab	8.3ab	14.2abc	4.3bc
Drumhead (4kg/m^2)	193.0abc (46)	13.4cde	4.2cde	9.8bcd	2.4bc
Glory of enkhuizen (2kg/m^2)	190.5abc (47)	12.6cde	5.3cd	9.7bcd	4.1bc
Drumhead (6kg/m^2)	166.0abc (54)	7.6de	3.6de	4.7cd	1.3c
Glory of enkhuizen (4kg/m^2)	157.0abc (56)	3.5e	2.0e	2.3d	0.5c
Glory of enkhuizen (6kg/m^2)	101.0bc (72)	3.3e	2.1e	0.3d	0.1c
Forage rape (6kg/m^2)	101.0bc (72)	8.5de	3.0de	3.3cd	1.1c
Fenamiphos (2 liter/ha)	72.0c (80)	17.0bcd	6.2bc	8.0bcd	2.3bc

Each value is the mean of 6 replicates. Means in each column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test. * Ranked gall indices followed by the same letter do not differ significantly at $P \leq 0.05$ according to the Kruskal-Walis test. Figures in brackets represent % decrease compared to the untreated control.

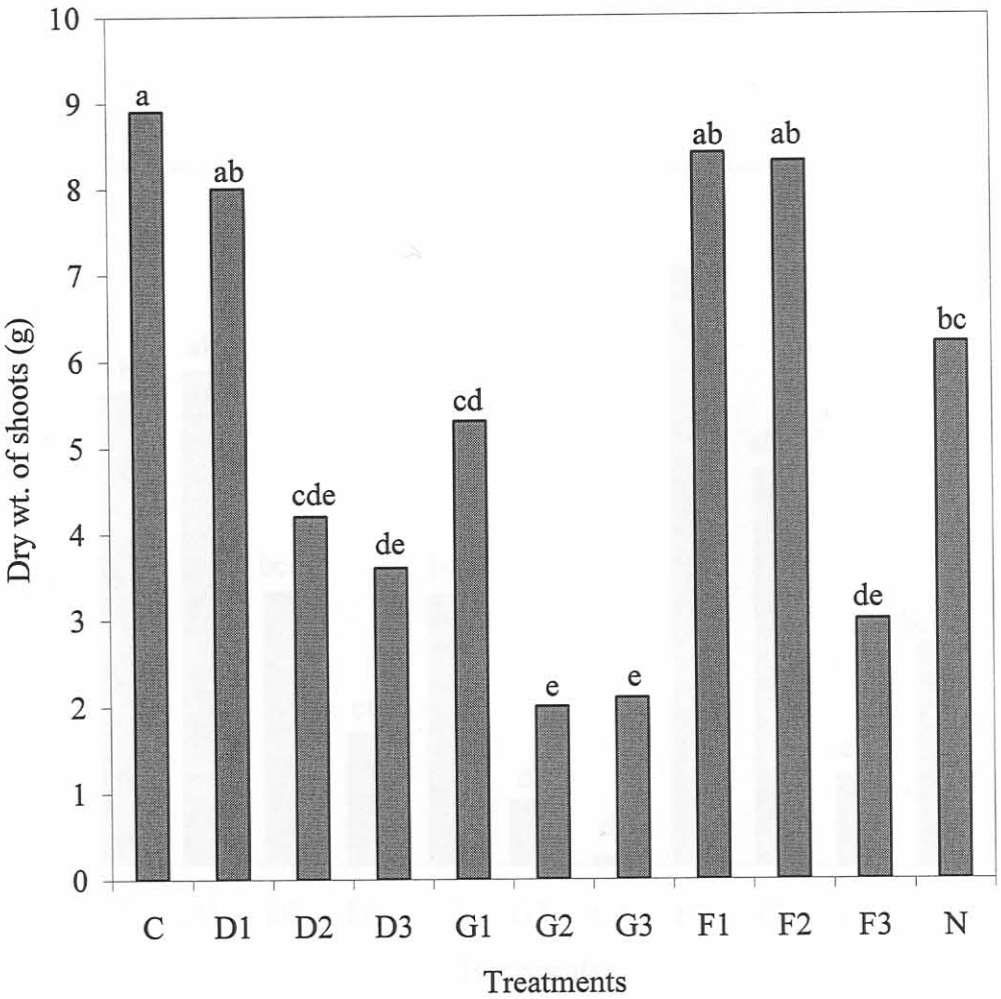

Fig. 4.6: Effect of soil amendment with different *Brassica* species on the dry wt. of shoots of eight-week old *Vigna subterranea* plants grown in the greenhouse in soil infested with *M. incognita* race 2. Bars followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test. C = Control, D = Drumhead, G = Glory of Enkhuizen, F = Forage rape, N = Fenamiphos. 1 = 2 kg/m^2, 2 = 4 kg/m^2, 3 = 6 kg/m^2.

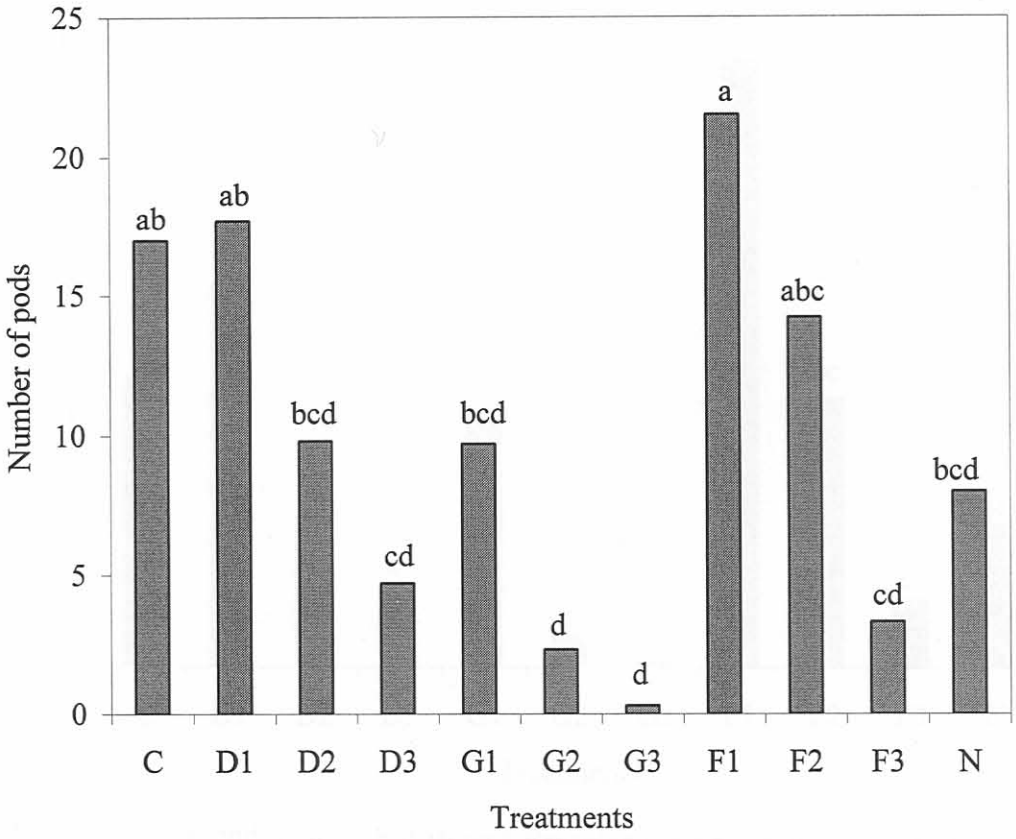

Fig. 4.7: Effect of soil amendment with different *Brassica* species on the number of pods of eight-week old *Vigna subterranea* plants grown in the greenhouse in soil infested with *M. incognita* race 2. Bars followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test. C = Control, D = Drumhead, G = Glory of Enkhuizen, F = Forage rape, N = Fenamiphos. 1 = 2 kg/m^2, 2 = 4 kg/m^2, 3 = 6 kg/m^2.

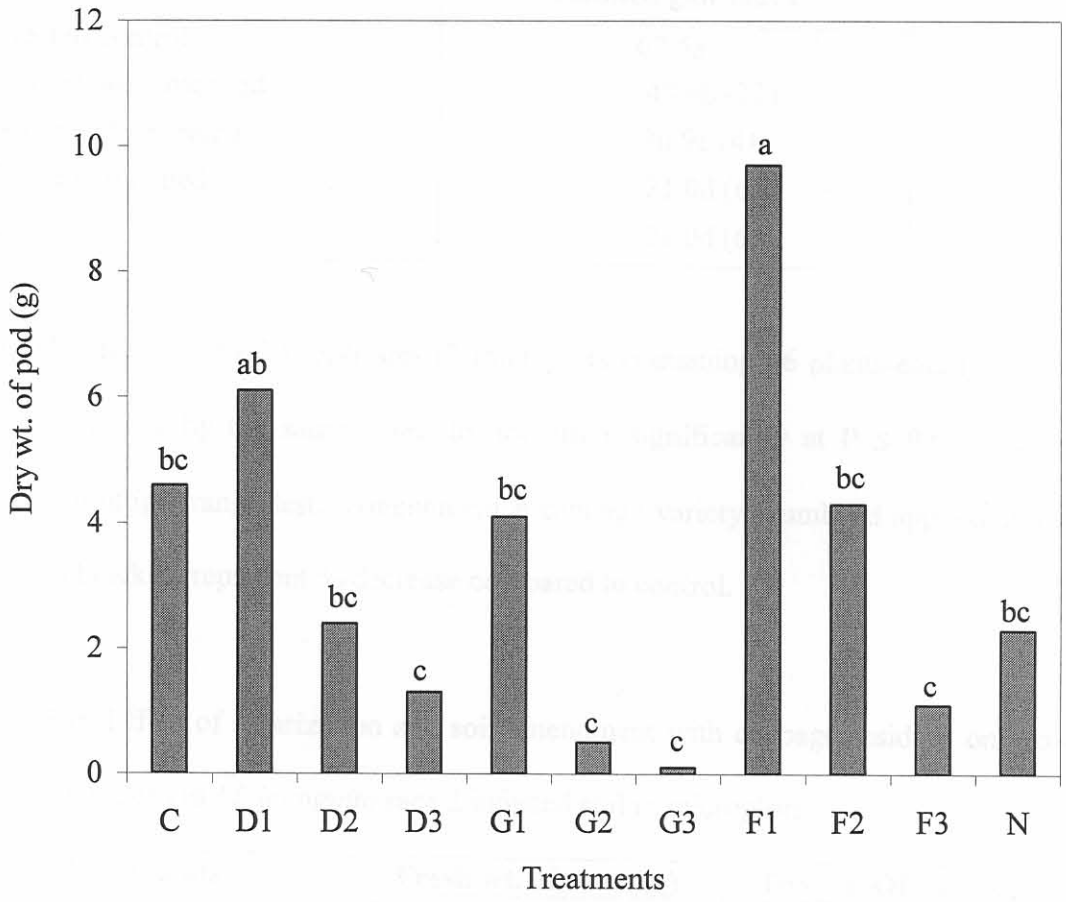

Fig. 4.8: Effect of soil amendment with different *Brassica* species on the dry wt of pods of eight-week old *Vigna subterranea* plants grown in the greenhouse in soil infested with *M. incognita* race 2. Bars followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test. C = C0ntrol, D = Drumhead, G = Glory of Enkhuizen, F = Forage rape, N = Fenamiphos. 1 = 2 kg/m^2, 2 = 4 kg/m^2, 3 = 6 kg/m^2

Table 4.3c: Effect of biofumigation and solarization on galling of *Vigna subterranea* roots and on incidence of *M. incognita* race2 egg masses after eight weeks in microplots.

Treatments	Ranked gall index
Untreated control	62.5a -
Solarized non-amended	48.6b (22)
Unsolarized amended	36.9c (41)
Solarized amended	21.0d (66)
Aldicarb	21.0d (66)

Each value is a mean of 3 replicates (3 microplots containing 16 plants each). Means in a column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. Amendment = cabbage variety drumhead applied at 4 kg/m^2. Figures in brackets represent % decrease compared to control.

Table 4.3 d: Effect of solarization and soil amendment with cabbage residues on growth of *Vigna subterranea* in *M. incognita* race 2 infested soil in microplots.

Treatments	Fresh wt. of roots (g)	Dry wt. Of shoots (g)
Aldicarb	5.5a (50 %)	2.1a (54 %)
Untreated control	3.5b -	1.4bc -
Solarized non-amended	2.2c (-59 %)	1.1c (-21 %)
Unsolarized amended	2.0cd (-47 %)	1.7ab (26 %)
Solarized amended	1.0d (-71 %)	2.0a (46 %)

Each value is the mean of 3 replicates (3 microplots containing 16 plants each). Means in each column followed by the same do no differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. Amendment = cabbage variety drumhead applied at 4 kg/m^2. Figures in brackets represent % increase compared to control.

Table 4.3e: Soil temperature* (°C) at depths of 15 and 30 cm depths in solarized-amended and unsolarized-amended soil in microplots in the field.

Week	Mean temperature (°C) in solarized-amended soil				Mean temperature (°C) in unsolarized- amended soil				Differences in temperature between solarized and unsolarized soil			
	Maximum		Minimum		Maximum		Minimum		Maximum		Minimum	
Soil Depth	**15**	**30**	**15**	**30**	**15**	**30**	**15**	**30**	**15**	**30**	**15**	**30**
1	30.6	27.5	22.9	24.7	37.5	31.8	26.7	27.3	6.9	4.3	3.8	2.6
2	30.2	27.1	23.2	24.6	36.0	30.9	27.3	27.5	5.8	3.8	4.1	2.9
3	34.6	29.9	24.4	26.1	40.0	33.9	28.4	28.7	5.4	4.0	4.0	2.6
4	31.8	29.1	24.0	26.1	36.7	32.6	28.2	28.7	4.9	3.5	4.2	2.6
Average	31.8	28.4	23.6	25.4	37.6	32.3	27.7	28.1	5.8	3.9	4.0	2.7

*Temperature was measured by means of a 1200 series Grant squirel digital logger.

Table 4.3f: Soil temperature* (°C) 15 and 30 cm depths in solarized non-amended and unsolarized non-amended soil microplots in the field.

Week	Mean temperature (°C) in solarized non-amended soil				Mean temperatuer(°C) in solarized Non-amended soil				Differences in temperature between solarized and unsolarized soil			
	Maximum		Minimum.		Maximum.		Minimum.		Maximum.		Minimum	
Depth	15	30	15	30	15	30	15	30	15	30	15	30
1	29.6	27.0	22.2	23.8	35.7	30.1	24.8	26.0	6.1	3.1	2.6	2.2
2	29.5	26.7	22.5	24.0	34.5	29.4	25.0	26.3	5.0	2.7	2.5	2.3
3	34.0	29.9	23.9	25.4	38.5	32.9	26.3	27.4	4.5	3.0	2.4	2.0
4	31.4	28.9	23.5	25.3	35.8	31.8	26.3	27.9	4.4	2.9	2.8	2.6
Ave.	31.1	28.1	23.0	24.6	36.1	31.1	25.5	26.9	5.0	2.9	2.6	2.3

* Temperature was measured by means of a 1200 series Grant squierel digital data logger.

CHAPTER 5

COMPARISON OF SOIL AMENDMENTS, BIOFUMIGATION AND BIOLOGICAL CONTROL AGENTS FOR CONTROL OF *MELOIDOGYNE INCOGNITA* RACE 2 ON BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA*).

ABSTRACT

A study was conducted in the greenhouse to compare a range of non-chemical control measures for the control of *M. incognita* race 2 on bambara groundnut. Treatments consisted of soil amendments with cabbage applied at the rate of 4 kg/m^2, cattle manure at 4 kg/m^2, and a commercial biocontrol agent Biostart® 2000, aldicarb, fertilzer supplement of KNO_3 plus $Ca(NO_3)_2$, and a fertilizer supplement of $(NH_4)_2NO_3$. The effects of the treatments on gall and egg mass indices, and on growth of infected bambara groundnut were evaluated eight weeks after planting. All treatments reduced galling and egg mass index to some degree. There was no significant difference in gall and egg mass indices between plants treated with cattle manure, cabbage, Biostart® 2000, and fertilizer supplements. Aldicarb resulted in the greatest reduction of the nematode population.

5.1 Introduction

Root knot nematode, *Meloidogyne incognita* race 2 on bambara groundnut was successfully controlled in previous experiments by various control methods such as soil organic amendments, biofumigation, solarization and the use of nematicides. These control methods

gave various degrees of success in controlling *Meloidogyne* species on other crops such as tomato (Chindo & Khan, 1990; Kaplan & Noe, 1993; Stapleton & Duncan, 1998), citrus (McLeod & Da Silva, 1994), potato (Motjahedhi, Santo, Hang & Wilson, 1991; Motjahedhi, Santo, Wilson & Hang, 1993), and water melon (Keinath, 1996) to mention a few. However it was observed that some of these control methods were not very effective when used individually and needed to be complemented by other methods in order to improve control (Keinath, 1996). It is therefore necessary to evaluate these control methods together and compare their effects in order to determine how they can be used in an IPM program for *M. incognita* race 2 on bambara groundnut.

The objective of this study was therefore to determine the effectiveness of a range of non-chemical control measures in comparison with a commercial nematicide for control of *M. incognit*a race 2 on bambara groundnut.

5.2 Materials and methods

The study was conducted in the greenhouse with temperatures maintained between 20 and 30 °C. The soil used was a steam pasteurized sandy loam soil (80 % sand, 4 % silt, 14 % clay and pH 6.0) prepared by mixing topsoil with river sand at the ratio of 2:1. The volume of soil contained in a 25 cm diameter plastic pot was weighed and the amount of plant material required as amendment per pot calculated accordingly. The leaves and stems of three and a half months old cabbage (*Brassica oleracea capitata* L.), variety Drumhead were cut into 1cm long pieces and weighed to determine the amount of amendment needed per pot. Cattle manure was ground into a fine powder and sifted to remove large particles. The

manure was subsequently added to dry soil at the rate of 0.8 kg/m^2. A commercial biological control product Biostart® 2000 was obtained from Microbial Solutions (Pty) Ltd (P.O. Box 103, Kya, Sand, 2163, South Africa). The product contains *Bacillus laterosporous*, *Bacillus chitinosporous*, and *Bacillus licheniformis*.

The experiment comprised of the following six treatments replicated six times and arranged in a completely randomized design:

- Soil amended with cabbage variety Drumhead at the rate of 4 kg/m^2
- Soil amended with cattle manure at the rate of 0.4 kg/m^2
- Biostart® 2000 (1:1 mixture of biostart and microboost dissolved in 100 ml water) applied at the rate of 5 ml/pot
- Aldicarb applied at the rate of 5 g/m^2
- Supplement of KNO_3 and $Ca(NO_3)_2$ applied at the rate of 60 kg N/ha
- Supplement of $(NH_4)_2NO_3$ applied at the rate of 60 kg N/ha.

The two fertilizer supplements were included as controls to compare the effects of the different nitrogen sources on the nematode population as was previously reported by (Rodriguez-Kabana, 1986). For the biofumigation treatment, Quantities of 9 kg of soil were thoroughly mixed with cabbage residues, watered to field capacity, put in plastic bags and placed on the floor in the greenhouse. The cattle manure was incorporated into the soil and put in 25 cm diameter plastic pots and after thorough mixing watered to field capacity. The pots were placed on benches in the greenhouse. Each treatment was applied separately to *M. incognita* race 2 inoculated and non-inoculated soil.

The *Meloidogyne* inoculumn consisted of a suspension of eggs and juveniles prepared as follows: tomato roots heavily infected with *M. incognita* race 2 (egg mass index = 5) were washed free of soil, cut into 1 cm long pieces and incubated in water for 12 hours to allow for hatching of the eggs. A 5ml suspension of eggs and juveniles was poured into a recess in the soil and recesses were subsequently covered with soil. The nematode inoculum was applied at the time of planting except for the soil amendments where inoculum was incorporated into the soil together with the amendment to allow for biofumigation and biocontrol activities to take effect.

Three seeds of bambara groundnut variety DIPC were planted in each pot and thinned to one seedling per pot four weeks after emergence. The Biostart®2000 treatment was applied every two weeks and terminated three weeks before harvest. Plants were harvested ten weeks after planting and assessed for nematode damage. The fresh root mass was determined prior to evaluating roots for gall and egg masses. Roots were stained in a 0.15g/liter aqueous solution of Phloxine B (Hussey & Barker, 1973), and evaluated for gall and egg mass indices using a rating scale of 0-5 (Taylor & Sasser, 1978). Shoots were dried in an oven at 60 °C for three days and the dry mass determined. All data were analyzed statistically by ANOVA and means separated using Duncan's multiple range test.

5.3 Results

There were significant differences between treatments with regard to gall and egg mass indices. Plants from the control supplemented with $(NH_4)_2NO_3$ had the highest gall and egg mass indices while the lowest indices were recorded in the aldicarb treatment. Gall and egg

mass indices were reduced by 8-58 % in plants treated with Biostart® 2000, cattle manure, fertilizer, cabbage and aldicarb respectively, compared to the control supplemented with $(NH_4)_2NO_3$ (Table 5.3 a). There was no significant difference between the Biostart®2000 treatment and the $(NH_4)_2NO_3$ control in terms of gall and egg mass indices. No significant differences in gall and egg mass indices occurred between treatments with a combination of KNO_3 plus $Ca(NO_3)_2$, cattle manure and Biostart® 2000 (Table 5.3a).

The nematode had a dramatic effect on the root mass of inoculated plants compared to the non-inoculated control plants (Fig 5.1). Plants grown in non-inoculated soil had greater root mass than the *M. incognita* race 2-infected plants. (Table 5.3b). In plants treated with KNO_3 plus $Ca(NO_3)_2$, cattle manure, and $(NH_4)_2NO_3$, root mass was greater than in infected plants treated with cabbage, Biostart ® 2000 and aldicarb There were significant differences between the fresh weight of roots of inoculated and non-inoculated plants from KNO_3 plus $Ca(NO_3)_2$ treatment and the $(NH_4)_2NO_3$ control. No significant differences in weight occurred between roots of inoculated and non-inoculated plants receiving cattle manure. There were significant differences between root weight of inoculated and non-inoculated plants treated with Biostart® 2000, and aldicarb. No significant differences in weight occurred between roots of inoculated and non-inoculated plants from cabbage treatments (Table 5.3b). Phytotoxicity symptoms ocurred on plants treated wih cabbage, Biostart® 2000 and aldicarb. Roots of plants receiving these treatments were rotten and stunted compared to the other treatments.

Significant differences occurred in the dry mass of shoots between inoculated and non-inoculated plants from soil treated with KNO_3 combined with $Ca(NO_3)_2$ and the $(NH_4)_2NO_3$ control (Table 5.3b). No significant differences in dry mass were observed in other treatments. The dry mass of shoots of plants inoculated with *M. incognita* race 2 in soil treated with KNO_3 combined with $Ca(NO_3)_2$, and cattle manure, was significantly different from that of the other treatments. Dry mass of shoots of inoculated plants treated with cattle manure, aldicarb, and control supplemented with $(NH_4)_2NO_3$ did not differ significantly. However, plants treated with cattle manure were significantly different from those treated with cabbage and Biostart® 2000 (Table 5.3b).

5.4 Discussion

All the treatments evaluated in this study were able to give some degree of control of *M. incognita* race 2 on bambara groundnut although the effect of Biostart® 2000 was insignificant. Although the control obtained was not dramatic, the results confirmed the potential of using cabbage residues, cattle manure, fertilizer (KNO_3 plus $Ca(NO_3)_2$, Biostart® 2000, and aldicarb as IPM components for *M. incognita* race 2 on bambara groundnut. Incorporation of cabbage residues into the soil reduced galling of bambara groundnut roots and incidence of *M. incognita* race 2 egg masses by 33 % compared to the $(NH_4)_2NO_3$ control, whereas aldicarb resulted in a 58 % reduction. Previously, *M. incognita* population was successfully controlled by the incorporation of cabbage residues into the soil on other crops (McLeod & Da Silva, 1994; McLeod, Somers & Gendy, 1995). It has been reported previously that this practice on its own is not very effective unless it is combined with other control methods to enhance its efficacy (Gamliel & Stapleton, 1997).

Aldicarb has been used successfully to control nematodes on cowpea, a relative of bambara groundnut, but it has not been tested on bambara groundnut before. The 58 % control achieved in this study warrants further evaluation of aldicarb on this crop.

Addition of KNO_3 plus Ca $(NO_3)_2$ to the soil reduced galling of bambara groundnut roots and egg mass index of *M. incognita* race 2 by 22 % compared to the $(NH_4)_2NO_3$ control confirming previous reports that nitrogenous fertilizer have detrimental effects on nematodes (Rodriguez-Kabana, 1986). According to Rodriguez-Kabana (1986), inorganic and organic fertilizers containing ammoniacal nitrogen have the greatest effect on nematode populations. The nitrogenous fertilizer used in this study was applied at a very low rate hence it can not be accurately concluded that the reduction in nematode population was due to the nitrogen released into the soil. Riegel, Fernandez & Noe (1996) evaluated KNO_3 against *M. incognita* on cotton and observed an increase in juvenile population in plants receiving this treatment. In this study, the combination of KNO_3 with Ca $(NO_3)_2$ resulted in a 22 % reduction in *M. incognita* race 2 population. According to Rodriguez-Kabana (1986), inorganic nitrogenous fertilizers are effective against nematodes when they are applied at levels far in excess of those required for normal fertilization. Since such levels are toxic to plants, in this study, fertilizers were applied at rates required for normal fertilization and resulting in only 22 % reduction in nematode population

In the present study, soil amendment with 0.4 kg/m^2 cattle manure reduced gall formation by *M. incognita* with 21 % compared to the control. This reduction was similar to that obtained when the soil was treated with a combination of the two mineral fertilizers (KNO_3 and

$Ca(NO_3)_2$. Cattle manure was previously reported to be effective against *Meloidogyne* species (Poswal & Akpa, 1991). As in the case of mineral fertilizers, the control achieved could be as a result of nutrients available to the plant and release of chemicals such as ammonia that are detrimental to the nematodes (Stirling, 1991).

Treatment of soil with Biostart ®2000 only reduced galling of bambara groundnut roots and incidence of *M. incognita* race 2 egg masses by 8 % compared to the $(NH_4)_2NO_3$ control According to the suppliers of Biostart® 2000, the product acts by dissolving the chitin of the eggshell and this inhibits egg development. The inoculum used in this study consisted of egg masses, which were incubated for 12 hours to allow eggs to hatch so that the final inoculumn could include some juveniles. It is possible that most eggs hatched and resulted in more juveniles than eggs, hence the Biostart® 2000 treatment being ineffective. The Biostart® 2000 treatment was applied every second week. Since the life span of the bacteria is approximately eight days, the population probably declined to small numbers before the next application. Ideally, the product should have been applied weekly.

Addition of cattle manure and fertilizer to the soil improved the plant growth, corresponding with previous reports (Poswal & Akpa, 1991; Riegel et al., 1996). Although the mode of action of organic matter is complex and a number of mechanisms appear to be involved, this improvement in plant growth could be attributed to the changes in the nutrient status and physical characteristics of the soil (Stirling, 1991). It common knowledge that addition of inorganic fertilizer to the soil results in improvement of plant growth (Riegel et al., (1996)

Treatment with cabbage, Biostart® 2000, and aldicarb did not improve plant growth. This could be attributed to phytotoxicity that was observed in plants receiving these treatments confirming earlier findings in this study. Although Biostart® 2000 is reported to promote plant growth (G. Limmerick, P. O. Box 103, Kya Sand, 2163, South Africa, personal communication), it was not confirmed in this study.

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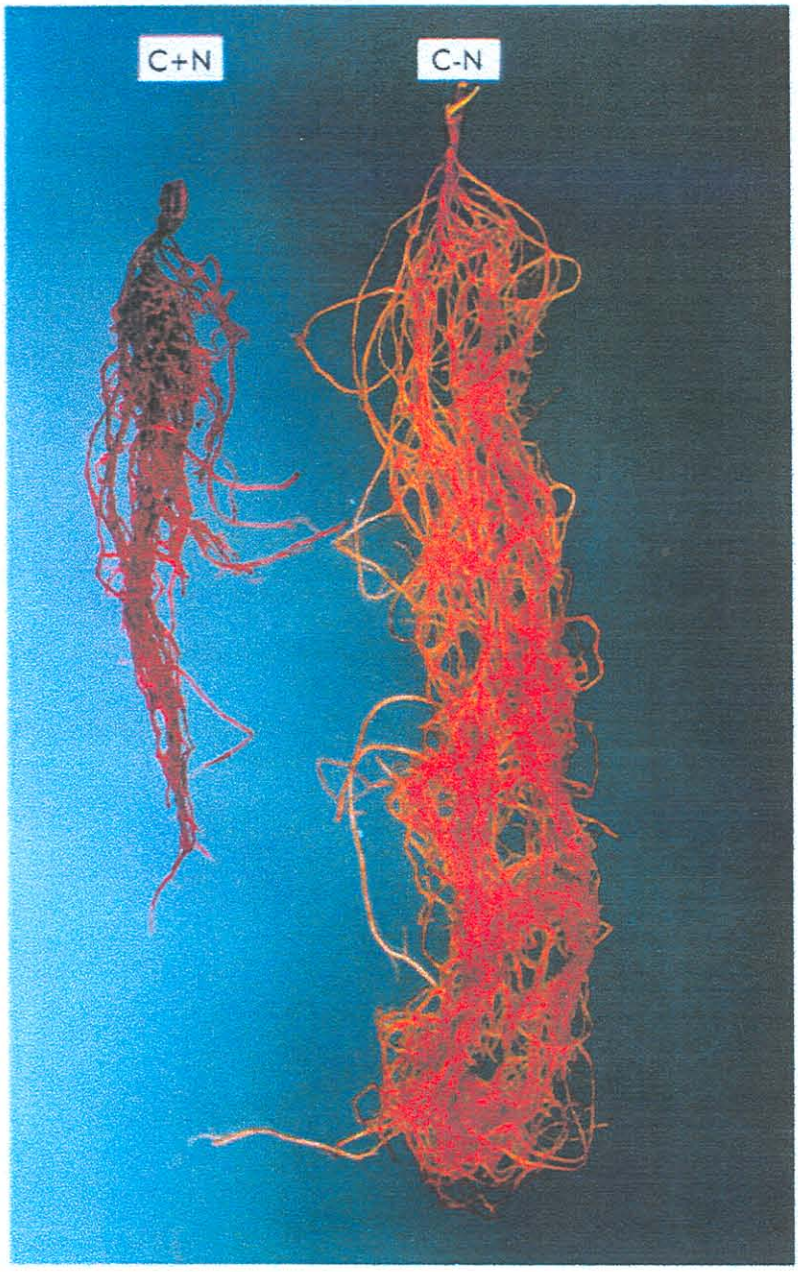

Fig. 5.1: Effects of *M. incognita* infection on roots of bambara groundnut after ten weeks in the greenhouse. C + N = Control with nematodes, C – N = Control without nematodes. Roots were stained with Phloxine B to enhance egg masses.

Table 5.3a: Effect of different soil treatments on galling of *Vigna subterranea* ten weeks after planting in the greenhouse in soil inoculated with *M. incognita* race 2.

Treatments	Ranked gall index
$(NH_4)_2NO_3$ (Control 1)	67.0a
Biostart ®2000	61.9ab (8)
Cattle manure	52.6bc (21)
KNO_3 and $Ca(NO_3)_2$ (Control 2)	52.0bc (22)
Cabbage	44.6c (33)
Aldicarb	28.3d (58)

Each value is the mean of 6 replicates. Means in each column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test. Figures in brackets represent the % increase or decrease compared to the $(NH_4)_2SO_4$ control.

Table 5.3b: Effect of different soil treatments on growth of *Vigna subterranea* inoculated with *M. incognita* race 2, ten weeks in the greenhouse.

Treatment	Fresh weight of roots (g)		Dry weight of shoots (g)	
	Inoculated	Non-inoculated	Inoculated	Non-inoculated
KNO_3 and $Ca(NO_3)_2$ (Control 2)	7.35aA	10.83aB	1.82aA	2.37aB
Cattle manure	6.93aA	7.61abcA	1.62abA	1.57bcA
$(NH_4)_2NO_3$ (Control 1)	4.38abA	7.92abB	1.19bcA	1.79abB
Cabbage	3.50bA	4.24cA	1.13cA	0.93cA
Biostart®2000	3.27bA	6.11bcB	0.91cA	1.43bcA
Aldicarb	3.25bA	7.56abcB	1.24bcA	1.49bcA

Each value is the mean of 6 replicates. Means in each vertical column followed by the same lower case letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. Means within each parameter followed by the same upper case letter do not differ significantly according to the t test.

CHAPTER 6

GENERAL DISCUSSION

Despite reports that *Meloidogyne* spp. attack bambara groundnut in most areas where the crop is grown, very little research has been done on this problem. Perhaps this could be due to the fact that bambara groundnut is a small-scale farmer crop and its commercial potential has not been explored. Statistics on crop loss due to pests and diseases including root-knot nematodes in bambara groundnut is also not available. It is only recently that research on bambara groundnut has received some attention (Azam-Ali, 1992). Unfortunately the current research on bambara groundnut focuses on agronomic practices that will improve production of the crop under small-sacle farming systems and does not include the disease component. The root-knot nematode problem is no doubt increasing under small-scale farmer conditions and control methods suitable for the resource limited small-scale farmers must be investigated and developed.

The *Meloidogyne* spp. attacking bambara groundnut in Botswana was identified in this study (Chapter 2). Previous reports identified *M. javanica* as the species attacking bambara groundnut in South Africa (Mc Donald & De Waele, 1989), while in Nigeria both *M. javanica* and *M. incognita* were reported (Ogbuji, 1979). Identification of the species has provided valuable information that will be useful in studies on control methods aimed at this species. on bambara groundnut.

Generally, control of *Meloidogyne* species. is best achieved through the use of nematicides. However, currently no nematicide has been tested on bambara groundnut in South Africa or Botswana. Even if suitable nematicides were available, small-scale farmers could probably not afford them. Hence non-chemical methods of control were investigated in this study.

Results in Chapter 2 showed that resistance/tolerance of bambara groundnut to *M. incognita* does not exsist in the fifty bambara groundnut landraces evaluated in this study. Previously, similar results were obtained with fifteen landraces evaluated in South Africa (Mc Donald & De Waele, 1989) and seven in Nigeria (Ogbuji, 1979). There were some borderline cases involving landraces such as HVA 38-3, SB 4-4C, CLDRE and Swazi V4 where the R factors were slightly above 1 and the gall indices were less than 2. According to Canto Saenz's (1986) designations, a landrace is tolerant if the R factor is less than 1 and the gall index is less than 2. The variation in yield encountered among landraces in this study may be of genetic origin and may not imply existence of tolerance as previously suggested by Mc Donald & De Waele (1989) against *M. javanica*.

Although both cattle and poultry manure were effective in reducing *M. incognita* populations on bambara groundnut, cattle manure had an advantage over poultry manure in that it was less phytotoxic. Addition of cattle manure also increased plant growth and yield. Soil amendment with cattle manure could be useful to small-scale farmers in Botswana where cattle manure is abundant and readily available. An application rate of 4 ton/ha is feasible and less expensive for the small-scale farmer who may have insufficient capital to buy

nematicides. Poultry manure could also be useful in areas where cattle manure is not available.

The success of biofumigation using *Brassica* spp. to control *Meloidogyne incognita* was confirmed in this study (Chapter 4). However, this practice would not be viable for small-scale farmers in most parts of Botswana and South Africa where cabbage is produced under irrigation for consumption. Farmers can not afford the luxury of irrigating cabbage only for it to be incorporated into the soil. They can however use the residues from pruning and the residues from insect damaged crops for biofumigation although the recommended application rate of 4 kg/ha requires a substantial amount of material. For biofumigation to be viable in small-scale farming systems, a brassica crop could be considered. Unfortunately forage rape was not very effective in this study because of the phytotoxicity problem.

The control achieved with soil solarization alone was not as effective as when it was combined with biofumigation confirming previous reports by Katan (1981) and Keinath (1996). In Chapter 4 of this thesis it was demonstrated that combination of solarization and biofumigation could give better control of the nematode than when either method is used alone, and that the effect is comparable to that obtained with aldicarb. Gamliel & Stapleton (1997) reported similar findings. Although this approach seems attractive, the length of time required and its dependency on weather conditions are likely to make it less attractive to small-scale farmers. The practice would be more successful in areas where temperatures are high.

Results obtained with Biostart® 2000 were not impressive and more work is needed to evaluate this product on *M. incognita* on bambara groundnut. The product could benefit small-scale farmers because it is easy and safe to use (G. Limmerick, Microbial Solutions, Kya, Sand, Personal communication).

Application of nitrogenous fertilizers to bambara groundnut resulted in increased plant growth and reduced *M. incognita* galling on bambara groundnut. Previous reports suggested that nitrogenous fertilizers have detrimental effects on nematodes if they are applied in large quantities (Rodriguez-Kabana, 1986). The dosages used in this study were not high enough to have any significant effect on the nematode. It could be that the plants were able to tolerate nematode damage because of their healthy condition and the favourable nutrient status of the soil (Stirling, 1991).

Comparison of non-chemical and chemical methods of control for *M. incognita* on bambara groundnut (Chapter 5) proved the superiority of nematicides over other control methods. It has been demonstrated in this study that non-chemical methods of control provide some degree of control for *M. incognita*. Positive results obtained when solarization and biofumigation were combined indicated that integration of control methods is desirable in order to achieve improved control of nematodes. An integrated pest management (IPM) program for *M. incognita* race 2 on bambara groundnut could be developed on the basis of results obtained in this study. This approach mainly targets small-scale farming systems where bambara groundnut is an important crop.

Egunjobi (1987) suggested the following key steps for formulating an IPM strategy for *Meloidogyne* species:

1. Identifying the crop and nematode pest
2. Selecting the IPM components
3. Formulating the strategy based on the species identified and on selection of components.

The nematode species attacking bambara groundnut in Botswana was identified as *M. incognita* race 2. Non-chemical control methods were evaluated against this species for possible inclusion in an IPM program. A proposed IPM program for *M. incognita* on bambara groundnut is presented below:

1. Soil amendment with cattle manure. This component proved to be effective against nematode in the present study. Amendment of soil with cattle manure will reduce the nematode population while at the same time providing nutrition for the plant. An application rate of 4 ton/ha is recommended on the basis of the results obtained in this study.
2. Solarization. This should be combined with organic soil amendment to improve the efficacy of both methods. Solarization for four weeks will enhance beneficial microbial activity in the soil and increase volatile evolution from the decomposing manure (Gamliel & Stapleton, 1993).
3. Selection of seed. Although no resistance/tolerance was found among the bamabara groundnut landraces evaluated, some South African lines viz. HVA 38-3, SB 4-4C, CLDRE and Swazi V4 were borderline cases which could be used in an IPM program.

Among the Botswana lines, DIPC would be ideal because although it is susceptible to the nematode, it can still produce high yields even when infected. It is also very popular among small-scale farmers in Botswana.

The suggested IPM strategy could be adjusted to suit farmers in other localities with their particular cicumstances. For example, where cattle manure is not available, it could be substituted with poultry manure, or residues from *Brassica* crops. A nematicide such as aldicarb could be applied at the time of planting after solarization or be included the following season. Nitrogenous fertilizers could also be considered for inclusion depending on the nutrient status of the soil and notwithstanding the capability of bambara groundnut to fix its own nitrogen through rhizobium nodulation.

Although the discussions referred to small-scale farmers in Botswana, these proposals are also relevant to other countries. The approach is likely to be the same with minor deviations due to differences in climatic conditions that may have an effect on some methods such as solarization. For example, it may not be feasible for small-scale farmers in Botswana to adopt biofumigation using cabbage residues, but it may be feasible for farmers in South Africa to adopt this practice. The proposed strategy may therefore be modified, and tailor made to suit each situation.

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INTEGRATED PEST MANAGEMENT OF *MELOIDOGYNE INCOGNITA* ON BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA*)

by

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SUMMARY

In this study non-chemical methods of control were evaluated as possible components of integrated pest management of *Meloidogyne incognita* race 2 on bambara groundnut. The main findings were:

1. *Meloidogyne incognita* race 2 was identified as the species that attack bambara groundnut in Botswana. No resistance was found among the fifty bambara groundnut landraces screened in the greenhouse against *M. incognita* race 2. However, there were some borderline cases among the South African landraces showing slight tolerance to the nematode.

2. *M. incognita* race 2 population on bambara groundnut was decreased by application of poultry and cattle manure. Poultry manure applied at 0.8 kg/m^2 (8 ton/ha) was as effective as the nematicide fenamiphos applied at 2 liter/ha. The two treatments were however, phytotoxic. Shoot mass was increased by 132 % to 270 % in plants treated with cattle manure at the rates of 0.4 kg/m^2 (4 ton/ha) and 0.8 kg/m^2 (8 ton/ha) respectively compared to the untreated control. Cattle manure at 0.4 kg/m^2 was less phytotoxic than poultry manure applied at the same rate. An application rate of 0.4 kg/m^2 cattle or poultry manure was found to be more appropriate for effective nematode control.
3. Control of *M. incognita* race 2 population on bambara groundnut by means of biofumigation using *Brassica* species was effective. Cabbage residues applied at the rate of 6 kg/m^2 were as effective as the aldicarb treatment. Combination of biofumigation and solarization in the field resulted in 66 % reduction of *M. incognita* compared to 41 % when biofumigation was used alone.
4. Non-chemical methods could be integrated to improve their efficacy on *M. incognita* as it was demonstrated with a combination of biofumigation and solarization.
5. A proposed integrated pest management (IPM) strategy for *M. incognita* on bambara groundnut was developed targeting small-scale farmers, and using the Botswana situation as an example.

GEÏNTEGREERDE PLAAGEBEHEER VAN *MELOIDOGYNE INCOGNITA* OP BAMBARA GRONDBONE (*VIGNA SUBERRANEA*)

deur

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SAMEVATTING

In hierdie studie is nie-chemiese beheer geëvalueer as komponente van geïntegreerde plaagbeheer van *Meloidogyne incognita* ras 2 op bambara grondbone. Die hoof-bevindinge was die volgende:

1. *Meloidogyne incognita* ras 2 is geïdentifiseer as die spesie wat bambara grondbone in Botswana aanval. Geen bestandheid of toleransie is in enige van bambara grondboonlyne wat in die glashuis geëvalueer is gevind nie. Daar was egter enkele grensgevalle in die Suid-Afrikaanse lyne wat 'n mate van toleransie getoon het.

2. *M. incognita* ras 2 populasies is verlaag met toediening van pluimvee- en kraalmis. Pluimveemis toegedien teen 0.8 kg/m^2 (8 ton/ha) was net so effektief as die nematosied fenamiphos toegedien teen 2 liter/ha. Beide toedienings was egter fitotoksies. Loofmassa is verhoog met 132 % en 270 % in plante wat behandel is met kraalmis teen 0.4 kg/m^2 (4 ton/ha) en 0.8 kg/m^2 (8 ton/ha) onderskeidelik, in vergelyking met die onbehandelde kontrole. Kraalmis teen 0.4 kg/m^2 was minder fitotoksies as pluimveemis teen dieselfde dosis. 'n Toedieningsdosis van 0.4 kg/m^2 is meer toepaslik gevind vir effektiewe nematode beheer.

3. Beheer van *M. incognita* ras 2 op bambara grondbone deur middel van bioberoking met *Brassica* spesies was effektief. Kool residue toegedien teen 'n dosis van 6 kg/m^2 was net so effektief soos aldicarb toediening. Kombinering van bioberoking en solarisasie in die veld het 66 % afname in *M. incognita* populasie tot gevolg gehad in vergelyking met 41 % afname waar bioberoking alleen toegepas is.

4. Nie-chemiese maatreëls kan geïntegreer word om hul effektiwiteit teen *M. incognita* te verbeter soos gedemonstreer met die kombinering van bioberoking en solarisasie.

5. 'n Voorgestelde geïntegreerde plaagbeheer-strategie vir *M. incognita* op bambara grondbone is ontwikkel, gemik op klein-boer sisteme met die Botswana situasie as voorbeeld.