The efficacy of abamectin in reducing plant-parasitic nematodes in cotton.

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Abstract

Cotton production is worldwide hampered by infection of various pests and diseases, including plant-parasitic nematodes (PPN). Root-knot nematodes (RKN), in particular *Meloidogyne incognita* race 4 is the predominant nematode species and race that adversely affects the production of cotton in South Africa and thus result in substantial yield losses. Management strategies that are frequently used to minimize yield losses in cotton locally are limited to a few registered nematicides and to a lesser extent, crop rotation. In addition, no resistant cotton cultivars are available that are adapted to local climatic conditions.

The main objective of this study was to evaluate the efficacy of abamectin against PPN, particularly *M. incognita* race 4, in greenhouse and field trials. The host suitability of four cultivars Delta OPAL®, Nu OPAL®, Nu OPAL RR® and Delta OPAL RR® that were used in the greenhouse trial were concurrently also evaluated against *M. incognita* race 4. To conduct this study, mature RKN females that were present in roots of tomato (cv. Rodade) and produced egg masses from which eggs and J2 (used as inoculum source for the greenhouse trial) were identified using Deoxyribonucleic Acid (DNA)-based techniques. The same procedure was followed for females that were present in roots of cotton cultivars that were planted in field trials.

With regard to the greenhouse trial, two treatments namely abamectin at a dosage rate of 0.15mg a.i./seed as well as a non-abamectin (untreated control) treatment were used. Approximately 2 500 *M. incognita* race 4 eggs and J2 were inoculated per cotton seed at planting for four cultivars (Delta OPAL®, Nu OPAL®, Nu OPAL RR® and Delta OPAL RR®) that were used. Nematode parameters *viz*. numbers of eggs and J2, egg masses and galls per root system as well as egg-laying female (ELF) indices and reproduction (Rf) values were obtained during five sampling intervals. These intervals represented the major growth stages of cotton plants, namely first true leaf, square, flower and boll development as well as when 50% of the bolls were opening. In addition, root mass (g) and biomass (g) data per cotton plant were also obtained. The trial layout was a randomized complete split-plot design including the two treatments, five sampling intervals and the four cultivars, which were replicated six times. Nematode and plant growth stage data were subjected to a factorial analysis of variance (ANOVA) with treatments as factor 1, sampling intervals as factor 2 and cultivars as factor 3. Means were separated by the Tukey Test and degrees of freedom (error) > 18 were always pursued. Nematode data for nematode parameters (dependent variables) were non-linearly regressed on the various sampling intervals (independent variable) using polynomial models

(Genstat for Windows), while plant data (dependent variable) were linearly regressed using the sampling intervals (the independent variable).

The RKN species and race that was used as inoculum for the greenhouse trial proved to be *M. incognita* race 4 using the specific sequenced characterized amplified region (SCAR)-polymerase chain reaction (PCR) method. Although the 0.15mg abamectin a.s./seed treatment resulted in a significant ($P \leq 0.05$) reduction in *M. incognita* race 4 population levels in roots of the four cultivars, these levels were still relatively high. Significant differences ($P \leq 0.05$) were also evident among the sampling intervals for both the abamectin and non-abamectin treatments with regard to all nematode and plant growth parameters. Further, the four cultivars were identified as susceptible hosts to this RKN species and race and generally had similar non-linear regression lines for the non-abamectin treatment in terms of *M. incognita* race 4-population development during the duration of the trial. These cultivars did, however, differ significantly ($P \leq 0.05$) from each other in terms of particularly the eggs and J2/root system only for the abamectin treatment when data were pooled for the five sampling intervals. Cultivar Nu OPAL® maintained significant ($P \leq 0.05$) higher egg and J2 numbers/root system than those maintained by the other three cultivars. This cultivar was, however, still classified as being highly susceptible (like the other three cultivars) to *M. incognita* race 4 using Rf values. For the latter as well as other nematode parameters, namely egg mass and gall numbers/root system as well as ELF indices significant ($P \leq 0.05$) differences were only evident between the two treatments and the five sampling intervals, but not for the cultivars. With regard to interaction data those that were significant ($P \leq 0.05$) between the two treatments and five sampling intervals for all the nematode and plant parameters, were regarded as the most important. This indicated that the treatments reacted differently during these intervals for all parameters measured. Since this trial was conducted in a greenhouse under controlled conditions, nematode and plant growth data obtained should be verified in field trials throughout the cotton-producing areas of South Africa under natural occurring environmental conditions. Only then can final conclusions be made in this regard.

For evaluation and verification of the efficacy of abamectin as a seed treatment in reducing PPN populations particularly *M. incognita* race 4, field trials were conducted at five sites where cotton was commercially grown during the 2005/2006 and 2006/2007 growing seasons. Four trials were conducted at three sites that are located in commercially-grown cotton fields in the Marble Hall area (Limpopo Province), while the other trial was done in the Vaalharts area near Jan Kempdorp (Northern Cape Province). For abamectin, two dosage treatments,

namely 0.15mg a.s./seed and 0.30mg a.s./seed were used in all field trials. Standard treatments included were the classical nematicides aldicarb and fenamiphos that are registered on cotton in South Africa. An untreated control as well as a thiamethoxam 0.3mg a.s./seed treatment were also included for the 2005/2006 trials. In addition to these treatments, a seventh treatment containing abamectin 0.15mg a.s./seed + thiamethoxam 0.3mg a.s./seed was included during the 2006/2007 season. Cotton seed used to plant trials during the 2005/2006 season were those for cultivar Nu OPAL®, while Nu OPAL RR® was used during the 2006/2007 season. Trial layouts for all trials constituted a randomized complete block design with nine and six replicates during the 2005/2006 and 2006/2007 growing seasons, respectively. Both root and soil samples were taken for nematode extraction, counts and identification purposes from the outer two rows of each plot at 42 as well as 84 days after planting (DAP), except when excessive rainfall occurred. Nematode and yield data for all trials were subjected to analyses of variance (ANOVA). For yield estimation, cotton lint was also harvested for all trials, weighed and subjected to ANOVAS.

Meloidogyne incognita race 4 has been identified as the predominant PPN species and race being present at all trial sites, while low population levels of individuals from the Hoplolaimidae, *Criconema* spp., *Pratylenchus* spp. and *Paratrichodorus* spp. were also present. The standard nematicide treatments aldicarb and fenamiphos generally resulted in the lowest number of *M. incognita* race 4 eggs and J2/root system in all trials and differed significantly from those for the untreated control treatments for three trials. The 0.15mg abamectin dosage treatment in particular did generally not differ significantly ($P \leq 0.05$) from the untreated control treatments nor from the standard nematicide and the thiamethoxam 0.3mg treatment as well as for the abamectin 0.15mg a.s./seed + thiamethoxam 0.3mg treatment during sampling interval one for two of the trials and during sampling intervals one and two for the other. Yield for the abamectin 0.15mg a.s./seed treatment was significantly ($P \leq 0.05$) higher than that of the untreated control only for Trial A. In terms of the cost-effectiveness, the estimated cost of the 0.15mg abamectin a.s./seed treatment was calculated to be substantially lower than those for the two standard nematicide treatments for the latter trial. This scenario poses a potential benefit for producers when this abamectin dosage will be used.

Although the 0.15mg abamectin dosage treatment showed potential to reduce population levels of *M. incognita* race 4 during this study, data varied between trials and seasons for the field trials. It must, however, be emphasised that since *M. incognita* race 4 populations in roots of abamectin-treated cotton plants were comparable to those for the

standard nematicides as well as those of the untreated controls, additional management strategies should be used in combination with the abamectin treatment. It further accentuates that abamectin should preferably be used only where population levels of *M. incognita* race 4 are not particularly high.

Uittreksel

Katoenproduksie word wêreldwyd deur verskeie peste en plae bedreig. Plantparasitiese aalwurm (PPA) is onder andere een van die ekonomies belangrike parasiete wat dié gewas aanval en daarop parasiteer. In terme van knopwortelaalwurms (KWA) is *M. incognita* ras 4 die predominante spesie en ras wat ernstige oesverliese in katoen plaaslik tot gevolg het. Die beheer van PPA in katoen in Suid-Afrika is hoofsaaklik beperk tot die gebruik van enkele geregistreerde klassieke aalwurmdoders. In ŉ mindere mate word wisselbou ook deur produsente toegepas. Daar is egter geen aalwurmweerstandbiedende katoenkultivar(s) beskikbaar wat vir lokale klimaatsomstandighede aangepas is nie. Dus is *Meloidogyne incognita* ras 4 eiers en tweede jeugstadium (J2) wat *in vivo* vermeerder is in ŉ glashuis in tamatiewortels (kultivar Rodade) en as inokulum gebruik in ŉ glashuisloodsproef tydens hierdie studie, deur middel van deoksieribonukleose (DNA)-gebaseerde tegnieke geïdentifiseer. Voorts is KWA wyfies wat teenwoordig was in katoenwortels wat vanaf persele van die vyf veldproewe verwyder is tydens die 2005/2006 en 2006/2007 groeiseisoene, ook geïdentifiseer deur van bogenoemde tegnieke gebruik te maak.

Die hoofdoelwit van hierdie studie was egter om die effektiwiteit van abamektien evalueer ten opsigte van die verlaging van PPA se bevolkingsvlakke, spesifiek KWA, in katoenwortels in beide glashuis- en veldproewe. Vier kultivars, nl. Delta OPAL®, Nu OPAL®, Nu OPAL RR® en Delta OPAL RR® is in die glashuisproef gebruik en is tegelykertyd vir hul gasheerstatus ten opsigte van *M. incognita* ras 4 geëvalueer.

In terme van die glashuisproef is twee behandelings, nl. ŉ abamektien (0.15mg aktiewe bestanddeel/saad) en ŉ nie-abamektien (onbehandelde kontrole), vyf monsternemingsintervalle en die vier bg. kultivars ingesluit. Die monsternemingsintervalle het die vyf prominente groeistadiums van ŉ katoenplant, nl. eerste ware blaar-, vrugknop-, blom- en bolvorming verteenwoordig asook wanneer 50% van die bolle oop was. Terselfdertyd is die bevolkingsontwikkeling van *M. incognita* ras 4 in wortels van die vier kultivars tydens elk van die vier monsternemingsintervalle ook gemonitor om sodoende ŉ aanduiding te kry van die gasheerstatus van elke kultivar t.o.v. hierdie parasiet. Elke katoensaad is tydens plant met ongeveer 2 500 *M. incognita* ras 4 eiers en J2 geïnokuleer. Aalwurmparameters wat gemonitor is het die aantal eiers en J2, eierpakkies en galle/wortelstelsel asook J2/200ml grond ingesluit. Voorts is die eierproduserende wyfie- (ELF) indekse asook reproduksie-(Rf) waardes ook bepaal. Bykomend tot die plantparameters wat gemonitor is tydens elke monsternemingsinterval, is wortel- en biomassa (g)/plant ook bepaal vir elke kultivar tydens elke

interval. Die proefuitleg was ’n ten volle gerandomiseerde split-proef blokontwerp en beide aalwurm- en plantparameterdata is aan ’n faktoriaal analise van variasie (ANOVA) onderwerp. Aalwurmparameters is voorts ook as die afhanklike veranderlikes teenoor die monsternemingsintervalle (onafhanklike veranderlike) onderwerp aan polinome, nie-lineêre regressies. Wortel- en biomassadata (afhanklike veranderlike) vir elke kultivar is egter aan liniêre regressies onderwerp deur dit te plot teenoor monsternemingsintervalle (afhanklike veranderlike).

Die knopwortelaalwurmspesie en ras wat as inokulum gebruik is vir die glashuisproef is geïdentifiseer as *M. incognita*. Aangesien rasse een en drie nie voorheen in Suid-Afrika op katoen aangeteken is nie, is dit aanvaar dat hierdie spesie ras vier is. Alhoewel die 0.15mg abamektien-behandeling ’n betekenisvolle ($P \leq 0.05$) verlaging in die bevolkingsvlakke van *M. incognita* ras 4 tot gevolg gehad het vir al die aalwurmparameters wanneer dit vergelyk is met die nie-abamektien behandeling, was getalle vir hierdie KWA spesie en ras steeds relatief hoog in wortels van al vier die kultivars. Betekenisvolle ($P \leq 0.05$) verskille is ook tussen die vyf monsternemingsintervalle verkry vir alle aalwurmparameters en was betekenisvol laer tydens interval een in vergelyking met die wat tydens die ander intervalle verkry is. Hierdie neiging is vir al die aalwurmparameters waargeneem. Voorts is die vier kultivars ook almal geïdentifiseer as hoogs vatbare gashere vir hierdie knopwortelaalwurmspesie en ras deur hoofsaaklik van Rf waardes gebruik te maak. Die vier kultivars het egter ook betekenisvol ($P \leq 0.05$) van mekaar verskil wat die abamektien behandeling betref met kultivar Nu OPAL® wat betekenisvol meer eiers en J2/wortelstelsel onderhou het as die ander drie kultivars. Nu OPAL® is egter steeds hoogs vatbaar vir hierdie spesie en ras, net soos die geval is met die ander drie kultivars. Wat interaksies betref, word dié wat tussen behandelings en monsternemingsintervalle teenwoordig was vir beide aalwurm- en plantparameters as die belangrikste geag. Dit dui dus daarop dat die twee behandelings verskillend gereageer het tydens die verskillende monsternemingsintervalle. Betekenisvolle interaksies tussen behandelings en/of intervalle en kultivars is egter nie in ag geneem nie aangesien die vier kultivars geneties van mekaar verskil. Dit is egter belangrik dat inligting, insluitend vir interaksies wat in hierdie proef verkry is, geverifieer word in veldproewe onder bestaande omgewingstoestande voordat gevolgtrekkings in verband hiermee gemaak word.

Wat die veldproewe betref, is twee abamektien dosisse nl. 0.15mg en 0.3mg aktiewe bestanddeel/saad ingesluit in vyf proewe wat in die katoenproduksiegebiede in die Mpumalanga en Noord Kaap Provinsies geleë is. Vier van hierdie proewe is tydens die 2005/2006 en twee

tydens die 2006/2007 groeiseisoen uitgevoer in kommersiële lande van produsente. Kultivar Nu OPAL® en kultivar Nu OPAL RR® is tydens die 2005/2006 en 2006/2007 seisoene onderskeidelik gebruik. Voorts is die geregistreerde klassieke aalwurmdoders aldikarb en fenamifos ingesluit aangesien geen aalwurmdodersaadbehandeling beskikbaar/geregistreer was op katoen tydens die uitvoering van hierdie studie nie. Voorts is 'n onbehandelde kontrole behandeling asook 'n tiametoksam 0.3mg aktiewe bestanddeel/saad behandeling tydens die 2005/2006 seisoen ingesluit, terwyl 'n sewende behandeling wat abamektien 0.15mg a.i./saad + tiametoksam 0.3mg a.i./saad bevat het ook tydens die 2006/2007 seisoen ingesluit is. Die proefontwerp vir al vyf proewe was ten volle gerandomiseerde blokontwerpe met onderskeidelik nege en ses herhalings tydens die 2005/2006 en 2006/2007 seisoene. Beide wortel- en grondmonsters is geneem vir aalwurmekstraksies tydens twee monsternemingsintervalle, nl. 42 en 84 dae na plant. Laas genoemde monsternemingstye het net afgewyk wanneer oorvloedige reën voorgekom het. Oesopbrengs is vir al vyf proewe bepaal deur die katoenlint te oes en te weeg. Aalwurm- en oesdata is onderwerp aan ANOVAS.

M. incognita ras 4 is as die predominante PPA en KWA geïdentifiseer vir al vyf lokaliteite waar die proewe uitgevoer is. Ander PPA wat in lae bevolkingsvlakke teenwoordig was, het individue van die Hoplolaimidae, *Criconema* spp., *Pratylenchus* spp. en *Paratrichodorus* spp. ingesluit. Die 0.15mg abamektien behandeling het oor die algemeen nie betekenisvol ($P \leq 0.05$) verskil van beide die standaard aalwurmdoders aldikarb en fenamifos, wat die laagste *M. incognita* ras 4 eiers en J2/20g wortels onderhou het tydens die 2005/2006 seisoen vir drie van die proewe tydens die eerste monsternemingsinterval nie. Dieselfde tendens is waargeneem tydens die 2006/2007 seisoen vir twee van die proewe tydens beide die eerste en tweede monsternemings intervalle. Hierdie behandeling het egter ook nie betekenisvol ($P \leq 0.05$) verskil van die onbehandelde kontrole en die tiametoksam 0.3mg behandeling tydens die 2005/2006 seisoen sowel as die abamektien 0.15mg + tiametoksam 0.3mg behandeling tydens die 2006/2007 seisoen nie. Oesopbrengs vir die 0.15mg abamektien behandeling was betekenisvol ($P \leq 0.05$) hoër in net een proef wat wel 'n aanduiding is dat hierdie dosis die potensiaal het om beide aalwurmgetalle te verlaag en oesverlies betekenisvol te beperk. Wat betref die koste-effektiwiteit van veral hierdie dosis is die geskatte koste geraam om substansieël laer te wees as die van die twee standaard aalwurmdoders wat tydens hierdie studie gebruik is.

Alhoewel veral die 0.15mg abamektien dosis behandeling potensiaal getoon het tydens hierdie studie om veral *M. incognita* ras 4 bevolkingsvlakke te verlaag, het die data gevarieer tussen proewe en seisoene. Dus behoort die gebruik van abamektien as 'n addisionele strategie

oorweeg te word, veral waar getalle van hierdie aalwurmspesie en ras nie uitermatig hoog is nie. Belowend egter is dat hierdie abamektien dosis vergelykbare data getoon het as dié vir die twee klasieke aalwurmdoders wat gebruik is tydens hierdie studie. Verdere navorsing behoort dus gedoen te word om meer verteenwoordigende data in hierdie verband te genereer deur meer proewe op meer lokaliteite in te sluit.

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Chapter 1: Introduction

1.1. General introduction

This study focussed on the potential efficacy of abamectin as a seed treatment in reducing plant-parasitic nematode (PPN) population levels, particularly *Meloidogyne* spp. (root-knot nematodes; RKN) in cotton (*Gossypium hirsutum* L.). The first part of the study was conducted to identify the RKN species used in this study using deoxyribonucleic acid (DNA)-based techniques. This was done since *M. incognita* race 4 is regarded as the predominant and economically most important PPN parasite associated with cotton in South Africa at present. In addition a greenhouse trial was conducted to investigate the efficacy of abamectin as a seed treatment under controlled conditions. In addition, the effect of abamectin on population development of *M. incognita* race 4 was tested on four local commercially available cotton cultivars in the same greenhouse trial. Finally the efficacy of various abamectin dosage treatments was investigated in reducing populations of PPN in cotton field trials where natural occurring environmental conditions prevailed. In terms of the cotton crop, a general background, its importance as an economically important fibre crop and its status regarding pests and diseases will briefly be discussed in the introductory chapter. Interaction of *M. incognita* race 4 with the cotton crop as well as with other organisms will also be accentuated. Finally, current perspectives and practices concerning the identification of RNK as well as the management of PPN in cotton that is particularly grown locally will be emphasized and elaborated on.

1.2. *Gossypium hirsutum*

1.2.1. Origin and history

The *Gossypium* genus comprises 49 species that are generally planted in tropical and subtropical zones of the world (Brubaker *et al.*, 1999; Starr *et al*., 2005). These countries include north-western Australia, north-eastern Africa, the Arabian Peninsula and western and northern Mexico. Four different *Gossypium* species were domesticated to use cotton as a fibre crop (Brubaker *et al*., 1999). In four isolated regions of the New World (Americas and Australia) as well as the Old World (Europe, Asia and Africa) aboriginal groups discovered that the coarse hairs that cover the seeds of *G. arboreum*, *G. barbadense*, *G. herbaceum*, and *G. hirsutum* were useful for several life sustaining purposes (Brubaker *et al*., 1999; Oosterhuis & Jernstedt, 1999). Four of the 49 known cotton species have been domesticated, with *G. herbaceum* and *G. barboreum* being classified as “Old World” diploids and *G.*

hirsutum and *G. barbadense* as “New World” tetraploids (Brubaker *et al*., 1999). As a result of the importance of cotton as a textile fibre, the latter four cotton species were disseminated beyond their original areas of discovery to practically every region of the world (Wendel & Cronn, 2003). Human interest in the production of cotton led to selection of superior plants in terms of agronomical characteristics for breeding efforts. This way adverse, small, perennial, cotton plants were genetically transformed using traditional breeding techniques and developed into compact, annual plants with high yields in terms of the strong white fibre product that are ultimately used for commercial purposes (Percival *et al.*, 1999; Wendel & Cronn, 2003). Although four cultivated species of cotton are of economic importance at present, *G. hirsutum* also known as upland cotton dominates global cotton production and constitutes almost 90% of the annual cotton crop that is currently grown worldwide (Sacks & Robinson, 2009). The latter species has been introduced from where it originated in Mesoamerica to over forty countries worldwide (Brubaker *et al*., 1999). Planting of long staple cotton (*G. barbadense*) comprises the other 10% of world production (Percival *et al*., 1999). Currently four domesticated cotton species namely, *G. hirsutum*, *G. barbadense*, *G. arboreum* and *G. herbaceum* are planted worldwide (Wendel & Cronn, 2003).

1.2.2. Classification

The *Gossypium* genus belongs to the family Malvaceae (Sacks & Robinson, 2009), is diverse and includes a variety of herbaceous perennials (Percival *et al.,* 1999). Cotton seed varies in terms of the fibres they contain, from almost nonexistent to short, stiff, dense brown hairs to long fine white fibres that are typical of highly improved cultivars (Brubaker *et al.*, 1999).

1.2.3. Anatomy

1.2.3.1. Seed anatomy and germination

The appearance of cotton seed is typically ovoid, slightly pointed at the one end and has a dark brown colour (Oosterhuis & Jernstedt, 1999). Cotton seed typically consists of a testa (seed coat), an embryo with two well developed cotyledons and remains of the endosperm. Before delinting (ginning), the outer layer of the seed coat consists of two types of fibres, namely long and short lint fibres. The embryo of a cotton seed typically consists of a radicle, a hypocotyl, two cotyledons and a poorly developed epicotyl. The two cotyledons, which are also referred to as seed leaves finally develop into the first two leaves of the cotton plant (Oosterhuis & Jernstedt, 1999). Initially these leaves contain stored nutritional substances, which provide the

energy for seed germination and early growth of a cotton seedling. Approximately 8 000 acid-delinted seeds generally constitute one kilogram in terms of mass (Van Iersel & Oosterhuis, 1996; Oosterhuis & Jernstedt, 1999), but differ among cultivars due to the size of the seeds.

Germination of cotton seeds generally commences when available soil water is absorbed through the chalaza (base of the immature seed) as well as through other parts of the seed coat directly after planting (Dong *et al*., 2004). Oxygen uptake occurs and respiration increases as the stored food reserves are used by the germinating cotton plant and applied for energy and the formation of new cells and tissues. During water absorption cotton seed swells and forms a split at the pointed micropylar end, and the radicle emerges through the micropyle within two to three days after planting. The radicle ultimately develops into the primary/tap root that grows downwards into the soil (Fig. 1.1). Soil temperatures reported to be favourable for germination of cotton seed is above 18 °C (Oosterhuis & Jernstedt, 1999; Dong *et al*., 2007; Sawan *et al*., 2009).

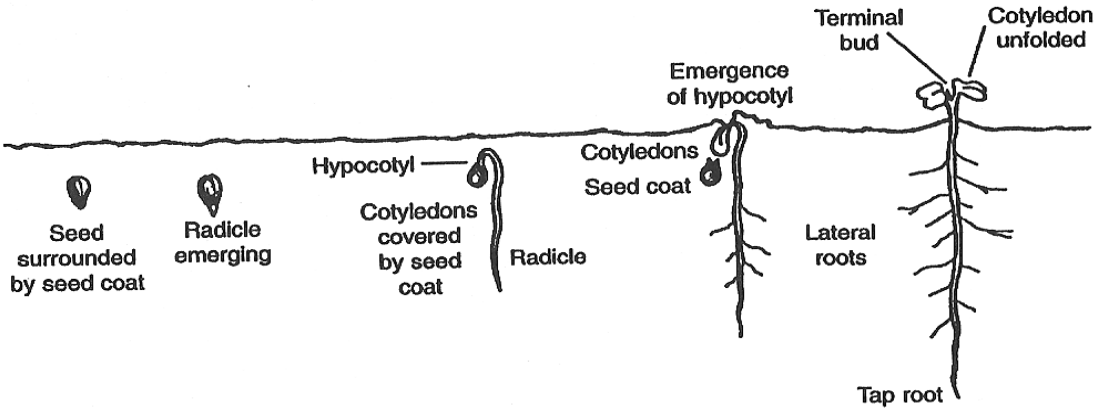


Figure 1.1. Germination and seedling development of cotton seed illustrating early development of the radicle, taproot and lateral roots (Illustration by Oosterhuis & Jernstedt, 1999).

1.2.3.2. Roots

Cotton root growth follows a typical sigmoidal curve and continues to grow and increase until maximum plant height is achieved, which is soon after flowering (Taylor & Klepper, 1974; Sawan *et al*., 2009). After flowering fruits (bolls) begin to form and as the boll load increases as carbohydrates, which acts as the terminal sink source for photosynthesis, are gradually translocated towards these fruits (Oosterhuis & Jernstedt, 1999). Cotton plants have a typical taproot with secondary lateral roots, which penetrates the soil rapidly and may reach a depth of 25cm at the stage that the cotyledons unfold (Oosterhuis & Jernstedt, 1999; Dong *et al.*, 2004). Depending on

soil conditions, a cotton plant with a height of 35cm may have a taproot of 100cm long (Oosterhuis & Jernstedt, 1999).

1.2.3.3. The shoot system

The shoot system of cotton plants consists of a main axis stem, leaves, buds, branches with floral buds, flowers and bolls developing as the plant develop to maturity (Oosterhuis & Jernstedt, 1999; Dong *et al.*, 2004). The protruding main stem results from the development of the shoot meristem and consists of a series of nodes and internodes where leaves are attached (Oosterhuis & Jernstedt, 1999; Dong *et al.*, 2004). The main stem has an erect, indeterminate monopodial growth habit (Oosterhuis & Jernstedt, 1999; Dong *et al.*, 2004). The number of nodes, length of the internodes and the number and location of branches is determined by genetics, environmental factors as well as cultural practices (Oosterhuis & Jernstedt, 1999; Dong *et al.*, 2004).

1.2.3.4. Reproductive structures

The flowering pattern of cotton plants is distinctive and predictable (Oosterhuis & Jernstedt, 1999; Dong *et al*., 2004). The first flowers, usually six or seven, are produced on the main stem nodes (Fig. 1.2) at the first flowering position along the fruiting branch (Oosterhuis & Jernstedt, 1999; Dong *et a*l., 2004). The time period between the openings of different flowers that are located at the same position on two different fruiting branches is usually three days, while it is six days for different flowers on the same fruiting branch (Oosterhuis & Jernstedt, 1999; Dong *et a*l., 2004). The flowering order on main stem nodes is spirally upward and outward and flowers continue to be formed until defoliation or frost occurs (Oosterhuis & Jernstedt, 1999; Dong *et a*l., 2004). Reproductive growth of cotton plants starts approximately four to five weeks after planting and appears as floral buds, referred to as squares (Oosterhuis & Jernstedt, 1999; Dong *et a*l., 2004). The time period between the appearance of the first squares and formation of white flowers (anthesis) is approximately 25 days (Oosterhuis & Jernstedt, 1999; Dong *et al*., 2004). Pollination occurs shortly after anthesis, which result in the development of bolls (Oosterhuis & Jernstedt, 1999; Hanan & Hearn, 2002; Hofs *et al*., 2006).

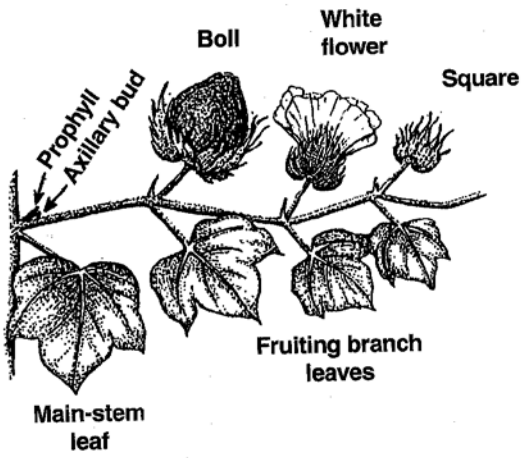


Figure 1.2. Cotton squares, a flower, and a boll that develops on a sympodial branch (Illustration by Oosterhuis & Jernstedt, 1999).

1.2.4. Economic and social importance

Cotton is grown in the tropical, subtropical and temperate regions of about 65 countries worldwide (Singh *et al.*, 2006). Hectares planted to cotton and yield of 10 countries that are regarded as the major producers of cotton are presented in Table 1.1. The latter information for South Africa is also listed in this table. Cotton is one of the crops that produces of the highest quantities of dry matter per amount of water applied during the growth period (Van Biljon, 2006: personal communication) and is the most important fibre crop grown globally (Starr *et al*., 2005). During 2005 the area under cotton production worldwide was 33.5 million hectares, with 46 million tonnes of cotton lint being produced (United States Department of Agriculture, 2009). From 2001 to 2003 cotton yield per production unit averaged 1 702kg/ha, ranging from less than 500kg/ha in some African countries to 4 317kg/ha in Israel. The world's biggest cotton producer, the Peoples Republic of China, delivered an average of 3 436kg/ha during 2005 (Oerke, 2006). Cotton production, from the raw fibre to the finished textile product, is a multibillion-dollar enterprise (May & Lege, 1999). Due to high costs and limited availability of labour in many parts of the world, technologies have been incorporated to stabilise and improve cotton production (Smith, 1999).

Cotton production peaked in the USA during 1926 with 18 million hectares planted to the crop (Koenning *et al*., 2004). The introduction and availability of improved, mechanised farming equipment, the cost-effective use of fertilizers, pesticides as well as improved cultivars facilitated higher yields per unit of land, which lead to a substantial reduction in hectares being planted with cotton (Koenning *et al*., 2004). Although the cotton industry in South Africa is relatively small when compared to a crop such as maize or potato, it employs approximately 20 000 people. In terms of production, approximately 30 000ha is planted to the cotton crop annually in a wide

range of localities that are situated within the cotton-producing areas of South Africa (Bruwer, 2009: personal communication; Fig. 1.3).

Table 1.1. Hectares planted to cotton, total cotton production (tonnes) for the 10 largest cotton-producing countries as well as relevant information for South Africa in this regard for the 2005 season (United States Department of Agriculture, 2009).

Country	Area planted (ha)	Total produce (t)
India	8 850 000	5 984 432
United States	5 545 000	6 415 966
Peoples Republic of China	5 060 000	12 522 106
Pakistan	3 150 000	3 337 748
Republic of Uzbekistan	1 450 000	1 501 877
Brazil	850 000	2 173 128
Burkina	630 000	374 925
Turkey	600 000	1 890 955
Mali	580 000	334 647
United Republic of Tanzania	510 000	175 706
Republic of South Africa	30 000	61 616

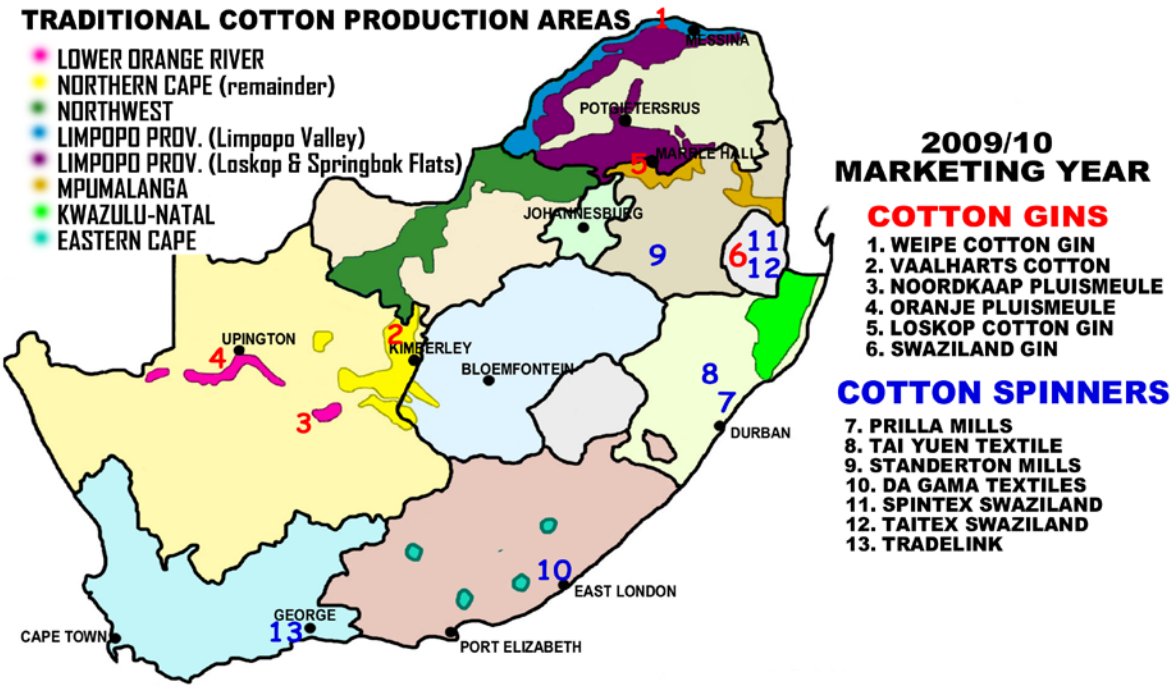


Figure 1.3. A graphic representation of the areas where cotton production areas are situated in South Africa as well as an indication of where cotton gins and spinners are located within these areas (Illustration by Bruwer, 2009: personal communication).

1.2.5. Agronomy

Important management options or decisions by cotton producers include cultivar choice, optimum but cost-effective application of fertilisers as well as insect and disease control and defoliation and timing of harvesting (Smith & Cothren, 1999). Planting dates for cotton in South Africa is from mid October to mid November, with harvesting normally being done from April to July (Van Biljon, 2006: personal communication). An important factor that determines planting time for the cotton crop is soil temperature, since cotton should not be planted before the soil temperature in the top 3cm layer has reached 16 °C to 18 °C for 10 consecutive days (Prinsloo, 2004). Producers might experience several production constraints such as hail, drought as well as insect and disease damage during the growing season (Robinson, 1999). In addition, specific water requirements for cotton production are fundamental. Optimal rainfall during the early-growing season is preferable since it favours vegetative growth of the crop (Taylor & Klepper, 1974). Occurrence of a successive dry midsummer in which bolls are formed from flowers is generally optimal for crop development. Cotton that is produced under irrigation usually produces higher yields than those grown under rain fed conditions (Fig. 1.4), (Van Iersel & Oosterhuis, 1996). Optimal temperatures for development of cotton plants are between 25 °C and 30 °C, with ample sunshine and 1 800 day degrees (Prinsloo, 2004; Van Biljon, 2006: personal communication).

The cotton crop is harvested either with a spindle picker or a stripper harvester before seed cotton is delivered to the gin where the seed and lint are separated. Cotton seeds are intended for food, seed and crushing industries that produce a variety of food products. Cotton fibres on the other hand are use as lint, which are twisted into wool threads of ranging sizes and is then processed into various textile materials (Smith & Cothren, 1999). Oil contained within cotton seed is extracted and purified to remove gossypol, which is a chemical that is toxic to humans and monogastric animals (Scheffler & Romano, 2008; Anonymous, 2008). Recent advances that favour cotton production includes the development of herbicide– and insect-resistant cultivars, the use of plant growth regulators, improved processing equipment and many other refining technologies (Smith & Cothren, 1999; Singh *et al.*, 2006; Dong *et al.*, 2007; Sacks & Robinson, 2009).

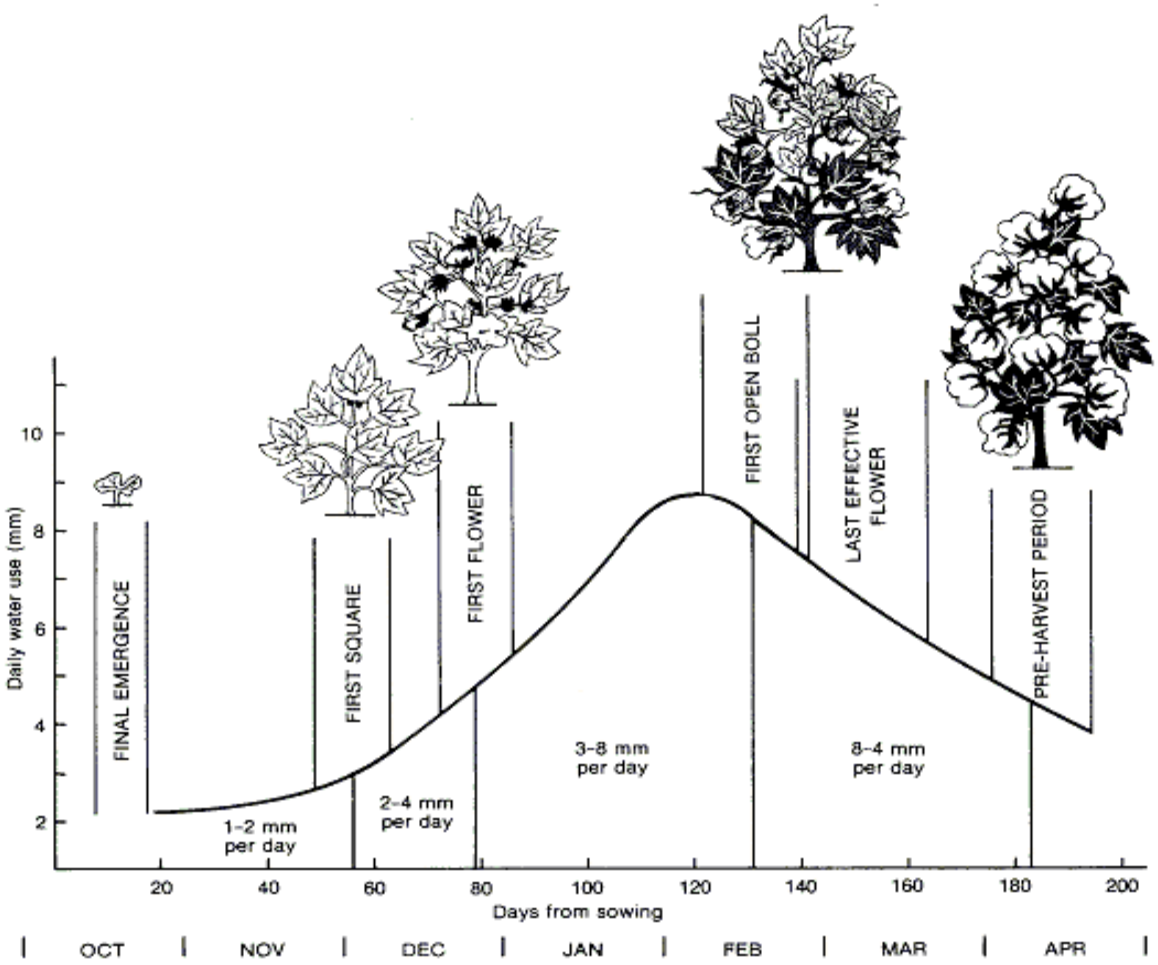


Figure 1.4. A graphic illustration of the development, growth period and water consumption of cotton grown in the southern hemisphere (Abdulmumin & Misari, 1990).

1.2.6. Diseases and pests

A range of pests and diseases limit cotton production wherever the crop is grown globally (Faske & Starr, 2006; Monfort *et al*., 2006a; Starr *et al*., 2007). Yield losses resulting from the latter constraints are in most cases difficult to assess and are probably underestimated (Bell, 1999). The principle reason for the inaccurate estimation of damage inflicted by pests and diseases is generally because of the nature of these diseases (Walker *et al*., 1998). Seedling-, wilt root rots and damage caused by PPN constitute the majority of all cotton yield losses and are caused by soil-borne pathogens that primarily attack the cotton roots and subsequently interrupt normal root function and development (Walker *et al*., 1998). Seedling diseases of cotton is generally caused by various *Pythium*, *Rhizoctonia* and *Fusarium* species, while wilt disease is mainly caused by the fungus *Verticillium dahliae*, which enters the root system and multiplies within the vascular tissue (Bell, 1999). Other diseases of cotton include fungal leaf spots and bacterial blights, but these are of minor importance in comparison to root diseases described above (Prinsloo, 2004).

1.2.6.1. Economically important PPN associated with cotton

The presence of a taproot in cotton plants is regarded as a reason why cotton is adversely affected due to parasitism by PPN (Starr *et al*., 2005). The latter scenario is aggravated when cotton plants experience stress conditions such as drought (Starr *et al*., 2005). In addition, cotton is frequently grown in monoculture and therefore facilitates the build-up of PPN in such soils (Van Biljon, 2004; Starr *et al.*, 2007). Management of plant-parasitic nematodes is expensive and complicated, while damage to successive crops is generally experienced in cotton-based cropping systems (Van Biljon, 2004). Furthermore, the rapid development of new cultivars that exhibit transgenic insect- and herbicide resistance has led to the use of cultivars of which limited data on nematode tolerance and/or resistance is available (Koenning *et al.*, 2003). The increase in estimated yield losses in cotton as a result of PPN infection can ultimately be ascribed to several contributing factors, namely i) the lack of resistant cultivars, ii) limited crop rotation practices/sequences, iii) the increased awareness of PPN being an important production constraint and iv) the banning/withdrawal of effective soil fumigants from the world market (Koenning *et al.*, 2004). Further, the lack of standard nematicide use together with practice of cotton monoculture has created conditions where the cumulative effect of seedling diseases as well as infection by PPN, as a result of interaction, synergistically increases crop damage (Monfort *et al.*, 2006a).

Globally the economically most important PPN that infect and cause damage to cotton include RKN, particularly *M. incognita* races 3 and 4 and *Rotylenchulus reniformis* (Robinson, 2008). One or both these latter species are usually present in soils in most cotton-producing areas and cause serious problems in cotton production (Robinson, 1999). Economically important PPN that infect cotton in South Africa are *M. incognita* race 4, *Pratylenchus* spp. (lesion nematodes) and *Paratrichodorus* spp. (stubby root nematodes). Other species that are associated with cotton worldwide are *Belonolaimus longicaudatus*, *Hoplolaimus columbus*, *P. brachyurus* and *M. acronea*. Of lesser importance in terms of damage caused to cotton growth and yield are infection by *Hoplolaimus aegypti*, *H. galeatus*, *H. indicus*, *H. seinhorsti*, *Longidorus* spp., *Paratrichodorus* spp., *R. parvus*, *Scutellonema* spp. and *Xiphinema* spp (Robinson, 2008).

Aboveground symptoms of nematode damage are not easily distinguished by producers, chemical representatives and other role players due to non-typical or the absence of symptoms and are generally ascribed to the presence of the semi woody

nature of the cotton plant (Starr *et al.*, 2005). Nematodes are known to aggregate in clusters (Robinson, 1999) and therefore occur in patches in cotton fields as is indicated by visibly damaged/stunted plants in Fig. 1.5A.

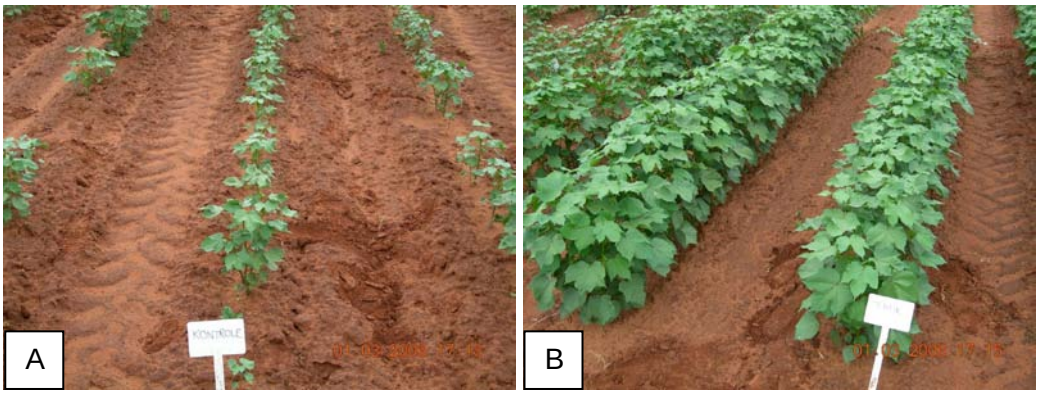


Figure 1.5. Cotton seedlings in an untreated control plot (A) indicating a typical patchy and stunted appearance due to infection by *Meloidogyne incognita* race 4 compared to visually healthy plants in a plot treated with the synthetic nematicide Temik® (B) in a field trial that was conducted in the Marble Hall area during the 2006/2007 growing season.

RKN-infested cotton plants may be discoloured or yellowish, stunted and wilted even when the available soil moisture content is optimal for plant growth and development (Bridge & Starr, 2007). In severe cases of RKN infestation the plant population of cotton seedlings may be affected severely due to the inability of the plants to germinate and develop properly (Van Biljon, 2004). An estimated average annual yield loss of 10.7% has been reported for cotton due to damage caused by PPN worldwide (Sasser & Freckman, 1987), while 10.2% was reported for South Africa (Keetch, 1989). For the USA a loss of 5 million tonnes of raw cotton lint has been experienced during the 2005 production season, with a calculated value of $2 billion US (Sasser & Freckman, 1987),

1.3. A general perspective on PPN

PPN are increasingly recognised as economically important parasites of cotton globally (Starr *et al.*, 2007). Nematodes that attack cotton are classified as root feeders and belong to both the ecto- and endoparasitic trophic groups (Decraemer & Hunt, 2006; Starr *et al*., 2005; Khan, 2008). Crop damage caused by PPN can manifest either as a reduction in yield, quality or both. RKN are the economically most important and predominant genus that infect a variety of crops, including cotton, worldwide (Thomas & Kirkpatrick, 2001; Starr *et al*., 2005; Bridge & Starr, 2007).

Annual losses caused by PPN on life sustaining crops such as grains, legumes, banana, cassava, coconut, potato, sugarbeet, sugarcane and sweet potato are estimated to be approximately 11% globally. Losses for most other economically important, life-sustaining crops such as a wide range of vegetables and fruit crops are estimated at approximately 14% (Agrios, 1997).

1.3.1. Morphology and biology

Although controversy exists in terms of the classification of PPN (Kleynhans *et al.*, 1996; Subbotin & Moens, 2006), these parasites are categorised in three orders within the phylum Nemata, namely Tylenchida, Triplonchida and Dorylaimida (Kleynhans *et al*., 1996).

Most nematodes live freely in fresh or salt water or in the soil and feed on other microorganisms (free-living), or plants (plant-parasitic), (Agrios, 1997; Kleynhans *et al*., 1996). Numerous nematode species on the other hand attack and parasitize humans and animals of which insects are also included (Agrios, 1997).

PPN obtain their food by injecting their feeding apparatus or stylets into plant cells of both above- and/or below-ground parts of host plants (Agrios, 1997; Decraemer & Hunt, 2006; Khan, 2008; Bridge & Starr, 2007). This group of parasites are thus classified as obligate biotrophic organisms that obtain nutrients only from cytoplasm of living plant cells (Decraemer & Hunt, 2006). These nematodes are small, eel-shaped, unsegmented roundworms and are usually invisible to the naked eye (Decraemer & Hunt, 2006). PPN can reach lengths ranging from 300–1 000µm, although some species may be up to 4 000µm long and 15µm to 35µm wide (Agrios, 1997). The nematode, both parasitic and non-parasitic body, lacks legs or other appendages and is mostly wormlike (Kleynhans *et al*., 1996). Sexual dimorphism occurs where females of some nematode species such as RKN and cyst (*Heterodera* and *Globodera* spp.) become swollen during their development towards maturity and are characterised by saccate-like bodies (Agrios, 1997; Decraemer & Hunt, 2006; Khan, 2008).

Non-parasitic or free-living nematodes on the other hand feed on viruses, bacteria, fungi, crustaceans, insects, mites and other nematodes (Kleynhans *et al.*, 1996). Non-parasitic nematodes do not possess a stylet, except for some genera of the Dorylaimida that use the latter to feed on other micro-organisms and other nematodes (Kleynhans *et al*., 1996).

1.3.1.1. Parasitic feeding strategies of plant-parasitic nematodes (PPN): Trophic groups

In terms of habitat and parasitism, PPN are either classified as ecto- or endoparasites (Decraemer & Hunt, 2006). Both of these categories are further subdivided into migratory or sedentary parasites (Agrios, 1997; Decraemer & Hunt, 2006; Khan, 2008).

Ectoparasitic nematodes remain in the soil, with only the anterior parts of their bodies attached to the plant tissue during feeding (Decraemer & Hunt, 2006). The anterior part of the bodies of ectoparasitic nematodes does not enter the root tissue during feeding (Decraemer & Hunt, 2006). The majority of ectoparasitic nematode species are migratory and move from one feeding site of the host plant to another (Hunt *et al.*, 2005; Decraemer & Hunt, 2006). The stylet that punctures plant cells differ among various PPN genera (Decraemer & Hunt, 2006). The longer the stylet, the deeper the penetration and feeding site inflicted by the particular nematode genus/species (Decraemer & Hunt, 2006; Hunt *et al*., 2005). Ectoparasites that have long stylets usually insert the feeding apparatus deep into root/tuber tissues of the host plant (Decraemer & Hunt, 2006; Hunt *et al*., 2005). These latter parasites generally penetrate the roots/tubers of a host plant at or near the growing tip. These ectoparasites are relatively immobile and feed at one specific site for a number of days (Bridge & Starr, 2007). Ectoparasites with short stylets mainly feed on the outer cells and root hairs of plant tissue of the host and include genera such as *Tylenchorynchus*, *Trichodorus, Paratrichodorus* and some *Helicotylenchus* species, which remains relatively mobile during infection and feeding (Decraemer & Hunt, 2006).

Endoparasitic nematode species on the other hand enter tissue of the host plant with their bodies being entirely enclosed within the root/tuber/pod tissue (Decraemer & Hunt, 2006). Endoparasitic species are classified as either migratory, which refer to their movement within the tissue of the host plant or they could be sedentary (Decraemer & Hunt, 2006). The latter PPN penetrate tissue of the host plant and become entirely enclosed within the host. In addition, such sedentary nematodes become sessile at their feeding sites and induce specialized feeding plant cells, referred to as giant cells in the case of RKN and syncytia in the case of cyst nematodes (Decraemer & Hunt, 2006). Migratory endoparasitic nematodes include *Pratylenchus* spp., *Radopholus* spp., *Hirschmaniella* spp., and others (Agrios, 1997; Bridge & Starr, 2007; Decraemer & Hunt, 2006). Sedentary nematode endoparasites

include genera such as *Meloidogyne*, *Heterodera*, *Globodera*, *Rotylenchulus*, *Tylenchulus* and others (Agrios, 1997; Bridge & Starr, 2007; Decraemer & Hunt, 2006; Perry & Moens, 2006). Semi-endoparasitic nematode genera on the other hand include *Scutellonema*, *Hoplolaimus*, *Rotylenchus* and others (Decraemer & Hunt, 2006). Feeding strategies of the latter trophic group of PPN are characterised by only the anterior part of the body being inserted within the host's tissue, while the posterior part remains in the soil (Hunt *et al*., 2005; Decraemer & Hunt, 2006).

1.3.2. Root-knot nematodes (RKN)

The most important nematode pest of cotton is the RKN *M. incognita* and in particular races 3 and 4 (Bridge & Starr, 2007). RKN are contained in the genus *Meloidogyne* Göldi, 1892, which refers to the apple-shaped form of the bodies of females and comprise a small but important polyphagous group of highly specialized obligate plant pathogens (Karssen & Moens, 2006; Moens *et al.,* 2009). In the subfamily Meloidogyninae several species are reported as being economical important parasites of a wide range of agricultural and horticultural crops, particularly in tropical, subtropical and warm temperate regions (Agrios, 1997; Luc *et al*., 2005; Moens *et al.,* 2009). The four RKN genera *M. javanica*, *M. incognita*, *M. hapla* and *M. arenaria* are known to be the most damaging nematode parasites of crop plants (Luc *et al*., 2005; Starr *et al*., 2005; Moens *et al.,* 2009). The latter species are distributed worldwide in soils and reduce crop quality and yield, especially in root and tuber crops where damage symptoms are directly inflicted on plant parts (Kleynhans *et al*., 1996; Karssen & Moens, 2006; Moens *et al.,* 2009). The cosmetic value of such RKN-infected tubers that are marketed for consumption are of great economic importance since the adverse effect of these parasites on tuber quality result in downgrading of such a food commodity and subsequently a lowered price for such a consignment (Kleynhans *et al*., 1996; Karssen & Moens, 2006).

1.3.2.1. Root-knot nematode (RKN) species associated with cotton in South Africa

The RKN genus that is of economical concern to local cotton production in terms of damage caused to the crop is *M. incognita* race 4 (Kleynhans *et al*., 1996; Keetch & Heyns, 1982). However, eggs and J2 of *M. arenaria*, *M. hapla* and *M. javanica* may be present in soils where cotton is grown in local production areas (Kleynhans *et al*., 1996; Keetch & Heyns, 1982). The same scenario applies for *M. acronea* (Van Biljon, 2004) which has been found as a major production constraint of cotton in the lower Shire valley of Malawi (Bridge *et al.*, 1976). *M. acronea* has not been reported to

cause a reduction in cotton yield in South Africa to date (Van Biljon, 2006: personal communication).

Since *M. incognita*, particularly and exclusively race 4, is regarded as the predominant PPN parasite of cotton locally, basic information on this species will be accentuated in the next part of this chapter.

1.3.3. *Meloidogyne incognita*

1.3.3.1. Biology, life cycle and population dynamics

M. incognita has like the majority of other PPN four juvenile stages between the egg and the adult (Shepherd & Huck, 1989; Robinson, 2008; Moens *et al*., 2009). After embryogenesis, the first moult occurs within the egg, the second-stage juvenile (J2) leaves the egg and moves through the soil in search of a plant host (Agrios, 1997; Moens *et al.,* 2009). The J2, which represent the only infective stage of *Meloidogyne* species, hatch from the eggs and penetrate cotton roots at the area of root elongation just behind the root cap (Faske & Starr, 2007). J2 are also attracted to apical meristems, which represents regions where lateral roots originate (Creech *et al.*, 1995; Karssen & Moens, 2006; Moens *et al*., 2009). After penetration J2 is reported to migrate intra- and intercellularly through the cortex towards the vascular cylinder where xylem and phloem tissues differentiate (Creech *et al.*, 1995; Karssen & Moens, 2006; Moens *et al*., 2009). Developing vascular or nearby parenchyma cells of the host plant are pierced with the stylet of a *M. incognita* J2 into which oesophageal gland secretions are injected. The formation of specialized feeding sites, known as giant cells, is subsequently induced (Sijmons *et al*., 1994; Karssen & Moens, 2006; Moens *et al*., 2009). These enlarged multinucleate giant cells become permanent feeding sites for the rest of the life cycle of the RKN (Karssen & Moens, 2006; Moens *et al*., 2009). RKN juveniles complete three additional molts within the root before they develop into adults (Sijmons *et al.*, 1994; Moens *et al*., 2009). J3 develop into J4, which ultimately develop into swollen, pear-shaped females (Sijmons *et al.*, 1994; Moens *et al*., 2009). Individuals of J3 and J4 developing stages do not possess a stylet and do not feed within the host tissue (Sijmons *et al.*, 1994; Moens *et al*., 2009). During the fourth and final molt, male nematodes may develop due to adverse conditions that include non-optimal temperature, insufficient food sources and others. RKN males migrate out of the root of a host plant without feeding (Creech *et al.*, 1995; Moens *et al*., 2009).

Reproduction in *M. incognita* occurs without fertilization and is referred to as obligatory mitotic parthenogenesis (Agrios, 1997; Karssen & Moens, 2006; Moens *et al*., 2009). Development of J1 within the eggs begins immediately after deposition of the eggs and J2s emerge within 14 days to continue the onset of the next generation (Shepherd & Huck, 1989). Soil temperature is the most important factor influencing egg development of *M. incognita* and the duration of the life cycle after hatching occurred is not dependent on a stimulus from plant roots (Robinson & Perry, 2006). Root diffusates may, however, stimulate hatching of RKN eggs (Karssen & Moens, 2006). At a temperature range of 25 °C to 30 °C, *M. incognita* females are able to complete one life cycle within 20 days (Shepherd & Huck, 1989). Each adult *M. incognita* female can produce 500 to 1 000 eggs that are enclosed in a gelatinous egg matrix (Karssen & Moens, 2006). The latter is deposited either on the surface, or sometimes inside the visibly galled roots/tubers of the host plant (Karssen & Moens, 2006). The life cycle of *M. incognita* and *M. acronea* is similar to that of other *Meloidogyne* species, with the only difference being that *M. incognita* reproduces by means of parthenogenesis only, while *M. acronea* reproduces almost always by means of amphimixis (Starr *et al.*, 2005). The latter is defined as cross fertilization, which is also known as sexual reproduction that takes place after male and female RKN mated (Evans, 1998; Hunt *et al*., 2005; Moens *et al*., 2009).

Limited information is available in terms of population development of *M. incognita* in roots of cotton cultivars. For local cultivars Van Biljon and Bleve (2005) reported that *M. incognita* race 4 J2 infected roots of commercially-available cultivars Delta OPAL®, Tetra®, Acala OR3® and Gamka®. In addition a decrease in root mass and plant height was observed early in the growth stage, namely at formation of the first true leaves. Reduction in root mass of the four cultivars ranged from 13% to 63%, while a 28% to 68% reduction was recorded at first square formation and appearance of the first flowers for the various cultivars (Van Biljon & Bleve, 2005). In terms of plant height, a reduction ranging between 17% and 37% were recorded during square formation, while first flower formation was reduced between 8% and 51% for the different cultivars. A decline in *M. incognita* female numbers was also recorded during first flower formation for all the cultivars except Tetra® (Van Biljon & Bleve, 2005). *M. incognita* race 4 showed a non-linear polynomial growth response in roots of Delta OPAL® and Gamka®, while the growth response for this parasite in roots of Acala OR3® and Tetra® were not representative of polynomial models (Van Biljon & Bleve, 2005).

1.3.3.2. Geographical distribution and host range of root-knot nematodes (RKN)

RKN are economically important parasites that attack and infect a wide range of crops that are produced in a diverse range of environmental conditions throughout the world (Starr *et al*., 2007). Although more than 70 *Meloidogyne* species has been described, only two are known to be pathogenic to cotton, namely *M. incognita* and *M. acronea* (Starr *et al*., 2005). *M. incognita* was first identified from cotton roots in 1889 in the Southern United States, but is distributed throughout all cotton producing areas of the world (Koenning *et al.*, 2004). In contrast to *M. incognita*, *M. acronea* has only been identified in cotton producing areas of Malawi and South Africa, where it is of much lesser importance than *M. incognita* and other PPN that are associated with cotton (Page & Bridge, 1994; Van Biljon, 2006: personal communication). Kirkpatrick and Sasser (1983) reported that *M. incognita* races 1 and 2 did not reproduce well on either resistant or susceptible cotton cultivars. Some root galling and reproduction did occur when populations of these host races infected cotton, indicating the possibility that genetic selection enable them to parasitize cotton (Kirkpatrick & Sasser, 1983). Although the latter authors reported race 3 to reproduce in cotton roots, population increases of race 4 were significantly higher than that of race 3 in the USA. Locally, *M. incognita* race 4 is also regarded as the most pathogenic and predominant PPN in cotton production areas, with race 3 not been found in soils in these areas to date (Van Biljon, 2009: personal communication).

1.3.3.3. Damage symptoms and yield losses

Distribution of *M. incognita* race 4 in cotton fields is in accordance with the universal phenomenon that nematode distribution in fields is uneven and patchy (Bridge & Starr, 2007; Khan, 2008). Yield losses in infested areas due to parasitism by this species may range between 75% and 100%, while other parts of cotton fields may show no typical or visible aboveground symptoms of nematode damage (Fig. 1.6), (Robinson, 1999; Bridge & Starr, 2007). Most damage caused by this RKN species and race is generally visible on crop plants that experience water stress and are usually present in high population levels in those parts of the field that contain soils with higher sandy contents (Robinson, 1999). Cotton plants that are severely infected by *M. incognita* race 4, are usually stunted, appear to be nitrogen deficient (e.g. yellow leaves) and may wilt when compared to uninfected or non-infected plants (Starr *et al*., 2005). Early in the cropping season when low to moderate population levels of *M. incognita* race 4-infection are present in cotton plants, root galls caused by feeding of these parasites are often indefinite and may not be clearly visible to the

naked eye (Koenning *et al*., 2004). Under such conditions galls are less than twice the diameter of non-infected cotton roots and are generally easier to be seen and identified on lateral roots (Starr *et al.*, 2005). During development of the cotton plant to maturity and the build-up of *M. incognita* race 4 numbers increases, root galls caused by these parasites increase in size and numbers (Starr *et al.*, 2005; Koenning *et al*., 2004). Galls on cotton roots caused by *M. incognita* race 4 are characteristically smaller than those inflicted this species in tomato and okra roots (Robinson, 1999). Infection by this species also causes tap- and lateral roots of the cotton plant to branch prematurely or to be aborted, with terminal galls being formed due to feeding of these parasites (Fig. 1.7), (Shepherd & Huck, 1989). *M. incognita* race 4-infected cotton root systems generally lack fine feeder roots and normally reach only half the length compared to healthy roots of cotton plants (Fig. 1.8). Females of *M. incognita* race 4 are generally fully embedded inside root tissue of cotton plants where they compete for nutrients that are vital for the plant to photosynthesise (Robinson, 1999).

Figure 1.6. A root system of a cotton plant that is not infected with *Meloidogyne incognita* race 4 root-knot nematode (RKN) individuals and indicates a typical taproot and adequate lateral feeder roots.

Figure 1.7. A cotton root system infected with *Meloidogyne incognita* race 4, resulting in typical gall formation (encircled area) that is particularly visible on the tip of lateral roots.

Figure 1.8. A cotton root system infected by *Meloidogyne incognita* race 4 individuals with typical root galling symptoms (encircled area) being visible.

Apart from only forming characteristic galls on cotton roots, *M. incognita* races 3 and 4 are known to be involved with several other diseases that form disease complexes

in this crop (Starr *et al*., 2005). Although lesions formed by penetrating *M. incognita* race 3 or 4 J2 were originally considered to be the only route of entry for fungal pathogens, recent research revealed that modifications of the host substrate plays a more important role in such interactions (Perry & Moens, 2006).

Bacterial infections in a range of crop plants, including cotton, are aggravated by RKN infection that cause wounds on roots of the host and feed inside it by means of specialized feeding strategies (Karssen & Moens, 2006). Walker *et al.* (1998) reported that increased seedling mortality and suppression of early seedling growth were more adversely affected when the bacteria *Thielaviopsis basicola* as well as *M. incognita* race 3 or 4 individuals were both present than when either one of these pathogens occurred singly within the roots of cotton. The primary effect of *T. basicola* is reported to inhibit early seedling survival and development, with concomitant infection by *M. incognita* race 3 or 4 also reported to adversely affect crop development during the rest of the growing season (Walker *et al.*, 1998). This scenario is illustrated by the occurrence of the number of sympodial branches that contains two bolls, percentage of bolls in the second fruiting position, days to first cracked boll occurrence as well as cotton yield (Walker *et al.*, 1998). Early plantings of cotton were more prone to *T. basicola* infection than cotton that was planted later during the season (Walker *et al.,* 1998). Both yield and timely harvest of cotton is affected by *T. basicola* and *M. incognita* race 3 or 4 co-infection (Walker *et al*., 1998). Plants infected with *M. incognita* race 3 or 4 seldom die, but concomitant infection with *Fusarium oxysporum* f. sp. *vasinfectum* generally result in the death of cotton plants (Fig. 1.9), (Robinson, 1999; Van Biljon, 2004).

In terms of *M. acronea* and its effect on cotton, Starr *et al*. (2005) reported that limited information is available for this parasite-host relationship. Symptoms induced by *M. acronea* on cotton roots are different from those caused by *M. incognita* race 3 or 4 (Page & Bridge, 1994; Starr *et al.,* 2005). Fewer root galls have been observed in *M. acronea*-infected cotton roots, with females of this species also often not being fully embedded within roots as opposed to those of *M. incognita* race 4. Root elongation is also generally terminated after infection by *M. acronea*, resulting in a flush of new growth of lateral roots (Page & Bridge, 1994). These familiar and typical symptoms caused by *M. acronea* infection in cotton is referred to as the “turned-aside” manifestation, which is due to an initial interruption of growth in the taproot followed by an aggressive, increased formation of lateral roots (Starr *et al.*, 2005).

Host-parasite interactions that include *M. acronea* and fungal diseases have not been reported to date (Bridge *et al*., 1976; Robinson, 2008).

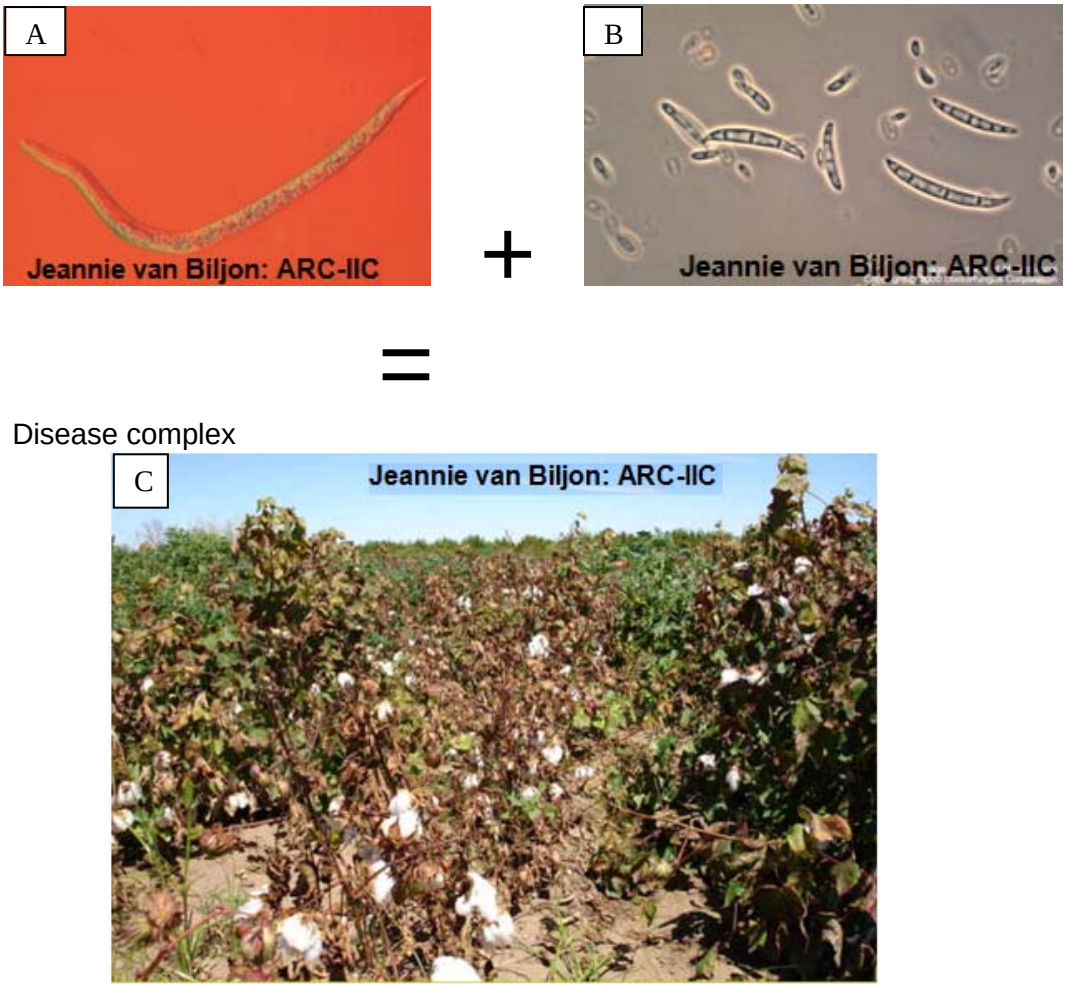


Figure 1.9. An illustration of the synergistic effect (C) of a concomitant infection by *Meloidogyne incognita* race 4 (A) and *Fusarium oxysporum* f. sp. *vasinfectum* (B) on cotton in a trial that was conducted during the 2004/2005 growing season in the Northern Cape, Loxtonvale area of South Africa (Van Biljon, 2004).

1.3.4. *Pratylenchus* species

Lesion nematodes are one of the PPN genera that have the broadest host range worldwide (Duncan & Moens, 2006). *Pratylenchus* that is associated with cotton globally are *P. brachyurus*, *P. neglectus*, *P. penetrans*, *P. scribneri*, *P. thornei*, *P. vulnus* and *P. zeae* (Kleynhans *et al*., 1996; Luc *et al*., 2005). In South Africa

Pratylenchus spp. is seldom reported as economically important parasites of cotton, but *P. brachyurus* en *P. teres* have been associated with this crop and should not be disregarded in terms of the pathogenic effect they may have on this crop (Van Biljon, 2009: personal communication). *Pratylenchus teres* (Khan & Singh, 1974; Carta *et al*., 2002) was first reported to parasitize cotton in the Jan Kempdorp area (Northern Cape Province) of South Africa during the 1995/1996 growing season.

1.3.5. *Paratrichodorus* species

Stubby root nematodes, namely *Paratrichodorus* spp., have also been reported to infect the cotton crop in certain areas within the local cotton producing areas, namely Northern Cape, Mpumalanga and Limpopo (Kleynhans *et al*., 1996; Luc *et al*., 2005; Van Biljon, 2006: personal communication; Moens *et al*., 2009). In South Africa high population levels of particularly *P. lobatus* and *P. minor* have been found in soil samples from localities in the Mpumalanga and Northern Cape Provinces (Kleynhans *et al*., 1996; Van Biljon, 2006: personal communication). Damage symptoms caused by infection of *Paratrichodorus* spp. individuals usually include poor cotton growth, wilting of developing plants as well as a stubby appearance of root systems (Kleynhans *et al*., 1996; Luc *et al*., 2005; Van Biljon, 2006: personal communication; Moens *et al*., 2009). Locally serious crop damage to cotton has seldom been reported due to parasitism by *Paratrichodorus* spp. (Van Biljon, 2006: personal communication).

1.3.6. *Rotylenchulus reniformis*

Two species of the *Rotylenchulus*, namely *R*. *parvus* and *R. reniformis* are regarded as parasites of cotton in all the major cotton producing areas in the US, India, Pakistan Egypt and Brazil (Robinson, 2008). Only *R. parvus* has been reported in local cotton producing areas to date (Van Biljon, 2009: personal communication). *R. reniformis* is regarded as the predominant species that causes more severe crop damage opposed to that caused by *R. parvus*, with only a few studies having been done to confirm the parasitic association between *R. parvus* and cotton (Starr *et al.*, 2005). Aboveground symptoms caused by *R. reniformis* in cotton are relatively ordinary and generally representative of those caused by the other PPN that infect and parasitize the crop (Koenning *et al*., 2004), namely stunted growth and chlorosis (Starr *et al*., 2005; Robinson, 2008). Conversely root symptoms caused by these parasites result in a subnormal developed of root systems with a dirty appearance, which are caused by soil particles that adhere to egg masses produced by individuals

of this PPN genus. This is particularly observed where high infestation levels of *R. reniformis* occur (Starr *et al*., 2005).

1.4. Molecular identification of plant-parasitic nematodes (PPN), particularly root-knot nematodes (RKN)

Techniques to facilitate the timely detection and correct identification of economically important PPN species are fundamental before research on nematode management can be conducted (Zijlstra *et al*., 2000; Donn *et al*., 2007; Blok & Powers, 2009). The use of molecular techniques to identify PPN are widely used, are becoming more popular and are used as a diagnostic tool on a routine basis in laboratories across the world (Luc *et al*., 2005; Perry & Moens, 2006; Khan, 2008; Blok & Powers, 2009). It is particularly popular since identification of PPN based on morphological characteristics requires highly experienced taxonomists and is frequently inconclusive due to significant variation within a PPN population (Jepson, 1987; Blok *et al.*, 1997; Zijlstra *et al.*, 2000; Otsen *et al*., 2001; Vuu *et al*., 2004; Luc *et al*., 2005; Perry & Moens, 2006; Donn *et al*., 2007; Khan, 2008; Blok & Powers, 2009).

Substantial progress has been made over the past 40 years by using systematics resulting from identification of nematodes using molecular techniques to explain and expand taxonomical knowledge about PPN (Benton & Myers, 1966; Dickson *et al.*, 1970; Evans, 1971; Hussey *et al.*, 1972; Starr, 1979; Ebenshade & Triantaphyllou, 1985; Blaxter, 2003; Luc *et al.*, 2005; Perry & Moens, 2006; Khan, 2008; Blok & Powers, 2009). Since the middle 1900's, electrophoresis was used to identify particularly RKN by means of their proteins (antibodies) and isozymes (Benton & Myers, 1966; Dickson *et al.*, 1970; Evans, 1971; Hussey *et al.*, 1972; Starr, 1979; Ebenshade & Triantaphyllou, 1985; Blaxter, 2003; Blok & Powers, 2009). The disadvantages of this technique are, however, that RKN females of a certain developmental stage(s) is needed (Ebenshade & Triantaphyllou, 1990; Blok & Powers, 2009) and that proteins only counts a minor fraction of the total nematode genome (McLain *et al.*, 1987; Da Rocha Fragoso *et al*., 2009). The nature of the proteins expressed depends on the life stage and vary between the life stages of RKN (Sasser *et al.*, 1978; McLain *et al*., 1987).

Improved DNA-based diagnostics were subsequently developed later during the 1980's and held attractive solutions to the limitations experienced when using electrophoresis identification of RKN (Blok & Powers, 2009). Such improved techniques do not rely on the expressed outcome of the RKN genome, are

independent of environmental influences and life stage of the nematode and are potentially very selective (Zijlstra *et al.*, 2000; Da Rocha Fragoso *et al*., 2009). As a result, several studies have been performed where polymorphisms of DNA banding patterns between and within RKN species have been investigated since the late 1980's until recently (Curran *et al.*, 1985 & 1986; Powers *et al.*, 1986; Garate *et al.*, 1991; Castagnone-Sereno *et al.*, 1991 & 1993; Cenis *et al.*, 1992; Piotte *et al.*, 1992; Xue *et al.*, 1992 Fargette *et al.*, 1996; Blaxter, 2003; Da Rocha Fragoso *et al*., 2009). Since restriction fragment length polymorphism (RFLP) techniques require relatively large amounts of DNA, the latest studies have used polymerase chain reaction (PCR-based) techniques that require minimal amounts of nematode DNA (Otsen *et al*., 2001). The exploration of a PCR-based method that use multicopy DNA sequences as target DNA, namely mitochondrial DNA (mtDNA), initially became popular from pioneering work that has been done by Harris *et al.* (1990) as well as Powers and Harris (1993) when they managed to discriminate between three *Meloidogyne* species using these techniques. Their results were later confirmed by Donn *et al*. (2007) when these authors reported that terminal restriction fragment length polymorphism (T-RFLP) based on a single enzyme digest was sufficient to discriminate between nematode communities of which individuals were extracted from different habitats. Since the PCR reaction has to be followed by a restriction enzyme digestion step that delay the identification process (Zijlstra *et al.*, 2000; Blok & Powers, 2009), random amplified polymorphic DNA (RAPD) fingerprinting was investigated and used as a rapid technique to distinguish successfully between *M. arenaria*, *M. incognita* and *M. javanica* (Cenis, 1993; Boum *et al.*, 1994; Blok *et al.*, 1997; Blok & Powers, 2009).

Improvement beyond RAPD fingerprinting of nematodes included the more powerful, quick and accurate sequenced characterized amplified region marker (SCAR) technique (Zijlstra *et al.*, 2000; Blok & Powers, 2009). A set of SCAR-primers direct the amplification of a single fragment with a specific size from the target DNA and since SCAR-primers are longer than RAPD-primers, higher annealing temperatures can be used (Zijlstra *et al.*, 2000; Blok & Powers, 2009). The desired SCAR-fragments can, therefore, be easily detected in PCR reactions and are independent of the amount and source of nematode DNA used (Zijlstra *et al.*, 2000; Blok & Powers, 2009). This method subsequently opened the possibility of controlling RKN effectively (Zijlstra *et al.*, 2000; Fourie *et al*., 2001; Hooper *et al*., 2005). Molecular identification of RKN species occurring in South Africa has also been done (Fourie *et al.*, 1999 & 2000), with *M. fallax* that is regarded as a quarantine organism in Europe

(Karssen, 1996; Zijlstra, 2000) being identified locally for the first time using the SCAR-PCR technique (Fourie *et al.*, 2000). Since the discovery of molecular techniques the identification of particularly RKN has become essential in nematode research, advisory and quarantine services and regulatory programmes worldwide (Hooper *et al*., 2005; Blok & Powers, 2009). Therefore, molecular techniques have also been used in this study to validate the presence of RKN that occur in soils where cotton trials were planted.

1.5. Management of plant-parasitic (PPN) nematodes in cotton

PPN that annually cause crop yield losses where no control measures are applied is considered a key pest (Karssen & Moens, 2006). *Meloidogyne* spp. frequently achieves this status since it is the economically most important nematode pest that parasitizes cotton (Karssen & Moens, 2006). As a result management strategies need to be developed, considering the habitat and behaviour (e.g. life cycle, temperature requirements, trophic group) of these parasites (Karssen & Moens, 2006; Bridge & Starr, 2007).

The management of PPN are generally aimed at preventing the economically most important species from reaching high population densities that cause reduction in crop yield and quality, followed by financial losses (Ferraz & Brown, 2002). Several strategies can be applied to manage PPN such as i) application of nematicides, ii) use of cultural practices and iii) use of host plant resistance (Koenning *et al*., 2004). The integration of the abovementioned strategies, where economically feasible, would favour the management of PPN to prevent severe damage, yield and quality losses (Jorgenson, 1978). Strategies that are used by most producers are largely dictated by the economics of cotton production rather than the ability to control PPN and therefore result in many of the effective control methods not being exploited optimally (Koenning *et al*., 2004). Future successes in terms of protecting cotton from PPN damage lies in the development of new sources of host plant resistance along with more effective and environmentally-safe nematicides (Starr *et al*., 2007).

1.5.1. Cultural practices and host plant resistance

While only a limited number of high-yielding cotton cultivars with resistance to particularly RKN are available and crop rotation is generally not an economically viable option in cotton cropping systems, both cultural practices and host plant resistance is not widely adopted by producers across the globe (Koenning *et al*., 2007). This is particularly the case in South Africa (Van Biljon, 2004). Germplasm

with genetic resistance to *M. incognita* race 4 had been identified and was incorporated into cotton breeding lines. A nematode-tolerant line was selected and quality characteristics were monitored at different localities (Botha-Greeff & van Biljon, 1999). Since planting of genetically-modified cotton cultivars by local producers steadily increased and their market share expanded, development of nematode-tolerant or –resistant lines was terminated.

1.5.2. Crop rotation

Crop rotation is one of the most used cultural practices to prevent nematode populations to reach damage threshold levels but requires the availability of sufficient suitable land for production of alternative crops that are either non- or poor hosts to the relevant nematode species (Nyczepir & Thomas, 2009). For crop rotation to be successful, the use of alternative crops in such a system must provide an adequate income to the grower and increase the yield of the following cotton crop to justify removing land from the cotton growing programme (Koenning *et al*., 2004). A prerequisite for the selection of a crop to be included in a cotton-based cropping system is that the nematode species present in the applicable field is known (Koenning *et al*., 2004; Coyne *et al*., 2009). The presence of only one PPN species is seldom experienced in a field, which complicates the crop selection process. Furthermore, alternative crops that are economically justifiable rarely exist and growers do not always possess the mechanical infrastructure to facilitate the planting of such crops. The use of crop production as a successful nematode-control strategy has thus seldom been documented globally and in particular in South Africa (Koenning *et al*., 2004; Van Biljon, 2004).

1.5.3. Chemical control

Although many strategies have been developed to manage PPN in cropping systems, the use of classical nematicides has been the major method used during the past 50 years to minimise damage in cotton production (Halbrendt & Lamondia, 2003). Nematicides that are most commonly used in cotton production worldwide are granular carbamates and organophosphates, which are both classified as non-fumigants (Bridge & Starr, 2007; Nel *et al*., 2007). In addition the fumigant nematicide 1, 3-dichloropropene is also used regularly in cotton production to control nematodes (Bridge & Starr, 2007; Nel *et al*., 2007).

1.5.3.1. Non-fumigant nematicides

These products are formulated as either liquid or granular compounds (Rich *et al*., 2003). According to their chemical composition, active ingredients can be categorised as carbamates such as aldicarb and/or oxamyl, organophosphates such as ethoprophos and/or fenamiphos and abamectin which constitutes of the avermectins (Rich *et al.*, 2003; Cochran, 2006: personal communication). Non-fumigant nematicides are exposed to accelerated microbial breakdown by soil micro-organisms, particularly in fields where it is used repeatedly over consecutive growing seasons (McLean & Lawrence, 2003). Subsequently, the efficacy of these products is non-optimal and limits management of PPN (McLean & Lawrence, 2003).

Carbamate and organophosphate compounds

Carbamate and organophosphate compounds are described as acetyl cholinesterase inhibitors in terms of their mode of action, which is known to be nemastatic rather than nematicidal (Rich *et al.*, 2003). Nematode control is thus achieved since hatching of J2 from eggs is delayed and penetration of J2 into host roots is inhibited (Hough & Thomason, 1975; Hough *et al*., 1975; Perry & Moens, 2006; Moens *et al.*, 2009). In addition, disruption of feeding of J2 and females as well as disorientation of males in terms of locating females for copulation occurs as a result of application of such products (Hough & Thomason, 1975; Hough *et al*., 1975; Perry & Moens, 2006; Moens *et al.*, 2009). As a result of their nemastatic effect, lower dosages are applied as a cost-effective strategy, resulting in the fact that these non-fumigants subsequently rarely kill PPN and reducing their numbers (Sikora *et al.*, 2005). PPN may also recover if they are no longer in contact with non-fumigant nematicides and become infective again (Rich *et al*., 2003). Non-fumigants thus need to be in contact with nematodes for a specific period of time to enable them to significantly reduce population levels of these parasites and this way contribute to healthy root growth after germination of the crop (Rich *et al*., 2003).

Non-fumigants that are used commonly by cotton producers including aldicarb, aldicarb plus oxamyl or fenamiphos have, however, not been reported to reduce final numbers of *R. reniformis* at harvest (Koenning *et a*l., 2007). The most widely used non-fumigant on cotton is aldicarb (Temik®) that is registered to be applied at a rate of 0.8kg to 1.2kg a.i./ha in furrows at planting (Nel *et al*., 2007). Another less common preferred strategy is application of a side dressing of aldicarb during the first 50 days after planting of cotton, which serves as a supplemental strategy to the initial in-furrow application at planting (Lawrence & McLean, 2000 & 2002). Nematicide

applications that are normally recommended for use on cotton in the USA are similar for *R. reniformis*, *M. incognita* and *H. columbus* and are only recommended when yield losses are expected to exceed 5% (Robinson, 1999). Registered dosage rates for aldicarb on cotton in the USA ranges from 0.7kg to 4.6kg a.i./ha at planting in the furrow, with two potential side dresses of 2.4kg a.i./ha during the formation of squares and flowering, respectively. Rates of aldicarb vary significantly between states in the USA (Anonymous, 2009).

In South Africa aldicarb is registered on cotton at 1.05kg to 1.5kg a.i./ha and fenamiphos at 1.5kg a.i./ha (Nel *et al*, 2007). This current registration does, therefore, not provide for any other applications than in-furrow application at planting (Nel *et al*., 2007). Except for aldicarb and fenamiphos, 1, 3-dichloropropene (Telone®) is currently the only other nematicides that is registered for use on cotton in South Africa (Nel *et al*., 2007). The future availability and use of particularly aldicarb is under serious threat due to the misuse of the product and its high toxicity levels (Class I nematicide) (Verdoorn, 2009: personal communication). Banning of fenamiphos from world markets is also expected since it is also classified as a Class I nematicide (Pienaar, 2008: personal communication).

1.5.3.2. Fumigant nematicides

Fumigant nematicides are highly effective in reducing PPN in soil (Starr et al., 2007; Koenning *et al*., 2004). The practical application thereof are limited due to difficulties experienced during its application, high cost of these products and concerns about their effect on human and animal health and the environment (Starr *et al*., 2007; Moens *et al*., 2009). Rich & Kinloch (2001) reported that soil treatment with the fumigant 1, 3-dichloropropene (Telone®) increased cotton yield in the USA from 349kg/ha to 504 kg/ha in *M. incognita* race 4-infested soil when applied at 16kg, 32kg and 48kg a.i./ha. The latter treatments also resulted in significantly lower post-harvest numbers of *M. incognita* race 4 (12 J2/100cm^3 soil) compared to untreated control plots (460 J2/100cm^3 soil). Thomas and Smith (1993) later reported a 20% to 50% increase in yield when susceptible cotton cultivars were planted on *M. incognita* race 4-infested soils after application of 1, 3-dichloropropene at a dosage rate of 56 ℓ/ha.

The use of soil fumigants such as 1, 3-dichloropropene or metam-sodium to reduce PPN population levels in soils where cotton is produced is currently the second most adopted strategy in the USA (Lawrence & McLean, 2000 & 2002; Koenning *et al*.,

2007). The dosage rate most frequently recommended for application of 1, 3-dichloropropene in the USA is 28 l/ha at 10 to 14 days before planting of cotton at a soil temperature range between 16 °C and 25 °C (Koenning *et al*., 2004). Such a treatment is usually followed by a lower, but registered rate of aldicarb at planting (Robinson, 1999; Koenning *et al.*, 2004). In South Africa, the control method that is used most by producers is the single in-furrow application of aldicarb at planting at a dosage rate of 1.5kg a.i./ha (Bredell, 2009: personal communication).

1.5.5.3. Avermectins

Evaluation of abamectin, which constitute a product that is derived from biologically-active avermectins, is the focus of this study and therefore its effect in reducing PPN will be accentuated below opposed to those of other nematicidal compounds that are applied on cotton.

Avermectins comprise a family of 16 macrocyclic lactones that are produced by the soil-inhabiting micro organism *Streptomyces avermitilis* (Faske & Starr, 2006). Abamectin is an 80:20 mixture of avermectin B1a and avermectin B1b and is formed during fermentation of these micro organisms (Maienfisch, 2007: personal communication). In terms of the mode of action of this group of chemicals, Fischer and Mrozik (1992) reported that avermectins effects nervous signal transmission by reducing the input resistance of muscle fibres of nematodes. Furthermore, avermectins results in opening of the chloride channels and also adversely affects the major inhibitory neurotransmitters in the central nervous system of nematodes gamma-aminobutyric acid-like (GABA) neurotransmitters (Maienfisch, 2007: personal communication).

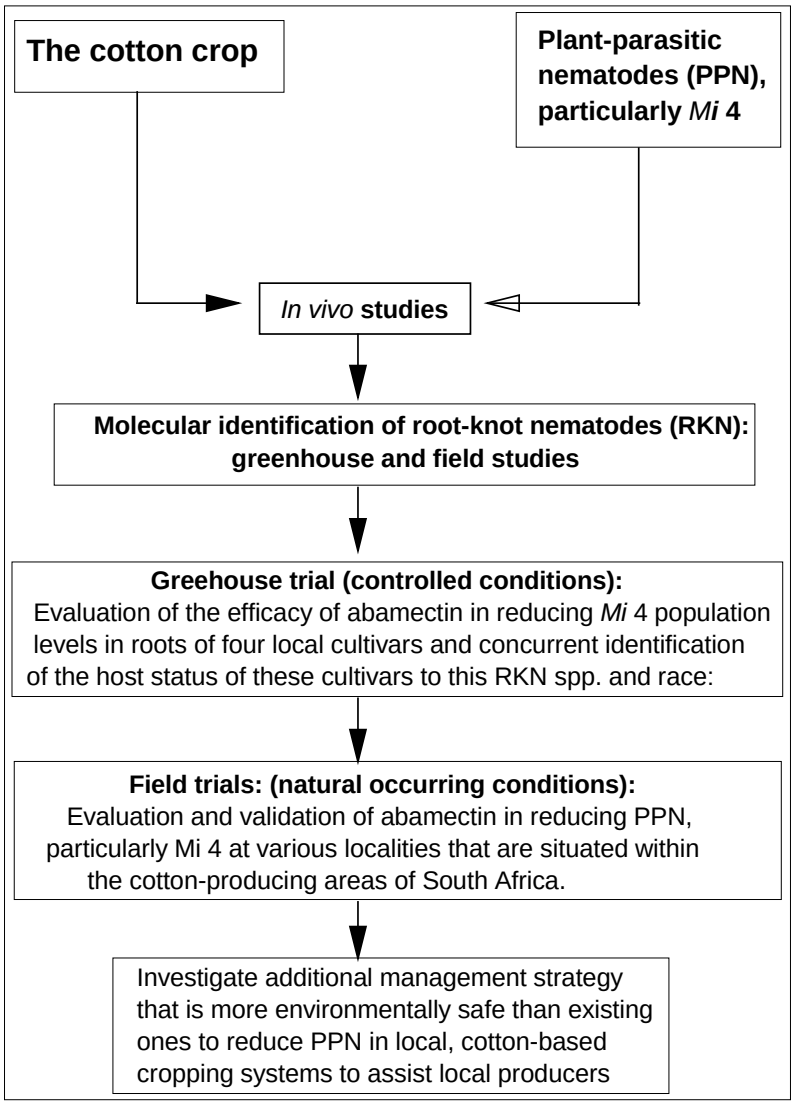
Neurophysiological research showed that avermectins acts at GABA-mediated synapses in both arthropods and nematodes (Wang & Pong, 1982; Mellin *et al*., 1983). These results were supported by the observations that avermectins limits nematode movement following the addition of GABA antagonists (Wang & Pong, 1982; Mellin *et al*., 1983). In general the response of *M. incognita* J2 to avermectin is described as triphasic (Wright *et al*., 1984). These three stages involve i) initial loss of locomotor activity where the J2 remain sensitive to contact by avermectins, ii) a recovery phase and iii) a final loss of activity where the J2 are relatively insensitive to contact by avermectins (Wright *et al*., 1983). The latter process is in contrast with the acetylcholinesterase inhibitor-effect caused by oxamyl, which initially results in hyperactivity of J2, followed by a gradual decline in movement of such juveniles (Wright *et al.*, 1983 & 1984).

In terms of its application, direct soil applications of abamectin proved to be more effective in reducing *M. arenaria* J2 penetrating tomato roots in greenhouse trials than foliar applications (Cayrol *et al*., 1993). However, abamectin is only recommended to be used on sandy or soils that contain low levels of organic materials due to its rapid decomposition in soils with high organic contents (Cayrol *et al*., 1993). The main disadvantage of abamectin is its low solubility in water and its rapid decomposition in particularly soils with high organic contents (Putter *et al.*, 1981; Garabedian & Van Gundy, 1982). Application of abamectin as a seed treatment is regarded as an attractive approach to reduce PPN in cotton due to its convenience and the relative low risk it poses in terms of its toxicity to humans, animals and the environment (Monfort *et al.*, 2006b). Commercialisation of abamectin as a nematicide has, however, been delayed during the late 1980's because of its limited foliar translocation, high affinity to attach to soil particles and rapid decompositions in soil and sunlight (Putter *et al*., 1981). At present abamectin is registered for use as a seed coat treatment on cotton and maize in the USA (Anonymous, 2007), Brazil and South America. In Europe, abamectin is also registered on canola as a seed coat treatment, while research on tomato and other vegetables are in progress (Dreyer, 2009: personal communication). Locally, abamectin has been evaluated on various crops such as cotton, maize, pumpkin and soybean since 2005 (Fourie & McDonald, 2005; 2006 & 2007). Registration of this seed coat product on maize is expected to be finalised during 2010 (Dreyer, 2009: personal communication). Investigations in terms of the potential of abamectin to reduce PPN are increasing worldwide since use of this product may offer a valuable contribution in terms of protecting crops against these parasites, particularly RKN (Coyne *et al*., 2009).

1.6. Rationale and aims of present study

PPN species that parasitize cotton occur wherever the crop is grown (Robinson, 1999; Starr *et al.*, 2005). In South Africa, cotton production is often not economical sustainable due the damaging effect of PPN since the cost of nematicides is relatively high (Van Biljon, 2006: personal communication). The importance of PPN as yield-limiting pathogens of cotton has recently created increased awareness in both the scientific and commercial sectors (Starr *et al*., 2007). Since cotton is mostly grown in monoculture systems in South Africa, a progressive build-up of PPN species threatens sustainable production of the crop. The efficacy and potential use of alternative, less toxic nematicides in cotton warrants investigation in order to protect the crop in a cost-effective way against infection by these parasites.

Therefore, the objectives (Fig. 1.10) of this study were to i) identify the RKN species that were used as inoculum as well as those that are present at field sites where trials were planted by using molecular techniques, ii) establish the efficacy of abamectin-treated cotton seed in reducing *M. incognita* race 4 in a pilot greenhouse study and concurrently iii) establish population growth curves for this RKN species and race for cotton cultivars in a greenhouse trial and iv) evaluate the efficacy of abamectin as a seed treatment in reducing PPN populations, particularly *M. incognita* race 4, in cotton roots in field trials where natural occurring environmental conditions prevailed.



Mi 4 = *Meloidogyne incognita* race 4.

Figure 1.10. A schematic representation to illustrate the key activities that were conducted during this study and the proposed outcome of this research.

Chapter 2: Molecular identification of root-knot nematode (RKN; *Meloidogyne*) species using deoxyribonucleic acid (DNA)-based techniques

2.1. Introduction

Methods to enable the timely detection and accurate identification of economically important PPN species, particularly RKN, are crucial before proper research on nematode resistance and management studies can be conducted (Piotte *et al*., 1992; Zijlstra *et al*., 2000; Blok & Powers, 2009). Until recently, available identification methods for RKN were generally based on morphological characters of mature females (Jepson, 1987), which required highly skilled taxonomists (Blok *et al*., 1997; Zijlstra *et al*., 2000; Blok & Powers, 2009). Identification of RKN species using the latter approach was often inconclusive due to conservative morphology, the presence considerable variation within populations of these parasites due to indistinct species boundaries or species complexes (Blok *et al*., 1997; Zijlstra *et al*., 2000; Blok & Powers, 2009). Therefore, molecular methods to identify *Meloidogyne* spp. have become more popular over the last decade and are currently generally done on a routine basis in laboratories worldwide (Blok *et al*., 1997; Zijlstra *et al*., 2000; Blok & Powers, 2009).

DNA-based diagnostics provide attractive and alternative solutions for reliable identification of RKN (Ziljstra *et al*., 2000; Blok & Powers, 2009) and have been used successfully in the past by several researchers (Ziljstra *et al*., 2000; Castagnone-Sereno *et al*., 1991 &1993; Cenis *et al*., 1992; Curran *et al*., 1985 & 1986; Fargette *et al*., 1996; Fourie *et al*., 2001; Garate *et al*., 1991; Piotte *et al*., 1992; Powers *et al*., 1986; Xue *et al*., 1992). The latter methods generally include isozymes, antibodies and DNA-based techniques (Blok & Powers, 2009). The increased popularity and benefit of using molecular-based identification techniques are that they do not rely on the expressed products of the nematode genome, are independent of environmental influences as well as the developmental stage of the nematode and are potentially extremely discriminating and accurate (McLain *et al*., 1987; Zijlstra *et al*., 2000; Blok & Powers, 2009). Therefore, the objective of this study was to extract DNA from mature RKN species females that were obtained from i) *in vivo* mass reared roots of tomato (cv. Rodade) that were used as inoculum for a greenhouse trial as well as those from ii) cotton roots that were obtained from the five different field sites where field trials were conducted during the 2005/2006 and 2006/2007 growing seasons and subject it to sequence characterised amplified regions (SCAR-PCR) assays.

2.2. Materials and methods

2.2.1. Deoxyribonucleic acid (DNA) extraction from root-knot nematode (RKN) females

For identification purposes, ten RKN females were randomly removed with a forceps from roots of the tomato cv. Rodade in which *M. incognita* race 4 populations were mass reared in a greenhouse at the Agricultural Research Council's (ARC) Grain Crops Institute (GCI) at Potchefstroom. A stereomicroscope was used to conduct this task. Although eggs and J2 from this *in vivo*-reared culture were used to inoculate the greenhouse trial in which the efficacy of an abamectin 15mg a.s/seed dosage treatment was evaluated for its potential to reduce populations of these parasites in roots of four local commercially-available cotton cultivars (Chapter 3), it also served as the *M. incognita* reference population. In addition, RKN females were also removed from roots of cotton plants 42 to 56 days after planting (DAP) from five field trials in which the effect of abamectin dosage treatments against these parasites were investigated under natural occurring environmental conditions in cotton-producing areas of South Africa (Chapter 4).

Ten mature females were also obtained from an *in vivo*-reared *M. javanica* population that are mass cultured in roots of the tomato cv. Rodade in a separate greenhouse at the ARC-GCI. The same procedure as described above was used to obtain these females. No reference populations were available for other economically important RKN such as *M. arenaria*, *M. acronea, M. chitwoodi, M. fallax* as well as for *M. hapla*. Therefore no DNA could be extracted and no SCAR-PCR reactions be done for these latter RKN species, resulting in either their presence or absence not being verified during this study.

The DNA of mature RKN females was extracted by placing the 10 RKN females from each source (greenhouse and field) in separate 0.5-mℓ micro centrifuge tubes. Females were crushed and 50µℓ ProteinaseK buffer (50 mMTris-HCl pH 8.0, 100 mM NaCl, 10mM EDTA, 1% SDS (m/v) and 20µg ProteinaseK (Roche) added. The tubes containing the females in the latter solution were then incubated at 65 °C for one hour. DNA was extracted from the 10 females within each tube using a Geneclean kit (Bio101, CA). DNA extraction was done by adding 150µℓ of a 6M NaI solution (150µℓ) as well as 5µℓ glass milk were added to each tube containing the 10 RKN females. The latter solution was mixed, incubated at room temperature for 5 minutes and then spinned for 5 seconds at 10 000rpm using a centrifuge. The supernatant was subsequently removed, the pellet containing the DNA resuspended in 200µℓ NEW

Wash solution spinned for 5 seconds. The latter process was repeated two more times. The supernatant was removed and the pellet containing the RKN DNA dried at room temperature. The pellet was subsequently resuspended in 20µℓ TE buffer and spinned for 30 seconds. Two microlitres of DNA of the RKN females from each tube was subsequently submitted to specific sequenced characterized amplified region (SCAR) as described in paragraph 2.2.2.

2.2.2. Specific sequenced characterized amplified region (SCAR)

The polymerase chain reaction (PCR) was conducted in a total volume of 25µℓ, containing 10ng DNA of the respective RKN females, 1 Uillustra Rdna Taq DNA polymerase (GE Healthcare), 200M dNTPs,1.5mM $MgCl_2$, reaction buffer at optimal concentrations and 5 pmol of the respective forward and reverse primers. The latter included the respective forward primers for *M. incognita* (FincDNA), *M. javanica* (FjavDNA) and reverse primers RincDNA (*M. incognita*), RjavDNA (*M. javanica*). SCAR-amplification for DNA from *M. incognita* females was done in a Thermo Thermal Cycler that has been programmed as follows: denaturation at 94 ^{o}C for two minutes (one cycle), followed by 40 cycles of denaturation at 94 ^{o}C for 30 seconds, annealing at 54 ^{o}C for 30 seconds and extension at 72 ^{o}C for one minute. After the latter processes a final extension step was done at 72 °C for five minutes (Zijlstra, 2000; Zijlstra *et al.,* 2000). The same programme was used for *M. javanica* with the exception of the annealing temperature being set at 64 °C (Zijlstra, 2000; Zijlstra *et al.,* 2000).

DNA products resulting from the amplification processes for each RKN female were subsequently analyzed by electrophoresis in a 3% agarose gel with 1x TBE running buffer (89mM Tris-acetate, 2.5mM EDTA, pH 8.3). Two microlitres of a 15% Ficol (0.75g Ficol + 0.012g Bromophenolblue) containing 3µℓ GelRed/1 000µℓ solution was used as loading buffer. One blank reaction that contained no RKN female DNA but only distilled water, one reference sample of the appropriate RKN species that were available and 4µℓ of FastRuler® DNA ladder (10-10 000bp; Fermentas) as molecular weight markers were also loaded on the gel in separate wells. This way the size of the DNA bands for each of the species that were represented by the mature RKN females could be obtained and the species identified. The gel containing DNA products of the respective females was subjected to electrophoresis for 2 hours at 80V and banding patterns were visualised using ultraviolet (UV) illumination. A photograph was taken of each gel containing the RKN DNA-banding patterns of *M.*

incognita and *M. javanica* and included in the results that are listed and described in paragraph 2.2.4.

2.3. Results

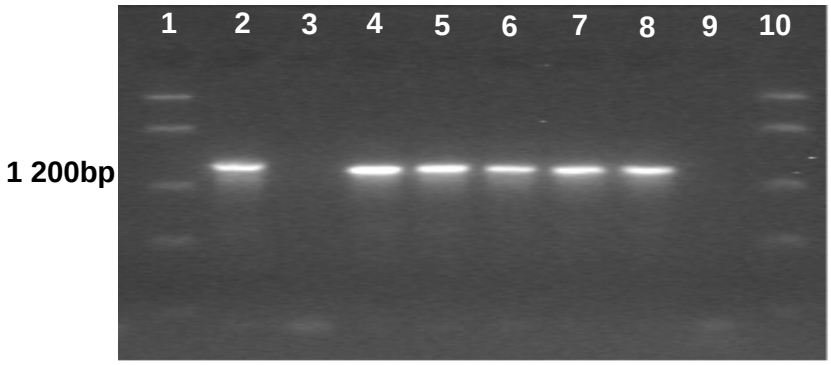


Figure 2.1. Amplification products of PCR reactions using forward FincDNA and reverse RincDNA primers for *Meloidogyne incognita* (lane 1 = DNA ladder – middle range); lane 2 = *M. incognita* reference (from *in vivo* greenhouse culture); lane 3 = *M. javanica* reference (from *in vivo* greenhouse culture); lane 4 = Trial A (E16; Marble Hall area 2005/2006); lane 5 = Trial B (J17; Marble Hall 2005/2006); lane 6 = Trial C (E6; Marble Hall 2006/2007); lane 7 = Trial D (686; Marble Hall 2006/2007); lane 8 = Trial E (Vaalharts 2006/2007); lane 9 = blank sample containing only water; lane 10 = DNA ladder – middle range).

PCR with the *M. incognita* specific SCAR-primer resulted in amplification of the *M. incognita* 1 200bp SCAR-fragment for the *M. incognita* race 4-females that were removed from tomato roots (cv. Rodade) from an *in vivo*-reared population in the greenhouse. The latter represented the reference population for *M. incognita* (Figure 2.1; lane 2) and were used as a source of inoculum for a greenhouse trial that was conducted during this study (See Chapter 3). The latter fragment was compared to that of the DNA bands of the middle range ladder that are visible in lanes one and 10. The 1 200bp SCAR-fragment were also amplified for the *M. incognita* females that were obtained from cotton roots from the five field sites where trials were conducted during this study (See Chapter 4). This was evident for the 2005/2006 growing season for Trial A (E6; Marble Hall area – lane 4), Trial B (J17; Marble Hall area – lane 5) and during the 2006/2007 growing season from Trial C (E6; Marble Hall area – lane 6), Trial D (686; Marble Hall area – lane 7) and Trial E (Vaalharts area – lane 8). Although the DNA-based methods cannot discriminate between races of this *Meloidogyne* species, it is accepted that race 4 was present at these trial sites since

it is reported to be the predominant race attacking cotton in South Africa (Keetch & Heyns, 1982; Van Biljon, 2006: personal communication).

No amplification of the 1 200bp SCAR-fragment was evident for the *M. javanica* reference population (lane 2) as well as for the blank sample that only contained distilled water (lane 9).

Figure 2.2. Amplification products of PCR reactions using forward FjavDNA and reverse RjavDNA primers for *Meloidogyne javanica* (lane 1 = DNA middle range ladder); lane 2 = *M. javanica* reference (from *in vivo* greenhouse culture); lane 3 = *M. incognita* reference (from *in vivo* greenhouse culture); lane 4 = Trial A (E6; Marble Hall 2005/2006); lane 5 = Trial B (J17; Marble Hall 2005/2006); lane 6 = Trial C (E6; Marble Hall 2006/2007); lane 7 = Trial D (686; Marble Hall 2006/2007); lane 8 = Trial E (Vaalharts 2006/2007); lane 9 = blank sample containing only water: lane 10 = DNA middle range ladder).

PCR with the *M. javanica* specific SCAR-primer resulted in amplification of the 670bp fragment only for DNA that were obtained from RKN females from the *in vivo* greenhouse culture (lane 2). The latter fragment was compared to that of the DNA bands of the middle range ladder that are visible in lanes one and 10. No amplification of a 670bp fragment that indicate the presence of *M. javanica* was, however, obtained for females from the *M. incognita in vivo* greenhouse culture (lane 3) as well as for those obtained from roots of cotton cultivars from the various field sites where trials were planted during the 2005/2006 and 2006/2007 growing season (lanes 4 to 8). No amplification of the 670bp SCAR-fragment was further evident for the blank sample that only contained distilled water (lane 9).

2.4. Discussion

DNA-based identification of RKN females that represented *M. incognita* race 4 populations by means of the SCAR-PCR method verified the presence of this predominant, economically important monoculture species. This was true for both samples obtained from an *in vivo* mass-reared population used as a source of inoculum for a greenhouse trial (see Chapter 3) and those from field sites where field trials were planted (see Chapter 4) during this study. Although available molecular methods used cannot yet discriminate among the RKN races, individuals from race 4 are the predominant parasites that infect and parasitize cotton in South Africa (Van Biljon, 2006: personal communication). Up to date races one and three have not been recorded to parasitize on cotton in South Africa (Kleynhans *et al*., 1996; Keetch & Heyns, 1982; Van Biljon, 2006: personal communication). Since it is known from the North Carolina Host Range Test that *M. incognita* race 2-individuals do not infect and parasitize cotton worldwide (Hartman & Sasser, 1985), this race particularly could also be excluded in this regard. Ultimately the race represented by RKN females investigated for identification purposes is thus proposed as four.

Molecular identification using the SCAR-based techniques proved to be valuable in confirming that both for the greenhouse as well as the field trials the target nematode species, *viz. M. incognita* race 4, was used or was present. DNA-based techniques thus proved to be reliable technique for routine diagnostic identification of RKN since it was used with success by other researchers (Blok *et al*., 1997; Fourie *et al*., 2001; Zijlstra, 2000; Zijlstra *et al*., 2001; Blok & Powers, 2009). It is, however, important to bear in mind that although RKN females that were identified during this study were not subjected to *M. arenaria*, *M. hapla*, *M. chitwoodi*, *M. fallax* and *M. acronea* primers for identification purposes, it is preferable to do the latter since, although it is highly unlikely that these species will infect cotton roots in local producing areas, the possibility should and could not be excluded.

Chapter 3: *In vivo* evaluation of the efficacy of abamectin in reducing *Meloidogyne incognita* race 4 population levels in four commercially available cotton cultivars in a greenhouse trial

3.1. Introduction

Avermectins constitute a group of natural substances that have an anthelmintic effect and were developed in 1975 in the USA for its potential use to control insects (Fischer & Mrozik, 1992).

Only recently has the commercially available product abamectin, which contain avermectins, stimulated new interest in the industrial and scientific community as a result of its ability to reduce PPN effectively when applied as a seed treatment (Monfort *et al.*, 2006b; Faske & Starr, 2006). The potential of abamectin to control PPN has already been reported in the early 1980's (Cayrol *et al*., 1993, Bull *et al*., 1984; Jansson & Rabatin, 1998). In contrast to seed treatment and soil drench applications, foliar applications of abamectin did not result in a reduction of PPN populations that parasitize underground parts of plants (Bull *et al.*, 1984). The poor efficacy of foliar applications of abamectin is particularly attributed to photodegradation of this product, which occur on leave surfaces after its application (Bull *et al.*, 1984; Cayrol *et al*., 1993). The low water solubility of abamectin is another constraint that limits the efficacy of the product when applied on aerial parts of plants (Bull *et al.*, 1984; Cayrol *et al*., 1993). The latter characteristic results in limited systemic translocation of the product to belowground parts of the plant, e.g. roots, tubers, pods and others (Bull *et al.*, 1984; Cayrol *et al*., 1993).

At present the four most popular cotton cultivars that are planted in local cotton producing areas are Delta OPAL®, Nu OPAL®, Nu OPAL RR® and Delta OPAL RR® (Olivier, 2009: personal communication). Comparative trials to investigate the efficacy of abamectin seed treatments against *M. incognita* race 4 as well as the host status of these cultivars to these parasites have received limited attention to date (Van Biljon, 2006: personal communication). Results from trials that were formerly conducted by Van Biljon (2005 & 2006: personal communication) have indicated significant differences among cotton cultivars Acala OR3®, Delta OPAL®, Gamka® and Tetra® in terms of *M. incognita* race 4 population. In addition, information on the efficacy of abamectin in reducing *M. incognita* race 4 numbers in the roots of these cotton cultivars are also lacking. Since transgenic lines that exhibit insect- and herbicide resistance (Koenning *et al.*, 2003) are at present most widely planted by local producers (Van Biljon, 2005; Olivier, 2009: personal communication), research in terms of these aspects warrants further investigation. The objectives of this study were therefore to i)

evaluate the efficacy of abamectin as a seed treatment against *M. incognita* race 4 using the four cotton cultivars Delta OPAL®, Delta OPAL RR®, Nu OPAL® and Nu OPAL RR® under controlled conditions in a greenhouse and ii) concurrently determine the population development of this RKN species and race in roots of these cultivars as well as the host status of this parasite.

3.2. Materials and methods

3.2.1. General trial procedures

Two-hundred-and-fourty-four-litre pots (Fig. 3.1) were filled with sandy soil (93.6% sand, 3.9% clay, 1.9% silt and 0.6% organic matter content) that had been fumigated with ethylene dibromide (EDB) at 55ℓ/ha and thereafter steam-sterilized at 50 ºC for 24 hours. The soil had a pH (H_20) value of 4.35. Nutrients were added according to a soil nutrient analysis, namely 2:3:4 (36) fertilizer at a rate of 4.05g (0.3g nitrogen (N); 0.5g phosphorous (P); 0.6g potassium (K) and 24g dolomitic lime per pot before planting. Limestone ammonium nitrate (28% N) at a rate of 1.5g per pot was incorporated at seedling emergence and repeated ten days later. The pots were placed in a greenhouse where an ambient temperature regime of 18 ºC to 20 ºC (minimum) and 26 ºC to 28 ºC (maximum) and a 14L:10D hour photoperiod were maintained for the duration of an experiment. Subsequently, abamectin treated cotton seeds at a dosage rate of 0.15mg a.i./seed as well as untreated seeds of four local commercially-available cotton cultivars, namely Delta OPAL®, Nu OPAL®, Nu OPAL RR® and Delta OPAL RR®, were planted in these pots. The trial was conducted on the premises of the Agricultural Research Council's (ARC) Grain Crops Institute (GCI) at Potchefstroom during 2008. Cotton seed of the four cultivars used were pre-treated with a standard multiple fungicide seed treatment used in South Africa, which include 0.05mg carboxin (oxathiin) + 0.05mg thiram (organo sulphide) + 7.8µg mefenoxam (phenylamide)/seed. Coating of seeds was done according to specifications by personnel of Syngenta, South Africa (Dreyer, 2005: personal communication).

Figure 3.1 Cotton seedlings of the four cultivars used in this study in 4ℓ-pots in a greenhouse trial.

One seed of each cultivar were planted per pot. Cotton seeds for both treatments and for the four cultivars used were subsequently inoculated with ±2 500 *M. incognita* race 4 eggs and J2 (described in paragraphs 3.2.3.& 3.2.4) that were mass reared *in vivo* (described in paragraph 3.2.2.) at planting.

3.2.2. Mass rearing of *Meloidogyne incognita* race 4-inoculum

Meloidogyne incognita race 4 populations were maintained separately *in vivo* in potted tomato plants (cv. Rodade) in a greenhouse. Temperature regimes of 19 ^{o}C to 27 ^{o}C and a 14:10 LD hour photoperiod were maintained for the duration of the mass rearing process. Plastic pots (25 000 cm^3 capacity) were filled with steam-pasteurized soil (4.5% clay, 92.8% sand, 2.4% silt and 0.3% organic matter content). The soil pH (H_20) was 4.35. Nutrients were added according to soil nutrient analyses, namely 24g dolomitic lime, 12g zinc (Zn), 1.68g 2:3:2 (NPK), 0.78g potassium chloride (KCl) and 14.04g super phosphate (10% phosphorus). The *M. incognita* race 4 population used during this study was originally obtained from Jan Kempdorp in the Northern Cape Province. The abovementioned species were identified by means of molecular techniques (Chapter 2) and were subsequently artificially reared *in vivo* to obtain adequate numbers of eggs and J2 for inoculation of a greenhouse trial that was done during this study.

3.2.3. Preparation of *Meloidogyne incognita* race 4-inoculum

M. incognita race 4-infected tomato roots were obtained from pots from the greenhouse where it had been artificially mass reared as described in paragraph 3.2.2. Eggs and J2 were extracted from these roots using the modified NaOCl method (Riekert, 1995). After extraction, the nematode egg and J2 water suspension was poured in a 500-ml glass beaker, placed on a magnetic stirrer and stirred for 5 minutes to ensure an even distribution of the eggs and J2 during division of the inoculum into six equal aliquots. The latter six aliquots were used to individually inoculate the six replicates of the trial. A Socorex nematode stepper (411 model) was used for this purpose. Each of the six suspensions containing the egg and J2 inoculum was topped up to approximately 200mℓ with tap water and was continuously stirred throughout the inoculum preparation process. Ten aliquots of 10mℓ each were collected from each of the six suspensions using a measured pipette. Each of these 10mℓ aliquots was then poured into a separate counting dish and counted to check for at least a 95% accuracy level of inoculum preparation. When this accuracy level was not achieved the whole procedure was repeated until the desired number of nematodes to be inoculated per plant was suspended in 10mℓ aliquots of tap water. This was achieved by diluting the nematode suspension to a volume that contained 2 500 *M. incognita* race 4 eggs and J2 per 5mℓ water.

3.2.4. Inoculation of cotton seeds with *Meloidogyne incognita* race 4 eggs and J2

Nematode inoculation was done prior to planting of the seeds of all four cotton cultivars used in this trial. A 2cm-deep hole was made in the soil in the middle of each pot and inoculation was done by pipeting 5mℓ aliquots of the egg and J2 water suspension containing ±2 500 eggs and J2 of *M. incognita* race 4 into the hole. Each sample bottle was rinsed with 5mℓ tap water to ensure that no eggs and J2 were left in the bottles. After inoculation, three cotton seeds of each cultivar were placed into the hole in each pot where the inoculum was poured in. Each seed was then covered with the soil that was initially removed to form the hole where the inoculum was poured in. This procedure was followed to ensure that the abamectin-coated seed stayed intact and that the product was not rinsed off during inoculation of eggs and J2.

3.2.5. Nematode and plant growth assessments

3.2.5.1. Nematode parameters

Various nematode parameters, *viz*. the number of eggs and J2/root system, J2/200ml soil as well as egg masses and gall numbers/root systems were obtained for each treatment and cultivar during each of the five prominent growth stages of the developing cotton seedlings. Subsequently egg-laying female (ELF) indices were calculated using the index of Hussey and Boerma (1981), which represents a scale of zero to 5 where 0 = no egg masses; 1 = 1 to 2 egg masses; 2 = 3 to 10 egg masses; 3 = 11 to 30 egg masses; 4 = 31 to-100 egg masses and 5 = more than 100 egg masses per root system. This was done by removing the root system of each cotton plant from the pots during each of the five sampling intervals as listed in paragraph 3.2.5.2. The root systems were subsequently rinsed free of adhering soil and debris with running tap water, blotted on towel paper and weighed. Staining of egg masses to facilitate counting was done during each of these sampling intervals by immersing the roots of each of the cotton plants in a 0.1% phloxine B solution for 20 minutes (Hussey & Boerma, 1981). Each root system was then removed from the staining solution, cut into approximately 1-cm pieces and transferred to a rectangular (20cm wide x 30cm long x 5cm deep), white plastic container that contained 200mℓ tap water. Each 1cm-piece of each root system was inspected individually for red-stained egg masses, which were counted using a commercial magnifying glass.

The number of egg masses present on each root system was subsequently rated according to the method of Hussey and Boerma (1981) as described in paragraph 3.2.5.1. Furthermore, Rf values were calculated using Oostenbrink's reproduction factor (Rf), where Rf = final egg and J2 numbers (Pf)/initial egg and J2 numbers (Pi) (Windham & Williams, 1987).

Ultimately *M. incognita* race 4 eggs and J2 were extracted using Riekert's (1995) modified NaOCl-method and counted using a dissection microscope at a 100x magnification. Using classification systems for ELF indices (Table 3.1) and Rf values (Table 3.2), different categories were assigned to the root systems of each of the four cultivars during each of the sampling intervals. This way the host suitability of the cotton seedlings for *M. incognita* race 4 were determined and the effect of the abamectin seed treatment evaluated in terms of its potential to reduce population-level development of these parasites.

Table 3.1. The classification system of Murray *et al*. (1986) using egg-laying female (ELF) indices, number of egg masses/root system to categorise crop cultivars in terms of their host status to root-knot nematodes (RKN).

Category	ELF rating index	Egg masses/root system	Host status of cultivar
1.	0	0	Resistant
2.	1.0 – 3.2	1 – 15	Moderately resistant
3.	3.3 – 3.7	16 – 25	Susceptible
4.	3.8 – 5.0	26 – 100+	Very susceptible

Table 3.2. The classification system of Windham and Williams (1988) using reproduction factor (Rf) values to categorise crop cultivars according to their host status for root-knot nematodes.

Category	Rf value	Host status of cultivar
1.	< 1	Resistant/poor host
2.	1 – 5	Good hosts
3.	> 5	Excellent hosts

3.2.5.2. Plant growth parameters

The five nematode sampling intervals represented the first true leaf (Fig. 3.2) 22 DAP and inoculation (Sampling Interval 1), first square (Figs. 3.3 & 3.4) 50 DAP and inoculation (Sampling Interval 2), first flower (Fig. 3.4) 67 DAP and inoculation (Sampling Interval 3), first boll opening 91 DAP and inoculation (Sampling Interval 4) and 50% boll opening 106 DAP and inoculation (Sampling Interval 5). During each of these sampling intervals counts were done for each of the respective growth stage parameters, i.e. the number of squares visible during sampling interval one, number of squares visible during sampling interval two, number of bolls that opened during sampling interval four. Sampling interval five was conducted when 50% of the bolls on each cotton plant were open.

Management of other pests and diseases that attacked the cotton plants during the duration of the experiment was done as needed using various contact chemicals that are listed in Table 3.3.

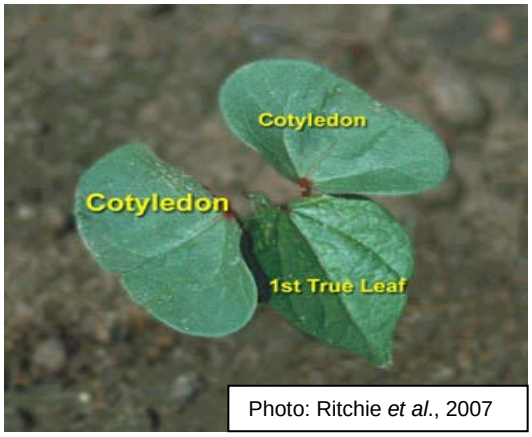


Figure 3.2. Aerial parts of a cotton seedling illustrating the visible difference between the cotyledons and the first true leaf stage.

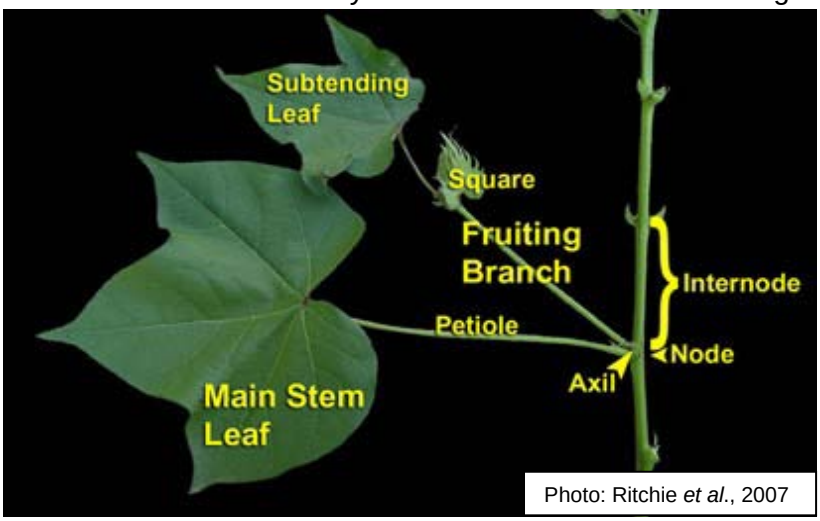


Figure 3.3. A photo showing the main stem of a cotton plant with the first true leaf and square that developed on the fruiting branch of a cotton plant.

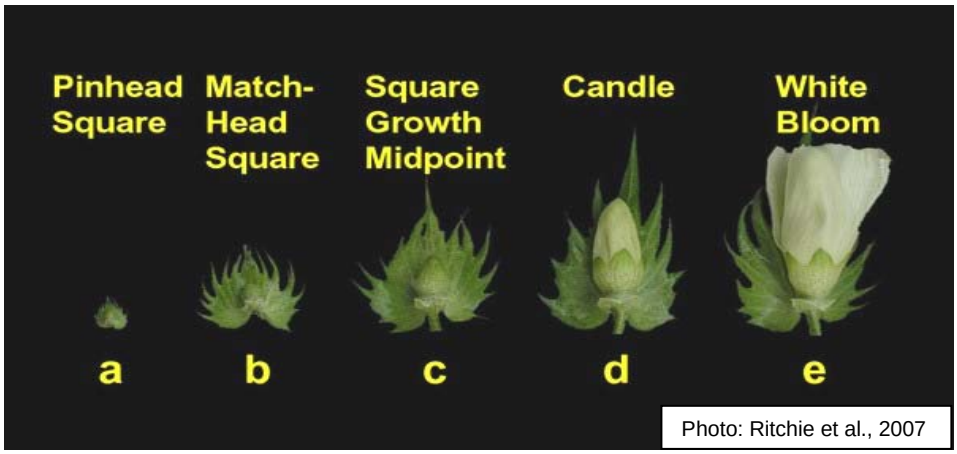


Figure 3.4. The first square (pin head) developing on a cotton plant (a) and subsequent development of such a square (b to e) into a flower**.**

Table 3.3. Information on contact insecticides used to control insects such as white flies and red spider mites during this study.

Sampling interval (growth stage)	Product used (active ingredient)	Dosage rate
1 (square formation)	Telstar® (Bifenthrin 100g a.i./ℓ)	0.02g a.i./ha
2 (boll formation)	Hunter® (Chlorphenapyr 360g a.i./ℓ)	72g a.i./ha

3.2.6. Experimental design and data analysis

The trial layout was a randomised complete split-plot design including the two treatments, five sampling intervals and four cotton cultivars (Fig. 3.5). Nematode and plant data were subjected to a factorial analysis of variance (ANOVA), with treatments as factor 1, cultivars as factor 2 and sampling intervals as factor 3. Means were separated by the Tukey Test (Statgraphics Plus 5 for Windows) and degrees of freedom (error) > 18 (Van Ark, 1981) were always pursued. In addition nematode data for *M. incognita* race 4 egg and J2/root system were non-linearly regressed on the various sampling intervals (independent variable) using polynomial models (Genstat for Windows). On the other hand, plant biomass data (dependent variable) were linearly regressed using the sampling intervals as the independent variable (Genstat for Windows). These rational functions are representative of the data obtained during the experiment.

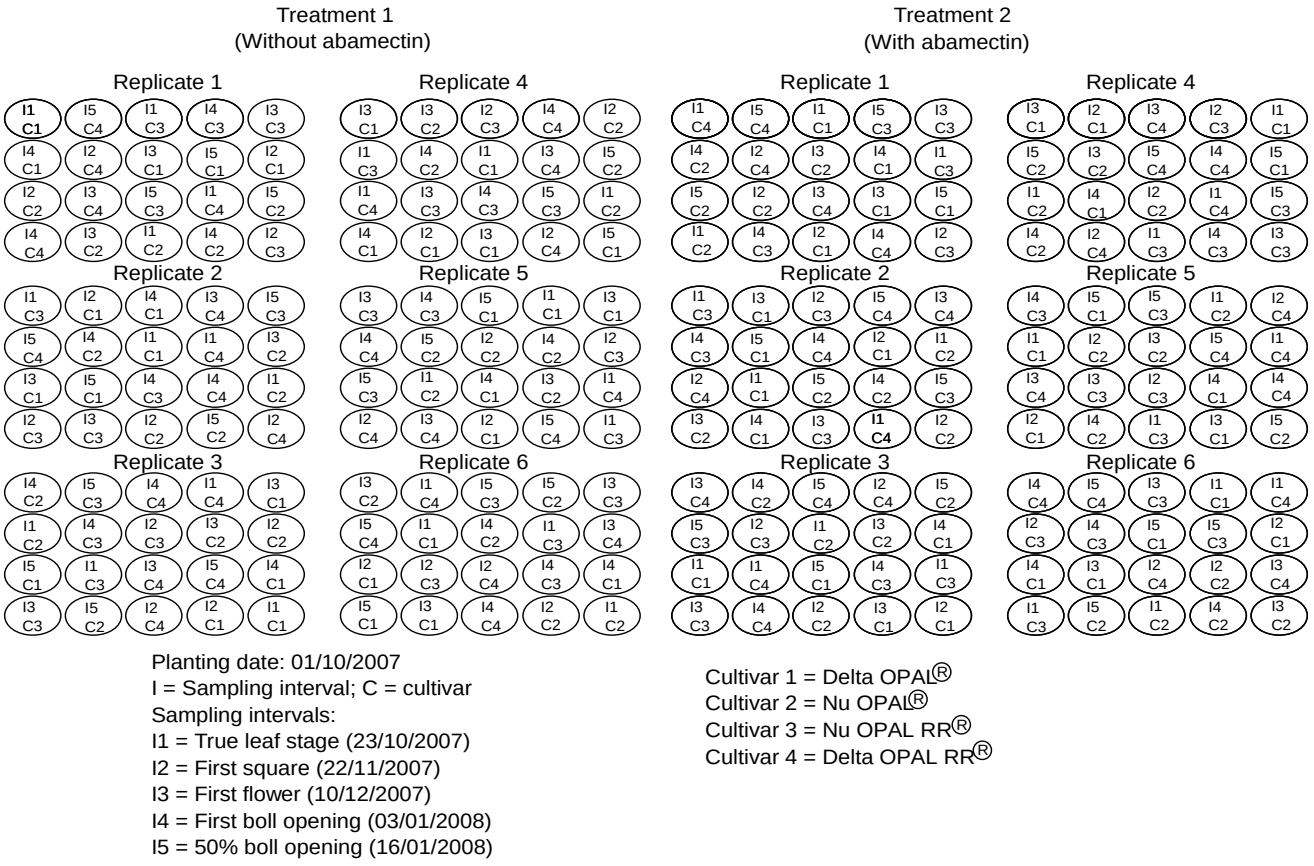


Figure 3.5. An illustration of the trial layout of the greenhouse trial that was conducted to evaluate the efficacy of abamectin on *Meloidogyne incognita* race 4 using four local cotton cultivars.

3.3. Results

3.3.1. Nematode data (pooled for cultivars)

The non-abamectin and abamectin treatment differed significantly ($P \leq 0.05$) from one another for egg and J2 numbers/root systems as well as for J2 numbers/200ml soil when data were pooled for the four cultivars (Table 3.4). The same applied for the five sampling intervals for both treatments, while significant differences were only evident among the four cultivars for the non-abamectin treatment. Detail information with regard to this significant difference in cultivars is listed and elaborated on in Tables 3.5 and 3.6.

Significant interactions ($P \leq 0.05$) existed between the treatments and sampling intervals, the cultivars and sampling intervals as well as for the treatments, cultivars and sampling intervals in terms of nematode egg and J2 numbers/root system when data were pooled for the four cultivars (Table 3.4).

Table 3.4 Significance and interaction data for *Meloidogyne incognita* race 4 parameters obtained during sampling intervals and pooled for four local commercially-available cotton cultivars [data were *ln*(*x*+1) transformed].

Parameter	Source	F-ratio	P-value
Number of eggs & J2/root system	Treatments	248.74	0.0000*
	Cultivars	4.62	0.0038*
	Sampling intervals	610.99	0.0000*
	Treatment x interval	14.05	0.0000*
	Treatment x cultivar	0.62	0.6027
	Cultivar x interval	1.91	0.0350*
	Treatment x cultivar x interval	2.05	0.0217*
J2 numbers/200mℓ soil	Treatments	120.69	0.0000*
	Cultivars	0.09	0.0957
	Sampling intervals	72.02	0.0000*
	Treatments x intervals	10.92	0.0000*
	Treatment x cultivar	0.96	0.4121
	Cultivars x intervals	2.09	0.0191*
	Treatment x cultivar x interval	0.87	0.5752
Number of egg masses/root system	Treatment	228.11	0.0000*
	Cultivar	2.62	0.0521
	Sampling interval	66.76	0.0000*
	Treatment x interval	22.51	0.0000*
	Treatment x cultivar	1.15	0.3292
	Cultivar x interval	0.93	0.5177
	Treatment x cultivar x interval	1.07	0.3868
Number of galls/root system	Treatment	337.06	0.0000*
	Cultivar	3.21	0.0242*
	Sampling interval	50.57	0.0000*
	Treatment x interval	3.31	0.0119*
	Treatment x cultivar	3.74	0.0121*
	Cultivar x interval	0.78	0.6663
	Treatment x cultivar x interval	1.16	0.3108
Number of egg-laying females (ELF)/root system	Treatment	295.91	0.0000*
	Cultivar	1.79	0.1500*
	Interval	186.55	0.0000*
	Treatment x interval	22.97	0.0000*
	Treatment x cultivar	0.89	0.4467
	Cultivar x interval	2.15	0.0157*
	Treatment x cultivar x interval	1.40	0.1681
Rf values/root system	Treatment	82.83	0.0000*
	Cultivar	0.97	0.4148
	Sampling interval	11.03	0.0000*
	Treatment x interval	8.02	0.0000*
	Treatment x cultivar	1.30	0.2437
	Cultivar x interval	1.30	0.2437
	Treatment x cultivar x interval	1.44	0.1726

*Differences are significant.

Significant interactions ($P \leq 0.05$) also existed between treatments and sampling intervals and between cultivars x sampling intervals for J2 numbers/200mℓ soil when data were pooled for cultivars (Table 3.4).

The two treatments differed significantly ($P \leq 0.05$) from each other with regard to the number of egg masses and galls/root system as well as for ELF indices and Rf values when data were pooled for cultivars (Table 3.4). Significant ($P \leq 0.05$) interactions were also recorded for all the latter nematode parameters for the five sampling intervals. The four cultivars did not differ significantly from one another for all these nematode parameters.

Significant ($P \leq 0.05$) interactions were evident for the number of egg masses and galls/root system as well as for ELF indices and Rf values between treatments and intervals, while an interaction between treatments and cultivars were only recorded for the number of galls/root system (Table 3.4). No significant interactions among treatments, cultivars and intervals were, however, recorded for these nematode parameters (Table 3.4).

3.3.1.1. Eggs and J2/root system as well as J2/200ml soil

In this section data for the eggs and J2/root system as well as the J2/200ml soil will be discussed, which will be followed by the number of egg masses and galls/root system, ELF indices and Rf values in section 3.3.2.2.

Table 3.5. Significance and interaction data for *Meloidogyne incognita* race 4 eggs and second-stage juveniles (J2)/root system as well as J2/200mℓ soil for four local commercially-available cotton cultivars and two treatments during five sampling intervals [data were *ln*(x+1) transformed].

Parameter	Source	F-ratio	P-value	F-ratio	P-value
		T1[1]		T2[2]	
Number of eggs & J2/root system	Cultivar	4.93	0.0040*	0.93	0.4332
	Sampling interval	441.65	0.0000*	225.03	0.0000*
	Cultivar x sampling interval	1.83	0.0637	2.19	0.0234*
Number of J2/200mℓ soil	Cultivar	0.61	0.6101	0.43	0.7335
	Sampling interval	84.58	0.0000*	13.42	0.0000*
	Cultivar x sampling interval	1.28	0.2567	1.46	0.1648

*Differences are significant.

[1] Treatment 1 = non abamectin treatment); [2] Treatment 2 = abamectin treatment).

Significant differences were evident among the four cultivars only for the non-abamectin treatment when the data were pooled for the five sampling intervals (Tables 3.5). The four cultivars did, however, not differ significantly from one another for the J2 numbers/200ml soil both for the non-abamectin as well as the abamectin treatments.

The five sampling intervals differed significantly ($P \leq 0.05$) from one another for both the number of eggs and J2/root system and J2/200ml soil for both treatments (Table 3.5).

A significant interaction was evident between cultivars and sampling intervals for the abamectin treatment only for the number of eggs and J2/root system, indicating that the cultivars reacted differently in terms of this parameter during the sampling intervals (Table 3.5).

Table 3.6. Data for *Meloidogyne incognita* race 4 eggs and second-stage juveniles (J2) numbers/root system for the four local commercially-available cotton cultivars pooled for the five sampling intervals for the non-abamectin treatment [data were *ln*(*x*+1) transformed; real means in parenthesis].

Cultivar number and name	Eggs and J2 numbers/root system
1.Delta OPAL®	7.8 (25 722) a[1]
2.Nu OPAL®	8.6 (29 025) b
3.Nu OPAL RR®	8.05 (24 769) a
4.Delta OPAL RR®	7.6 (20 136) a

[1]Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to the Tukey Test (Statgraphics Plus 5 for Windows).

Although cultivar Nu OPAL® maintained significantly higher egg and J2 numbers/root system compared to those for the other three cultivars used in this study (Table 3.6), it is highly susceptible to this *M. incognita* race 4 population. The other three cultivars used are also highly susceptible to this RKN species and race. For this reason, the effect of cultivar will not be accentuated further during this study.

Table 3.7. Data for *Meloidogyne incognita* race 4 egg and second-stage juveniles (J2) numbers/root system as well as J2/200ml soil for the five sampling intervals for both the non-abamectin and abamectin treatments when pooled for the four local commercially-available cotton cultivars [data were *ln*(*x*+1) transformed; real means in parenthesis].

Sampling interval	T1[1]		T2[2]	
	Eggs & J2/root system	J2/200ml soil	Eggs & J2/root system	J2/200ml soil
1	0.6 (7) a[3]	0.8 (3) a	0.07 (0.2) a	1.1 (7) a
2	10.4 (21 523) c	6.7 (1 278) c	7.5 (3 587) c	4.5 (169) d
3	9.9 (28 303) bc	5.1 (352) b	8.05 (5 668) c	2.8 (44) b
4	9.7 (28 673) b	7.1 (2 115) c	7.8 (3 602) c	3.7 (152) cd
5	9.7 (46 058) b	5.1 (566) b	6.2 (1 138) b	2.1 (45) bc

[1] Treatment 1 = non abamectin treatment; [2]Treatment 2 (abamectin treatment).

[3]Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to the Tukey Test (Statgraphics Plus 5 for Windows).

M. incognita race 4 eggs and J2/root system increased from sampling interval one to five when data were pooled for cultivars for both the non-abamectin and abamectin treatments (Table 3.7). Sampling interval one had significantly ($P \leq 0.05$) lower egg and J2/root system for both treatments compared to those for the other four intervals. While population levels gradually increased during the intervals for the non-abamectin treatment, it increased until interval three for the abamectin treatment and then decreased during intervals four and five.

Although J2 numbers/200ml soil were significantly ($P \leq 0.05$) lower during sampling interval one compared to those for the other sampling intervals for the non-abamectin treatment, no consistency was evident in terms of an increase in these numbers during the five intervals (Table 3.7). With regard to the non-abamectin treatment, significantly ($P \leq 0.05$) lower J2 were present during interval one, which differed significantly ($P \leq 0.05$) from those for the other four intervals. J2/200ml soil did not differ significantly during intervals two and four, but these latter intervals differed significantly ($P \leq 0.05$) from those for intervals three and five. The two latter intervals did, however, not differ significantly ($P \leq 0.05$) from each other in terms of J2 numbers/200ml soil. For the abamectin treatment J2 numbers/200ml soil for sampling intervals one were significantly lower compared to the others, while sampling intervals three and five did not differ from each other. J2/200ml numbers for sampling intervals two and three did, however, differ significantly ($P \leq 0.05$) from each other.

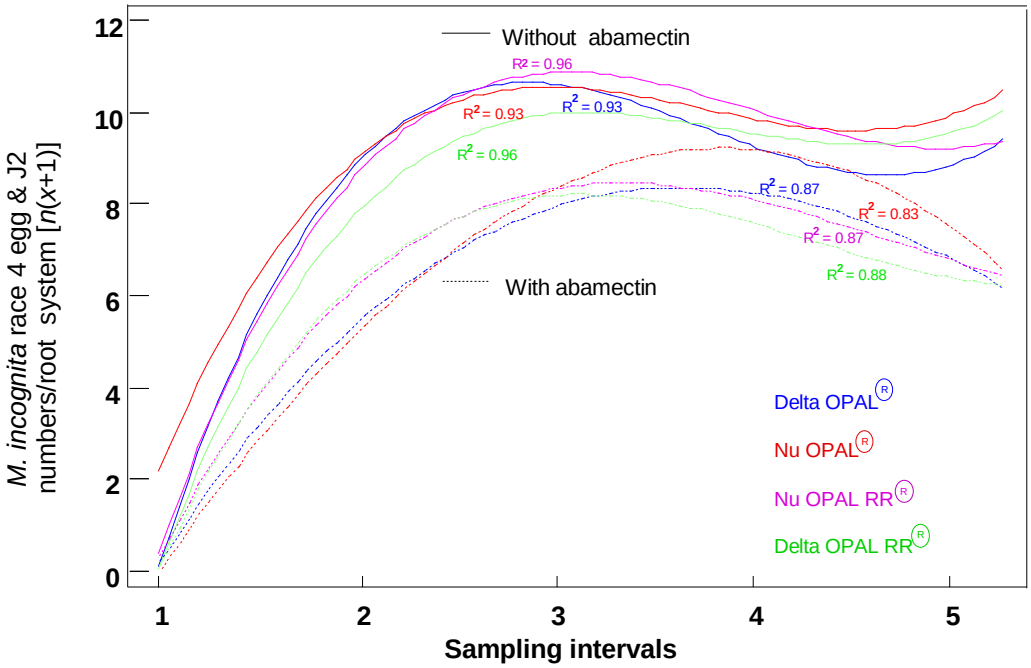


Figure 3.6. Population growth development of *Meloidogyne incognita* race 4 egg and second-stage juveniles (J2) in root systems of four local commercially available cotton cultivars during five samplings for non-abamectin and abamectin treatments.

Table 3.8. Equations for non-linear, polynomial regression lines that describe the relationships between *Meloidogyne incognita* race 4 eggs and second-stage juveniles (J2)/root system for cotton cultivars Delta OPAL®, Delta OPAL RR®, Nu OPAL® and Nu OPAL RR® during five sampling intervals for non-abamectin treatments [*ln*(*x*+1) transformed data].

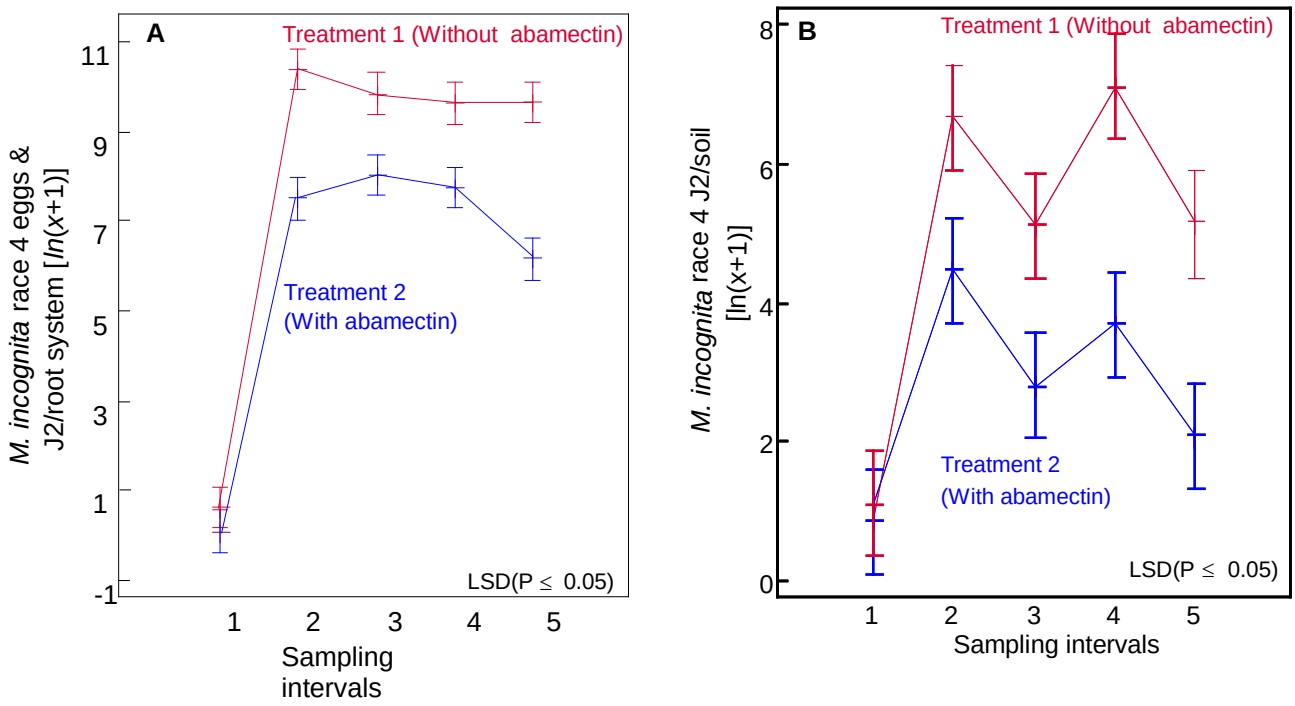
Cultivar name and number	Equation
1. Delta OPAL®	y = 0.071+0.6798*x-0.01366*x*x+0.0000816+x*x*x
2. Nu OPAL®	y = 2.168+0.5200*x-0.01022*x*x+0.0000616*x*x*x
3. Nu OPAL RR®	y = 0.390+0.6101*x-0.01115*x*x+0.0000613*x*x*x
4. Delta OPAL RR®	y = 0.045+0.5747*x-0.01063*x*x+0.0000616*x*x*x

Table 3.9. Equations for non-linear, polynomial regression lines that describe the relationships between *Meloidogyne incognita* race 4 eggs and second-stage juveniles (J2)/root system for cotton cultivars Delta OPAL®, Nu OPAL®, Nu OPAL RR® and Delta OPAL RR® during five sampling intervals for the abamectin treatment [*ln*(*x*+1) transformed data].

Cultivar name and number	Equation
1. Delta OPAL®	y = 0.125+0.3352*x-0.00392*x*x+0.0000097x*x*x
2. Nu OPAL®	y = 0.028+0.03036*x-0.00219*x*x+0.0000053*x*x*x
3. Nu OPAL RR®	y = 0.278+0.4091*x-0.00633*x*x+0.0000279*x*x*x
4. Delta OPAL RR®	y = 0.028+0.4513*x-0.000777*x*x+0.0000390*x*x*x

The relationships between *M. incognita* race 4 egg & J2 numbers/root system and the five sampling intervals for the four cotton cultivars for the two treatments were best described by strong, non-linear equations (Fig. 3.6; Tables 3.8 & 3.9). Significant R^2 values were obtained for all four cultivars for both treatments using polynomial models of the third order, explaining the significance ($P \leq 0.05$) in terms of the variation.

The four cultivars generally had similar regression lines for the non-abamectin treatment in terms of *M. incognita* race 4-population development during a period of 106 DAP and inoculation, which represented the five sampling intervals, (Fig. 3.6; Table 3.8). Regression lines for all four cultivars levelled off from the third sampling interval (first flower formation), but showed a slight increase during the fifth and final sampling interval. The latter trend was, however, not observed for nematode population development in roots of the same cultivars for the abamectin treatment. Although the four cultivars also had similar regression lines for the latter treatment (Table 3.8), the regression line for Delta OPAL RR® levelled off from the third sampling interval (first flower formation), while those for Delta OPAL® and Nu OPAL RR® levelled off from the fourth sampling interval (first boll opening), explaining the difference in the slopes of the regression lines. The general trends for all cultivars in terms of nematode population levels at 106 DAP and inoculation, which represents the fifth sampling interval when 50% of the bolls were opened, showed a decline for the abamectin treatment opposed to an increase being evident for all four cultivars for the non-abamectin treatment.



Figures 3.7 (A & B). The effect of abamectin- and non-abamectin treatments on *Meloidogyne incognita* race 4 numbers/root system (A) and J2/200ml soil (B) during five sampling intervals when pooled for four local commercially-available cotton cultivars that was planted in a greenhouse and artificially inoculated with approximately 2 500 eggs and J2/seed.

Significantly ($P \leq 0.05$) higher *M. incognita* race individuals were present in roots of cotton cultivars for the non-abamectin treatment compared to those for the abamectin treatment for sampling intervals two, three, four and five (Fig. 3.7A). No significant ($P \leq 0.05$) differences were, however, evident between the two treatments for sampling interval one.

M. incognita race 4 numbers peaked for the non-abamectin treatment in roots of all cultivars during the second sampling interval and then levelled off during sampling intervals three to five (Fig. 3.7A). The latter sampling intervals did not differ significantly ($P \leq 0.05$) from one another in terms of nematode eggs and J2/root system for this treatment. Nematode egg and J2 numbers/root system peaked during the third sampling interval for the abamectin treatment but were not significantly ($P \leq 0.05$) lower for the second, third and fourth sampling intervals. Significantly ($P \leq 0.05$) lower eggs and J2/root system were, however, maintained by roots of cotton cultivars during the first and fifth sampling intervals compared to those for sampling intervals two, three and four.

M. incognita race 4 J2 in 200ml soil samples collected from the rhizosphere of the four cultivars did not increase consistently over time for both treatments when data were pooled

for the cultivars (Fig. 3.7B). J2 numbers were significantly ($P \leq 0.05$) higher in soil samples for the non-abamectin treatment compared to those for the abamectin treatment, except for sampling interval one. In addition, J2 numbers were highest for both treatments during sampling intervals two and four, representing the first square and boll opening growth stages of the cultivars, respectively.

Table 3.10. Data for *Meloidogyne incognita* race 4 egg and second-stage juveniles (J2) numbers/root system as well as J2/200mℓ soil for four local commercially-available cotton cultivars and two treatments during five sampling intervals were conducted [*ln*(*x*+1) transformed data].

Sampling Interval	Cultivar number and name	Eggs & J2/root system		J2/200ml soil	
		T1[1]	T2[2]	T1	T2
1	1. Delta OPAL®	0 (0) a[3]	0 (0) a[3]	0.3 (1) a[3]	0.7 (3) a[3]
	2.Nu OPAL®	2.1 (27) a	0 (0) a	0.3 (1) a	0.6 (5) a
	3.Nu OPAL RR®	0.4 (1) a	0.3 (1) a	1.3 (4) a	1.8 (11) a
	4.Delta OPAL RR®	0 (0) a	0 (0) a	1.5 (6) a	1.4 (8) a
2	1.Delta OPAL®	10.9 (66 087) a	7.8 (3 873) a	7.0 (1 890) a	4.8 (133) a
	2.Nu OPAL®	10.5 (49 036) a	6.8 (2 700) a	7.0 (1 633) a	3.0 (63) a
	3.Nu OPAL RR®	10.5 (47 420) a	7.7 (3 616) a	6.8 (1 050) a	5.0 (181) a
	4.Delta OPAL RR®	9.7 (21 691) a	7.8 (4 161) a	6.0 (537) a	5.1 (298) a
3	1.Delta OPAL®	9.5 (28 531) a	6.9 (1 377) a	5.3 (247) a	2.8 (38) a
	2.Nu OPAL®	10.0 (27 058) a	9.2 (13 975) a	5.4 (486) a	3.6 (90) a
	3.Nu OPAL RR®	10.5 (44 423) a	8.3 (4 449) a	4.8 (276) a	3.6 (46) a
	4.Delta OPAL RR®	9.5 (14 678) a	7.8 (2 871) a	5.0 (399) a	1.2 (5) a
4	1.Delta OPAL®	9.3 (13 809) a	8.6 (5 695) a	7.5 (2 853) a	4.3 (302) a
	2.Nu OPAL®	10.0 (29 969) a	8.1 (4 881) a	7.5 (3 053) a	3.6 (86) a
	3.Nu OPAL RR®	9.6 (15 751) a	7.3 (1 896) a	6.5 (922) a	3.1 (162) a
	4.Delta OPAL RR®	9.7 (26 562) a	7.0 (1 937) a	7.0 (1 633) a	3.8 (57) a
5	1.Delta OPAL®	9.2 (20 183) a	5.6 (484) a	4.5 (355) a	1.3 (7) a
	2.Nu OPAL®	10.4 (39 032) a	6.6 (2 076) a	5.7 (560) a	2.1 (97) a
	3.Nu OPAL RR®	9.3 (16 248) a	6.3 (1 071) a	4.3 (271) a	2.1 (26) a
	4.Delta OPAL RR®	9.9 (37 748) a	6.1 (922) a	6.0 (1 076) a	2.8 (51) a

[1]Treatment 1 = non abamectin treatment; [2]Treatment 2 = abamectin treatment.

[3]Means in the same column followed by the same letter do not differ significantly (P ≤0.05) according to theTukey Test (Statgraphics Plus 5 for Windows) and applies only for each sampling interval.

Egg and J2 numbers/root system ranged from zero to 27 for the non-abamectin treatment during sampling interval one and between zero and one for the abamectin treatment (Table 3.10). From sampling interval two these numbers ranged from 21 691 to 66 087, for interval three from 14 678 to 44 423, for interval four from 13 809 to 29 969 and for interval five from 16 248 to 39 032. For the abamectin treatment, egg and J2 numbers ranged from 2 700 to 4

161 during interval two, from 1 377 to 13 975 for interval three, from 1 896 to 5 695 for interval four and from 484 to 2 076 for interval five.

J2 numbers/200ml soil were substantially lower than egg and J2 numbers/root system during each of the sampling intervals and ranged between one and six for the non-abamectin and from three to 11 for the abamectin treatment during sampling interval one (Table 3.10). J2 numbers did not increase or decrease consistently during intervals two to five for both treatments. For the non-abamectin treatment it ranged from 537 to 1 890 for interval two, from 247 to 486 for interval three, from 922 to 3 053 for interval four and from 271 to 1 076 for interval five. For the abamectin treatment it ranged between 63 and 298 for interval two, between 5 and 90 for interval three, between 57 and 302 for interval four and between seven and 97 for interval five.

3.3.2.2. Number of egg masses and galls/root system as well as egg-laying females (ELF) and Rf values

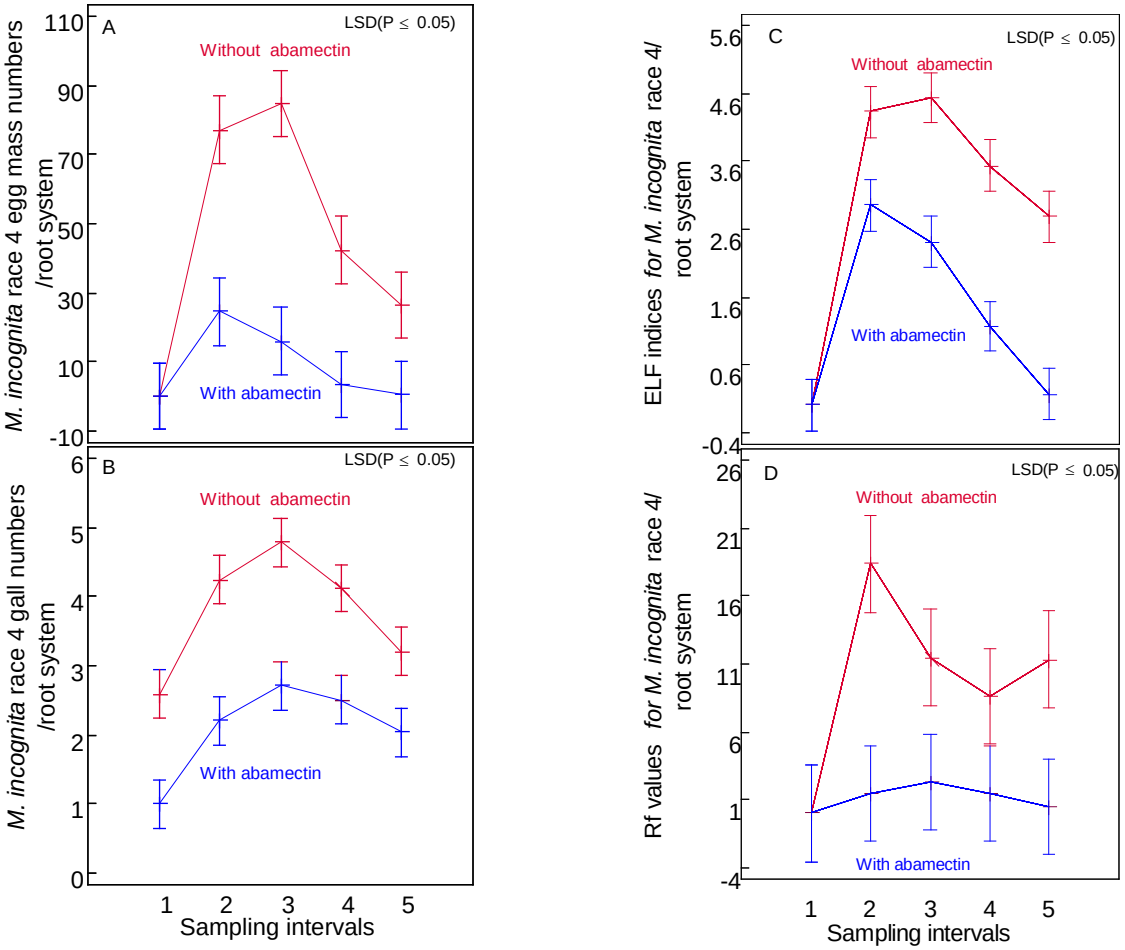


Figure 3.8 (A, B, C & D). The effect of abamectin and non-abamectin treatments on *Meloidogyne incognita* race 4 egg mass (A), gall numbers (B), egg-laying females (ELF; C) as well as reproduction factor (Rf values; D) per root system when data were pooled for four local commercially-available cotton cultivars during five sampling intervals.

Egg mass numbers were significantly ($P \leq 0.05$) higher during all sampling intervals for the non-abamectin treatment compared to those for the abamectin treatment, except for interval one (Fig. 3.8A). For the non-abamectin treatment egg mass numbers/root system peaked during sampling interval three and were significantly ($P \leq 0.05$) lower for sampling interval one compared to those for intervals two, three, four and five. For the abamectin treatment egg mass numbers peaked during sampling interval two, which did not differ significantly from those of sampling intervals three, four and five. Egg mass numbers/root system recorded for sampling interval two were, however, higher than those obtained for sampling interval one (Fig 3.8A).

Significant differences were evident for gall numbers/root system for both treatments and cultivars when data were pooled (Table 3.4 Fig. 3.8B). The non-abamectin treatment maintained significantly ($P \leq 0.05$) higher gall numbers/root system during all sampling intervals compared to those for the abamectin treatments. Gall numbers peaked for the non-abamectin treatment during sampling interval three and were significantly higher than those for intervals one and five, but not to those for intervals two and three. For the abamectin treatment gall numbers peaked during sampling interval three, with significantly ($P \leq 0.05$) lower gall numbers being recorded for sampling interval one, but not for intervals two, four and five.

ELF indices were significantly lower for the abamectin treatment during all the sampling intervals, except for interval one, when compared to those for the non-abamectin treatment (Table 3.4; Fig. 3.8C). For the non-abamectin treatment ELF indices peaked during sampling interval three and were significantly ($P \leq 0.05$) higher when compared to those for sampling intervals one, four and five, but not to those for interval two. ELF indices peaked during sampling interval two for the abamectin treatment and were significantly ($P \leq 0.05$) higher than those for intervals one, four and five.

Rf values were inversely related and were significantly ($P \leq 0.05$) lower for the abamectin treatment during sampling intervals two, three and five compared to those for the non-abamectin treatment (Fig. 3.8D). For the non-abamectin treatment Rf values for the first sampling interval were significantly ($P \leq 0.05$) lower compared to those for intervals two, three, four and five. Rf values did, however, not differ significantly ($P \leq 0.05$) for the five sampling intervals for the abamectin treatment.

Table 3.11. Significance and interaction data for *Meloidogyne incognita* race 4 numbers for the number of egg masses and galls/root system as well as egg-laying females (ELF) and Rf values for the four local commercially-available cotton cultivars and two treatments during five sampling intervals.

Parameter	Source	F-ratio	P-value	F-ratio	P-value
		T1[1]		T2[2]	
Number of egg masses/root system	Cultivars	1.83	0.1507	0.89	0.4512
	Sampling intervals	41.98	0.0000*	32.90	0.0000*
	Cultivars x sampling intervals	0.68	0.7623	2.88	0.0034*
Number of galls/root system	Cultivars	1.83	0.1507	3.54	0.0197*
	Sampling intervals	28.58	0.0000*	24.92	0.0000*
	Cultivars x sampling intervals	0.68	0.7623	1.69	0.0927
ELF indices	Cultivars	1.84	0.1498	0.61	0.6141
	Sampling intervals	118.72	0.0000*	79.36	0.0000*
	Cultivars x sampling intervals	1.18	0.3207	2.42	0.0124*
Rf values	Cultivars	0.57	0.6381	4.55	0.0061*
	Sampling intervals	9.32	0.0000*	10.97	0.0000*
	Cultivars x sampling intervals	1.30	0.2399	3.91	0.0002*

* Differences are significant

[1]Treatment 1 = non abamectin treatment; [2]Treatment 2 = abamectin treatment.

No significant ($P \leq 0.05$) differences were recorded among the four cultivars for both the non-abamectin and abamectin treatments in terms of number of egg masses and ELF indices/root system (Table 3.11). For the number of galls/root system as well as for Rf values, significant ($P \leq 0.05$) differences for cultivars were obtained for the abamectin treatment, but not for the non-abamectin treatment.

The five sampling intervals differed significantly ($P \leq 0.05$) from one another for both treatments and for all four nematode parameters measured and calculated (Table 3.11).

Significant ($P \leq 0.05$) interactions were evident between the cultivars and sampling intervals only for the abamectin treatment for the number of egg masses/root system as well as for ELF indices and Rf values (Table 3.11). This data indicated that the four cultivars did not react the same during the various sampling intervals.

Table 3.12. Data for *Meloidogyne incognita* race 4 number of galls/root system as well as Rf values for the abamectin treatment four local commercially-available cotton cultivars when pooled for the five sampling intervals [data were not *ln*(*x*+1) transformed)].

Cultivar number and name	Number of galls/root system	Rf values
1.Delta OPAL®	2 b[1]	1 a[1]
2.Nu OPAL®	1.7 b	2 b
3.Nu OPAL RR®	2 b	1 a
4.Delta OPAL RR®	1 a	1 a

[1]Means in the same column followed by the same letter do not differ significantly (P ≤0.05) according to theTukey Test (Statgraphics Plus 5 for Windows).

Significantly (*P* ≤ 0.05) lower gall numbers, but higher Rf values/root system was evident for cultivar Nu OPAL® compared to those for the other three cultivars for the abamectin treatment (Table 3.12).

Table 3.13. Data for *Meloidogyne incognita* race 4 number of egg masses and galls/root system as well as egg-laying females (ELF) and Rf values for the five sampling intervals for both the non-abamectin and abamectin treatments when pooled for the four local commercially-available cotton cultivars [data were not *ln*(*x*+1) transformed].

Sampling interval	T1[1]				T2[2]			
	Egg masses	Galls	ELF	Rf	Egg masses	Galls	ELF	Rf
1	0 a[3]	3 a[3]	0 a[3]	0 a[3]	0 a[3]	1 a[3]	0 a[3]	0 a[3]
2	77 d	4 b	4 c	18 b	24 c	2 bc	3 c	1.4 b
3	85 d	5 c	5 d	11 b	16 b	3 c	2 d	2.3 c
4	42 c	4 b	4 c	9 b	4 a	3 cd	1 b	1.4 b
5	26 b	3 a	3 b	11 b	0 a	2 b	0.2 a	0.5 a

[1]Treatment 1 = non abamectin treatment; [2]Treatment 2 = abamectin treatment.

[3]Means in the same column followed by the same letter do not differ significantly (P ≤0.05) according to theTukey Test (Statgraphics Plus 5 for Windows) and applies only for each sampling interval.

Significant (*P* ≤ 0.05) differences were evident among the sampling intervals for all four nematode parameters measured for both the non-abamectin and abamectin treatments when data were pooled for cultivars (Tables 3.13). Data for these nematode parameters were significantly (*P* ≤ 0.05) lower during sampling interval one compared to those for the other sampling intervals, except for gall numbers/root system where intervals one and five did not differ significantly (*P* ≤ 0.05) from each other for the non-abamectin treatment. The same applied for egg mass numbers/root system, ELF indices and Rf values for the abamectin treatment (Table 3.13). Data for all nematode parameters for both treatments generally

decreased from interval four, except for Rf values for the non-abamectin treatment that generally increased throughout the duration of the trial (Table 3.13).

Table 3.14. Data for *Meloidogyne incognita* race 4 number of egg masses and galls/root system as well as egg-laying females (ELF) indices and Rf values for four local commercially-available cotton cultivars and two treatments during five sampling intervals.

Sampling interval	Cultivar number and name	Egg masses/root System		Galls/root system		ELF indices		Rf values	
		T1[1]	T2[2]	T1	T2	T1	T2	T1	T2
1	1.Delta OPAL®	0 a[1]	0 a[3]	3 a[1]	0.8 a[1]	0 a[1]	0 a[1]	0 a[1]	0 a[1]
	2.Nu OPAL®	0 a	0 a	2 a	0.6 a	0 a	0 a	0 a	0 a
	3.Nu OPAL RR®	0 a	0 a	3 a	1 a	0 a	0 a	0 a	0 a
	4.Delta OPAL RR®	0 a	0 a	3 a	1 a	0 a	0 a	0 a	0 a
2	1.Delta OPAL®	81 a	24 a	5 a	2 a	4 a	3 a	26 a	2 a
	2.Nu OPAL®	85 a	14 a	5 a	1 a	5 a	2 a	20 a	1 a
	3.Nu OPAL RR®	74 a	34 a	5 a	3 a	5 a	3 a	19 a	1 a
	4.Delta OPAL RR®	69 a	25 a	4 a	3 a	4 a	3 a	9 a	2 a
3	1.Delta OPAL®	74 a	8 a	5 a	3 a	4 a	2 a	11 a	1 a
	2.Nu OPAL®	87 a	31 a	5 a	3 a	5 a	3 a	11 a	6 b
	3.Nu OPAL RR®	99 a	15 a	5 a	3 a	4 a	3 a	18 a	2 a
	4.Delta OPAL RR®	79 a	10 a	5 a	3 a	5 a	2 a	6 a	1 a
4	1.Delta OPAL®	36 a	5 a	4 a	3 a	4 a	2 a	6 a	2 a
	2.Nu OPAL®	58 a	6 a	4 a	2 a	4 a	2 a	12 a	2 a
	3.Nu OPAL RR®	39 a	1 a	4 a	3 a	4 a	1 a	6 a	1 a
	4.Delta OPAL RR®	36 a	1 a	4 a	2 a	3 a	1 a	11 a	1 a
5	1.Delta OPAL®	5 a	0 a	4 a	2 a	2 a	0 a	8 a	0.2 a
	2.Nu OPAL®	43 a	0 a	4 a	2 a	3 a	1 a	16 a	1 a
	3.Nu OPAL RR®	25 a	0 a	3 a	2 a	3 a	0 a	7 a	0.4 a
	4.Delta OPAL RR®	33 a	0 a	3 a	2 a	3 a	0 a	15 a	0.4 a

[1]Treatment 1 = non abamectin treatment; [2]Treatment 2 = abamectin treatment

[3]Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to the Tukey Test (Statgraphics Plus 5 for Windows) and applies only for each sampling interval since data were separately analysed for each of the treatments.

Egg mass numbers/root system of zero were recorded for all cultivars during sampling interval one (Table 3.14). Although no significant ($P \leq 0.05$) differences were evident for the non-abamectin treatment among the four cultivars in terms of egg mass numbers/root system throughout the duration of the experiment (Table 3.14). It ranged between 69 and 85 for interval two, 74 and 99 for interval three, 36 and 58 for interval four and five and 43 for interval five for the non-abamectin treatment. These egg mass numbers recorded resembles

the classification of moderately resistant, susceptible and very susceptible crops as categorised by the system of Murray *et al*. (1986) Table 3.1. For the abamectin treatment egg mass numbers/root system ranged between 14 and 34 for sampling intervals two, eight to 31 for interval three and one to six for interval four. No egg masses were recorded on roots of all four cultivars during interval five. Egg mass numbers recorded for the latter treatment resembles the classification of resistant, moderately resistant and susceptible crops (Murray *et al*., 1986; Table 3.1).

The numbers of galls/root systems ranged between two and three for the non-abamectin and 0.6 to one for the abamectin treatment during sampling interval one (Table 3.14). For the non-abamectin treatment gall numbers ranged between four and five for sampling interval two, was five galls/root system during interval three, four galls/root system during interval four and ranged between three and four during sampling interval five. For the abamectin treatment, it ranged from one to three during interval two, was three galls/root system during interval three, ranged from two to three during interval four and was two galls/root system during sampling interval five.

ELF indices of zero were recorded for all cultivars during sampling interval one (Table 3.14), but ranged between four and five for the non-abamectin treatment for sampling intervals two and three, three and four for interval four and two and three for interval five. These ELF indice-values resemble the classification of moderately resistant, susceptible and very susceptible crops according to the system of Murray *et al*. (1986). ELF indices for the abamectin treatment, however, ranged between two and three for intervals two and three, one and two for interval four and zero and one for interval five. For the latter treatment these values resembled the classification of resistant and moderately resistant crops as indicated by the system of Murray *et al.* (1986; Table 3.1).

Rf values of zero were calculated for all cultivars and for both treatments during sampling interval one (Table 3.14). For the non-abamectin treatment it ranged from nine to 26 during interval two, from six to 11 during interval three, from six to 12 during interval four and from seven to 15 during interval five. For the latter treatment Rf values generally resembled the classification of good as well as excellent hosts according to the system of Windham and Williams (1988; Table 3.2). For the abamectin treatment Rf values ranged between one and two during intervals two and four, one and six during interval three and 0.2 and one during interval five. These Rf values resembled the classification of resistant/poor host crops according to the system of Windham and Williams (1988; Table 3.2).

3.3.2. Plant growth parameter data

Table 3.15. Significance and interaction data for plant growth parameters for cotton seedlings inoculated with *Meloidogyne incognita* race 4 eggs and second-stage juveniles (J2) numbers/root system during five sampling intervals for a non-abamectin and abamectin treatment and pooled for cultivars [data were not *ln*(*x*+1) transformed].

Parameter	Source	F-ratio	P-value
Number of squares/plant	Treatment	8.13	0.0048*
	Cultivar	0.66	0.5752
	Sampling interval	474.18	0.0000*
	Treatment x interval	4.58	0.0015*
	Treatment x cultivar	1.13	0.1482
	Cultivar x interval	1.80	0.3348
	Treatment x cultivar x interval	1.70	0.7000
Number of flowers/plant	Treatment	2.23	0.1369
	Cultivar	0.40	0.7529
	Sampling interval	122.21	0.0000*
	Treatment x interval	0.71	0.5842
	Treatment x cultivar	0.47	0.7030
	Cultivar x interval	0.99	0.4559
	Treatment x cultivar x interval	0.93	0.5154
Number of bolls/plant	Treatment	0.69	0.4062
	Cultivar	0.20	0.8949
	Sampling interval	355.81	0.0000*
	Treatment x interval	0.69	0.5977
	Treatment x cultivar	1.36	0.2573
	Cultivar x interval	1.37	0.9186
	Treatment x cultivar x interval	0.74	0.7077
Biomass (g)/plant	Treatment	44.54	0.0000*
	Cultivar	1.21	0.3084
	Sampling interval	815.68	0.0000*
	Treatment x interval	6.18	0.6484
	Treatment x cultivar	0.55	0.0001*
	Cultivar x interval	1.37	0.1843
	Treatment x cultivar x interval	1.05	0.4038
Root mass (g)/plant	Treatment	17.44	0.0001*
	Cultivar	0.23	0.8745
	Sampling interval	223.30	0.0000*
	Treatment x interval	4.53	0.0029*
	Treatment x cultivar	0.43	0.7292
	Cultivar x interval	1.02	0.4438
	Treatment x cultivar x interval	0.65	0.7866

*Differences are significant.

Plant growth parameters were not compared among the four cultivars since each cultivar exhibits its own intrinsic features and therefore differs genetically from one another. In contrast each cultivar was compared against itself in this regard particularly in terms of its performance for the non-abamectin and the abamectin treatment. Although F-ratio's and P-values for cultivars are listed in Table 3.15, it will not be considered for the purpose of this study.

Significant differences existed for treatments when data were pooled for the four cultivars only for the number of squares/plant, biomass (g)/plant and root mass (g)/plant (Table 3.15). In terms of sampling intervals, all plant growth parameters measured differed significantly ($P \leq 0.05$) among the five intervals.

Significant interactions ($P \leq 0.05$) existed between treatments and sampling intervals for the number of squares/plant and root mass when data were pooled for the four cultivars (Table 3.15). The two treatments thus reacted differently during the different intervals for these two parameters. A significant interaction between treatments and cultivars only existed for biomass (g)/plant, indicates that the cultivars did not react the same in terms of biomass production with regard to the two treatments. No three-way interaction existed between treatments, cultivars and sampling intervals for any of the plant growth parameters measured during this study.

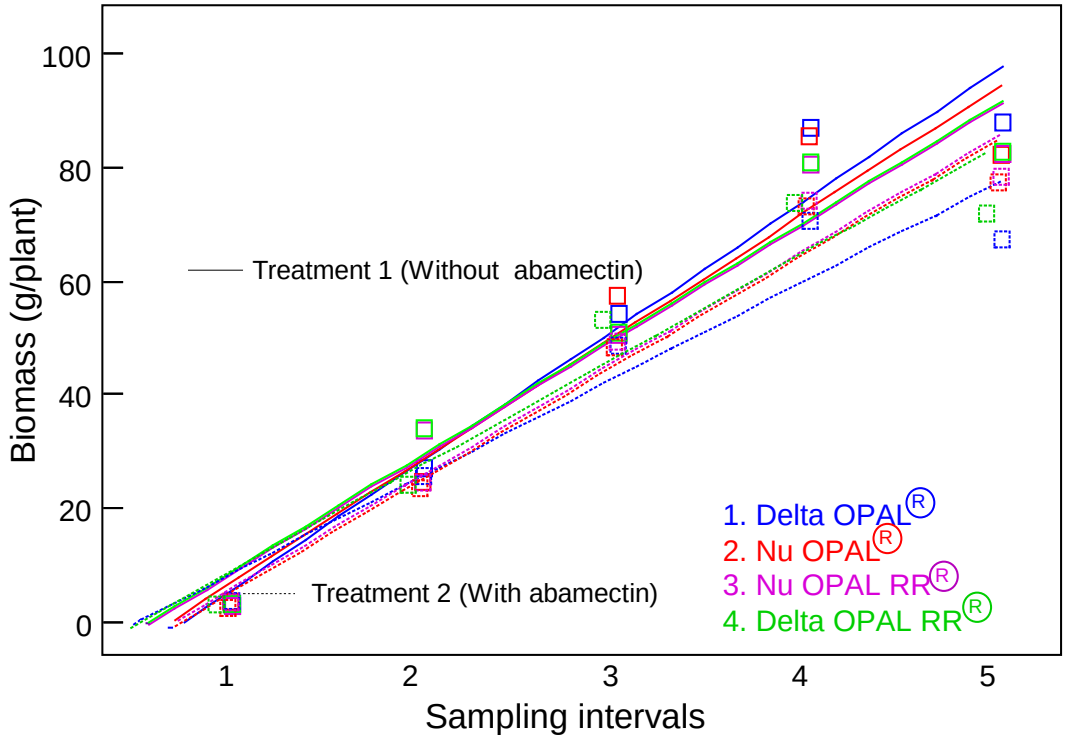


Figure 3.9. Biomass data for four commercially available cotton cultivars infected with *Meloidogyne incognita* race 4 egg and second-stage juveniles (J2) during five samplings for a non-abamectin and abamectin and treatment [data were not *ln*(*x*+1) transformed].

Table 3.16. Equations for linear regression lines that described the relationships between biomass (g)/plant that were inoculated with approximately 2 500 *Meloidogyne incognita* race 4 eggs and second-stage juveniles (J2)/seed for four cotton cultivars during five sampling intervals for a non-abamectin and abamectin treatment [data were not *ln*(*x*+1) transformed].

Cultivar name and number	Equation	
	Non-abamectin (T1)	Abamectin (T2)
1. Delta OPAL®	y = -17.2+23*X	y = -8.3+17.3*X
2. Nu OPAL®	y = 15.5+21.9*X	y = -14.4+20*X
3. Nu OPAL RR®	y = -11.7+20.7*X	y = -8.6+17.4*X
4. Delta OPAL RR®	y = -10.6+21.6*X	y = -10.2+18.8*X

The relationships between biomass (g)/plant and five sampling intervals for four cotton cultivars were best described by strong, linear equations for all four cultivars used for both the non-abamectin and abamectin treatments (Fig. 3.9; Table 3.16). Significant R^2 values (Fig. 3.9) were obtained for all four cultivars by means of polynomial models for both treatments, which significantly ($P \leq 0.05$) explained the variation.

Although the four cultivars could not be compared to one another in terms of its biomass (g) production/plant, the cultivars had similar regression lines for the non-abamectin treatment when inoculated with *M. incognita* race 4 (Fig. 3.9). Regression lines for all four cultivars showed a constant increase during the five sampling intervals. The latter scenario also applied for the abamectin treatment with the four cultivars used during this study showing similar regression lines for the latter treatment (Table 3.16). Biomass (g)/plant were significantly lower for all four cultivars for the abamectin treatment compared to those for the non-abamectin treatment.

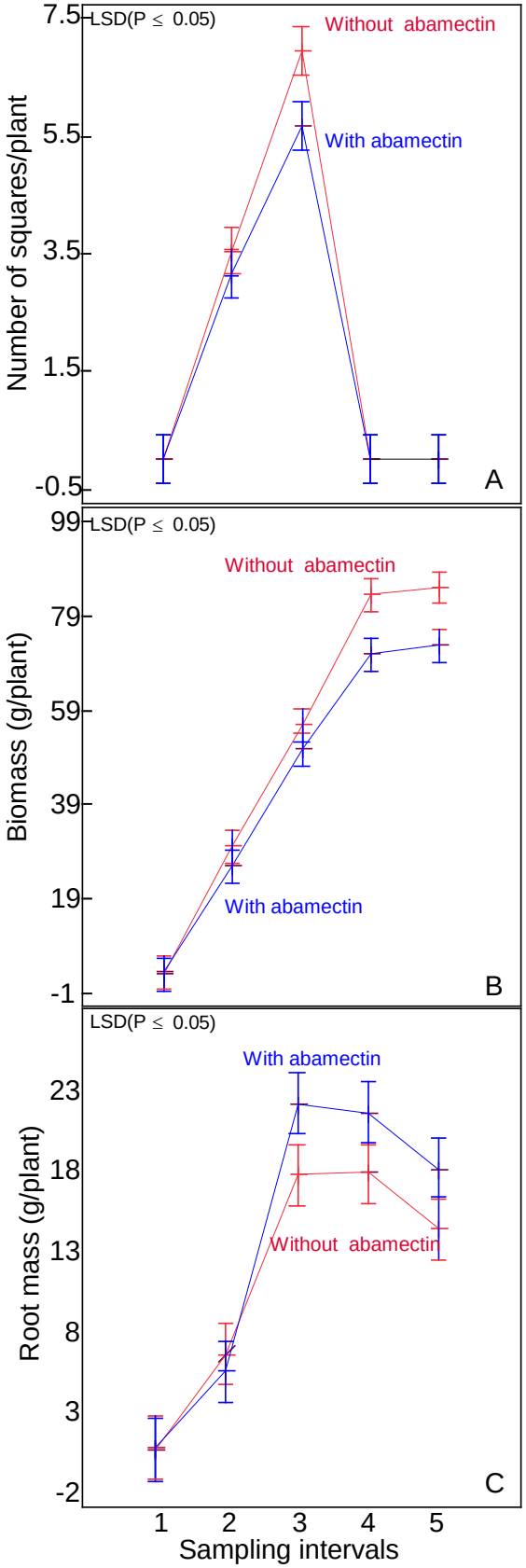


Figure 3.10 (A, B & C). The effect of treatments when data for cultivars were pooled during five sampling intervals with regard to the number of squares/plant (A), biomass (g)/plant (B) and root mass (g)/plant (C) for cotton plants infected with approximately 2 500 *Meloidogyne incognita* race 4 eggs and J2 in a greenhouse trial [data were not *ln*(*x*+1) transformed].

When data were pooled for the four cultivars, the number of squares/plant peaked during sampling interval three and were significantly ($P \leq 0.05$) higher in both treatments during the latter interval compared to those that developed during sampling interval two (Fig. 3.10A). No square development was recorded during intervals one, four and five for all four cultivars and for both treatments.

Biomass of cultivar data (when pooled) were significantly ($P \leq 0.05$) higher for the non-abamectin treatment than those for the abamectin treatment during sampling intervals four and five (Fig. 3.10B). The latter two intervals also contained cotton plants that produced significantly ($P \leq 0.05$) higher biomass than those during sampling intervals one, two and three. The same scenario applied for the abamectin treatment. Biomass of plants of all four cultivars gradually increased during sampling intervals one, two and three, which differed significantly ($P \leq 0.05$) from each other, and then levelled off for sampling intervals four and five. This trend was recorded for both treatments.

Root mass (g)/plant data when pooled for the four cultivars were only significantly ($P \leq 0.05$) higher for the abamectin treatment during sampling interval three, compared to those for the non-abamectin treatment (Fig. 3.10C). Root mass (g)/plant were highest for the abamectin treatment during sampling interval three, did not differ significantly ($P \leq 0.05$) from that for the fourth interval but from those from the first, second and fifth intervals. The same scenario applied for the non-abamectin treatment. Root mass gradually increased during sampling intervals one and two and levelled off from sampling interval three for both treatments.

Table 3.17. Significance and interaction data for number of squares, flowers, bolls as well as biomass (g) and root mass (g)/cotton plant for the four local commercially-available cultivars that were inoculated with approximately 2 500 *Meloidogyne incognita* race 4 eggs and second-stage juveniles (J2)/seed for both a non-abamectin and abamectin treatment during five sampling intervals [data were not *ln*(*x*+1) transformed].

Parameter	Source	F-ratio	P-value	F-ratio	P-value
		T1[1]		T2[2]	
Number of squares	Cultivar	1.41	0.2446	0.84	0.4758
	Sampling intervals	234.75	0.0000*	214.45	0.0000*
	Cultivar x interval	1.34	0.2212	1.31	0.2384
Number of flowers	Cultivar	0.73	0.5410	0.42	0.7397
	Sampling intervals	90.00	0.0000*	65.90	0.0000*
	Cultivar x interval	0.57	0.8543	1.49	0.1539
Number of bolls	Cultivar	0.50	0.6858	1.68	0.1844
	Sampling intervals	146.81	0.0000*	307.43	0.0000*
	Cultivar x interval	0.20	0.9989	1.78	0.0273
Root mass (g)	Cultivar	0.07	0.9760	0.69	0.5611
	Sampling intervals	82.53	0.0000*	166.36	0.0000*
	Cultivar x interval	0.84	0.6121	0.95	0.5097
Biomass (g)	Cultivar	0.66	0.5773	1.84	0.1496
	Sampling intervals	334.79	0.0000*	781.32	0.0000*
	Cultivar x interval	0.87	0.5849	2.69	0.0058*

*Differences are significant.

[1] Treatment 1 (non abamectin treatment); [2] Treatment 2 (abamectin treatment).

Significant ($P \leq 0.05$) differences were evident for all plant growth parameters measured among the five sampling intervals both for the non-abamectin and abamectin treatments (Table 3.17).

Although a significant interaction existed for biomass (g)/plant between cultivars and sampling intervals for the abamectin treatment (Table 3.17), this data warrants no further discussion.

Table 3.18. Plant parameter data (real means; data were not *ln*(*x*+1) transformed) of four cotton cultivars inoculated with approximately 2 500 *Meloidogyne incognita* race 4 eggs and second-stage juveniles (J2)/seed for a non-abamectin and abamectin treatment during the five sampling intervals.

Sampling Interval	**T1[1]**					**T2[2]**				
	Squares	**Flowers**	**Bolls**	**Root mass (g)**	**Biomass**	**Squares**	**Flowers**	**Bolls**	**Root mass (g)**	**Biomass**
1	0 a	0 a	0 a	1 a	3 a	0 a	0 a	0 a	1 a	3 a
2	4 b	0 a	0 a	7 b	30 b	3 b	0 a	0 a	6 b	26 b
3	7 c	2 c	0 a	18 d	56 c	6 c	2 b	0 a	22 d	51 c
4	0 a	0.4 b	3 b	18 d	84 d	0 a	1 c	3 b	22 d	71 d
5	0 a	0 a	3 b	15 c	85 d	0 a	0.1 a	3 b	18 c	73 d

[1] Treatment 1 (non abamectin treatment); [2] Treatment 2 (abamectin treatment).

Data for all plant growth parameters differed significantly with regard to the five sampling intervals for both treatments when data were pooled for cultivars (Fig. 3.18). Biomass (g)/plant increased gradually throughout the duration of the trial for both the non-abamectin and abamectin treatments. Root mass for the four cultivars peaked during sampling interval four and then decreased during sampling interval five for both treatments.

Table 3.19. Plant growth parameter data (real means; data were not *ln*(*x*+1) transformed) of four local commercially-available cotton cultivars inoculated with approximately 2 500 *Meloidogyne incognita* race 4 eggs and second-stage juveniles (J2)/seed during the five sampling intervals (SI) for four cultivars and two treatments.

SI	Cultivar number and name	Number of first squares		Number of flowers		Number of bolls		Biomass (g/plant)		Root mass (g/root)	
		T1[1]	T2[2]	T1	T2	T1	T2	T1	T2	T1	T2
1	1.Delta OPAL®	0 a[3]	0 a[1]	0 a[1]	0 a[1]	0 a[1]	0 a[1]	3 a[1]	4 a[1]	0.7 a[1]	0.9 a*1*
	2.Nu OPAL®	0 a	0 a	0 a	0 a	0 a	0 a	3 a	3 a	0.8 a	0.9 a
	3.Nu OPAL RR®	0 a	0 a	0 a	0 a	0 a	0 a	3 a	3 a	0.8 a	0.9 a
	4.Delta OPAL RR®	0 a	0 a	0 a	0 a	0 a	0 a	3 a	4 a	0.7 a	0.9 a
2	1.Delta OPAL®	3 a	3 a	0 a	0 a	0 a	0 a	27 a	26 a	6 a	6 a
	2.Nu OPAL®	3 a	3 a	0 a	0 a	0 a	0 a	24 a	24 a	6 a	5 a
	3.Nu OPAL RR®	4 a	4 a	0 a	0 a	0 a	0 a	34 a	29 a	7 a	7 a
	4.Delta OPAL RR®	4 a	3 a	0 a	0 a	0 a	0 a	35 a	25 a	7.7 a	6 a
3	1.Delta OPAL®	7 a	5 a	2 a	2 a	0 a	0 a	54 a	49 a	16 a	20 a
	2.Nu OPAL®	7 a	6 a	2 a	2 a	0 a	0 a	57 a	49 a	20 a	23 a
	3.Nu OPAL RR®	6 a	6 a	2 a	2 a	0 a	0 a	51 a	50 a	17 a	22 a
	4.Delta OPAL RR®	8 a	5 a	2 a	3 a	0 a	0 a	61 a	54 a	20 a	24 a
4	1.Delta OPAL®	0 a	0 a	0 a	1 a	4 a	3 a	87 a	71 a	19 a	23 a
	2.Nu OPAL®	0 a	0 a	0 a	1 a	3 a	3 a	85 a	74 a	17 a	24 a
	3.Nu OPAL RR®	0 a	0 a	1 a	1 a	3 a	3 a	81 a	63 a	19 a	19 a
	4.Delta OPAL RR®	0 a	0 a	0 a	0 a	3 a	3 a	81 a	75 a	17 a	21 a
5	1.Delta OPAL®	0 a	0 a	0 a	0 a	4 a	2 a	88 a	68 a	16 a	18 a
	2.Nu OPAL®	0 a	0 a	0 a	0 a	3 a	3 a	82 a	78 a	14 a	20 a
	3.Nu OPAL RR®	0 a	0 a	0 a	0 a	3 a	4 a	83 a	73 a	16 a	17 a
	4.Delta OPAL RR®	0 a	0 a	0 a	0 a	3 a	3 a	87 a	73 a	13 a	18 a

[1]Treatment 1 = non abamectin treatment; [2] Treatment 2 = abamectin treatment.

[3]Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to the Tukey Test (Statgraphics Plus 5 for Windows) and applies only for each sampling interval.

Squares developed from 50 DAP and inoculation and were evident during the second and third sampling intervals, with numbers ranging from three to eight for the non-abamectin treatment and from three to six for the abamectin treatment (Table 3.19). No significant ($P \leq 0.05$) differences were recorded among the cultivars for square development for both treatments.

Flowers developed form the 67th DAP and inoculation for all four cultivars both for the non-abamectin and abamectin treatments and were observed and counted during the third and fourth sampling intervals (Table 3.19). The numbers of flowers/plant ranged from zero to two for the non-abamectin treatment and from zero to three for the abamectin treatment during the latter sampling intervals.

Bolls developed from the 91th DAP and inoculation for all four cultivars both for the non-abamectin and abamectin treatments and were observed and counted during the fourth and fifth sampling intervals (Table 3.19). The number of bolls/plant ranged between three and four for the non-abamectin treatment and between two and four for the abamectin treatment.

Root mass (g) increased consistently and ranged from 0.7g/plant to 20g/plant for the non-abamectin treatment for the four cultivars during the five sampling intervals and from 0.9g/plant to 24g/plant for the abamectin treatment during the same period (Table 3.19).

Biomass (g) increased consistently and ranged from 3g/plant to 87g/plant for the non-abamectin treatment during the five sampling intervals and from 3g/plant to 78g/plant for the abamectin treatment during the same period (Table 3.19).

3.4. Discussion

The potential of the 0.15mg abamectin a.i./seed treatment in reducing *M. incognita* race 4 population levels in roots of four local commercially-available cotton cultivars proved to exist as a result of this preliminary study. In addition, the four cultivars Delta OPAL®, Delta OPAL RR®, Nu OPAL® and Nu OPAL RR® that were used during this study and concurrently evaluated for their host suitability against *M. incognita* race 4, were also identified as susceptible hosts to this root-knot nematode species and race. Although the abamectin treatment generally resulted in significantly lower numbers of all nematode parameters measured for the four cultivars compared to those for the non-abamectin treatment, population levels of these parasites were still relatively high. This trend emphasizes the importance of additional nematode control strategies being used in combination with or supplemental to this dosage rate treatment of abamectin. It further accentuates that abamectin may be considered particularly where lower populations of RKN are present in cotton fields.

Contrary to reports by Faske and Starr (2007), but in accordance to results obtained during this study, Monfort *et al*. (2006b) reported that an abamectin treatment at a dosage rate of 0.1mg a.i./seed reduced infection of *M. incognita*. According to the latter authors, less severe root galling due to infection by *M. incognita* race was visible during the early growth stages of cotton plants treated with abamectin in both greenhouse and microplot trials 14 DAP. However, Faske and Starr (2007) reported no significant difference being evident between abamectin-treated seed at a dosage rate of 0.15mg a.i./seed and a non-abamectin treatment in terms of root galling and nematode reproduction due to infection by *M. incognita* race. Important, however, is that abamectin-treated cotton seeds used in their study were additionally coated with three fungicides (azoxystrobin, fludioxinil and mefenoxam) and an insecticide (thiamethoxam). According to Faske and Starr (2007) interactions that occurred among these chemical treatments may have contributed to the small portion of abamectin that were suspected to be transferred along the developing cotton root systems of the plants. Cotton seeds of the cultivars used in our study was also coated with the two fungicides carboxin and thiram prior to the abamectin treatment and resulted in significant data being obtained in terms of a reduction in RKN population levels. Faske and Starr (2007), however, also reported that reduced penetration by *M. incognita* was most significant when the taproot length of cotton plants were 5cm compared to 15-cm long roots. This further supports the probability of dilution that may occur when abamectin is transferred along the developing root system of a cotton plant. The potential dilution effect of abamectin during development of the roots of cotton plants, however, warrants further investigation to substantiate this trend.

The classification of the four cultivars used during this study as being susceptible to the *M. incognita* race 4 population used, is based particularly on data that were recorded for the non-abamectin treatment with regard to egg and J2, egg mass and gall numbers/root systems as well as for ELF indices and Rf values. Results from this study with regard to cultivar Delta OPAL® also corresponds with reports from Van Biljon and Bleve (2005). These authors reported that the latter cultivar as well as Gamka® and Acala OR3® were identified as susceptible to the *M. incognita* race 4 population used in their study. The polynomial population development curve for the latter cultivar that was reported by the latter authors was also confirmed for the four cultivars that were used in this present study.

It is, however, important to bear in mind that significant differences that were evident among the four cultivars for egg and J2 numbers/root systems during this study should not be accentuated. Although cultivar Nu OPAL® maintained significant higher numbers of eggs and J2/root systems than those maintained by the other three cultivars (when data for the five sampling intervals were pooled) it is still highly susceptible to this RKN species and race, like the other three cultivars used in this study. Producers could, however, be recommended to rather plant Delta OPAL®, Delta OPAL RR®, or Nu OPAL RR® than Nu OPAL® to reduce the build-up of *M. incognita* race 4 numbers in their fields. The ultimate is that all four these cultivars showed potential to maintain *M. incognita* race 4 individuals and may result in a build-up of these parasites in local cotton fields infested with this species and race. Furthermore, the two Round-Up Ready (RR) cultivars Delta OPAL RR® and Nu OPAL RR® used in this study are popular and currently dominate the cotton market due to its favourable characteristics in terms of insect- and herbicide resistance (Olivier, 2009: personal communication). No reports on the host suitability of these cultivars have been made prior to this study. It is thus important to emphasise that the planting of these cultivars may cause problems in cotton-based crop systems if *M. incognita* race-4 infections are not controlled by using nematode management strategies such as nematicides or others. Results from this study provide further proof that it is important to consider as many as possible nematode parameters when investigating aspects such as the suitability of crop cultivars to a given RKN. Rf values were used in this study as the main criteria for host suitability since the primary objective in a crop rotation system is to apply the management strategy that will result in the lowest number of PPN maintained by the current crop. However, root galling and ELF-indices are also used widely as criteria to determine root-knot nematode resistance, especially under field conditions where laboratory infrastructure are not available to extract eggs and J2 (Hussey & Boerma, 1981). These indices are, however, not as accurate as Rf values.This trend has been indicated in other studies on soybean cultivars (Hussey and Boerma, 1981; Fourie *et al*., 2001, 2006) and on cotton (Shepherd, 1979). In

some of these latter cases crop cultivars exhibited low gall ratings but high ELF indices and high numbers of eggs per plant. Use of for example gall ratings would, therefore, lead to misinterpretation of results obtained during this study. This trend was also evident during this study when no egg masses/root system, ELF indices or Rf values were recorded during sampling interval one. However, galls per root system were recorded for both treatments. This phenomenon illustrates that although galls were formed, the females within these galls were not mature yet and therefore no egg masses were produced during sampling interval one.

The effect of the prominent growth stages of cotton plants in terms of their development for both the non-abamectin and abamectin treatments with regard to all nematode and plant growth parameters is another important aspect that has been illustrated during this study. Results from this study showed that these growth stages should not be disregarded in terms of RKN development. It is important to bear in mind that these prominent growth stages of cotton plants, which represented the five sampling intervals used during this study, generally corresponds with the major metabolic processes that occur within the cotton plant (Ritchie *et al*., 2007). Since RKN development has a substantial impact on the metabolic processes within plant cells (Moens *et al*., 2009), the range of these growth stages in terms of time are most probably the period when RKN populations should be controlled optimally within cotton roots to prevent the build-up of high levels.

Furthermore, data from this study showed that when data were pooled for cultivars the two treatments were significantly affected by sampling intervals. This illustrates that the non-abamectin and the abamectin treatments did not react the same during the five sampling intervals and was substantiated since data for *M. incognita* race 4 parameters were generally significantly higher for the non-abamectin treatments during intervals two to five. In terms of the four cultivars, a significant interaction between them and sampling intervals particularly for the abamectin treatment for eggs and J2/root system, showed that the cultivars reacted differently during the intervals. Since the four cultivars are genetically different, interactions between neither treatments and/or intervals with cultivars were considered valuable. Therefore, the latter data will not be discussed although interaction data in terms of treatments and intervals are valuable, this trial was conducted in a greenhouse under controlled conditions. Verification of such data in field trials under natural occurring environmental conditions will result in more accurate and reliable conclusions being made. Furthermore, since soil- and climatic conditions that occur in fields in local cotton-producing areas differ from those in the greenhouse where this study was conducted, nematode and plant growth data recorded during this study should be substantiated by field-

trial data. Greenhouse conditions may be sub-optimal for sustaining particularly the natural growth and development of cotton plants, which is in need of a high level of sunlight and heat units (Bredell, 2009: personal communication). Furthermore, the presence of a wide variety of micro-organisms in soils as well as prevailing physical and environmental factors may influence the efficacy of abamectin in reducing PPN populations and should thus be investigated in further research.

Although the use of abamectin as a nematicide shows potential as a result of this study, more sophisticated and accurate application techniques of abamectin on seeds of crops should be investigated. Such information may contribute to the optimum amount of abamectin being transferred to the developing cotton root system. The potential use of abamectin to reduce *M. incognita* race 4 population levels in roots of four most popular cultivars currently used by producers, however, adds value to both the cotton industry as well as the scientific community. Use of this product currently receives increased interest for its potential as an alternative nematicide (Olivier, 2009: personal communication; Coyne *et al*., 2009).

Chapter 4: Evaluation of the efficacy of abamectin as a seed treatment in reducing population levels of plant-parasitic nematodes (PPN) in field trials

4.1. Introduction

Standard nematicides that are currently available and registered for use on cotton in South Africa are expensive, highly toxic, pose health risks to humans and animals and have an adverse impact on the environment (Nel *et al*., 2007; Monfort *et al*., 2006b). In contrast, a novel seed-treatment nematicide such as abamectin that is derived from a natural occurring biotic source is a convenient and practical alternative to classical nematicides (Monfort *et al.*, 2006b; Jansson & Rabatin, 1998).

Ultimately, use of abamectin poses reduced environmental risks due to its poor leaching potential and rapid degradation in the soil profile (Monfort *et al.*, 2006b; Jansson & Rabatin, 1998). Therefore, market adoptability in terms of using such a product is favourable (Monfort *et al.*, 2006b; Jansson & Rabatin, 1998). In terms of its decomposition, abamectin rapidly breaks down in soils that contain high levels of organic materials (Cayrol *et al.*, 1993). Optimal control of PPN using this product has been reported to be more effective in sandy soils where low levels of organic matter generally occur (Cayrol *et al.*, 1993). Furthermore, reports from field trials in which the efficacy of abamectin was evaluated against *M. incognita* in the USA showed variable results when compared with those that were conducted in sterilized soil under controlled and semi-controlled conditions in greenhouse and microplots, respectively (Monfort *et al*., 2006b). This trend was confirmed by Faske and Starr (2007), who reported that numerous factors may influence the efficacy of abamectin in reducing PPN in cotton under field conditions.

It is important to bear in mind that the actual toxicity of abamectin is comparable to that of Class 1 classical nematicides such as aldicarb and others (Nel *et al.*, 2007). Exposure of PPN to abamectin results in irreversible paralysis of *M. incognita* and *R. reniformis* individuals in cotton roots (Faske & Starr, 2006). Reports by Faske and Starr (2006) indicated that application of abamectin at 0.15mg a.i./cotton seed exceeds the LD_{50} values for *M. incognita*. Although the presence of low concentrations of abamectin in the spermosphere and rhizosphere of cotton plants demonstrated irreversible paralysis of *M. incognita* and *R. reniformis* and further inhibited subsequent infection by these nematodes, these dosages have not yet been properly investigated and verified (Faske & Starr, 2006). Therefore, evaluation of various dosage rates of abamectin as a

seed treatment of cotton warrants further investigation in terms of its efficacy in reducing population levels of PPN and were addressed during this study.

4.1.1. Specific objective

The specific objective of this study was to evaluate the efficacy of abamectin as a seed treatment in reducing PPN populations, particularly those of *M. incognita* race 4, in field trials at various sites where cotton was commercially grown during the 2005/2006 and 2006/2007 growing seasons. Classical nematicides that are registered on cotton in South Africa (Nel *et al*., 2007) were included in these trials as standard treatments to compare their efficacy to that of abamectin. Although abamectin should rather be compared to another seed treatment, no such treatment(s) are currently registered and the inclusion of classical nematicides was the only option.

4.2. Materials and methods

4.2.1. Procedures

Trial sites were selected at localities that are situated in the Marble Hall (Limpopo Province) and Vaalharts (Northern Cape Province) areas where cotton is grown in soils where high infestations of *M. incognita* race 4 exist. Crops that are traditionally grown in rotation systems in the Marble Hall area include potato, maize, wheat, beans, tobacco and cotton (Bredell, 2009: personal communication). These crops are susceptible to particularly RKN (Keetch & Heyns, 1982; Mc Donald & Nicol, 2005) that predominantly occur in soils at these sites and generally result in a build-up of these parasites when used in cropping systems.

In total five field trials were conducted over two consecutive growing seasons, namely 2005/2006 and 2006/2007. These trials were planted at five different sites (Table 4.1), which were all located in commercially-grown cotton fields. In the Marble Hall area four trials were conducted, which included plots E6 (Trials A & C), J17 (Trial B) and 686 (Trail D). The fifth trial (Trial E) was conducted in the Vaalharts area near Jan Kempdorp. Trial A constitutes the only site where a trial was repeated during the two consecutive seasons within the same field. However, the trial area used during 2006/2007 was moved to an alternative site in the same field to avoid planting on the specific site that was used during the previous season. This way the potential residual effects from the nematicides that were evaluated during the 2005/2006 season were eliminated.

Soil samples were taken before the onset of these trials to determine values of parameters that are used as an indication of soil fertility and structure. These soil samples were analysed at the Institute for Industrial Crops (IIC) of the Agricultural Research Council (ARC) at Rustenburg (North-West Province). Soil nutrients were applied at the individual trial sites at the rates that are indicated in Table 4.1. Cotton seed used to plant the four trials were certified and obtained from Monsanto (Pty) Ltd., South Africa. During the 2005/2006 growing season trials were planted using cultivar Nu OPAL®, while cultivar Nu OPAL RR® was used in all trials that were conducted during the 2006/2007 growing season. The reason for the use of these two different cultivars was to adhere to the farmers' practices, which implied the overall broad application of the non-selective glyphosate herbicide. Therefore, the Roundup-Ready® (RR) cultivar Nu OPAL RR® was used during the latter season. Irrigation was provided at all trial sites by means of centre pivots, except for Vaalharts where sprinkler irrigation was used. Irrigation and supplementary rain figures for all trial sites are provided in Table 4.1.

Table 4.1. Information on trial sites where abamectin seed treatments were evaluated for its potential to reduce populations of plant-parasitic nematodes (PPN) during two consecutive growing seasons as well as soil nutrients applied, irrigation provided as well as rainfall figures.

Growing season	Trial number, site and planting dates	Clay %	Treat-ment	Product names for nematicides and other chemicals used	Dosage rates of nematicides and other chemicals applied (a.i. = active ingredient)	Soil nutrients (kg/ha) applied per trial	Irrigation and rainfall figures (mm) for each trial site
2005/2006	Trial A: E6 – Marble Hall (11/11/2005) 29°16'29.36"E	9	1	Avicta® 400 FS	0.15mg a.i. abamectin/seed	Nitrogen: 200	220.0 + 685.0
			2	Avicta® 400 FS	0.3mg a.i. abamectin/seed		
			3	Temik® 150 GR	1.35kg a.i. aldicarb/ha	Nitrogen: 200	255.4 + 685.0
	Trial B: J17 – Marble Hall (15/11/2005) 25°01'41.97"S 29°12'19.61"E	22	4	Nemacur® 100 GR	1.5kg a.i. fenamiphos/ha		
			5	Untreated control	-		
			6	Cruiser® 350 FS	0.3mg a.i. thiamethoxam/seed		
2006/2007	Trial C: E6 – Marble Hall (07/11/2006),	9	1	Avicta® 400 FS	0.15mg a.i. abamectin/seed	Nitrogen: 160	260.0 + 490.0
			2	Avicta® 400 FS	0.3mg a.i. abamectin/seed		
			3	Temik® 150 GR	1.35kg a.i. aldicarb/ha	Nitrogen: 200	266.0 + 490.0
	Trial D: 686 – Marble Hall (31/10/2006) 25°04'47.90"S 29°17'34.45"E	15	4	Nemacur® 100 GR	1.5kg a.i. fenamiphos/ha		
			5	Untreated control	-	Nitrogen: 180 Phosphorous: 20 Potassium: 60	190.0 + 577.7
			6	Cruiser® 350 FS	0.3mg a.i. thiamethoxam/seed		
	Trial E: Vaalharts (08/11/2006) 29°01'29.36"S 23°58'34.79"E	8	7	Avicta® 400 FS + Cruiser® 350 FS	0.15mg a.i. abamectin/seed + 0.3mg a.i. thiamethoxam/seed		

Except for the abamectin seed treatments, dosage rates for standard nematicides were used as indicated in the application guidelines of agrochemical chemicals as registered for use on cotton (Nel *et al*., 2007). Rates for abamectin were applied at the dosages recommended by Van Heerden (2005: personal communication). Van Heerden (2005: personal communication) also recommended inclusion of thiamethoxam in the 2005/2006 and 2006/2007 trials in combination with abamectin treatments to determine the role of insect damage on cotton yield. Dosage rates, application times/intervals and cost of products for the various trials are provided in Table 4.2. All trials constitute randomized complete block designs (Figs. 4.1 & 4.2). Each treatment as well as the untreated control was replicated nine times during the 2005/2006 season and six times during the 2006/2007 season. Simultaneously, degrees of freedom (error) of ≥ 18 (Van Ark, 1981) were always pursued. During the 2005/2006 growing season six treatments and nine replicates were included with degrees of freedom (error) 40 [(treatments - 1) × (replicates - 1)]. In the 2006/2007 season seven treatments and six replicates were included with degrees of freedom (error) 30. Each plot was clearly marked with durable plastic markers, which were placed at both ends of each plot (Fig. 4.3).

For the 2005/2006 trials four rows of cotton were planted per plot, while each plot had six rows during the 2006/2007 season. Cotton was planted at all trial sites using an Earthway Precision Garden Seeder® (Fig. 4.4) to obtain a final stand of 7 plants per metre. Each planted row was 5m long. The outer row for each plot served as a border row to adjacent plots. Nematode samples were collected from the outer two rows, while yield data were collected from the inner two rows. During sampling of cotton roots and soil for nematode extraction, counts and identification purposes, six cotton plants were removed from the outer two rows of each plot at both 42 and 84 DAP (days after planting). Due to heavy rainfalls during the proposed sampling intervals, samples were taken at a later stage for some of the trials as is indicated in the results.

Table 4.2. General information on nematicides and other chemicals used during this study as well as the estimated cost of each product when it is used by producers.

Common name	Trade name	Formulation	Active ingredient concentration (dosage rate)	Chemical classification	Registration designation and application	Product cost (ZAR)	Trial numbers/locality
Nematicides							
Abamectin FS	Avicta®	Flowable concentrate for seed treatment	400g/ℓ (0.15mg or 0.3mg a.i./seed)	Avermectin	Nematicide, seed treatment	2 500/ℓ	Trials A, B, C, D & E (Marble Hall & Vaalharts)
Aldicarb GR	Temik®	Granule	150g/kg (1.35kg a.i./ha)	Plant systemic carbamate	Nematicide /insecticide, at planting application	85/kg	Trials A, B, C, D & E (Marble Hall & Vaalharts)
Fenamiphos GR	Nemacur®	Granule	100g/kg (1.5kg a.i./ha)	Plant systemic organophosphate	Nematicide/ insecticide, at planting application	45/kg	Trials A, B, C, D & E (Marble Hall & Vaalharts)
Other pesticides							
Carboxin/ thiram FS	Vitavax 7® FS	Flowable concentrate for seed treatment	200g/ℓ & 200g/ℓ (0.05g & 0.05g a.i./seed respectively).	Oxathiin and organo sulphide respectively	Fungicide, seed treatment.	145/ℓ	Trials A, B, C, D & E (Marble Hall & Vaalharts)
Glyphosate SL	Round Up Ready®	Soluble liquid	540g/ℓ (0.675kg a.i./ha × 2 appl.)	Systemic glycine	Herbicide	55/ℓ	Trials A, C & D (Marble Hall)
Lambda-cyhalothrin EC	Karate®	Emulsifiable concentrate	50g/ℓ (0.006kg a.i./ha × 3 appl.)	Contact and stomach action pyrethroid	Insecticide	200/ℓ	Trials A, B, C, D & E (Marble Hall & Vaalharts)
Mepiquad chloride SL	Pix®	Soluble liquid	50g/ℓ (0.06kg a.i./ha in total)	Plant systemic growth regulator	Plant growth regulator	120/ℓ	Trials A, B, C, D & E (Marble Hall & Vaalharts)
Mefenoxam FS	Apron® XL	Flowable concentrate for seed treatment	350g/ℓ (8.75g a.i./100kg seed)	Phenylamide	Fungicide	2 800/ℓ	Trials A, B, C, D & E (Marble Hall & Vaalharts)
Thiamethoxam FS	Cruiser®	Flowable concentrate for seed treatment	350g/ℓ (0.3mg a.i./seed)	Neonicotinoid	Insecticide, seed treatment	957/ℓ	Trials A, B, C, D & E (Marble Hall & Vaalharts)
Thidiazoron/ diuron SC	Ginstar®	Suspension concentrate	360g/ℓ & 180g/ℓ (0.09kg a.i./ha and 0.045kg a.i./ha respectively)	Phenylurea compound and urea compound	Defoliant and leaf regrowth suppressor	2100/ℓ	Trials A, B, C, D & E (Marble Hall & Vaalharts)
Trifluralin EC	Treffer®	Emulsifiable concentrate	480g/ℓ (720g a.i./ha)	Dinitroanaline	Herbicide	56/ℓ	Trials A, B, C, D (Marble Hall)
Endosulfan SC	Thioflo®	Suspension concentrate	475g/ℓ (0.37kg a.i./ha × 3 appl.)	Contact and stomach action sulfurous acid ester of a chlorinated cyclic diol	Insecticide	60/ℓ	Trials A, B, C, D & E (Marble Hall & Vaalharts)

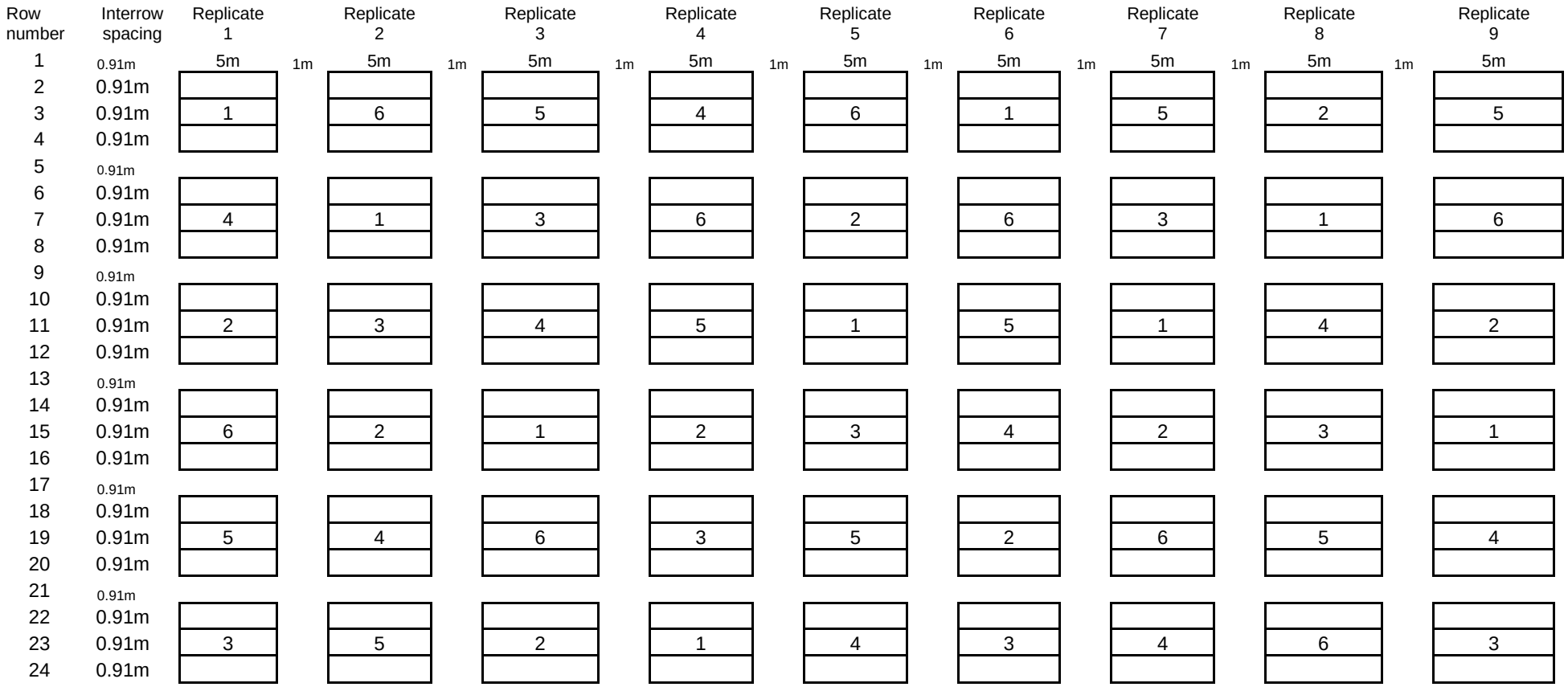


Figure 4.1. An illustration of a randomised complete block design layout used for the evaluation of abamectin seed treatments against plant-parasitic nematodes (PPN) in cotton trials A and B that were conducted during the 2005/2006 growing season in the Marble Hall area using the cultivar Nu OPAL®. (Intrarow-spacing = 14cm, interrow-spacing = 0.91m, row length = 5m. Treatments: 1 = abamectin 0.15mg a.i./seed, 2 = abamectin 0.3mg a.i./seed; 3 = aldicarb 1.35kg a.i./ha, 4 = fenamiphos 1.5kg a.i./ha, 5 = untreated control, 6 = thiamethoxam 0.3mg a.i./seed).

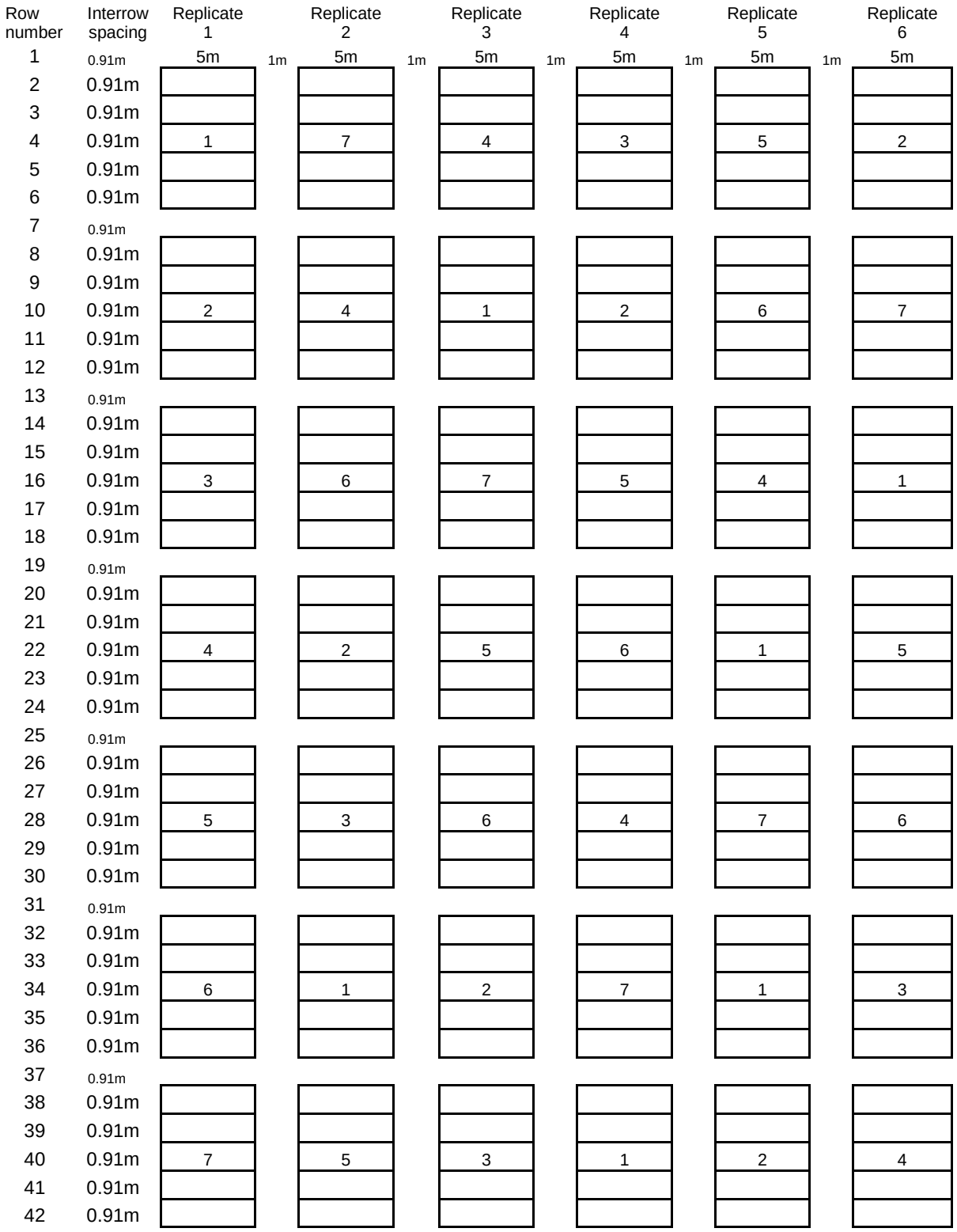


Figure 4.2. An illustration of a randomised complete block design layout used for the evaluation of abamectin seed treatments against plant-parasitic nematodes (PPN) in cotton Trials C, D and E that were conducted during the 2006/2007 growing season in the Marble Hall and Vaalharts areas using the cultivar Nu OPAL RR®. (Intrarow-spacing = 14cm, interrow-spacing = 0.91m, row length = 5m. (Treatments: 1 = abamectin 0.15mg a.i./seed, 2 = abamectin 0.3mg a.i./seed, 3 = aldicarb 1.35kg a.i./ha, 4 = fenamiphos 1.5kg a.i./ha, 5 = untreated control, 6 = thiamethoxam 0.3mg a.i./seed and 7 = abamectin 0.15mg a.i./seed + thiamethoxam 0.3mg a.i./seed).

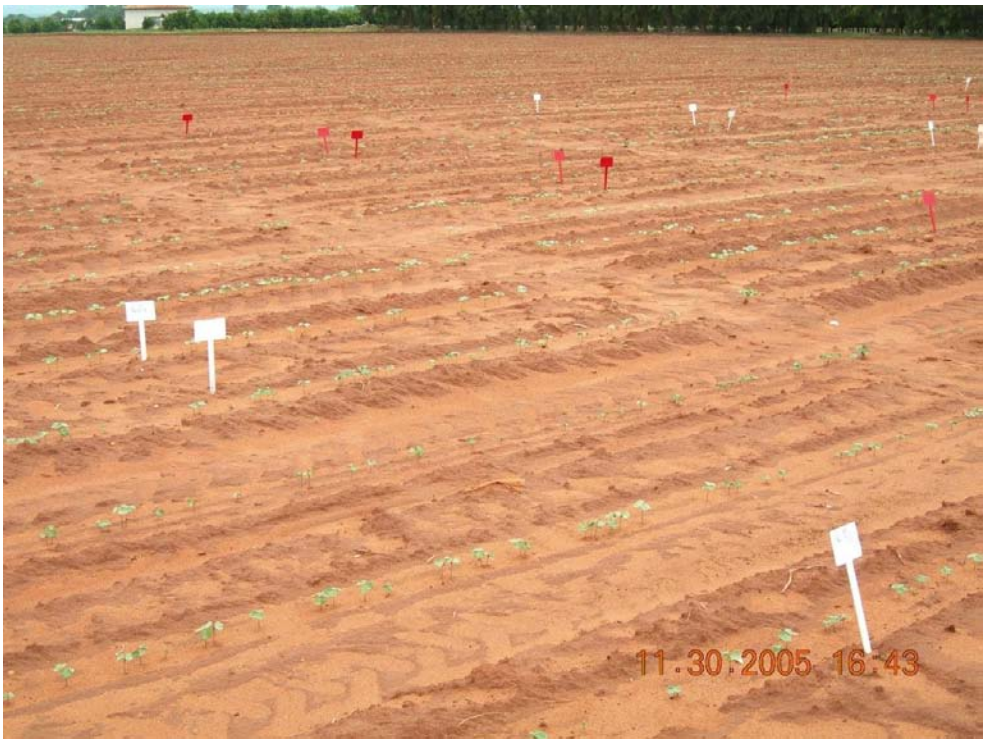


Figure 4.3. A view of plots included in Trial A near Marble Hall at 19 DAP where cotton was planted during the 2005/2006 growing season to evaluate the efficacy of various abamectin dosage treatments for its potential to reduce population levels of plant-parasitic nematodes (PPN), particularly *Meloidogyne incognita* race 4.

Figure 4.4. A lateral view of an Earthway Precision Garden Seeder® that was used to plant cotton seeds in field trials during this study.

Figure 4.5. A lateral view of a Microband Applicator® that was used to apply nematicides at the registered dosage rates in cotton field trials during this study.

All granular nematicide formulations were applied to soil in the field trials during the seasons the trials were conducted using a calibrated Microband Applicator® (Fig. 4.5) according to standard practices (Nel *et al*., 2007). The Microband Applicator® is manually operated. A bicycle wheel on the ground with a chain and sprocket drives the axle in the channel at the bottom of the hopper bin. Removable aluminium cogwheels of various thicknesses are mounted on the driven axle fitting between round spacers, which facilitate different calibration settings. Cavities in the rotating cogwheels carry granules downward to the outlet chamber beneath the driven axle from where gravity force causes a free flow into the outlet pipe. The amount of granules applied per metre is determined by the thickness and number of cogwheels loaded on the driven axle. Since the size, shape and dosage rate of the two granular products differs, the applicator had to be calibrated separately for each product. A plastic bottle was mounted beneath the outlet pipe to accumulate released granules, while the applicator was pushed over a 50m-measured distance in the applicable field. Cogwheels were added and removed until the desired rate was achieved for each product. The set of cogwheels fitted for each separate rate were marked to ensure that the calibrating process was not repeated. Application of granular products was done alongside rows. The calibrated amount of granules was applied in a ±9cm band resulting from an outlet pipe with a splash-pad ending, moving 15cm above ground level. The applied granules were immediately lightly incorporated into the top 1cm of soil with a harrow. Seed treatments on the other hand were applied by personnel of Syngenta (Pty) Ltd. to ensure accuracy and precision. All seeds have been pre-treated by Monsanto SA (Pty) Ltd. with the standard fungicide seed treatment: 0.05mg a.i. carboxin (oxathiin) + 0.05mg a.i. thiram (organo sulphide) + 7.8µg a.i. mefenoxam (phenylamide)/seed. In this study the cotton cultivars used had a seed count of 11 200 seeds per kilogram.

4.2.2. Soil and root samples

Root samples were obtained by removing three cotton plants with a spade from each of the two outer rows of every plot and replicate. Soil samples were taken from the corresponding cotton plants by obtaining the soil surrounding the roots of these plants. Both root and soil samples were taken at 56 and 84 DAP during the 2005/2006 growing season for Trials A and B (Marble Hall) for nematode extraction, counting and identification purposes.

During the 2006/2007 growing season soil and root samples were taken at 42 and 84 DAP for Trials C and D (Marble Hall) and Trial E (Vaalharts). The delay in sampling in the 2005/2006 growing season was due to heavy rains, resulting in postponement of basic trial procedures. Root and soil samples were obtained for nematode extraction, counting and identification purposes as described above.

4.2.2.1. Soil samples

Nematodes were extracted from soil samples using the adapted decanting and sieving method that was originally described by Cobb (1918) and recently updated and described by Hooper *et al*. (2005), followed by the sugar centrifugal-flotation method (Caveness & Jensen, 1955) as modified and described by Hooper *et al*. (2005) and Khan (2008).

Decanting and sieving method

This method is based on the size and density of nematodes. Approximately 20% of the nematodes originally present in a sample will be lost during this extraction procedure (Cobb, 1918; Hooper *et al*., 2005).

Two hundred millilitres of soil collected from the rhizosphere from six cotton plants was soaked in a one litre-beaker that was filled with tap water. Soil particles larger than 1mm were removed by passing the sample through a 710-µm-mesh sieve mounted on a 5-ℓ bucket. The residue on the sieve was washed through with tap water for 2 minutes and then discarded. The 5-ℓ bucket containing the nematodes was filled up to a volume of 5ℓ, the content of this bucket thoroughly stirred and allowed to settle for 30 seconds. The mixture containing the nematodes was then decanted through a 25-µm sieve leaving the sediment behind that has settled at the bottom of the bucket. Hereafter the 5-ℓ bucket was filled again with tap water and the process described above repeated one more time. The nematodes and fine soil particles from each soil sample that were subsequently retained on the 25-µm-mesh sieve were washed into separate 50-mℓ centrifugal tubes. The fine soil and nematode mixture in the centrifuge tubes was subsequently centrifuged for 5 minutes with a RCF (relative centrifugal force) of 1800g. After centrifugation the supernatant was carefully decanted and discarded. Due to the centrifugal force, nematodes

were collected at the bottom of the centrifuge tubes. This process was then followed by the sugar centrifugal-flotation method to complete the nematode extraction process.

Sugar centrifugal-flotation method

This method is based on the specific gravity of nematodes. Terrestrial nematodes have a specific gravity of 1.08 (Hooper, 1986). After centrifugation in water, only organic materials with a specific gravity lower than 1.0 will remain in suspension and could be discarded. When centrifuged in a solution with a higher specific gravity than the density of nematodes, the nematodes will remain in suspension and can be separated from soil particles with a specific gravity larger than 1.15 (Hooper, 1986).

A sucrose solution was, therefore, added to the centrifuge tubes containing the nematodes. This solution was prepared by adding 624g sugar to 1000mℓ water. The sucrose solution and the sediment containing the nematodes were thoroughly stirred with a spatula and centrifuged for one minute at a RCF of 1800g. When the centrifugation was completed, the supernatant containing the nematodes was decanted on a 250-µm-mesh sieve and gently rinsed with tap water to remove the sucrose as quickly as possible. The nematodes were washed into a sampling bottle for counting and identification. Since the sucrose solution is osmotically active the nematodes suspended in it can plasmolyse. It was therefore critical that the time period during which the nematodes were kept in the sucrose solution was limited, not exceeding a total of four minutes.

4.2.2.2. Root samples

Extraction of RKN eggs and second stage juveniles (J2) were done by using the adapted NaOCl-method of Riekert (1995), while PPN were extracted from 5g-root samples using the adapted sugar-flotation method as adapted and described by De Waele *et al*. (1987).

Adapted NaOCl-method

A 20-g root sample was obtained from the root systems of six cotton plants sampled for each treatment, cut into 1-cm pieces, mixed thoroughly and then liquidised in a 1-ℓ capacity Waring Stainless Steel Blender®. The root sample was then shaken in 800mℓ of a 1% -NaOCl solution for four minutes using an orbital shaker. The weak bleach solution breaks down the gelatinous matrix surrounding the eggs and releases them from the roots. The mixture containing the root-knot nematode eggs and J2 was decanted through a series of mounted sieves, consisting of a 710-µm, 250-µm, 75-µm, 63-µm, 45-µm, 25-µm and a 10-µm-mesh sieve in order from top to bottom. This specific arrangement of mesh sieves results in less congestion at the 10-µm-mesh sieve. A vacuum pump was connected to the 10-µm-mesh sieve to facilitate the passing of

water. The process to extract root-knot nematode eggs and J2 from roots of each sample using this range of mesh sieves took approximately 5 minutes. Nematode eggs and J2 were collected on both the 25-µm and 10-µm-mesh sieves by rinsing it from these sieves into a 100-mℓ sample bottle.

Centrifugal-flotation method for the extraction of nematodes from plant roots

One of the most successful methods to extract a wide range of PPN from plant roots is the centrifugal-flotation method described by Coolen and D'Herde (1972). A modified version of this method by De Waele *et al*. (1987) is described below. This method is based on maceration and centrifugal-flotation.

A 5g subsample was taken from the six cotton root systems sampled from each treatment and replicate at each of the localities and were cut into 1cm pieces. Each combined root sample was then macerated in 250mℓ tap water at high speed in a domestic blender for 90 seconds to release plant-parasitic nematodes from the root tissue. The suspension containing nematodes and root fragments were decanted on a 710 µm-mesh sieve, which was nested on a 25-µm-mesh sieve. Root pieces on the 710 µm-mesh sieve were rinsed thoroughly with running tap water and the residue on the 25 µm-mesh sieve containing the nematodes was washed into a 50mℓ centrifuge tube. Kaolin ($2cm^3$) was added to the tube and stirred well. The kaolin-water mixture containing the nematodes was then centrifuged at 1800g for 1 minute to ensure settlement of the nematodes at the bottom of each tube. The supernatant was subsequently decanted and each tube was filled with a sucrose solution (specific gravity = $1.15g/cm^3$). The mixture was stirred well and centrifuged at 1800g for 1 minute. The supernatant was decanted onto a 25-µm-mesh sieve and rinsed well with tap water to remove the sucrose. The residue containing the nematodes was collected in a sample bottle for examination and counting of the nematodes.

The importance of kaolin

Kaolin is a clay mineral with a specific gravity of 2.6 and consists of particles, which are 2-3 µm in size. Although the density of kaolin is greater than that of the nematodes, kaolin particles are small and flat and sink to the bottom of the centrifuge tube more slowly than the nematodes. This way kaolin forms a layer over the loose sediment and nematodes and seals it off when the supernatant is decanted. When the sucrose solution is added to the sediment the mixture must be stirred thoroughly to break the kaolin layer and bring the nematodes into suspension within the sugar solution. Another advantage of kaolin is that it precipitates during the second centrifugation, thus preventing re-mixing of the sedimented debris when the sugar solution is

decanted. Finally, a suspension of nematodes in clear water is obtained (Coolen & D'Herde, 1972).

Counting of nematodes

Nematode population levels were determined in a counting dish under a stereo/dissection microscope and expressed either as the number of nematodes per 5g roots, 20g roots or 200mℓ soil. Nematode data were *ln*(*x*+1) transformed and subjected to an Analysis of Variance (ANOVA) using Statgraphics 5 Plus for Windows.

4.2.3. Harvesting and yield of cotton lint

Cotton lint was harvested by hand from the 70 plants of the two inner rows in each of the field plots for all the trials (Table 4.3), weighed, subjected to data analysis (Statgraphics 5 Plus for Windows) and presented in t/ha.

Table 4.3. Dates during which harvesting of cotton field trials was done at the various trial sites.

Growing season	Trial number (locality)	Harvest date (DAP)
2005/2006	A (Marble Hall)	09/06/2006 (210)
	B (Marble Hall)	19/06/2006 (216)
2006/2007	C (Marble Hall)	17/06/2007 (222)
	D (Marble Hall)	08/06/2007 (220)
	E (Vaalharts)	16/06/2007 (220)

4.3. Results: Nematode data: Root and soil samples

4.3.1. 2005/2006 Growing season

4.3.1.1. Trial A (Marble Hall): First sampling (56 DAP)

Table 4.4. Significance data for *M. incognita* race 4 population levels in 5g and 20g root as well as in 200mℓ soil samples [data were *ln*(*x*+1) transformed].

Parameter	Significance data	
	F-ratio	P-value
M. incognita race 4 J2/5g roots	2.67	0.0357*
M. incognita race 4 egg & J2/20g roots	4.16	0.0039*
M. incognita race 4 J2/200mℓ soil	5.19	0.0009*

* Differences are significant.

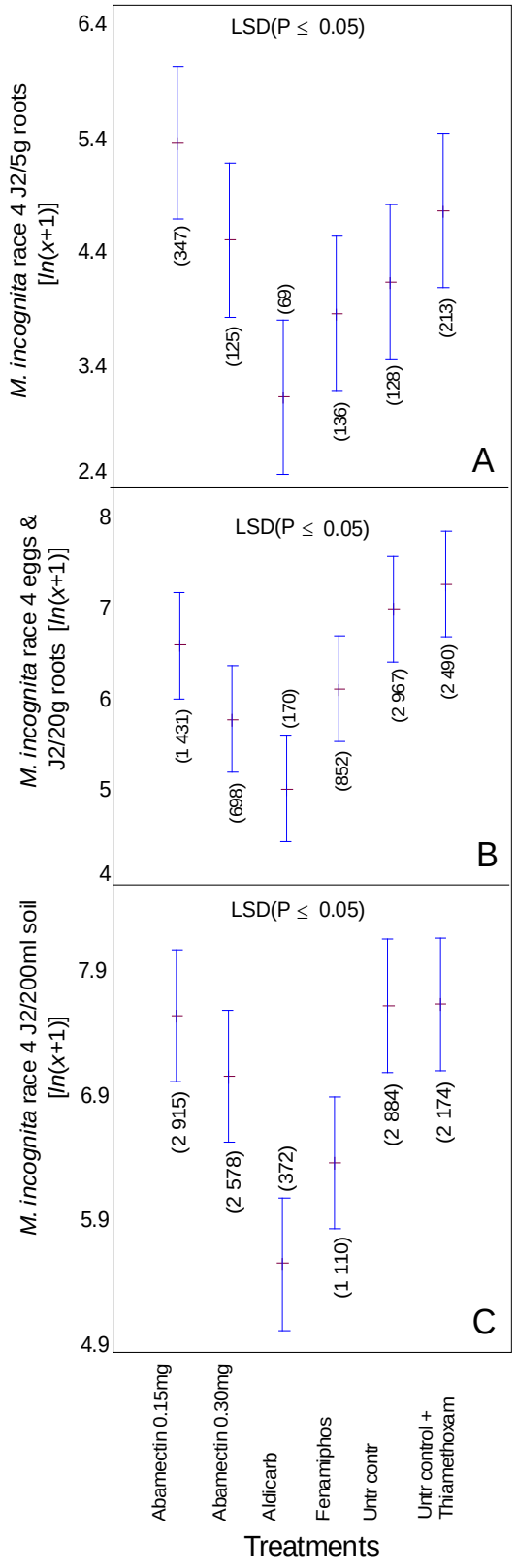


Figure 4.6. *Meloidogyne incognita* race 4 population levels in 5g (A) and 20g (B) root as well as in 200mℓ soil (C) samples [data were *ln*(*x*+1) transformed] of a root-knot susceptible cotton cultivar Nu OPAL® 56 DAP from field Trial A in the Marble Hall area during the 2005/2006 growing season.

4.3.1.2. Trial A (Marble Hall): Second sampling (84 DAP)

Table 4.5. Means (actual numbers in parenthesis) for *Meloidogyne incognita* race 4 individuals per 5g and 20g roots as well as per 200mℓ soil [data were *ln*(*x*+1) transformed].

Treatment	Parameter		
	5g roots	**20g roots**	**200mℓ soil**
1. Abamectin 0.15mg a.i./seed	6.5 (716)	8.5 (6 113)	7.7 (2 754)
2. Abamectin 0.3mg a.i./seed	5.4 (241)	7.3 (2 290)	7.0 (1 958)
3. Aldicarb 1.35kg a.i./ha	5.3 (274)	7.6 (3 852)	7.1 (1 606)
4. Fenamiphos 1.5kg a.i./ha	6.0 (519)	7.5 (2 926)	6.9 (1 733)
5. Untreated control	5.6 (467)	7.8 (4 517)	7.5 (2 436)
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	6 (515)	8.0 (3 536)	7.5 (1 929)
F-ratio	2.28	1.27	0.97
P-value	0.0651	0.2944	0.4465

No significant differences.

Table 4.6. Means (actual numbers in parenthesis) for *Criconema* spp. individuals/200mℓ soil [data were *ln*(*x*+1) transformed].

Treatment	Parameter
	200mℓ soil
1. Abamectin 0.15mg a.i./seed	1.8 (120)
2. Abamectin 0.3mg a.i./seed	1.0 (23)
3. Aldicarb 1.35kg a.i./ha	1.0 (50)
4. Fenamiphos 1.5kg a.i./ha	0.5 (12)
5. Untreated control	0.8 (6)
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	0 (0)
F-ratio	1.5
P-value	0.2112

No significant differences.

Meloidogyne incognita race 4 was identified as the predominant PPN species and race that infected cotton roots at Trial site A (See Chapter 2; paragraph 2.2.4). The only other PPN genus that occurred at this site was *Criconema*, which were present in relative low numbers (Table 4.6).

A substantial build-up of *M. incognita* race 4 population levels was evident from the first (56 DAP) to the second sampling interval (84 DAP) at this trial site where cotton was planted during the 2005/2006 season (Fig. 4.6; Table 4.4 & 4.5).

Trial A: First sampling (56 DAP)

Significant ($P \leq 0.05$) differences were obtained among the various treatments with regard to *M. incognita* race 4 numbers per 5g and 20g roots as well as J2 numbers/200mℓ soil (Table 4.4; Fig. 4.6). The highest population levels of *M. incognita* race 4 were generally recorded in soil samples at this trial site during this specific sampling interval.

The standard nematicide aldicarb had the lowest RKN egg and J2 numbers/20g roots and differed significantly ($P \leq 0.05$) when compared to both the untreated control treatments (Fig. 4.6). Both abamectin treatments, namely the 0.15mg and 0.3mg a.i./seed as well as the other standard nematicide treatment fenamiphos did not maintain significantly ($P \leq 0.05$) lower *M. incognita* race 4 numbers when compared to the untreated control treatments. On the other hand, both abamectin dosage treatments did not differ significantly ($P \leq 0.05$) from either the aldicarb and/or fenamiphos treatments.

For the 5g root samples only the aldicarb treatment resulted in significantly ($P \leq 0.05$) lower *M. incognita* race 4 numbers compared to the untreated control + thiamethoxam. None of the other nematicide treatments differed significantly ($P \leq 0.05$) from each other or from both the untreated control treatments. In contrast, the abamectin treatment 0.15mg a.i./seed had higher RKN numbers than the untreated control.

With regard to the RKN J2 numbers/200mℓ soil, aldicarb and fenamiphos were the only treatments that maintained significantly ($P \leq 0.05$) lower numbers than both the untreated control treatments.

Trial A: Second sampling (84 DAP)

During this sampling interval no significant ($P \leq 0.05$) differences were recorded for *M. incognita* race 4 individuals as well as other plant-parasitic nematodes that were extracted from both root and soil samples (Table 4.5 & 4.6).

In terms of yield, significant ($P \leq 0.05$) differences were evident among the different treatments used in this study (Table 4.7). The aldicarb treatment had the highest yield and differed significantly ($P \leq 0.05$) from both the untreated control treatments as well as the fenamiphos and abamectin treatments. Although the abamectin 0.15mg a.i./seed dosage

treatment had a significantly ($P \leq 0.05$) higher yield than the untreated control, it was similar to the untreated control + thiamethoxam 0.3mg a.i./seed treatment.

Table 4.7. Cotton yield for the various nematicide treatments as well as the untreated control treatments for Trial A that was conducted in the Marble Hall area during the 2005/2006 growing season using cultivar Nu OPAL®.

Treatment	Mean yield (t/ha)*
1. Abamectin 0.15mg a.i./seed	5.5 b
2. Abamectin 0.3mg a.i./seed	5.3 ab
3. Aldicarb 1.35kg a.i./ha	7.3 c
4. Fenamiphos 1.5kg a.i./ha	5.5 b
5. Untreated control	4.6 a
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	5.5 b
F-ratio	12.74
P-value	0.0000*

* Differences are significant.

4.3.1.3. Trial B (Marble Hall): First sampling (56 DAP)

Table 4.8. Means (actual numbers in parenthesis) for *Meloidogyne incognita* race 4 individuals in 5g and 20g root as well as in 200mℓ soil samples [data were *ln*(*x*+1) transformed].

Treatment	Parameter		
	5g roots	20g roots	200mℓ soil
1. Abamectin 0.15mg a.i./seed	2 (49)	1.7 (11)	0.0 (0)
2. Abamectin 0.3mg a.i./seed	0.3 (0.1)	0.4 (3)	0.0 (0)
3. Aldicarb 1.35kg a.i./ha	0.5 (9)	1.7 (12)	0.0 (0)
4. Fenamiphos 1.5kg a.i./ha	0.5 (7)	2.4 (25)	1 (8)
5. Untreated control	0.8 (28)	3.2 (67)	0.3 (1.3)
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	0.1 (0.2)	3.1 (97)	0.5 (6.3)
F-ratio	1.62	1.93	1.08
P-value	0.1843	0.1191	0.3874

No significant differences.

Table 4.9. Means (actual numbers in parenthesis) for Hoplolaimidae and *Paratrichodorus* spp. individuals/200mℓ soil [data were *ln*(*x*+1) transformed].

Treatment	Parameter (200mℓ soil)	
	Hoplolaimidae	***Paratrichodorus* spp.**
1. Abamectin 0.15mg a.i./seed	1.7 (14)	0.4 (2)
2. Abamectin 0.3mga.i./seed	1.8 (12)	0.0 (0)
3. Aldicarb 1.35kg a.i./ha	0 (0)	0.0 (0)
4. Fenamiphos 1.5kg a.i./ha	0.9 (7)	0.0 (0)
5. Untreated control	1.5 (24)	0.0 (0)
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	1.9 (20)	0.4 (2)
F-ratio	2.13	1.07
P-value	0.0873	0.3970

No significant differences.

4.3.1.4. Trial B (Marble Hall): Second sampling (84 DAP)

Table 4.10. Means (actual numbers in parenthesis) for *Meloidogyne incognita* race 4 individuals per 5g and 20g root as well as for 200mℓ soil samples [data were *ln*(*x*+1) transformed].

Treatment	Parameter		
	5g roots	**20g roots**	**200mℓ soil**
1. Abamectin 0.15mg a.i./seed	3.2 (49)	1.1 (9)	3.7 (328)
2. Abamectin 0.3mg a.i./seed	3.0 (108)	0.9 (18)	2.0 (30)
3. Aldicarb 1.35kg a.i./ha	2.2 (65)	0.4 (6)	1.7 (50)
4. Fenamiphos 1.5kg a.i./ha	3.5 (294)	0.6 (17)	3.8 (207)
5. Untreated control	1.7 (53)	2.5 (110)	3.7 (336)
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	2.1 (33)	0.7 (59)	3.3 (172)
F-ratio	1.33	1.27	1.88
P-value	0.2698	0.2964	0.1199

No significant differences.

Table 4.11. Means (actual numbers in parenthesis) for *Pratylenchus* spp. individuals/200mℓ soil [data were *ln*(*x*+1) transformed].

Treatment	Parameter
	200mℓ soil
1. Abamectin 0.15mg a.i./seed	0.8 (4)
2. Abamectin 0.3mg a.i./seed	1.2 (8)
3. Aldicarb 1.35kg a.i./ha	0.0 (0)
4. Fenamiphos 1.5kg a.i./ha	0.74 (3)
5. Untreated control	0.5 (8)
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	0.3 (1)
F-ratio	1.22
P-value	0.3191

No significant differences.

Table 4.12. Significance data for Hoplolaimidae individuals in 5g root and 200mℓ soil samples [data were *ln*(*x*+1) transformed].

Parameter	Significance data	
	F-ratio	**P-value**
Hoplolaimidae individuals/5g roots	2.77	0.0308*
Hoplolaimidae individuals/200mℓ soil	0.0308	0.0000*

* Differences are significant.

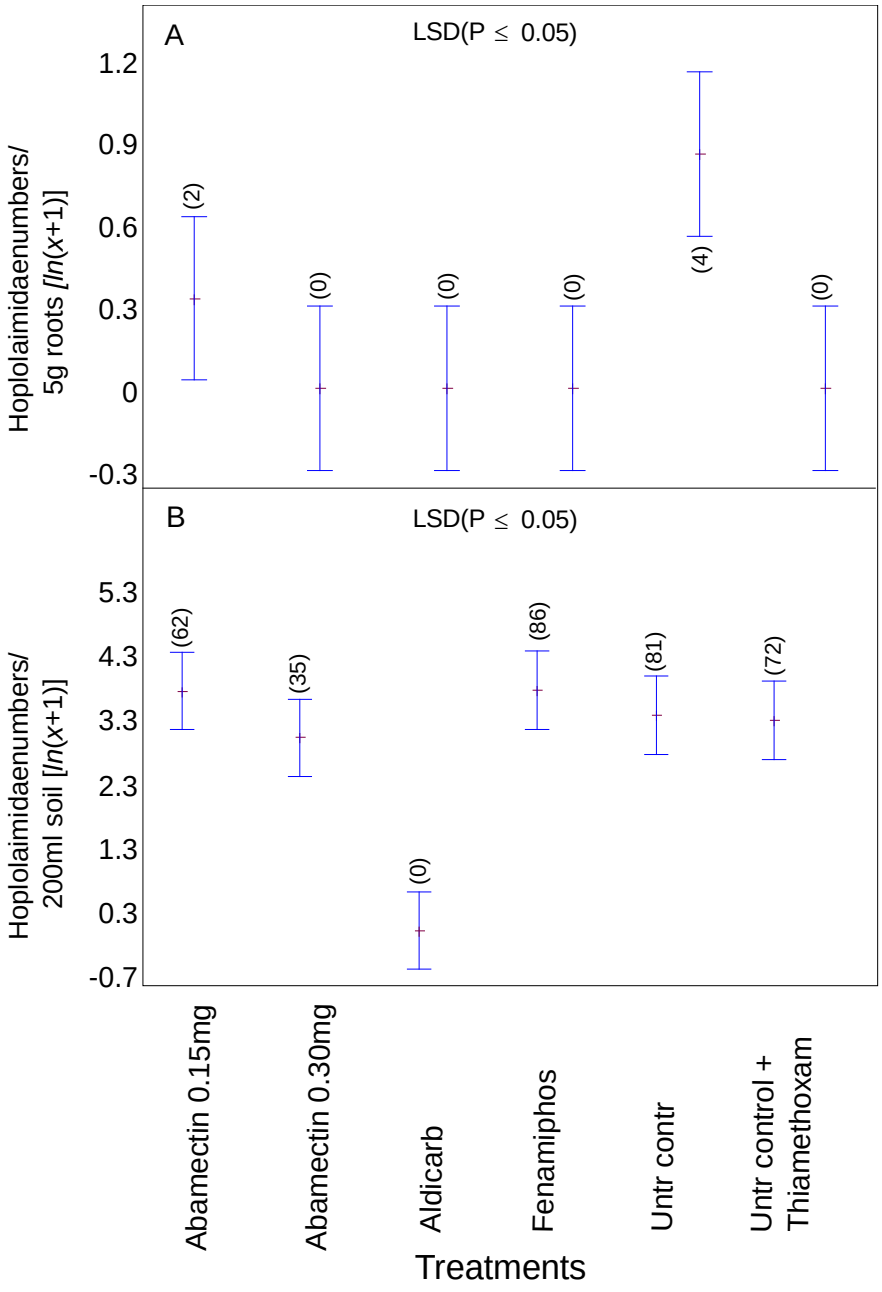


Figure 4.7. Population levels of Hoplolaimidae individuals in 5g root (A) and 200mℓ soil (B) samples [*ln*(*x*+1) transformed] from field Trial B that was planted in the Marble Hall area during the 2005/2006 growing season using the root-knot susceptible cotton cultivar Nu OPAL®.

Table 4.13. Means (actual numbers in parenthesis) for *Paratrichodorus* spp. individuals/200mℓ soil [data were *ln*(*x*+1) transformed].

Treatment	Parameter
	200mℓ soil
1. Abamectin 0.15mg a.i./seed	0.0 (0)
2. Abamectin 0.3mg a.i./seed	0.5 (2)
3. Aldicarb 1.35kg a.i./ha	0.0 (0)
4. Fenamiphos 1.5kg a.i./ha	0.4 (3)
5. Untreated control	0.0 (0)
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	0.0 (0)
F-ratio	1.21
P-value	0.3204

No significant differences.

Table 4.14. Cotton yield for the various nematicide treatments as well as the untreated control treatments for Trial B that was conducted in the Marble Hall area during the 2005/2006 growing season using cultivar Nu OPAL®.

Treatment	Mean yield (t/ha)
1. Abamectin 0.15mg a.i./seed	4.7 a
2. Abamectin 0.3mg a.i./seed	4.8 a
3. Aldicarb 1.35kg a.i./ha	5.4 b
4. Fenamiphos 1.5kg a.i./ha	4.7 a
5. Untreated control	4.7 a
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	4.7 a
F-ratio	3.01
P-value	0.0214*

* Differences are significant.

Meloidogyne incognita race 4 was identified as the predominant PPN species and race that infected cotton roots of Trial B (See Chapter 2; paragraph 2.2.4). Other PPN genera that were present at this trial site were *Paratrichodorus* spp., *Pratylenchus* spp. as well as *Rotylenchus* and *Helicotylenchus* spp. (Tables 4.9 & 4.11). Individuals from the latter genera were grouped under the family Hoplolaimidae.

Trial B: First sampling (56 DAP)

Although population levels of *M. incognita* race 4 in both root and soil samples increased over time, they were very low throughout the growing season at this trial site (Tables 4.8 & 4.9). No significant ($P \leq 0.05$) differences in numbers of these parasites were evident among the various treatments for both root and soil samples (Tables 4.8, 4.9 & 4.10).

Trial B: Second sampling (84 DAP)

Only Hoplolaimidae numbers in 5g root and 200mℓ soil samples differed significantly ($P \leq 0.05$) among the treatments at this trial site at 84 DAP (Table 4.12 & Fig. 4.7). Population levels of these parasites were, however, very low in both 5g root and 200mℓ soil samples and warrant no further discussion.

The seed cotton yield for the aldicarb treatment was significantly ($P \leq 0.05$) higher compared to those for the other treatments (Table 4.14).

4.3.2. 2006/2007 Growing season

4.3.2.1. Trial C (Marble Hall): First sampling (42 DAP)

Table 4.15. Significance data for *Meloidogyne incognita* race 4 population levels in 5g and 20g root as well as in 200mℓ soil samples [data were *ln*(x+1) transformed].

Parameter	Significance data	
	F-ratio	P-value
Meloidogyne spp. J2/5g roots	3.9	0.0054*
Meloidogyne spp. egg & J2/20g roots	3.5	0.0096*
Meloidogyne spp. J2/200mℓ soil	5.09	0.0010*

* Differences are significant.

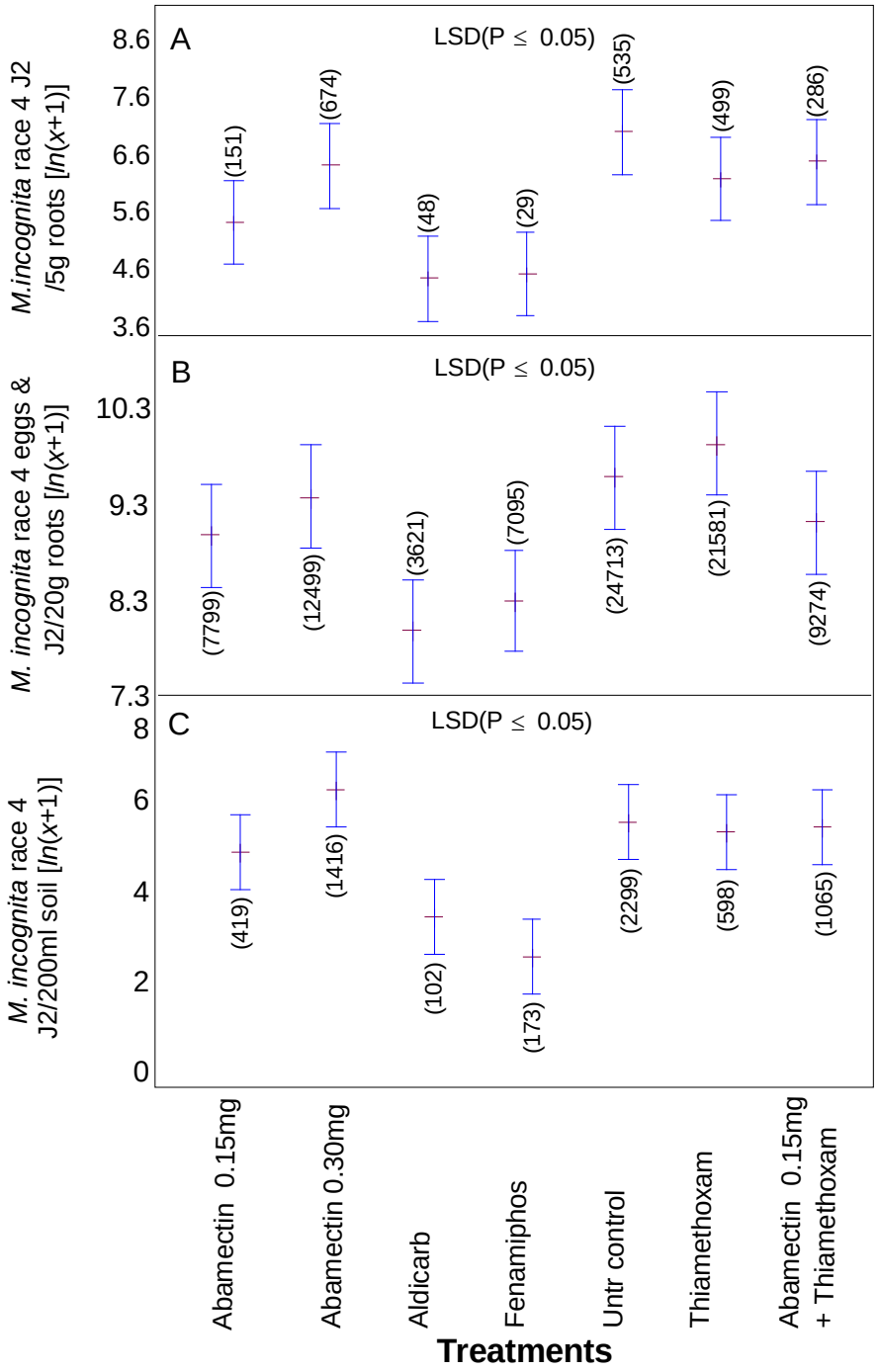


Figure 4.8. *Meloidogyne incognita* race 4 population levels in 5g and 20g root as well as 200mℓ soil samples [data were *ln*(*x*+1) transformed] of a root-knot susceptible cotton cultivar Nu OPAL RR® 42 DAP from field Trial C in Marble Hall area during the 2006/2007 growing season.

Table 4.16. Means (actual numbers in parenthesis) for Hoplolaimidae and *Tylenchorynchus* spp. individuals/200mℓ soil samples [data were *ln*(*x*+1) transformed].

Treatment	Parameter (200mℓ soil)	
	Hoplolaimidae	***Tylenchorhynchus* spp.**
1. Abamectin 0.15mg a.i./seed	0.0 (0)	0.9 (6)
2. Abamectin 0.3mg a.i./seed	0.7 (3)	0.5 (4)
3. Aldicarb 1.35kg a.i./ha	0.0 (0)	0.0 (0)
4. Fenamiphos 1.5kg a.i./ha	0.0 (0)	0.5 (3)
5. Untreated control	0.0 (0)	0.0 (0)
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	0.4 (1.5)	0.0 (0)
7. Abamectin 0.15mg a.i./seed + Thiamethoxam 0.3mg a.i./seed	0 (0)	0.5 (3)
F-ratio	1.59	0.76
P-value	1.858*	0.6083

No significant differences.

4.3.2.2. Trial C: Second sampling (84 DAP)

Table 4.17. Means (actual numbers in parenthesis) for *Meloidogyne incognita* race 4 individuals in 5g and 20g root as well as 200mℓ soil samples [data were *ln*(*x*+1) transformed].

Treatment	Parameter		
	5g roots	**20g roots**	**200mℓ soil**
1. Abamectin 0.15mg a.i./seed	4.8 (171)	8.0 (7 351)	5.5 (553)
2. Abamectin 0.3mg a.i./seed	5.3(269)	7.6 (3 731)	5.8 (563)
3. Aldicarb 1.35kg a.i./ha	4.7 (115)	7.2 (2 613)	6.0 (692)
4. Fenamiphos 1.5kg a.i./ha	5.2 (396)	8.2 (9 199)	6.1 (604)
5. Untreated control	5.5 (265)	8.0 (4 139)	5.5 (453)
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	4.9 (274)	7.1 (4 446)	6.0 (826)
7. Abamectin 0.15mg a.i./seed + Thiamethoxam 0.3mg a.i./seed	5.4 (321)	7.5 (6 921)	6.3 (770)
F-ratio	0.80	0.59	0.47
P-value	0.5760	0.7333	0.8221

No significant differences.

Table 4.18. Means (actual numbers in parenthesis) for Hoplolaimidae individuals/200mℓ soil [data were *ln*(*x*+1) transformed].

Treatment	200mℓ soil	
	Hoplolaimidae	***Tylenchorhynchus* spp.**
1. Abamectin 0.15mg a.i./seed	0.0 (0)	2.2 (50)
2. Abamectin 0.3mg a.i./seed	1.1 (9)	2.0 (29)
3. Aldicarb 1.35kg a.i./ha	0.0 (0)	1.9 (24)
4. Fenamiphos 1.5kg a.i./ha	0.0 (0)	2.4 (61)
5. Untreated control	1.1 (9)	2.4 (56)
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	0.4 (2)	1.5 (32)
7. Abamectin 0.15mg a.i./seed + Thiamethoxam 0.3 a.i.mg/seed	0.5 (3)	0.8 (21)
F-ratio	1.30	0.35
P-value	0.2886	0.9066

No significant differences.

Table 4.19. Cotton yield data for the various nematicide treatments as well as the untreated control treatments for Trial C that was conducted in the Marble Hall area during the 2006/2007 growing season using for the cultivar Nu OPAL RR®.

Treatment	Mean yield (t/ha)
1. Abamectin 0.15mg a.i./seed	2.6
2. Abamectin 0.3mg a.i./seed	2.6
3. Aldicarb 1.35kg a.i./ha	3.1
4. Fenamiphos 1.5kg a.i./ha	2.9
5. Untreated control	2.9
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	2.9
7. Abamectin 0.15mg a.i./seed + Thiamethoxam 0.3mg a.i./seed	3.0
F-ratio	1.24
P-value	0.3143

Replicates differed significantly, therefore data are not reliable.

Meloidogyne incognita race 4 was identified as the predominant PPN species and race that infected cotton roots of Trial C (See Chapter 2; paragraph 2.2.4). Other PPN genera that were present at this trial site were *Tylenchorhynchus* spp. as well as *Rotylenchus* spp. and

Helicotylenchus spp. (Tables 4.18). Individuals from the latter two genera were grouped under the family Hoplolaimidae. Low population levels of these latter PPN genera were present at this site.

Trial C: First sampling (42 DAP)

Significant ($P \leq 0.05$) differences were recorded for *M. incognita* race 4 egg and J2 numbers in 5g and 20g root samples as well as for J2 numbers in 200ml soil samples at this locality 42 DAP (Table 4.15 & Fig. 4.8). For the 5 and 20g root as well as the 200ml soil samples both standard nematicide treatments aldicarb and fenamiphos had significantly ($P \leq 0.05$) lower *M. incognita* race 4 numbers than the untreated control and untreated control + thiamethoxam treatments. The two abamectin dosage treatments, namely 0.15 and 0.3mg a.i./seed did not differ significantly ($P \leq 0.05$) from both the two untreated control treatments. The abamectin 0.3mg a.i./seed dosage treatment did maintain significantly ($P \leq 0.05$) higher numbers of this nematode race than the standard nematicide treatment aldicarb (Fig. 4.8). The abamectin 0.15mg a.i./seed treatment did not differ significantly ($P \leq 0.05$) from the two standard nematicide treatments.

Trial C: Second sampling (84 DAP)

A general decline in *M. incognita* race 4 population levels was evident from the first to the second sampling interval (Table 4.17 & Fig. 4.8).

The cotton yield for this trial was less compared to those for the other trials during the previous season and the general growing period shorter than that for the corresponding trial that was planted at the same site during the 2005/2006 season. No significant ($P \leq 0.05$) differences in *M. incognita* race 4 numbers were recorded amongst the various treatments used in this trial (Table 4.17).

4.3.2.3. Trial D (Marble Hall): First sampling (42 DAP)

Table 4.20. Significance data for *Meloidogyne incognita* race 4 egg and second-stage juvenile (J2) numbers in 20g root samples [data were *ln*(x+1) transformed].

Parameter	Significance data	
	F-ratio	P-value
Meloidogyne spp. egg & J2/20g roots	3.87	0.0056*

***** Differences are significant.

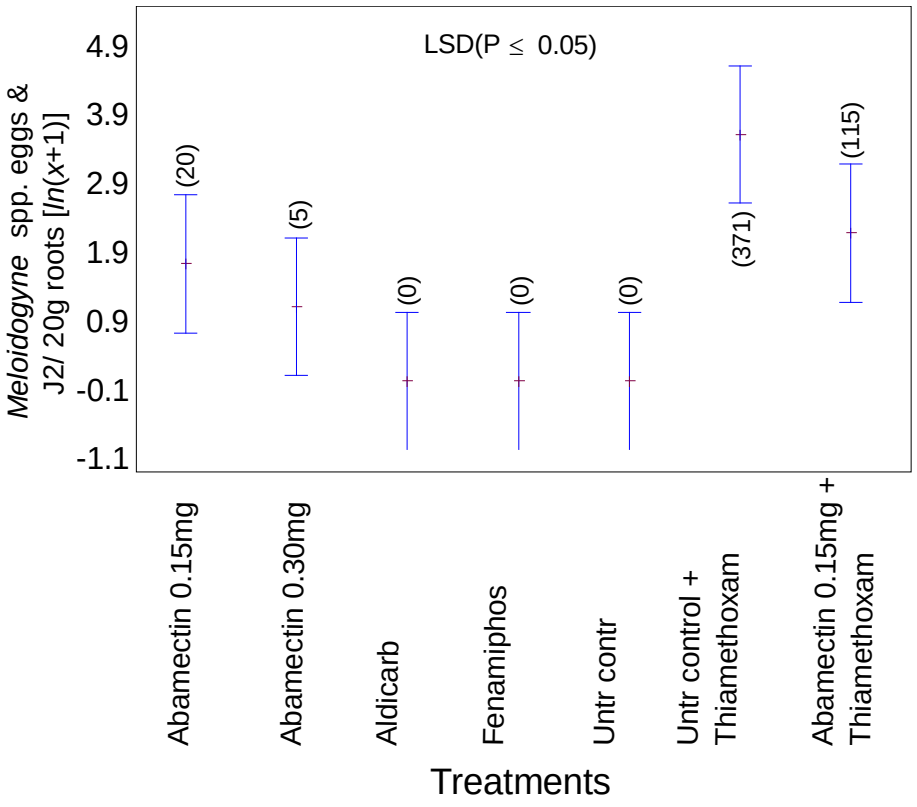


Figure 4.9. *Meloidogyne incognita* race 4 egg and second-stage juvenile (J2 numbers) in 20g root samples [data were *ln*(*x*+1) transformed] of the root-knot susceptible cotton cultivar Nu OPAL RR® 42 DAP from field Trial C in the Marble Hall area during the 2006/2007 season.

Table 4.21. Means (actual numbers in parenthesis) for *Meloidogyne incognita* race 4 individuals in 5g root and 200mℓ soil samples [data were *ln*(*x*+1) transformed].

Treatment	Parameter	
	5g roots	**200mℓ soil**
1. Abamectin 0.15mg a.i./seed	3.1 (102)	2.5 (116)
2. Abamectin 0.3mg a.i./seed	2.6 (39)	1.9 (64)
3. Aldicarb 1.35kg a.i./ha	0.9 (4)	1.4 (11)
4. Fenamiphos 1.5kg a.i./ha	0.3 (1)	1.3 (9)
5. Untreated control	2.6 (90)	3.1 (124)
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	2.7 (46)	3.0 (114)
7. Abamectin 0.15mg a.i./seed + Thiamethoxam 0.3mg a.i./seed	0.95 (6)	3.0 (97)
F-ratio	2.39	0.6
P-value	0.0526	0.7312

No significant differences.

Table 4.22. Means (actual numbers in parenthesis) for *Pratylenchus* spp. and Hoplolaimidae individuals/200mℓ soil samples [data were *ln*(*x*+1) transformed].

Treatment	200mℓ soil	
	***Pratylenchus* spp.**	**Hoplolaimidae**
1. Abamectin 0.15mg a.i./seed	0.0 (0)	2.2 (23)
2. Abamectin 0.3mg a.i./seed	0.0 (0)	1.5 (12)
3. Aldicarb 1.35kg a.i./ha	0.0 (0)	1.9 (12)
4. Fenamiphos 1.5kg a.i./ha	0.0 (0)	0.4 (2)
5. Untreated control	0.0 (0)	0.4 (2)
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	0.3 (0.7)	0.4 (2)
7. Abamectin 0.15mg a.i./seed + Thiamethoxam 0.3mg a.i./seed	0.0 (0)	1.9 (9)
F-ratio	1.0	2.22
P-value	0.4435	0.0684

No significant differences.

4.3.2.4. Trial D (Marble Hall): Second sampling (84 DAP)

Table 4.23. Significance data for *Meloidogyne incognita* race 4 individuals in 5g and 20g root as well as 200mℓ soil samples [data were *ln*(*x*+1) transformed].

Parameter	Significance data	
	F-ratio	**P-value**
M. incognita race 4 J2/5g roots	1.06	0.4056
M. incognita race 4 egg & J2/20g roots	5.34	0.0008*
M. incognita race 4 J2/200mℓ soil	2.89	0.0242*

***** Differences are significant.

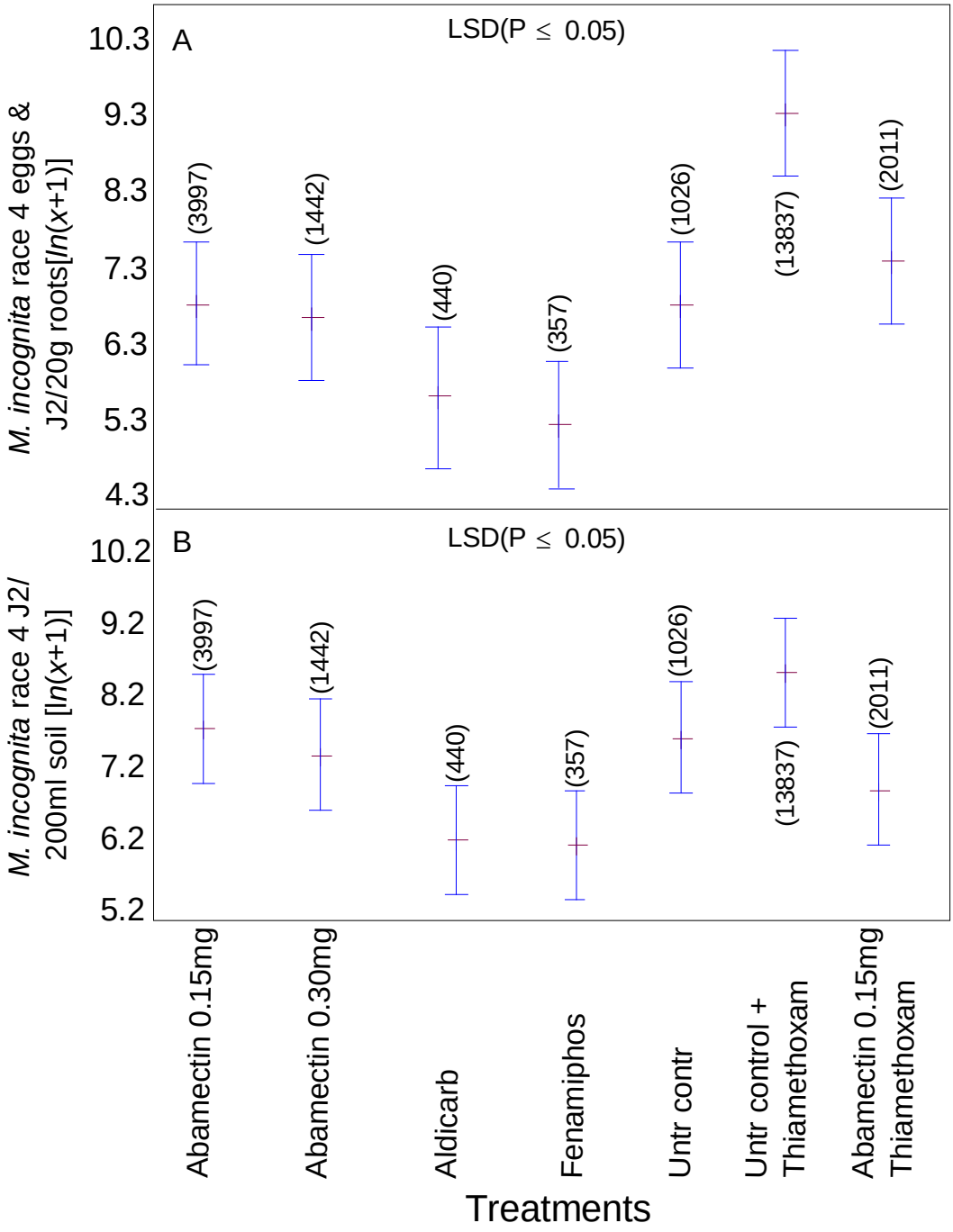


Figure 4.10. *Meloidogyne incognita* race 4 egg and second-stage juvenile (J2) numbers in 20g root (A) and J2 numbers in 200mℓ soil (B) samples [data were *ln*(*x*+1) transformed] of the root-knot susceptible cotton cultivar Nu OPAL RR® 84 DAP that was planted in field Trial C in the Marble Hall area during the 2006/2007 growing season.

Table 4.24. Cotton yield data for the various nematicide treatments as well as the untreated control treatments for Trial D that was conducted in the Marble Hall area during the 2006/2007 growing season using cultivar Nu OPAL RR®.

Treatment	Mean yield (t/ha)
1. Abamectin 0.15mg a.i./seed	6.0
2. Abamectin 0.3mg a.i./seed	6.1
3. Aldicarb 1.35kg a.i./ha	6.2
4. Fenamiphos 1.5kg a.i./ha	6.1
5. Untreated control	5.6
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	5.7
7. Abamectin 0.15mg a.i./seed + Thiamethoxam 0.3mg a.i./seed	6.0
F-ratio	0.78
P-value	0.5921

No significant differences.

Meloidogyne incognita race 4 was identified as the predominant PPN species and race that infected cotton roots at Trial site D (See Chapter 2; paragraph 2.2.4). *Pratylenchus* spp. as well individuals from the Hoplolaimidae family were also present in samples from this trial site, but at low population densities. *Rotylenchus* and *Helicotylenchus* spp. were identified as genera belonging to the Hoplolaimidae family that occurred at this site (Table 4.22).

Trial D: First sampling (42 DAP)

Significant ($P \leq 0.05$) differences were only obtained for *M. incognita* race 4 egg & J2 numbers in 20g roots at this locality (Fig. 4.9). The two standard nematicide treatments had the lowest *M. incognita* race 4 numbers and differed significantly ($P \leq 0.05$) from the untreated control + thiamethoxam and the abamectin 0.15mg a.i./seed + thiamethoxam 0.3mg a.i./seed treatments, but not from the untreated control and the two abamectin treatments (0.15 and 0.3mg a.i./seed). Therefore this data merits no further discussion.

Trial D: Second sampling (84 DAP)

Significant ($P \leq 0.05$) differences were obtained among the treatments for *M. incognita* race 4 egg & J2 numbers in 20g roots as well as 200ml soil (Fig. 4.10). Only the standard nematicides fenamiphos and aldicarb maintained significantly ($P \leq 0.05$) lower *M. incognita* race 4 J2/200ml soil than those for the untreated control and untreated control +

thiamethoxam treatments (Fig. 4.10). The fenamiphos treatment maintained the lowest *M. incognita* race 4 J2/200mℓ soil as well as eggs & J2/20g roots.

4.3.2.5. Trial E (Vaalharts): First sampling (42 DAP)

Table 4.25. Significance data for *Meloidogyne incognita* race 4 individuals in 5g and 20g root as well as 200mℓ soil samples [data were *ln*(*x*+1) transformed].

Parameter	Significance data	
	F-ratio	P-value
M. incognita race 4 egg & J2/20g roots	2.52	0.0426*
M. incognita race 4. J2/5g roots	5.04	0.0011*
M. incognita race 4 J2/200mℓ soil	5.42	0.0007*

***** Differences are significant.

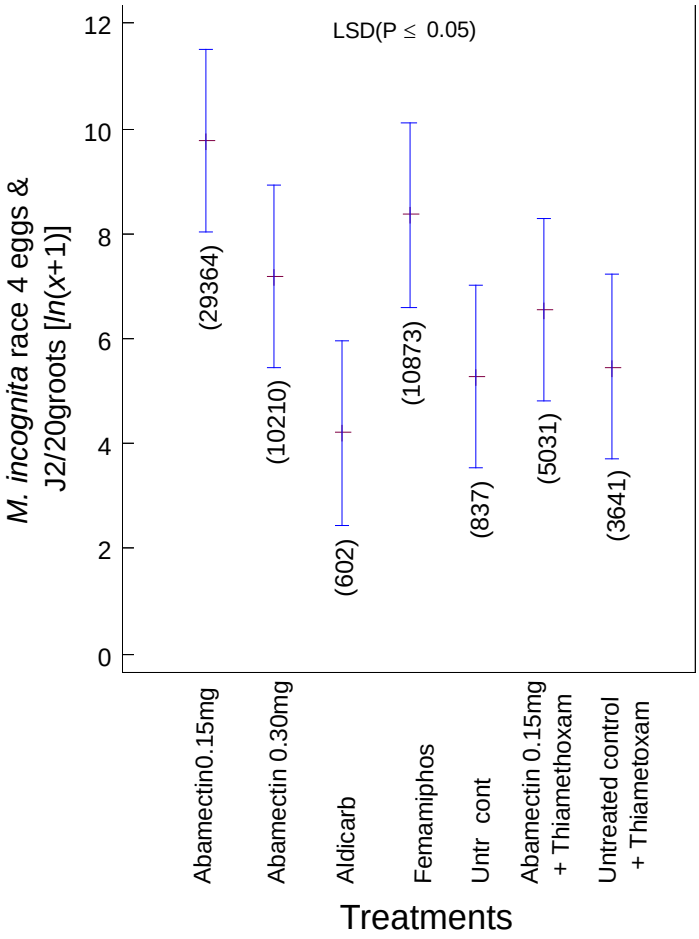


Figure 4.11. *Meloidogyne incognita* race 4 egg and J2 numbers in 20g roots [data were *ln*(*x*+1) transformed] of a RKN susceptible cotton cultivar Nu OPAL RR® 42 DAP from field Trial E that was planted in the Vaalharts area.

4.3.2.6. Trial E (Vaalharts): Second sampling (84 DAP)

Table 4.26. Significance data for *Meloidogyne incognita* race 4 individuals in 5g and 20g root as well as 200mℓ soil samples [data were *ln*(*x*+1) transformed] as well as means (actual numbers in parenthesis) of this parasite for the various parameters.

Treatment	5g roots	20g roots	200mℓ soil
1. Abamectin 0.15mg a.i./seed	4.8 (217)	8.8 (18015)	2.9 (50)
2. Abamectin 0.3mg a.i./seed	5.5 (399)	9.4 (23 799)	3.7 (60)
3. Aldicarb 1.35kg a.i./ha	4.1 (254)	6.7 (2935)	1.8 (26)
4. Fenamiphos 1.5kg a.i./ha	5.2 (546)	7.4 (3042)	0.5 (4)
5. Untreated control	5.1 (285)	7.8 (9462)	2.5 (34)
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	5.1 (303)	8.4 (9044)	3.6 (147)
7. Abamectin 0.15mg a.i./seed + Thiamethoxam 0.3 a.i.mg/seed	3.6 (130)	8.1 (8302)	1.9 (18)
F-ratio	1.51	1.56	2.67
P-value	0.2089	0.1940	0.0340*

Significant differences were recorded but the untreated control maintained low population levels.

Table 4.27. Cotton yield data for the various nematicide as well as the untreated control treatments for Trial E that was conducted in the Vaalharts area during the 2006/2007 growing season using for the cultivar Nu OPAL RR®.

Treatment	Mean yield (t/ha)
1. Abamectin 0.15mg a.i./seed	1.5 a
2. Abamectin 0.3mg a.i./seed	1.9 ab
3. Aldicarb 1.35kg a.i./ha	1.9 ab
4. Fenamiphos 1.5kg a.i./ha	2.1ab
5. Untreated control	1.5 a
6. Untreated control + Thiamethoxam 0.3mg a.i./seed	3.6 b
7. Abamectin 0.15mg a.i./seed + Thiamethoxam 0.3mg a.i./seed	1.7 a
F-ratio	1.37
P-value	0.2590

Differences are not significant

Meloidogyne incognita race 4 was identified as the predominant PPN species and race that infected cotton roots at Trial site E (See Chapter 2; paragraph 2.2.4). Individuals of no other nematode genera or race was present at this trial site during 42 DAP and 84 DAP.

Trial E: First sampling 42 DAP

Although significant ($P \leq 0.05$) differences were evident among the various nematicide treatments used in this trial (Fig. 4.11; Tables 4.25 & 4.26), both untreated control treatments had the lowest *M. incognita* race 4 individuals in both root and soil samples. For this reason this data are not reliable and warrants no further discussion. No other PPN genera and/or species were recorded at this site during this sampling interval.

Trail E: Second sampling 84 DAP

The same tendency applied as mentioned in the above paragraph.

4.4. Discussion

M. incognita race 4 population levels varied substantially in soil and root samples of cotton sampled from the five trial sites as well as over the two growing seasons during which this study was conducted. Infestation levels of this nematode race were adequate for trials to be conducted in order to obtain results in terms of the efficacy of abamectin in reducing the numbers of these parasites.

Although the Avicta 400® FS treatments were not effective in reducing RKN numbers significantly when compared to the untreated control treatment(s) in five cotton trials that were conducted during this study, it resulted in similar reduction of these parasites as the aldicarb and fenamiphos dosage treatments used in at least two trials. In addition yield of the abamectin 0.15mg a.i./seed treatment was significantly higher than that of the untreated control in Trial A, which indicates the potential of this product to be considered as an alternative control option for particularly *M. incognita* race 4 in local cotton fields.

In terms of the cost-effectiveness of using abamectin as a seed treatment, the estimated cost of the 0.15mg a.i./seed treatment is 160-00 ZAR/ha, while it is 320-00 ZAR/ha for the 0.3mg a.i./seed treatment. When extrapolated to gross profit margin, it is calculated that an increase of at least 0.04t/ha of cotton should be realised to cover the cost of the abamectin treatment at 0.15mg a.i./seed. Compared to aldicarb and fenamiphos applications, which are 765-00 ZAR/ha and 675-00 ZAR/ha, respectively, abamectin application showed potential to earn its rightful place in the market in terms of the management of PPN in cotton. Further illustration of the contribution of nematicides to cotton yield was done in this study by using

the following formula: Yield of a nematicide treatment – yield of the untreated control treatment. These calculations resulted in 1.8t/ha for aldicarb and 0.9t/ha for the abamectin 0.15mg a.i./seed treatment when calculated for Trial A that was done during 2005/2006 in the Marble Hall area. Although these calculations clearly showed that use of aldicarb is superior to that of abamectin, it must be borne in mind that abamectin could not be classified in the same range as aldicarb due to the difference in origin (active ingredient) and classification of these two products.

Inconsistency in terms of the efficacy of the abamectin dosage treatments evaluated during this study in terms of reducing PPN, in this case *M. incognita* race 4, has also been reported elsewhere. In the USA Faske and Starr (2007) as well as Monfort *et al*. (2006b) reported inconsistent results when abamectin has been applied at dosage rates of 0.1 to 0.15mg a.i./seed in field trials where *M. incognita* posed problems to cotton production. Van Biljon (2005) reported that the 0.3mg a.i./seed abamectin cotton seed treatment showed superior efficacy in reducing *M. incognita* race 4 population levels in cotton root and soil samples with a corresponding yield increase when planted in sandy soils under local environmental conditions in the Jan Kempdorp area. These results could, however, not be verified during a follow-up growing season when a lower dosage rate of 0.15mg a.i./seed treatment was evaluated against this nematode race (Van Biljon, 2005). In addition Monfort *et al*. (2006b) reported that *M. incognita* numbers in soil samples were the same for all treatments eight DAP when an abamectin-treated cotton seed at a dosage rate of 0.1mg a.i./seed was planted in a trial in the USA. He also reported that root-gall ratings due to infection by these parasites 30 DAP were similar to those on cotton roots that were treated with aldicarb. The latter trend also corresponded with cotton roots sampled from the untreated control treatment, which had a gall index comparable to those for the aldicarb and abamectin treated plots. Monfort *et al*. (2006b) further recorded that *M. incognita* numbers in cotton roots were similar for all the latter treatments at midseason and at harvest, while root-galling and yield did not differ amongst these treatments. However, in greenhouse trials, significant differences were obtained amongst various nematicide treatments that were evaluated against *M. incognita* race 4, which explains the contributional effect of a controlled environment (i.e. steam pasteurized soil and temperature controlled conditions) in terms of the efficacy of abamectin (Monfort *et al.*, 2006b).

Inconclusive results in terms of the reduction of *M. incognita* race 4 population levels in cotton using abamectin was also reported by Faske and Starr (2007). The latter authors recorded that root gall rating and nematode reproduction were similar for cotton seed treated with abamectin plus three fungicides (azoxystrobin, fludioxinil and mefenoxam) and one

insecticide (thiamethoxam) in comparison with an untreated control. This observation suggests that interaction amongst these five concomitant treatments may have contributed to the small amount of abamectin transferred to the developing radicle (Faske & Starr, 2007). Furthermore in this study it was found that in abamectin seed treatments, root penetration by *M. incognita* was reduced optimally at early stages of root elongation and development. Since abamectin pose poor water solubility and is, therefore, not regarded as systemic product, successful use of this products ultimately requires the placement of abamectin in such a way that it is directly in contact with PPN (Faske & Starr, 2006). Thus as a seed treatment the amount of abamectin transferred from the seed coat to the radicle is fundamental, with only a small portion of abamectin being reported to be transferred to the radicle (Faske & Starr, 2007). A strong dilution effect of the product is thus suggested by the latter authors during elongation of cotton roots through the soil profile. Abamectin is further adsorbed by organic materials present in the soil and is also metabolized by soil-invading micro-organisms (Faske & Starr, 2006 & 2007; Fisher & Mrozik, 1992; Garabedian & Van Gundy, 1983). The abovementioned data corresponded with ours since no significant differences in PPN numbers and cotton yield were recorded between the abamectin treatments and the abamectin + thiamethoxam in our study.

The rational in adding thiamethoxam to an abamectin-seed treatment during the second year of this study was aimed in explaining the impact of insect parasitism in terms of yield. Thiamethoxam was added to abamectin coated seed since aldicarb and fenamiphos both are systemic nematicides and insecticides. The two standard nematicides, aldicarb and fenamiphos, showed higher efficacy in terms of PPN control throughout the replicates. Aldicarb, particularly, has shown superior efficacy and yield response. Aldicarb further has characteristics of controlling PPN, early season thrips and sucking insects and stimulates plant growth (Jones, 2005: personal communication). This latter finding might indicate the possible effect of insects during the growth season since thiamethoxam is designated to suppress early damaging insect populations in cotton fields.

Results from this study again illustrated that variability in terms of the efficacy of nematicides occur (Mc Donald, 1998; Haydock *et al*., 2006; Van Biljon, 2006: personal communication). Therefore, additional field trials that are conducted preferably on a commercial scale should be done to verify the effect of abamectin in terms of reducing PPN and in particular *M. incognita* race 4 in local cotton fields. The potential of abamectin in reducing *M. incognita* race 4 numbers has been illustrated during this study and this product can contribute to the local cotton industry, particularly where lower infestations of these parasites occur.

Chapter 5: Conclusions and recommendations

The need exists to evaluate the efficacy of alternative nematode management strategies, particularly those which are more human, animal and environmentally-friendly. Optimising crop yield and reducing populations of PPN populations, particularly *M. incognita* race 4, below damage threshold levels are thus the ultimate objectives for local cotton producers. Therefore, the efficacy of a biologically-derived product, namely abamectin, applied as a seed dressing on cotton seed has been investigated during this study in both a greenhouse and follow-up field trials. The identification of the RKN species and race that has been used in the greenhouse trial as well as those that were present in field trial sites, was done using DNA-based techniques. The host suitability of four local commercially-available cotton cultivars for *M. incognita* race 4 was also identified concurrently during the greenhouse study. Ultimately this study was aimed at providing practical, but also potentially applicable contributions to sustainable crop production in local cotton-based systems.

DNA-based SCAR-PCR methods applied during this study successfully resulted in the identification of a monospecific *M. incognita* race 4 population that has been reared *in vivo* and used as a source of inoculums for the greenhouse trial. Furthermore, RKN populations that were present in roots of cotton that were collected from the five field trial sites were also identified as *M. incognita* race 4.

Although the 0.15mg abamectin a.i./seed treatment resulted in significant lower *M. incognita* race 4 population levels in roots of the four cultivars, these levels were still relatively high. Significant differences among the five sampling intervals for both the abamectin and non-abamectin treatments further showed that all nematode and plant growth parameters generally had lower values during the first interval compared to the others. The four cultivars used were identified as susceptible hosts to this RKN species and race and generally had similar non-linear regression lines for the non-abamectin treatment in terms of *M. incognita* race 4-population development. Although cultivar Nu OPAL® maintained significant higher egg and J2 numbers/root system than those maintained by the other three cultivars, it was still classified as highly susceptible (like the other three cultivars) to *M. incognita* race 4 using Rf values. In addition, significant interactions among the two treatments and five sampling intervals for all the nematode and plant parameters, were regarded as the most important. The latter indicated that the treatments reacted differently during these intervals for all parameters measured. Nematode and plant growth data obtained during this greenhouse study should be verified in field trials throughout the cotton-producing areas of South Africa

under natural occurring environmental conditions. Only then can final conclusions could be made in this regard.

The predominant PPN that were present at all five field trial sites were identified as the RKN *Meloidogyne incognita* race 4. Except for the latter nematodes, low population levels of individuals from the Hoplolaimidae, *Criconema* spp., *Pratylenchus* spp. and *Paratrichodorus* spp. were also generally present. The 0.15mg abamectin treatment did not generally differ significantly from the untreated control treatment nor from the two standard nematicides, the thiamethoxam 0.3mg as well as from the abamectin 0.15mg a.i./seed + thiamethoxam 0.3mg treatment with regard to eggs and J2/root system in at least three of the five trials. Therefore, its efficacy is comparable to that of the two standard classical nematicides. Since yield for the abamectin 0.15mg a.i./seed treatment was significantly higher than that of the untreated control for one of the trials, the cost-effectiveness of abamectin application as a seed treatment was calculated to be substantially lower than those for the two standard nematicide treatments. Reduction of *M. incognita* race 4 populations as well as increase in yield using abamectin thus poses a potential benefit for producers. Results from these field trials showed that additional nematode management strategies should be used in combination with the abamectin treatment. Furthermore, although the potential of this product to be considered as an alternative control option for particularly *M. incognita* race 4 in local cotton fields has been demonstrated, it is recommended that abamectin should preferably be used only where population levels of *M. incognita* race 4 are not particularly high.

Chapter 6: References

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