OCCURRENCE, BIOLOGY, DAMAGE POTENTIAL AND MANAGEMENT OF *HETERODERA SCHACHTII* (NEMATODA: HETERODERIDAE) IN SMALL-SCALE FARMING IN THE WESTERN CAPE PROVINCE, SOUTH AFRICA

by

JACQUES VAN ZYL

Dissertation presented for the Degree of

Doctor of Philosophy in Agriculture

at the University of Stellenbosch

Promotor: Prof A J Meyer

Copromotor: Dr K L Pringle

December, 2003

DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

ABSTRACT

During a survey in the greater Cape Flats *Heterodera schachtii* was found to be widespread on cauliflower, Brussels sprouts, beetroot and cabbage. The numbers present were above two eggs and juveniles per gram of soil, generally regarded as the economic threshhold level of infestation and requiring control.

The damage potential of *H. schachtii* on vegetables, as well as the ability of certain weeds to serve as a source of infection on subsequent crop plantings was studied under greenhouse conditions and resulted in a reduction of yield and root weight of crops. Population densities of *H. schachtii* increased significantly under favourable hosts like cabbage where densities of 198 eggs and juveniles per gram of soil were reached. The most commonly occurring weeds maintained nematode development and increased their population densities. They can thus serve as alternative hosts in the absence of susceptible hosts and should be routinely controlled.

The life cycle and biology of *H. schachtii* was also studied. Penetration of plant tissue and subsequent development on vegetables, weeds and trap crops were observed. Penetration was successful on all crops tested reaching 37% and 52% at inoculum levels of 22 and 11 juveniles per gram of soil, respectively. Subsequent development of *H. schachtii* on weeds and vegetables was similar, but in the case of cauliflower and black nightshade as hosts, their life cycle was shorter in comparison to other crops.

The possible existence of varying susceptibility of crops to different populations of *H. schachtii* was examined by comparing the rates of penetration in crops and reproduction of geographically isolated populations of *H. schachtii* in the greater Cape Flats. When

root penetration, virulence and juvenile emergence were examined, populations from Lynedoch and Philippi were distinct from the other populations. Subsequently, representative individuals of these populations were subjected to PCR-RFLP, but with these techniques real differences between the various populations could not be adequately detected.

The environmental parameters such as soil texture, temperature and pH on *H. schachtii* were investigated as to their influence on the root weight and yield of crops. Reductions in the yield of beetroot and cabbage were observed with soil temperatures ranging between 15 to 30°C. Migration and penetration of *H. schachtii* juveniles declined with an increase in clay and silt content of the soil. Above a 34% silt and clay content of soil, no migration and penetration took place. Root penetration levels of 30% and higher were reached with pH varying between 4.5 and 7.4. This resulted in a significant reduction in yield of crops.

Crop rotation is an essential component of non-chemical control. In the case of *H. schachtii,* it required one host crop in four non-host rotational cycles to maintain the population of the nematode in the soil less than three eggs and juveniles per gram of soil. The inclusion of a trap crop reduced the population densities to below two eggs and juveniles per gram of soil. It therefore also forms an integral part of a control strategy.

Solarization proved successful as a physical control method. Best results were obtained in summer with clear polyethylene which led to a 97% reduction of infective juveniles. This method can be applied during the late summer in the greater Cape Flats, just before the onset of winter. This may safeguard future spring plantings.

The need for effective control strategies in order to reduce the numbers of *H. schachtii* is of the utmost importance to ensure vegetable production in the future. Small-scale farmers should therefore be educated in this respect.

UITTREKSEL

Heterodera schachtii het wydverspreid in 'n opname in die groter Kaapse Vlakte voorgekom op beet, blomkool, Brusselse spruite en kopkool. Die nematode getalle by alle lokaliteite was bo die algemeen aanvaarbare ekonomiese drempelwaarde van twee eiers en larwes per gram grond wat beheer regverdig.

Die skadepotensiaal van *H. schachtii* op groente, sowel as die vermoë van sekere onkruide om as infeksie bronne te dien vir opvolgende gewasse, is in glashuise ondersoek en het tot 'n verlaging in opbrengs en wortelmassa by gashere gelei. Die populasie digthede van *H. schachtii* het met die aanplant van geskikte gashere tot vlakke van 198 eiers en larvae per gram grond gestyg. Die mees algemeen voorkomende gasheer-onkruide het nematode ontwikkeling in stand gehou en selfs tot 'n populasie verhoging gelei. Hierdie onkruide is 'n beperkende faktor vir die verbouing van groente aangesien die onkruide as alternatiewe gasheer kan dien in die afwesigheid van gashere en onkruidbeheer moet dus op 'n gereelde basis toegepas word.

Die lewenssiklus en biologie van *H. schachtii* is ondersoek deurdat die penetrasie van gasheer wortels en die daaropvolgende ontwikkeling op groente, onkruide en vanggewasse vergelyk is. Penetrasie, vyf dae na inokulasie, is met alle gashere verkry met 37% en 52% penetrasie met inokulum vlakke van 22 en 11 larwes per gram grond onderskeidelik. Daaropvolgende ontwikkeling van *H. schachtii* was soortgelyk op groente en onkruide, maar blomkool en nastergal het as gashere 'n verkorte lewenssiklus tot gevolg gehad.

Die moontlikheid van verskille in die virulensie van *H. schachtii* is ondersoek deur die penetrasie van gewasse en reproduksie vlakke van nematodes van nege verskillende

geografies geskeide populasies in the groter Kaapse Vlakte te vergelyk. Die Lynedoch en Philippi populasies het onderskeibare resultate gelewer ten opsigte van die populasies uit die ander lokaliteite, maar geen verskille kon met PKR-RFLP aangetoon word nie.

Die invloed van omgewings parameters, grondtekstuur, temperatuur en pH, is op *H. schachtii* ondersoek ten opsigte van opbrengste en wortelmassa van gewasse. Grondtemperature tussen 15°C - 30°C het tot die grootste daling in opbrengs gelei op kopkool en beet. Migrasie en penetrasie het afgeneem met 'n toename in klei en slik inhoud tot en met 'n klei en slik inhoud van 34%, waarna geen penetrasie en migrasie voorgekom het nie. Wortelpenetrasie van 30% en hoër het voorgekom by pH vlakke van tussen 4.5 - 7.4 met die gepaardgaande verlaging in opbrengs van gewasse.

Afwisseling van gewasse is 'n essensiële metode van nie-chemiese beheer van nematode getalle in die grond. Die mees optimale rotasie ten opsigte van *H. schachtii* beheer is met die aanplanting van een gasheer gewas in vier gewas aanplantings verkry. Die insluiting van 'n vanggewas in die gewas rotasie siklus het die nematode populasievlakke tot onder twee per gram grond laat daal. Solarisasie is suksesvol uitgevoer met deurskynende poli-etileen in die groter Kaapse Vlakte gedurende die somer met gevolglik 'n 97% vermindering van die getalle infektiewe nematodes.

Effektiewe beheermaatreëls ten opsigte van *H. schachtii* moet in die groter Kaapse Vlakte ingestel word om groente-produksie in hierdie gebied te verseker. Kleinboere moet in hierdie tegnieke opgelei word.

DEDICATION

This dissertation is dedicated to my mother, Mabel Rosemarie van Zyl, for her love, support and encouragement and for giving me the opportunities she did not have.

CONTENTS

Chapter 1.

Introduction to and literature review of *Heterodera schachtii.*

Heterodera schachtii, also known as the sugar beet nematode, beet cyst nematode or the beet eelworm, was first observed by Schacht in 1859 near Halle Germany (Franklin 1951) and was the first cyst nematode to be described. In 1871 Schmidt named it after its discoverer and gave a comprehensive description of its morphology. Due to its intensive cultivation practices severe yield losses were found on sugar beet which subsequently led to the rapid spread of this nematode. In 1876 *H. schachtii* caused the closure of 24 sugar beet processing factories in Germany. During the late 19th and early 20th century, most cyst nematode species were assigned to the species *H. schachtii.* It is suggested that the Mediterranean area is the epicenter for the evolution of *H. schachtii* with the weed, goosefoot (*Chenopodium*), as the primary host, from where the nematode migrated to other species of *Chenopodiaceae* and *Cruciferae* (Krall & Krall 1978).

Heterodera schachtii is distributed worldwide and is found at high densities in areas where ever sugar beet is grown (Steele 1965). Most occurrences of *H. schachtii* are in the temperate zones, mainly north of the equator. Bello & Romero (1973) listed their presence in more than 40 countries and in 1968 it was reported from the Cape Flats, South Africa (Coetzee 1968).

In the cyst stage the eggs become embryonated and the first stage juveniles moult into the second stage infective juveniles (J_2). These may remain dormant in the eggs for several years or hatch almost immediately when stimulated by exudates from the roots of host and some non-host plants (Raski 1949). Hatching, to a lesser extent also takes place in the absence of plants. The infective juveniles attracted to the roots enter the root tips proximally and take up a feeding position close to the stele. If the plant is a suitable host, the nematode forms a syncytium on which the juvenile feeds and develops to maturity. The juvenile undergoes a second moult, six to seven days after penetration which is the third stage. The fourth stage female increases greatly in size, rupturing the root tissues, but remains fixed at the head position to the root. It moults again and becomes flask-shaped and white and at this stage the subcrystalline layer begins to form. Males are attracted to the females (Green 1967) by pheromones excreted in a gelatinous material in which some of the eggs are deposited. When feeding ends, the female dies and the cuticle becomes brown protecting the eggs inside her body from desiccation. The cysts are typically lemon-shaped.

The cysts of *H. schachtii* can remain dormant in the soil for long periods. Nickle (1984) found that in microplots, under fallow conditions, the eggs survived for 12 years. The cyst contained up to a maximum of 600 eggs in the form of coiled second stage juveniles (Raski 1949). Once the eggs have hatched, the nematode is susceptible to environmental extremes and has a rapid energy consumption. Under favourable conditions the juvenile uses its stylet to pierce the egg shell to escape from the cyst via the vulval or oral opening. After emerging from the cyst, the nematode moves through the soil in search of

a host plant root. Large proportions of *H. schachtii* eggs readily hatch in water. Cooke (1985) found that 21% of the juveniles hatched in distilled water at 20°C. The optimal temperature for hatching was 25°C and the minimum 10°C (Cooke 1985). Gleissl & Bachthaler (1989) found that root diffusates of favoured hosts promoted the orientation and penetration of juveniles. Perry & Trett (1986) found that hatching agents appeared to change the structure of the lipoprotein membrane of the egg shell, thereby allowing leakage of trehalose. Bound calcium was displaced from the egg shell by artificial hatching agents which may be relevant to egg shell permeability (Clarke & Perry 1985). Females of *H. schachtii* deposited some of their eggs in a gelatinous egg sac and these eggs hatched earlier and in greater percentages than those in the cysts. The egg sac remained up to three months without a significant contribution to the persistence of populations between host crops (Bowen *et al.* 1986). Maximum hatch of eggs occurred in soil with a water content close to field capacity, ensuring the availability of water to the hatching juveniles (Wallace 1958a).

Once the eggs hatch, the infective juveniles migrate through the soil and are vulnerable to environmental extremes and need to locate a root of a host plant to commence feeding. The results obtained by Wallace (1958b) indicated that the optimal temperature for juvenile activity was 15°C.

Wallace (1958b) found that, after removing the host plant with its roots, the juveniles still accumulated at the site of former root growth and concluded that this attraction was due to a concentration gradient of some chemical substance secreted by the roots. Bird

(1960) and Johnson & Viglierchio (1961) showed that juveniles accumulated at sources of carbon dioxide, quantitatively the most important root exudate. Klinger (1965) concluded that the carbon dioxide gradient was responsible for the orientation of *H. schachtii* juveniles. Dusenbury (1987) argued that a carbon dioxide gradient would not be effective in attracting the nematodes to the roots, but to have a more general function, such as guiding the nematodes to an suitable soil depth. Several gradients such as that of amino acids, pH and sugar gradients exist around physiologically active roots. Some of these compounds may constitute general attractants for long distance migration of juveniles to the roots of host plants. According to Perry (1996) other chemicals could also be responsible for orientation. Phytohormones could possibly be the trigger for the induction of nematode secretions (Duncan *et al.* 1995) and that auxin could act as a host cue has been supported by the evidence of a putative auxin binding protein associated with the amphids (Duncan *et al.* 1996). The electrical potential gradients in the region of root elongation may also play a role in attracting the juveniles to the actively growing root tip. According to Robertson & Forrest (1989) nematode movement can be orientated by redox potential or an electrical field created by the roots.

The juvenile nematodes move to a host root, exploring its surface by moving along the root and occasionally testing the surface with its lips and by stylet probing (Doncaster & Seymour 1973). The optimal temperature for root penetration was 20-25°C (Shepherd & Wallace 1959). The maximum penetration was observed at low inoculation levels using first hatched juveniles (Johnson & Viglierchio 1969). The growing tip of roots and sites of lateral root emergence were the preferred invasion sites (Mildenberger & Wartenberg

1958, Mankau & Linford 1960, Wyss & Zunke 1986). The speed of invasion is host dependant (Steinbach 1972b). Dickinson (1959) found that the hydrophobicity of a surface played a role in selecting an invasion site as waxed nitrocellulose membranes of roots were chosen most frequently by the nematodes.

The speed of stylet probing increased with the onset of attack to a speed of 150 probes per minute (Wyss & Zunke 1986). The selected invasion areas were perforated along a line into the cell surface by several perforations caused by rhythmical stylet thrusts (Wyss & Zunke 1986). This action leads to the formation of a slit which enables the juvenile to enter and likewise to penetrate subsequent layers. After entering a plant cell, the juvenile investigates the area by moving its head from side to side and by stylet probing (Wyss & Zunke 1986). The inner walls of the epidermal cells are penetrated easier and the cortex can be penetrated ten times faster than the outer walls (Steinbach 1972a). Steinbach (1973) and Wyss & Zunke (1986) found that the juveniles prefered to penetrate the inflexible corners of a cell. The age of the host influenced invasion, younger hosts being more prone to invasion than older hosts.

Intracellular migration of juveniles takes place in the plant tissue (Wyss & Zunke 1986, Wyss & Grundler 1992). The juvenile migrated straight into the vascular cylinder (Dropkin 1955, Endo 1964) or within the cortical tissue parallel to the root axis. The juvenile could migrate for two weeks in the cortical tissue of a less suitable host (Mankau & Linford 1960). The selection of a feeding site took place when the J_2 positioned itself next to or within the vascular system in the region of cell differentiation (Steinbach

1973). The feeding cell initiation was reported in different tissues, i.e. in the vascular cylinder (Wyss *et al.* 1984, Bleve-Zacheo & Zacheo 1987, Golinowski & Magnusson 1991), in the endodermal cells (Endo 1987) and in the cortex (Endo 1964). Golinowski *et al.* (1996) found that the J_2 selected a procambial or cambial cell as the initial syncytical cell.

After penetration into the initial syncytical cell the J_2 was subjected to a preparation period. This enabled the J_2 to enter the sedentary parasitic stage and this stage lasted between six and 18 hours (Wyss 1992). After the preparation period, the J_2 could not leave the root and it was suspected that atrophy of the locomotory muscles occurred during this period (Wyss 1992). Wyss (1992) found that if a J_2 was disturbed by another penetrating juvenile during the preparation period, the J_2 would select a new cell nearest to its anterior end and be able to start feeding earlier on this cell due to the partial completion of the preparation period.

Within a few hours after the onset of feeding an enlarged nucleus and an increase in cytoplasmic streaming and density was noted in the initial syncytical cell. Within one day the cell was transformed to a multinucleate syncytium with high metabolic activity. Cell wall dissolution took place and the merging of protoplast was noted 48 hours after initial syncytical cell initiation (Endo 1991). Protuberances adjacent to the xylem were formed by the thickening of the cell walls near the head of the nematode (Mankau & Linford 1960). These protuberances were associated with the transfer of nutrients from

the conductive tissues to the syncytium. The nematode could be considered a nutrient sink (Jones & Northcote 1972).

In order to reproduce a juvenile female *H. schachtii* must feed on a single transfer cell during its entire life. At large population densities, the available space for the formation of syncytia is limited and less syncytia are produced. In conjunction with the physiological condition of the host the sex ratio will also be influenced. According to Steele (1975) the development of males and females were disproportionately influenced by the nematode inoculum level and by the root size, these two factors determining the number of invading larvae.

The volume of food consumed by the males relative to that of females, at small to medium nematode densities was negligible. At high densities there could be eight to ten times as many males as females requiring 20% of the amount of food needed by the females (Trudgill 1967). *H. schachtii* produced more than one generation per year and in most temperate areas *H. schachtii* completed either two (Jones 1950, Müller 1979) or three (Vinduska 1969, Greco *et al.* 1982) generations per year. In the Imperial Valley, California, with a growing season of ten months and with relatively high soil temperatures, up to five generations per year were recorded by Thomason & Fife (1962).

The multiplication rates are density dependent and decrease with an increase in initial density due to intraspecific competition. Jones (1956) found that the final population on sugarbeet reached a maximum of 140 eggs per gram of soil in a fen skirt soil, but found

up to 230 eggs per gram of soil in a black fen soil. A logistic curve described the final population level in relation to the initial population level (Seinhorst 1970). Olthoff *et al.* (1974) found that the number of cysts per root system and number of cysts per gram of root in a range of vegetable crops were positively correlated with pre-plant densities.

H. schachtii is capable of moving only short distances by active migration. Dispersal is primarily by passive transport. *H. schachtii* eggs remained viable even after having passed through the digestive system of cattle, a possible source of infection if cattle are moved to uninfested fields (Kontaxis *et al.* 1976). Flooding, as well as irrigation with waste-water, can also disperse the nematode. The cysts can also be transported by wind and with the potential of rapid dissemination.

The soil environment influences survival, hatch, host finding, movement, penetration and mating and thus plays a critical role in the persistence of this nematode. The soil texture also plays an important role in plant growth and nematodes by influencing the nutrient and water holding capacity of the soil, rate of gas exchange and nematode movement. The greatest movement was detected in soils with particle sizes of 150-250 microns. At particle sizes smaller than 150-250 microns, downward motility was much less than with larger particles (Wallace 1958a). The soil structure influenced the bulk density of the soil which, in turn, impacted on the population densities of nematodes. Tyler *et al.* (1983) found that population densities were lower under a no-till cultivation system, probably due to the associated increase in soil bulk density.

The vertical distribution of *H. schachtii* in a sandy loam soil was investigated by Thorne & Giddings (1922). They found that 98% of the nematodes were present in the 0-35cm soil profile and only 2% in the 35-76 cm profile. Goffart (1954) found that the vertical distribution was influenced by the age of infestation. In a young infestation 83% of the cysts were present in the upper 10 cm of soil and in an older infestation cysts were uniformly distributed up to a depth of 50 cm. Korab (1929) found cysts up to a depth of 140 cm in soil.

Soil water relations and gas exchange played an important role in the survival and multiplication of *H. schachtii.* Nematode activity was negatively influenced by the lack of oxygen (Wallace 1963) and anaerobic conditions in the soil resulted in the formation of inorganic and organic acids and alcohols potential toxic to the nematodes. Wallace (1955) found that hatching of *H. schachtii* was optimal at a soil moisture level close to field capacity.

A factor that affects the importance of *H. schachtii* as plant parasites is their ability to survive within the cysts for long periods in the soil in the absence of host plants as cysts. The survival strategy in the cysts is achieved by a low metabolic activity of dormant juveniles, diapause and the response to root diffusates forming a complex pattern of behaviour. A decline in egg viability of 13% per year over a period over eight years was found by Hijner (1952). Olthof *et al.* (1974) found a decline of 36% in viable eggs in the first year and 60% in the subsequent year. The population declined in the soil due to

spontaneous hatching in the absence of a host leading to a seasonal decline in fallowed soil (Wallace 1958b).

Raski & Johnson (1959) found that the optimal temperature for the development of *H. schachtii* ranged between 21-26°C, with overall development taking place between 18-29.5°C. Santo & Bolander (1979) reported that development at 24°C was significantly greater than at 16, 18 and 21°C. Johnson & Viglierchio (1969) found the optimum development temperature of *H. schachtii* on axenic *Beta vulgaris* to be 25°C and only males were observed in cultures maintained at 30°C. Thomason & Fife (1962) found maximum reproduction of *H. schachtii* to be at 27.5°C.

Winslow & Williams (1957) observed amoeboid organisms attacking *H. schachtii* juveniles. *Mononchus papillatus* (Bastian 1965) and *M. sigmaturus* (Cobb 1917) were found to consume larvae (Thorne 1927). Polychronopoulos & Lownsbery (1968) found that heavy infestations of *H. schachtii* delayed and reduced emergence and caused stunting, necrosis and hairy root symptoms in affected hosts. They also found that some of the seedlings failed to emerge or died just after emergence. The appearance of poor or reduced growth of crops in areas in a field and premature wilting of the outer leaves were the first indicators of an infestation (Cook *et al.* 1983). Such areas may appear sporadically or homogenous if the entire field is infested. Macroscopic symptoms showed an elongation of the petioles together with a reduction in leaf size, chlorophyll content and photosynthetic activity of hosts (Kitsno *et al.* 1980). The tap roots of heavily infested plants were stunted with excessive lateral root development and poor yields. The

leaves were normally pale or lighter green in colour than those of uninfested plants. The stunted appearance of plants affected by *H. schachtii* remained untill the end of the growth cycle of the surviving plant. The extent of damage caused to the host by the nematodes was related to the concentration of amino acids in the syncytia due to their influence on nematode development, as *H. schachtii* development was influenced by the concentration of certain amino-acids (Betka *et al.* 1991). Glutamine supported female development, whereas methionine, phenylalanine, lysine and tryptophan inhibited female development (Betka *et al.* 1991). The concentration change of these amino acids in the syncytium could influence nematode development and thus indirectly the amount of damage caused to the host plant.

The damaged root tissue may serve as infection ports for secondary infections. The damage done to the vascular tissue of plants was permanent and impeded normal elongation and development of roots with loss of optimal root vigour. The photosynthetic rate of plants under high levels of infection was suppressed, probably due to the damage caused by nematodes to the root system. The damage caused a decrease in water uptake, resulting in moisture stress conditions in the plant which in turn suppressed the photosynthetic rate.

Interactions between soil pathogens are common, but there are relatively few reports of interactions involving *H. schachtii.* Müller (1980) found that increases in sugar beet yield caused by a fungicide plus nematicide treatments were higher than the sum of the increases from separate nematicide and fungicide treatments. This was attributed to

synergism between *H. schachtii* and soil fungi. Sugarbeet losses due to *Cercospora beticola* were aggravated by infections of *H. schachtii* (Nickle 1984). Polychronopoulos *et al.* (1969) found that *Rhizoctonia solani* killed infested sugarbeet seedlings more rapidly than uninfested seedlings. Following the root invasion by nematodes, the syncytical cells became infected by the fungus which subsequently also spread to adjacent cells. Necrosis was increased by the presence of both pathogens. Whitney (1974) found that the damping-off of seedlings caused by *Pythium ultimum* increased in *H. schachtii*- infested soil.

Aphanomyces cochlioides and *H. schachtii* also showed a synergistic effect at high inoculum levels, but at lower levels there was an indication that the organisms were antagonistic (Whitney & Doney 1973). Jorgenson (1970) found antagonism between *H. schachtii* and *Fusarium oxysporum.* The simultaneous inoculation of these organisms simultaneously did not alter the population dynamics of either (Jatala & Jenson 1976). They found that by first inoculating the sugarbeet with *H. schachtii* and then by *Meloidogyne hapla* (Chitwood 1949), the production of root-knot galls was reduced.

Heterodera schachtii has a wide host range that spans 23 plant families, including at least 218 plant species (Steele 1965). The most important cultivated host is sugar beet, hence its vernacular name as the sugar beet nematode. The host range consists mainly of *Amaranthaceae, Capparidaceae, Caryophyllaceae, Chenopodiaceae, Cruciferae, Labiatae, Leguminosae, Phytolaccaceae, Polygonaceae, Portulacaceae, Primulaceae, Resedaceae, Scrophulariaceae, Solanaceae, Tropaeolaceae, Umbelliferae* and

Urticaceae (Steele 1965). The economically most important hosts are *Beta vulgaris* L. and *B. oleraceae* L.

Crop rotation is an effective and practical management tool for the control of *H. schachtii.* The hosts should be rotated with non-hosts such as beans, lucerne, onions, maize and grain for various periods, depending on the nematode infestation levels. The control of weeds in the crop rotation cycle played an important part in the success of this control method. Müller (1986) stated that growing a host crop once every five years will provide effective control, but for the production of vegetable crops this long period was not economically viable. The population densities of *H. schachtii* could be reduced by growing resistant radish and mustard before the host crop was grown (Müller 1991). Steudel & Müller (1983) found that the natural population decline in nematodes, after the host crop was removed, was 53% in the first year with a non-host crop and 30% in the second year of non-host crops. Heijbroek (1977) found a similar decrease of *H. schachtii* during a three-year rotation cycle. The rotation system was modified to include a resistant green manure crop after sugar beet (Steudel & Muller 1983) which reduced the population to 17% of the initial population density (P_i) value. Based on work by Seinhorst (1970), Jones & Kempton (1978) and Thielemann & Steudel (1973) the general recommendation was that a specific host crop should not be grown more than every three years.

Kuhn (1881) investigated the effect of growing radish as a trap crop to induce hatch of eggs of *H. schachtii*. The biggest danger with trap cropping was that the timing had to be

precise otherwise the trap crop would rather increase the population levels (Balandras 1986). Trap crops of radish (*Raphanus sativus* L. Pegletta) and white mustard (*Sinapsis alba* L. Tilney) were utilized for the management of *H. schachtii.* Cultivation of nematode resistant trap crops in nematode-infested soils triggered the nematode eggs to hatch altough the adults were not able to reproduce (Lubberts & Toxopeus 1982).

The percentage hatch of one-year old eggs in leachates of resistant mustard and radish and that of susceptible rape and sugar beet, ranged from 62 – 84% and was greater than the normal water hatch of 42% (Heijbroek, 1982). Cooke (1985) found the highest hatch percentage at 20°C with progressively lesser hatchings at 15, 10 and 5°C. Nematode resistant trap crops were tested in Idaho and it was found that Pegletta and Nemex oil radish and white mustard reduced the number of *H. schachtii* eggs by 67, 23 and 87%, respectively (Hafez 1999). Fallowed soil only reduced the eggs by 28%. Similar results were obtained in Wyoming where yield increases of between 3.8 and 9.5 tons per acre were obtained when sugar beet was planted subsequent to trap crops (Hafez 1999). Planting trap crops in rows alternating with the host (sugar beet) to stimulate hatch and to attract large numbers of juveniles was investigated by Müller (1985). He found no significant reduction of the nematodes in the sugar beet and the possible competition between the trap crops led to reduced yields of the host.

H. schachtii is subject to predation or parasitism by a number of soil organisms during the four distinct phases of its life cycle. According to Jones & Kempton (1978) a soil organism that destroys second stage juveniles (J_2) could cause a reduction in plant

damage, but because of the pyramidal age structure of the population, it probably removed those individuals excessive to the carrying capacity of the host, with little or no effect on the population numbers of nematodes at the end of the season.

Resistant crops have a major impact on integrated control systems, lowering the population and allowing for a shorter rotation period. Pawelska-Kozinska & Szota (1970) and Korol'chuk *et al.* (1971) attempted to select resistance in cultivated and wild strains of *B. vulgaris.* Müller (1992) suggested that whenever new sources of resistand hosts were deployed, new cyst nematode pathotypes were selected. The existence of *H. schachtii* pathotypes was demonstrated by Steele (1975), Griffen (1981) and Müller (1992). Should *H. schachtii* be managed by resistance or crop rotation, the genetic variation within and among populations must be characterized (Caswell & Roberts 1987, Opperman *et al.* 1994). Californian *H. schachtii* populations of different localities showed genetic diversity between populations (Caswell-Chen *et al.* 1992).

Chemical control of cyst nematodes at present is effective, but the highly successful nematicides and fumigants are being phased out due to environmental concerns and dangerous nature of the products (Roberts 1993). After the success of 1.3-dichloropropene + 1,2-dichloropropane (D-D), various chemicals were used for nematode management. These included 1,2-dibromo-3-chloropropane (DBCP), ethylene dibromide (EDB), trichloronitromethane, methyl bromide and aldicarb. Aldicarb found to be very effective when applied correctly, but that the effectiveness was adversely affected by unfavourable environmental conditions (Smith *et al.* 1991). The control of cyst

nematodes having more than one generation per year, such as *H. schachtii*, was more difficult than of those having one generation. The recommended application rates were 150 l/ha 1,3-dichloropropene or 250 l/ha 1.3-dichloropropene-1,2-dichloropropane mixture. Metham sodium was recommended at 300 l/ha. In France 1.3-dichloropropene-1,2-dichloropropane mixture at a rate of 250 l/ha was recommended when the population of *H. schachtii* exceeded 6 eggs/g soil (Richard-Molard 1984).

References

Balandras C M 1986. Limits to methods for management of potato cyst nematodes in western France, in *Cyst Nematodes* (eds F Lamberti and C E Taylor), Plenum Press, New York, pp. 229-34.

Bello A & Romero D 1973. *Heterodera schachtii* (Schmidt, 1871) en los suelos de las Islas Canarias. *Anales de Edafologia y Agrobiologia* 32: 887-892.

Betka M, Grundler F & Wyss U 1991. Influence of changes in the nurse cell system (syncytium) on the development of the cyst nematode *Heterodera schachtii*: Single amino acids. *Phytopathology* 81: 75-79.

Bird A F 1960. Additional notes on the attractiveness of roots to plant parasitic nematodes. *Nematologica* 5: 217.

Bleve-Zacheo T & Zacheo G 1987. Cytological studies of the susceptible reaction of sugar beet roots to *Heterodera schachtii*. *Physiological and Molecular Plant Pathology* 30: 13-25.

Bowen S A, Storey G W & Evans K 1986. The contribution of egg sacs to the population dynamics of *Heterodera cruciferae* and *H. schachtii*. *Revue dé Nematologie* 9: 290.

Caswell-Chen E P & P A Roberts 1987. Nematode population genetics. In: J A Veech and D W Dickson, eds. *Vistas on nematology*. Maryland: Society of Nematologists. pp. 390-397.

Caswell-Chen E P, Williamson V M & Wu F F 1992. Random amplified polymorphic DNA analysis of *Heterodera cruciferae* and *H. schachtii* populations. *Journal of Nematology* 24: 343-351.

Clarke A J & Perry R N 1985. Egg-shell calcium and the hatching of *Globodera rostochiensis. International Journal for Parasitology* 15: 511-516.

Coetzee V 1968. The distribution of the family *Heterodera* (Filipjev, 1934) in South Africa and some host records of *Meloidogyne* species. *South African Journal of Agricultural Science* 11: 775-788.

Cooke D A 1985. The effect of resistant cultivars of catch crops on the hatching of *Heterodera schachtii*. *Annals of applied Biology* 106: 111-120.

Cooke D A & Southey J F 1983. Beet cyst nematode. *Ministry of Agriculture, Fisheries and Food, Leaflet* 233.

Dickson S 1959. The behaviour of larvae of *Heterodera schachtii* on nitrocellulose membranes. *Nematologica* 4: 60-66.

Doncaster C C & Seymour M K 1973. Exploration and selection of penetration site by *Tylenchida. Nematologica* 19: 137-45.

Dropkin V H 1955. The relations between nematodes and plants. *Experimental Parasitology* 4: 282-321.

Duncan L H, Robertson W M & Kusel J R 1995. Induction of secretions in *Globodera pallida. Proceedings of the British Society for Parasitology.* Edinburgh. p.13.

Duncan L H, Robertson W M, Kusel J R & Phillips M S 1996. A putative nematode auxin binding protein from the potato cyst nematode *Globodera pallida*. *Proceedings of the Third International Nematology Congress*. Guadeloupe, p. 116

Dusenbery D B 1987. Prospects for exploiting sensory stimuli in nematode control, in *Vistas on Nematology* (eds J A Veech and D W Dickson), Society of Nematologists, Hyattsville, Maryland, pp. 131-35

Endo B Y 1964. Penetration and development of *Heterodera glycines* in soybean roots and related anatomical changes. *Phytopathology* 54: 79-88.

Endo B Y 1987. Histopathology and ultrastructure of crops invaded by certain sedentary endoparasitic nematodes. In: *Vistas on Nematology* (eds. J A Veech & D W Dickson), Society of Nematologists, Hyattsville, Maryland, pp196-210.

Endo B Y 1991. Ultrastructure of initial responses of susceptible and resistant soybean roots to infection by *Heterodera glycines*. *Revue dé Nematologie* 14: 73-94.

Franklin M T 1951. The cyst-forming species of *Heterodera*. Commonwealth Agricultural Bureaux, Franham Royal. UK.

Gleissl W & Bachthaler G 1989. Influence of physiological properties of weeds on hatching, orientation and penetration of larvae as well as sex differentiation of *Heterodera schachtii* Schmidt. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 96: 187-96.

Goffart H 1954. Gegenwartsfragen zum Rübennematodenproblem. *Zucher* 7: 130-137.

Golinowski W, Grundler F M W & Sobczak M 1996. Changes in the structure of *Arabidopsis thaliana* during female development of the plant parasitic nematode *Heterodera schachtii*. *Protoplasma* 194: 103-16.

Golinowski W & Magnusson C 1991. Tissue response induced by *Heterodera schachtii* in susceptible and resistant white mustard cultivars. *Canadian Journal of Botany* 69: 53-62.

Greco N, Brandonisio A & Marinis G 1982. Investigation on the biology of *Heterodera schachtii* in Italy. *Nematologia Mediterranea* 10: 201-214.

Green C D 1967. The attraction of male cyst-nematodes by their females. *Nematologica* 13: 173-173.

Griffen G D 1981. Pathological differences in *Heterodera schachtii* populations. *Journal of Nematology* 13: 191-195.

Hafez S L 1999. Sugar beet cyst nematode: Impact on Sugar beet production in Idaho and Eastern Oregon. *Idaho Agricultural Experiment Station Bulletin* 792.

Heijbroek W 1977. Mogelijkheden om de schade door het bietenzystenaaltje te voorkomen. *Bedrijfontwikkeling* 8: 451.

Heijbroek W 1982. The influence of resistant cruciferous green manure crops on beet cyst nematodes. *Mededelingen van het Instituut voor Rationele Suikerproductie. Bergen op Zoom Nr.* 58, 20 pp.

Hijner J A 1952. De gevoeligheid van wilde bieten voor het bietencystenaaltje. *Mededelingen van het Instituut voor Rationele Suikerproductie. Bergen op Zoom* 21: 1-13.

Jatala P & Jenson H J 1976. Parasitism of *Beta vulgaris* by *Meloidogyne hapla* and *Heterodera schachtii* alone and in combination. *Journal of Nematology* 8: 200-205.

Johnson R N & Vilglierchio D R 1961. The accumulation of plant parasitic nematode larvae around carbon dioxide and oxygen. *Proceedings of the Helminthological Society of Washington* 28: 171-174.

Johnson R N & Vilglierchio D R 1969. Sugar beet nematode *Heterodera schachtii* reared on axenic *Beta* root explants. I. Selected environmental factors affecting penetration. *Nematologica* 15: 129 – 143.

Jones F G W 1950. Observations on the beet eelworm and other cyst-forming species of *Heterodera*. *Annals of Applied Biology* 37: 407-440.

Jones F G W 1956. Soil population of beet eelworm (*Heterodera schachtii* Schm.) in relation to cropping. II. Microplot and field plot results. *Annals of Applied Biology* 44: 25 – 26.

Jones F G W & Kempton R A 1978. Population dynamics, population models and integrated control. In *Plant Nematology*. Ministry of Agriculture, Fisheries and Food, GDI (replaces Technical Bulletin 7). (J F Southey Ed) pp 333 – 361. HMSO, London.

Jones M G K & Northcote D H 1972. Nematode-induced syncytium – A multinucleate transfer cell. *Journal of Cell Science* 10: 789-809.

Jorgenson E C 1970. Antagonistic interaction of *Heterodera schachtii* Schmidt and *Fusarium oxysporum* (Woll) on sugar beets. *Journal of Nematology* 2: 393 – 398.

Kitsno L V, Borisyuk VA, Medvedev A A & Linnik L I 1980. (Translated title) Formation of the assimilation apparatus and yield capacity in sugarbeet growing in soil infected with *Heterodera schachtii*. *Doklady Akademii Nauk Ukrainskoi USSR. Series, B* No. 3. 87-90.

Klinger J 1965. On the orientation of plant nematodes and of some other soil animals. *Nematologica* 11: 4-18.

Kontaxis D G, Lofgreen G P, Thomason I J & McKinney H E 1976. Survival of the sugar beet cyst nematode in the alimentary canal of cattle. *California Agriculture* 30 (3): 15.

Korab I I 1929. Materials for the study of the beet nematode *Heterodera schachtii* Schm. From data of investigations carried out at the nematode laboratory of the Veltserkov Selection Station S.S.U. Preliminary report. *Ukrainian res. Inst. Sugar Indus. Kiev* 8: 20-67.

Korol'chuk V V, Nesterov P I, Bryushkova G I & Lizogubova L P 1971. (Translated title) Evaluation of the resistance of local varieties of sugarbeet to *Heterodera schachtii*. In: Parazity zhivotnykh I rastenii. Kishinev: Izdatel'stvo 'Shtiinsta' 7: 150-155.

Kühn J 1881. Die Ergebnisse der Versuche zur Ermittlung der Ursache der Rübenmüdigkeit und zur Erforschung der Natur der Nematoden. *Berichte aus dem physiologischen Laboratorium und der Versuchsanstalt des landwirthschaftlichen Institutus der Universitat Halle* 3: 1-153.

Krall EL & Krall K A 1978. (Translated title) Revision of the plant nematodes of the family *Heteroderidae* on the basis of the trophic specialization of these parasites and their co-evolution with their hosts plants. *Filogelmintologicheskie issledovaniya Moscow, USSR, "Nauka"* 39-56.

Lubberts J H & Toxopeus H 1982. Het ontwikkelen van bietencystenaaltjes-resistentie op bladrammenas en gele mosterd. *Zaadbelangen* 36: 66-69.

Mankau R & Linford M B 1960. Host-parasite relationships of the clover cyst nematode, *Heterodera trifolii* Goffart. Agriculture Experimental Station, University of Illinois. *Bulletin* 667, pp50

Mildenberger G & Wartenberg H 1958. Histologische Untersuchungen der Nematodengallen in den Wurzeln der Kartoffelpflanze, (*Heterodera rostochiensis* Woll. und *Meloidogyne* spec. in *Solanum tuberosum* L) *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 65: 449-64.

Müller J 1979. Über die jährliche Generationszahl von *Heterodera schachtii* unter Feldbedingungen an Zuckerrüben. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, Braunschweig* 31: 92-95.

Müller J 1980. Wechselwirkungen zwischen *Heterodera schachtii* und Bodenpilzen an Zuckerrüben. *Phytopathologische Zeitschrift* 97: 357-363.

Müller J 1985. Der Einfluss der Wirtspflanze auf die Geschlechtsdeterminierung bei *Heterodera schachtii. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem* 226: 46-63.

Müller J 1986. Integrated control of the sugar beet cyst nematode, in *Cyst Nematodes* (eds. F Lamberti & C E Taylor), Plenum Press, New York, pp. 235-250.

Müller J 1991. Catch cropping for population control of *Heterodera schachtii. Proceedings of the 54th Winter Congress of the International Institute of Sugar Beet Research*, pp 179-96.

Müller J 1992. Detection of pathotypes by assessing the virulence of *Heterodera schachtii* populations. *Nematologica* 38: 50-64.

Nickle W R 1984. *Plant and insect nematodes.* Marcel Dekker, Inc. New York.

Olthoff T H A, Potter J W & Peterson E A 1974. Relationship between population densities of *Heterodera schachtii* and losses in vegetable crops in Ontario. *Phytopathology* 64: 549-554.

Opperman C H, Dong K & Chang S 1994. Genetic analysis of the soybean – *Heterodera glycines* interaction. In: Lamberti F, DeGeorgi C, and McK Bird D (eds), *Advances in Molecular Plant Nematology.* pp.65-75.

Pawelska-Kozinska K & Szota Z 1970. (Translated title) Studies on the degree of tolerance to the sugar-beet nematode (*Heterodera schachtii* Schm.) in some varieties and strains of sugar beet. *Hodowla Roslin, Aklimatyzacja I Nasiennictwo* 14: 39-48.

Perry R N 1996. Chemoreception in plant parasitic nematodes. *Annual Review of Phytopathology* 34: 181-99.

Perry R N & Trett M W 1986. Ultrastructure of the eggshell of *Heterodera schachtii* and *H. glycines*. (Nematoda: *Tylenchida*). *Revue dé Nematologie* 9: 399-430.

Polychronopoulos A G & Lownsberry B F 1968. Effect of *Heterodera schachtii* on sugar beet seedlings under monoxenic conditions. *Nematologica* 14: 526-534.

Polychronopoulos A G, Houston B R & Lownsbery B F 1969. Penetration and development of *Rhizoctonia solani* in sugar beet seedlings infected with *Heterodera schachtii*. *Phytopathology* 59: 482-485.

Raski D J 1949. The life history and morphology of the sugar-beet nematode, *Heterodera schachtii*. *Phytopathology* 40: 135-152.

Raski D J & Johnson R T 1959. Temperature and the activity of the sugar beet nematode as related to sugar beet production. *Nematologica* 4: 131-141.

Richard-Molard M 1984. Nematode control in sugarbeet. *Sugar Journal* 46: 15-16.

Roberts P A 1993. Influence of nematicides on nematode pathogens and their host plants. In: *Pesticide Interactions in Crop Production: Beneficial and Deleterious Effects* (ed J Altman), CRC Press, Boca Raton, Florida, pp. 335-52.

Robertson W M & Forest J M S 1989. Factors involved in host recognition by plant-parasitic nematodes. *Aspect of Applied Biology* 22: 129-33.

Santo G S & Bolander W J 1979. Interacting effects of soil temperature and type on reproduction and pathogenicity of *Heterodera schachtii* and *Meloidogyne hapla* on sugar beet. *Journal of Nematology* 11: 289-291.

Schmidt A 1871. Über den Rüben-nematoden. *Zeitschrift des Vereins für die Rübenzucker-industrie im Zollverein* 22: 67-75.

Seinhorst J W 1970. Dynamics of populations of plant nematodes. *Annual Review of Phytopathology* 8: 131-156.

Shepherd A M and Wallace H R 1959. A comparison of the rates of emergence and invasion of beet eelworm *Heterodera schachtii* Schmidt and pea root eelworm *Heterodera göttingiana* Liebscher. *Nematologica* 4: 227-235.

Smith G S, Niblack T L & Minor H C 1991. Response of Soyabean cultivars to aldicarb in *Heterodera glycines*-infested soils in Missouri. *Supplement to the Journal of Nematology* 23: 693-98.

Steinbach P 1972a Eine Lebendbeobachtungmethode für Verhaltensstudien an *Heterodera rostochiensis*-Larven. *Biologische Rundschau* 10: 57-59.

Steinbach P 1972b. Untersuchungen über das Verhalten von Larven des Kartoffelzystenälchens (*Heterodera rostochiensis* Wollenweber, 1923) an und in Wurzeln der Wirtspflanze *Lycopersicon esculentum* Mill. II Das Eindringen von Kartoffelnematodenlarven in die Wirtswurzel. *Biologisches Zentralblatt* 91: 743-56.

Steinbach P 1973. Untersuchungen über das Verhalten von Larven des Kartoffelzystenälchens (*Heterodera rostochiensis* Wollenweber, 1923) an und in Wurzeln der Wirtspflanze *Lycopersicon esculentum* Mill. III Die Nahrungsaufnahme von Kartoffelnematodenlarven. *Biologische Zentralblatt* 92: 563-82.

Steele A E 1965. The host range of the sugar beet nematode *Heterodera schachtii* Schmidt. *Journal of the American Society of Sugar Beet Technologists* 13: 573-603.

Steele A E 1975. Population dynamics of *Heterodera schachtii* on tomato and sugar beet. *Journal of Nematology* 7: 105-111.

Steudel W & Müller J 1981. Der Einfluss resistenter Ölrettichlinien auf die Abundanzdynamik von *Heterodera schachtii* Schmidt. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, Braunschweig* 33: 97-103.

Steudel W & Müller J 1983. Untersuchungen und Modellrechnungen zum einfluß pflanzenverträglicher und nematodenresistenter Zwischenfrüchte auf die

Adundanzdynamik des Zuckerrübennematoden (*Heterodera Schachtii*). *Zuckerrübenfruchtfolgen, Zuckerind* 108: 365.

Thielemann R & Steubel W 1973. Neunjährige Erfahrungen mit Monokultur von Zuckerrüben auf mit *Heterodera schachtii* (Schmidt) verseuchtem Boden. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, Braunschweig* 25: 145-149.

Thomason I J & Fife D 1962. The effect of temperature on development and survival of *Heterodera schachtii* Schm. *Nematologica* 7:139-145.

Thorne G 1927. The life history, habits and economic importance of some *Mononchus*. *Journal of Agricultural Research* 34: 265-283.

Thorne G & Giddings L A 1922. The sugar beet nematode in the Western States. *U.S. Departement of Agriculture Bulletin no.* 1248.

Trudegill D L 1967. The effect of environment on sex determination in *Heterodera rostochiensis*. *Nematologica* 13: 263-72.

Tyler D D, Overton J R & Chambers A Y 1983. Tillage effects on soil properties, diseases, cyst nematodes, and soybean yields. *Journal of Soil Water Conservation* 38: 374-76.

Vinduska L 1969. Pocet generaci hadatka repneho *Heterodera schachtii* Schmidt v nasich podminkach. *Rostlin Vyoba* 15: 757-763.

Wallace H R 1955. The influence of soil moisture on the emergence of larvae from cysts of the eelworm, *Heterodera schachtii* Schmidt. *Annals of Applied Biology* 43: 477-84.

Wallace H R 1958a. Movement of eelworms. 1. The influence of pore size and moisture content of the soil on the migration of larvae of the beet eelworm, *Heterodera schachtii* Schmidt. *Annals of Applied Biology* 46: 74-85.

Wallace H R 1958b. Observation on the emergence from cysts and the orientation of larvae of three species of the genus *Heterodera* in the presence of host plant roots. *Nematologica* 3: 236-243.

Wallace H R 1963. *The Biology of Plant-Parasitic Nematodes*. Edward Arnold, London.

Whitney E D 1974. Synergistic effect of *Pythium ultimum* and the additive effect of *P aphanidermatum* with *Heterodera schachtii* on sugarbeet. *Phytopathology* 64: 380-383.

Whitney E D & Doney D L 1973. The effects of *Heterodera schachtii* and *Aphanomyces cochlioides* on root-rot of sugar beet. *Journal of the American Society of Sugar Beet Technologists* 17: 240-245.

Winslow R D & Williams T D 1957. Amoeboid organisms attacking larvae of potato root eelworm (*Heterodera rostochiensis* Woll.) in England and the beet eelworm (*H. Schachtii* Schm.) in Canada. *Ziekten* 63: 242-243.

Wyss U 1992. Observations on the parasitic behaviour of *Heterodera schachtii* throughout its development, including events during molt. *Fundamental and Applied Nematology* 15: 75-89.

Wyss U & Grundler F M W 1992. Feeding behaviour of sedentary plant parasitic nematodes. *Netherland Journal of Plant Pathology* 98: 165-73.

Wyss U, Stender C & Lehmann H 1984. Ultrastructure of feeding sites of the cyst nematode *Heterodera schachtii* Schmidt in roots of susceptible and resistant *Raphanus sativus* L var. oleifornis Pers. Cultivars. *Physiological Plant Pathology* 25: 21-37.

Wyss U & Zunke, U 1986. Observation on the behaviour of second stage juveniles of *Heterodera schachtii* inside host roots. *Revue dé Nematologie* 9: 153-65.

Chapter 2.

Distribution and host range of the sugar beet nematode, *Heterodera schachtii* (Schmidt, 1871), in the greater Cape Flats, South Africa

Introduction

The sugar beet nematode or beet cyst nematode (*Heterodera schachtii* Schmidt, 1871) was discovered by Schacht at Halle in Germany in 1859. It was primarily a parasite of sugar beet, *Beta vulgaris* L., and responsible for more than 90% of the damage caused to this crop by nematodes (Steele, 1984). Total crop loss of beet grown in the USA was not uncommon and the damage caused to beet by this nematode was estimated at 25%. In central Europe it has long been regarded as the most serious pest of sugar beet where it was implicated in numerous crop failures and resulted in the closure of beet sugar factories (Steele, 1984).

The first record of *H. schachtii* in South Africa was by Coetzee (1968). Apart from the observations of Daiber (1990, 1992), no extensive study of any aspect of this important nematode has been undertaken in South Africa.

The aim of the present study was to gain information on the distribution and host range of *H. schachtii* in the greater Cape Flats and its immediate vicinity.

Materials and methods

A survey to determine the presence of *H. schachtii* on various host plants in the vegetable-growing areas of the greater Cape Flats was conducted during September 1997 to May 1998. A total of 728 soil and plant samples were randomly collected in a vegetable production area of approximately 325 hectares (Fig. 1). A soil sample consisting of 10 sub-samples of 1000 g each was collected from the upper 200 mm of soil. Plant samples collected consisted of entire plants. These were placed separately in numbered plastic bags and transported in a cool-bag to the laboratory at Elsenburg.

To collect cysts quantitatively, each soil sample was processed as follows: each bulked sample, consisting of 10 sub-samples, was passed through a 4 mm aperture sieve to retain organic material and large stones. The soil was then mixed by hand to a homogeneous consistence from which a representative sample of 500 g was taken. The sample was air-dried at room temperature.

Fifty grams of the 500 g air-dried soil was placed in a 750 ml Erlenmeyer flask half filled with water and shaken vigorously for half a minute. After five minutes the flask was filled almost to the brim with water and left for a further 5 minutes to allow the cysts and light debris to rise to the surface. The floating material was decanted through a pair of nested sieves, the upper with pore size of 850 µm and the lower with pore size of 150 µm. This procedure was repeated twice. The residue on the 150 µm aperture sieve was rinsed onto fluted filter paper in a funnel and the residue was left until the water had

drained completely. The filter paper with the material was removed and dried at room temperature. The cysts on the filter paper were then counted using a microscope.

The number of eggs and juveniles in 50% of the cysts of *H. schachtii* was determined when 30 or more cysts/sample were encountered, while the entire population was counted when fewer than 30 cysts/sample were present. Cysts were crushed to release the eggs and juveniles, which were then counted and expressed as average number of juveniles and viable eggs per gram of soil (Daiber, 1992). Meyer's cyst grinder was used to crush the cysts. This device consists of a glass cylinder, 100 mm long, inner diameter of 28 mm, and a plunger with a metal shaft and a perspex disc that fits tightly inside the tube. The plunger is held in position by a perspex top around the shaft. Moist cysts are placed in 4 ml water in the tube and the plunger pushed down with a twisting motion to crush the cysts. The suspension in the tube is then decanted and the eggs and juveniles counted. The results obtained with the device compare well with those of Reid (1955).

The area surveyed was, based on geographical positions of infested sample sites, subdivided into three regions: Phillipi/ Mitchell's Plain/ Guguletu/Khayelitsha, Kraaifontein/ Durbanville, and Kuilsriver/ Stellenbosch (Fig. 1). The sample sites were all irrigated on a regular basis.

The presence of fully developed cysts on the root system of a plant was considered proof of a positive host. The root system of every plant was examined and the severity of the infestation was rated as follows:

0 cysts	=	no infestation
1 – 10 cysts	=	light infestation
11 – 30 cysts	=	medium infestation
> 30 cysts	=	heavy infestation

The pH was determined with a pH meter, 5 ml of soil was mixed with 30 ml KCl and left for 20 minutes before determination. The soil texture was determined according to the method described by Bouyoucos (1962).

Results and discussion

Distribution

No relationship between the presence of *H. schachtii* and pH of the soil was found. *Heterodera schachtii* was present on the roots of host crops and in the soil of 35 samples and in 10 samples from non-host crops (Tables 1,2). The soil texture of the samples was sandy, and ranged in pH from 5.1 – 6.6.

Soil samples from Region 1 (Table 1) had the highest incidence of *H. schachtii*, with 47% of the sites infested. In Region 2 (Table 1) 18% of the sites were infested and the distribution was limited to a small area. The farmers in this region applied an effective nematode control program by using crop rotation. The infestations found were probably due to absence of crop rotation.

Soil samples from Region 3 (Table 1) indicated 35% of the sites being infected with *H. schachtii*. Farms in this area are predominantly planted to vineyards, with vegetables as cash crops, but a small number of farmers do operate as full-time vegetable growers. The latter farms showed the highest incidence of *H. schachtii* due to being small-scale enterprises and as a consequence the continuous planting of cruciferous crops. Rapid spread of the nematode is presently occurring in this region due to frequent movement of equipment and labourers between infested and non-infested fields.

Population densities

Population numbers of *H. schachtii* are given in Tables 1 and 2. The highest population density of *H. schachtii* was found in Region 1 (average 15.30 eggs and juveniles/g soil) followed by Region 2 (average 7.95 eggs and juveniles/g soil) and Region 3 (7.21 eggs and juveniles/g soil) (Table 1). These are high population densities when compared to the findings of Daiber (1990) at Rondebult, Gauteng. Two exceptionally high densities were found at Khayalitsha, viz. 61.2 and 52.1 eggs and juveniles/g of soil where the crop failed in both instances. The average density of *H. schachtii* for all three regions was 11.09 eggs and juveniles/g soil. The high densities in the surveyed area resulted in crop reduction, similar to the results of Olthof *et al.* (1974). The threshold population density for crop damage by *H. schachtii* in the greater Cape Flats has not yet been established, but according to Griffen (1981) 2,9 eggs and juveniles per gram of soil reduced yields of sugar beet by 12%. The economic threshold of two eggs and juveniles/gram soil used by Daiber (1992) was lower than any infestation found in the greater Cape Flats.

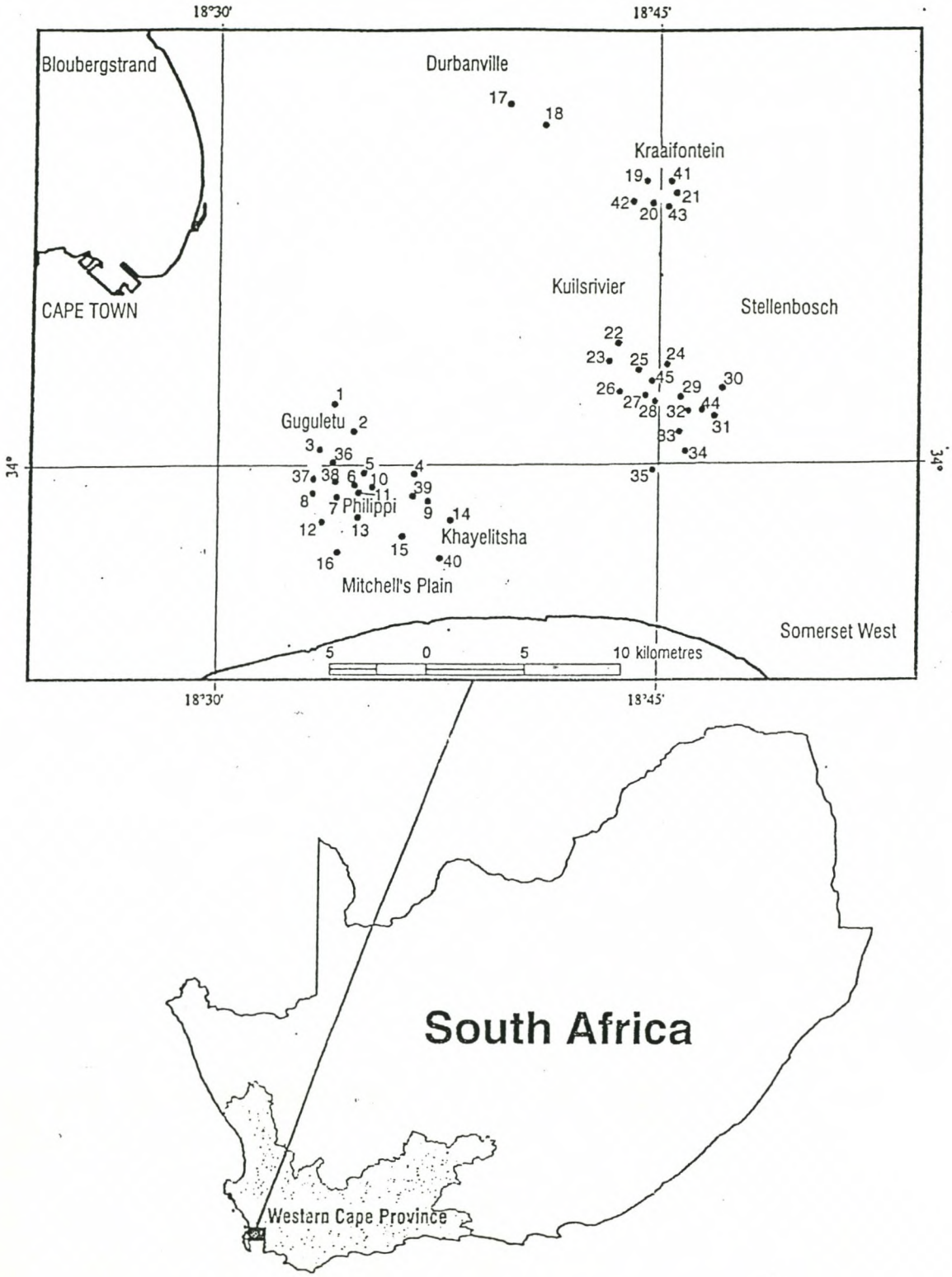

Fig. 1 Distribution of *Heterodera schachtii* in the greater Cape Flats, South Africa, 1997-1998.

Table 1. Numbers of *Heterodera schachtii* and infected host crops in the greater Cape Flats, South Africa (1997 – 1998).

Locality	Position see Fig.1	Eggs and juveniles/gram soil	Host crop	Previous crop
Region 1	1	9.3	cabbage	broccoli
	2	15.4	Brussels sprouts	cabbage
	3	12.4	cabbage	lettuce
	4	17.8	beetroot	cabbage
	5	52.1	cabbage	cabbage
	6	12.3	Brussels sprouts	carrot
	7	12.1	cabbage	cabbage
	8	8.9	cabbage	pumpkin
	9	61.2	cabbage	cabbage
	10	8.2	cauliflower	cauliflower
	11	12.2	cabbage	onion
	12	11.2	cabbage	cabbage
	13	12.7	beetroot	cabbage
	14	15.3	cauliflower	cabbage
	15	10.2	cabbage	cabbage
	16	16.5	beetroot	tomato
Region 2	17	12.8	cabbage	cabbage
	18	3.1	cauliflower	carrot
	19	3.1	cabbage	carrot
	20	12.2	cabbage	cauliflower
	21	9.2	cabbage	cabbage
Region 3	22	9.3	cabbage	cauliflower
	23	9.2	cabbage	cabbage
	24	3.8	cauliflower	lettuce
	25	7.2	beetroot	carrot
	26	7.2	cauliflower	sweetcorn
	27	4.3	broccoli	pepper
	28	4.1	cabbage	bean
	29	5.5	beetroot	pepper
	30	2.1	cabbage	lettuce
	31	12.2	Brussels sprouts	cauliflower
	32	4.1	broccoli	lettuce
	33	3.8	cauliflower	carrot
	34	8.2	broccoli	cabbage
	35	14.1	cauliflower	cabbage

The highest densities in nematode numbers were recorded when cabbage was followed by cabbage as cultivated hosts. High densities also occurred where beetroot was grown following cabbage. In fields where host crops were alternated with non-hosts, the average

density of *H. schachtii* was 6.74 eggs and juveniles/g of soil. A crop rotation system, as suggested by Mai & Abawi (1980), should be encouraged to reduce the population density and to prevent the build-up of populations of *H. schachtii*.

Host range

Host range studies, based upon field observations, have the disadvantage that infected soils may contain cysts from previous grown crops. The criterion used to determine host susceptibility was therefore the presence of fully developed cysts on the roots of plants. *H. schachtii* has a wide host crop range consisting of 23 plant families (Steele, 1965). The host range of cultivated vegetables found in the survey is given in Table 1 and corresponds to those of Daiber (1990). During the survey five cultivated vegetable hosts were found to be infected: cabbage, cauliflower, beetroot, Brussels sprouts and broccoli. Cabbage was most frequently infested as 62.3% of the plants had a medium infestation and 18.4% a heavy infestation. Only two percent of cauliflower was heavily infested and 73.9% a light infestation. In the case of beetroot 75.6% showed a light and 23.1% medium infestation levels. Broccoli and Brussels sprouts had predominantly light infestations of *H. schachtii*.

Table 2. Numbers of *Heterodera schachtii* in soil in which cultivated non-hosts were growing in the greater Cape Flats, South Africa (1997 – 1998).

Locality	Position see Fig.1	Eggs and juveniles/gram soil	Crop	Previous crop
Region 1	36	5.8	lettuce	cauliflower
	37	8.7	carrot	cabbage
	38	6.1	onion	cabbage
	39	5.8	lettuce	cauliflower
	40	6.2	butternut	cabbage
Region 2	41	8.7	carrot	cabbage
	42	7.2	onion	broccoli
	43	7.3	potato	cauliflower
Region 3	44	12.1	lettuce	cauliflower
	45	8.2	potato	cabbage

It can therefore be concluded that *H. schachtii* is widely distributed in all vegetable-growing areas in the greater Cape Flats occurring in numbers well above the generally accepted threshold level adopted for economic production of these crops. It must therefore be regarded as an important pest to the farming community of the Cape Flats.

References

Bouyoucos G J 1962. Hydrometer method improved for making particle size analysis of soils. *Agronomy Journal* 54: 464-465.

Coetzee V 1968. The distribution of the family *Heteroderidae* (Filipjev, 1934) in South Africa and some host records of *Meloidogyne* species. *South African Journal of Agricultural Science* 11: 775-778.

Daiber K C 1990. *Heterodera schachtii* in South African vegetable fields. *Phytophylactica* 22: 133-134.

Daiber K C 1992. The decline of *Heterodera schachtii* populations under conditions of vegetable production in South Africa. *Phytophylactica* 24: 321-324.

Griffen G D 1981. The relationship of plant age, soil temperature and population density of *Heterodera schachtii* on the growth of sugar beet. *Journal of Nematology* 13: 184-190.

Mai W F & Abawi G S 1980. Influence of crop rotation on spread and density of *Heterodera schachtii* on a commercial vegetable farm in New York. *Plant Disease* 64: 302-305.

Olthof T H A, Potter J W & Peterson E A 1974. Relationship between population densities of *Heterodera schachtii* and losses in vegetable crops in Ontario. *Phytopathology* 64: 549-554.

Reid E 1955. A rolling method for opening cysts of potato-root eelworm. *Plant Pathology* 4: 28-29.

Schmidt A 1871. Über den Rubennematoden. *Zeitschrift des Vereins fur die Rubenzucker-industrie im Zollverein* 12: 1-19.

Steele A E 1965. The host range of the sugar beet nematode *Heterodera schachtii* Schmidt. *Journal of the American Society of Sugar Beet Technologists* 13: 573-603.

Chapter 3.

The effect of *Heterodera schachtii* (Schmidt, 1871) on vegetable and weed hosts

Introduction

The first record of *Heterodera schachtii* in South Africa was by Coetzee in 1968. A recent survey (Van Zyl & Meyer 2000) revealed that it occurred widespread in high numbers in the greater Cape Flats on especially cabbage, cauliflower, beetroot and Brussels sprouts. Apart from Daiber (1990, 1992a, 1992b), no comprehensive study of this nematode has been undertaken in South Africa. Economic losses due to *H. schachtii* were common on vegetables (Jones 1957) in Europe and California and producers in the Cape Flats are also experiencing serious losses due to this nematode. The host range, which includes 23 plant families, including weeds, is well documented (Steele 1965), with the potential of posing a serious threat to vegetable farming, especially at small-scale levels, in general in the Western Cape Province. Effective management of *H. schachtii* under South African conditions requires a thorough knowledge of the biology of this nematode. According to Seinhorst (1965) initial population density was the main factor that determined eventual damage.

The aim of this study was to determine the damage potential of varying population numbers of *H. schachtii* on six vegetable hosts. The potential of five weeds to maintain

H. schachtii populations and to serve as a source of infection on vegetable crops was also investigated.

Material and methods

Experiment 1: H. schachtii, used as inoculum, was collected from an infested cabbage field at Lynedoch, Stellenbosch, in June 1999. The inoculum was increased on cabbage in a greenhouse at Elsenburg in seedbeds. Cyst extraction and determination was as described by Van Zyl & Meyer (2000). Mature brown cysts were surface sterilized with a 0.5% sodium hypochloride solution, rinsed in distilled water and hatched in a $ZnCl_2$ solution at 25°C in an incubator for five days, the procedure as described by Griffen (1982).

Sandy loam soil was steam sterilized at 104°C for 48 hours and, to improve water absorption, was mixed with sterile vermiculite in a 3:1 ratio. Five kilogram of this soil was placed in six-liter plastic pots, with the drainage holes covered with a fine mesh. In December 1999 the larval suspension was incorporated into the medium by pipetting the aqueous suspension into the soil surrounding plant stems to yield concentrations of 0, 0.66, 2, 6 and 18 juveniles per g of soil. One five week-old cabbage (*Brassica oleracea* L. var *capita* L. Green Coronet), cauliflower (*Brassica oleracea* var. botrytis L. Hunter), beetroot (*Beta vulgaris* var. *conditiva* L. Red Ace), Brussels sprouts (*Brassica oleraceae* var. *gemmitera* L. Odette), broccoli (*Brassica oleraceae* var. *cymosa* L. Viking) and

turnip (*Brassica rapa* L. Snowball) seedlings were transplanted in each of the inoculated pots.

A completely randomized design was followed with 20 replications for each treatment of the six different vegetable hosts. Analysis of variance was performed using SAS version 8.1 (SAS 1999). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk 1965). Student's t-least significant difference was calculated at the 5% confidence level to compare treatment means (Ott 1998). Moisture and temperature sensors were placed at a depth of 10 and 25 cm in 40 pots to indicate the need for extra moisture. Daily fertigation provided the plant with a balanced nutrient solution (Chemicult^{R}). The day/night temperature in the greenhouse at Elsenburg was maintained at 25/16 °C ± 2°C.

To determine the population densities of *H. schachtii* 30 days after inoculation, soil samples were taken at a depth of 10 cm from 20 replications per crop with a small auger as described by Townshend (1963). Growth and population data were determined at crop maturity, being 56 days after planting for cauliflower, 62 days for cabbage, 60 days for beetroot, 65 days for broccoli, 120 days for Brussels sprouts and 65 days for turnip, respectively. The fresh weight of marketable yield was determined by amount of the produce of a suitable quality for human consumption. The fresh weight of tops included all above-ground material produced per plant. The number of juveniles per gram of root was determined by staining the roots in an acid fuschsin-lactophenol solution according to the method of Byrd *et al.* (1983). The final population of eggs and juveniles of *H.*

schachtii was determined as described by Van Zyl & Meyer (2000). The reproduction ratio (P_f/P_i) was calculated as a quotient from the initial (P_i) and final (P_f) population densities of *H. schachtii* per gram of soil.

Experiment 2: This experiment was conducted on host weeds under greenhouse conditions as described before. The seed of white goosefoot (*Chenopodium album*), black nightshade (*Solanum nigrum*), shepherd's purse (*Capsella bursa-pastoris*), wild radish (*Raphanus raphanistrum*) and purslane (*Portulaca oleracea*) was collected from infested fields in the Stellenbosch and Cape Flats during April 1999 and stored for 6 months at 5°C (Anderson 1968). The seeds were then scarified and germinated on captan-treated filter paper at 25°C and planted in a steam-sterilized sand-filled seedbed at Elsenburg. Twenty-eight day-old seedlings were each transplanted during December 1999 to a six liter pot, as described before, with initial inoculum levels (P_i) of 0.66, 2 and 6 juveniles per gram of soil. A complete randomized design, using 15 replications, was used. Analysis of variance was performed using SAS version 8.1 (SAS 1999). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk 1965). Student's t-least significant difference was calculated at the 5% confidence level to compare treatment means (Ott 1998). The weeds were compared to each other in regard to the number of juveniles per gram of root and eggs and juveniles per gram of soil. The P_f/P_i ratio was calculated for each of the inoculum levels to determine the host and reproduction potential 90 days after inoculation.

Results and discussion

Experiment 1: Nematode population densities, 30 days after inoculation, are presented in Table 1.

Table 1. Population densities of *Heterodera schachtii* on vegetables 30 days after inoculation with different initial densities (P_i) of juveniles (December 1999).

Vegetable	P_i (juveniles/gram of soil)				
	0.00	0.66	2.00	6.00	18.00
Beetroot	0.00	0.26	0.21	1.18	5.02
Broccoli	0.00	0.12	0.12	0.94	4.87
Brussels	0.00	0.16	0.47	1.53	4.23
Cabbage	0.00	0.16	0.64	0.69	4.60
Cauliflower	0.00	0.12	0.31	1.82	3.85
Turnip	0.00	0.10	0.23	0.82	3.51
Mean	0.00	0.15	0.33	1.16	4.35
S	0.00	0.06	0.19	0.44	0.59

The change 30 days after inoculation population densities of *H. schachtii* in the soil resulted in a reduction in numbers of juveniles in the soil for all vegetables. Olthoff *et al.* (1974) obtained similar results. The lower densities were due to the penetration of nematodes into the roots and insufficient time for the completion of a generation. This must be considered when soil samples are taken during the season to determine nematode populations, as low densities during this stage do not necessarily show the existence of nematode problems. Cabbage and cauliflower showed signs of wilting and slight discoloration at P_i levels of six and 18 juveniles/g of soil, but the hosts survived to the end of the trial. Most of the vegetable crops exhibited signs of damage at 30 days after

inoculation due to the high penetration levels of the nematodes. Olthoff *et al.* (1974) also observed damage to rutabagas, cabbage, cauliflower and beetroot at mid-season of the plant growth period. The variability in the susceptibility to *H. schachtii* depended on host and nematode population density.

The vegetables used in this experiment showed little resistance to *H. schachtii* as the yield was reduced at most pre-plant inoculation densities (Fig.1) except for Brussels sprouts with a P_i of 0.66 and cabbage with a P_i of 2. There was no difference between initial P_i levels of 0.66, 2 and 6 (P=0.05) on the yield of cauliflower. Yields for all vegetables at initial densities of 18 were significantly (P=0.05) lower when compared to the other initial densities (0.66-6). Turnip was highly sensitive to *H. schachtii* infestations as even the lowest population densities of *H. schachtii* resulted in stunted plants with less foliage than normal. Losses of plant material on turnip reached 75% when compared to the control plots. The sensitivity of turnips was observed during a survey in the greater Cape Flats when severely damage by the presence of *H. schachtii* was noted (Van Zyl & Meyer, 2000). In some cases total crop failures of turnips occurred. The reduction in yield for broccoli, Brussels sprouts and cabbage became evident at P_i of 6 and higher. At the lower concentrations the other vegetables showed a larger reduction in yield. Lear *et al.* (1966) found reduced yields of cabbage and other vegetables associated with high *H. schachtii* infestations and Daiber (1992) found losses of up to 60% on beetroot in the Rondebult area. Cabbage and beetroot showed under greenhouse conditions, reduced yields of 71% and 34%, respectively (Mai *et al.* 1972).

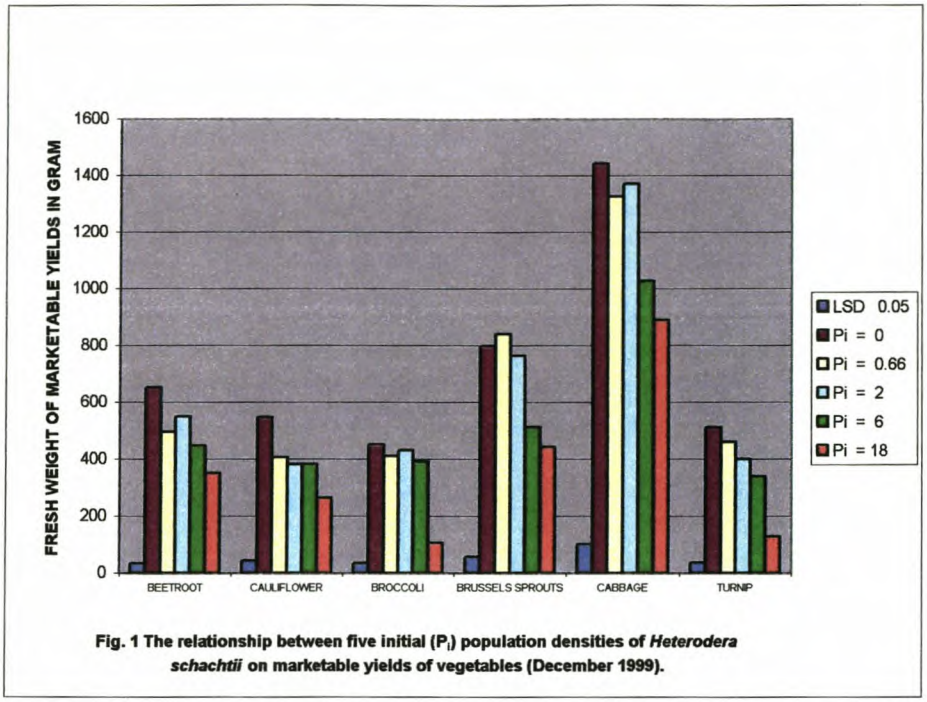

Fig. 1 The relationship between five initial (P_i) population densities of *Heterodera schachtii* on marketable yields of vegetables (December 1999).

The marketable percentage of the yield (Table 2) ranged from 28% on turnip to 97% on Brussels sprouts with Pi levels of 18 and 0.66 respectively. The marketable percentage of the yield of cabbage and beetroot was markedly lower than that found in Ontario by Olthoff *et al.* (1974), where they reported losses of 24% and 30% for cabbage and beetroot respectively. The higher losses observed were probably be due to different pathological races of *H. schachtii* or different vegetable varieties used in South Africa having less tolerance to *H. schachtii*.

The fresh weight of tops of cauliflower, beetroot, broccoli and turnip decreased with an increase in initial population densities, but Brussels sprouts and cabbage needed a density of at least six eggs and juveniles per gram of soil to show a significant decrease in the weight of tops (Fig. 2). The P_i level of 0.66 led to a marked decrease in the top weight of beetroot, broccoli and cauliflower.

Table 2. The marketable percentage of the yield of vegetables at five initial inoculum levels

P_i	Beetroot	Broccoli	Brussels sprouts	Cabbage	Cauliflower	Turnip
0	100 a	100 a	100 a	100 a	100 a	100 a
0.66	79 b	84 c	97 a	91 b	71 b	87 b
2	88 c	92 b	93 b	98 a	67 b	76 c
6	66 d	82 c	66 c	76 c	67 b	62 d
18	56 e	38 d	53 d	64 d	49 c	28 e
LSD	3.9	3.25	3.5	4.6	5.1	4.8

Values in colums followed by the same letter do not differ at the 5% level (P=0.05).

This is a cause for concern, as these infestation levels occurred widely in the affected areas of the greater Cape Flats resulting in yield losses. Daiber (1992b) found a reduction of 20% in the yield of cabbage and 30% in cauliflower when crops were grown in infected soil. The tops of cauliflower in infected soil were visibly smaller than normal.

The largest reduction in root weight was observed on cauliflower, broccoli and turnip with big reductions even at the lowest density of 0.66 juveniles per gram of soil (Fig. 3). The infestations manifested as small, branched root systems with excessive root hairs in all vegetables. Griffen (1981) found similar results on sugar beet root growth, with the highest reduced root systems at high pre-plant densities. Significant reductions in yield at P_i levels of six and 18 were found.

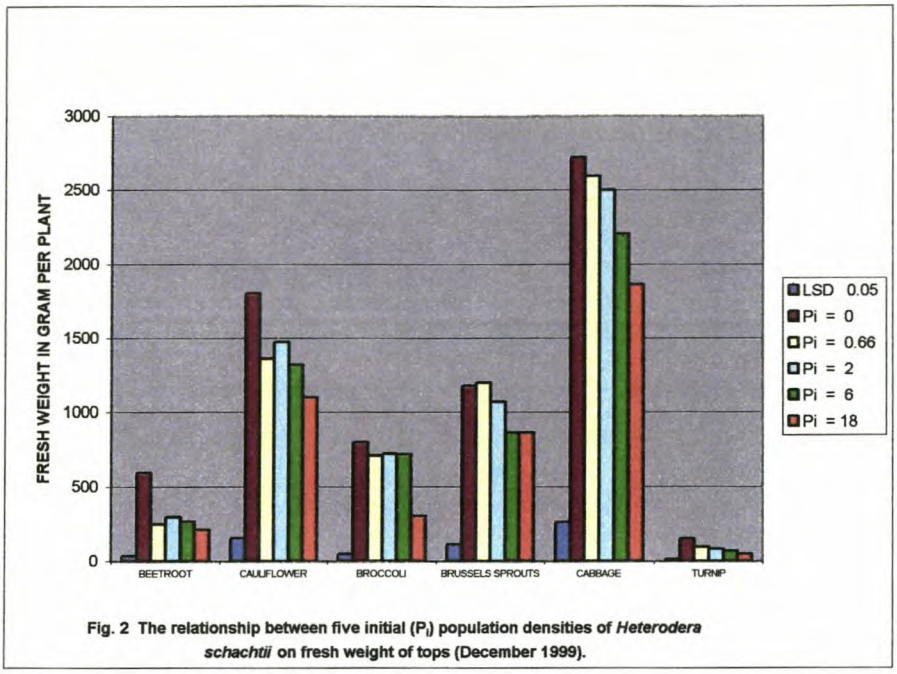

Fig. 2 The relationship between five initial (P_i) population densities of *Heterodera schachtii* on fresh weight of tops (December 1999).

The absence of lateral root growth of cabbage and cauliflower resulted in the stunted appearance. Rhoades (1972) found similar results associated with *H. schachtii* infestations on cabbage and cauliflower in Florida. That leaves and root systems of infected cabbage were much smaller than the control was also found by McCann (1981). This suggested that the smaller root systems of the infected plants restricted nutrient and water uptake, thereby reducing the growth rate of the plants. Due to the restricted nutrient uptake, vegetables experienced nutrient deficiencies, leading to reduced yields. The cabbage roots were discoloured at Pi levels of six and 18 juveniles/g of soil. The discoloration was probably due to a nutrient deficiency, also observed on cabbage by Abawi & Mai (1980). Limited necrosis of the roots of all vegetables tested, occurred. Root tissue necrosis associated with penetration and syncytium degeneration played an important role in the disfunction of the roots. Under field conditions it could also lead to infection ports for secondary infections. The damage caused by *H. schachtii* on

vegetable roots impeded normal root elongation, plant development as well as water and nutrient uptake.

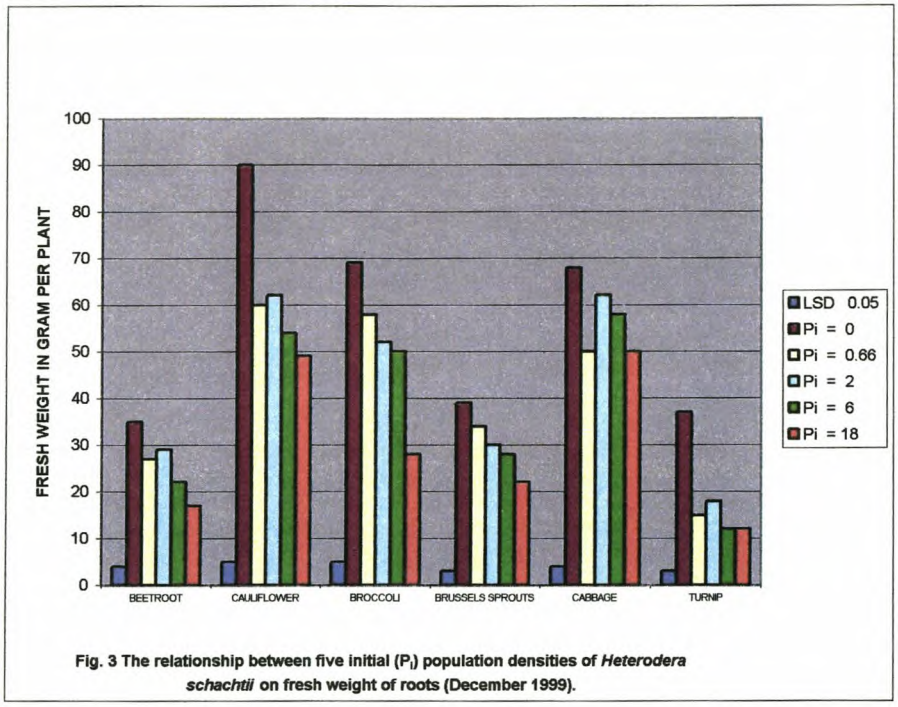

Fig. 3 The relationship between five initial (P_i) population densities of *Heterodera schachtii* on fresh weight of roots (December 1999).

The number of juveniles per gram of root increased (P=0.05) with increasing pre-plant densities (Fig. 4). Cabbage at tested initial densities supported the highest number of juvenile *H. schachtii* per gram of root compared to the other vegetables. Turnip supported the lowest densities of *H. schachtii*. The high densities of the latter found in the roots, impeded normal root function due to the formation of large numbers of syncytia. There was a difference in the final number of eggs and juveniles per gram of soil (P=0.05) between all pre-plant densities for all vegetable crops tested (Fig. 5).

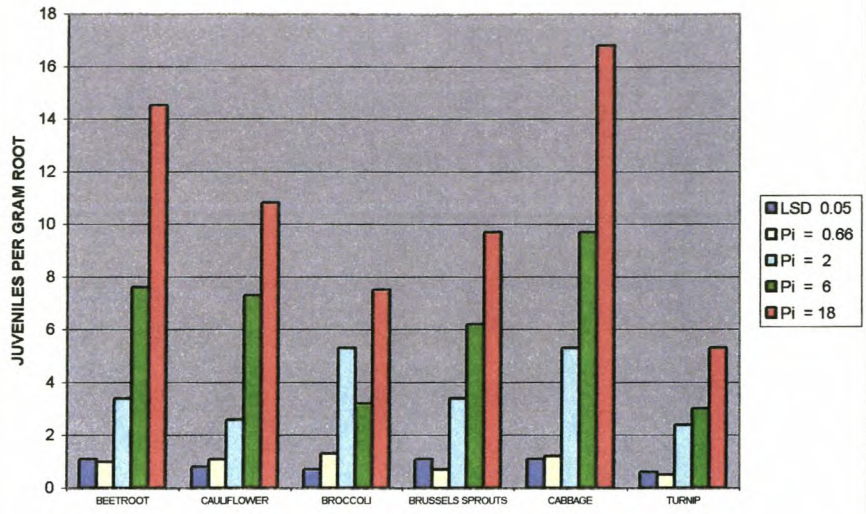

Fig. 4 The relationship between four initial (P_i) population densities of *Heterodera schachtii* on juveniles per gram root (December 1999).

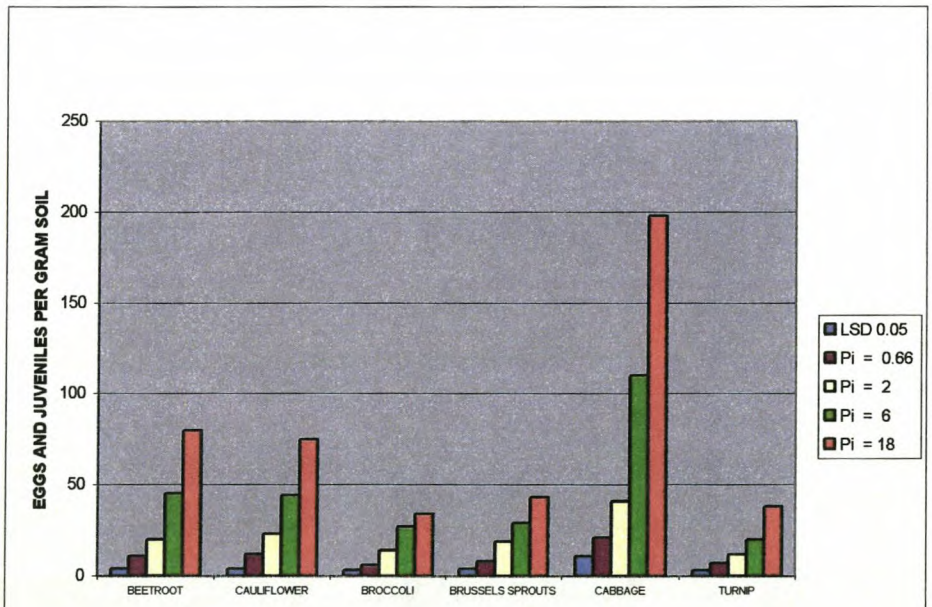

Fig.5 The relationship between four initial (P_i) population densities of *Heterodera schachtii* on the final population of eggs and juveniles (December 1999).

The increase in population numbers of *H. schachtii* corresponded with the increase in pre-plant densities of hosts. The highest density of 198 eggs and juveniles per gram of soil was found on cabbage and the lowest of six on broccoli. The largest variation was found on cabbage, ranging from 20.74 to 198.00 juveniles per gram of root. An increase in the final number of juveniles per gram of soil was also found by Olthoff *et al.* (1974), reporting a correlation between final densities and initial densities.

The reproduction potential (P_f/P_i) decreased ($P=0.05$) as a function of increasing P_i (Fig. 6). The highest P_f/P_i ratio of 31.4 was found on cabbage with an initial level of 0.66 and the least of 1.9 on broccoli with a P_i of 18. These results were expected as at low population densities the intraspecific competition is minimized and the population increase will be at maximum. The P_f/P_i ratio was high, probably because the reproduction rate was promoted by optimal temperature conditions and the soil type (Santo & Bolander 1976).

The reduction in plant mass and root systems of vegetables were directly related to the feeding of nematodes when at high infestation levels. This, in turn, led to higher reductions in yield as more photosynthate was drained from the plant by the nematodes and resultant insufficient nutrient uptake by the plant roots. The phloem is the primary source of organic compounds for the syncytium (Brockenhoff & Grundler 1994) and the amount of food withdrawn daily by this cyst nematode from the plant was equivalent to four times the volume of the syncytium (Sijmons *et al.* 1991).

The most extensive damage to the vegetables was caused by females needing 40 times the amount of food in comparison to that of males required to develop into the adult (Muller *et al.* 1981). The syncytia associated with males were also smaller than those of females. The time of infection played an important role in the reduction of plant growth, due to variation in photosynthetic production in different growth stages of the plant. A young plant with a low photosynthetic production will not be able to sustain plant growth and to support development of high nematode numbers.

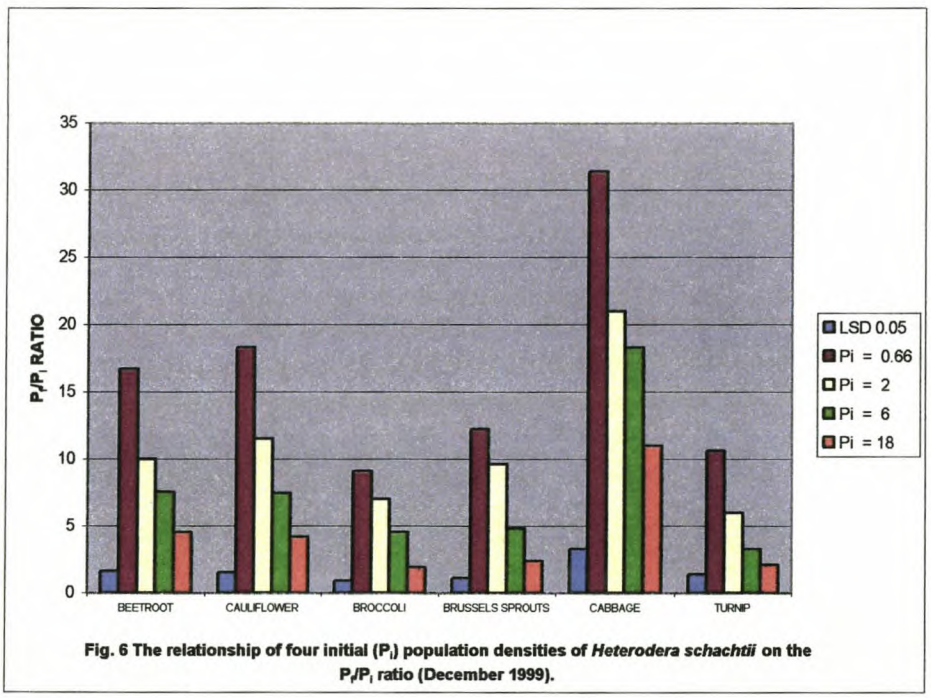

Fig. 6 The relationship of four initial (P_i) population densities of *Heterodera schachtii* on the P_f/P_i ratio (December 1999).

Koenning & Barker (1995) found a correlation between the photosynthetic rate of soybean and the initial *H. glycines* population densities. Likewise, Schans & Arntzen (1991) found that *Globodera pallida* at high inoculum densities suppressed the photosynthetic rate of potatoes.

The amount of damage caused is also expressed by the amount of amino acids in the syncytia as a consequence of nematode presence. Fibrous roots parasitized by *H. schachtii* contain significantly increased amounts of total amino acids, aspartic acid, glutamic acid and glutamine in the syncytia (Doney *et al.* 1970).

The symptoms associated with nutrient deficiency found on the infected vegetables were common in plants infected with *H. schachtii.* Prince *et al.* (1982) found that potassium and phosphorus uptake, compared to uninfected plants, increased in oat roots when infected with *H. avenae*. The roots of soybean infected with *H. glycines* showed lower concentrations of potassium and magnesium and higher concentrations of calcium than uninfected plants (Blevins *et al.* 1995). They found that the translocation of plant nutrients was altered by cyst nematode infection. Magnesium, calcium and phosphorus translocation was also increased. The body composition of nematodes contains substantial amounts of calcium and phosphorous and thus the syncytium may function as a sink for these nutrients (Blevin *et al.* 1995).

Experiment 2, Weeds: The variability in the susceptibility to *H. schachtii* depends on the plant species and the initial nematode population densities. The most obvious host response was shown by shepherd's purse and wild radish. The final population density of juveniles per gram root at P_i of 0.66 eggs and juveniles per gram soil for shepherd's purse and wild radish were higher ($P=0.05$) than those for the other weed species (Fig. 7). Shepherd's purse and wild radish also had the highest number of eggs and juveniles per gram of soil with all initial P_i levels (Fig. 8) as well as the highest P_f/P_i ratios (Fig. 9).

Griffen (1982) found that white goosefoot, purslane and black nightshade were good hosts. The results of the present study showed that shepherd's purse and wild radish are even better hosts.

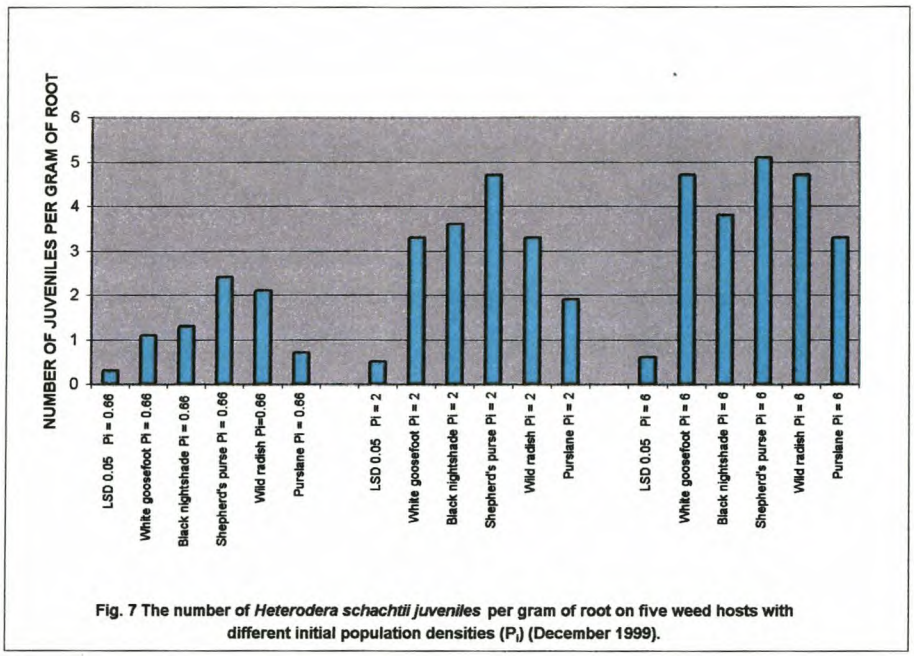

Fig. 7 The number of *Heterodera schachtii juveniles* per gram of root on five weed hosts with different initial population densities (P_i) (December 1999).

This also confirmed previous findings on the importance of weeds as hosts of *H. schachtii* on vegetable production (Steele 1965). When the P_f/P_i ratio was considered, it was evident that vegetable production should be free from weeds, even when a non-host crop was planted. This was to prevent reproduction of nematodes that serving as inoculum for future plantings. Shepherd's purse and wild radish supported the highest number of eggs and juveniles per gram soil and the highest P_f/P_i ratio. Purslane and black nightshade supported the lowest number of eggs and juveniles per gram soil, but even their management is important, as at even the lowest initial densities, a reproduction potential of 5.7 was reached. Shepherd's purse and wild radish must be considered favoured hosts of *H. schachtii* when considering vegetable production.

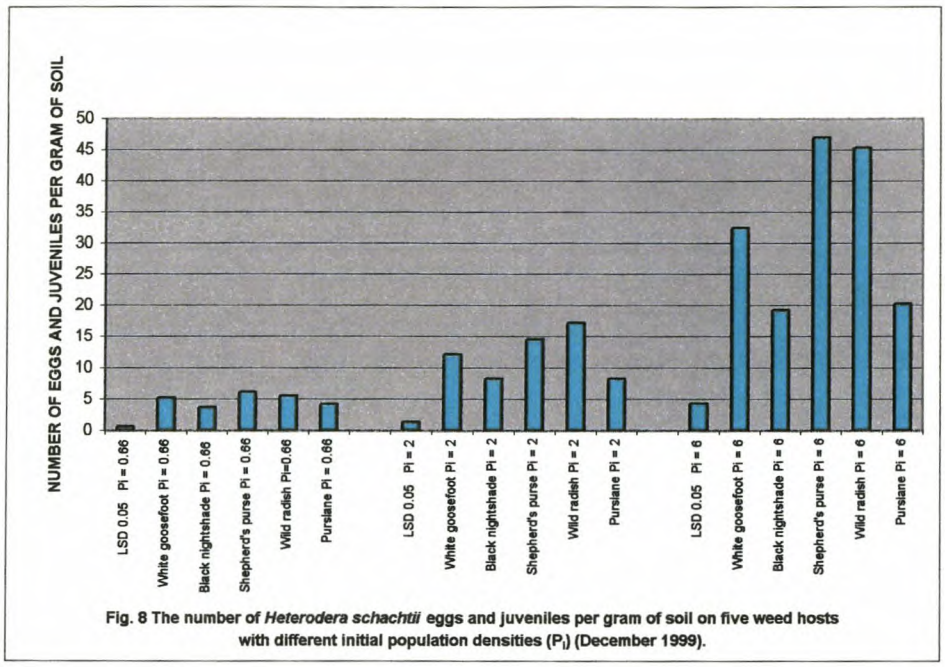

Fig. 8 The number of *Heterodera schachtii* eggs and juveniles per gram of soil on five weed hosts with different initial population densities (P_i) (December 1999).

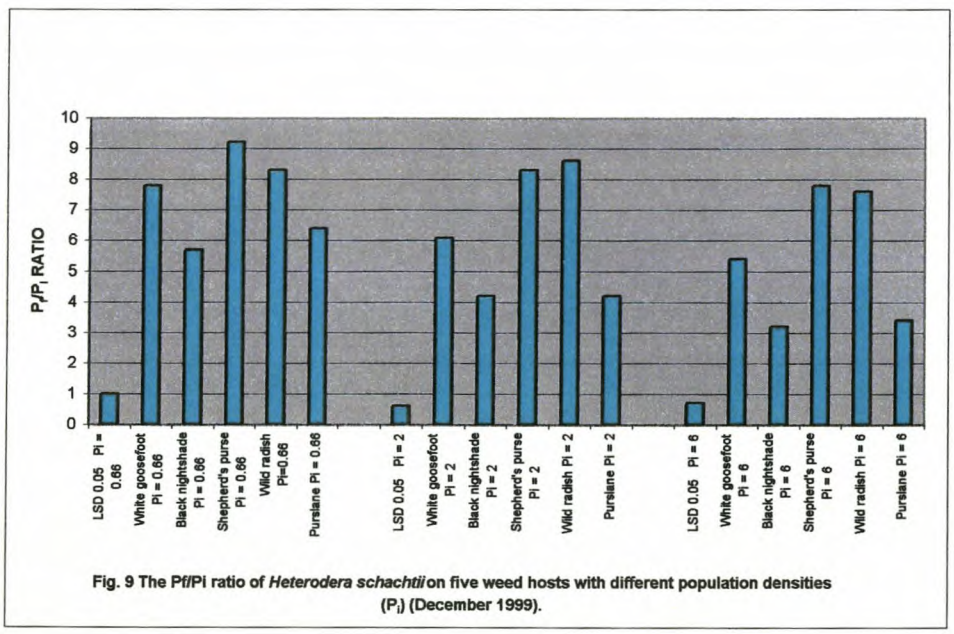

Fig. 9 The Pf/Pi ratio of *Heterodera schachtii* on five weed hosts with different population densities (P_i) (December 1999).

These were the most commonly found weeds in a general survey of the greater Cape Flats, Western Cape, as reported by Van Zyl & Meyer (2000). Agricultural producers do not realize the potential danger of such weeds, as they are commonly observed in vegetable fields and usually tolerated. Griffen (1982) observed differences in the

response of certain weed host populations from different geographic locations to *H. schachtii*. Apparently weed host suitability is dependant on genetic differences in the nematode population as well as an weed biotypes.

The present study has shown that most of the commonly grown vegetables were susceptible to *H. schachtii*. Damage occurred even at low initial population densities, in the greater Cape Flats. *H. schachtii* must be controlled, because the occurring population densities are capable of causing economic losses to small scale vegetable producers. The ability of commonly occurring weeds to support multiplication of *H. schachtii* demonstrates the need of an awareness program for effective weed management to keep *H. schachtii* populations under economic threshold levels.

References

Abawi G S & May W F 1980. Effects of initial population densities of *Heterodera schachtii* on yield of cabbage and table beets in New York State. *Phytopathology* 70: 481-485.

Anderson R N 1968. *Germination and establishment of weeds for experimental purposes.* Geneva, New York: W.F. Humphrey Press.

Blevins D G, Dropkin V H & Luedders V G 1995. Macronutrient uptake, translocation and tissue concentration of soybeans infested with the soybean cyst nematode and elemental composition of cysts isolated from roots. *Journal of Plant Nutrition* 18: 579-591.

Bockenhoff A & Grundler F M W 1994. Studies on the nutrient uptake by the beet cyst nematode *Heterodera schachtii* by *in situ* microinjection of fluorescent probes into the feeding structures in *Arabidopsis thaliana. Parasitology* 109: 249-255.

Byrd D W Jr., Kirkpatrick T & Barker K R 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology* 15: 142-143.

Coetzee V 1968. The distribution of the family Heterodera (Filipjew, 1934) in South African and some host records of *Meloidogyne* species. *South African Journal of Agricultural Science* 11: 775 – 788.

Daiber K C 1990. *Heterodera schachtii* in South African vegetable fields. *Phytophylactica* 22: 133 – 135.

Daiber K C 1992a. The decline of *Heterodera schachtii* populations under conditions of vegetable production in South Africa. *Phytophylactica* 24: 321 – 324.

Daiber K C 1992b. Insects and nematodes that attack cole crops in Southern Africa. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 4: 430 – 440.

Doney D L, Fife JM & Whitney E D 1970. Effect of the sugar-beet nematode *Heterodera schachtii* on the free amino acids in resistant and susceptible *Beta* species. *Phytopathology* 60: 1727-1729.

Griffen G D 1981. The relationship of plant age, soil temperature and population density of *Heterodera schachtii* on the growth of sugar beet. *Journal of Nematology* 13: 184 – 190.

Griffen G D 1982. Differences in the response of certain weed host populations to *Heterodera schachtii*. *Journal of Nematology* 14: 174 – 182.

Jones F G W 1957. Soil populations of beet eelworm (*Heterodera schachtii Schm*) in relation to cropping III. Further experiments with microplots and with pots. *Nematologica* 11: 257 – 272.

Koenning S R & Barker K R 1995. Soybean photosynthesis and yield as influenced by *Heterodera glycines*, soil type and irrigation. *Journal of Nematology* 27: 51-62.

Lear B, Miyagawa S T, Johnson D E & Atlee J R 1966. The sugar beet nematode associated with reduced yields of cauliflower and other vegetable crops. *Plant Disease Reporter* 50: 611 – 612.

Mai W F, Abawi G S & Becker F R 1972. Population levels of *Heterodera schachtii* in New York and damage to red table beet and cabbage under greenhouse conditions. *Plant Disease Reporter* 56: 434 – 437.

McCann J 1981. Threshold populations of *Heterodera cruciferae* and *H. schachtii* causing damage to cabbage seedlings. *Plant Disease* 65: 264 – 266.

Muller J, Rehbock K & Wyss U 1981. Growth of *Heterodera schachtii* with remarks of amounts of food consumed. *Revue dé Nematologie* 4: 227-234.

Olthof T H A , Potter J W & Peterson E A 1974. Relationship between population densities of *Heterodera schachtii* and losses in vegetable crops in Ontario. *Phytopathology* 64: 549 – 554.

Ott R L 1998. *An introduction to statistical methods and data analysis.* Belmont, California. Duxbury Press.

Prince N S Clarkson D T & Hague N G M 1982. The uptake of potassium and phosphorus in oats infested with the cereal cyst nematode, *Heterodera avenae* Woll. *Revue dé Nematologie* 5: 321-325.

Rhoades H L 1972. Pathogenicity and control of a Florida population of sugarbeet nematode, *Heterodera schachtii*, on cabbage. *Proceedings of the Florida State Horticultural Society* 84: 139 – 142.

SAS 1999. SAS/STAT User's Guide, Version 8, 1 st printing, Volume 2. SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.

Santo G S & Bolander W J 1976. Effects of soil temperature and soil type on the reproduction of *Heterodera schachtii* and *Meloidogyne hapla* on sugar beets. *Journal of Nematology* 8: 301 – 302.

Schans J & Arntzen F K 1991. Photosynthesis, transpiration and plant growth characters of different potato cultivars at various densities of *Globodera pallida*. *Netherlands Journal of Plant Pathology* 97: 297-310.

Shapiro SS & Wilk M B 1965. An analysis of variance test for normality. *Biometrika* 52: 591-611.

Seinhorst J W 1965. The relationship between nematode density and damage to plants. *Nematologica* 11: 137 – 154.

Sijmons P, Grundler F M W, Von Mende N, Burrows P R & Weiss U 1991. *Arabidopsis thaliana* as a new model host for plant parasitic nematode. *The Plant Journal* 1: 245-254.

Steele A E 1965. The host range of sugar beet nematode, *Heterodera schachtii* Schmidt. *Journal of the American Society of Sugar Beet Technologists* 13: 574 – 603.

Steele L E 1984. Nematode parasites of sugar beet. In: *Plant and Insect nematodes*, 507-569 (Ed. W R Nickle). Marcel Dekker, New York.

Townshend J L 1963. A modification and evaluation of the apparatus for the Oostenbrink direct cottonwool filter extraction method. *Nematologica* 9: 106-110.

Van Zyl J & Meyer A J 2000. Distribution and host range of the sugar beet nematode, *Heterodera schachtii* in the Western Cape Province, South Africa. *African Plant Protection* 6: 47 – 51.

Chapter 4.

Penetration, development and life cycle of *Heterodera schachtii* (Schmidt, 1871) from the greater Cape Flats, South Africa

Introduction

Coetzee in 1968 reported the first *Heterodera schachtii* in the greater Cape Flats. Observations since then have revealed that this nematode presents a serious threat to vegetable production in the Cape Flats and surrounding areas (Van Zyl & Meyer 2000). The host status of vegetables and weed plants for *H. schachtii* in the greater Cape Flats showed that this nematode can multiply on a wide range of hosts and weeds occurring in the area. Since it can survive on weeds during the fallow period, the effective control of *H. schachtii* on vegetables and weeds requires detailed information on the life cycle of the nematodes on these crops. The life cycle of *H. schachtii* on sugar beet was described by Raski (1949).

In view of the potential threat to vegetable production and severe economic losses, the aim of this study was to observe the penetration, development and life cycle of *H. schachtii* on vegetables, weeds and trap crops. The possible differences in stages of the life cycle of *H. schachtii* on crops can then be considered when making recommendations to vegetable growers.

Materials and methods

Penetration:

Weed hosts used were shepherd's purse (*Capsella bursa-pastoris*), purslane (*Portulaca oleracea*) and black nightshade (*Solanum nigrum*). The vegetable hosts were broccoli (*Brassica oleraceae* var. *cymosa*), turnip (*Brassica rapa*), beetroot (*Beta vulgaris* var. *conditiva*), cabbage (*Brassica oleraceae* var. *capitata*), cauliflower (*Brassica oleraceae* var. *botrytis*), Brussels sprouts (*Brassica oleraceae* var. *gemmifera*) and lettuce (*Lactuca sativa*). The respective seed stocks have been used as in chapter 2. Two trap crops, radish (*Raphanus sativus*) and mustard (*Sinapsis alba*), as indicated by (Gardner & Chaswell-Chen 1993), were also included in the trial.

The seeds of the weeds were collected in the previous season during April 2000 and stored at 18°C at Elsenburg. Seeds on filter paper were in Petri dishes and germinated at 25°C in an incubator. The seedlings were planted in plastic vials, each containing 400 cm^3 of steam-sterilized sand.

The *H. schachtii* population used in this trial was originally collected at Lynedoch (18.65E;34.00S), Western Cape, South Africa, in April 1999 and cultured on cabbage in a greenhouse at Elsenburg. The cysts of *H. schachtii* were extracted by the method as described by Van Zyl & Meyer (2000). The cysts were hatched in a $ZnCl_2$ solution (408 mg/l) (Muller 1992). One plant was planted in each glass vial and pipetted with a suspension of infective juveniles to yield concentrations of 22 l_2/cm^3 of sand in trial 1 and

11 l_2/cm^3 sand in trial 2. The plants in the vials were incubated at a constant temperature of 25°C. The root systems were washed free of sand after five days and stained with acid fuchsin following the methods of Byrd *et al.* (1983). The number and growth stages of nematodes were determined by clamping the root systems between two glass plates and examining the roots. The juvenile growth stages were classified as l_2 (early l_2) or swollen (late l_2, l_3) as described by Gardner & Chaswell-Chen (1993). There were 10 replicates in the first and 15 in the second experiment. Analysis of variance was performed using SAS version 8.1 (SAS 1999). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk 1965). Student's t-least significant difference was calculated at the 5% confidence level to compare treatment means (Ott 1998).

Development in roots:

The treatments consisted of the same host plants, except for lettuce, as used in the penetration trials (see above). Similar inoculation procedures, but now in vials containing 600cm^3 steam-sterilized sand, were used. The vials were inoculated with 22 l_2 /cm^3 sand in the first trial and 10 l_2/cm^3 in the second trial during November 2000. After inoculation, the vials were packed in a plastic container and kept in a greenhouse at a constant temperature of 25°C. The vials were irrigated daily for the duration of the experiment. Thirty-eight days after inoculation, the roots were washed free of sand and nematode numbers and life stages were determined and statistically analyzed as described before.

Life cycle:

The life cycle of *H. schachtii* was studied during November 2001, using the weeds and vegetables, except for lettuce, as described in the previous two trials. The seeds were germinated and grown for two weeks on White's agar medium in one-liter black plastic vials. The vial was covered at soil level with black plastic surrounding the stem of the plants. The cysts were hatched in $ZnCl_2$; (408 mg/l) and the plants were infected with *H. schachtii* using a pipette at concentrations of 50 l_2 per plant into the medium. After 24 hours the plants were placed in fresh medium to prevent any further penetration by the nematodes in the roots. The plants showed limited root development and were cut free under sterile conditions of the growing medium. The plant roots were then washed in distilled water. The roots were pushed into the new medium with sterilized tweezers enabling normal development to continue. For each host 500 plants were used. Ten plants from each treatment were removed daily and their root systems stained with acid fuchsin (Byrd *et al.* 1983). Roots were cleared in lactophenol and fuchsin-stained nematodes were teased from the tissue. The specimens were mounted on slides and examined. The first occurrence of each moult and developmental stage was the criterium used to determine the stages of the life cycle sequence. Continued root growth and nematode development were maintained by adding a thin overlay of fresh sterile agar medium to the cultures. The time for cysts to turn brown was determined in plants, which had been planted in sterilized sand in three liter plastic pots kept in a greenhouse at a temperature of 25 ± 2 °C.

Results and discussion

Penetration: In Trail 1 the highest number of nematodes in the roots was on cabbage and the lowest on purslane (Fig. 1). There was no difference (P=0.05) between the average number of larvae in broccoli, shepherd's purse and radish roots.

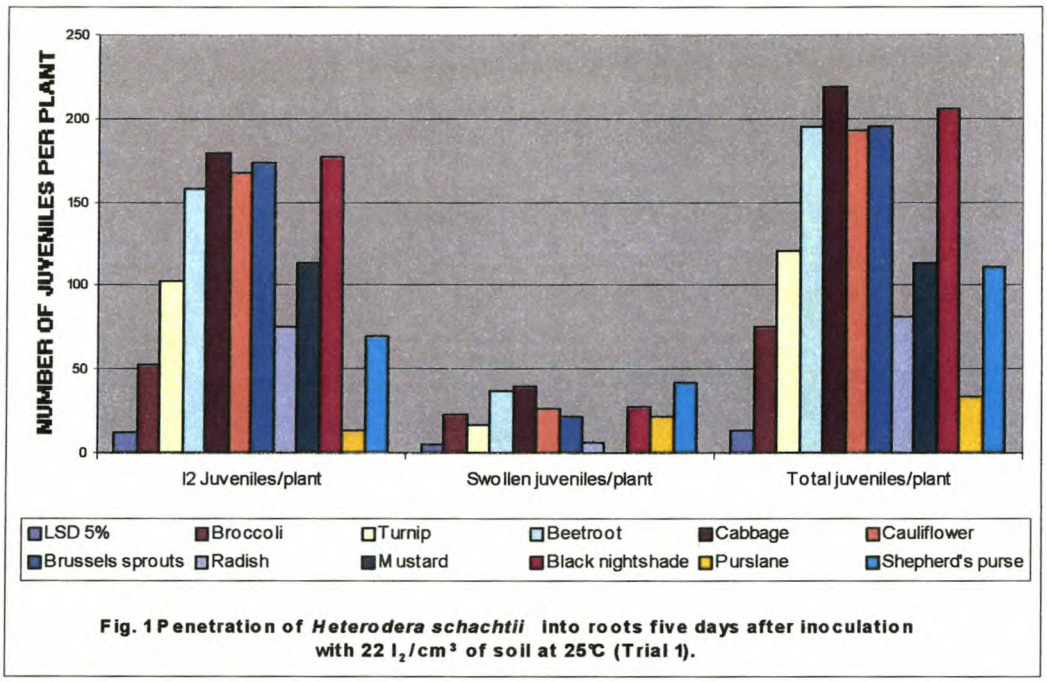

Fig. 1 Penetration of *Heterodera schachtii* into roots five days after inoculation with 22 l_2/cm³ of soil at 25°C (Trial 1).

In Trial 2 the number of l_2 per plant ranged from 155.8 on cabbage to 21.7 on radish, the latter as the trap crop (Fig. 2). The two trap crops (radish and mustard) had the lowest number of l_2 per plant. It was unexpected as it was surmised that the trap crops would have high penetration levels and thereafter to inhibit further nematode development. The two trap crops also showed significantly lower numbers of nematodes than the other treatments.

Lettuce had consistent zero values obviating a statistical analysis in comparison to the other treatments. The total lack of penetration was biologically important, as it

establishes lettuce as a definite non-host crop for *H. schachtii.* This fact should be taken into account when determining crop rotation cycles in the Cape Flats to prevent build-up of *H. schachtii* populations in agricultural settings.

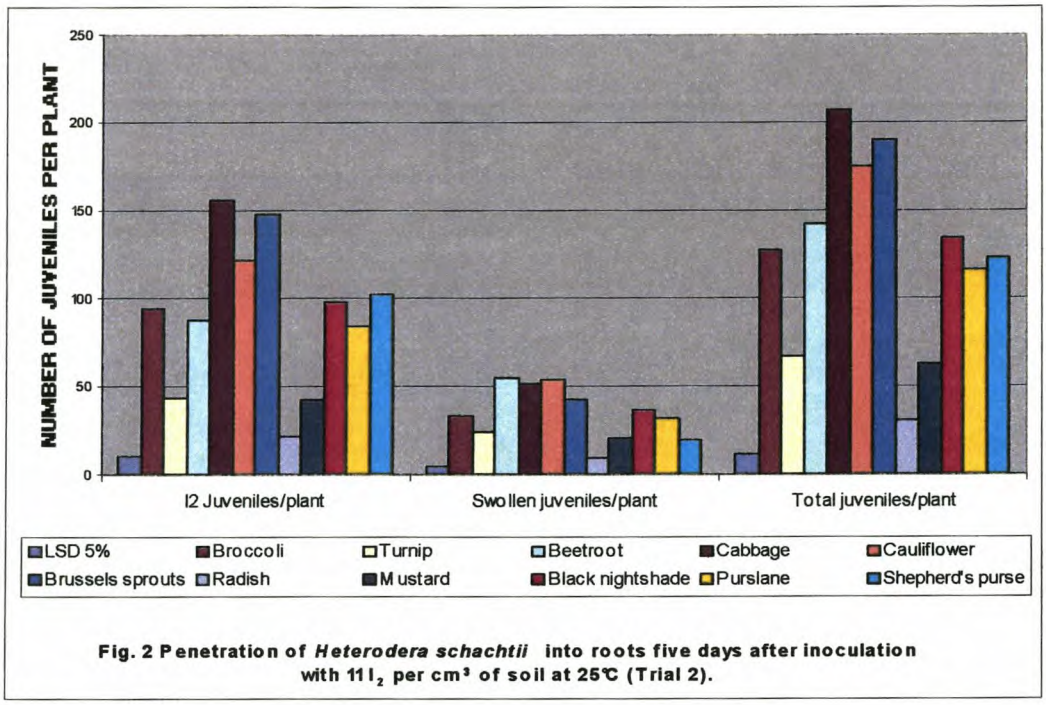

Fig. 2 Penetration of *Heterodera schachtii* into roots five days after inoculation with 11 l_2 per cm^3 of soil at 25°C (Trial 2).

All vegetables (except lettuce), weeds and trap crops were penetrated by *H. schachtii* within five days after inoculation. Although radish and mustard experienced penetration, larvae of *H. schachtii* failed to develop into adults, confirming the success of the two trap crops (Cooke 1984). At optimum temperature of 25°C, high penetration levels of *H. schachtii* were reported by Johnson & Viglierchio (1969). The average penetration was 37% in the high (22l_2/g soil) inoculum trial and 52% in the low (11l_2/g soil) inoculum trial. The lower penetration associated with the high inoculum level could be the result of decreased competition between juvenile nematodes. Similar penetration levels of *H. schachtii* on sugar beet roots were found by Sheperd & Wallace (1959). Bridgeman &

Kerry (1980) found penetration levels of up to 60% with single juvenile inoculations of *H. schachtii* on sugar beet. Cooke (1984) obtained similar results with high inoculation levels which led to lower penetration in younger roots when compared to lower inoculum levels. In the two trap crops, only a small number of swollen juveniles formed. Thus the effectiveness of these trap crops prevented the completion of the life cycle (Golinowski & Magnusson 1991). They also found that dysfunctional syncytia possibly inhibited the development of the nematode in resistant crops. The early retardation of *H. schachtii* in mustard and radish indicates a similar mode of action. The numbers of swollen nematodes corresponded to the findings of Steele (1975), who suggested that normal development took place during the first two to three days after penetration and subsequently impeding development. The juvenile invasion resulted in the production of additional lateral roots on all tested hosts, giving the roots of these crops a bearded appearance. Most of the juveniles invaded the root region just proximal to the root tips and a few invasions occurred 1-2 cm from the root tip.

Development in the roots:

The numbers of l_2 per plant in Trial 1 ranged between 0.5 on mustard to 37.2 on shepherd's purse (Fig. 3). In Trail 2 the numbers of l_2 ranged from 2.2 on broccoli to 55.2 on cauliflower (Fig. 4). The two trap crops (radish and mustard) had the lowest number of swollen larvae after 38 days which. This was significant as nematodes past this stage would not develop in these plants. The number of cysts per plant in Trial 1 ranged from none on mustard to 179.2 on cabbage. Black nightshade and shepherd's purse had high numbers of cysts per plant with 173.5 and 118.2, respectively, which

could have an impact on the subsequent alternative crops, especially in the case of a suitable or preferred host.

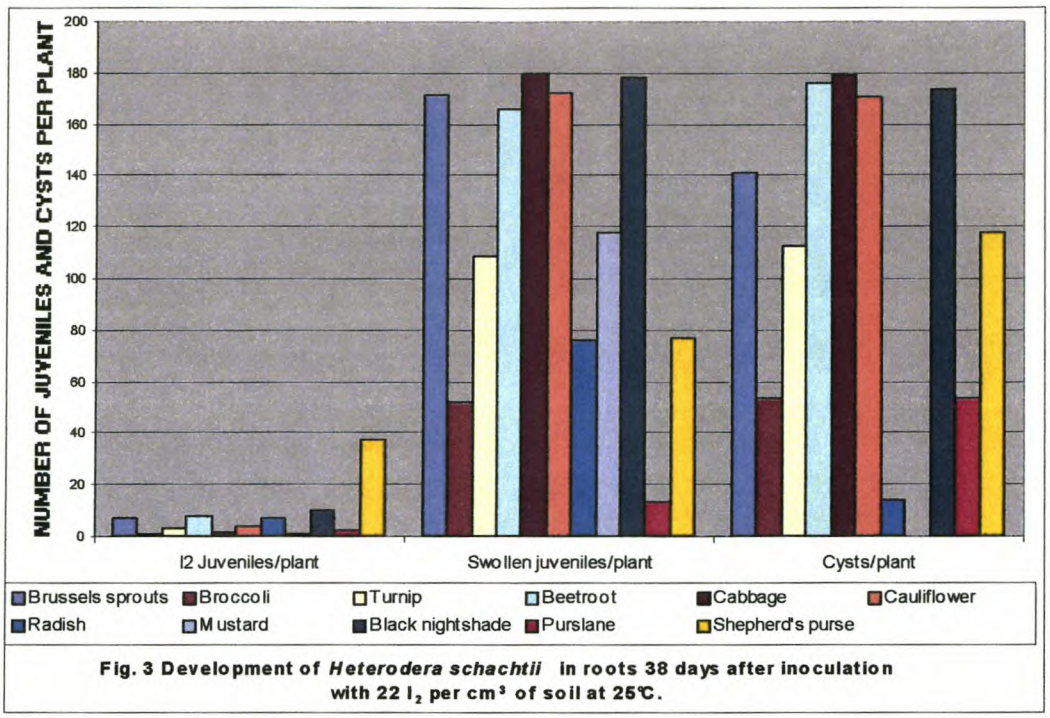

Fig. 3 Development of *Heterodera schachtii* in roots 38 days after inoculation with 22 l_2 per cm^3 of soil at 25°C.

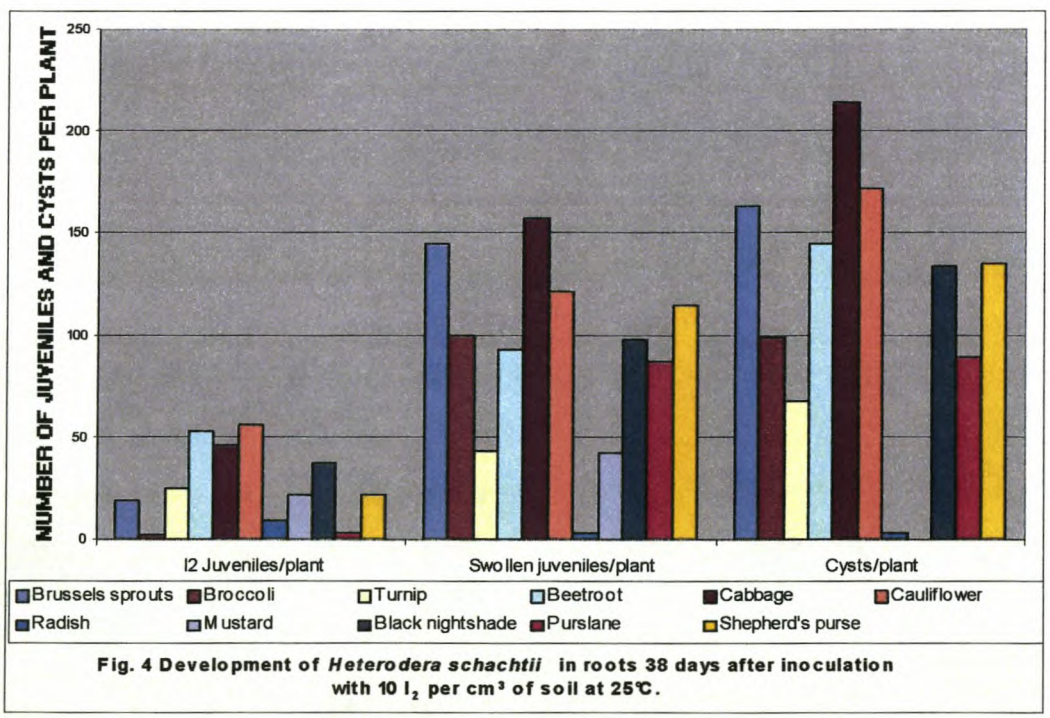

Fig. 4 Development of *Heterodera schachtii* in roots 38 days after inoculation with 10 l_2 per cm^3 of soil at 25°C.

Mustard had no cysts and radish produced 13.8 cysts per plant. In Trial 2 the number of cysts per plant ranged from none on mustard to 214.1 on cabbage. Shepherd's purse and black nightshade had high numbers of cysts per plant of 135.2 and 133.4, respectively. This was considered high compared to a commercially grown crop such as broccoli or turnip. It should be emphasized that vegetable producers should keep their fields free of these commonly occurring weeds, due to their positive impact on population numbers of *H. schachtii*.

There was a higher recovery rate of *H. schachtii* with a lower initial density. The high inoculum led to a smaller number of swollen larvae per plant, possibly due to competition for entrance space or resources. Mustard produced no cysts per plant, although a high number of swollen nematodes per plant occurred. It can therefore be included in a rotation cycle to limit multiplication of this nematode. Radish after 38 days produced 13.8 and 2.8 cysts respectively in Trial 1 and Trial 2 and should be destroyed before the swollen nematodes can develop into cysts. If not destroyed early enough, radish will cause an increase in nematode numbers.

Penetration and development of *H. schachtii* were variable in replicates within treatments and could be caused by heterozygosity within crops, as reported by Muller (1986). This may explain the substantial suppression, but not total prevention of cyst development occurring in radish. Some radish plants had no cysts and in others it ranged from four to 18 cysts per plant. Genetic variation in *H. schachtii* over a succession of generations may have led to the development of pathotypes capable of reproducing on radish (Muller

1992, Griffen 1981, Steele 1975). All of the crops tested, except for radish and mustard, led to the successful completion of the life cycle of *H. schachtii* in the roots of hosts.

Life cycle:

Microscopic studies of the penetration of *H. schachtii* infective juveniles into cabbage roots was at a maximum (42%) within 24 hours after inoculation (Fig. 5). The nematodes penetrated proximal to the root tip in both lateral and main roots which led to the formation of numerous root hairs in all hosts tested. According to Raski (1949) the general life cycle of *H. schachtii* had five stages. After the elongation of the embrio, the first moult occurred inside the egg. The second stage larvae exited from the egg after complete formation and it penetrated a suitable host root, where it underwent three moults before reaching the adult stage. The second moult in this experiment occurred four days after inoculation (DAI) up to ten DAI on the various hosts tested. Cauliflower demonstrated an early onset of the second moult. The l_3 juveniles were fully developed after 11 to 14 DAI. Sexual differentiation could be detected during the third stage of development, the larvae appearing more robust, but still elongate. The third moult occurred five days after the second one, but with broccoli and black nightshade as hosts, it occurred seven and six days after the second moult respectively. Raski (1949) found that the third moult on sugar beet occurred four days after the second moult. The third moult of the male occurred within the third larval exuvium and completing its development in this cast skin. From this stage onwards the male and female nematodes differed in speed of development. The fourth moult two to five days after the third one of the males and females occurred simultaneously. The males were fully developed after

three to four days emerging from the third larval skin and leaving the roots. During the first five days the males left the roots rapidly, but thereafter the males left the roots at a reduced rate for up to 47 DAI as in turnip.

The female juvenile developed into a rounded flask shape after the third moult and the typical lemon–shaped adult female form was discernable after the fourth moult. During the period from day 22 to 28, the female posteriori bursted through the root tissue and became visible from the exterior. Most of the female's bodies were filled with eggs by day 31 to 34, except for those on beetroot which were filled from day 38 onwards. Brown cysts were identified on the respective hosts from day 34 to day 50. Black nightshade had brown cysts from day 38. Cabbage, cauliflower, Brussels sprouts and black nightshade formed brown mature cysts up to 10 days before the other crops. This indicates a shorter generation time for *H. schachtii* on these crops, leading, in turn to high population densities. At least two generations/year can be completed under the prevailing conditions in the greater Cape Flats. It can thus be concluded that the development of the life cycles on the different hosts are dissimilar and should be considered when recommendations on crop rotation are made to growers. The importance of weed plants as hosts should be emphasized, as the duration of life cycles on these are of the same duration than on vegetable hosts. This is undesirable due to post-harvest multiplication of the nematodes. In the case of black nightshade, the life cycle is even shorter than on some of the vegetable hosts with similar consequences.

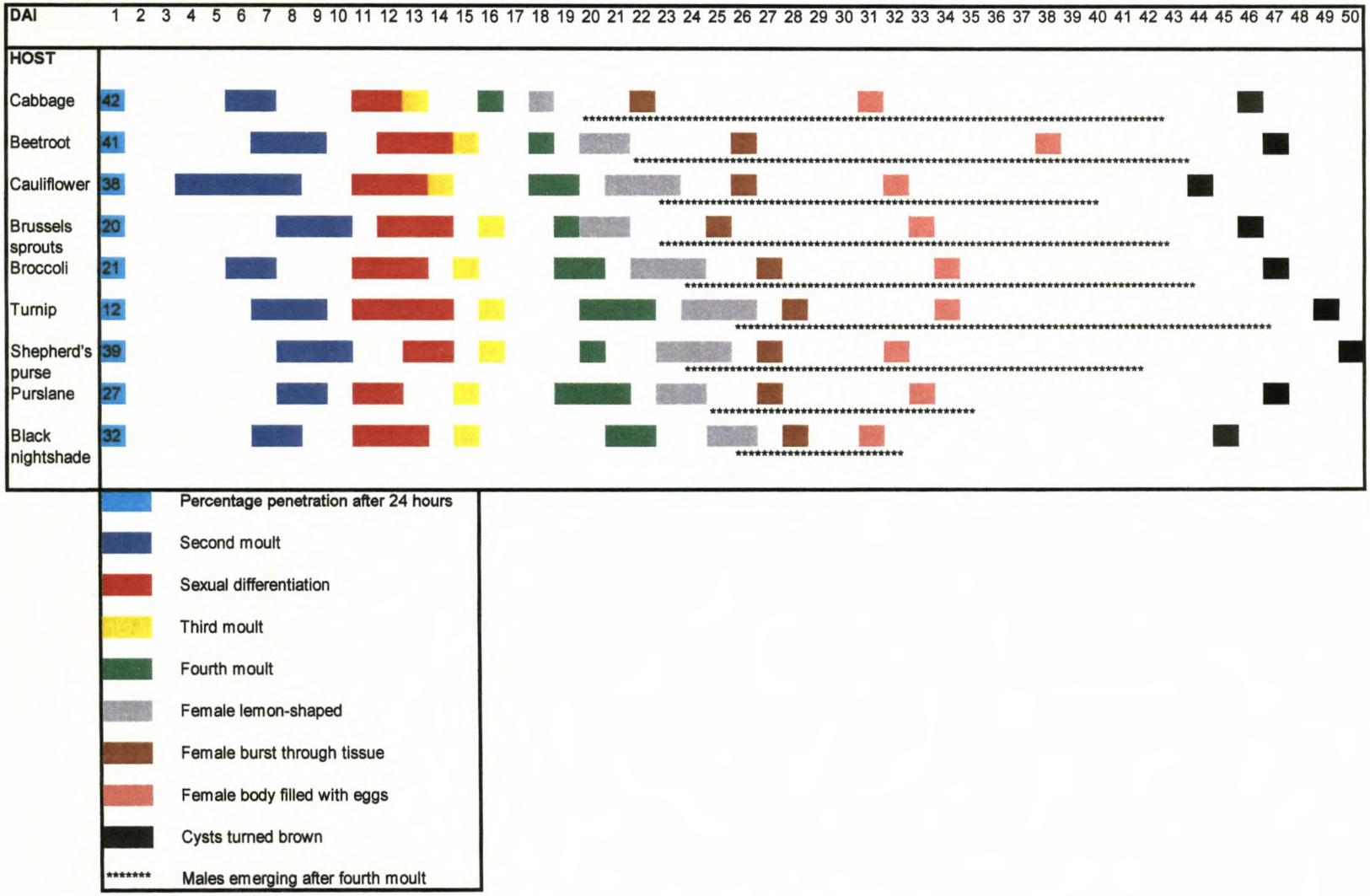

Fig. 5. The penetration and life cycle of *Heterodera schachtii* on different host plants up to 50 days after inoculation.

References

Bridgeman M R & Kerry B R 1980. The sex-rates of cyst-nematodes produced by adding single second-stage juveniles to host roots. *Nematologica* 26: 209 – 213.

Byrd D W Jr. Kirkpatrick T & Barker K R 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology* 15: 142 – 143.

Coetzee V 1968. The distribution of the family *Heteroderidae* (Filipjev, 1934) in South Africa and some host records of *Meloidogyne* species. *South African Journal of Agricultural Science* 11: 775-778.

Cooke D A 1984. The relationship between numbers of *Heterodera schachtii* and sugar-beet yields on a mineral soil. *Annals of Applied Biology* 104: 121 – 129.

Gardner J & Caswell-Chen E P 1993. Penetration, development and reproduction of *Heterodera schachtii* on *Fagopyrum esculentum, Phacelia tanacetifolia, Raphanus sativus, Sinapsis alba* and *Brassica oleracea. Journal of Nematology* 25: 695 – 702.

Golinowski W & Magnusson C 1991. Tissue response induced by *Heterodera schachtii* (Nematoda) in susceptible and resistant white mustard cultivars. *Canadian Journal of Botany* 69: 53 – 62.

Griffen G D 1981. Pathological differences in *Heterodera schachtii* populations. *Journal of Nematology* 13: 191 – 195.

Johnson R N & Viglierchio D R 1969. Sugar-beet nematode (*Heterodera schachtii*) reared on axenic *Beta vulgaris* root explants. Selected environmental and nutritional factors affecting development and sex-ratio. *Nematologica* 15: 144 -152.

Muller J 1986. Integrated control of the sugar beet cyst nematode. In: *Cyst Nematodes*, pp. 235-250 (Eds. F Lamberti & C E Taylor). Plenum Press, New York.

Muller J 1992. Detection of pathotypes by assessing the virulence of *Heterodera schachtii* populations. *Nematologica* 38: 50 – 64.

Ott R L 1998. *An introduction to statistical methods and data analysis.* Belmont, California. Duxbury Press.

Raski D J 1949. The life history and morphology of the sugar-beet nematode, *Heterodera schachtii*. *Phytopathology* 40: 135 – 152.

SAS 1999. SAS/STAT User's guide, Version 8.1, 1 st printing, Volume 2. SAS Institute Inc. SAS Campus Drive, Cary, North Carolina, 27513.

Shapiro S S & Wilk M B 1965. An analysis of variance test for normality. *Biometrika* 52: 591-611.

Shepherd A M & Wallace H R 1959. A comparison of the rate of emergence and invasion of beet eelworm, *Heterodera schachtii* Schmidt and pea root eelworm, *Heterodera göttingiana* Liebster. *Nematologica* 4: 227 – 235.

Steele A E 1975. Population dynamics of *Heterodera schachtii* on tomato and sugar-beet. *Journal of Nematology* 7: 105- 111.

Van Zyl J & Meyer A J 2000. Distribution and host range of the sugar beet nematode, *Heterodera schachtii,* in the Western Cape Province, South Africa. *African Plant Protection* 6: 47 – 51.

Chapter 5.

Pathological reactions to host plants of nine *Heterodera schachtii* (Schmidt 1871) populations in the Western Cape

Introduction

Population variability (Caswell & Roberts 1987) and genetic diversity exists within nematode species (Wallace 1973). The difference in species may be subtle, for example the ability to adapt to environmental stimuli (Thomason 1962, Croll 1970, Griffen 1974) or more pronounced true genetic variability (Thorne 1961, Olthof 1968, Sidhu & Webster 1981, Griffen & Gray 1990). Intraspecific variability in *Heterodera schachtii* was first suspected when chemical control in Utah failed. Control even failed when normal recommendations relating to soil temperature, soil moisture, population densities and application rates were followed (Griffen & Gessel 1973, Griffen 1977). This led to the speculation that an unusually virulent physiological strain existed and, after further research confirmed by Griffen (1981).

To effectively manage and control *H. schachtii* in the Western Cape, it is important to determine whether different populations exhibit differences in virulence. Plant breeders, growers and seed importers also have to consider population variability of nematodes in developing resistant and tolerant cultivars. This study was initiated to determine whether

pathological differences as to their effect on host plants within the nine populations of *H. schachtii* found in the greater Cape Flats did exist.

Materials and methods

Soil samples were obtained from nine localities in the greater Cape Flats during April 2001. These localities were as follows: Durbanville (18.65E;33.83S), Guguletu (18.56E;33.98S), Khayelitsha (18.65E;34.00S), Kraaifontein (18.66E;33.81S), Kuilsriver (18.69E;33.93S), Lynedoch (18.85E;33.96S), Mitchell's Plain (18.63E;34.06S), Philippi (18.61E;34.06S) and Stellenbosch (18.86 E;33.94S). Cysts were collected from each locality and extracted as described by Van Zyl & Meyer (2000). Each population was cultured separately on cabbage (*Brassica oleracea*) in three liter plastic pots in a temperature controlled greenhouse (25 ± 2°C) at Elsenburg. Sterilized sand and vermiculite was used as medium for the multiplication. Cysts were surface sterilized with a 0.5% sodium hypochloride solution, rinsed in distilled water and second stage juveniles were hatched from these cysts in a $ZnCl_2$ (408 mg/l) solution (Muller 1992).

An analysis of variance was performed using SAS version 8.1 (SAS 1999). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk 1965). Student's t-least significant difference was calculated at the 5% confidence level to compare treatment means (Ott 1998).

Virulence: Difference in virulence between the nine populations was investigated on beetroot (*Beta vulgaris* var. *conditiva* L. Hunter) and cabbage (*Brassica oleracea* var. *capitata* L. Green Coronet). Two-week old seedlings of beetroot and cabbage were planted in December 2001 in 14 cm diameter plastic pots, one plant per container, in

bromomethane fumigated sandy-loam soil and inoculated with 500 *H. schachtii* juveniles per plant in December 2001. The juveniles were pipetted into 3 cm deep holes surrounding each plant and the holes were filled with soil after inoculation. Treatments were replicated 18 times and plants grown in a temperature-controlled greenhouse (25 ± 2°C). The root and top weight of the host plants were determined after 50 days

In a further experiment, cabbage seedlings were before also planted in three liter plastic pots and infected with 500 juvenile *H. schachtii*. After 90 days the experiment was terminated and cysts were extracted. The cyst contents and number of cysts per plant were determined as described by Muller (1992). The reproduction ratio (P_f/P_I) was calculated as the quotient of the final number of eggs and the initial number of inoculated juveniles.

Root penetration: During December 2001 two-week old seedlings of beetroot and cabbage were planted in 14 cm plastic containers, inoculated with 250 juveniles per plant and grown in a greenhouse (25 ± 2 °C) at Elsenburg. Treatments were replicated 18 times and plants were harvested after 14 days. The roots were rinsed free of sand and the entire root systems were stained in an acid fuchsin-lactophenol solution (Byrd *et al.* 1983). Juvenile penetration for each plant was determined microscopically.

Juvenile emergence: To determine possible differences between the nine sampled *H. schachtii* populations in the rate of juvenile emergence from the cysts, two-week old cabbage seedlings were planted in 14 cm plastic containers and inoculated with 500

juveniles per plant during February 2002. Treatments were replicated 10 times and plants were grown at 25 ± 2°C for 50 days. Twenty brown cysts were hand picked at random from each root system. The cysts were hatched in a $ZnCl_2$ solution at 25°C and juveniles counted daily. The $ZnCl_2$ solution was replaced daily with a fresh solution for 42 days.

Polymerase chain reaction (PCR)-discrimination between populations:

DNA isolation: Cysts from the different populations of *H. schachtii* were placed in separate reaction tubes, each containing 100µl Sabax water. A Greiner P-20 pipette tip was used to crush the eggs which were then incubated at 55°C for 3 hours. The tubes containing the crushed eggs were centrifuged for 5 minutes at 13 000 revolutions per minute and the supernatant transferred to a clean reaction tube and stored at – 20°C. This work was performed in the Department of Genetics at the University of Stellenbosch during 2002.

Restriction analysis of internally transcribed spacer 1 (ITS) amplification products (RFLP): The two primers used in this study were ITS1 – R380 (5'-CCA GTC AGT GTG TTA TGT GC-3') and ITS1 – F40 (5'-GTT GGG CTA CGC TTG GCA CC-3') (Szalanski *et al.* 1997). The final concentrations of the components used in the polymerase chain reaction (PCR) mixture were 1 ng/µl of nematode genomic DNA, 10 µM of each dNTP (Promega), 0.5 µM of each primer (IDT), 1.5 µM $MgCl_2$ (Bioline), 1X PCR buffer (Bioline) and 0.0025 U/µl DNA polymerase (Bioline). The mixture was made up to 20 µl with double distilled water. Fragment amplification was on a GeneAmp PCR system 9600 (Applied Biosystems). Thermal cycling performed

consisted of an initial five minutes at 94°C, followed by 15 cycles consisting of 30 seconds at 94°C, 30 seconds at 58°C and 30 seconds at 72°C and 15 cycles consisting of 30 seconds at 94°C, 30 seconds at 55°C and 30 seconds at 72°C and completed with a final extension period 72°C for ten minutes. The ITS1 – F40 / ITS1 – R380 amplicons (5-10 ng/µl) were digested for five hours at 37°C using 1 U/µl *Fok* I (Roche) restriction endonuclease. A 1.2% agarose gel was used to analyse the PCR products and a 1% agarose (Whitehead Scientific) gel was used for the *Fok* I digestions.

Results and discussion

Virulence: The top and root weight of beetroot infected with the Lynedoch and Philippi populations of *H. schachtii* were significantly (P=0.05) lower than those of the other populations (Table 1). The top weight of beetroot infected with the Philippi juveniles was 49.5% of that of the Guguletu population. This reduction was expected, considering that the root weight was 34.9% of beetroot infected with the juveniles from the Guguletu population. The *H. schachtii* originating from Guguletu was the least virulent in regard to both top and root weight on beetroot.

The *H. schachtii* from Lynedoch and Philippi also showed the lowest (P=0.05) top and root weight of cabbage compared to the nematodes from the other areas (Table 1). The root weight of cabbage infected with the nematodes from Philippi was 44% of that of the least virulent population originating in Kraaifontein. Griffen (1981) also found that one

population, from six geographically separated populations tested, produced in lower top and root weights of sugar beet.

Table 1. The effect of nine populations of *Heterodera schachtii* on top and root weight of beetroot and cabbage 50 days after inoculation

Population	Top Weight (g)		Root Weight (g)	
	Beetroot	Cabbage	Beetroot	Cabbage
Durbanville	233.0 d	2108.1 c	28.3 c	58.8 b
Guguletu	328.2 a	2218.1 c	36.7 a	58.3 b
Khayelitsha	276.8 c	2611.0 a	36.0 a	58.7 b
Kraaifontein	287.3 c	2192.1 c	27.2 c	67.3 a
Kuilsriver	218.2 e	2415.0 b	22.3 d	61.2 b
Lynedoch	172.3 f	1500.0 d	15.2 e	32.4 d
Mitchell's Plain	310.2 b	2713.0 a	32.7 b	49.3 c
Philippi	162.4 f	1498.1 d	12.8 e	29.6 d
Stellenbosch	221.9 de	2100.2 c	27.2 c	59.8 b
LSD 5%	13.93	132.19	2.57	3.15

Values in columns followed by the same letter do not differ significantly (P=0.05).

The highest number of cysts/plant was recorded from *H. schachtii* populations originating from Philippi and Lynedoch (Table 2). The number of eggs/cyst (Table 2) ranged from 152 to 270 in all nine populations, with a variation of 42 eggs/cyst in a given population. Multiplication rates (Table 2) of the nematodes from Lynedoch and Philippi were 24 and 27, respectively, with the other populations ranging from 12 to 20. Muller (1992) compared seven geographically separated *H. schachtii* populations from Germany and found considerable differences in the mean number of cysts produced per plant. He also found significant differences in multiplication rates and numbers of eggs/cyst between some of the populations.

Root penetration: Significantly (P = 0.05) more *H. schachtii* juveniles from the Lynedoch and Philippi populations penetrated the seedling roots of beetroot and cabbage (Table 3). The Kraaifontein population had the lowest penetration level (57 juveniles per seedling) on beetroot and the Mitchell's Plain population the lowest penetration on

Table 2. The effect of nine populations of *Heterodera schachtii* on the number of cysts per plant, eggs per cyst and multiplication rate on cabbage, 90 days after inoculation

Population	Cysts per plant	Eggs/cyst	Pf/Pi
Durbanville	179 b	193 cd	13 ed
Guguletu	153 c	172 e	14 ed
Khayelitsha	169 b	200 c	15 d
Kraaifontein	125 e	183 de	20 c
Kuilsriver	125 e	152 f	12 e
Lynedoch	193 a	225 b	24 b
Mitchell's Plain	143 c	195 cd	18 c
Philippi	197 a	270 a	27 a
Stellenbosch	139 d	183 de	18 c
LSD 5%	12.8	16.9	2.4

Values in columns followed by the same letter do not differ significantly (P=0.05).

cabbage (49 juveniles per seedling). Griffen (1981) comparing root penetration of six American populations, found that juveniles of one population penetrated seedlings significantly more than the other populations. Similar results with *H. schachtii* from Lynedoch and Philippi populations were obtained. Griffen (1981) also reported a wide variation in penetration rates, with some populations showing rates twice that of others.

Juvenile emergence: The number of juveniles emerging from cysts from Lynedoch and Philippi were higher (P=0.05), over a shorter period of time, especially during the first seven days (Table 4). After seven days 67.6% of the total number of juveniles emerged from the Lynedoch population and 65.5% from the Philippi population. The population from Guguletu had the lowest emergence of 19.2% during the first week. The Philippi population had the highest (P=0.05) total number of larval emergence per cyst of 267.

Table 3. Penetration of beetroot and cabbage seedlings by larvae from nine *Heterodera schachtii* populations 14 days after inoculation

Population	Beetroot	Cabbage
Durbanville	72 b	59 c
Guguletu	63 cd	52 de
Khayelitsha	62 de	58 cd
Kraaifontein	57 e	64 bc
Kuilsriver	68 bc	68 b
Lynedoch	98 a	108 a
Mitchell's Plain	71 b	49 e
Philippi	93 a	112 a
Stellenbosch	59 de	70 b
LSD 5%	5.2	6.4

Values in columns followed by the same letter do not differ significantly (P=0.05).

The percentage emergence of the three most virulent populations after seven, 14 and 21 days were 65.5%, 83.1% and 93.6% for the Philippi population, 21.5%, 57.9% and 99.1% for the Stellenbosch population and 67.6%, 83.8% and 84.6% for the Lynedoch population, respectively. The higher percentage of juvenile emergence of the Philippi and Lynedoch populations in the first two weeks, could result in earlier root penetration

and severe pathological reaction by the host. Griffen (1981) also identified a population with a rapid juvenile emergence that resulted in earlier root penetration.

Table 4. Emergence of juveniles from nine populations of *Heterodera schachtii* cultured on cabbage for 42 days

Population	Number of larvae emerged per cyst.				
	Days				Total
	7	14	21	42	
Durbanville	47 cd	53 d	72 c	18 b	190 d
Guguletu	28 e	43 fg	59 d	16 b	146 f
Khayelitsha	42 d	65 c	60 d	17 b	184 d
Kraaifontein	47 cd	91 a	81 b	4 a	223 c
Kuilsriver	33 e	37 h	52 e	15 b	137 f
Lynedoch	163 b	39 gh	21 g	18 b	241 b
Mitchell's Plain	32 e	51 de	64 d	16 b	163 e
Philippi	175 a	47 ef	28 f	17 b	267 a
Stellenbosch	49 c	83 b	94 a	2 a	228 bc
LSD 5%	5.3	5.5	4.8	6.2	16.11

Values in columns followed by the same letter do not differ significantly (P=0.05).

PCR-RFLP discrimination between populations: The amplification products from all nine samples were approximately 380 bp in length (Fig. 1) and fragments of approximately 210 bp and 170 bp were obtained from all the isolates after *Fok* I digestions (Fig. 2). This result was similar to the finding of Szalanski *et al.* (1997) as to the identification of *H. schachtii* using PCR-RFLP of the ITS-1 region. Caswell-Chen *et al.* (1992) detected differences between *H. schachtii* populations, using RAPD-analysis to determine the genetic variation among and within different geographic populations. The RFLP indicated that the restriction endonuclease used, did not reveal polymorphisms in

the specific amplified fragment and that the isolates were identical to one another using this PCR-RFLP marker.

The evidence presented here, demonstrated physiological variation in *H. schachtii* populations as far as virulence, root penetration and larval emergence from cysts on hosts were concerned. The physiological differences were apparently due to a faster larval penetration and a shorter period of larval emergence, contributing to earlier root penetration. This was also found by Griffen (1981) in one of the populations tested. A reduced generation time can be suspected when yields are lower than normal, after the use of cultural and chemical control practices, but further investigation is needed. Biological differences in individuals within a population exist and as the population continually undergoes genetic re-combinations, it could lead to the development of physiological races of nematodes. This may be the result of monocultural practices, favouring certain gene combinations while suppressing others in the nematode (Griffen 1991).

The results indicate that the Lynedoch and Philippi populations were different from the other populations in their effect on selected hosts. The physiological variation between populations will influence the host-parasite relationship and new management strategies must be considered to control of *H. schachtii* in these areas. Control should be determined in line with the most virulent population, as such populations will eventually spread into the cultivation areas.

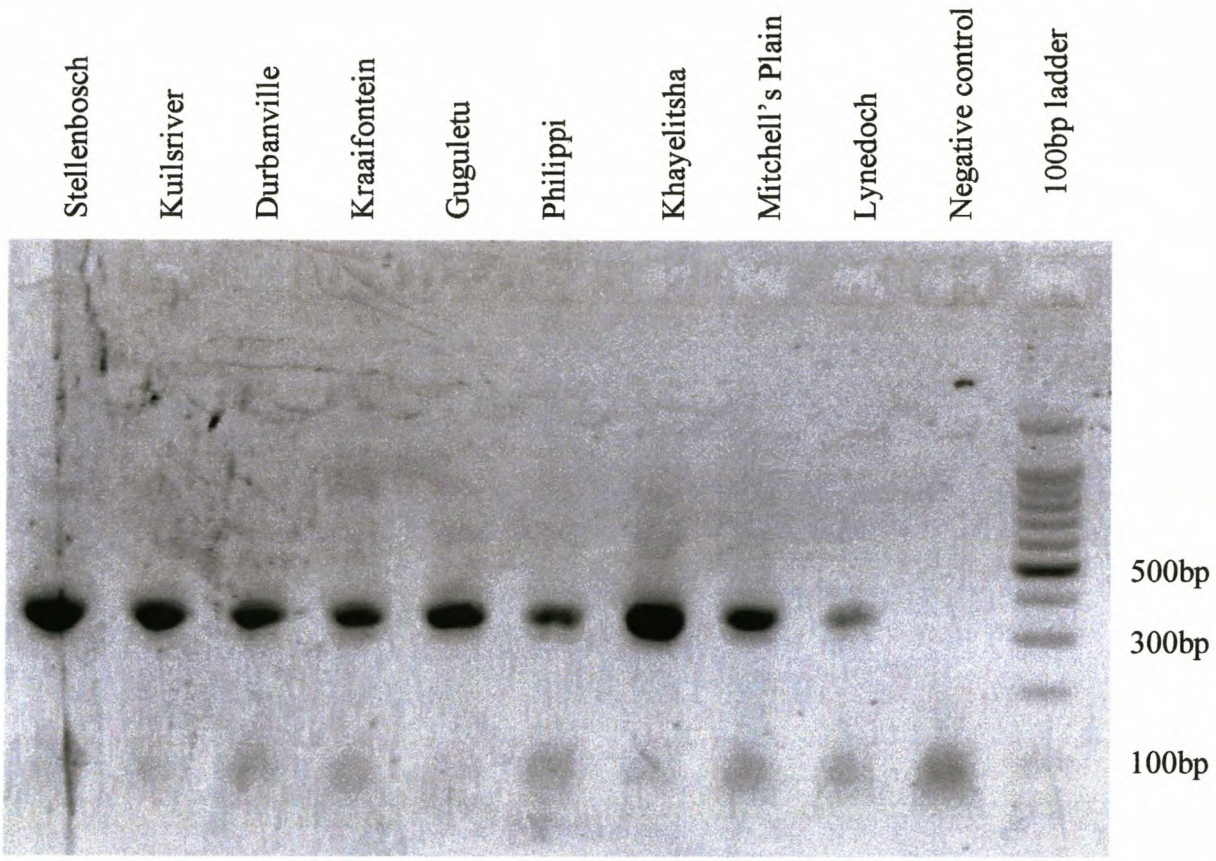

Fig. 1. ITS1-F40/ITS1-R380 fragments obtained from the different *Heterodera schachtii* populations.

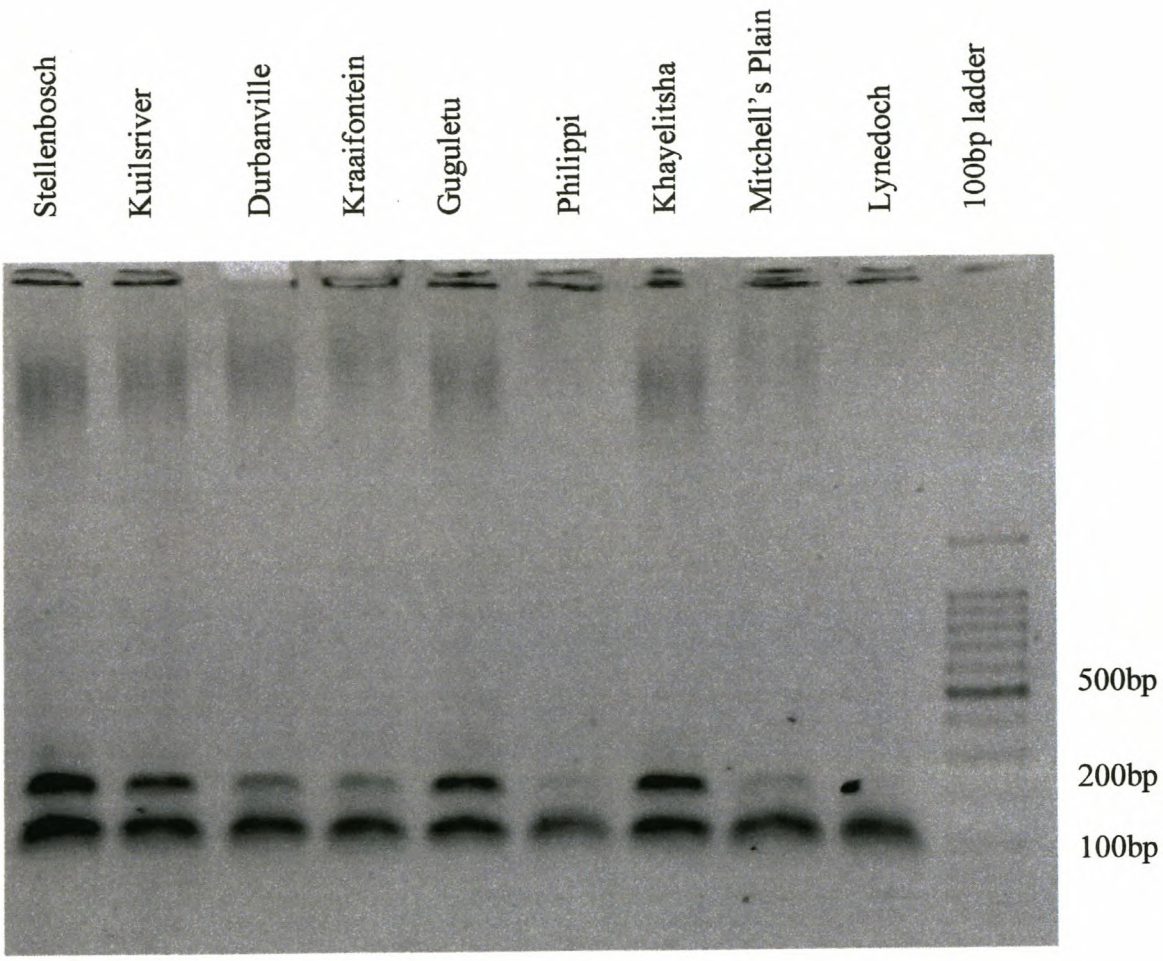

Fig. 2. PCR-RFLP analysis using *Fok* I digest of the ITS1-F40/ITS1-R380 amplicon.

References

Byrd D W, Jr., Kirkpatric T & Barker K R 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology* 15: 142–143.

Caswell E P & Roberts P A 1987. Nematode population genetics. In. J A Veech and D W Dickson, eds. *Vistas on nematology*. Society of Nematologists.

Caswell-Chen E P, Williamson V M & Wu F F 1992. Random amplified polymorphic DNA analysis of *Heterodera cruciferae* and *H. schachtii* populations. *Journal of Nematology* 24: 343-351.

Croll N A 1970. Acclimatization in the eccritic thermal response of *Ditylenchus dipsaci. Nematologica* 13: 385-389.

Griffen G D & Gessel T G 1973. Systemic nematicide control of *Heterodera schachtii* on sugarbeet. *Plant Disease Reporter* 57: 942-945.

Griffen G D 1974. Effect of acclimation temperature on infection of alfalfa by *Ditylenchus dipsaci. Journal of Nematology* 6: 57-59.

Griffen G D 1977. Effect of soil moisture on control of *Heterodera schachtii* with aldicarb. *Journal of Nematology* 9: 211-215.

Griffen G D 1981. Pathological differences in *Heterodera schachtii* populations. *Journal of Nematology* 13: 191-195.

Griffen G D & Gray F A 1990. The biology and pathogenicity of *Pratylenchus neglectus* on alfalfa. *Journal of Nematology* 22: 546-551.

Griffen G D 1991. Differential pathogenicity of four *Pratylenchus neglectus* populations on alfalfa. *Journal of Nematology* 23: 380-385.

Muller J 1992. Detection of pathotypes by assessing the virulence of *Heterodera schachtii* populations. *Nematologica* 38: 50-64.

Olthof H A 1968. Races of *Pratylenchus penetrans* and their effect on black root rot resistance of tabacco. *Nematologica* 14: 482-488.

Ott R L 1998. *An introduction to statistical methods and data analysis.* Belmont, California. Duxbury Press.

SAS 1999. SAS/STAT User's guide,Version 8.1. Volume 2. SAS Institute Inc. SAS Campus drive, Cary, North Carolina 27513.

Shapiro S S & Wilk M B 1965. An analysis of variance test for normality. *Biometrika* 52: 591-611.

Sidhu G S & Webster J M 1981. The genetics of plant-nematode parasitic systems. *Botanical Review* 47: 387-419.

Szalanski A L, Sui D D, Harris T S & Powers T O 1997. Identification of cyst nematodes of agronomic and regulatory concern with PCR-RFLP of ITS1. *Journal of Nematology* 29: 255-267.

Thorne G 1961. *Principals of nematology*. New York: McGraw-Hill Book Company.

Thomason I J 1962. Reactions of cereals and sudan grass to *Meloidogyne* spp. and the relation of soil temperature to *M. javanica* populations. *Phytopathology* 52: 787-791.

Van Zyl J & Meyer A J 2000. Distribution and host range of the sugar-beet nematode, *Heterodera schachtii* in the Western Cape Province, South Africa. *African Plant Protection* 6: 47-51.

Wallace H R 1973. *Nematode ecology and plant disease*. New York, Crane Russak

Chapter 6.

The effect of soil temperature, soil texture and pH on *Heterodera schachtii* (Schmidt, 1871)

Introduction

Varying environmental conditions in areas where vegetables are grown resulted in considerable variation in the degree of pathogenicity of *Heterodera schachtii* to hosts (Seinhorst 1965, Olthof 1978, Cook & Thomason 1979). The variation in soil temperature in geographically separated vegetable growing areas of the Western Cape could influence the effect of the nematodes on vegetables.

The soil texture and pore size of the soil influences the migration and penetration of juveniles into plant roots. Migration of *Globodera rostochiensis* (Wollenweber 1923) towards potato plants was optimum in sandy, intermediate in loamy and minimal in clay soils (Rode 1962). *Radopholus similis* (Cobb 1893) moved more effectively in sandy soil than in heavily textured soil Tarjan (1971). Apparently, an optimal soil particle size exists for each species of nematode Wallace (1958).

The influence of soil pH on *H. schachtii* has not been studied extensively. Several authors found that the numbers of *Heterodera avenae* (Wollenweber 1924), *Criconemoides xenoplax* (Raski 1952) and *Tylenchulus semipenetrans* (Cobb 1913) increased as soil alkalinity increased (Duggan 1963, Van Grundy & Martin 1961, Schmitt

1969). Morgan & Mac Lean (1968) found that greater numbers of *Pratylenchus penetrans* were recorded from vetch grown in soil with a pH 5.8 rather than at 4.8 or 7.5.

The objective of this study was to investigate the effects of soil temperature, various climatic conditions, soil texture and pH on *H. schachtii* in order to predict the response to vegetables under the conditions prevailing in the greater Cape Flats.

Material and Methods

Temperature and P_i: The effect of initial nematode inoculum density and soil temperature on the growth of beetroot (*Beta vulgaris* var. *conditiva* L. Red Ace) and cabbage (*Brassica oleracea* var. *capita* L. Green Coronet) were investigated under controlled temperature conditions in a greenhouse (25±2°C) at Elsenburg. Cysts originating from Lynedoch, were also reared on cabbage in a greenhouse at Elsenburg. Cysts were surface sterilized with a 0.5% sodium hypochloride solution, rinsed in distilled water and hatched in $ZnCl_2$ solution. The hatched juveniles were added to previously steam sterilized sand to represent initial population densities of 0, 2, 6 and 18 juveniles per gram of soil. The study was performed during September to December 2002 in a greenhouse. Four waterbaths were set to produce soil temperatures of 7, 14, 21 and 28 °C (±1°C). Two week-old cabbage and beetroot seedlings were planted in a complete random design in four litre plastic pots in the water-baths. Ten replicates per treatment were planted. The soil in the pots was fertigated (Chemicult^R) to field capacity and all nutrients needed for normal plant growth were supplied. The data collected after 90 days were top and root weight of the host plant, numbers of eggs and juveniles per

gram of soil and the P_f/P_i ratio where P_f was the final density and P_i the initial population density. Multiple regression was performed with top weight, root weight, initial number of juveniles per gram of soil and the P_f/P_i ratio on dependent variables. The independent variables were temperature and initial population densities (Table Curve 3D 2001).

Field trial: A second trial under field conditions was conducted on cabbage in micro-plots at George, Stellenbosch and Vredendal during September 2002 - December 2002 to test the effect of climatical conditions in geographically separated vegetable growing regions in the Western Cape. Micro-plots of 3m x 4m with a depth of 1.2 m were constructed with pre-fabricated cement slabs and filled with a sandy loam soil to eliminate the effect of different soil types at each locality. Cysts of *H. schachtii* were mixed into the soil to yield initial inoculum densities of 0, 2, 6 and 18 eggs and juveniles per gram of soil. The egg and juvenile densities were randomly assigned to the micro-plots, with 10 replicates per treatment. An analysis of variance was performed using SAS version 8.1 (SAS 1999). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk 1965). Student's t-least significant difference was calculated at the 5% confidence level to compare treatment means (Ott 1998). Two-week old cabbage seedlings were planted in each micro-plot. The micro-plots at the three locations received similar fertigation and pest management regimes simultaneously. All plants in the plots were harvested after 90 days, yield of crops and eggs/juveniles per gram of soil determined. Soil samples were taken as one sample per m^2. Samples were bulked to determine the final number of eggs and juveniles/gram of soil. The final number of eggs and juveniles was determined as described by Van Zyl & Meyer (2000).

Soil texture: The juveniles used in this study originated from the same source and were treated as described in the temperature trails. The vertical migration of *H. schachtii* in five soil types was studied in November 2002 in a modified apparatus as described by Prot & Van Gundy (1981). A polyvinyl chloride (PVC) tube of 12 cm long, with an internal diameter of two cm was filled with different soil types and closed at the bottom with polyethylene film. Four hundred juveniles, hatched in $ZnCl_2$, were inoculated at the bottom 2 cm of soil through a cavity in the side of the tube. The cavity was sealed afterwards. The top of the tube was inserted in a cavity made in the centre of the bottom of a styrofoam cup. The cups were filled with 120 cm^3 the same soil as was in the tube and kept at field capacity. A two-week old cabbage seedling was planted in each cup. Ten replicates of each of the five soil types were used. Their composition varied between 9% silt and clay content and 37%. The International Society of Soil Science regards sand as having particles bigger than 0.02 mm, but less than 2 mm, silt particles being less than 0.02 mm, but bigger than 0.002 mm, and particles smaller than 0.002 mm are considered to be clay (Buol *et al.* 1980). A regression with penetration as the dependant variable and soil type as the independent variable was fitted (Table curve 2D 2001).

The experiment was conducted in a growth chamber with a controlled temperature of 27°C ± 2°C for 10 days at Elsenburg. After 10 days the roots were washed to remove the sand and roots stained with acid fuchsin (Byrd *et al.* 1983) to establish the penetration levels of the nematodes.

pH: The effect of soil pH on nematode penetration and damage was determined in two experiments at Elsenburg during December 2002 to March 2003. A sandy-loam soil of pH 4.2 was used and adjusted to the desired pH levels. The soil was fumigated with

methyl bromide at a rate of 454g/500g of soil. A 3:2 mixture of powdered $CaCo_3$ and $MgCo_3$ was added to create pH levels of the soil of 4.2, 5.2, 6.2 and 7.2. The amount of salts added are shown in Table 1. A commercial fertilizer (Chemicult^R) was added and mixed with the soil, without affecting the pH, to provide the plants with sufficient nutrients for the 90 days growth period. Pots were initially watered by weight to field capacity of the soil and watered again when soil moisture content of the soil reached 70% of field capacity. After 10 days the roots were washed free of sand and penetration of *H.* schachtii determined as described before. Regression analysis with penetration, yield and final numbers of juveniles per gram of soil as the dependant variables and Ph as the independent variable was performed on this data (Table Curve 2D 2001).

Table 1. Soil pH after adding $CaCO_3$ and $MgCO_3$

3:2 Mixture of $CaCO_3$ + $MgCO_3$ added (g/kg)	pH
0	4.2
1.2	5.2
3.4	6.2
8.4	7.2

The pH was determined with a pH meter. Five ml of soil was mixed with 30 ml KCl (1 mol) and left for 20 minutes before the pH value was determined. The soils with different pH levels were placed in six litre plastic pots in a temperature controlled greenhouse at 25°C ±2°C at Elsenburg. The juveniles were handled and allowed to hatch as described before. The juveniles were added to the soil in a water suspension and mixed with the soil to give an inoculum level of 6 juveniles per gram of soil. Two-week old cabbage seedlings were planted in each pot. Each treatment was replicated 10 times

and the experiment was repeated three times. The cabbages were harvested after 90 days to determine the yield.

Results and Discussion

Temperature and initial densities on Cabbage:

The quadratic term in relationship between top weight of cabbage and temperature was not significant indicating that the relationship between temperature and top weight was linear (Table 2; Fig. 1). Top weight of cabbage and number of juveniles per gram of soil had a quadratic trend. Yield decreased a minimum at 17.5 juveniles per gram of soil. The lower temperatures resulted in higher yields at initial densities of 0 to 10 juveniles, but at higher densities temperature had a less pronounced effect on yield. The slightly higher yields at initial densities of above 17.5 juveniles per gram of soil were due to the formation of a mass of root hairs due to the infestation.

The relationship between root weight and temperature was linear, while the relationship between root weight and juveniles per gram of soil was quadratic (Table 2; Fig. 2). The increase in root weight at population densities higher than 12.5 juveniles per gram of soil was due to the formation of the root hairs as reported earlier. Root weight increased at the lower temperature range. This was expected as the optimum temperature for *H. schachtii* penetration was 25 °C and the minimum temperature for penetration was 10°C. The relationship between reproduction ratio (P_f/P_i) and temperature was quadratic, with high P_f/P_i values between temperatures of 12°C and 26°C (Table 2; Fig. 3). Neither the

linear nor the quadratic relationships between P_f/P_i and juveniles per gram of soil were significant.

The relationship between the number of eggs and juveniles per gram of soil and both initial populations in the soil and temperature of soil was quadratic (Table2; Fig. 4). The regression models on cabbage are described in Table 2.

Temperature and initial populations on beetroot:

There was a linear trend between temperature and top weight (Table 3; Fig. 5). The relationship between initial numbers of juveniles and top weight was quadratic with a steep increase in top weight at initial densities below five juveniles per gram of soil. The slight increase in top weight with initial levels of above 17.5 juveniles per gram of soil was due to enhanced nutrient uptake because of excessive hair root formation. The relationship between root weight and temperature was linear and the relationship between temperature and initial nematode densities was quadratic (Table 3). Excessive hair root formation was observed with initial densities of 18 juveniles per gram of soil (Fig. 6).

The reproduction ratio was quadratic in relation to temperature with maximum P_f/P_i ratios achieved between temperatures of 15°C to 28°C (Table 3; Fig. 7). The relationship between reproduction ratio and initial population density was quadratic as well.

The relationship between final population densities of eggs and juveniles per gram of soil and initial population densities was linear. There was a quadratic trend in the relationship between temperature and final numbers of juveniles per gram of soil due to optimal

Table 2. Relationship between top weight (z), root weight (z), reproduction ratio (z) and final numbers of nematodes per gram of soil (z) against temperature (x) and initial densities of *H. schachtii* (y) on cabbage

Relationship between top weight, temperature and initial population densities.

$z = a+bx+cy+dx^2+ey^2+fxy$ $r^2 = 0.782$

Parm	Value	t	P
a	3517.453	29.478	0.00000
b	-41.869	-2.919	0.00403
c	-165.532	-11.421	0.00000
d	0.407	1.023	0.30799
e	4.699	6.959	0.00000
f	1.085	3.039	0.00279

Relationship between root weight, temperature and initial population densities.

$z = a+bx+cy+dx^2+ey^2+fxy$ $r^2 = 0.786$

Parm	Value	t	P
a	102.693	24.970	0.00000
b	-1.948	-3.940	0.00012
c	-4.087	-8.181	0.00000
d	0.014	1.051	0.29510
e	0.183	7.881	0.00000
f	-0.029	-2.358	0.01964

Relationship between reproduction ratio (P_f/P_i), temperature and initial population densities.

$z = a+bx+cy+dx^2+ey^2+fxy$ $r^2 = 0.761$

Parm	Value	t	P
a	7.877	4.903	0.00000
b	1.213	6.362	0.00000
c	-0.535	-1.058	0.29249
d	-0.025	-4.751	0.00001
e	-0.096	-1.378	0.17102
f	-0.033	-2.511	0.01342

Relationship between final number of eggs and juveniles per gram of soil, temperature and initial populations.

$z = a+bx+cy+dx^2+ey^2+fxy$ $r^2 = 0.917$

Parm	Value	t	P
a	-16.319	-1.633	0.10531
b	6.771	5.708	0.00000
c	31.661	10.051	0.00000
d	-0.170	-5.177	0.00000
e	-2.333	-5.379	0.00000
f	0.282	3.432	0.00830

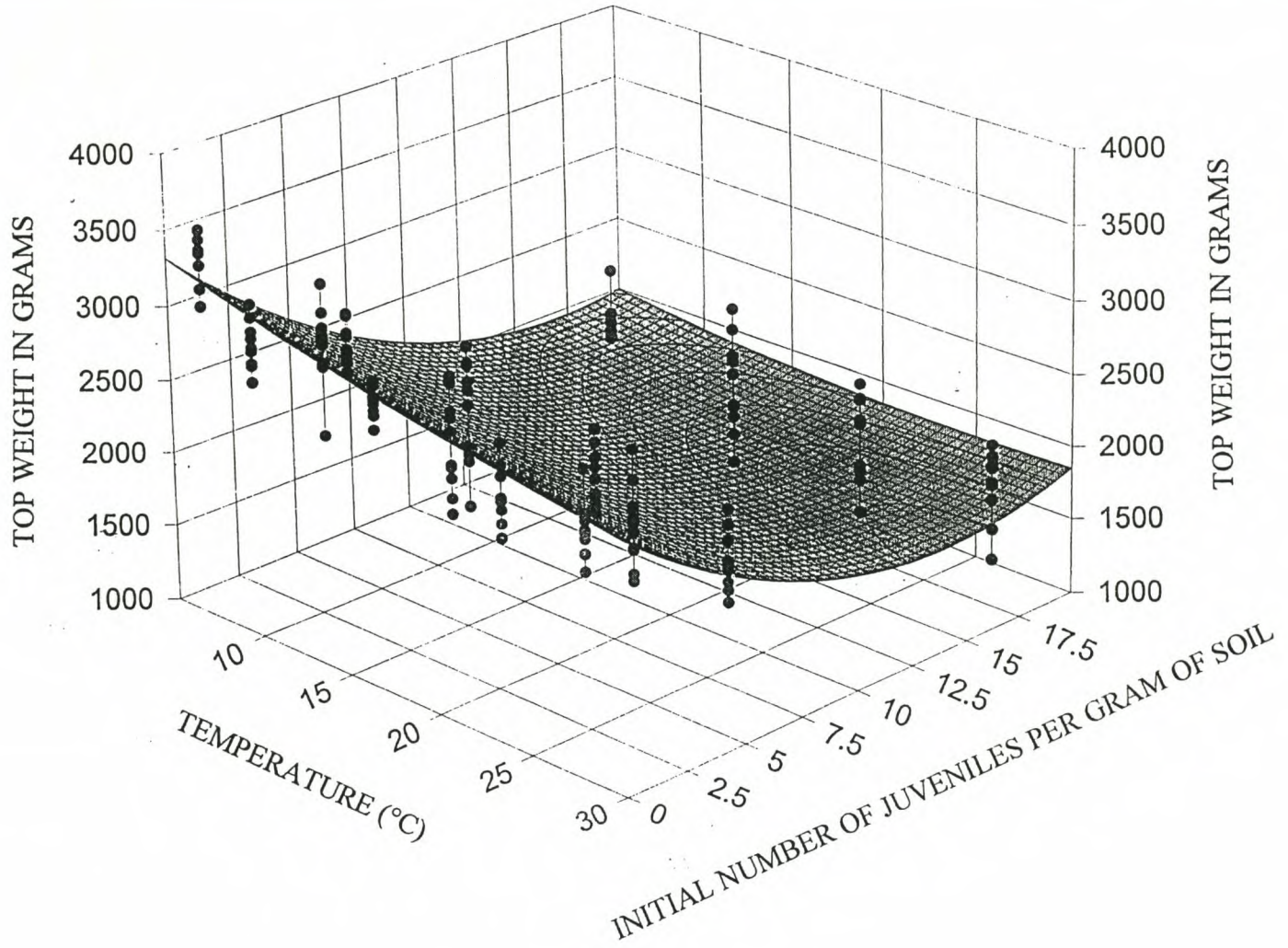

Fig. 1 The relationships between cabbage top weight, soil temperature and initial numbers of *Heterodera schachtii* juveniles per gram of soil

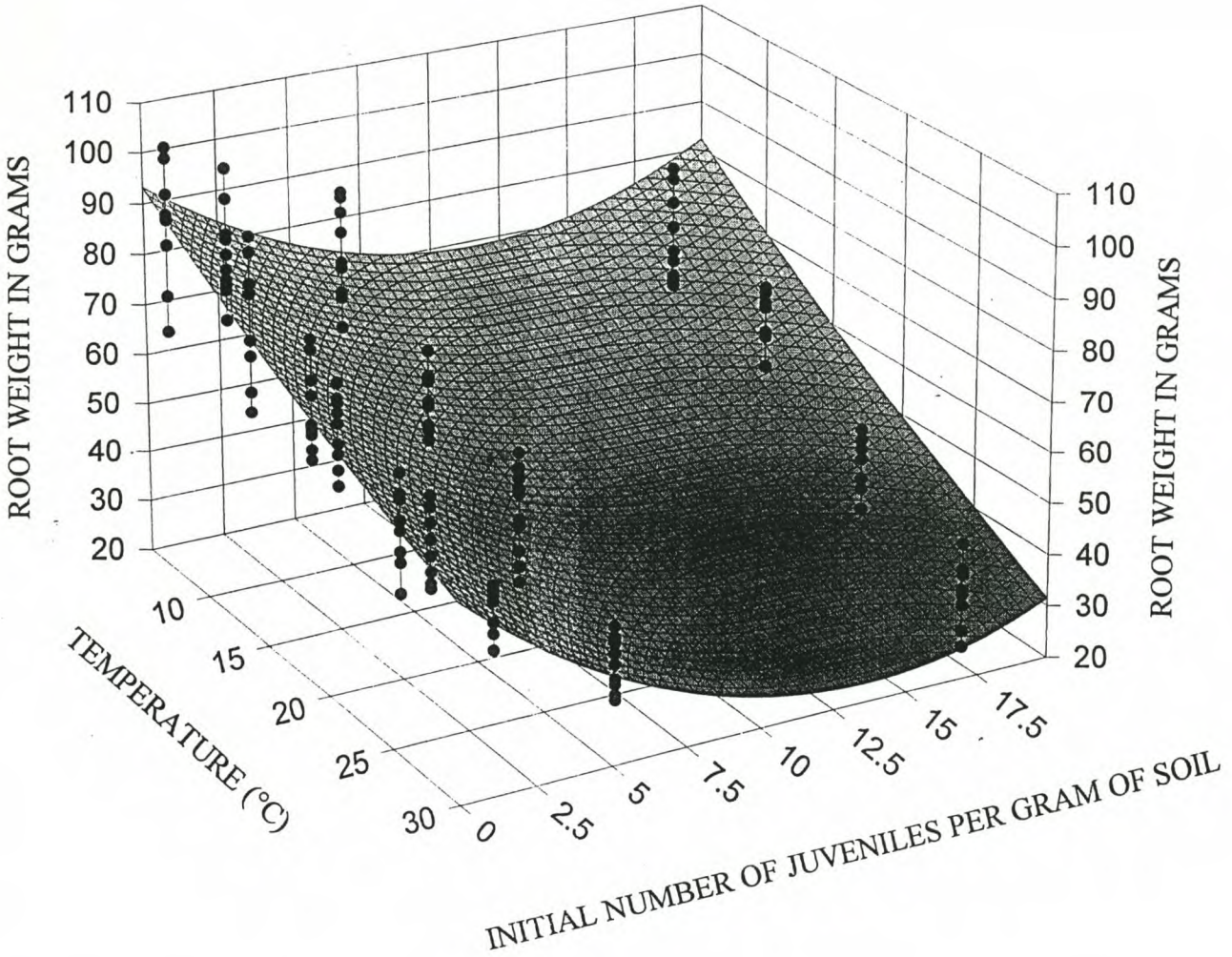

Fig. 2 The relationships between cabbage root weight, soil temperature and initial numbers of *Heterodera schachtii* juveniles per gram of soil

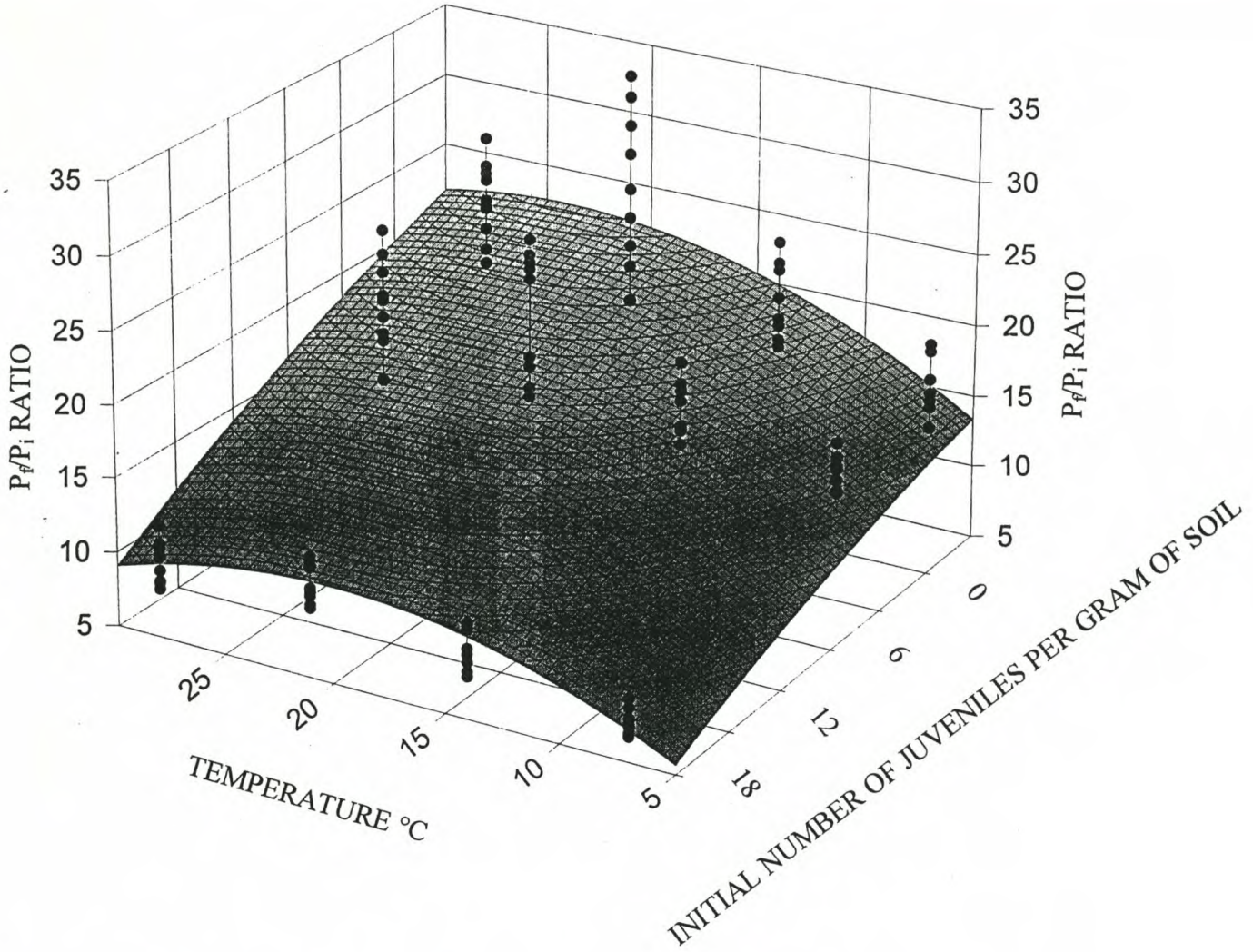

Fig. 3 The relationships between cabbage reproduction ratio (P_f/P_i), soil temperature and initial numbers of *Heterodera schachtii* juveniles per gram of soil

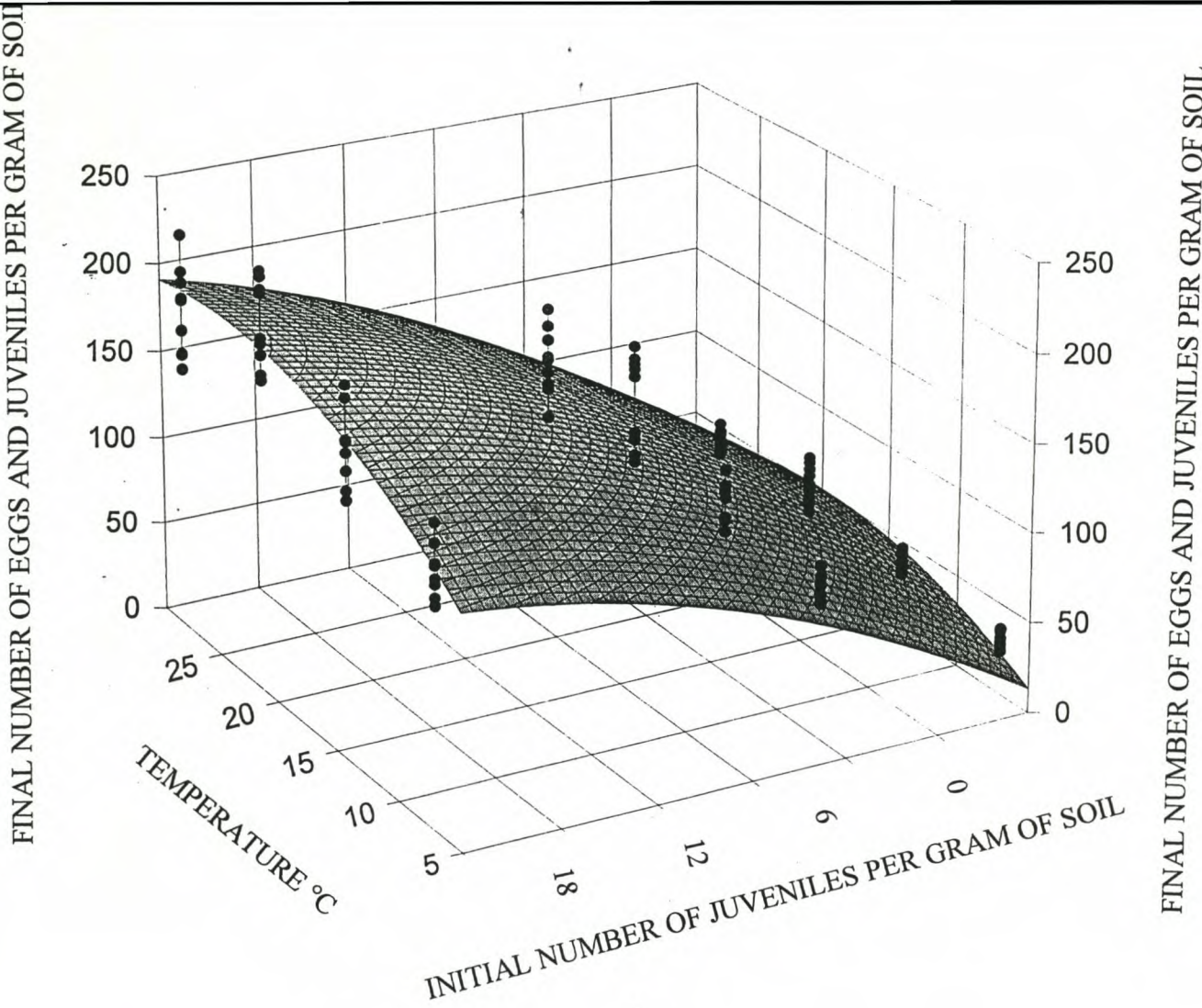

Fig. 4 The relationships between cabbage final number of eggs and juveniles per gram of soil, soil temperature and initial numbers of *Heterodera schachtii* juveniles per gram of soil

Table 3. Relationship between top weight (z), root weight (z), reproduction ratio (z) and final numbers of nematodes per gram of soil (z) against temperature (x) and initial densities of *H. schachtii* (y) on beetroot

Relationship between top weight, temperature and initial population densities.

$z = a+bx+cy+dx^2+ey^2+fxy$ $\quad r^2 = 0.946$

Parm	Value	t	P
a	715.324	37.762	0.00000
b	-4.821	-2.117	0.03583
c	-72.161	-31.362	0.00000
d	-0.039	-0.610	0.54303
e	2.642	24.645	0.00000
f	0.156	2.750	0.00675

Relationship between root weight, temperature and initial population densities.

$z = a+bx+cy+dx^2+ey^2+fxy$ $\quad r^2 = 0.782$

Parm	Value	t	P
a	55.515	23.029	0.00000
b	-0.978	-3.374	0.00094
c	-3.305	-11.287	0.00000
d	0.003	0.333	0.73953
e	0.110	8.085	0.00000
f	0.018	2.558	0.01151

Relationship between reproduction ratio (P_f/P_i), temperature and initial populations densities.

$z = a+bx+cy+dx^2+ey^2+fxy$ $\quad r^2 = 0.846$

Parm	Value	t	P
a	4.242	6.090	0.00000
b	0.509	6.156	0.00000
c	-1.891	-8.613	0.00000
d	-0.009	-4.094	0.00008
e	0.212	7.026	0.00000
f	-0.024	-4.223	0.00005

Relationship between final number of eggs and juveniles per gram of soil, temperature and initial populations.

$z = a+bx+cy+dx^2+ey^2+fxy$ $\quad r^2 = 0.931$

Parm	Value	t	P
a	0.256	0.087	0.93101
b	2.088	5.965	0.00000
c	7.382	7.940	0.00000
d	-0.047	-4.886	0.00000
e	-0.234	-1.831	0.06977
f	0.069	2.821	0.00564

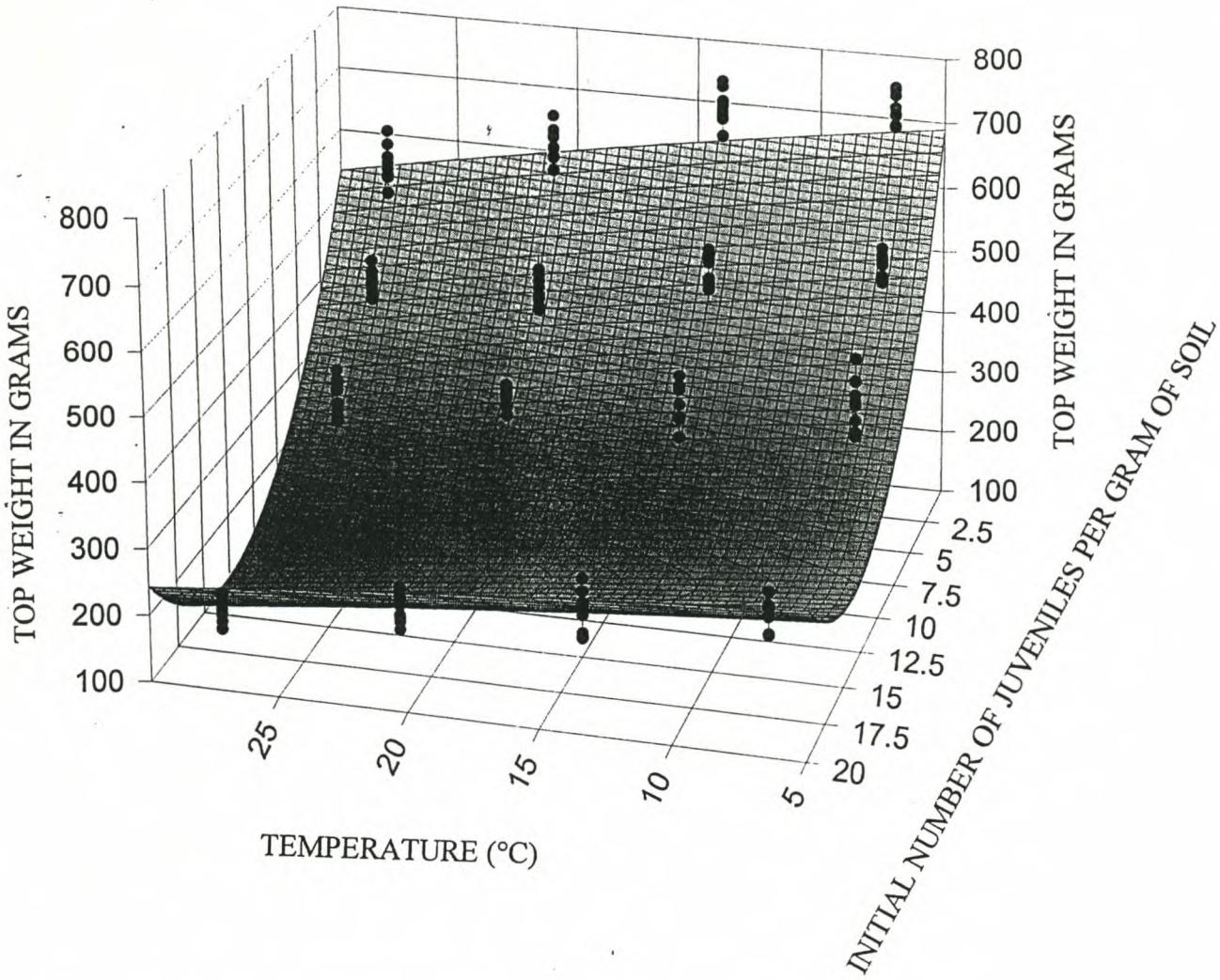

Fig. 5 The relationships between beetroot top weight, soil temperature and initial numbers of *Heterodera schachtii* juveniles per gram of soil

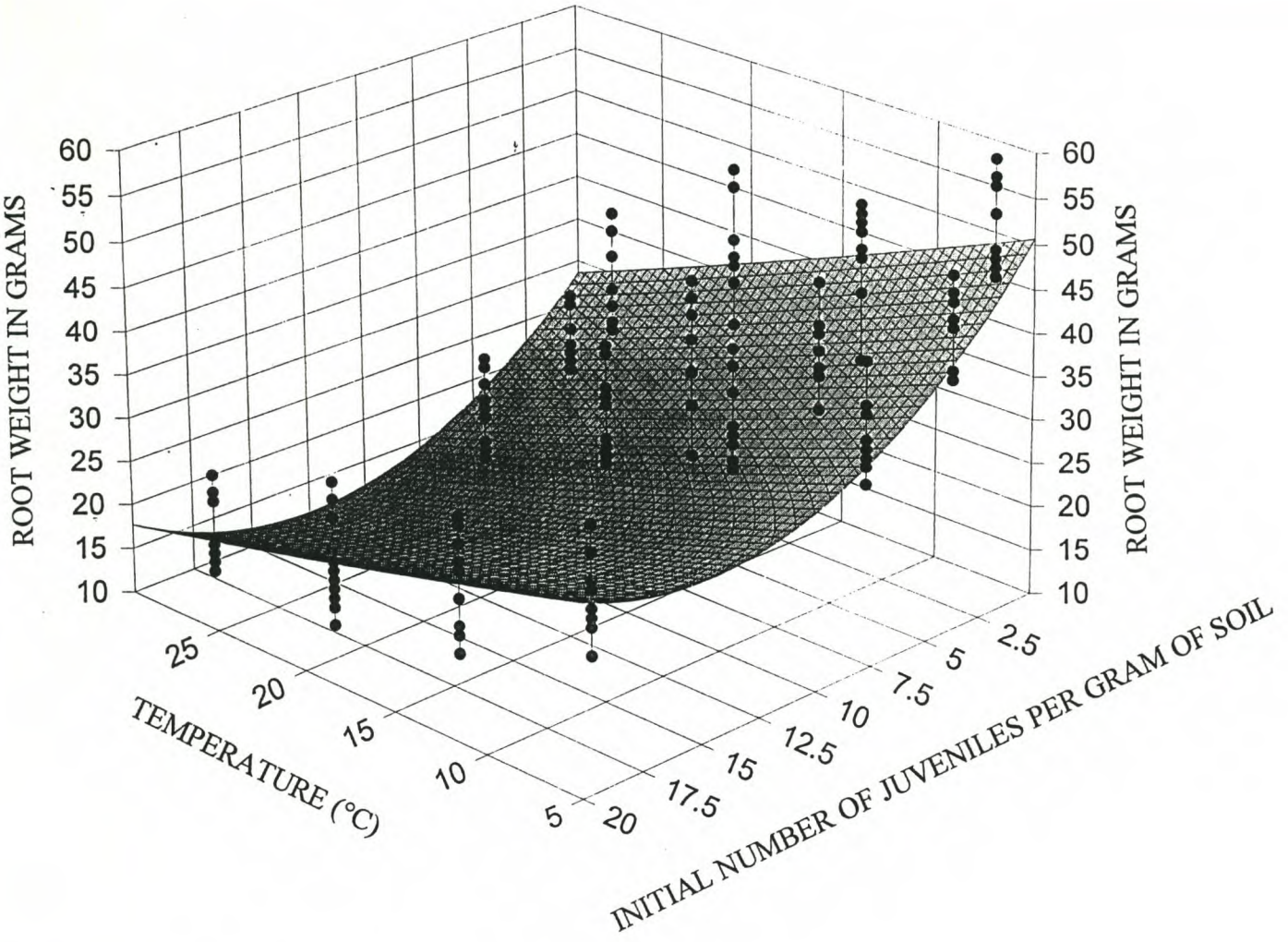

Fig. 6 The relationships between beetroot root weight, soil temperature and initial numbers of *Heterodera schachtii* juveniles per gram of soil

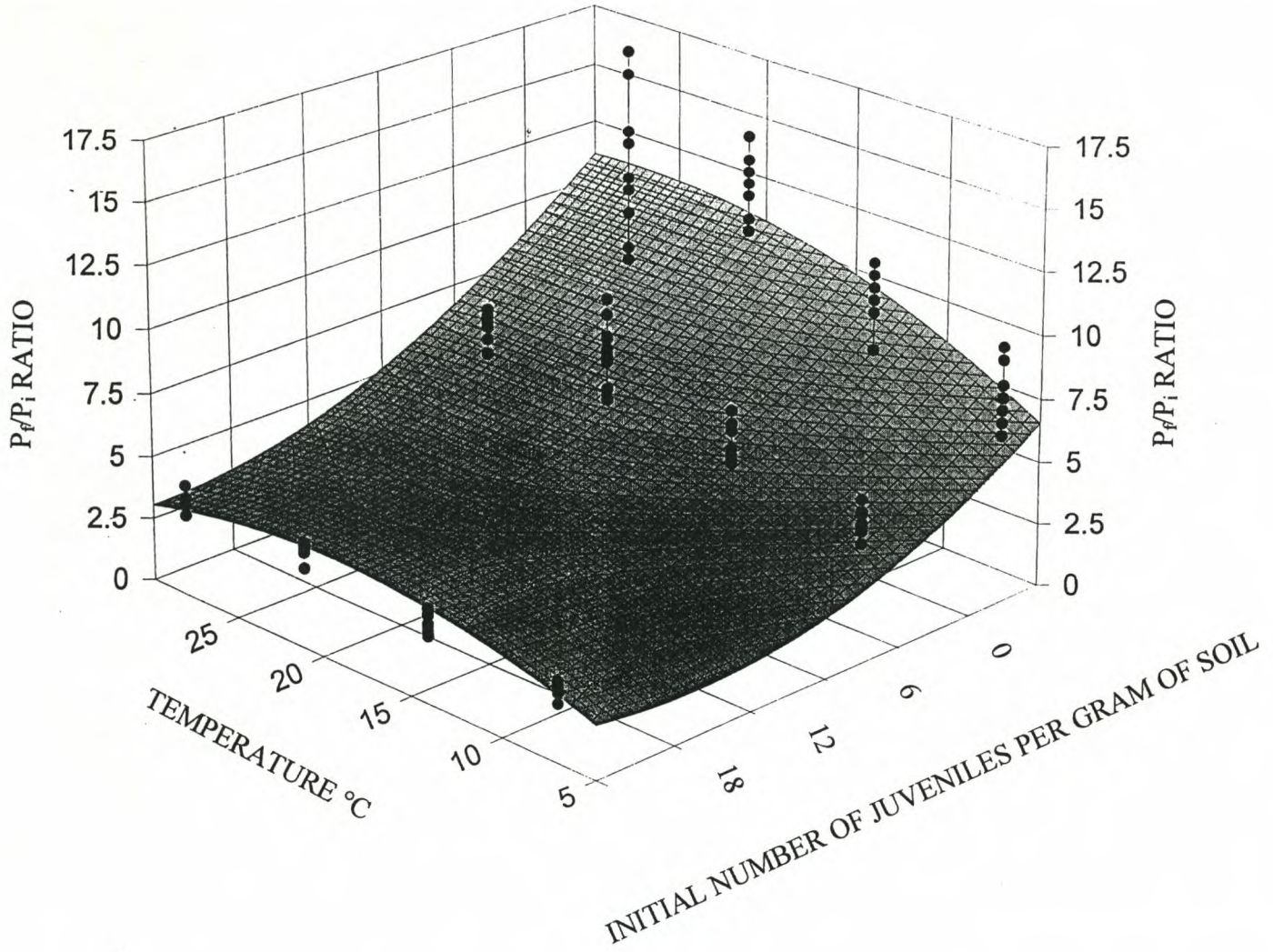

Fig. 7 The relationships between beetroot reproduction ratio (P_f/P_i), soil temperature and initial numbers of *Heterodera schachtii* juveniles per gram of soil

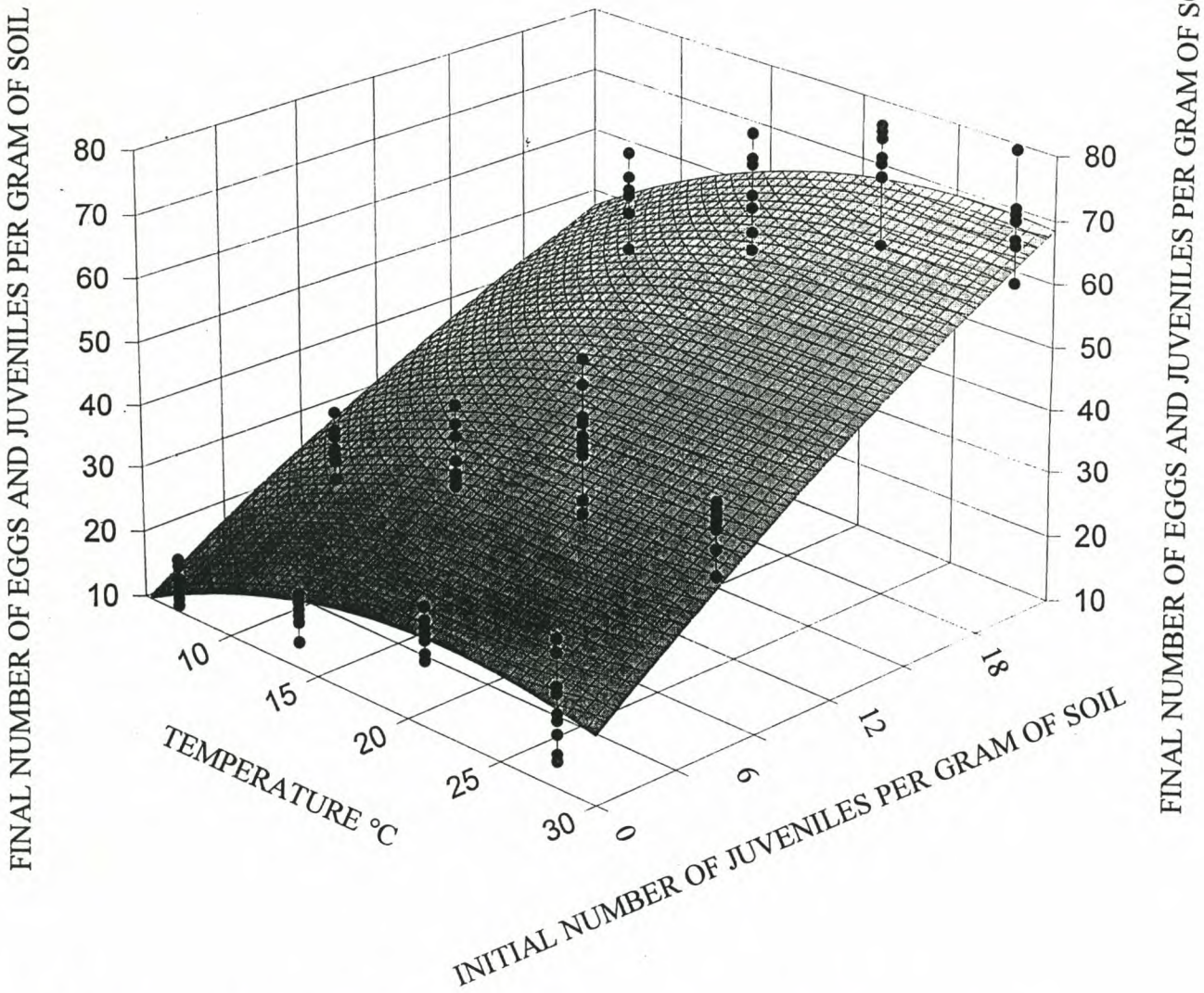

Fig. 8 The relationships between beetroot final number of eggs and juveniles per gram of soil, soil temperature and initial numbers of *Heterodera schachtii* juveniles per gram of soil

temperatures for *H. schachtii* development (Fig. 8). The linear trend was expected because at high initial juvenile densities there will be higher final numbers of eggs and juveniles per gram of soil if the host plant can survive up to the completion of one life cycle of the nematode. If the plant dies the final numbers of nematodes will be lower than the initial numbers.

Field trial (Micro-plots): At all initial densities the Vredendal trial had the lowest cabbage (P=0.05) yield compared to the other two localities (Table 4). At all locations an increase in inoculum density led to a decrease in yield with a reduction of 60.3% (relative to the control) at Vredendal with a P_i of 18.

Table 4. The yield of cabbage on three localities with four P_i levels of *Heterodera schachtii*

Locality	P_i (eggs & larvae per gram of soil)			
	0	2	6	18
George	3440 a	3011 a	2680 a	1986 a
Stellenbosch	3122 a	2580 b	2440 a	2154 a
Vredendal	2369 b	1778 c	1287 b	940 b
LSD 5%	326.4	287.7	245.8	249.8

Columns followed by the same letter do not differ significantly (P=0.05).

The population densities of *H. schachtii* increased with an increase in inoculum numbers (Table 5). The numbers of eggs and juveniles at the George location was significant lower (P=0.05) at all inoculum levels. The numbers of eggs and juveniles per gram of soil at Vredendal were higher than at the other two sites. This is supported by data on the lower yields recorded at this site. Vredendal had the highest maximum temperatures (Table 6) compared with the other two localities, favouring *H. schachtii* development.

Table 5. The final number of eggs and juveniles per gram of soil on cabbage at three P_i levels of *Heterodera schachtii* at three locations

Locality	P_i (eggs & juveniles per gram of soil)		
	2	6	18
George	15 b	29 b	52 c
Stellenbosch	20 a	35 a	63 b
Vredendal	23 a	41 a	72 a
LSD 5%	3.7	5.9	6.4

Values in columns followed by the same letter do not differ significantly (P=0.05).

Increasing soil temperatures led to an increase in juvenile penetration (Griffen 1981) and nematode metabolism (Raski & Johnson 1959, Thomason & Fife 1962, Santo & Bolander 1976). This resulted in reduced plant growth as observed in this study with regard to top and root growth of cabbage and beetroot. The number of eggs and juveniles per gram of soil

Table 6. Climatological data for the period September 2002 to December 2002.

Location	Month	Rainfall(mm)	Temperature Maximum °C	Temperature Minimum °C
George	September	106.6	20.4	9.9
	October	3.41	20.4	9.8
	November	42.5	22.2	10.5
	December	48.1	24.5	15.3
Stellenbosch	September	39.9	22.5	9.1
	October	30.7	23.1	9.1
	November	15.8	25.7	9.7
	December	14.3	29.7	16.1
Vredendal	September	24.5	26	8.9
	October	9.9	27.1	8.7
	November	4.5	29.5	9.2
	December	42.8	32.1	15.2

and the P_f/P_i ratio was less at 28°C than at 21°C. This was probably because nematode development was inhibited at 28°C due to stress induced by the high temperatures in the plants and reduction in plant growth due to the nematode infestations. Temperatures higher than 23°C induced stress in cabbage and in beetroot temperatures above 25°C

caused stress (Lorenz & Maynard 1980). Thomason & Fife (1962) found at temperatures above 32.5 °C no development of *H. schachtii*. This was an important aspect because the soil temperature in the sandy soils of the greater Cape Flats is above these levels for extended periods during the day in the summer, thus impeding development of *H. schachtii*. However, the soil temperatures were not high enough to kill the nematodes. Temperatures of 45°C in the top 5 cm of soil would be needed to kill *G. rostochiensis* and similar temperatures would be required to kill *H. schachtii* (LaMondia & Brodie 1984). There was a correlation between soil temperature and both top weight and root weight as well as between inoculation density and both top weight and root weight of sugar beet (Raski & Johnson 1959). Results of Griffen (1981) supported these findings. The results obtained in the temperature range of 7 to 28°C and with initial levels of 2 to 6 juveniles per gram of soil indicated the devastating effect of *H. schachtii* in the greater Cape Flats. This was due to the fact that the temperature range is not always most suitable to the vegetable hosts and that nematode densities of 2 to 6 per gram of soil exists at most of the infected sites.

The results of the micro-plot experiments indicated that the soil temperature during the season played an important role. High temperatures at Vredendal resulted in the lowest crop yields and highest numbers of nematode eggs and juveniles per gram of soil at the end of the growing season. When soil moisture was controlled by irrigation, the relationship between population densities and yields were closely related and the yield in relation to soil temperature more predictable. It thus seems possible to predict the host-parasite relationship, including yields that can be obtained with certain temperatures and nematode population densities. Soil temperatures of 19.5°C, 14.8°C and 10.6°C at planting at Vredendal, Stellenbosch and George respectively, could also have influenced

the yields and population densities. Griffen (1981) found that high soil temperatures at planting reduced the root weight of sugar beet. This probably caused the higher reduction in yield of cabbage at Vredendal in compared to the other locations.

Soil texture:

The relationship between percentage migration and penetration of juveniles of *H. schachtii* in five soil textures was linear: $y = 111.147 - 18.979x^{0.5}$ ($r^2 = 0.92$, $P = 0.05$) and it was evident that soil texture played an important role in the migration of *H. schachtii*. It declined with an increase in clay and silt percentage up to 34%, but increased with a higher sand particle composition of above 64% (Fig. 9).

High penetration and migration rates were observed in soils with a clay and silt content below 10%. No penetration was observed in soils with more than 34% clay and silt content. The differences in migration in soils with different textures occurred because nematodes generally have an optimum particle size for movement (Wallace 1958). This could partially explain the greater pathogenicity of *H. schachtii* in sandy soils. Rapid re-infestation by the upward migrating nematodes of *H. schachtii* was observed in sandy soils after poor fumigation (Prot & Van Gundy 1981). The fine particle size of clay and silt also appeared to be obstacles for the migration of *H. schachtii* in soils containing a clay and silt percentage higher than 23%. Wallace (1956) found that there was little evidence that juveniles were capable of constriction to enable them to pass through pore sizes less than their own diameter. The juveniles were thus confined to spaces between soil particles in the soil and the mobility of the juveniles related to the size of such spaces. Wallace (1958) found that maximum migration was attained when the channels

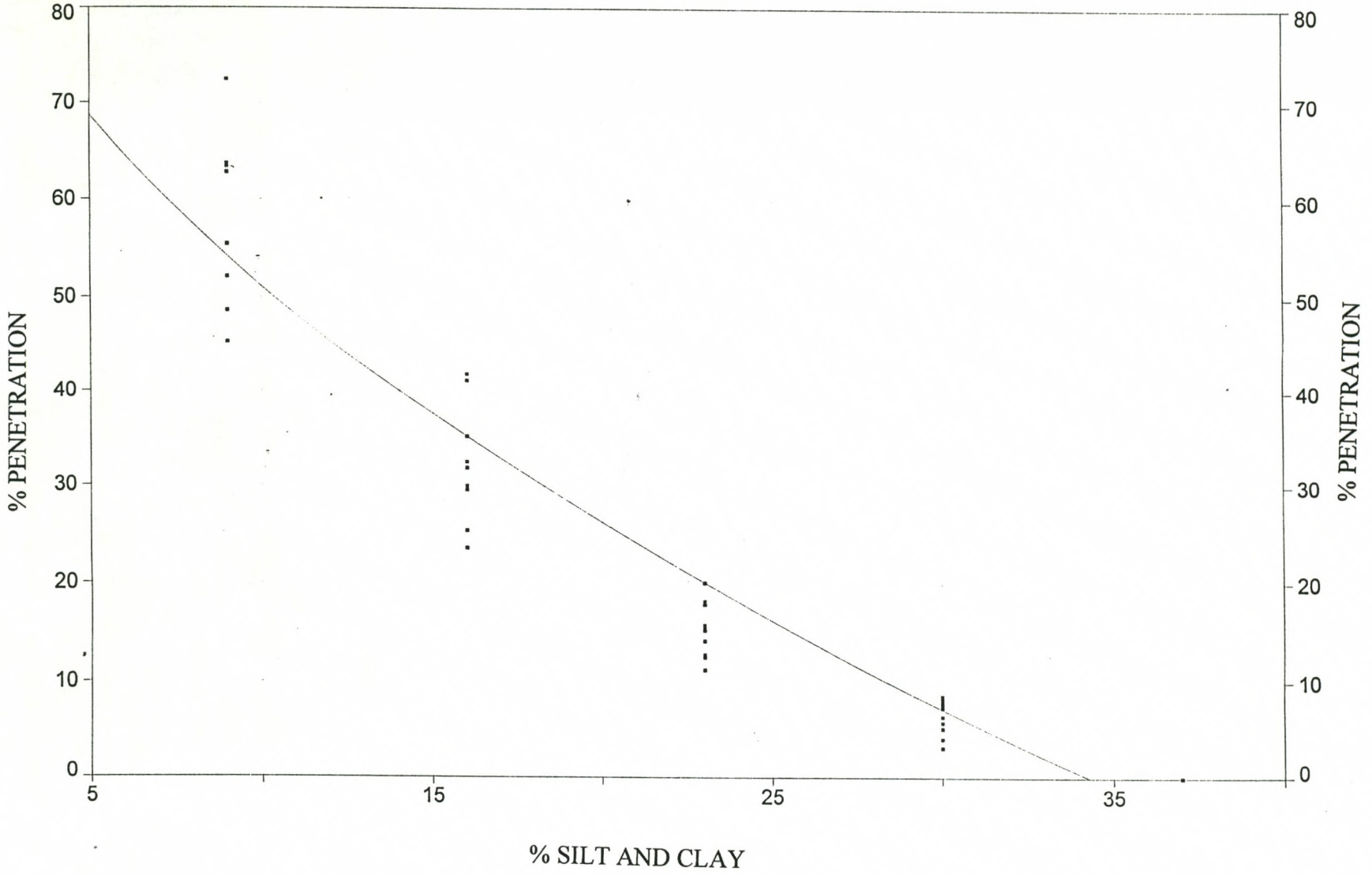

Fig. 9 The relationship between percentage penetration into roots and percentage clay and silt

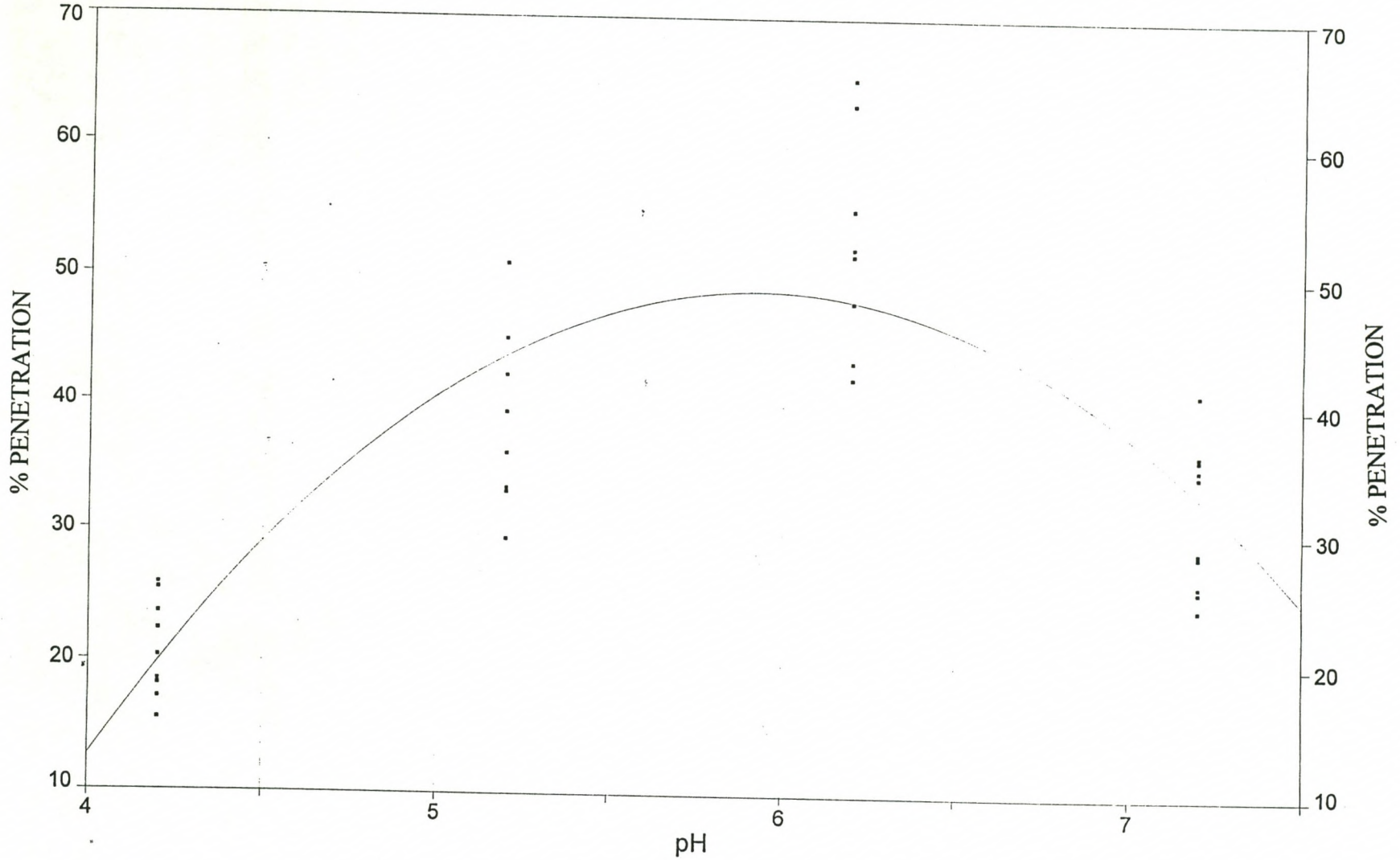

Fig. 10 The relationship between percentage penetration into roots and pH levels

were about the same diameter as that of the nematode. In soil particle densities less than 150 – 250 µm, the upward and downward mobility of nematodes was much lower than with larger sized particles. *H. schachtii* has been identified in a wide range of soil textures (Caveness 1958), but the most favourable texture is course textured soils (Wallace 1956, Whitney & Doney 1973). Wallace (1956) indicated that soil aeration was the most important factor associated with soil texture and that the emergence of juveniles from cysts was related to soil texture. Most of the soils in the greater Cape Flats have a texture with more than 84% sand (Van Niekerk 2001), leading to the high nematode migration percentages. These soils are thus conducive to the migration of *H. schachtii,* a fact to be considered in designing control strategies.

pH:

A pH of between 4.5 and 7.3 led to the penetration levels of above 30% (Fig. 10) and the relationship between pH and penetration levels was quadratic: $y = -294.23 + 115.76\ x - 9.76\ x^2$ ($r2 = 0.71$, $P = 0.05$). The relationship between yield per plant and pH had a quadratic trend with minimum levels at pH of 5.7 (Fig. 11) with the regression model: $y = 23187.85 - 7680.65\ x + 670.75\ x^2$ ($r^2 = 0.955$, $P = 0.05$). The relationship between final numbers of eggs and juveniles per gram of soil and pH was quadratic resulting in levels higher than 10 eggs and juveniles per gram of soil at pH levels of between 4.1 and 7.1 (Fig. 12). This relationship was described by the regression model: $y = -126.73 + 52.85\ x - 4.75x^2$ ($r^2 = 0.78$, $P = 0.05$). High numbers of eggs and juveniles per gram of soil at pH levels of between 4.1 and 7.1 compared positively with the results reported for *M. incognita* (Chitwood 1949) and *M. javanica* (Treub 1885) as found by Loewenberg *et al.* (1960) and Wallace (1966). They also found the highest numbers of nematodes at

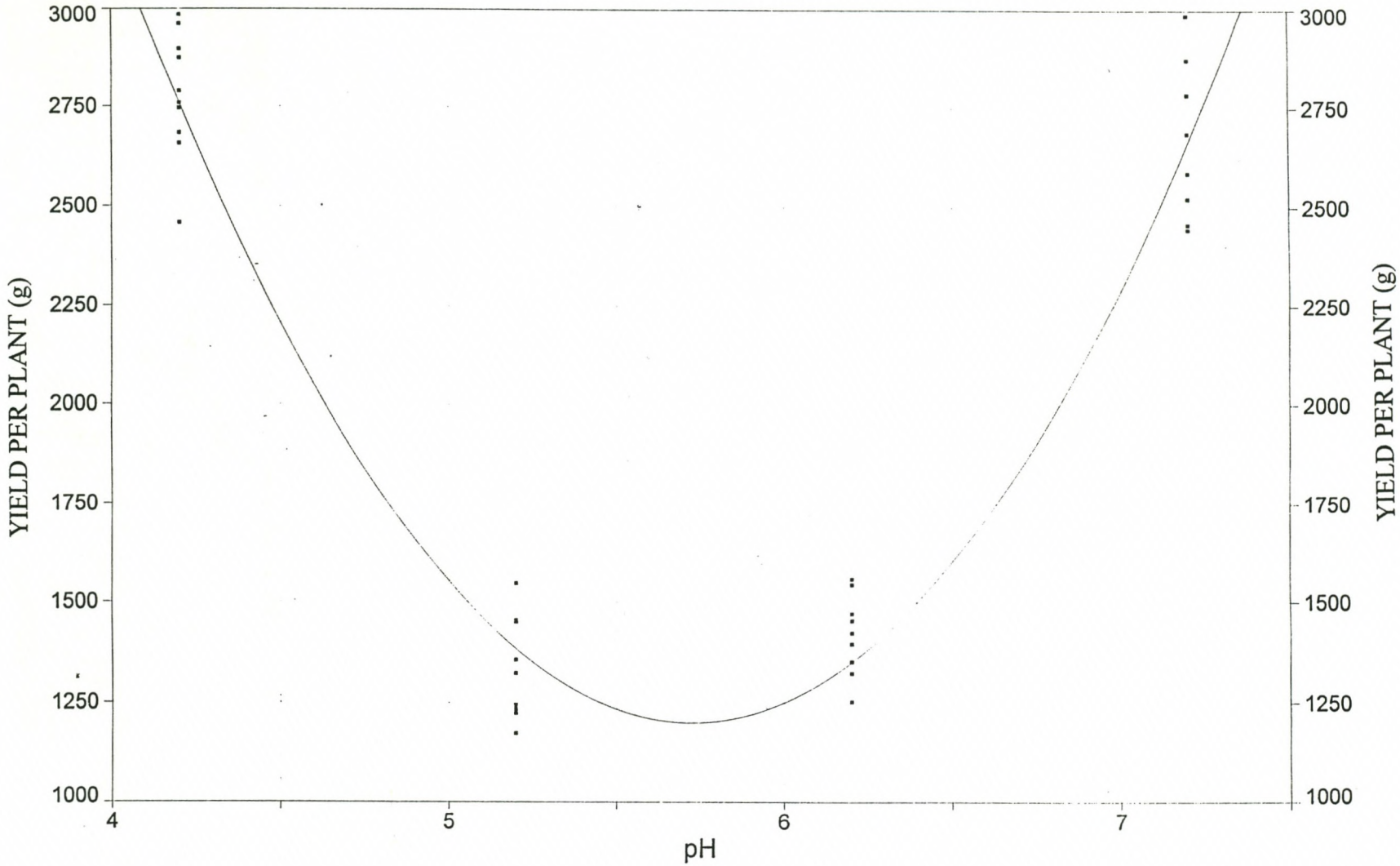

Fig. 11 The relationship between yield per plant and pH levels

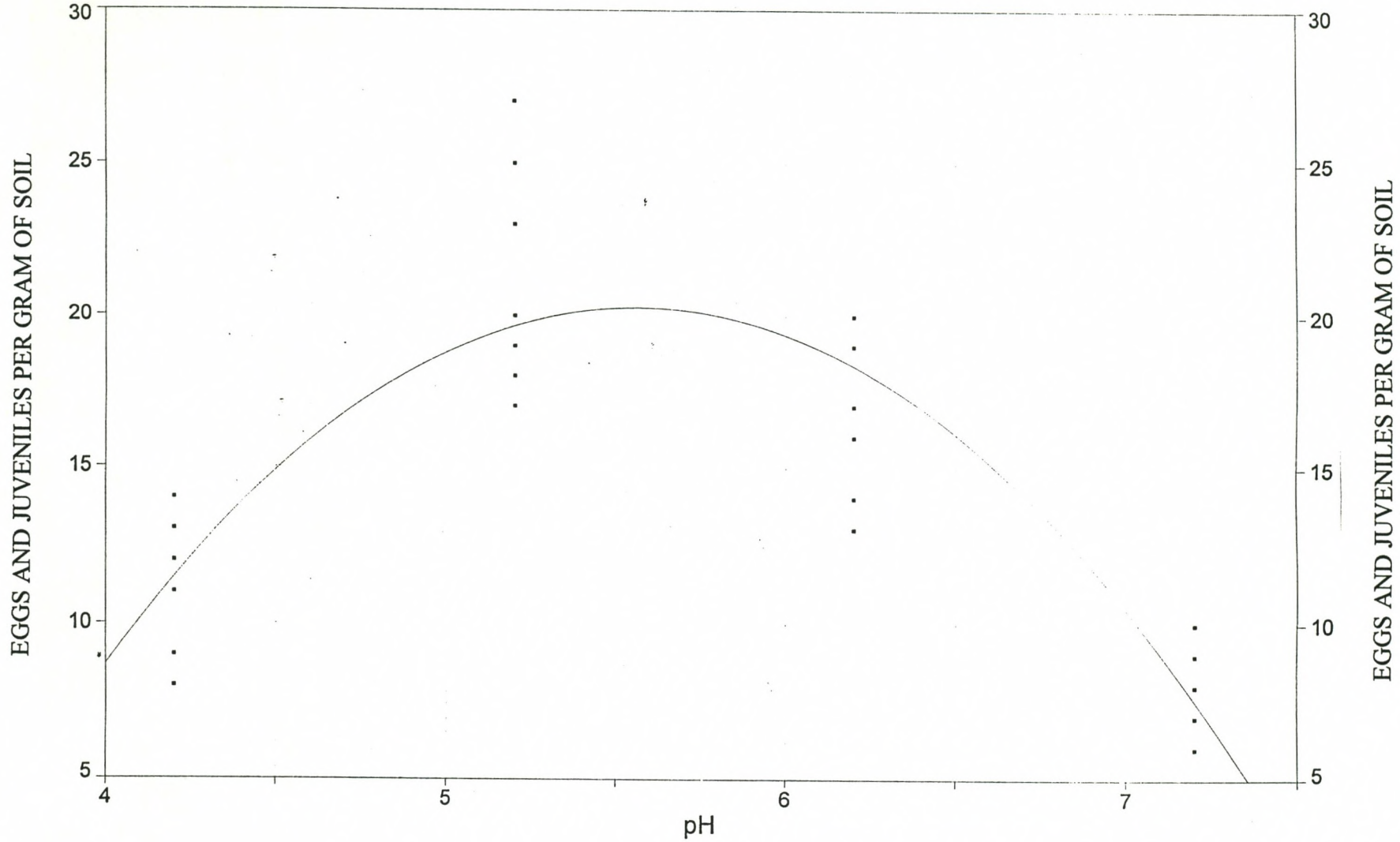

Fig. 12 The relationship between number of eggs and juveniles per gram of soil and pH levels

intermediate pH levels. Morgan & Mac Lean (1968) showed that pH levels varying from 5.2 to 6.4 resulted in higher numbers of *P. penetrans* (Cobb 1917) per gram of soil. This supported the findings on *H. schachtii* in this study. Similar results were found by Burns (1971) for *P. alleni* (Ferris 1961) in soybean roots. The influence of soil pH on vegetable production in relation to the influence on nematodes is an important factor when considering crop locations, especially considering the fact high penetration percentages and yield reductions with pH levels 5.2 and 6.2 were found. This value represents the pH levels of most of the sandy soils in the Cape Peninsula (Van Niekerk 2001). The pH range between 6 and 7.5 was also the optimum range for the production of most vegetables (Lorenz and Maynard 1980).

The results showed that the environmental conditions in the greater Cape Flats were conducive for penetration and multiplication of *H. schachtii.* It is therefore important that control strategies, such as crop rotation, for the management of *H. schachtii* on vegetables in the greater Cape Flats be implemented. This will prevent build-up of higher populations and inevitable economical losses to growers of vegetable crops.

References

Buol S W, Hole F D & McCracken R J 1980. *Soil Genesis and Classification.* Iowa State University Press, Ames, Iowa, USA.

Burns N C 1971. Soil pH effects on nematode populations associated with soybeans. *Journal of Nematology* 3: 238-245.

Byrd D W, Kirkpatrick T & Barker K R 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology* 15: 142-143.

Caveness F E 1958. The incidence of *Heterodera schachtii* soil population densities in various soil types. *Journal of the American Society for Sugar Beet Technology* 10: 177-180.

Cook D A & Thomason I J 1979. The relationship between population density of *Heterodera schachtii*, soil temperature and sugarbeet yields. *Journal of Nematology* 11: 124-128.

Duggan J J 1963. Relationship between intensity of cereal root eelworm (*Heterodera avenae* Wollenweber 1924) infestation and pH value of soil. *Irish Journal of Agricultural Research* 2: 105-110.

Griffen G D 1981. The relationship of plant age, soil temperature and population density of *Heterodera schachtii* on the growth of sugarbeet. *Journal of Nematology* 13: 184-190.

LaMondia J A and Brodie B B 1984. Control of *Globodera rostochiensis* by solar heat. *Plant Disease* 68: 474-476.

Loewenberg J R, Sullivan T & Schuster M L 1960. The effect of pH and minerals on the hatching and survival of *Meloidogyne incognita* larvae. *Phytopathology* 50: 215-217.

Lorenz O A & Maynard D N 1980. *Knott's Handbook for vegetable growers.* John Wiley & Sons, New York.

Morgan G T & Mac Lean A A 1968. Influence of soil pH on an introduced population of *Pratylenchus penetrans. Nematologica* 14: 311-312.

Olthof H A 1978. Influence of population densities of *Heterodera schachtii* on sugarbeets grown in microplots. *Journal of Nematology* 10: 255-258.

Ott R L 1998. *An introduction to statistical methods and data analysis.* Belmont, California. Duxbury Press.

Prot J C & Van Gundy S D 1981. Effect of soil moisture and the clay component on migration of *Meloidogyne incognita* second stage juveniles. *Journal of Nematology* 13: 213-217.

Raski D J & Johnson R T 1959. Temperature and the activity of the sugarbeet nematode as related to sugarbeet production. *Nematologica* 4: 136-141.

Rode H 1962. Untersuchungen über das Wandervermögen von Larven des Kartoffelnematoden (*Heterodera rostochiensis* Woll.) in Modellversuchen mit verschiedenen Bodenarten. *Nematologica* 7: 74-82.

Santo G S & Bolander W J 1976. Effect of soil temperature and soil type on the reproduction of *Heterodera schachtii* and *Meloidogyne hapla* on sugar beets. *Journal of Nematology* 8: 301-302.

SAS 1999. SAS/STAT User's guide, statistics, Version 8.1, Volume 2. SAS Institute, Inc. SAS Campus Drive, Cary, North Carolina.

Schmitt D 1969. Population patterns of some stylet-bearing nematodes in a native Iowa prairie. *Journal of Nematology* 1: 304.

Seinhorst J W 1965. The relation between nematode density and damage to plants. *Nematologica* 11: 137-154.

Shapiro S S & Wilk M B 1965. An analysis of variance test for normality. *Biometrika* 52: 591-611.

Table Curve 2D 2001. Automated curve fitting and equation discovery, Version 5.0 for Windows. User's manual. SYSTAT Software INC, Canal Boulevard, Richmond, California, 94804.

Table curve 3D 2001. Automated surface fitting and equation discovery, Version 3.0 for Windows. User's manual. SYSTAT Software INC, Canal Boulevard, Richmond, California, 94804.

Tarjan A C 1971. Migration of three pathogenic citrus nematodes through two Florida citrus soils. *Soil Crop Science Society of Florida Proceedings* 31: 253-255.

Thomason I J & Fife D 1962. The effect of temperature on development and survival of *Heterodera schachtii* Schm. *Nematologica* 7: 139-145.

Van Grundy S D & Martin J P 1961. Influence of *Tylenchulus semipenetrans* on the growth and chemical composition of sweet orange seedlings in soils of various exchangeable cation ratios. *Phytopathology* 51: 146-151.

Van Niekerk S P 2001. Soil survey of the Western Cape. Dept. of Agriculture Western Cape, Elsenburg. Government Press.

Van Zyl J & Meyer A J 2000. Distribution and host range of the sugar-beet nematode, *Heterodera schachtii* in the Western Cape Province, South Africa. *African Plant Protection* 6: 47-51.

Wallace H R 1956. Soil aeration and the emergence of larvae of cysts of the beet eelworm, *Heterodera schachtii* Schm. *Annals of applied Biology* 43: 477-484.

Wallace H R 1958. The influence of pore size and moisture content of the soil on the migration of larvae of the beet eelworm, *Heterodera schachtii* Schmidt. *Annals of applied Biology* 46: 74-85.

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

Whitney E D & Doney D L 1973. The effects of soil types, inoculum levels, fertilizers and water regimes on the development of *Heterodera schachtii* on lines of sugar beet. *Journal of the American Society for Sugar Beet Technology* 17: 309-314.

Chapter 7.

The effect of crop rotation, non-hosts and solarization on the control of *Heterodera schachtii* (Schmidt, 1871)

Introduction

The sugar beet nematode, *Heterodera schachtii*, has a wide host range which includes over 23 plant families (Steele 1965). Coetzee (1968) reported this nematode for the first time in the Cape Flats. In a subsequent survey conducted by Van Zyl and Meyer (2000), *H. schachtii* occurred widely in the vegetable production areas in the greater Cape Flats. The nematode caused significant damage to a range of vegetables (Radewalt *et al.* 1971, Olthof *et al.* 1974, Abawi & Mai 1980).

Crop rotation and the planting of non-hosts and catch crops are probably the most effective and economical means to control this nematode. Due to high production costs vegetable production in the greater Cape Flats is under economic threat. Researchers have investigated several host and non-host combinations to test their effectiveness in reducing the population densities of *H. schachtii* in the soil (Steele & Price 1965, Nusbaum & Ferris 1973). The rotations and combinations of hosts employed are not always practical under local conditions, due to of different market requirements and the availability of crops used in these rotations.

Solarization of the soil with plastic mulches could also be cost effective during the warm dry summers in the Western Cape. It is a hydrothermal method of soil disinfection using solar heat trapped beneath the mulch. This practice has been used effectively for the management of soilborne pests and diseases, including nematodes (Grinstein *et al.* 1979, Barbercheck & Von Broembsen 1986, Gaur & Perry 1991). The use of solarization as an alternative to steam or chemical sterilization was recommended by Giblin-Davies & Verkade (1988). The lethal time/temperature requirements for cyst nematodes in water has been determined by various researchers (Lewis & Mai 1960, Endo 1962, Thomason & Fife 1962, Miller 1969, Slack *et al.* 1972, Steele 1973, Stone & Webley 1975, Brodie 1976).

The objective of this study was to evaluate the efficacy of certain crop rotation systems and to compare the ability of locally available non-host crops to reduce *H. schachtii* populations. The effectiveness of soil solarization in reducing nematode populations under conditions prevailing in the Western Cape was also evaluated.

Materials and methods

Crop rotation: In April 1999 crop rotation trails were conducted using microplots of 12 x 12 m at Elsenburg, Stellenbosch, South Africa. The microplots consisted of cement slabs placed vertically up to a height of 1.2 m. A sandy loam soil with an initial *H. schachtii* population of 1.5 eggs and juveniles per gram soil were placed in each microplot. Five crop rotation cycles were evaluated over a period of four years. The crop rotation systems are summarized in Table 1.

Table 1. Succession of crops over a period of four years for five crop rotation cycles

H = Host **N = Non-host** **T = Trap crop**

Rotation	Crops
N-H	carrot-beetroot-lettuce-cabbage-onion-cauliflower-carrot-beetroot
N-N-H	carrot-lettuce-beetroot-onion-bean-cabbage-carrot-lettuce
N-N-N-H	carrot-lettuce-onion-cabbage-bean-carrot-lettuce-beetroot
N-T-H	carrot-radish-beetroot-lettuce-radish-cabbage-onion-radish
N-N-T-H	carrot-lettuce-radish-cabbage-onion-bean-radish-beetroot

The hosts used were beetroot (*Beta vulgaris* var. *conditiva* L. Red Ace), cabbage (*Brassica oleracea* L. var *capita*, Green Coronet) and cauliflower (*Brassica oleracea* var. *botrytis* L. Hunter). The non-hosts were beans (*Phaseolus vulgaris*, Newton), carrot (*Daucus carota*, Fancy), lettuce (*Lactuca sativa*, Victory) and onion (*Allium cepa*, Hojem). Radish (*Raphanus sativus* L. Pegletta) was used as trap crop. The rotation cycles were assigned at random to the 50 microplots with 10 replicates per rotation cycle. The first crops were planted in October 1998 and the final data collected in December 2002.

After the harvest of each crop soil samples were taken and nematodes extracted as described by Van Zyl & Meyer (2000). A total of 50 sub-samples of 100 grams were taken from each plot. Sub-samples of soil from each plot were bulked and air-dried. The number of cysts and the number of eggs and juveniles per cyst were determined by crushing 100 randomly selected cysts per sample. The data was used to determine the number of nematode eggs and juveniles per gram soil and to determine their population changes caused by the various crop rotation cycles.

Non-hosts: The effect of non-hosts on the population dynamics of *H. schachtii* was determined during July 2002 in two greenhouse studies at Elsenburg. The soil was steam-sterilized and inoculated to yield 20 juveniles per gram of soil in six liter plastic containers. The temperature in the greenhouse was maintained at 25°C ± 2°C and daily fertigation provided the plants with a balanced nutrient solution. Non-host crops used were wheat (*Triticum vulgare*, SST 75), oats (*Avena sativa*, Sederberg), marigold (*Tagetes erecta*) and sweetcorn (*Zea mays* var. *saccharata*, Renown), radish (*Raphanus sativus* L. Pegletta) and the host crop was cabbage (*Brassica oleracea* L. var *capita*, Green Coronet). Each treatment had 10 completely randomized replicates. From each container ten soil samples were collected after 10, 60, 90 and 120 days after planting. The 10 sub-samples were bulked and the number of larvae per gram soil was determined as before. After a period of 120 days the plants were removed from the containers and the soil of each pot transferred into two smaller pots. Two-week old cabbage seedlings were planted in each smaller pot to determine the effect of the previous crop on the population numbers of *H. schachtii*. After 14 days the degree of penetration into cabbage roots was determined by removing the entire plant and staining the root system with acid fuchsin-lactophenol (Byrd *et al.* 1983) to detect the juveniles. The remainder of the cabbage plants was grown for 60 days after which period the root and top weights as well as the numbers of juveniles per gram root were determined.

Solarization: Field trials were conducted at Elsenburg, Stellenbosch, to investigate the effect of clear polyethylene, black polyethylene and no polyethylene in microplots during

the summer months of December 2001 to February 2002 on the viability of juveniles in a sandy-loam soil. The plots were set out in an area infested with *H. schachtii.* The plots were 2 x 8m with a fallow buffer zone of 3 m surrounded each plot. A complete randomized design with ten replicates per treatment was used. The initial numbers of viable juveniles per cyst were determined by taking 50 cm^3 of soil to a depth of 15 cm for each square meter of a plot. The samples from each plot were bulked and 25 cysts were selected at random. The viability of juveniles in the cysts for a period of eight weeks was determined by hatching the cysts in a $ZnCl_2$ solution (Griffen, 1982). A fresh solution was provided daily after determining the numbers of hatched juveniles. Bags containing 100 g of soil and cysts of equal size were placed in permeable nylon bags and buried at depths of 5, 10 and 15 cm in the center of each plot. The soil under polyethylene as well as that of the control was kept at field capacity by drip irrigation. The plastic covers were removed after 10 weeks and the viability of the juveniles in the cysts per plot and in the buried nylon bags were determined as before. Soil temperatures were recorded at depths of 5, 10 and 15 cm in one replication using copper-constantan thermocouples with a Campbell Scientific CR 5 data-logging device.

An analysis of variance was performed using SAS version 8.1 (SAS 1999). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk 1965). Student's t-least significant difference was calculated at the 5% confidence level to compare treatment means (Ott 1998).

Results and discussion

Crop rotation: The final population densities of *H. schachtii* varied in response to the crop rotation cycle. Variable results were found regarding the effectiveness of a crop rotation to reduce or maintain the *H. schachtii* at levels less than two eggs and juveniles per gram soil, the threshold suggested by Daiber (1992). The rotation cycles that included a non-host and a trap crop before the host (N-T-H) and the cycle that incorporated two non-hosts and a trap crop before the host (N-N-T-H) were the only rotations in which nematode numbers were lower than two eggs and juveniles per gram soil at the end of the trail period (Fig. 1). The P_f/P_i ratio of the N-T-H and the N-N-T-H were significantly (P=0.05) lower from the other rotation cycles with 0.33 and 0.07 respectively (Fig. 2). Rotation cycles of a host following by a non-host led to P_f/P_i levels of 8.4 and were ineffective. However, this is the host-non-host cycle that most producers are willing to adopt. Severe damage was noted on sugar beet when grown as a monoculture or in short rotations with non-hosts (Mai & Abawi 1980).

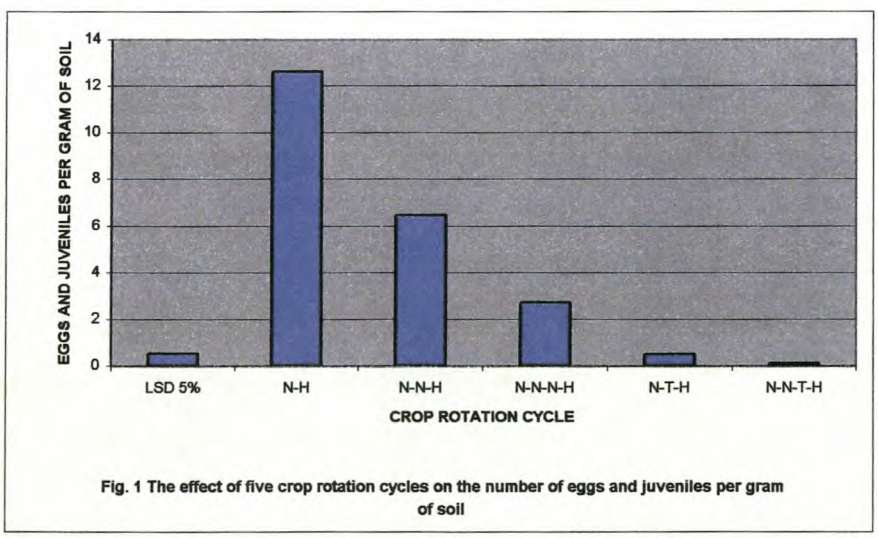

Fig. 1 The effect of five crop rotation cycles on the number of eggs and juveniles per gram of soil

Crop rotation is considered an important and effective control measure for plant parasitic nematodes and soilborne pests (Abawi & Mai 1980). Crop rotations also influence the

physical, biological and chemical properties of the soil and may have many beneficial effects. It also plays a major part in integrated control programs (Abawi & Mai 1980).

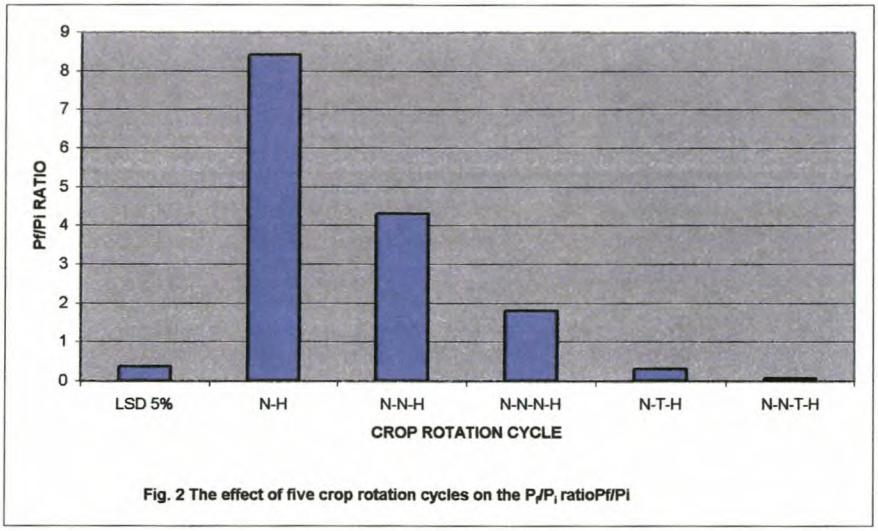

Fig. 2 The effect of five crop rotation cycles on the P_f/P_i ratioPf/Pi

Due to the high cost of fumigation, this practice is not always possible under South African conditions and crop rotation seems to be a more practical and feasible option. This was also shown by the results above. The population numbers of *H. schachtii* in the vegetable fields in the greater Cape Flats are currently on the increase and it is therefore important to include a catch crop such as radish to lower the population densities of *H. schachtii* . A crop rotation cycle of one susceptible host in four successive plantings should be encouraged, as such a rotational sequence will keep the nematode numbers at acceptable levels. It is also important to bear in mind that crop rotations are more effective in preventing nematode population increases to reach damaging levels, than what it is in reducing high population densities (Jones & Petherbridge 1947). A crop rotation cycle should be initiated not only in fields known to be infested with the nematode, but also in fields with no detectable infestation. At the current rate of spread, it is likely that infestation by this nematode in fields presently free from *H. schachtii* will take place in the future. The level of infestation will determine the duration of a crop

rotation cycle required for effective control. Jones & Petherbridge (1947) found that 10 years of non-host crops were needed in heavily infested fields before sugar beet could again be grown successfully. Lightly infested fields required only a two to three year rotation with non-hosts. The control of host weeds is considered essential for the effectiveness of a rotation cycle as a control measure.

Non-hosts: A reduction of the number of juveniles per gram of soil after 10 days was noted in all crops (P=0.05) with the highest reduction recorded on cabbage (Fig. 3), followed by marigold and radish with no significant differences between them.

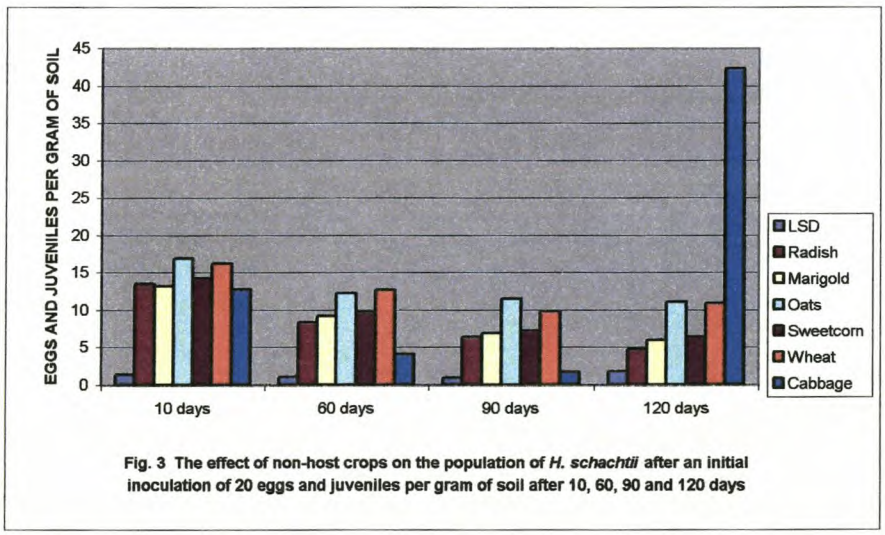

Fig. 3 The effect of non-host crops on the population of *H. schachtii* after an initial inoculation of 20 eggs and juveniles per gram of soil after 10, 60, 90 and 120 days

Cabbage caused a sharp increase in numbers of eggs and juvenile in the period ranging from 90 to 120 days with 42.3 juveniles per gram of soil at the end of this period. This high population density on cabbage was due to the increase in reproduction of the nematode on the host plant. After 120 days the reproductive index was calculated (Fig. 4). Radish produced the lowest (P=0.05) P_f/P_i ratio of 0.24, followed by marigold and

sweetcorn. The implication to producers is that marigold and sweetcorn can be planted in a rotation with good nett returns. Sweetcorn is always in high demand and marigold is currently utilized as an industrial crop as a colourant and therefore to be promoted for use in crop rotations.

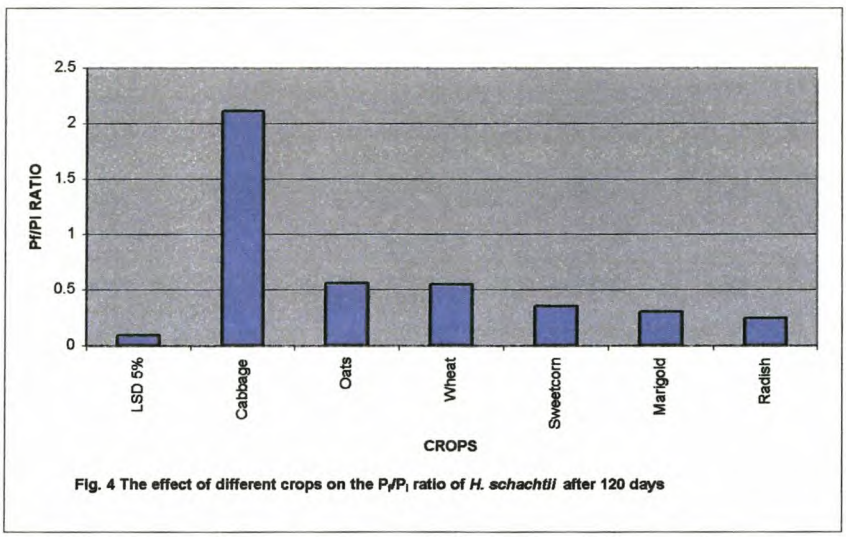

Fig. 4 The effect of different crops on the P_f/P_i ratio of *H. schachtii* after 120 days

The penetration of cabbage by *H. schachtii* in soil previously planted to cabbage had the highest (P=0.05) rate of 25.7 juveniles per seedling (Table 2). The biggest reduction in cabbage top and root weight was achieved when cabbage was grown in soils previously grown to cabbage. This illustrates the importance of a crop rotation system and the effect of non-host crops to control *H. schachtii* numbers. The highest number of females per gram of root was also found in soil previously grown to cabbage. The highest (P=0.05) cabbage production, tops and roots, was achieved in soils previously planted to radish, marigold and sweetcorn. This resembled the P_f/P_i ratios found in cabbage and therefore these crops must be promoted in rotation systems of crop production. Radish triggers the hatching of nematode eggs via their chemical root exudates. The penetrating juveniles can, however, not develop into reproductive females and thus the population densities of the nematode are reduced.

Soil type, aeration and pH have an effect on the hatching and spreading of nematodes and should be considered when a crop rotation cycle is designed (Wallace 1956, Shepherd 1959, Shepherd 1962).

Table 2. The effect of previous crops on the juveniles per seedling, females per gram of root and root and top weight of cabbage grown subsequently

Previous crop	juveniles/ seedling	females/g root	Cabbage weight g	
			Roots	Tops
Radish	8.9	3.7	126	3197
Marigold	9.3	3.2	146	3131
Oats	19.2	8.9	70	2094
Sweetcorn	12.8	5.2	116	2653
Wheat	13	6.7	90	2614
Cabbage	25.7	10.2	63	1375
LSD 5%	1.6	1.3	17.4	252.4

Influential factors such as host-effect differences in nematode populations, length of growth season of crop and geographical positions affect the effectiveness of a crop rotation cycle (Griffen 1977). The results obtained in this study showed that a crop rotation cycle that included a trap crop, like radish, is an effective management strategy under South African conditions. The rotation cycle of two non-host crops followed by a trap crop and then a host was found to be the optimal, because low levels of infestation were found after this rotational cycle and should be recommended to growers, especially using sweetcorn and marigold. Muller (1986) stated that effective control was achieved by growing a host plant once in five years. This is not economically viable under the conditions with the relatively small vegetable farms and the limited choice of vegetables to produce economically in the Western Cape. Growers usually resist crop rotation

because the alternative crops are not as profitable as their monoculture crops. The rotations investigated here proved, however, that a rotation system incorporating profitable crops led to a major decrease in nematode numbers. This findings should change the mind set of growers to implement crop rotation as indicated here. Intercrops such as mustard could also be incorporated in such a control strategy. To enhance root penetration when soil temperatures are high the trap crop is best planted as soon as possible after harvesting the summer crop.

The results indicate that *H. schachtii* population dynamics are affected differently by different non-host crops and suggest that yield losses can be reduced with effective crop rotations. This should be initiated not only in fields known to be infested with *H. schachtii*, but also in fields with no detectable infestation. The combination of a nematicide and crop rotation may shorten the required rotation time, but further investigation is needed on this aspect.

Solarization: Soil solarization utilizing transparent, clear polyethylene was the most effective method to reduce the infective population of *H. schachtii*, compared to black and no polyethylene (Table 3). The clear polyethylene caused in a reduction of 97.6% in nematode numbers over 10 weeks. No nematodes hatched under the clear polyethylene at a depth of 5 cm, indicating that the temperature and duration was sufficient to eliminate all the infective juveniles (Table 3). At 10 and 15 cm depth, the clear polyethylene had significantly less infective juveniles in the soil compared to black or no polyethylene treatments.

Table 3. Hatch and percentage reduction of encysted juveniles of *Heterodera schachtii* subjected to ten weeks of solarization.

Treatment	No. of juveniles emerged per cyst.			Percent reduction
	Depth			
	5 cm	10 cm	15 cm	
Clear	0 a	59.1 a	111.1 a	97.6 a
Black	157.6 b	191.3 b	182.5 b	65.2 b
No cover	242.3 c	193.9 b	196.4 c	21.7 c
LSD	16	11.3	12.8	4.7

The soil temperatures (Table 4) under polyethylene indicated that clear polyethylene was the most effective in raising the soil temperature to the levels where it caused the nematodes to lose its infective potential. Evans & Perry (1976) found similar results. It was found by LaMondia & Brodie (1984) that temperatures must reach or approach 45°C to effectively reduce the survival of *Globodera rostochiensis.* From this study it appears that *H. schachtii* needs similar temperatures for control. The reduced hatch may be due to high temperatures alone, but factors such as the effect of long-term exposure to sub-lethal temperatures and the effect of fluctuating temperatures may also play a part.

Table 4. Average maximum soil temperatures (°C) during solarization field trails.

Depth/cm	Clear/°C	Black/°C	No plastic/°C
5	45.5	39.5	37.5
10	40.5	35.4	32.4
15	37.2	32.5	27.6

The reduction in numbers of *H. schachtii* in the deeper soil layers may have been due to the sub-lethal heating of the eggs and larvae in the soil, resulting in reduced pathogenic

potential, lower egg hatching and possibly induced bio-control (Katan 1981, Stapleton & De Vay 1983 and Stapleton & De Vay 1986). Soil solarization reduced the pre-plant population densities in the soil and thus ensured less crop damage. It was, however, not completely effective. A combination of solarization and other practices such as crop rotation and trap cropping may result in low or lowering population densities of *H. schachtii* acceptable for vegetable production in the Western Cape. Due to the hot summers solarization may be of great use to control *H. schachtii* in the Western Cape, but further research is needed to determine the optimum conditions for solarization.

References

Abawi G S & Mai W F 1980. Effect of initial densities of *Heterodera schachtii* on yield of cabbage and table beets under field conditions in New York State. *Phytopathology* 70: 481-485.

Barbercheck M E & Von Broembsen S L 1986. Effects of soil solarization on plant-parasitic nematodes and *Phytophthora cinnamomi* in South Africa. *Plant Disease* 70: 945-950.

Byrd D W Jr., Kirkpatric T & Barker K R 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology* **15:** 142-143.

Brodie B B 1976. Effects of birds ingesting *Heterodera rostochiensis* cysts on viability of eggs and larvae. *Journal of Nematology* 8: 318-322.

Coetzee V 1968. The distribution of the family *Heteroderidae* (Filipjev, 1934) in South Africa and some host records of *Meloidogyne* species. *South African Journal of Agricultural Science* 11: 775-778.

Daiber K C 1992. The decline of *Heterodera schachtii* populations under conditions of vegetable production in South Africa. *Phytophylactica* 24: 321-324.

Endo B Y 1962. Lethal time-temperature relationships for *Heterodera glycines. Phytopathology* 52: 992-997.

Evans A A F & Perry R N 1976. Survival strategies in nematodes. In: *The Organization of Nematodes*, 383-424 (ed Croll N A). London, UK: Academic Press.

Gaur H S & Perry R N 1991. The use of soil solarization for the control of plant parasitic nematodes. *Nematological Abstracts* 60: 153-167.

Griffen G D 1977. Pathological differences in *Heterodera schachtii* populations on sugar beet (Abs.). *Proceedings of the American Phytopathological Society* 4: 206.

Griffen G D 1982. Differences in response to certain weed host populatons to *Heterodera schachtii. Journal of Nematology* 14: 174-182.

Giblin-Davies R M & Verkade S D 1988. Solarization for nematode disinfestation of small volumes of soil. *Annals of Applied Nematology* 2: 41-45.

Grinstein A, Orion D, Greenburger A & Katan J 1979. Solar heating of the soil for the control of *Verticillium dahliae* and *Pratylenchus thornei* in potatoes, in *Soil-borne plant pathogens*, pp 431-438 (Eds.B Chippers & W Gams). London, UK: Academic Press.

Jones F G W & Petherbridge F R 1947. Beet eelworm. *Sugar Beet Review* 16: 31-36.

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

LaMondia J A & Brodie B B (1984). Control of *Globodera rostochiensis* by solar heat. *Plant Disease 68: 474-476.*

Lewis F J & Mai W F 1960. Survival of encysted and free larvae of the golden nematode in relation to temperature and relative humidity. *Proceedings of the Helminthological Society Washington* 27: 80-85.

Mai W F & Abawi G S 1980. Influence of crop rotation on spread and density of *Heterodera schachtii* on a commercial vegetable farm in New York. *Plant Disease* 64: 302-305.

Miller P M 1969. Injury to *Heterodera tabacum* by high soil temperature. *Plant Disease Reporter* 53: 191-193.

Muller J 1986. Integrated control of the sugar beet cyst nematode, In: *Cyst Nematodes* (Eds. F Lamberti & C E Taylor), Plenum Press, New York, pp 179-196.

Nusbaum C J & Ferris H 1973. The role of cropping systems in nematode population management. *Annuals Reviews of Phytophythology* 11: 423-439.

Olthof T H A, Potter J W & Peterson 1974. Relationship between population densities of *Heterodera schachtii* and losses in vegetable crops in Ontario. *Phytopathology* 64: 549-554.

Ott R L 1998. *An introduction to statistical methods and data analysis.* Belmont, California. Duxbury Press.

Radewald D J, Hall F S & Nelson J 1971. Results of a preplant fumigation trail for the control of sugar beet nematode on cabbage. *Plant Disease Reporter* 55: 841-854.

SAS 1999. SAS/STAT User's guide, statistics, Version 8.1, Volume 2. SAS Institute, Inc. SAS Campus Drive, Cary, North Carolina.

Shapiro S S & Wilk M B 1965. An analysis of variance test for normality. *Biometrika* 52: 591-611.

Shepherd A M 1959. The invasion and development of some species of *Heterodera* in plants of different host status. *Nematologica* 4: 253-267.

Shepherd A M 1962. The emergence of larvae from cysts of the genus *Heterodera. Technical Communication No.32 Commonwealth Bur. Helminthology.*

Slack D A, Riggs R D & Hamblen J L 1972. Effect of temperature and moisture on the survival of *Heterodera glycines* in the absence of a host. *Journal of Nematology* 4: 263-266.

Stapleton J J & De Vay J E 1983. Response of phytoparasitic and free-living nematodes to soil solarization and 1,3-dichloropropene in California. *Phytopathology* 73: 1429-1436.

Stapleton J J & De Vay J E 1986. Soil solarization: A non-chemical approach for management of plant pathogens and pests. *Crop Protection* 5: 190-198.

Steele A E 1965. The host range of the sugar beet nematode *Heterodera schachtii* Schmidt. *Journal of the American Society of Sugar Beet Technologists* 13: 573-603.

Steele A E & Price C 1965. The effect of five legume crops on soil populations of the sugar beet nematode (*Heterodera schachtii* Schmidt). *Journal of the American Society of Sugar Beet Technology* 13: 314-317.

Steele A E 1973. The effects of hot water treatments on survival of *Heterodera schachtii*. *Journal of Nematology* 5: 81-84.

Stone L E W & Webley D P 1975. The effect of heat on the hatch of potato cyst eelworms. *Plant Pathology* 24: 74-76.

Thomason I J & Fife D 1962. The effect of temperature on development and survival of *Heterodera schachtii*. *Nematologica* 7: 139-145.

Van Zyl J & Meyer A J 2000. Distribution and host range of the sugar beet nematode, *Heterodera schachtii*, in the Western Cape Province, South Africa. *African Plant Protection* 6(2): 47-51.

Wallace H R 1956. Soil aeration and the emergence of larvae from cysts of the beet eelworm, *Heterodera schachtii*. *Applied Biology* 44: 57-66.

Chapter 8.

Summary and conclusions

A survey in the greater Cape Flats revealed that *Heterodera schachtii* is widely distributed in the area. It occurred in numbers in excess of two eggs and juveniles per gram of soil. This is accepted worldwide as the economic threshold level on beetroot, Brussels sprouts, cabbage and cauliflower. Should the present population levels prevail, it will probably lead to the demise of vegetable production in the region with resultant loss in food security for many small-scale farmers.

Investigations on the damage potential of *H. schachtii* on beetroot, broccoli, Brussels sprouts, cabbage, cauliflower and turnip revealed that all vegetables were negatively influenced at nematode densities as low as one egg and juvenile per gram of soil. The most common weeds on the greater Cape Flats were all suitable hosts and could serve as a ready source of infection to a subsequent host crop. Such weeds should not be tolerated in vegetable fields and the producers should be educated on the importance of weed control when cultivating crops.

The duration of the life cycle, in terms of penetration and development of *H. schachtii,* was compared on vegetables and weeds. On black nightshade, the nematode completed its life cycle in the shortest time. The Philippi and Lynedoch populations were distinct from the populations from other localities in regard to penetration of crops and reproduction potential, but these differences could not be detected by PCR-RFLP.

However, the virulence of the *H. schachtii* populations from Lynedoch and Philippi must be considered when crop rotation systems are developed and also when breeding for resistance in these vegetables.

The behaviour of local populations of *H. schachtii* under different soil temperatures, soil textures and pH levels revealed that the nematode had a high reproduction ratio at soil temperatures between 10°C and 26°C, penetrated well in sandy soils with a sand composition of above 90% and soil pH levels between 4.5 and 7.4. The varying conditions investigated as to their effect on *H. schachtii* resembled those prevailing in the greater Cape Flats and demonstrated that this area presents favourable conditions for the development of *H. schachtii.*

A successful control strategy should be built around a four-year rotation cycle that includes a non-host crop and trap crops before a host is planted as such practices led to the production of less than two eggs and juveniles per gram of soil, a level below the economic threshold for crop production. The available trap crops have no commercial value and alternatives should be investigated.

Solarization of soil for 10 weeks under clear polyethylene during summer proved successful in lowering the population densities of *H. schachtii* by up to 97%. This physical control method should be promoted and further investigated and refined as it is cheap, safe and has enormous utility in a region with a warm climate, such as the Western Cape.

The problem of *H. schachtii* in the greater Cape Flats is serious due to the high densities encountered, for example, in Kayelitsha where levels of up to 61 eggs and juveniles per gram of soil were encountered. *H. schachtii* has the potential to seriously disrupt vegetable production in the greater Cape Flats within eight to ten years unless effective control measures are implemented. The producers should be educated in regard to the threat posed by *H. schachtii* and its means of control. This will prevent a situation where a nursery was found to supply infected seedlings to small-scale vegetable producers in Kayelitsha.

Further research is also needed to determine threshold levels for the different vegetable hosts and to evaluate other biological control measures, including the use of parasitic fungi. Development of resistant cultivars should also be undertaken to ensure continued production of vegetables in the greater Cape Flats. The introduction of any new crop is under threat if it is susceptible to *H. schachtii*. Sugar beet has been considered for introduction in this area, but with the present population levels of the nematode in the soil, it is not feasible.

H. schachtii has proved to have the status of a agricultural pest serious enough to warrant quarantine measures to limit the distribution and spread of this nematode. In addition, an urgent campaign should be lodged amongst growers to inform them of the potential danger posed by the nematode, and of the results as presented in this dissertation.

Acnowledgements

Prof. A. J. Meyer for his support and constructive criticism and for supervising this project as promotor.

Prof. H. Geertsema for his critical reading of this manuscript.

Dr. A. P. Malan for her support and criticisms.

Dr. K. L. Pringle and M. Booyse for valuable discussions and support with the statistical aspects of this work.

M. Groenewald and G. J. de Jager for their guidance and assistance with the PCR determinations.

The Department of Agriculture, Western Cape, for the use of their facilities.

My wife Corlia and daughter Logan for their love and understanding.