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CONTROL OF ROOT-KNOT NEMATODE (*MELOIDOGYNE* SPP.) ON TOMATOES BY ORGANIC, CHEMICAL AND BY PLANT RESISTANCE TECHNIQUES

MSc(Agric) UP 1991

CONTROL OF ROOT-KNOT NEMATODE (*MELOIDOGYNE* SPP.) ON TOMATOES BY ORGANIC, CHEMICAL AND BY PLANT RESISTANCE TECHNIQUES

by

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D E C L A R A T I O N

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

....13/2/92.....

SIGNATURE DATE

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ABSTRACT

Techniques for controlling root-knot nematode (Meloidogyne spp.) in tomato include the use of organic amendments, chemicals such as nematicides as well as using resistant tomato plants. It is essential to study control techniques so that a choice of controls can be identified which will be of benefit to the tomato farmer. In this study the three control techniques were studied in detail so that their effectiveness could be determined.

Compost, being an organic amendment gave significant control over nematode larvae when the volume of compost in the soil mixture reached 40%. This amount of compost would be recommended for horticultural potting mixtures. The agent or entity within the compost could not be determined as being a bacterium.

The use of nematicides in both pot and field trials gave different degrees of control. Fenamiphos (Nemacur) gave excellent control in both pot and field trials whilst aldicarb

(Temik) only gave control in the pot trial. Oxamyl (Vydate) fared poorly in both the pot and field trials. Compost, when incorporated at quantities of $450m^3$ per hectare in the field trial, did reduce root galling but this was still insignificant. The only nematicide which would be recommended for root-knot nematode control in field tomatoes would be fenamiphos (Nemacur).

Resistant tomato cultivars offer excellent control on soils infested with root-knot nematodes.

All three techniques which were tested, namely compost, nematicides and plant resistance were found to effect control over root-knot nematode infestation. The degree and mechanism of control differed for the three techniques but excellent control was determined within each technique. The only technique which would, however be recommended for small-scale tomato farmers would be the use of root-knot resistant tomato cultivars.

UITTREKSEL

Tegnieke vir die beheer van knopwortel-aalwurm (Meloidogyne spp.) in tamaties sluit in die gebruik van organiese stowwe, chemikalië soos aalwurmdoders asook die gebruik van verdraagsame tamatiekultivars. Dit is noodsaaklik dat beheertegnieke bestudeer word sodat 'n keuse van beheer tot voordeel van die kleinskaalse tamatiekweker geïdentifiseer kan word. In hierdie studie was die drie beheertegnieke in detail bestudeer sodat die doeltreffendheid daarvan bepaal kon word.

Kompos wat 'n organiese stof is, het betekenisvolle beheer oor aalwurmlarwes uitgeoefen namate die volume kompos in die grondmengsel 40% bereik het. Die middel of entiteit binne die kompos kon nie as 'n bakterie bewys word nie.

Die gebruik van aalwurmdoders in beide pot- en veldproewe het verskillende grade van beheer uitgeoefen. Fenamifos (Nemacur) het uitstekende beheer in beide pot- en veldproewe gegee terwyl aldikarb (Temik) net beheer in die potproef gegee het. Oxamiel (Vydate) het swak beheer in beide pot- en veldproewe gelewer.

Kompos teen 450m^3 per hektaar in die veldproef het 'n vermindering van wortelgalle tot gevolg gehad maar dit was nie betekenisvol nie. Die enigste aalwurmdoder wat aanbeveel word vir die beheer van knopwortel-aalwurm in tamatielande is fenamifos (Nemacur).

Weerstandbiedende tamatiekultivars bied uitstekende beheer teen knopwortel-aalwurm op besmette gronde.

Al drie tegnieke wat getoets was, naamlik kompos, aalwurmdoders en plant verdraagsaamheid het beheer oor knopwortel-aalwurm besmetting uitgeoefen. Die graad en meganisme van beheer het vir die drie tegnieke gewissel maar uitstekende beheer was binne-in elk van die tegnieke verkry. Die enigste tegniek wat vir kleinskaalse tamatiekwekers aanbeveel kan word is die gebruik van weerstandbiedende knopwortel-aalwurm tamatie-kultivars.

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1. Introduction

Tomatoes are one of the major vegetable crops cultivated in South Africa with 253 601,54 tons worth R186,9M being sold on the 15 national fresh produce markets during 1990. This represents 26,5% of the total sales of all vegetables on these markets (Anon, 1991).

The yield of tomatoes can, however, be drastically reduced by pests and diseases and in 1949 Chitwood's paper provided conclusive evidence that Meloidogyne spp. and other plant nematodes were economically important plant parasites (Taylor & Sasser, 1978). The reduction due to root-knot nematodes, determined as a percentage, can be as high as 80% (Barker, Shoemaker & Nelson, 1976) although Taylor & Sasser (1978) state that the percentage is between 25 and 50%, whilst the loss in yield on a world basis has been estimated at 5%. Unfortunately, this loss is not divided equally amongst the world's farmers, but the greater part of the loss is borne by those least able to afford it, namely the small scale farmers of underdeveloped countries. Nematodes were found to be widespread and frequently abundant in tropical and subtropical soils (Netscher, 1978).

The yield loss is not only due to the effects of the feeding nematode, but also to the effects of invading fungi and other

pathogenic organisms which enter the roots together or after the nematode (Sasser & Jenkins, 1960).

2. Literature review

To help prevent yield losses brought about by the rootknot nematode, researchers have concentrated on the following aspects:

2.1 Integrated control methods

With these methods of control, the numbers of parasites are reduced as much as possible so that at planting, few of the infective nematodes are present in the soil to cause damage.
The methods include crop rotation, desiccation, flooding, organic amendments, pH changes, electricity, fumigants, nematicides and plant resistance (Taylor & Sasser, 1978).

2.1.1 With crop rotation, the method of control is based on the fact that the nematode larvae, being obligate parasites, only travel short distances through the soil. The larvae have a limited amount of food energy and must therefore find a suitable host or die of starvation. The most profitable crop grown by the farmer is the must susceptible whilst the non-susceptible one is used to reduce the numbers of infective larvae by starvation. Rotational experiments set out by Johnson & Campbell (1980) however, showed that a resistant crop for Meloidogyne spp. was not effective in reducing other nematode species and in this case

increased numbers of Pratylenchus sp. resulted when Crotalaria sp. was used in rotation with tomato. This nematode was also a parasite of the tomato.

2.1.2 The desiccation of soil by ploughing was shown by Waldmann (1971) to increase yields by 72%. Taylor & Sasser (1978) also mention that if lands were ploughed during the dry season so that the upper soil layers dried out completely, significant yield increases could be obtained. The larvae could not withstand desiccation.

2.1.3 Flooding of lands to a depth of 10 cm for several months in countries having plentiful water would control root-knot nematodes as the larvae could not survive extended periods of submergence (Taylor & Sasser, 1978).

2.1.4 Organic amendments in the form of adding oat straw and sawdust were shown to significantly reduce the galling on tomato roots (Johnson & Leander, 1962 and Singh, Singh & Beniwal, 1967).

With the addition of organic matter to the soil, increased microbial activity would reduce the nematode numbers be it fungus, bacteria, actinomycete or some chemical compound within the compost (Mankau, 1980).

Other mechanisms of control by the application of organic matter were a reduction in the parasitic nematode larvae numbers during the anaerobic decomposition of organic material (Wallace, 1973).

2.1.5 A decrease in the pH of the soil to 4.7, significantly reduced the number of egg masses and galls on the roots of tomato plants in an experiment conducted by Obbuj & Jensen (1974). On the other hand, Johnson & Leander (1962) showed that at pH's greater than 7, there was also a significant decrease in nematode numbers.

2.1.6 Although Taylor & Sasser (1978) mention that electricity was tried in Zimbabwe to control root-knot nematodes, the reduction in nematodes was brought about by increased soil temperature. The power requirement would be beyond the capabilities of normal farm equipment for effective control.

2.1.7 Fumigants which include volatile gasses were used by various researchers to kill nematodes. The fumigants used in various experiments show excellent results with methyl bromide and ethylene dibromide achieving significant reductions (McLeod, 1977 and Brodie, Good, Jaworski & Glaze, 1968). The reduction in soil populations of root-knot nematodes was also accompanied by control of soil insects, bacteria, fungi and most

weed seeds if methyl bromide was applied. Beneficial fungi and bacteria would also be killed (Taylor & Sasser, 1978).

These fumigants have major disadvantages however, especially for the small scale farmer as the first, methyl bromide is highly toxic (Bot, Sweet & Hollings, 1988), requires specific conditions to be effective which include correct soil preparation, moisture, temperature and slope as well as at application; correct timing and dosage, dispensation and distribution, tarp type and sealage, aeration period, correct irrigation for leaching and strict safety precautions (Marx, undated). Ethylene dibromide has disadvantages which include (McLeod, 1977):

(a) Specialised equipment required to inject the liquid into the soil.

(b) Rain and low temperatures after fumigation would reduce the effectiveness of the treatment.

(c) The soil moisture status must be optimal for diffusion of the gas. Dry soil caused irregular control.

2.1.8 Nematicides as against fumigants are applied to the planting row either just prior to planting or can be applied to plants which are already established (Bot et al, 1988).

The effects of the registered nematicides on the control of root-knot nematodes were tested by various researchers. McLeod & Khair (1975) looked at the effect of two granular nematicides namely aldicarb (Temik) and fenamiphos (Nemacur) on the hatching of eggs, migration and development of the root-knot nematode. In all three cases, aldicarb showed better control than did fenamiphos. According to McLeod (1977) the same tendency was observed in an experiment using amongst other nematicides, aldicarb, fenamiphos and oxamyl (Vydate). Oxamyl controlled nematodes less than the aforementioned two. Johnson (1985), Mariwah & Khera (1987), Salem (1979) also found that fenamiphos controlled root-knot nematodes significantly whilst Brodie et al, (1968) determined that aldicarb controlled the nematode well.

2.1.9 Plant resistance to Meloidogyne spp. may be defined as some characteristic or characteristics of the plant which inhibits reproduction of one or more Meloidogyne spp. The amount of inhibition should be 90% or more measured in plants before a plant is classed as being resistant (Taylor & Sasser, 1978).

The resistance in plants is of two kinds

(a) Passive, where pre-existing resistance factors already occur.

(b) Active or provoked resistance where nematodes give off enzymes and other substances and a condition of hypersensitivity occurs. A violent and rapid change occurs where necrosis causes isolation of the infection (Wallace, 1973).

When a plant is injured or invaded by various pathogens, oxidised compounds produced by the plant often show considerable biological activity and are a common mechanism of resistance towards the pathogens (Hung & Rohde, 1973).

It has been observed that after resistant plants were invaded, browning of tissues occurred. The larvae died within the necrotic areas and no giant cells (syncytia) were formed. In the partially resistant cultivars, the tissues browned less whilst in susceptible cultivars this was far lower and necrosis much less (Hung & Rohde (1973).

It is mentioned by Taylor & Sasser (1978) that the number of larvae entering roots in both resistant and susceptible cultivars was equal and that an immune reaction occurred after the larvae had penetrated the roots.

The controlling mechanism for resistance lies within the chromasomes of resistant plants as a single gene (Taylor & Sasser, 1978). This single resistant gene, which is dominant was derived from a wild species of tomato Lycopersicum peruvianum. This gene operates mainly against Meloidogyne incognita, M. javanica and M. arenaria. As yet it has not been shown that more than one resistant gene exists in resistant tomato cultivars. The resistance of the tomato plant is associated with the ambient temperature and Dropkin (1969) showed that if the day temperature remained at 32°C for longer than two consecutive days, the plants' resistance to nematode attack was broken and larvae were observed to complete their life cycle. If more than one gene were present at separate loci, resistance would be better as a broader spectrum of resistance could be attained (Sidhu & Webster, 1973).

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3. THE EFFECT OF VARIOUS CONCENTRATIONS OF COMPOST ON INFESTATION BY THE ROOT-KNOT NEMATODE Meloidogyne incognita OF TOMATO ROOTS.

3.1 Introduction

At the New Forest Irrigation Scheme in Gazankulu, tomatoes are grown by Black farmers during the cooler months of the year (March to July). The soils are sandy and as tomatoes are grown year after year on the same land, root-knot nematodes, Meloidogyne incognita (Kofoid and White) Chitwood are found in large numbers. This is in line with the views of Taylor & Sasser (1978) who reported a build-up of nematodes especially in sandy soils if no crop rotation was practiced. These root-knot and other nematodes are responsible for losses world-wide to various crops and the magnitude can vary from 25 - 50% (Taylor & Sasser, 1978) to as high as 80% (Barker, Shoemaker & Nelson, 1976).

A lack of capital amongst peasant farmers in the New Forest area restricts the use of nematicides and fumigants and an alternative to these substances was therefore sought. Compost was chosen as an alternative, as Wallace (1973) reported that one of the benefits of organic matter added to soils which contained nematodes was a reduction in the larval numbers during the decomposition of such matter. Two groups of researchers, Johnson & Leander (1962) using oat straw and

Singh, Singh & Beniwal, (1967) using sawdust, achieved significant reductions of galling on tomato roots.

When compost was added in combination with marigold straw, significant control of nematodes was achieved and yield increases were recorded in susceptible tomatoes (Ruelo, 1983).

The effect brought about by adding compost must have been brought about by an agent, be it fungus, bacteria, actinomycete or some chemical compound within the compost (Mankau, 1980).

The object of this investigation was to determine the effect, volume and economic value of compost when added to a sandy soil in pots for the benefit of nematode control.

3.2 Material and methods:

To obtain the objectives of this study, namely the effect of compost on nematode control the following research approach was used:-

3.2.1. An inoculum of nematode larvae was required for the pot experiment. This inoculum was obtained by bulking-up an initial sample obtained at New Forest.

3.2.2. The nematode larvae were then identified down to the species level using the identification charts of Taylor & Sasser (1978).

3.2.3. The pot experiment consisted of various concentrations of compost (Culterra) added to the nematode inoculated sand medium. Young tomato plants; cultivar Napoli were planted in the pots and after a period of four weeks, removed to determine the effect of the various treatments (six compost concentrations) on nematode numbers was.

The inoculum was initially bulked-up by inoculating 16 susceptible seedlings (cultivar Napoli) with infested soil from New Forest. These were then grown in a pasteurised sandy soil in two, one metre long asbestos troughs for two months. The soil sample for nematode larvae determination was obtained by firstly mixing the soil of the two troughs thoroughly to obtain a uniform distribution of nematodes. Ten sub-samples were then taken, mixed and one representative sample taken from these. The counting procedure was a modified technique from that of Townshend (1963) and entails the following:

A 50 ml soil sample was placed on a paper towel, suspended inside a course meshed ring (10 cm dia.) in a larger bowl of water. After 24 hours, the water was transferred into a 100 ml beaker and allowed to stand for 30 minutes. Second instar larvae settled at the bottom. A 10 ml pipette was then used three times to remove 3 X 7 ml of a random sample of settled larvae plus water which was poured into a standard calibrated dish. The larvae were examined microscopically and numbers recorded.

Table 1: Statistical layout of pot experiment with five blocks and having six compost treatments

	Blocks				
	A	D	E	C	B
	5	3	1	4	1
	3	1	4	2	6
Treatment	4	5	2	6	2
	1	6	3	1	5
	6	4	6	5	3
	2	2	5	3	4

Seed of Napoli was sown in Speedling trays in sandy soil. This was previously pasteurised at 60°C for 30 minutes to kill possible nematodes as referred to in the book by Hartmann & Kester (1975). Here, a temperature of 48,9°C is given as the threshhold survival temperature for parasitic nematodes.

Ninety seedlings at the two and a half expanded leaf stage were chosen for use in the experiment which was a randomised blocks design of six treatments replicated five times. These tomato seedlings were planted per 15 cm pot diameter (1,5 litre capacity). The experimental treatments were as follows:

Treatment:
1 = 6 vol. Sand + 1 vol. Inoculum (Inoculum was sandy soil containing an average of 85 larvae/50 ml soil)
2 = 5½ vol. Sand + ½ vol.Compost and 1 vol.Inoculum
3 = 5 vol. Sand + 1 vol. Compost + 1 vol. Inoculum
4 = 4 vol. Sand + 2 vol. Compost + 1 vol.Inoculum
5 = 2 vol. Sand + 4 vol. Compost + 1 vol.Inoculum
6 = 6 vol. Compost + 1 vol. Inoculum.

The treatments were based on a volume basis with Treatment 1 = 0% compost, 2 = 7,1% compost, 3 = 14,3% compost, 4 = 28,6% compost, 5 = 57,1% compost and 6 = 85,7% compost.

The statistical layout is presented in Table 1.

Table 2: Soil analysis from experimental plot at New Forest

FACTOR	UNITS
Phosphorus Bray 1	18 ppm
Potassium	33 ppm
Calcium	222 ppm
Magnesium	23 ppm
pH	6,0 in water
Resistance	4000 ohms
Clay	7%
Silt	5%
Sand	88%
Textural class	LoSa

LoSa = loamy sand according to the textural chart (MacVicar, De Villiers, Loxton, Verster, Lambrechts, Merryweather, Le Roux, Van Rooyen & Von M. Harmse, 1988).

Table 3: Average number of nematode root galls per 24 cm root length and per 0,5 g root mass.

Treatments			Blocks A	B	C	D	E	Av.
1	a	24 cm	54	30	64	24	30	40,4
	b	0,5g	67	36	62	111	42	63,6
2	a	24 cm	25	12	36	55	57	37,0
	b	0,5g	73	55	70	63	102	72,6
3	a	24 cm	9	22	21	17	29	19,6
	b	0,5g	43	46	62	25	55	46,2
4	a	24 cm	0	12	9	6	6	6,6
	b	0,5g	5	11	7	5	4	6,4
5	a	24 cm	0	0	0	0	0	0
	b	0,5g	0	2	0	0	0	0,4
6	a	24 cm	0	0	0	0	0	0
	b	0,5g	0	0	0	0	0	0

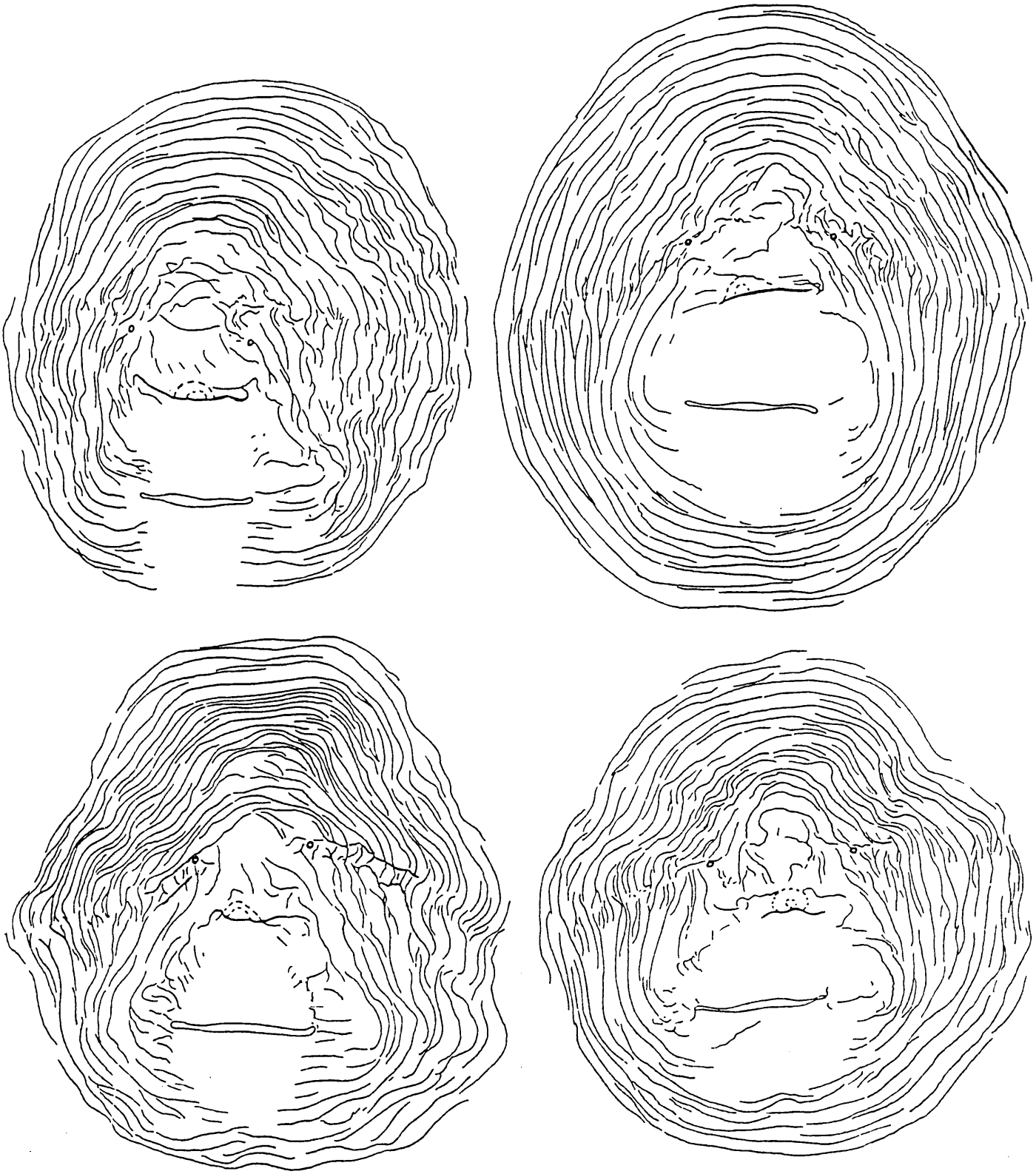

Figure 1. Meloidogyne incognita. Perineal patterns as observed on the posterior of adult females.

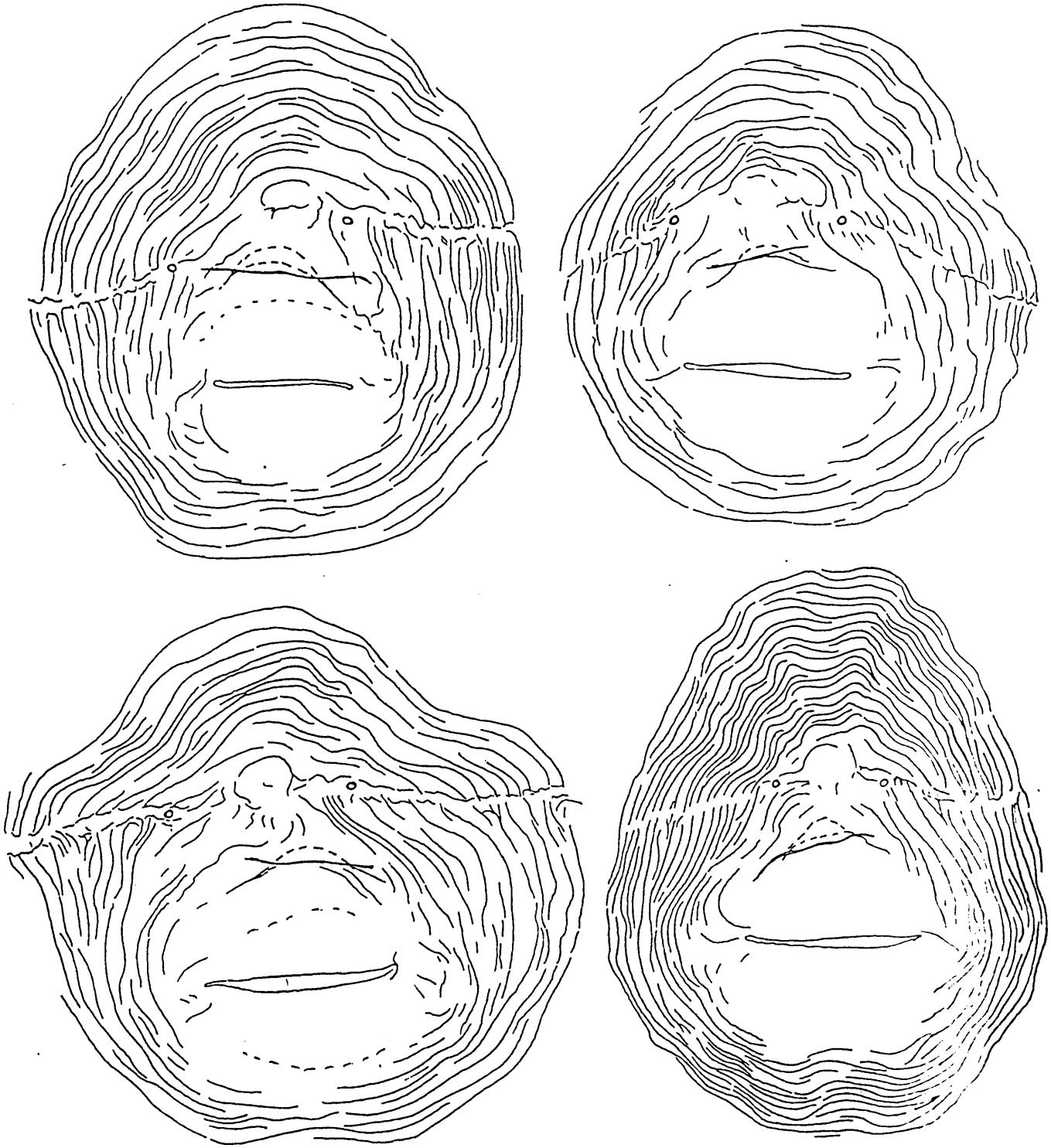

Figure 2. Meloidogyne javanica. Perineal patterns as observed on the posterior of adult females.

The compost used was that of a commercial firm which is freely available in South Africa. The soil used was a sandy loam.

The pot experiment was conducted in an evaporative cooled glasshouse of the Horticultural Department at the experimental farm in Pretoria.

3.3 Results and Discussion:

The soil analysis presented in Table 2 shows that the soil used was a sandy loam with 80% sand, 12% clay and 8% silt. From observations of mature females removed from the Napoli roots, Figures 1 and 2 perineal ring patterns showed that the nematodes were mostly M. incognita although a few M. javanica were also observed. These patterns are views of the posterior end of females. Identification as depicted in the book by Taylor and Sasser (1978) and by Kleynhans 1991 was used in this instance.

In Table 3 results are presented showing the average number of root galls per 24 cm root length and number of root galls for 0,5 root mass.

To analyse the results statistically a Log transformation was used because of inherent variation. For the 24 cm root lengths and 0,5g root masses, the model Log $Y = 4,127 - 0,085\ X + 0,00058\ X^2$ was used.

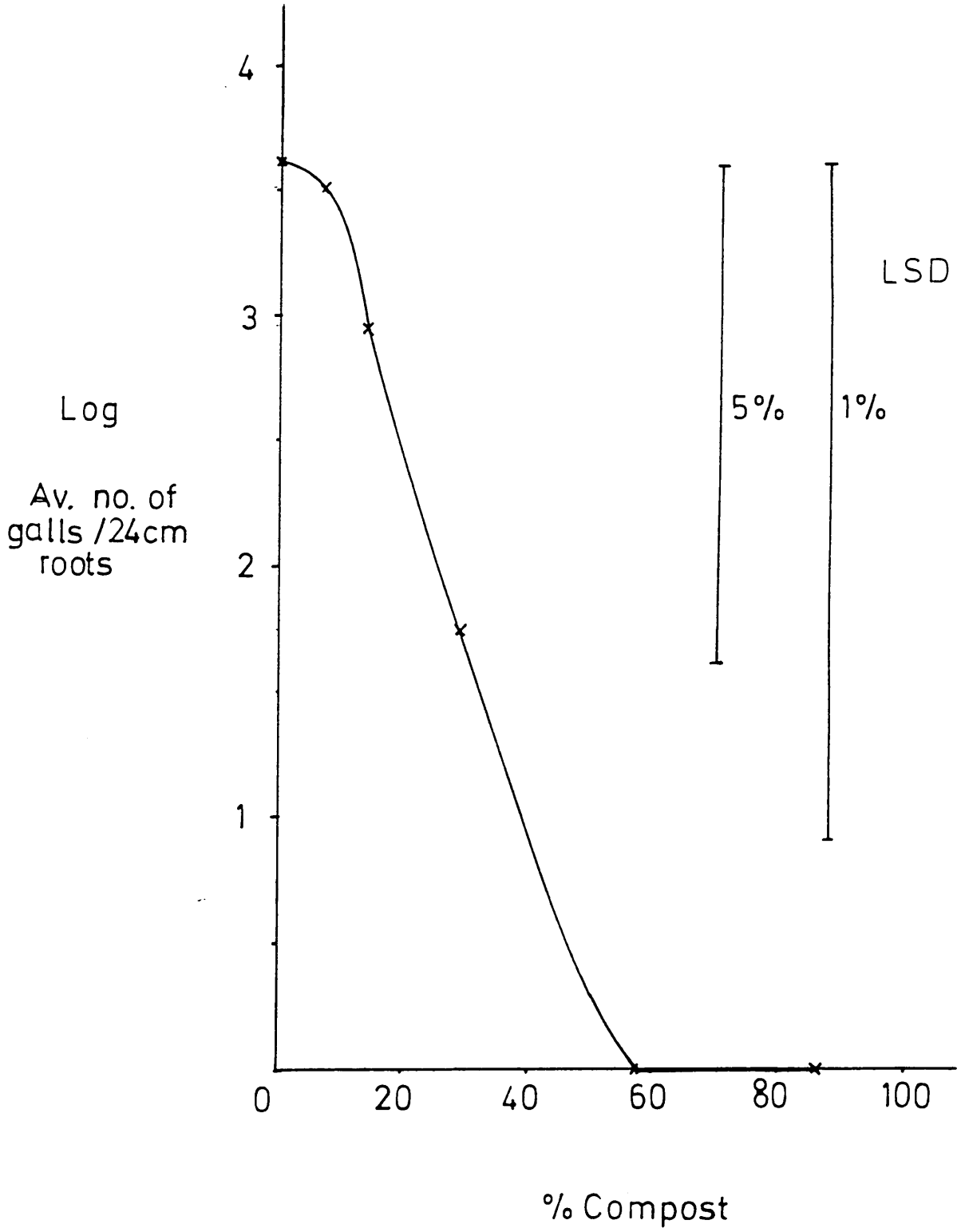

Figure 3 Effect of increasing compost concentration on root gall numbers

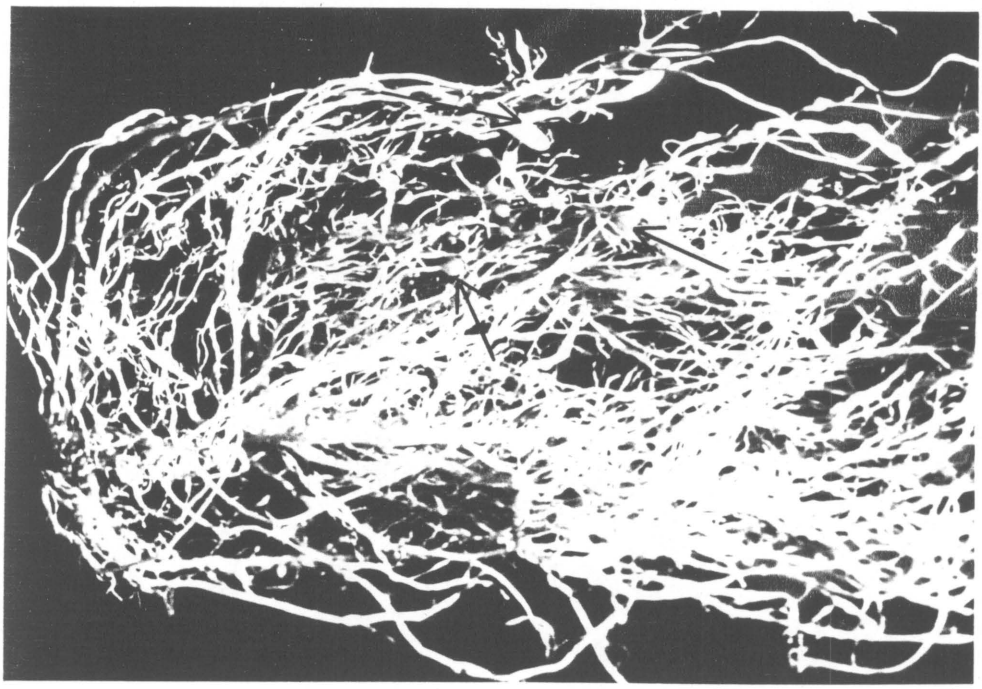

Figure 4 Effect of 28,6% compost on root gall development in tomato plants

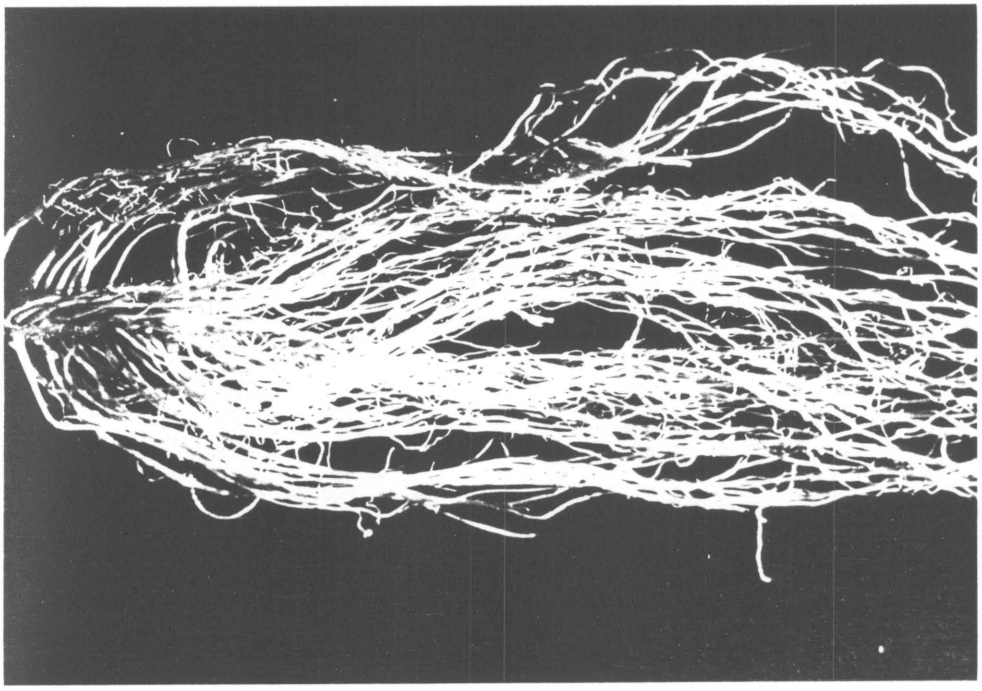

Figure 5 Effect of 57,1% compost on root gall development in tomato plants

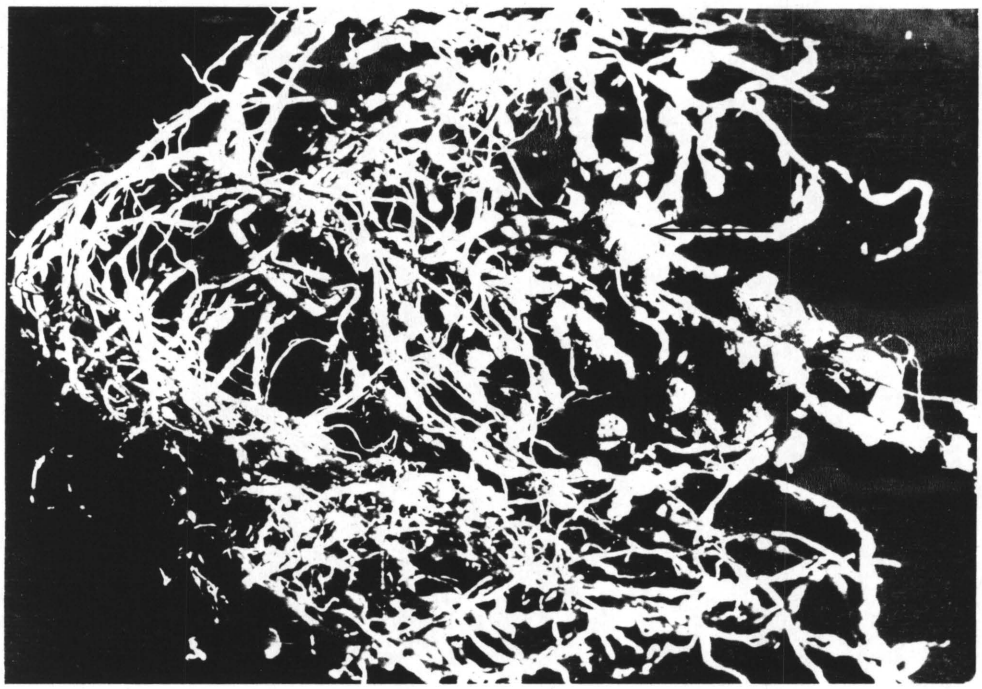

Figure 6 Control, treatment (no compost) on root gall development in tomato plants

The main effect of compost concentration on the ability to reduce nematode galls was found to be significant at both the 5% and 1% level of significance. The interaction between lengths of roots and the mass of roots was non-significant as there was a significant relationship between N-galls and compost percentage for both types. Section 1 of Addendum contains the full statistical analysis.

Figure 3 illustrates graphically the effect of compost concentration on root galling for root length. There was a significant decrease in the number of galls at the 5% level when the compost concentration was increased from 0 to 30% and at 40% this is significant at the 1% level. At this concentration, a factor within the compost began to exert a dramatic influence on the nematode larvae numbers. This caused them to become non-infective or killed them outright. From examination of roots (Figure 4) it was found that at 28.6% compost addition the galls formed later at the root tips thus suggesting an effect where the larvae were kept at bay by the factor within the compost and with time, this factor breaks down. Figure 5 shows complete control of the nematodes at 57,1% compost and the larvae became permanently non-effective. Figure 6 shows the effect if no compost was added and infestation was severe.

A hectare of soil which has 450 m^3 of compost incorporated into the top 150 mm would have a 30% compost concentration and would give a significant decrease in root galling on tomato roots.

The cost of transporting this quantity of organic matter even within the farm situation would cost R8,56 for a 9 m^3 load towed at 5 km/hr over 1 km by a 52 kw tractor (Van der Merwe, 1991). The total cost of bringing the 450 m^3 to the land would amount to R428.00 if the organic matter was transported over a 1 km distance. The availability of this vast quantity of organic matter from most individual South African farms would be doubtful. This cost does not include loading or unloading the organic matter from the trailer. The organic matter could contain inherent macro- or micro-nutrients which at 450 m^3/ha could be potentially harmful to certain crops. Certain micro-nutrients could become toxic at the elevated concentrations with significant crop losses (Tisdale, Nelson & Beaton, 1985).

Conclusion

This trial on a nematode susceptible tomato cultivar demonstrates that compost has a powerful positive influence on reducing root-knot nematode larvae infestation. It appears as if a compost concentration of between 12 and 16% is critical for dramatic nematode decline and a concentration of 60% leads to virtual nematode-free plants.

The results further show that where 40% of a potting medium consists of compost, an appreciative benefit for nurserymen who

grow plants can be achieved, especially where root-knot nematodes are known to be a problem.

It appears at this stage that a compost concentration of 30% by volume would not benefit farmers as the cost of applying this concentration would not warrant the cost of this input.

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4. BACTERIA AS A POSSIBLE AGENT FOR THE CONTROL OF ROOT-KNOT NEMATODES (Meloidogyne spp.) IN SOIL POTTING MIXTURES.

4.1 Introduction

In undeveloped countries, small scale farmers suffer crop losses of up to 80% due to the effects of nematodes (Barker, Shoemaker & Nelson, 1976). In these areas, Taylor & Sasser (1978) state that loss in yields as the direct result of root-knot and other nematodes would be between 25 - 50%.

The loss in yields due to nematode attack can thus have serious implications for all susceptible plants. In an earlier experiment (refer to 3,3) carried out the addition of compost brought about a significant drop in the amount of root galls in tomatoes caused by the root-knot nematode, an agent, be it fungus, bacteria, actinomycete or some chemical compound must have been present according to Mankau, (1980). This agent or combination is thus responsible for the reduction of infective larvae in the medium or an inhibitive agent prevents the emergence of the second-stage larvae from the eggs.

The fungi include two types, nematode-trapping and endozoic parasitic. Numerous attempts by using these fungi to control nematodes have led to little success although when organic matter is added together with the fungi, results were better (Taylor & Sasser, 1978).

Although little is known about the importance of soil and compost-bacteria in controlling nematodes Bacillus penetrans was found to be the most specific obligate parasite of nematodes (Mankau, 1980). It was decided that bacteria could be of significant importance in controlling second-stage root-knot nematode larvae.

To be able to determine what effect bacteria would have on nematode larvae, the following approach was followed:-

(a) Eggs containing second-stage larvae had to be obtained.

(b) The plating out of bacteria obtained from compost was to be undertaken.

(c) Eggs, bacteria and tomato seedlings were to be incubated together.

(d) The tomato seedlings were then to be allowed to develop normally for three weeks whereafter root galls would be counted.

4.2 Material and methods

An inoculum of nematode eggs was obtained from 1000 nematode egg sacs removed from the root galls of previously inoculated susceptible tomato roots. The 300 - 500 eggs contained within each egg sac were separated from the gelatinous egg sac as well as being surface sterilised by washing them in a 0,5% NaOCl

Figure 7 First stage root-knot nematode larva curled up within an egg.

solution for three minutes (Hussey and Barker, 1973). The eggs were then immediately washed using sterile water, filtered through a 75 µm sieve to remove sand particles and then a 26 µm sieve was used to retrieve the eggs (Byrd, Ferris & Nusbaum, 1972). Figure 7 shows a first stage larva within an egg.

To be able to obtain mostly 2nd stage larvae as referred to by Jenkins & Taylor (1967), the eggs were incubated on moist filter paper in three sterile petri dishes at 25°C for one week. This resulted in the non-infective first stage larvae developing to the infective, second stage which is the stage which could infest the young tomato roots.

To be able to obtain varioius bacteria a standard commercial compost (Culterra) was used as source of infection for four petri dishes containing a standard agar preparation as set out by Thornton (1922).

The plating out of bacteria continued by isolating colonies until six different genera (with one genera containing 3 species) had been isolated. These were then cultured separately. The bacteria were transferred onto moist filter paper in 32 sterilised petri dishes, each genus being replicated four times.

Four control petri dishes without bacteria were included, these contained only nematode eggs. The nematode eggs in 50 ml

Table 4 Layout of Randomised blocks design with Bacteria as main treatment

	Blocks				
	C	A	B	D	
	7	1	3	5	1
	1	3	7	6	2
	3	2	9	2	3 Rows
Treatments	5	9	8	1	4
	8	6	1	7	5
	2	7	5	9	6
	4	4	4	3	7
	6	5	2	8	8
	9	8	6	4	9

Treatment 1 = Bacillus sp. 1
2 = Bacillus sp. 2
3 = Bacillus sp. 3
4 = Arthrobacter sp.
5 = Pseudomonas sp.
6 = Azotobacter sp.
7 = Aerobacter sp.
8 = Radiobacter sp.
9 = Control.

Table 5 The number of root galls on tomato roots in the presence of various bacteria.

Genera of bacteria	Number of galls per replication 1	2	3	4	Av.
Bacillus sp. 1	0	2	1	0	0,75
Bacillus sp. 2	0	1	3	0	1,00
Bacillus sp. 3	3	1	2	1	1,75
Arthrobacter sp.	0	0	1	0	0,25
Pseudomonas sp.	6	1	2	3	3,00
Azotobacter sp.	0	1	1	1	0,75
Aerobacter sp.	1	2	2	2	1,75
Radiobacter sp.	0	0	1	0	0,25
Control (no bacteria)	5	3	1	4	3,25

water (now in their second stage) were then equally divided by using a pipette between the 36 petri dishes and incubated at 25°C for one week. The seed of the tomato cultivar Floradade was surface sterilised prior to germination in the same manner as for the nematode eggs using 0,5% NaOCl for three minutes whereafter sterile water was used to remove all traces of the NaOCl. Three germinated, bacterial and fungal free susceptible tomato seedlings were then placed into each petri dish and allowed to develop for a further five days.

The seedlings, together with the contents of the petri dishes were then planted out into 7,5 cm diameter plastic pots containing sterilised soil. The 36 pots laid out in a randomised blocks design (Table 4) of 9 treatments replicated 4 times were subsequently placed in an evaporatively cooled controlled glass-house for a further three weeks to allow for root gall development.

At the end of this period, the plants were carefully removed and the soil washed from the roots where-after nematode galls were counted on all roots.

4.3 Results and Discussion

From Table 5 it was not statistically possible to rank the effects of different bacteria to the amount of suppression each genus had on the infective larvae. There was no significant

difference at the 5% and 1% level between the various treatments. The analysis is contained in Section 2 of the Addendum. The low infestation in the control showed that conditions for infestation and development of the nematode larvae had been unfavourable.

The technique whereby the nematode eggs were surface sterilised could not be a contributing factor for the low infective rate obtained as this was the standard technique for obtaining nematode eggs.

The contributing factor could have been the fluctuations in the soil temperature within the pots. A soil temperature of 48,9°C (Hartmann & Kester, 1975) is sufficient to kill nematodes if this temperature persists for 30 minutes. Here, a far lower temperature which could be in the higher 30's could make the larvae unable to develop further in the tomato roots. (Taylor & Sasser, 1978). The air temperature, although kept lower than the air outside would not prevent the soil temperature within the pots from rising. This would come about by direct solar radiation on the brown soil surface and pot sides.

This exercise could then identify bacteria which either immobilise the second stage larvae before these hatch or make the larvae non-infective after hatching. The chemical substances produced by the bacteria, if identified, could have a far rea-

ching impact on modern nematicides as these would be of an organic nature and more acceptable to the environment.

4.4 Conclusion

The results reported here are only the beginning of what could be an interesting topic for further research. The temperature could also be more accurately controlled by the use of larger pots, i.e. the temperature of a larger volume of soil is less likely to fluctuate and the use of polystyrene beads on the soil surface would further insulate the soil from temperature variations. A temperature-controlled hothouse would also help prevent the rise in soil temperatures.

In this experiment, the bacteria isolated did not have a suppressive effect on the root infestation by root-knot nematode larvae. The low infestation of even the control plots shows that a refinement of the plant growing conditions would be necessary for determining whether bacteria are the causal agents in compost which gave excellent nematode control in a previous experiment.

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5. THE EFFECT OF VARIOUS COMMERCIAL NEMATICIDES ON THE GALLING INCIDENCE OF POTTED SUSCEPTIBLE AND RESISTANT TOMATO CULTIVARS.

5.1 Introduction

According to experiments conducted on tomato plants, both fenamiphos- (Nemacur) (Johnson, 1985; McLeod & Khair, 1975; Mariwah & Khera, 1987 and Salem, 1979) and aldicarb- (Temik) (Brodie, Good, Jaworski & Glaze, 1968 and McLeod, 1977) reduced the incidence of root-knot nematodes significantly on tomato roots. Oxamyl- (Vydate) (McLeod, 1977) was less effective although still significantly better than the control of no nematicide.

Fenamiphos and oxamyl are registered for the control of root-knot nematodes (Meloidogyne spp.) on tomatoes in South Africa although aldicarb is not (Bot, Sweet & Hollings, 1988). The experiment was laid out during January 1989.

A comparison with these nematicides was undertaken on two resistant (Rossol VFN, Ace VFN) and two susceptible tomato cultivars (Roma VF, Heinz 1370) being one resistant and susceptible cultivar of both a processing and fresh tomato type. The effect of the nematicides could thus be determined for both resistant and susceptible processing and fresh tomato cultivars.

Table 6: Layout of pot experiment using 4 cultivars of tomato and 3 nematicides.

Treatments	Cultivars			
	I - a	IIII - b	III - e	II - c
	I - b	IIII - d	III - d	II - d
	I - e	IIII - a	III - b	II - a
	I - c	IIII - c	III - c	II - e
	I - d	IIII - e	III - a	II - b

Resistant tomato plants were included as Dropkin (1969) showed that the resistance was easily broken by day temperatures exceeding 32°C and the plants would react as normal susceptible cultivars.

5.2 Material and methods

An inoculum of infective larvae, numbering an average of 43 larvae per 50 ml. soil was obtained via prior bulking-up before the experiment commenced (refer to 3.2). The determination of larvae was according to the procedure by Kleynhans (1990) modified from Townshend (1963). Eighty, 15 cm plastic pots (1,5 l capacity) were used for the randomised blocks design of four cultivars, undergoing five treatments and replicated four times. The layout is given in Table 6.

Seedlings of the four cultivars were grown in sterilised sandy soil in Speedling trays. Three plants per pot were used in this experiment and the nematicides applied according to standard recommendations, (Bot, et al, 1988). The treatments and cultivars were as follows:

Cultivars:	Treatments
I = Rossol VFN	a = aldicarb 15% granules @ 5 g/m^2 = 0,09g/15 cm pot.

II = Ace VFN

III = Roma VF

IIII = Heinz 1370

b = fenamiphos 40% EC @ 7,5 ml/m^2 = 0,13 ml/15 cm pot.

c = fenamiphos 10% granules @ 30 g/m^2 = 0,54 g/15 cm pot.

d = oxamyl @ 0,5 l/m^2 seed trays (conc. 500 ml/100 l water) & 2 weeks later 0,5 l/m^2 (conc. 1 l /100 l water)

e = Control.

At the two-leaf stage, the seedlings were transplanted, three to a pot and the various treatments applied. Aldicarb was applied according to recommendations for potato plantings (Bot, et al, 1988).

The tomato plants were allowed to grow for four weeks in the evaporatively cooled glass-house before the plants were inspected for root galling. The individual plants were carefully rinced in water to remove the soil before a 0,5 g sample of lateral roots was taken for analysis. The 0,5 g sample was chosen as it was determined previously that this amount of root gave a good parameter for infestation measurement (refer to 3.3).

Table 7. The galling incidence on two resistant and two susceptible tomato cultivars using various nematicides.

	Cultivars			
Treatments	Rossol VFN	ACE VFN	Roma VF	Heinz 1370
A Av.	0	0	0,25	0,25
B Av.	0	0,25	0,25	0,25
C Av.	0	0,25	0,5	0,75
D Av.	0	0	8	12,75
E Av.	0,25	0,25	18,25	20,75

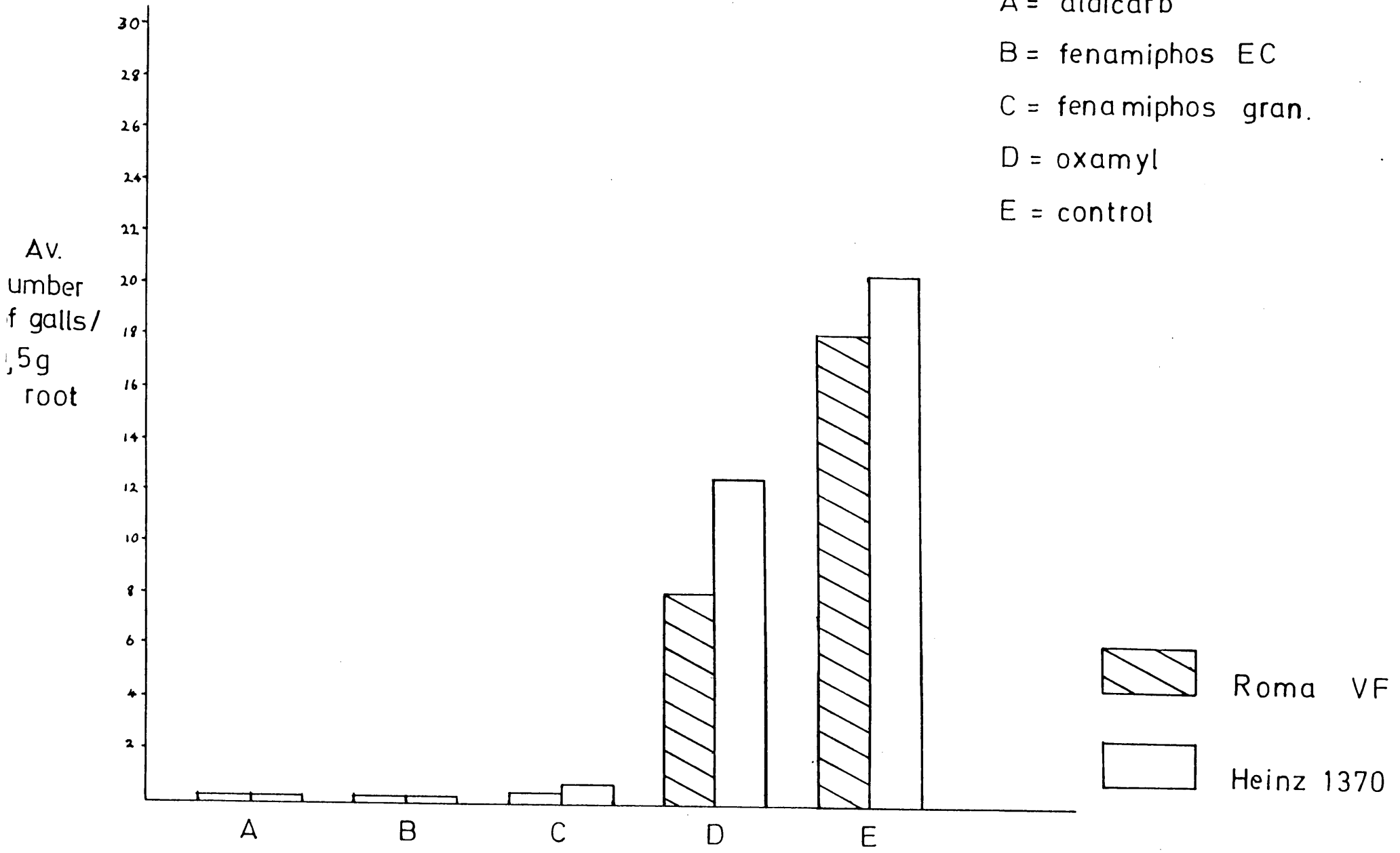

Figure 8 The effect of three nematicides on nematode galls for two tomato cultivars

5.3 Results and Discussion

From the results in Table 7, the following becomes apparent:

The Rossol VFN and Ace VFN both show a significant degree of resistance to attack by the root-knot nematode even without the addition of any nematicides. This resistance, as is described by Taylor & Sasser (1978) is gene linked and thus gives sufficient protection against nematode attack at no additional cost. This has great significance especially for the farmer, but as the author states, this resistance is due to only one gene and a wider resistance base would be preferable. This resistance can however, due to its being governed by a single gene, be affected by stress factors including environmental changes of high or low temperatures or by different races of root-knot nematodes. Temperatures above 30°C could cause the nematode resistant plants to become susceptible (Sasser & Taylor, 1978). The nematicides, aldicarb and fenamiphos when applied to Roma VF and Heinz 1370 gave excellent control against nematode infestation. This reduction is illustrated graphically for the two cultivars in Figure 8.

There is no significant benefit of using the various forms of fenamiphos as the results are almost identical. The EC formulation could be fed through a drip irrigation system if this was installed. This would save on the application cost if a choice of granules or EC was to be considered.

The oxamyl treatment, although giving somewhat better control than no nematicide, would not be recommended as an effective nematicide for tomatoes grown by farmers as the two previous nematicides gave far superior control. In the statistical analysis of the results, the number of plots where complete control was achieved (i.e. 12 for Roma VF & Heinz 1370 where aldicarb and fenamiphos were applied) led to difficulties in the analyses. A 0 result for many plots (complete control) cannot easily be used in normal analyses and the Friedman analysis of variance (non-parametric) for non homogenous variations using the rank totals as against actual counts had to be used instead. See Section 3 of Addendum. The LSD using this method was 27,09 whilst the difference in rank totals between the first and last was 26. Although there was no significant difference at the 5% level, the difference was significant at just above this level.

The pot experiment gave results that agree with those of McLeod & Khair (1975) and McLeod 1977 in that both aldicarb and fenamiphos controlled root-knot nematodes effectively whilst oxamyl was less effective.

The application of both fenamiphos and aldicarb would only be recommended at this stage for tomatoes under conditions where these were grown in containers (i.e. in pots in tunnels or hothouses).

A field trial using the various nematicides would probably give more definite answers to the most effective nematicide for farmer use.

When the two cultivars, Heinz 1370 & Roma VF were compared to one another, there was also no difference between these cultivars as to nematode resistance.

With the almost complete control of nematodes by aldicarb and fenamiphos (12 out of 16 plots) the reasons behind the nematicidal action of these chemicals should be examined. The chemicals caused the reduction of hatching of 2nd stage larvae as well as the prevention of the migration of the second stage larvae towards the tomato roots as was indicated by McLeod and Khair (1975). Was this due to the systematic poison effect of the two nematicides or are the effects brought about by other inhibiting factors?

5.4 Conclusion

The two resistant tomato cultivars, Rossol VFN and Ace VFN in this experiment offered excellent resistance to the root-knot nematode without the additional protection of a nematicide. This resistance could, however, due to its being governed by a single gene be affected by stress factors (i.e. higher or lower temperatures) resistance could be broken.

Both aldicarb and fenamiphos gave excellent control in the pot experiment whilst oxamyl cannot be recommended. Field trials using these nematicides would determine which chemical would be preferable for farmers.

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6. THE EFFECT OF VARIOUS NEMATICIDES AND ORGANIC MATTER UNDER FIELD CONDITIONS ON THE INFESTATION OF TOMATOES BY THE ROOT-KNOT NEMATODE, Meloidogyne incognita.

6.1 Introduction

The effects of various registered nematicides has been well documented in the literature (Brodie, Good, Jaworski & Glaze, 1968; Johnson, 1985; McLeod & Khair, 1975 and Mc Leod, 1977. These experiments are often carried out under controlled conditions in pots and results are not, however, always attained in practice. A field trial to determine the effects of three nematicides fenamiphos- (Nemacur), aldicarb- (Temik) and oxamyl- (Vydate) and compost was thus considered.

The same nematicides used by the author in a pot experiment would be used as a basis to substantiate these results under field conditions (refer to 5,3). Whilst testing the effect of root-knot nematodes with soil/compost mixtures, excellent results were achieved in pots under controlled conditions (refer to 3,3). The most effective method, either by a certain nematicide or by natural means in the form of a compost was to be determined to help control Meloidogyne incognita in field plantings of tomatoes.

Table 8. Field layout of experiment with five treatments replicated four times

	Blocks			
Treatments	C	A	B	D
	3	2	5	1
	4	3	3	2
	5	1	2	4
	1	4	1	5
	2	5	4	3

6.2 Material and methods

A field site on Plot 84 Waterval, Pretoria was used during December 1989 where the previous crop of potatoes had failed two years previously due to the total infestation of the tubers by M. incognita.

This site had not been cultivated for two seasons and had a pioneer grass cover. The soil was a red sandy loam . All four control plots were sampled (5 subsamples per plot) before planting, for nematode determination according to the method of Kleynhans (1990).

The experimental design consisted of a randomised blocks design of five treatments and four replications with the susceptible tomato cultivar, Floradade being used as the host plant. The field layout is given in Table 8. The plot size was 1,2 m X 1,2 m with plants spaced 30 cm apart giving 25 plants/plot. The border row consisting of 16 plants was not included in the determinations so as to reduce border effects whilst the remaining central nine plants were. A one metre border strip between plots was also left so that the influence of the one treatment by lateral movement could be minimised. The treatments were as follows:

1. aldicarb 15%G : 3 g/linear metre based on 50 cm row centres, at planting incorporated into soil and watered. Per plot 13,5 g.

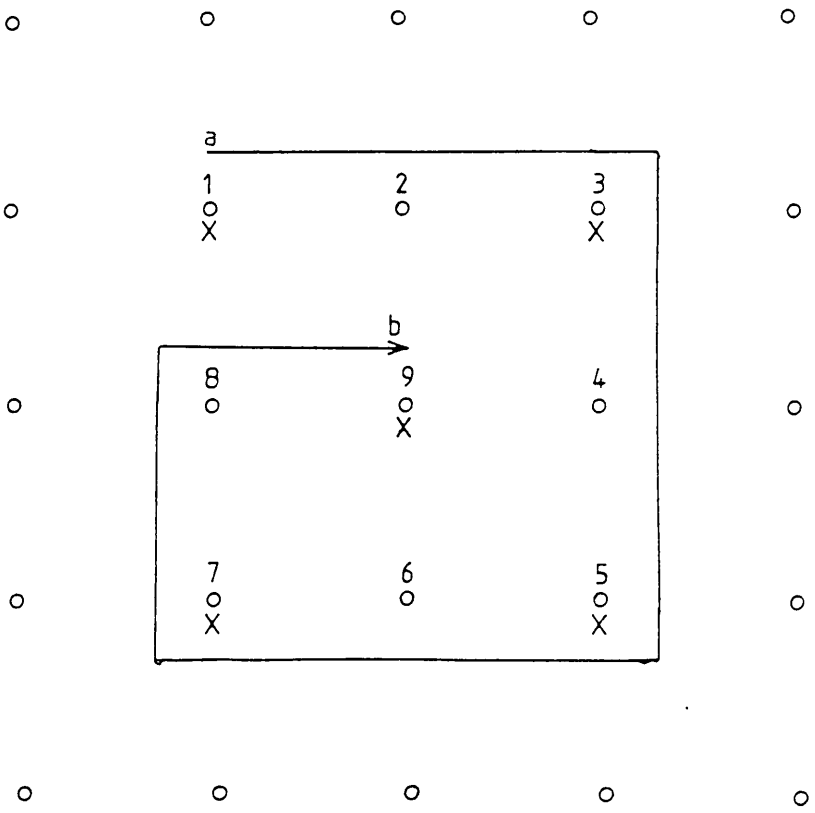


Figure 9. Plot sampling sequence for initial soil nematode determination as well as for final root-gall counts for the four control plots

Key:

Initial nematode sampling indicated at sites marked x.

Final root-gall determination sequence beginning at a (plant number 1 and continuing till b (plant 9).

2. fenamiphos 10% G: 10 g/linear metre in 30 cm band over planting row, 2 weeks before planting and incorporated into soil. Per plot 48g.

3. oxamyl 245 g/l: One day before transplanting (0,5 l/m^2) sprayed, 500 ml/100 l water of formulation. Repeat again 3 weeks later. Per plot 0,72 l of mixture formulated as aforementioned.

4. Compost: 30% volume of compost added to top 15 cm of soil two weeks before planting. Per plot 0,0648 m^3.

5. Untreated control.

Figure 9 indicates the sequence of sampling for initial soil nematode determination as well as for final root-gall counts for the control plots. The final root-gall counting sequence for the remaining treatment plots was identical to that for the control plots.

Table 9. Soil analysis of experimental plot

P	33 ppm
K	14 ppm
Ca	322 ppm
Mg	43 ppm
pH (water)	5,4
resistance (Ω)	4700
clay	12%
silt	8%
sand	80%
Textural class	SaLo*

* SaLo - Sandy Loam - According to textural class by Mac Vicar, De Villiers, Loxton, Verster, Lambrechts, Merryweather, Le Roux, Van Rooyen & Von M. Harmse (1988).

Table 10. Number of root-gall counts for 5 treatments on tomato plants

Treatment	Block A	B	C	D	Total for nine plants	Av.
1 aldicarb	2422	716	707	1394	5239	1317,25
2 fenami-phos	3	47	37	3	90	22,50
3 oxamyl	1170	1661	1456	109	4396	1099
4 compost	368	195	721	1119	2403	600,75
5 control	1645	1707	1788	317	5473	1368,25

The CV and LSD applied only to rank totals, see Section 4 of Addendum.

The middle nine plants were to be removed after 60 days, roots washed and eight lateral roots of 12 cm each were to be taken per plant for root-knot gall determination.

6.3 Results and Discussion

The soil analysis is given in Table 9. The soil was a sandy loam with 80% sand, 12% clay and 8% silt.

From the results, presented in Table 10 and in Section 4 of the Addendum it becomes apparent that the fenamiphos treatment brought about almost complete control whilst the compost treatment reduced the infestation over that of the control by 56%. The efficacy of fenamiphos agrees with that in the literature (Johnson, 1985) in that this chemical has good nematicidal properties. The reduction caused by compost also agrees with earlier work done by the author as well as with results by Johnson & Leander (1962). The 56% reduction in galling caused by the compost could lead to appreciable tomato yield increases. Refer to 3,3.

One problem found in natural populations of nematodes is that the numbers found in the soil are not uniform and it is thus often difficult to analyse the results statistically without first transforming the data due to the non homogenous variations.

Table 11 Nematode counts taken from control plots before planting

Plot A5 -	5
Plot B5 -	20
Plot C5 -	10
Plot D5 -	2

In the statistical analysis of the results, the non-parametric Friedman analysis of variance had to be used where only rank totals are used instead of actual values. Treatment 2, that of the Nemacur application was found to be significantly better than the control and almost significantly better than Temik at 5%

The high degree of variation of numbers of larvae in the soil is a natural trend but is difficult to quantify due to sampling techniques which only give counts at that point of extraction. The number of infective larvae observed before the experiment was conducted (by soil sampling) is an example in this connection. The numbers were counted using the technique by Kleynhans (1990) modified from Townshend (1963) where 50 ml of soil is tested for infective larvae. The numbers of larvae counted using the technique are presented in Table 11.

A value of 2 would be interpreted as soil being almost free of nematodes even although the third plant out of the nine plants in this plot (D5) had 99 galls on the eight roots. The A5 plot also showed a low count of 5 infective larvae per 50 ml of soil although plants five and six showed total infestation (greater than 350 root galls/plant).

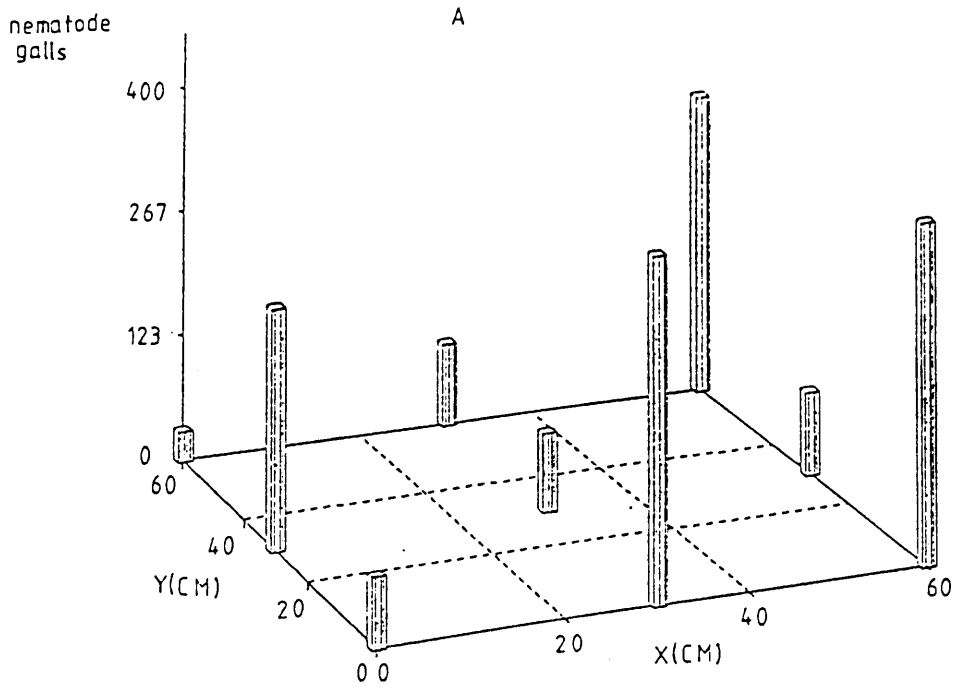
A reason for these low counts which were observed (Table 11) before the experiment commenced could be explained in that they were still in the 2nd stage within the eggs (Taylor & Sasser, 1978) or the sampling sequence missed many of the larvae.

Mention is also made by these authors that attractants are given off by susceptible plants and these result in the larvae hatching later and then moving to the roots.

The present method of determining the amount of nematodes in the soil is very misleading as the A5 plot shows. Although this is the standard method used by the Department of Agricultural Development (Kleynhans, 1990) results are not a true reflection of the real numbers of nematodes in the soil.

This is especially true with, as in this case, most susceptible plants had been replaced by an antagonistic grass cover which would be unsuitable as a food source by the nematode larvae and the larvae would thus remain within the eggs until a suitable host plant becomes available (Taylor & Sasser, 1978). Fewer larvae would thus be observed from the initial soil samples.

A more expensive and involved method would be to use the soil sample obtained i.e. 100 ml. as a growing medium and to use indicator plants which are susceptible to the various nematodes (Kleynhans, 1990). The nematodes would hatch out and infest the susceptible plant roots. After three weeks the degree of galling would be measured and a standard number of roots would



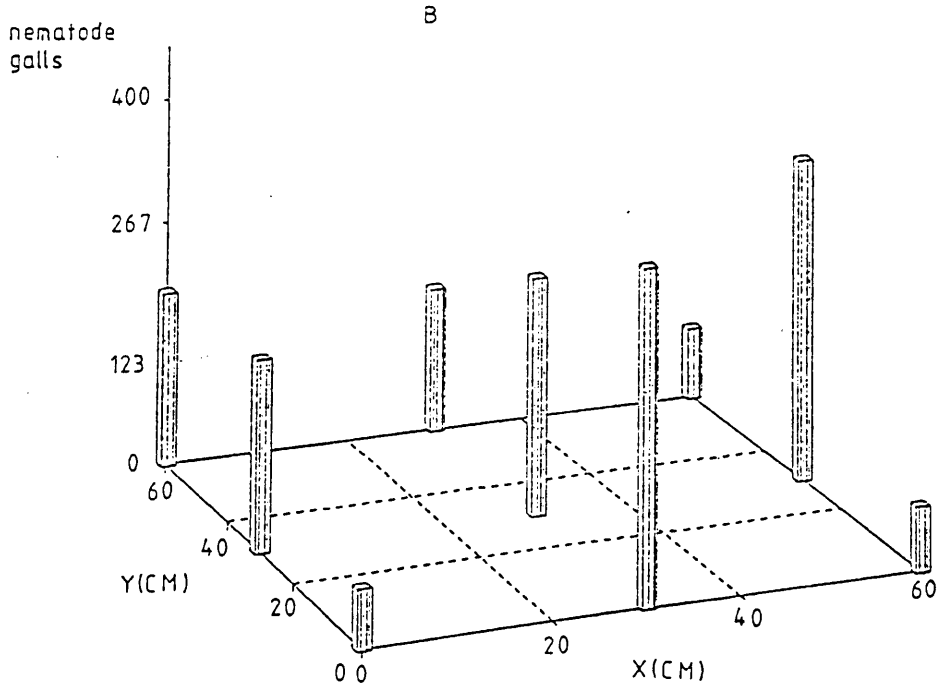
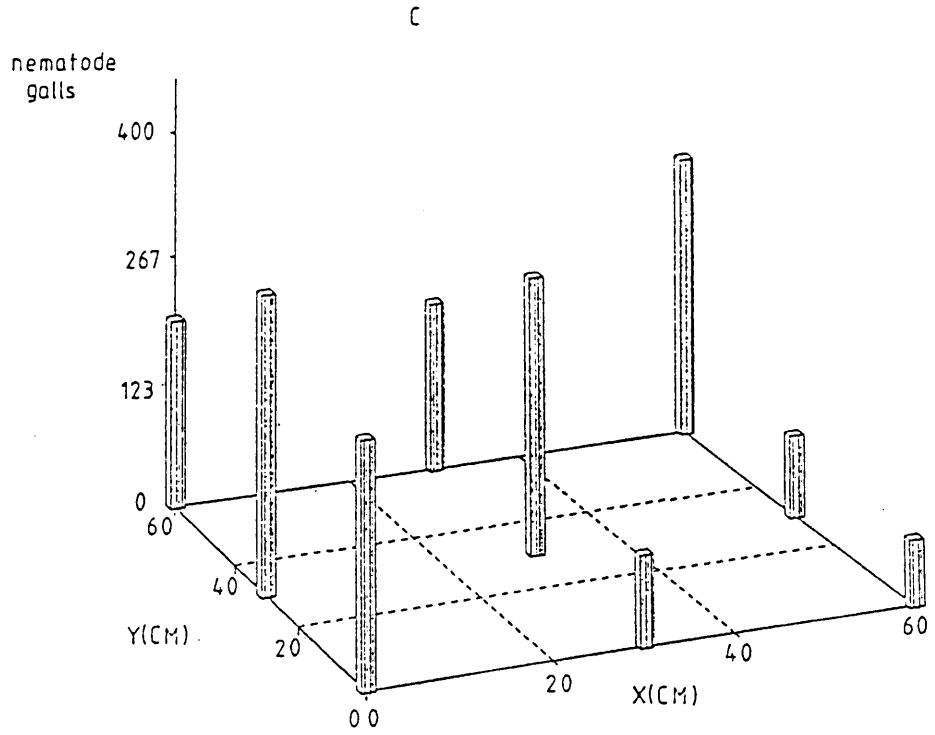


Figure 10 Nematode galls for individual tomato plants in control plots illustrating soil populations after conclusion of the experiment (plots A & B)



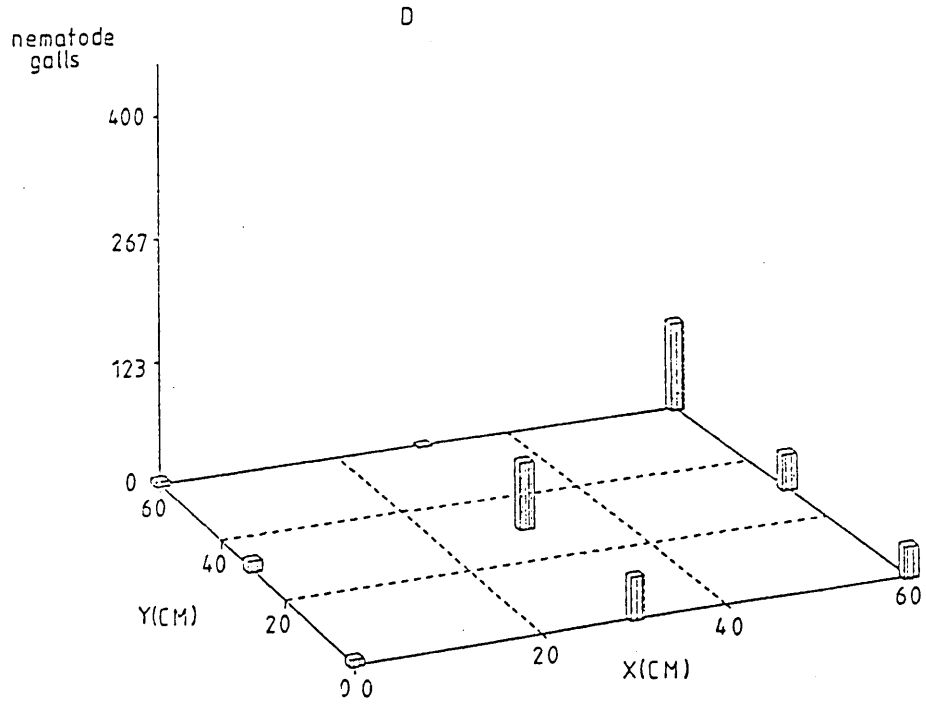


Figure 11 Nematode galls for individual tomato plants in control plots illustrating soil populations after conclusion of the experiment (plots C & D)

be used as a norm. Four lateral roots, six cm long would serve as a standard sampling norm for galling counts. A predetermined norm for low, medium and high nematode soil counts would be determined. These values would then be used when measuring soil for root-knot infestation. Other species of plant parasitic nematodes could be tested using other susceptible indicator plants. Although more expensive and time consuming, this method would give a more accurate picture of the soil nematode population especially of soil from commercial nurseries and fields to be planted to a specific crop.

The individual counts of root galls for each tomato plant in the control plots were plotted to form a series of 4 sets of histograms presented in Figures 10 and 11. The distribution of the nematode galls is clearly determined by the number of eggs and larvae in the immediate vicinity of the individual plants. (Taylor & Sasser, 1978). The A5 control plot shows this graphically as adjacent plants to the totally infested 5th plant (more than 350 galls) have only 89 and 83 galls respectively. Even with the small size of larvae (± 0,4 mm length), movement between the sand and clay particles is not rapid and the larvae move to the nearest susceptible root, here being the nearest tomato root (Taylor & Sasser, 1978).

The aldicarb treatment did not reduce the number of galls per plant over that for the control even though complete control was achieved in an earlier pot experiment by the same author. Taha & Salem (1979) also found that fenamiphos controlled root-knot

nematodes better than aldicarb in a sandy soil field trial. Here, a reason could be that the aldicarb acts as a fumigant and under the field conditions (present at the time) did not give effective control. The pot experiment, however, had conditions where the plastic pot kept the fumigant action working long enough to either kill the larvae or to make the larvae non-infective. The infestation by larvae was observed in the field trial plants to occur twenty millimetres further away from where the larvae entered other treatment plants. This could be explained by a temporary control by aldicarb before this chemical became ineffective.

The oxamyl did cause a slight reduction in the galling incidence although the 20% reduction could be ascribed to the normal variability of larvae in the soil besides not being significant over the control and would in this case not be recommended for controlling root-knot nematodes under field conditions.

6.4 Conclusion

The aldicarb- (Temik) only had a temporary effect on nematode infestation due to its possible action as a soil fumigant. This control in the field situation was poor and according to the statistical analysis no better than if no nematicide was used.

Oxamyl (Vydate) did cause a 20% reduction in galling but this was insignificant and could have been due to the normal variability of the nematode larvae within the soil.

Fenamiphos- (Nemacur) brought about almost complete control of root-knot nematodes and was significantly better using the Friedman analysis of variance at the 5% level over the control.

The compost which was also included as a treatment brought about a 56% decrease in nematode galls over that for the control, but was still statistically non-significant whilst the application of 450 m^3 per hectare would be prohibitive.

The soil numbers of root-knot nematodes were found to be non-uniformly distributed in the soil. Susceptible tomato plants planted in the soil also showed non-uniform infestation patterns which resulted in problems with the analysis of the results of treatments applied to the area.

The method whereby soil root-knot nematode numbers are determined at present is not accurate enough due to many nematodes remaining within the eggs as 2nd stage larvae and also by not being present in the soil samples taken.

The only nematicide which proved to be significantly better than no nematicide and would be recommended for tomato plantings on lands infested with root-knot nematodes would be fenamiphos-(Nemacur).

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7. RESISTANCE OF TWO TOMATO CULTIVARS TO THE ROOT-KNOT NEMATODE, *Meloidogyne incognita*.

7.1 Introduction

To help protect plants from the attacks of pests, resistance of one form or another is introduced through breeding so that the pest either finds the plant unpalatable, difficult to attack, unattractive or chemical changes within the plant itself lead to the pest either starving to death or being held at bay (Van Emden, 1976).

In the tomato, resistance against nematode attack is governed by a single gene (Taylor & Sasser, 1978). To date, it has not been shown that more than one resistant gene is present in resistant tomato cultivars. Dropkin (1969) showed that the plants' resistance to root-knot nematode could be broken if the day temperature remained at 32°C for longer than two consecutive days. The resistant plant would be invaded by nematode larvae and they would complete their life cycle within the root. If more than one gene were present at separate loci, resistance would be better as a broader spectrum of control would be attained over variables (Sidhu & Webster, 1973).

To be able to determine whether nematode larvae enter resistant tomato cultivar roots an experiment was laid out. If larvae did

enter the roots, they would die inside and this would be observed. In this case, the normal syncytia would not develop (Dropkin & Webb, 1967), necrosis of tissue would be observed and the larvae would die of starvation.

If no larvae could be found within the root tissue, the resistance exhibited by the plants would be predetermined and the larvae would be kept at bay.

Two susceptible tomato cultivars were also included so that normal entry, syncytial development and nematode growth could also be observed.

7.2 Material and methods

Seeds of susceptible Napoli VF (processing cultivar) and Floradade (fresh market cultivar) together with seeds of the resistant processing cultivar Rossol VFN and fresh market cultivar ACE VFN were sown in Speedling trays. The soil mixture used in the Speedling trays was nematode free due to prior heat treatment (Hartmann & Kester, 1975).

Two asbestos troughs each 1 m long (capacity 67,5 l), which contained an average of 30 nematode larvae/50 ml of soil were used as an inoculation and growing medium for all tomato cultivars. Larval numbers were counted according to Kleynhans (1990). At the two leaf stage, the tomato seedlings were trans-

Figure 12 Meloidogyne incognita larva observed between the mesophyll cells of tomato roots.

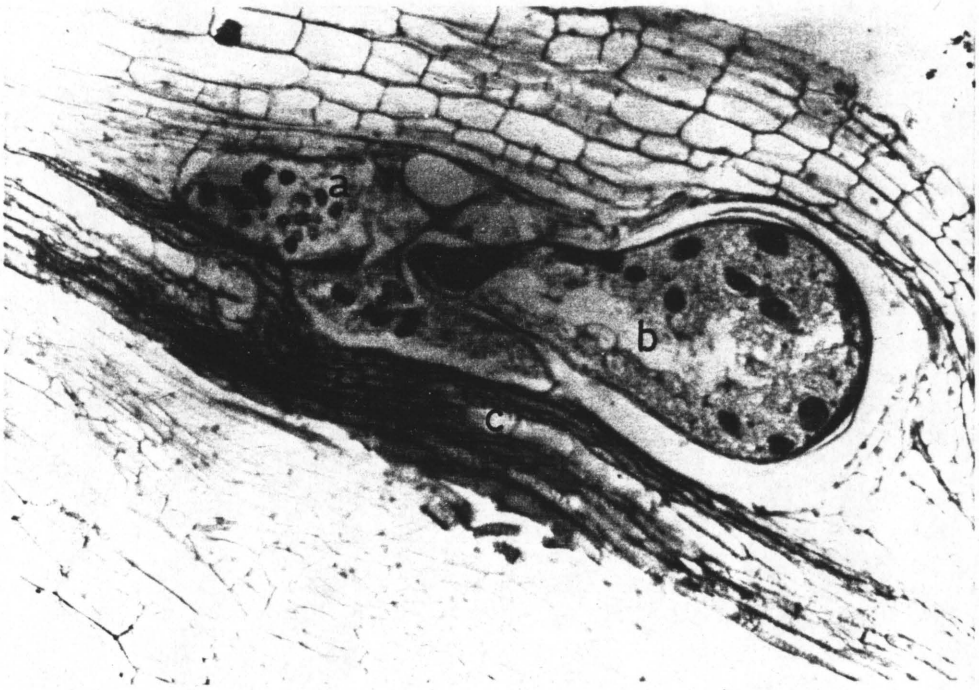

Figure 13 Female root-knot nematode lying alongside the vascular stele within the tomato root.
a = syncytia (giant cells), b = female nematode
c = vascular stele.

ferred to the troughs (two cultivars per trough) and then two seedlings of each cultivar were removed at 2, 4, 6, 8, 10, 14 and at 21 days after transplanting. The roots of the plants were carefully washed to remove soil, placed in FAA, embedded in wax, were sectioned, stained and mounted on slides according to the procedure by Holtzhausen (1972).

7.3 Results and discussions

The life cycle of the root-knot nematode begins with the hatching of the second-stage nematode larva from the egg, which then enters the root behind the root cap (Taylor & Sasser, 1978).

The larva then moves between the mesophyll cells (Figure 12) before settling near the central axis of phloem and xylem (Heyns, 1971).

In this study, nematodes only settled and developed successfully in the two susceptible cultivars, Napoli VF and Floradade. In figure 13, the female nematode can be observed lying alongside the vascular stele of the tomato root. This particular female has already caused the characteristic enlargement of cells (hypertrophy) of the vascular stele by secretions which were injected earlier (Taylor & Sasser, 1978).

These giant cells, also called syncytia contain numerous nuclei Figure 13) and in Figure 14 an enlargement of these cells shows

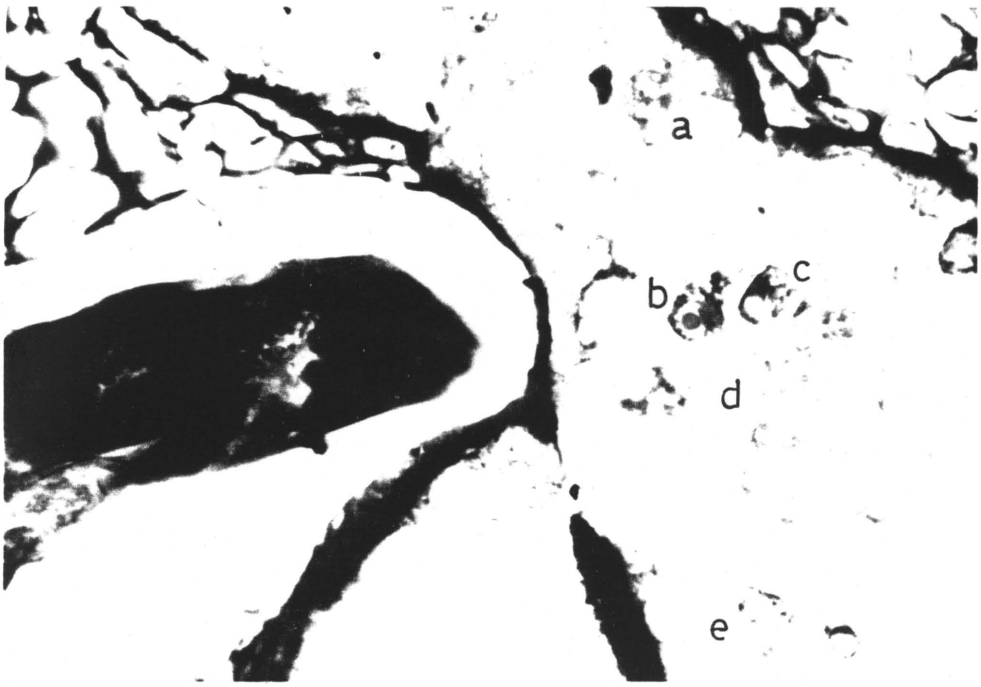

Figure 14 Nuclei within syncytia (giant cells) within the tomato root a,b,c,d & e = nuclei

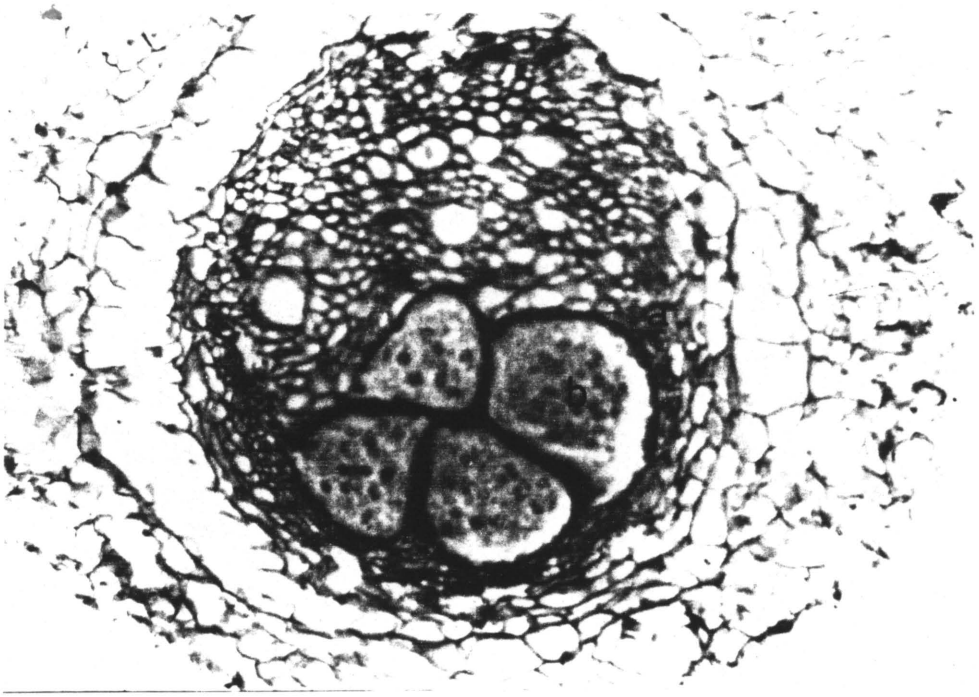

Figure 15 Syncytia (giant cells) occupying 40% of the vascular stele within the tomato root
a = vascular stele, b = giant cell.

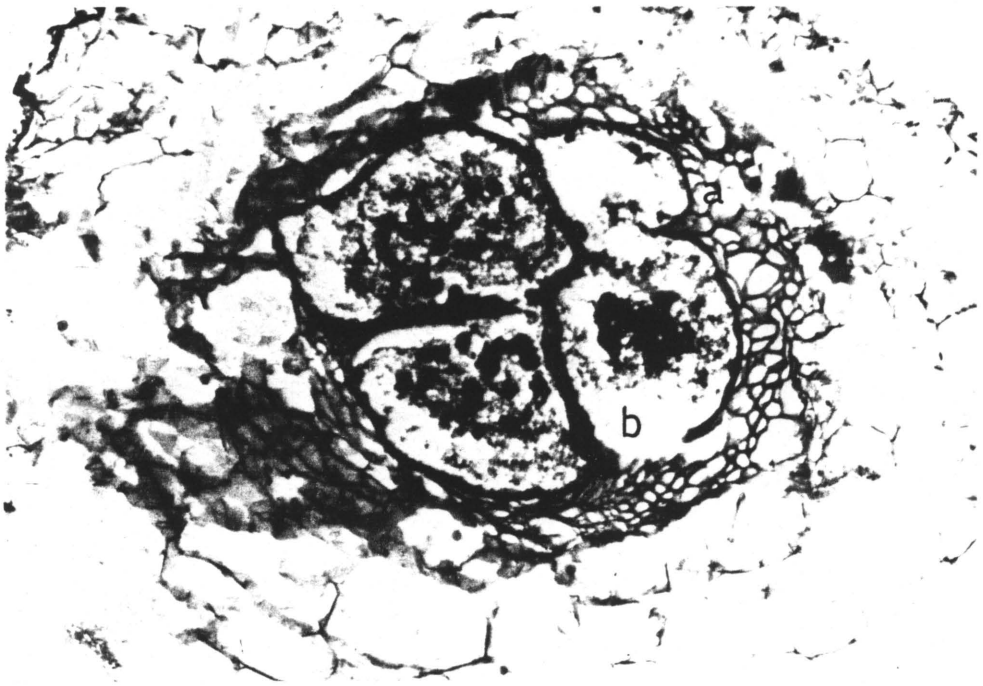

Figure 16 Syncytia (Giant cells) occupying 80% of vascular stele within the tomato root

a = vascular stele, b = giant cell

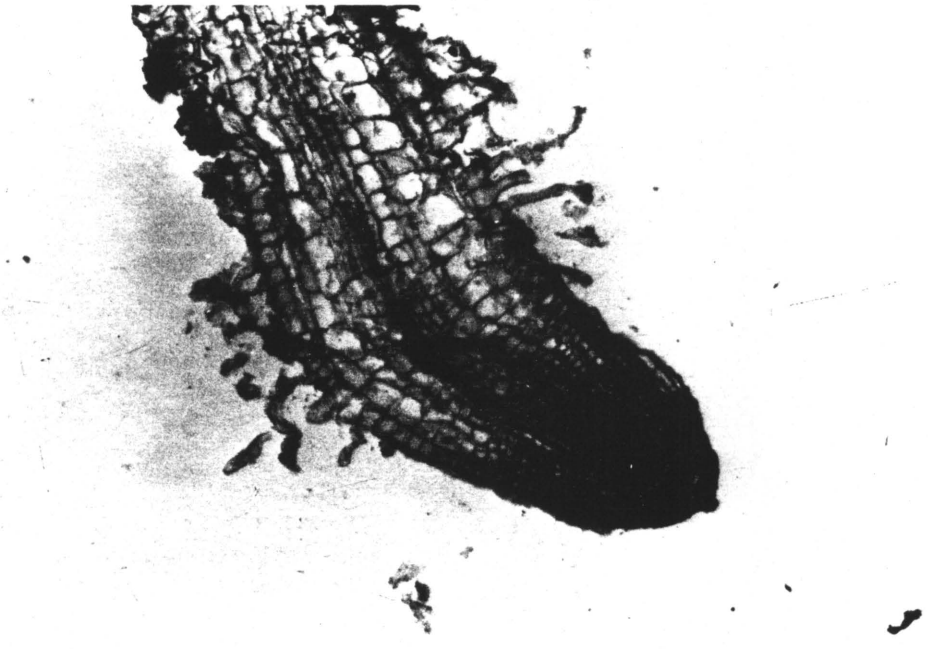

Figure 17 Umdamaged root of resistant tomato cultivar showing no evidence of entry by root-knot nematode larvae.

the nuclei which help to transform the cells into nutrient sinks (Ishibashi & Simizu, 1970). The nematode can thus feed on the enriched cell contents and develop to maturity.

The dramatic increase in the giant cell volume can be clearly seen in the Figures 15 & 16. In Figure 15 the area of the vascular stele already occupied by the giant cells is already around 40% whilst in Figure 16 this is near to 80%. The vascular stele contains the xylem and phloem and when 80% of this area is occupied by giant cells, it will have a profound effect on the translocation of water and of soil nutrients upwards to the leaves. The remaining xylem vessels can also be malformed (Figure 13), also depicted by Meon, Wallace & Fisher, (1978), and thus cannot function efficiently. A typical symptom of nematode infestation is premature wilting and nutrient deficiency symptoms (Taylor & Sasser, 1978).

The resistant tomato cultivar's roots showed no sign of penetration by the second stage larvae as a typical longitudinal section in Figure 17 depicts. The roots were healthy without any visible necrosis of internal tissue as suggested by Dropkin & Webb (1967). Although the initial inoculum of 30 larvae/50 ml of soil is not particularly high, some evidence of nematode larvae penetration should have been observed in the 400 samples examined. The susceptible cultivars' roots were, however, severely infested at this same inoculum strength.

The theory that the nematode larvae were prevented from entering the roots of the resistant tomato cultivars would be upheld in this case.

7.4 Conclusion

The two resistant tomato cultivars, Rossol VFN and ACE VFN showed no signs of entry by second stage root-knot nematode larvae even although 400 slides were inspected. The larvae were therefore prevented from entering these roots in this experiment.

Although in this experiment, resistance was confirmed, it has been found that the resistance to root-knot nematodes is easily broken by stress factors including temperatures above 32°C. This must be kept in mind especially in areas where day temperatures exceed 32°C.

The susceptible tomato cultivars, Napoli VF and Floradade demonstrated the crippling effect nematodes cause especially if their actions prevent the normal flow of water and nutrients to the leaves. The vascular stele becomes distorted by the giant cells and these cells prevent water and nutrient movement upwards to the leaves. The process of entry and development of the root-knot nematode conforms to that already documented in the literature.

The two cultivars of resistant tomato would be ideal for the farmer who farms in an area where air temperatures do not exceed 32°C and who experiences losses in tomato yields due to root-knot nematodes.

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8. Final Conclusion

The three methods of controlling Meloidogyne spp. which were investigated in tomatoes, namely the use of organic amendments, nematicides and plant resistance gave interesting results as well as scope for future research in a field which is often overlooked.

A compost concentration of 12 - 16% (volume basis) was critical for nematode decline and at 40% there was a significant reduction in nematode numbers which would benefit nurserymen who had a root-knot problem in plants attacked by this pest. At 30% compost concentration it was found that there was re-infestation by the nematodes in both pot and field trials and that the control was only of a temporary nature. The practicability and cost of applying vast amounts of compost (450 m^3/ha) would be prohibitive to most farmers especially as the control effect is only temporary.

The agent within the compost which brought about the dramatic decline in root-knot nematode numbers could have been a bacterium or combination of bacteria. The experiment laid out to verify this theory unfortunately could not give conclusive evidence in this regard. The problem was probably due to fluctuations in soil temperatures which made the nematode larvae non-infective or prevented them from developing further within the tomato roots.

The use of nematicides in controlling root-knot nematode infestation of tomatoes gave different degrees of control when pot verses field experiments were analysed. The pot experiments showed that both aldicarb (Temik) and fenamiphos (Nemacur) gave excellent results whilst in the field trial, only fenamiphos gave significant control. Oxamyl (Vydate) proved unable to effect meaningful control in both pot and field trial.

The technique as well as results obtained by the present method whereby soil root-knot nematode numbers are presently determined is questioned as this method does not give an accurate enough picture of the real soil population. The technique whereby host plants are used for root-gall counts for specific species of root-knot nematodes would be a more accurate although more consuming method of determining soil populations.

Root-knot nematode-resistant tomato cultivars displayed excellent resistance to infestation even though resistance was governed by a single resistant gene. The resistance exhibited by two resistant tomato cultivars prevented nematode larvae from entering the roots. The susceptible tomato cultivars were infested with the nematode larvae with the life cycle conforming to previous observations.

It is finally concluded that excellent control of root-knot nematodes can be achieved for specific situations and conditions by organic matter, the nematicide, fenamiphos as well by using nematode resistant tomato cultivars. For the small scale tomato farmer however, the use of resistant cultivars offers the best option at present.

ADDENDUM

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1          Section 1                1
 Genstat 5  Release 1.2  (IBM-PC/DOS)
 Copyright 1987, Lawes Agricultural Trust (Rothamsted Experimental Station)

    1  JOB 'ROOTLENGTH'
    2  "Average nematode root galls per 24cm root length and 0.5g root mass
   -3        LOG TRANSFORMATION "
    4  UNIT    [NVALUE=6]
    5  VARI    [VALUES=0,7.1,14.3,28.6,57.1,85.7]C_LEVEL
    6  VARI    [VALUES=3.654,3.504,2.964,1.752,0.0,0.0]MEAN[1]
    7  VARI    [VALUES=4.091,4.277,3.812,1.951,0.220,0.0]MEAN[2]
    8  VARI    [VALUES=3.872,3.891,3.388,1.852,0.110,0.0]MEAN[3]
    9  CALC    CQUADR=C_LEVEL**2  &  CCUBE=C_LEVEL**3
   10  FOR     Y=MEAN[1,2,3]
   11  MODEL   Y;FITTED=FITTED
   12  FIT     C_LEVEL,CQUADR
   13  GRAPH [NR=20;NC=45] Y,FITTED;C_LEVEL;METH=P,C;SYMB='*','.'
   14  FIT     C_LEVEL,CQUADR,CCUBE
   15  GRAPH [NR=20;NC=45] Y,FITTED;C_LEVEL;METH=P,C;SYMB='*','.'
   16  DELETE FITTED
   17  ENDFOR
1

 17.....................................................................


 ***** Regression Analysis *****

  Response variate: MEAN[1]
      Fitted terms: Constant, C_LEVEL, CQUADR


 *** Summary of analysis ***

                d.f.          s.s.          m.s.
 Regression        2       13.6642        6.8321   **
 Residual          3        0.3217        0.1072
 Total             5       13.9859        2.7972

 Percentage variance accounted for 96.2


 *** Estimates of regression coefficients ***

                  estimate          s.e.  cv%          t
 Constant            3.986         0.250  6.27     15.98  **
 C_LEVEL           -0.0976        0.0173 17.73     -5.64  **
 CQUADR          0.000580      0.000197 34.0       2.95  **

1
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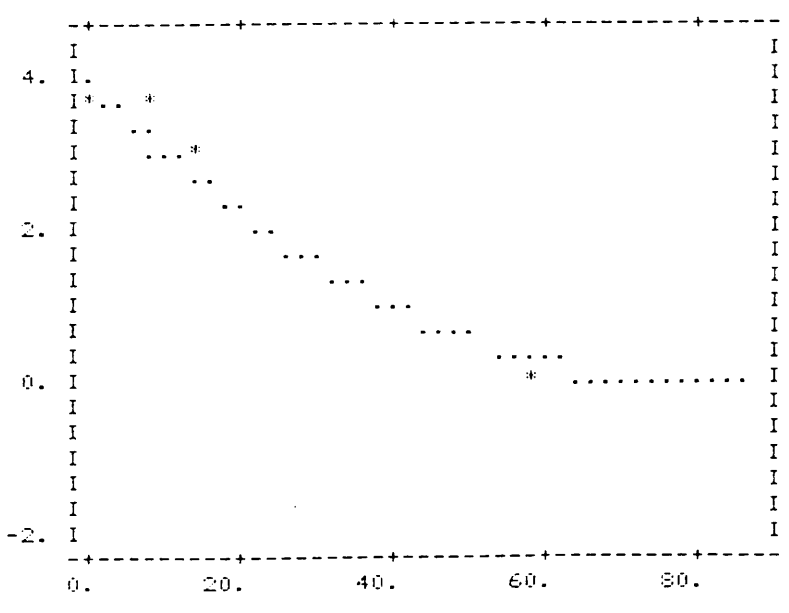



```
                  MEAN[1]  v. C_LEVEL using symbol *
                  FITTED   v. C_LEVEL using symbol .
```

***** Regression Analysis *****

MASS

Response variate: MEAN[2]
Fitted terms: Constant, C_LEVEL, CQUADR

*** Summary of analysis ***

	d.f.	s.s.	m.s.	
Regression	2	18.2226	9.1113	**
Residual	3	0.8673	0.2891	
Total	5	19.0900	3.8180	

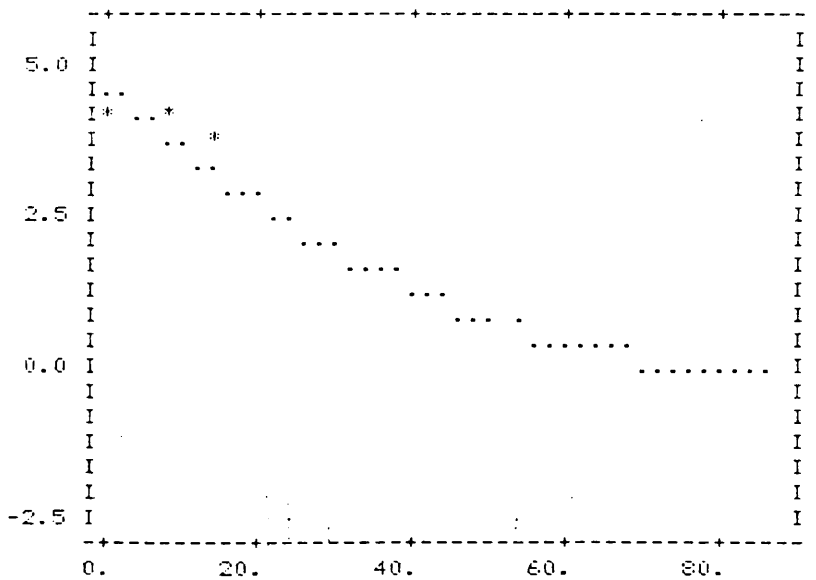
Percentage variance accounted for 92.4

log(N_GAUS) = 4.796 − 0.0982 COMPOST% + 0.000579 COMPOST%²

*** Estimates of regression coefficients ***

	estimate	s.e.	cv%	t	
Constant	4.655	0.410	8.81	11.36	**
C_LEVEL	-0.1055	0.0284	26.9	-3.71	**
CQUADR	0.000579	0.000323	55.8	1.79	NS

1



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Genstat 5  Release 1.2  (IBM-PC/DOS)
Copyright 1987, Lawes Agricultural Trust (Rothamsted Experimental Station)

    1  JOB 'ROOTLENGTH'
    2  "Average nematode root galls per 24cm root length and 0.5g root mass
   -3         LOG TRANSFORMATION "
    4  UNIT    [NVALUE=6]
    5  VARI    [VALUES=0,7.1,14.3,28.6,57.1,85.7]C_LEVEL
    6  VARI    [VALUES=3.654,3.504,2.964,1.752,0.0,0.0]MEAN[1]
    7  VARI    [VALUES=4.091,4.277,3.812,1.951,0.220,0.0]MEAN[2]
    8  VARI    [VALUES=3.872,3.891,3.388,1.852,0.110,0.0]MEAN[3]
    9  FOR     Y=MEAN[1,2,3]
   10  GRAPH [NR=20;NC=45] Y:C_LEVEL
   11  ENDFOR
1
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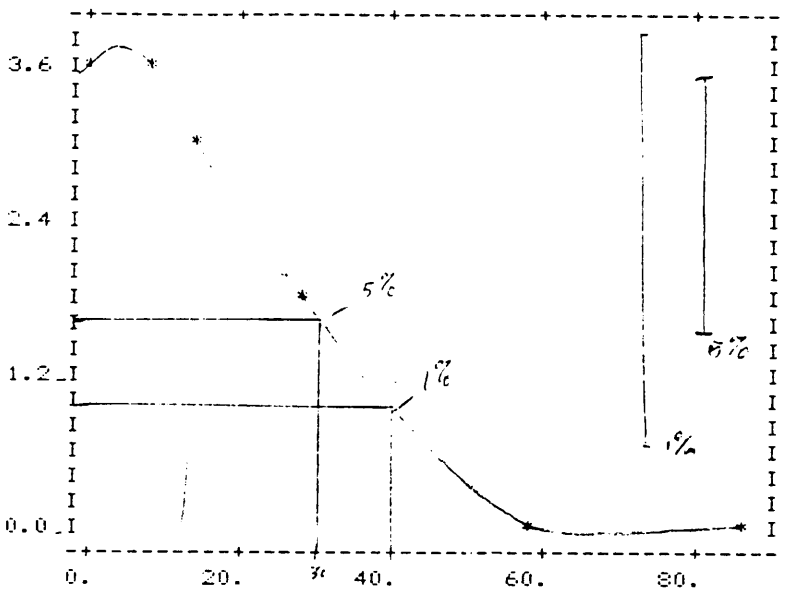
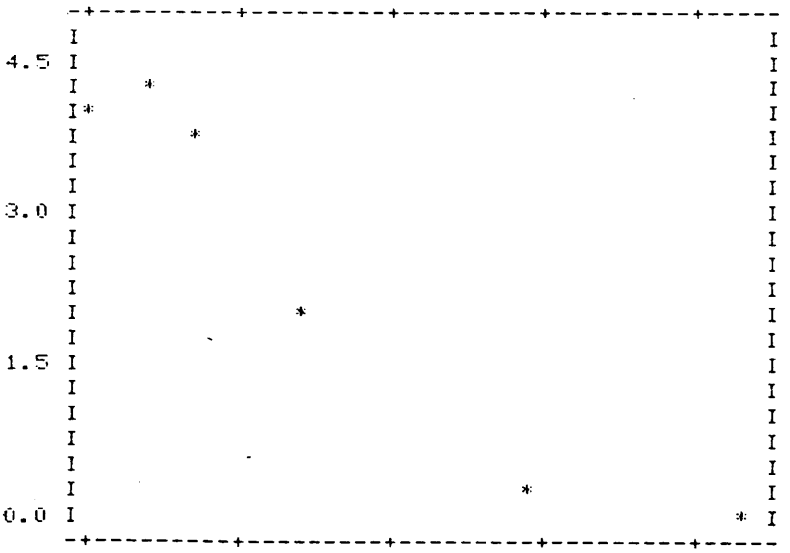


MEAN[1] v. C_LEVEL using symbol *

log(LENGTH)

t_{4DF} = 2,0154 (5%)
= 2,6423 (1%)

1



Log(MASS)

Section 2

: INFESTATION IN THE PRESENCE OF VARIOUS BACTERIA

NO.OF TREATMENTS = 9
NO.OF BLOCKS = 4
REQUEST.TEST LEVELS ARE:
GENERAL: 0.050
ADDITIVITY: 0.050
HOMOGENEOUS VARIANCES: 0.050
BETA FOR HARRIS ET AL: 0.800

FORMAT FOR DATA IS (F1.0,3F2.0)

INPUT DATA

TREATMENT NUMBER	BLOCK NUMBER 1	2	3	4
1	0.0000	2.0000	1.0000	0.0000
2	0.0000	1.0000	3.0000	0.0000
3	3.0000	1.0000	2.0000	1.0000
4	0.0000	0.0000	1.0000	0.0000
5	6.0000	1.0000	2.0000	3.0000
6	0.0000	0.0000	1.0000	1.0000
7	1.0000	2.0000	2.0000	2.0000
8	0.0000	0.0000	1.0000	0.0000
9	5.0000	3.0000	1.0000	4.0000

FRIEDMAN ANALYSIS OF VARIANCE (NON-PARAMETRIC) ← agv nie-homogene variansies.

TREATMENT NO.	1	2	3	4	5	6	7	8
RANK TOTALS	16	20	25	11	29	14	23	11

CHI-SQUARED VALUE= 15.9167 * (AT P=0.050 AND 8 D.F.)
(TE. 507 Tabel χ^2)

COMPARISON OF RANK TOTALS

LSD VALUE AT ALPHA=0.05 IS 24.02859

	2	3	4	5	6	7	8	9
1								
2								
3								
4								
5								
6								
7								
8								

RANK TOTALS IN ASCENDING ORDER

TREATMENT NUMBER	4	8	6	1	2	3	7	5
RANK TOTAL	11	11	14	16	20	25	23	29

29 - 11 = 18 ≪ LSD (24.03)

= geen betekenisvolle verskille nie

* FOR EXPERIMENT USING 2 VARIETIES OF TOMATO & 3 NEMATICIDES *

NO.OF TREATMENTS = 10
NO.OF BLOCKS = 4
REQUEST.TEST LEVELS ARE:
GENERAL: 0.050
ADDITIVITY: 0.050
HOMOGENEOUS VARIANCES: 0.050
BETA FOR HARRIS ET AL: 0.800

FORMAT FOR DATA IS (8F3.0)

INPUT DATA

	TREATMENT NUMBER	BLOCK NUMBER 1	2	3	4	Rank totals
Cult 1	1	1.0000 5	0.0000 3	0.0000 2	0.0000 3	13
	2	0.0000 2	0.0000 3	1.0000 4.5	0.0000 3	17.5
	3	0.0000 2	1.0000 6	0.0000 2	1.0000 6	16
	4	14.0000 7	4.0000 7	2.0000 7	2.0000 7	28
	5	18.0000 8.5	30.0000 10	10.0000 9	15.0000 9	36.5 →
Cult 2	6	0.0000 2	0.0000 3	1.0000 4.5	0.0000 3	12.5
	7	1.0000 5	0.1000 3	0.0000 2	0.0000 3	13
	8	1.0000 5	0.1000 3	2.0000 6	0.0000 3	17
	9	13.0000 8.5	4.0000 8	15.0000 8	12.0000 8	32.5
	10	23.0000 10	10.0000 9	20.0000 10	30.0000 10	39

[illegible]

FRIEDMAN ANALYSIS OF VARIANCE (NON-PARAMETRIC) → agv nie-homogene variansies

TREATMENT NO.	1	2	3	4	5	6	7	8	9	10
RANK TOTALS	13	13	16	28	36	13	13	17	34	39

CHI-SQUARED VALUE= 22.4455 * (AT P=0.050 AND 9 D.F.)

COMPARISON OF RANK TOTALS

LSD VALUE AT ALPHA=0.05 IS 27.09142

	2	3	4	5	6	7	8	9	10
1									*
2									*
3									
4									
5									
6									*
7									*
8									
9									

RANK TOTALS IN ASCENDING ORDER

TREATMENT NUMBER	6	2	1	7	3	8	4	9	5	10
RANK TOTAL	13	13	13	13	16	17	28	34	36	39

39 - 13 = 26 dit is nie kleiner as LSD.

* TOMATO FIELD EXPERIMENT

NO.OF TREATMENTS = 5
NO.OF BLOCKS = 4
REQUEST.TEST LEVELS ARE:
GENERAL: 0.050
ADDITIVITY: 0.050
HOMOGENEOUS VARIANCES: 0.050
BETA FOR HARRIS ET AL: 0.000

FORMAT FOR DATA IS (4F7.2)

INPUT DATA

TREATMENT NUMBER	BLOCK NUMBER			
	1	2	3	4
1	154.8900	78.5600	79.5600	269.1100
2	0.3300	4.1100	5.2200	0.3300
3	12.1100	161.7800	184.5600	130.0000
4	124.0000	20.1100	21.6700	40.8900
5	35.2200	198.6700	191.8900	182.5600

FRIEDMAN ANALYSIS OF VARIANCE (NON-PARAMETRIC)

TREATMENT NO.	1	2	3	4	5
RANK TOTALS	13	4	13	11	17

CHI-SQUARED VALUE= 10.0000 * (AT P=0.050 AND 4 D.F.)

COMPARISON OF RANK TOTALS

LSD VALUE AT ALPHA=0.05 IS 12.20007

	2	3	4	5
1				
2			*	
3				
4				

RANK TOTALS IN ASCENDING ORDER

TREATMENT NUMBER	2	4	3	1	5
RANK TOTAL	4	11	13	13	17