THE RESPONSE OF TOBACCO VARIETIES TO INFECTION BY *MELOIDOGYNE* SPECIES IN RELATION TO NEMATODE OCCURRENCE AND INFESTATION IN BUSIA AND BUNGOMA DISTRICTS OF KENYA.

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A thesis submitted to the University of Nairobi in partial fulfilment of the requirements for the degree of Master of Science in Plant Pathology.

SEPTEMBER 1984.

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signed _____ Date 22-11-84

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This thesis has been submitted for examination with our approval as University supervisors.

Signed _____ Date 22nd Nov. 1984.

Dr. E.M. Gathuru

Signed _____ Date 22nd Nov 1984

Prof. D.M. Mukunya

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Dedications

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A B S T R A C T

The occurrence and infestation of plant-parasitic nematodes on tobacco, *Nicotiana tabacum* L., in Busia and Bungoma districts of Kenya was investigated during 1983. Samples of tobacco roots and soil from 238 different sites were examined. The nematodes were extracted by the Baermann's pan and filter method, and then, identified to genus, except for rootknot nematode, *Meloidogyne* Goeldi, which was identified to species level.

The response of tobacco varieties; air-cured "Burley 181", fire-cured "Heavy Western" and flue-cured "Speight G28", to increasing infestation levels of rootknot nematodes, were determined in 60 different plots of either small-holder farmers or the tobacco company, B.A.T. (Kenya) Limited. Similar studies were also done on potted plants under glasshouse.

Some seven genera of plant-parasitic nematodes were commonly found associated with tobacco roots. They were; rootknot nematode, *Meloidogyne* spp., root lesion nematode, *Pratylenchus* spp., stubby root nematode, *Tylenchorhynchus* spp., spiral nematodes; *Scutellonema* spp., *Helicotylenchus* spp. and *Rotylenchus* spp., and sheath nematode, *Hemicycliophora* spp. The rootknot nematode caused conspicous galls on tobacco roots, and severely

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infected plants were considerably stunted in growth. The nematode was found to be present in all the sites sampled, and it contributed upto about 85% of the total population count of parasitic nematodes within and around tobacco roots at postharvest sampling. The specie *Meloidogyne javanica* (Treub.) Chitwood was predorminant with an incidence of 85%. *M.incognita* (Kofoid and White) Chitwood made up the remaining 15%.

On field plots where the preplant infestation levels of rootknot nematodes ranged from 5.9 to 10.2 larvae per 100 g of soil, the tobacco plants on sandy or gravel soils were more severely galled and stunted, relative to those on clay soils. Nematicide trials with phenamiphos at 10 Kg, a.i., per ha, on paired plots of tobacco always gave significantly ($P = 0.05$) better plant growth on sandy soils but not always on clay soils. Inspite of considerable galling of roots, the nematodes apparently caused relatively less reduction of growth and yields of tobacco plants in clay soils. Similar results occurred on potted plants as well.

Tobacco variety "Speight G28" was more tolerant to infection by rootknot nematodes at increasing infestation levels, relative to varieties "Burley 181" and "Heavy Western". The latter two were usually equally galled and stunted at similar inoculum levels.

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1. INTRODUCTION

1.1 The plant-parasitic nematodes

Plant-parasitic nematodes are transparent worms of microscopic size. Most species are vermiform in shape and measure 1-2 mm in length and less than 0.05 mm in width. In some genera such as Meloidogyne Goeldi 1887, Heterodera Schmidt 1871, and Tylenchulus Cobb 1913; the mature females are enlarged and nearly spherical (Thorne, 1961).

The generalised life cycle of plant-parasitic nematodes starts from an egg which hatches into a larva. The larva passes through four moulting stages before it becomes an adult. The second stage larva is often the infective one. While the females are parasitic at all stages thereafter, the males are usually only parasitic upto the last stage of moulting. Mature males are often found freeliving in soil (Christie, 1959).

Plant-parasitic nematode are majorly soil-borne pathogens whereby they attack roots of plants. Examples of the root attackers are Meloidogyne spp., Pratylenchus spp. and Xiphinema spp. (Jenkins and Taylor, 1967). Relatively few species of nematodes

attack aerial parts of plants. Examples are Ditylenchus spp. and Aphelenchoides spp. (Christie, 1959).

The nematodes feed on plant tissues by means of a buccal stylet which is thrust into the cortical cells. Salivary secretions consisting of enzymes and hormones are used to digest the cell metabolites extracellularly; which are subsequently ingested by the nematodes (Doncastes, 1971). The host plants are thus robbed of their nutrients and starved. Qualitative and quantitative injuries to plant tissues varies somehow with different nematode species. Meloidogyne spp. induce gall formation on roots thus hindering mechanical translocation of water and nutrients through the roots. Pratylenchus spp. cause necrotic lesions as they move through the cells. Plants which are heavily infected with nematodes are usually stunted, chlorotic and wilt temporarily in warm weather (Bird, 1974).

Nematode attack has been widely reported to make host plants more susceptible to other fungal and bacterial pathogens (Powell et al, 1971). Nematode infection wounds are easy infection points for soil-borne pathogens like Fusarium spp., Phytophthora spp. and Pseudomonas spp.

Certain plant-parasitic nematodes have been recorded as vectors of plant viruses. For example, Trichodorus spp. are the main vectors of Tobacco rattle virus (Caldman, 1963), and Xiphinema americanum transimits Tobacco ringspot virus (Rush, 1970).

Plant-parasitic nematodes attack nearly every crop plant, and the extent of damage varies with the host plants susceptibility to the attacking nematode species. Tobacco, Nicotiana tabacum L. is very susceptible to several nematodes which include rootknot nematodes, Meloidogyne spp. Goeldi 1887; root lesion nematodes, Pratylenchus spp. Filipjev 1939; and stunt nematodes, Tylenchorhynchus spp. Cobb 1913 (Sasser and Nusbaum, 1955; Milne 1972; Lucas, 1975). The rootknot nematodes have been considered among the very important disease problems of tobacco in the tropics (Milne, 1972). On average, they cause about 10% reduction of tobacco leaf yield, on world basis.

1.2 Tobacco production in Kenya.

The commercial production of tobacco in Kenya was started in 1975 by the British American

Tobacco (Kenya) Limited, BAT (K) Ltd (Anon, 1982). Since then, the crop has rapidly become an important cash crop among several small scale farmers in areas of Busia, Bungoma, South Nyanza and Meru districts. The BAT (K) Company mostly contracts the small scale farmers and provide them with major farm inputs, extension and marketing services.

In 1982, there were 10,200 tobacco farmers who planted some 5,000 ha and produced about 5,211 tonnes of cured leaf (Anon. 1983). The financial earnings for the period 1980 to 1982 are presented in Table 1.

Table 1. National financial earnings from tobacco for the years 1980, 1981 and 1982.

Item	1980 Kshs '000	1981 Kshs '000	1982 Kshs '000
1. Value of grown tobacco	27,653	38,914	61,662
2. Net income to farmers	20,364	29,001	46,027
3. Export earnings	1,643	6,180	17,470
	(219)*	(773)*	(1,547)*
4. Taxation	588,000	670,819	794,120

*US dollars at 1982 exchange rate.

Source: BAT (K) Ltd. Statement of Accounts and Annual report, 1982.

Three types of tobacco are being grown in Kenya. These are the Virginia leaf which is flue-cured, dark tobacco which is fire-cured and Burley which is air-cured. Virginia and dark tobacco types require wood fuel for curing. To ensure the continued supply of wood fuel, BAT (K) is actively promoting agroforestry among the tobacco growers (Anon. 1982).

BAT (K) agriculturalists had reported that rootknot nematodes are causing serious damages on tobacco in most farms (1982, personal communication). Field trials of nematode control with systemic nematicides; Phenamiphos (Nemacur) and Carbofuran (Furadan), had been conducted around Malakisi in Bungoma District. Crop response was found to vary from site to site between paired treated and untreated plots. There was need therefore to investigate the factors which could have contributed to the variable crop responses.

The BAT (K) is keen on establishing a comprehensive nematode control programme on tobacco growing areas. And to that effect, there was a request for a nematode survey in the main tobacco growing areas of Busia and Bungoma Districts (1982, personal communication). Hence, this thesis reports

the research project which was undertaken to fulfil those needs.

1.3 Objectives of the study.

1. Survey of the plant-parasitic nematodes in the tobacco growing areas of Busia and Bungoma districts, highlighting the spatial distribution patterns, levels of population densities and nematode genera. There was special emphasis on rootknot nematodes, Meloidogyne species.

2. Monitoring of nematode population densities versus crop damage in tobacco field plots.

3. Response of commercial tobacco varieties (Burley 181, Heavy Western, and Speight G28) to increasing initial inoculum densities of Meloidogyne javanica and M.incognita, concomitantly, on potted clay and sandy loam soils.

2. LITERATURE REVIEW

2.1 Nematode problems of tobacco.

Plant parasitic nematodes have been reported as among the most common and leading disease problems in the production of tobacco, Nicotiana tabacum L, world wide (Sasser and Nusbaum, 1955; Akehurst, 1968; Milne, 1972; Lucas, 1975).

More than ten nematode genera have been recorded to parasitise roots of tobacco; with root-knot nematodes, Meloidogyne spp., as the most economically important one. Others, in approximate descending order of importance, are; root lesion nematodes, Pratylenchus Filipjev 1934; stem break nematodes Ditylenchus Filipjev 1936; cyst nematodes, Heterodera Schmidt 1871; stunt nematodes, Tylenchorhynchus Cobb 1931; stubby root nematodes, Trichodorus Cobb 1913; dagger nematodes, Xiphinema Cobb 1913; sheath nematodes, Hemicycliophora de Man 1921; ring nematodes, Criconemoides Taylor 1936; spiral nematodes, Rotylenchus Filipjev 1936; Scutellonema Andrassy 1958, Helicotylenchus Steiner 1945; and lance nematodes, Hoplolaimus Daday 1905; and others of low frequency of occurance (Martin, 1962; Saka and Siddiqi, 1969; Milne, 1972; Lucas, 1975).

Meloidogyne spp. are world wide in distribution, though being more predominant in the tropics and subtropics (Sasser and Taylor, 1978). The species which are most commonly reported on tobacco are M. incognita and M. javanica. M. arenaria, M. hapla and M. grahami have also been reported on tobacco but less frequently. Specie M. javanica is considered as the most aggressive and damaging on tobacco, followed closely by M. incognita (Arens, 1979; Baker et al, 1981).

The second stage larvae of Meloidogyne spp. penetrates plant roots just behind the root cap to become sedentary endoparasites. They pierce cell walls with stylets and inject into the cells secretions from their oesophageal glands. The secretions are a mixture of enzymes and hormones which induce hypertrophy and hyperplasia of cortical cells in the vascular cylinder (Doncaster, 1977). The resultant enlarged or giant cells are also referred to as syncytia. The increased cell multiplication in the vicinity of the larva is usually accompanied by enlargement of the root and formation of distinct galls or knots (Taylor and Sasser, 1978).

Tobacco roots which are heavily infected by Meloidogyne spp. at young stages of development are

usually heavily galled and have fewer branches and root hairs (Arens et al, 1981). The infected root system would therefore not be able to utilise water and nutrients from as large a volume of soil as uninfected roots. Vascular vessels in galls are usually deformed and the translocation function is mechanically hindered. Heavily infected plants are usually stunted, chlorotic and do wilt readily in warm weather even when soil moisture is still adequate for plant growth. In some cases, heavy nematode infection results in the necrosis of tobacco leaf margins; a phenomenon known as rimfiring or tip burn (Milne, 1972).

In a field of tobacco, plants which are damaged by rootknot nematodes tend to occur in scattered random patches; a distributive pattern which is characteristic of the nematodes infestation (Baker and Imbriani, 1983).

Interaction of Meloidogyne species and other pathogens to cause disease complexes on tobacco has been demonistrated by several workers (Sasser and Nusbaum, 1955; Powell, 1971; Powell et al, 1971) Crop damage and yield reduction in such multiple infections are usually much higher than when a pathogen is acting singly. Some fungal and

bacterial diseases of tobacco in which Meloidogyne infection is an important predisposing factor are; fusarial wilt by Fusarium oxysporum f. nicotiane, black shank by Phytophthora parasitica var. nicotianae, brown leaf spot by Alternaria tenuis, and Granville wilt by Pseudomonas solanacearum.

Powell et al (1971) found that some soilborne fungi which are usually non-pathogenic became pathogenic on tobacco after the roots had been invaded by Meloidogyne incognita. The fungi were, Curvularia trifolii, Botrytis cinera, Aspergillus ochraceus, Pencillium martensisii, Trichoderma harzianun and Pythium ultimum. P. ultimum causes damping off on tobacco seedlings, but on its own it does not attack mature tobacco in the field.

Daulton (1963) estimated the loss of tobacco yield attributable to rootknot nematodes in Zimbambwe at 6-10%, annually. But on individual plots of small scale farmers, the loss could be as much as 25-50%. Taylor and Sasser (1978) estimated that 10% of the world's tobacco crop is lost due to rootknot nematodes.

Meloidogyne species generally occur in a wide range of soil types. However, the extent of crop damage has been observed to be greater in sandy soils

or sandy patches within a field (Whitehead, 1969; Ferris and Mckerny, 1977; Taylor and Sasser, 1978). Tobacco grown in fertile clay loam soil was able to tolerate high numbers of the nematodes without significant economic yield loss (Milne, 1972; Baker and Imbriani, 1983). It is apparent that stresses which plants experience in sandy soils, which are usually less fertile, accentuates nematode damage on them.

Decomposing organic matter in the soil has been reported to have inhibitory influence on the multiplication of plant parasitic nematodes (Webster, 1972; Wallace, 1973). Organic matter favours abundant occurance of fungi, bacteria and other microorganisms which are antagonistic to nematodes. Sayre et al (1971) found that decomposition byproducts of rye and timothy were about ten times toxic to Meloidogyne incognita than to the freeliving nematode, Panagrellus redivivus. Butyric acid was identified as one of the decomposition byproducts. Ammending soil with decomposing organic matter therefore helps to reduce nematode damage on plants.

Other soil factors like temperature, moisture and pH are important in the survival and development

of nematodes (Wallace, 1973). However, they are of secondary importance with regard to nematode damage on a susceptible crop since soil factors that will allow plant growth, will always allow nematode development.

Oostenbrink (1966) reported that the quantitative relationship between plant-parasitic nematodes and growth or yields of annual crops is primarily a function of preplant nematode densities. He argued that this is so because of nematodes' negligible mobility and their relatively low reproductive rate. Seinhorst (1965; 1970; 1973 and 1978) had also asserted that damage by nematodes ocurred only when their density exceeded the tolerance limit of the attacked plant. When the initial density of the nematodes was lower than the tolerance limit, they only became damaging when the subsquent density exceeded the limit. The tolerance limit of any host plant of a given genotype against a nematode species of a given pathogenecity will however vary with ecological factors (Wallace, 1973; Ferris, 1978).

The root lesion nematodes, Pratylenchus spp. are considered as the second economically important nematodes on tobacco in the tropics (Milne, 1972). They are migratory endoparasites and cause brown

rootrot disease on tobacco. The species most commonly reported on tobacco are Pratylenchus bra chyurus, P. zea, P. hexincicus, and P. neglectus.

The tobacco cyst nematodes, Heterodera spp. and stem break nematodes, Ditylenchus spp.; have not been reported as important nematodes which parasitise tobacco in the tropics (Lucas, 1975).

All the other nematode genera which were mentioned earlier as parasites of tobacco, are migratory ectoparasites of usually low economic importance. In the field, their population densities are rarely high enough to warrant concern (Milne, 1972).

2.2 Control of nematodes on tobacco

Nematodes, Meloidogyne species in particular, are usually controlled on tobacco plots through an integrated approach which includes crop rotation, farm hygiene, resistant cultivars and soil treatment with nematicides (Lucas,1975; Sasser and Taylor, 1978). Small scale farmers usually eradicate nematodes from the top soil of the prepared seedbeds by burning a pile of firewood over it (Milne, 1972).

Under crop rotation, ideally tobacco should be rotated with crops which are immune or resistant to the nematodes. But, due to the wide host range of Meloidogyne sp. and occurance of pathogenic races, that ideal situation is almost impossible. Alternative crops are often not sufficiently profitable, or they might build up other pests and diseases which attack tobacco also. Nevertheless, cereal crops and grass fallows have been used with good success in tobacco rotation (Daulton, 1963).

Several varieties of tobacco have been bred for resistance against Meloidogyne incognita. They include, "NC 95" "Speight G28", "Florida 22", and "Japanese RK 70" (Lucas, 1975). So far, there is

no commercial tobacco variety resistant to *M. javanica* (Sasser and Kirby, 1979).

Nematicides are being used effectively to reduce nematode populations in infested soils before tobacco is planted. Conventional nematicides have been liquid or gas fumigants which are injected beneath the soil surface (Daulton, 1964). They evaporate to produce fumes which kill nematodes. Common ones are; Ethylene dibromide (EDB); 1, 3, dichloropropene and 1, 2, dichloropropane (DD); and Methyl bromide (MBr). MBr is an "all rounder", which also kills soil-borne fungi, bacteria, insects and weed seeds. It is therefore commonly used on seedbeds. Fumigants are phytotoxic and require about two weeks of waiting period before the treated soil is planted (Lucas, 1975).

Newer nematicides are non-fumigants which can be applied as granules or emulsifiable liquids (Taylor and Sasser, 1978). Of growing importance are Systemic nematicides which are not phytotoxic and can be absorbed by growing plants through roots or foliage. They can therefore kill nematodes both in the soil and within the plant tissues. Common examples are phenamiphos (Nemacur), carbofuran (Furadan) and aldicarb (Temik) (Bunt, 1975).

It is a common practice by tobacco farmers to uproot and burn tobacco stubbles after harvest. The practice serves to reduce the source of disease inoculum for the next crop (Milne, 1972).

Due to the high susceptibility of tobacco to nematodes and to its similarly high value per unit of land, there has been spectacular economic returns from controlling nematodes on "problem fields". Under an integrated nematode control programme on tobacco farms in North Carolina, U.S.A., the following results have been reported:-

a. Sanitary procedures such as ploughing-up tobacco roots at the end of season, increased crop value above control plots by 68%.

b. Rotation with resistant crops added 105%.

c. Use of resistant tobacco varieties added 90%.

d. Use of soil chemicals, particularly combined nematicides and incecticides, added 132%.

The total increase was 395% per hectare and the total value was almost five times as much as without nematode and disease control (Taylor and Sasser, 1978).

2.3 Nematology in Kenya

Systematic studies of plant parasitic nematodes in Kenya started in the late fifties (Whitehead, 1958; Whitehead and Kariuki, 1960; Grisse, 1960). Those early workers conducted nematode surveys in the commercial farmlands of colonial settlers. Root knot nematodes, Meloidogyne incognita and M. javanica were noted as of wide distribution in the country and were causing economic losses on several crops such as tomatoes, potatoes, pyrethrum, beans, coffee and miscellaneous vegetables. M. hapla was found to be prevalent in the cool, high altitude areas where it attacked pyrethrum and several vegetable crops.

Whitehead (1959) did record a new species of Meloidogyne - M. africana, on coffee roots in Meru district. Grisse (1960), also recorded a new species - M. kikuyensis, on Kikuyu grass (Pennisetum clandestinum) in Muguga area. Hainsworth (1962) and Hollis (1962) wrote review papers in which they highlighted the importance of nematodes on the Kenyan agriculture.

Since the early seventies, there has been a boost in nematode research, mainly by the indigenous Kenyan scientists. Njoroge and Gichure (1973) carried

out host range studies of various plant parasitic nematodes on several crops which included pyrethrum and beans. Gichure (1973) conducted a nationwide nematode survey on bananas and found that over 90% of the banana stands were heavily infected with important nematodes like Meloidogyne spp., Pratylenchus spp., and Radopholus similis. The last one was recorded exclusively in Western Kenya. Kanyagia (1973) conducted host range studies on Meloidogyne hapla and showed that the species does attack several vegetable and field crops.

Very comprehensive studies on the host-parasite relations of Meloidogyne incognita and M. javanica on beans and, screening of several bean varieties for resistance to East Africa races of the rootknot nematodes have been done by Ngundo (1973, and 1977) Adeniji and Muigai (1978) conducted intensive studies on the distribution and crop association of plant parasitic nematodes in Kiambu district. Most of the commonly known plant parasitic nematodes were recorded.

The plant pathology section of National Agricultural laboratories in the Ministry of Agriculture offers advisory services to farmers on the diagnosis and control of nematode diseases.

The rootknot nematodes, Meloidogyne incognita and M. javanica are the most commonly reported nematode species, and they do cause economic damage on crops like tomatoes, okra, egg plants, bananas, beans and tobacco (Gatumbi, 1976).

Since commercial tobacco production in Kenya started rather recently, around 1975, there had been so far no studies on the nematode problems of tobacco. As such, the study herein reported would be an important milestone in understanding this very important problem.

3. MATERIALS AND METHODS

3.1 Nematode survey

3.1.1 Location and field description.

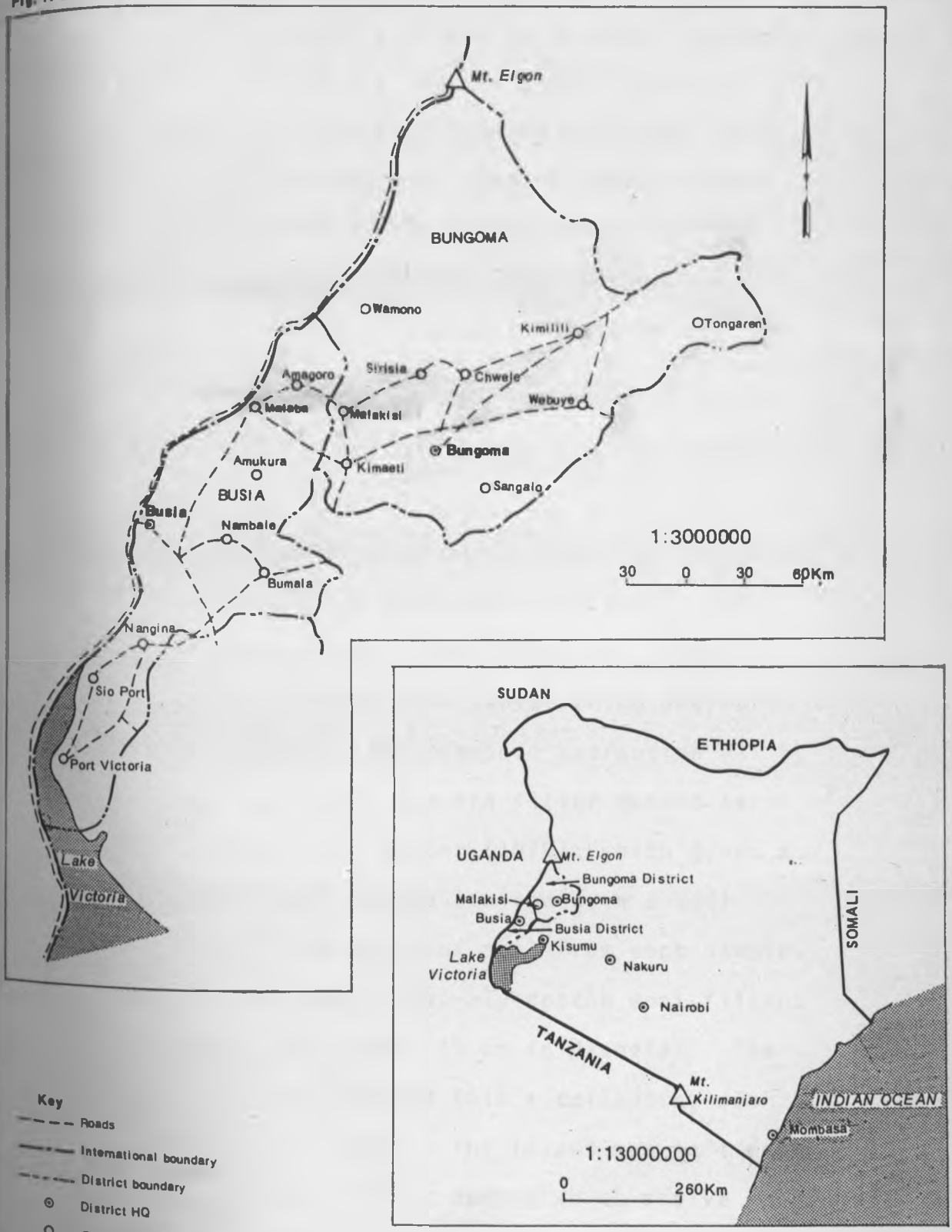
The nematode survey was conducted in the tobacco growing areas of Busia and Bungoma districts (Figure 1). The area covered was about 4,000 km^2, and lies between the equator (0^o) to 1^o N, and 34^o to 35^oE. The altitude is approximately 2,500 m and the region receives an annual rainfall of about 1,300 mm. The soil is predominantly sandy loam, and the main crops being grown are maize, millet, sorghum, beans, cowpeas, cassava, cotton, sunflower and tobacco (Sombroek et al, 1980).

3.1.2 Collection of soil samples.

Soil samples were collected from 238 different sites during the months of December 1982 and January 1983. The plots had been ploughed and were to be planted with tobacco. Each plot was about 0.5 ha.

From every plot, soil samples were dug from 10 random points. A soil auger which scooped 250 ml of soil up to a depth of 20 cm was used. The 10 soil

Fig. 1. Busia and Bungoma Districts (The tobacco growing areas under study)



samples were thoroughly mixed and a 250 ml subsample was sealed in a plastic bag and labled appropriately. Random samples of roots of tobacco and other crops of the previous season were examined for rootknot nematodes infection. Infected specimens were despatched to Plant Pathology Laboratories, University of Nairobi; for use in nematode species identification.

3.1.3 Nematode extraction.

The soil samples were kept moist at 30^{o}C for two weeks prior to nematode extraction. Such treatment was expected to facilitate the hatching of nematode eggs into active larvae which are more readily recoverable. The nematode extraction was by the modified Baermann's pan and filter method as described by Flegs and Hopper (1970); which gives a relative estimate of active nematodes in a soil sample. One hundred grams of soil from each sample was evenly spread over a two-ply cotton wool filters supported on a wire sieve; 15 cm in diameter. The sieve was carefully lowered into a collecting dish containing 90 ml of water. The layout was left on laboratory bench for 36 hrs during which active nematodes were expected to swim through into the

collecting dish.

The resulting nematode suspensions were collected in beakers. However, the suspensions were often too muddy for microscopic examination. They were clarified by repeated decanting and refilling the beakers with tap water. Each refil was left to stand for 20 min, then slowly decanted threequarter way.

3.1.4 Identification and enumeration of the plant-parasitic nematodes.

The plant parasitic nematodes were identified to genus level on the basis of morphological characteristics as described by Heyns (1971), and Mai and Lyon (1975). Individual specimens were closely examined under a compound light microscope. Population counts were done from a counting dish under a disecting microscope.

The genus Meloidogyne was identified further to species by examination of the cuticular perineal patterns of mature females, according to Taylor et al (1955). The female nematodes were obtained from galled roots of tobacco, beans, sunflower and of two weeds; Oxygonium sinuatum and Galinsoga parviflora. The roots were washed in water and then

chopped into pieces of about 2 cm long. A 100 g of the root pieces were macerated in a Waring blender for 10 seconds at 10,000 r.p.m. One hundred swollen Meloidogyne females were picked at random from the macerated pulp and their posterior cuticular parts were cut off and carefully cleaned under a dissecting microscope. The parts were stained in a solution of cotton-blue and lactophenol for 12 hours. The cuticular perineal patterns were examined under oil immersion lens of a compound light microscope.

3.2 Potted plants experiment: the response of commercial tobacco varieties to increasing population densities of *Meloidogyne javanica* and *M. incognita* concomitantly (6:1), in clay and sandy soils.

3.2.1 Preparation of soils.

Clay loam soil was prepared by thoroughly mixing red clay soil, builder's sand and horse manure in the ratio of 3:2:1, respectively. The sandy loam soil was from a mixture of red clay soil and builder's sand in the ratio of 1:1. The prepared soils were steam-sterilised in an autoclave at 120^{o}C for one hour . The sterilised soil was aerated, while being guarded against recontamination, in a glasshouse for 15 days before use.

3.2.2 Test plants

Seeds of three commercially grown tobacco varieties were obtained from BAT (K) at Malakisi leaf Center, Bungoma. The varieties were; air-cured "Burley 181", fire-cured "Heavy Western" and flue-cured "Speight G28".

The seedlings were raised in potted steam-

Sterilised clay loam soil inside a glasshouse, at a relative humidity of 90% and average daily temperature of 24°C. The pots were watered daily; always keeping the soil moist. The seedlings were ready for transplanting after 30 days, when they measured 10-15 cm in height and about 0.5 cm in stem diameter.

3.2.3 Preparation of nematode inoculum and inoculation of test plants.

The sources of nematode inocula were galled tobacco roots which were collected in tobacco growing areas of Busia and Bungoma districts. The rootknot nematode species causing the galls were Meloidogyne javanica and M. incognita. The former species was predominant with a relative occurance frequency of 85% (section 4.1; preliminary survey results).

About 10 kg of the galled roots were washed in running tap water and then chopped into pieces of about 2 cm long. The pieces were then mixed thoroughly with steam-sterilised sandy loam soil. The mixture was kept moist at 30°C for two weeks to facilitate the harching of the eggs and for the

nematode larvae to emerge from the disintegrating root tissues into soil. The population density of the Meloidogyne larvae in the prepared "inoculum soil" was determined as in section 3.1.3.

Each of the clay and sandy loam steam-sterilised soils were divided into five batches which were to be infested with the nematodes in the "inoculum soil". Five initial inoculum densities of 0 (control), 10, 20 and 40 nematodes per 100 g of soil; were prepared by thoroughly mixing appropriate amounts of the two soil sets (inoculum and sterilised soils).

The nematode infested soils were filled into plastic pots of 25 cm in diameter and 30 cm in deptth. Into each pot was applied fertilizers; 15 g of NPK (17-17-17) and 30 g sulphate of ammonia. Physical and chemical analysis of the two soil preparations were done in order to obtain their characteristics. A 10 week old tobacco seedlings of 10-15 cm in height was planted in each pot.

A split-split plot experimental design was used. The two soil preparations of clay and sandy loams were the main units. The three commercial tobacco varieties were the subunits, and the five inoculum levels of 0, 5, 10, 20 and 40 nematodes/100 g

of soil were the sub-subunits. The sub-sub units were replicated four times, hence there were a total of 120 experimental units of potted plants. Randomisation of the units was done at the variety and inoculum levels. The potted plants were of four blocks placed inside a large glasshouse.

3.3.4 Maintenance of potted plants.

The plants received one litre of water per pot at the earliest signs of wilting. The frequency of watering varied with soil type, tobacco variety and stage of growth. Incidences of powdery mildews on the plants were controlled with Propneb (Antracol) at 5 g/100 plants. Sprays of "Dimethoate" 40% EC, at 2 ml/100 plants were used to control infestation by aphids, whiteflies and spidermites.

3.2.5 Determination of rate of tobacco growth, leaf yields and rootgall indices.

The rates of growth of the tobacco plants were determined by measuring plant heights on the 30th, 45th and 60th days, after transplanting.

Ninety days after transplanting, the leaves were harvested by stripping them off the stalk. The leaves were oven-dried at $50^{o}C$ for 48 hrs. They were weighed and the dry-leaf weights recorded per treatment.

After harvest, all the plants were carefully uprooted and the soil washed off the roots under running tap water. The extent of root galling was rated against a scale of 0-4 indices, as shown below:-

0: No traces of galling

1: Slight galling (1-25%)

2: Moderate galling (26-50%)

3: Severe galling (51-75%)

4: Very severe galling (76-100%).

3.3 Monitoring of nematode population densities on tobacco fields.

After the preliminary nematode survey, 60 tobacco plots were selected on which nematode population density fluctuations and their damage on tobacco plants were determined. The plots were of three tobacco varieties and were grouped in 6 categories as described below:-

1. Air-cured, variety "Burley"
   a Farmers' plots in Moding area of Busia district. Ten plots, each measuring 0.5 ha, were sampled.

   b BAT (K) Ltd. variety trial plots in Malakisi area. Five plots, each measuring 0.25 ha, were sampled. Both a and b plots were treated against nematodes with a systemic nematicide, "Nemacur" (O-ethyl-O-3 (3-methylthiophenyl)-isopropyl-amido-phosphate). It was applied at transplanting time, at the rate of 10 kg/ha (active ingredient).

2. Fire-cured, variety "Heavy Western".
   a Nemacur-treated plots. 8 plots were sampled.

b Untreated plots. 8 plots were sampled.

Both a and b were farmers' plots in Amukura and Bumula areas. The treated and untreated plots were adjacent to each other, and each measured 0.25 ha.

3. Flue-cured, variety "Speight G28".

a Farmers' plots in Busia Training and Research (T and R) area. The plots received no special treatment against nematodes. They were 0.5 ha each, and 6 were sampled.

b BAT (K) Ltd. Repeated Cultivation trial plots. The plots were cultivated either twice or thrice at different intervals during the dry season of October 1981 to January 1983. Such treatments were intended at exposing the eggs and larvae of nematodes within the top soil to solar dessication, which would lead to reduction of initial nematode inoculum. The plots were in Malakisi area and 27 were sampled.

Tobacco seedlings were transplanted from late February to early March, 1983; being "dry-

planted". The plants were watered individually for two to three weeks till the rains came. Agronomic practises as recommended for tobacco production (Akehurst, 1968), were followed in all plots. The crops were sprayed with "Bayleton" to control fungal and bacterial diseases, and with "Orthene" to control insect pests. The application rates and timings, were as recommended by the manufacturers.

The nematode population densities in the soil were determined progressively; at preplant in January, at midseason in late March and finally at postharvest in early August, 1983. The plots were sampled and the soil samples analysed as was described in section 3.1.2 to 3.1.4.

At postharvest sampling, the root galls caused by Meloidogyne spp. on tobacco roots were rated. In every plot, ten plants were chosen at random and their root systems were carefully dug up. The extent of rootgalling was rated on a scale of 0-4 indices, as was described in section 3.2.5.

3.4 Preharvest estimation of rootknot nematodes damage on tobacco in the field.

The preharvest estimation of rootknot nematodes damage on tobacco in the field was done about ten weeks after transplanting. The plants had just been topped, that is, removal of terminal inflorescence.

The damage estimates were based on plant stunting; the most damaging effect of nematodes on tobacco. Other symptoms like chlorosis and root galls were noted as further evidence of rootknot nematodes infection on a particular plant. There were three categories of nematode damage based on plant height measurements. The categories were; severe stunting, moderate stunting and no apparent stunting. The last category of plants were not neccessarily uninfected by nematodes.

In each plot, a block comprising of 100 tobacco plants and situated in the middle of the plot was marked out. All the tall, healthy-looking plants were categorised as unstunted, and the average of their heights was adopted as a control height. Plants whose heights were less than two-thirds but more than one third of the control height were

categorised as moderately stunted. Plants whose heights were one third or less the control height were categorised as severely stunted.

Each plot was assessed independently of the others in order to exclude variations on plant growths due to soil fertility, tobacco varieties and other local factors. The number of tobacco plants falling in each category of stunting per block in a plot were expressed in percentages.

4. RESULTS AND DISCUSSION

4.1 Distribution and population density levels of rootknot nematodes.

Soil samples were collected from 238 plots which were widely distributed in the tobacco growing areas of Busia and Bungoma districts (Fig. 1). Rootknot nematodes, Meloidogyne spp. were detected in 144 plots (61%) from the preplant soil samples (Table 2). However, all the 60 tobacco plots which were sampled progressively at midseason and postharvest were found to be infested with the nematodes (Table 7). Among the 60 were also 10 plots in which the rootknot nematodes were not detected at the preplant soil sampling.

The failure to detect the presence of rootknot nematodes in the preplant soil samples from plots which were infact infested, was attributed to the following factors:

a The preplant soil population density of rootknot nematodes was too low to be detected by the extraction method used. Since the soil samples were collected at a relatively dry period, the

nematode population density in the soil was likely to be low. Baker and Imbriani (1983) reported that due to seasonal fluctuations, the nematode populations in the soil at preplant are usually very low and often escape detection by mechanical methods.

b. Spots infected by the nematode in the plots might have been missed out during the random soil sampling. This was likely because of the tendency of the nematodes to occur in nonuniform distribution patterns within a field (Taylor and Sasser, 1978).

The preplant soil infestation levels of Meloidogyne larvae in the plots which were sampled ranged from 0 to 40 nematodes per 100 g of soil, according to Baermann's pan and filter extraction method (Table 3). The infestation levels and spatial distribution of the nematode was random over the whole area of study. Quite often, two neighbouring plots were found to be at the extreme ends of the range of nematode infestation levels. Such variations were probably due to the cropping history of individual plots. Plots with high infestation levels

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could have been planted with crops which favoured increase of Meloidogyne spp. population.

It was therefore concluded that Meloidogyne spp. was extensively distributed over the tobacco growing areas of Busia and Bungoma districts. It was not found necessary to mark out geographical differences in the nematode infestation levels on the map of the area.

Identification of the Meloidodyne species was done from 100 perineal patterns of mature females (section 3.1.4). Meloidogyne javanica (Treub) Chitwood constituted 85% of the specimens. M. incognita (Kofoid and White) Chitwood consistituted the other 15% of the specimens.

M. javanica was therefore predominant in the area of study. The species has been reported on tobacco and many other crops from warm areas like the one under study (Milne, 1972; Lucas, 1975; Taylor and Sasser, 1978).

M. javanica has been reported as the most aggressive of all nematodes which attack tobacco (Arens, 1979; Baker et al, 1981). Attempts to produce a commercial tobacco variety resistant to it has so far not been successful (Sasser and Kirby, 1979).

Table 2. Distribution of Meloidogyne spp. in the tobacco growing areas of Bungoma and Busia districts, at preplant soil sampling.

Production Area	No. of plots	Meloidogyne larvae/100 g Soil				
		0	1-5	6-10	11-20	21+
		Frequency				
1. West Siboti	10	4	2	3	-	-
2. East Siboti	10	2	5	2	1	-
3. Kanduyi	10	1	6	1	2	1
4. Kibuke	10	3	6	1	-	-
5. Mwalie A	10	6	4	-	-	-
6. Mwalie B	10	4	5	1	-	-
7. Kulisiru	10	4	4	2	-	-
8. Chepkhutum	10	4	4	-	1	1
9. Kolani	10	3	2	3	1	1
10. Sirisia	10	5	5	-	-	-
11. Machakha	10	2	5	1	2	
12. Bumula	10	3	6	-	-	1
13. Amukura	11	3	4	3	-	1
14. Kocholya	10	6	4	-	-	-
15. Awata	10	4	6	-	-	-
16. Moding	10	2	3	2	2	1
17. Wamono	10	4	2	1	1	1
18. Adomoru	8	5	2	-	1	-
19. Angurai	10	7	3	-	-	1
20. Aboloi	10	6	3	-	-	-
21. Busia T and R	10	7	3	-	-	-

Table 2. cont.

Production Area	No. of plots	Meloidogyne larvae/100 g Soil				
		0	1-5	6-10	11-20	21+
		Frequency				
22. Bungoma T and R	10	5	2	2	1	-
23. Malakisi	18	4	5	2	2	3
Absolute frequency	238	94	91	24	14	12
Relative frequency, %	100	39	38	10	6	5

4.2 Occurrence of non-galling plant-parasitic nematodes on tobacco fields.

Several genera of the known plant-parasitic and saprophytic nematodes were identified from the soil samples. From the preplant soil samples, 14 genera of the non-galling plant-parasitic nematodes were identified and enumerated (Table 3).

However, only six genera of the listed nematodes were found persistently around tobacco roots during the midseason and postharvest samplings. They were *Pratylenchus*, *Tylenchorhynchus*, *Scutellonema*, *Rotylenchus*, *Helicotylenchus* and *Hemicycliophora* species (Table 9). These nematodes could be regarded as having had depended on tobacco roots for their livelihood.

The saprophytic nematodes which were recovered in the soil samples included; Rhabditids, Diplogasterids Mononchids, Dorylaimids and *Aphelenchus avenae*.

Table 3. *The non-galling plant-parasitic nematode genera; their occurance and relative frequency in preplant soil samples from the tobacco growing areas of Busia and Bungoma districts.*

Nematode genera	Common name	Relative frequency*, %	Abundance+
1. Pratylenchus	Rootlesion nematode	54	Low
2. Tylenchorhynchus	Stunt nematode	40	Low
3. Hemicycliophora	Sheath nematode	20	Low
4. Criconemoides	Ring nematode	12	Low
5. Trichodorus	Stubby root nematode	23	Low
6. Xiphinema	Dagger nematode	35	Low
7. Rotylenchus	Spiral nematode	60	Moderate
8. Helicotylenchus	"	72	Moderate

Table 3. cont.

Nematode genera	Common name	Relative frequency*, %	Abundance+
9. *Scutellonema*	Spiral nematode	75	High
10. *Radopholus*	Burrowing nematode	15	Low
11. *Tylenchulus*	Citrus nematode	20	Low
12. *Hoplolaimus*	Lance nematode	85	High
13. *Tylenchus*	-	78	High
14. *Psilenchus*	-	90	High

*Relative frequency: = $\frac{\text{(No. of samples with the nematode genus} \times 100)}{\text{(Total no. of samples (= 238)}}$

+Abundance: mean no. of nematodes/100 g of soils:-
Low: 0-5, Moderate: 6-10, High: 11 +.

4.3 Potted plants experiment: responses of commercial tobacco varieties to increasing initial inoculum densities of Meloidogyne javanica and M. incognita concomitantly (6:1) in clay and sandy loam soils.

Tobacco plants which were grown in the sandy loam soil were progressively stunted at increasing initial inoculum densities, Pi, of 0, 5, 10, 20 and 40 nemadotes /100 g of soil (Fig. 2, Plates 1-3). The reduction of dry leaf yield weights at the increasing Pi were in similar trend (Fig. 3).

Thirty days after transplanting the heights of all the plants in sandy loam soil were significantly different ($P=0.05$) with increasing Pi, while there were no significant differences in the heights of the plants grown on clay loam soil. However, at the 45th and 60th days after transplanting, plants of variety "Burley 181" were significantly stunted with increasing Pi in the clay loam soil. Significant stunting was also recorded in variety Heavy Western on the 60th day. There were no significant stunting of variety "Speight G28" on clay loam soil at any time of measurements.

On sandy loam soil, all the varieties were severely stunted by the 60th day at increasing Pi.

4.3 Potted plants experiment: responses of commercial tobacco varieties to increasing initial inoculum densities of *Meloidogyne javanica* and *M. incognita* concomitantly (6:1) in clay and sandy loam soils.

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On sandy loam soil, all the varieties were severely stunted by the 60th day at increasing Pi.

At the highest inoculum density of Pi = 40; the relative stunting was, 63%, 60% and 52%, on varieties "Burley" 181, "Heavy Western" and "Speight G28," respectively (Table 4).

The weights of dry leaf yields of the tobacco plants grown in sandy loam soil were greatly reduced at increasing Pi while there were no significant differences for the plants in clay loam soil (Fig. 3). The relative leaf yield reductions were 89%, 78% and 65% for the varieties "Burley 181," "Heavy Western" and "Speight G28," respectively (Table 5).

There was enhanced growth of plants grown in clay loam soil at the low inoculum density of Pi = 5. The plants were slightly taller and yielded more leaves than the uninoculated controls, Pi = 0 (Plate 5). On the 60th day after transplanting, their relative increase in heights were 14%, 13% and 4%, on varieties "Burley 181", "Heavy Western" and "Speight G28," respectively (Table 4). Corresponding increases in dry leaf yields were 9%, 4% and 5% for the varieties in the above order (Table 5).

The galling of tobacco roots due to infection by the Melodogyne species increased with increasing Pi, but not in constant ratios (Table 6). The root gall indices were often one or two scale(s) higher among the

plants grown in sandy than in the clay loam soils; at corresponding nematode densities. All the varieties ("Burley 181", "Heavy Western" and "Speight G 28") were galled nearly equally per Pi in sandy loam soil. But in clay loam soil, variety "Speight G28" was less galled relative to the other two varieties.

The correlation between the rootgall indices and the dry leaf yields was low and variable for the two soil types combined. In the sandy loam soil where there was a strong inverse relationship; the correlation coefficient was -0.7.

The results so far presented indicate that root knot nematodes caused greater damage on tobacco plants which were grown in sandy loam soil than in clay loam soil. This conformsto the reports of many workers (O'Bannon and Reynolds, 1961; Whitehead, 1969; Ferris and McKenry, 1977).

It is deducible that the physical and biological characteristics of sandy soils favour reproductive potentials and pathogenecity of the nematodes on host plants. The good aeration and porosity of sandy soils do favour the hatching of eggs and larvae movements. Hence, nematode inocula have higher chances of locating and infecting their host plants in sandy soils than in the more

packed clay or silty soils (Wallace, 1973).

Secondly, plants growing in sandy soils are more prone to water stress and nutrient deficiencies which lowers their resistance and tolerance to nematode attack. The soil has ppor water-holding capacity and low inherent fertility. Even applied nutrients may quickly get lost through leaching.

Plants grown in clay loam soil did tolerate increasing levels of rootknot nematode infections and root galling without any significant leaf yield reductions. Such tolerance to nematode attack could be due to better nutritional status offered by the soil. Baker and Imbriani (1983) reported that plants grown in fertile, well-watered clay loam soils were able to tolerate high numbers of Meloidogyne incognita infections without suffering significant yield reduction.

The organic matter which was added to the soil also helped to suppress the population of the nematodes in soil. Sayre (1971) reported that products of decomposing organic matter were found to be toxic to M. incognita. High organic matter also favours abundant occurance of soil-borne micro-organisms which are antagonistic to nematodes. Such suppression of the plant-parasitic nematode population did not

however occur in the experimental soils since they were steam-sterilised, and there was no recontamination other than the introduced test nematodes.

At the low initial nematode inoculum density of 5 larvae per 100 g of soil on clay loam soil, there was enhanced plant growth relative to the uninoculated controls. Low populations of nematodes had been observed to promote production of secondary roots subsequent to initial root injury (Baker and Olthof, 1978). The secondary roots apparently overcompensated the initial root injury by the nematodes. Baker and Imbriani (1983) reported that low populations of nematodes (below tolerant limit) resulted in tobacco yield increase on fertile clay loam soils during good weather conditions.

Though all the three tobacco varieties were infected by rootknot nematodes variety "Speight G28" was less damaged relative to both Heavy Western and Burley 181. Variety "Speight G28" had been bred for resistance against M. incognita (Lucas, 1975). Results of this study did show that the variety has, at least, some tolerance against M. javanica as well. Other workers, Baker et al,

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(1981), among them, had also observed that variety "Speight G28" was usually less damaged by infections of M. javanica.

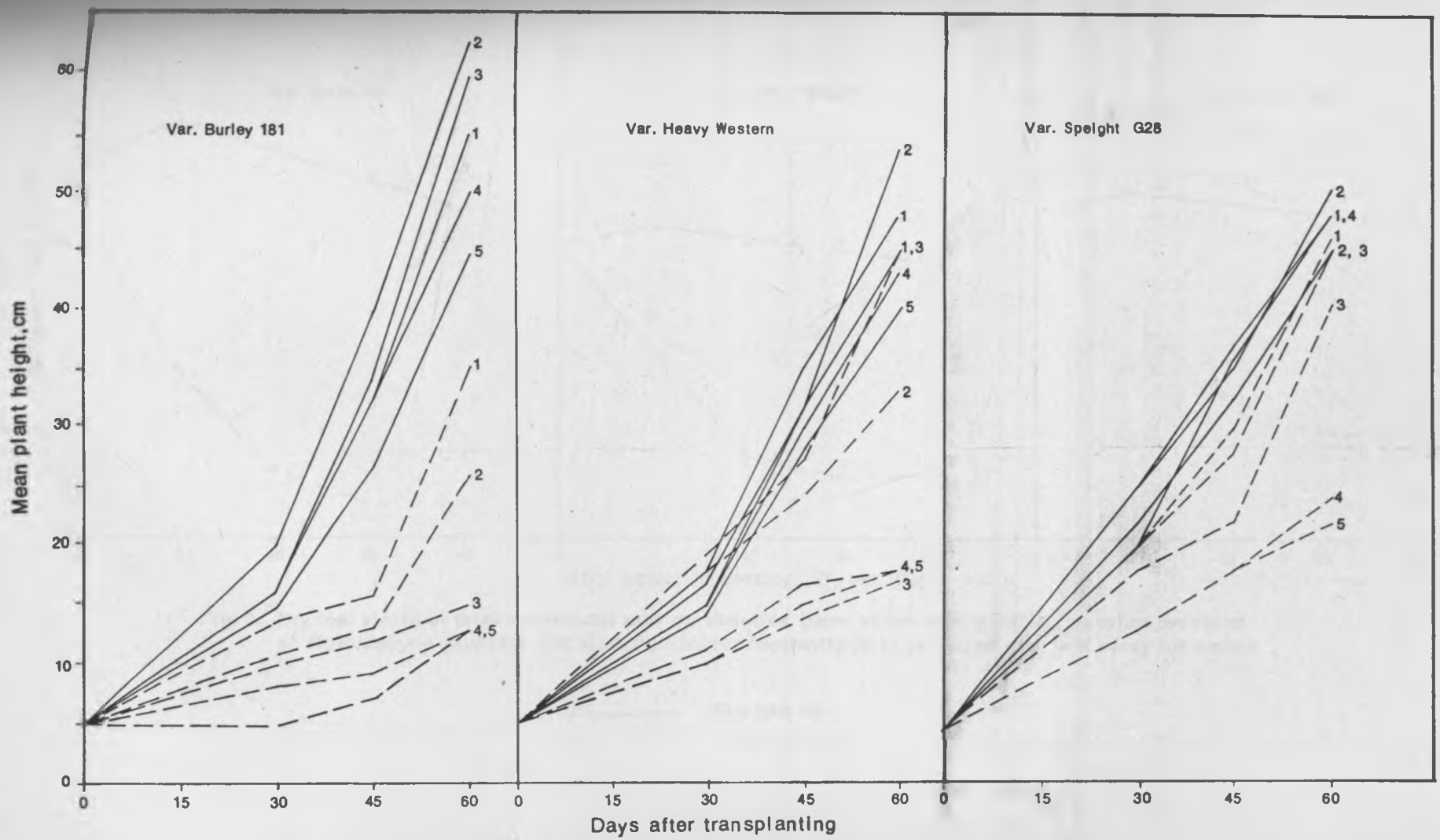

Fig. 2-Growth response of three commercial tobacco varieties to increasing nematode densities of *Meloidogyne javanica and M. incognita* concomitantly(6:1), in potted clay and sandy loam soils

——— Clay loam soil

– – – – – Sandy loam soil

1, 2, 3, 4 and 5 – initial nematode density of 0, 5, 10, 20, 40 per 100g of soil, respectively

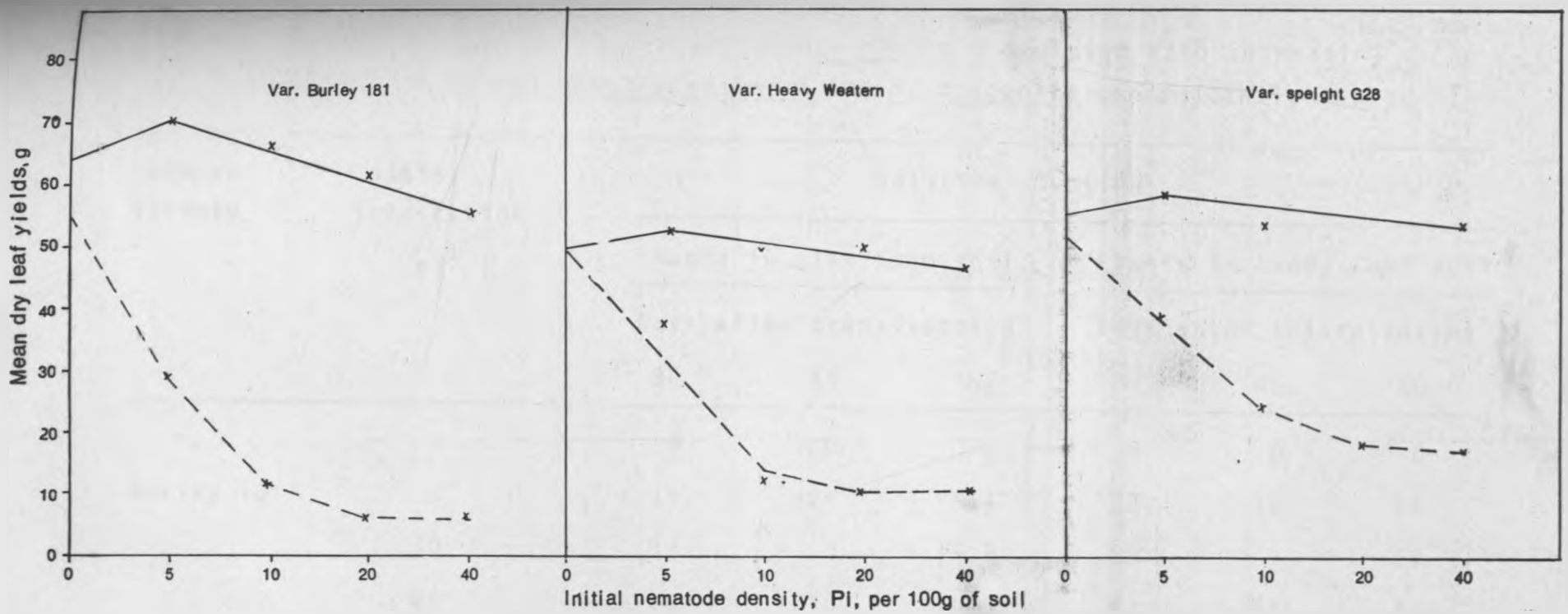

Fig. 3. Dry leaf yields of three commercial tobacco varieties grown at increasing initial inoculum densities of *Meloidogyne javanica and M. incognita*, concomitantly (6:1), in potted clay and sandy loam soils

———— Clay loam soil

— — — — Sandy loam soil

Table 4. Relative stunting of potted tobacco plants inoculated with increasing densities of Meloidogyne javanica and M. incognita concomitantly (6:1)

Tobacco Variety	Initial infestation pi[1]	Relative stunting[2]					
		Plants in clay loam soil			Plants in sandy loam soil		
		Days after transplanting			Days after transplanting		
		30	45	60	30	45	60
Burley 181	0	0	0	0	0	0	0
	5	-11	-21	-14	22	19	26
	10	19	- 3	- 9	29		57
	20	19	0	9	43	44	63
Heavy Western	0	0	0	0	0	0	0
	5	6	19	-13	5	14	27
	10	22	20	6	47	50	62
	20	17	19	10	42	39	60
	40	17	14	17	47	46	60

Table 4. cont.

Tobacco Variety	Initial infestation Pi[1]	Relative stunting[2]					
		Plants in clay loam soil			Plants in sandy loam soil		
		Days after transplanting			Days after transplanting		
		30	45	60	30	45	60
Speight G28	0	0	0	0	0	0	0
	5	-25	3	- 4	0	7	2
	10	-10	11	4	9	27	13
	20	-25	- 3	0	35	40	48
	40	0	3	4	35	40	52

[1] Pi = Initial density of rootknot nematodes larvae/100 g of soil.

[2] Relative stunting = $100 - \left\{\frac{\text{Mean plant height at } Pi \times 100}{\text{Mean plant height at } Pi = 0 \text{ (control)}}\right\}$

Table 5. Relative reduction on leaf yield of potted tobacco plants inoculated with varying densities of *Meloidogyne javanica* and *M. incognita* concomitantly (5:1).

Tobacco variety	Nematode Inoculum density, Pi	Relative stunting, %	
		Clay loam soil	Sandy loam soil
Burley 181	0	0	0
	5	-9	48
	10	-3	80
	20	5	89
	40	16	89
Heavy Western	0	0	0
	5	4	22
	10	0	76
	20	0	78
	40	6	78
Speight G28	0	0	0
	5	-5	23
	10	4	54
	20	0	63
	40	4	65

Pi = Initial density of rootknot nematodes /100 g of soil.

$$\text{Relative stunting} = 100 - \left(\frac{\text{(Mean weight of dry leaves at } Pi) \times 100}{\text{(Mean weight of dry leaves at Po (control)}}\right)$$

Table 6. Root gall indices on commercial tobacco varieties grown at increasing initial inoculum densities of *Meloidogyne javanica* and *M. incognita* combined (6:1) in sandy and clay loam soils of the pot plant experiment.

Tobacco variety	Nematode inoculum density, Pi[1]	Root gall indices[2]	
		Clay soil	Sandy soil
	0	0	0
	5	1	2
Burley 181	10	1	3
	20	2	4
	40	2	4
	0	0	0
	5	1	2
Heavy Western	10	1	3
	20	2	4
	40	3	4

Table 5. cont.

Tobacco varieties	Nematode inoculum density, Pi[1]	Root gall indices[2]	
		Clay soil	Sandy soil
Speight G28	0	0	0
	5	0	2
	10	1	3
	20	1	3
	40	2	4

1. Pi - Initial density of M. javanica + M. incognita larvae/100 g of soil.

2. Root gall indices:- 0 - no galls, 1 - slight galling (1-25%), 2 - moderate galling (26-50%), 3 - severe galling (51-75%) 4 - very severe galling (76-100%).

Plate 1: Growth response of potted tobacco varieties "Speight G28" (back row) and "Heavy Western" (front row), on sandy loam soil at increasing densities of Meloidogyne javanica and M. incognita, combined (6:1), 30 days after transplanting.

Initial inoculum densities, as determined by Bearmann's pan and filter method; from right to left: 0, 5, 10, 20 and 40 (two sets) nematodes per 100 g of soil.

Plate 2: Growth response of potted tobacco variety "Burley 181", on sandy loam soil at increasing densities of Melodogyne javanica and M. incognita, combined (6:1), 30 days after transplanting.

Initial inoculum densities, as determined by Baermann's pan and filter method; from right to left: 0, 5, 10, 20 and 40 (two sets) nematodes per 100 g of soil.

Plate 3: Growth response of potted tobacco varieties "Speight G28" (three back rows) and "Burley 181" (three front rows), on sandy loam soil at increasing densities of Meloidogyne javanica and M. incognita, combined (6:1), 50 days after transplanting.

Initial inoculum densities as determined by Baermmann's pan and filter method; from left to right: 0, 5, 10, 20 and 40 (two sets) nematodes per 100 g of soil.

Plate 4: Response of potted tobacco variety "Speight G28", on clay loam soil at increasing densities of *Meloidogyne javanica* and *M. incognita*, combined (6:1), 60 days after transplanting.

Initial inoculum densities, as determined by Baermann's pan and filter method; from left to right: 0, 5, 10, 20 and 40 (two sets) nematodes per 100 g of soil.

Plate 5: Growth response of potted tobacco variety "Burley 181", on clay loam soil at increasing densities of Meloidogyne javanica and M. incognita, combined (6:1), 60 days after transplanting.

Initial inoculum densities, as determined by Baermann's pan and filter method; from left to right; 0, 5 and 10 nematodes per 100 g of soil.

4.4 Population densities of *Meloidogyne* spp. and their damage on tobacco in the field.

The population densities of rootknot nematodies on tobacco plots were found to be very variable from plot to plot. And the nematode damage on tobacco plants tended to depend mainly on the soil types and crop management factors rather than the nematode population densities.

4.4.1 Air-cured variety "Burley 181" plots.

The plots of air-cured tobacco under the two categories of management; 1) BAT Company trial plots and, 2) Small-scale farmer plots; were under nematode control with "Nemacure" at 10 kg (a.i) per hactare (Table 2).

There was greater rootknot nematode infestation on the BAT plots than on the farmers plots at the midseason and postharvest samplings (Table 7). The mean nematode reproduction factors at postharvest were 133.3 and 22.1 on the BAT and farmers plots, respectively. The root-gall indices were closely correlated to the postharvest rootknot nematode population densities.

Nematode damage on tobacco plants were however greater on the farmers' plots than in the BAT's. During the preharvest estimation of the damages, there were 14% moderately and 16% severely stunted tobacco plants in the farmers plots. The corresponding figures in the BAT plots were 3% and 1% only, respectively (Table 7).

The observed anomally in relationship between nematode population densities and crop damage could be due to the differences in crop managements between the two plot categories. While the small scale farmers used oxen-plough for cultivation, the BAT Co. used tractor which achieved greater plough depth. Tobacco plants grown in the BAT plots had therefore greater freedom for root growth and nutrient uptake. From casual observations, the plants were found to be much more deeply rooted than those in the farmers plots. Such plants with extensive root systems were therefore able to tolerate nematode infection. Other causes for the variation could have been the differences in attention given to young transplants, application of fertilizers and weed control.

The incidence of relatively higher nematode population densities on BAT trial plots could be attributed to the potentials for nematode

reproduction in either plot category. The extensive root systems of tobacco plants on BAT plots offered abundant food source to the nematodes and hence a greater multiplication rate.

More than a half of the tobacco plants which were examined in either category of the plots had moderately galled roots yet they appeared quite healthy. The plants, though infected, attained the genetic potential height of 2 meters. Besides plant tolerance, another influencing factor could have been disease escape due to late infection. The nematicide which was applied on the plots apparently reduced the initial nematode inoculum density in the soil substantially. The young tobacco transplants therefore escaped the critical early infection. The observed root galls could be due to late infection by Meloidogyne larva which were shielded from the nematicide action by plant tissues or in egg masses.

Brodie and Dukes (1972) reported that if tobacco was protected from heavy nematode infection for one month after transplanting.late infection even if heavy, did not cause economic yield loss.

It was observed that most of the late planted refil transplants were heavily galled and severely stunted. The refils could have been

heavily infected by the nematodes immediately they were transplanted.

4.4.2 Fire-cured variety "Heavy Western" plots.

The plots of fire-cured tobacco were paired "Nemacur"-treated (10 kg. a.*i.*/ha) and untreated half-plots (Table 2).

Mean final population densities of rootknot nematodes on the no-nematicide half plots were about four times higher than on the nematicide treated ones (Table 7). Nematode damage on tobacco plants was also greater in the no-nematicide half-plots. Preharvest estimations of the damages revealed that 25% and 24% of the plants were moderately and severely stunted, respectively, in the no-nematicide half-plots. Corresponding damage levels in the treated half-plots were 22% and 8%, respectively (Table 7).

It was again observed that most of the severely stunted plants were the late-planted refils.

The significantly different nematode population densities between the Nemacur-treated and untreated half-plots did not always correspond to the crop performance. In most plots which had clay loam soils, the crop performances were about equal between the

treated and untreated half-plots. Yet on sandy soils, the tobacco damage by nematodes was more serious on the untreated half-plots. On two of the untreated sandy half-plots, about 50% of the plants were severely stunted. The observation re-emphasized the results and conclussions presented in section 4.3; that nematode damage on crops is often more serious on sandy soils.

In section 4.1, the physical and biological characteristics of clay loam soils were presented as the factors which contributed to the host plants tolerance to nematode infection. It was again observed that there were not always significant differences in tobacco performance between the nematicide-treated and untreated plots on clay loam soils. Other workers have also reported similar findings (O'Bannon and Reynolds, 1961).

4.4.3 Flue-cured variety "Speight G28" plots

The plots of flue-cured tobacco which consisted of; 1) small-scale farmer plots, and 2) BAT Co. trial plots of repeated cultivations as a means of nematode control, were all not treated with Nemacur (Table 2).

In the small-scale farmer plots, the mean population densities of rootknot nematodes were very low and had a mean potharvest reproduction factor (P_f/P_i) of 13.8 only (Table 6). Note that the P_f/P_i for other two tobacco varieties ranged from 22.1 (Burley 181 in small-scale farmer plots) to 136.0 (Heavy Western in no-nematicide plots). Estimates of preharvest tobacco damage by nematodes were similarly low for the flue-cured variety "Speight G28," than for the other varieties (Table 6).

The relatively low nematode population densities and tobacco damage by root knot nematodes on plots of variety "Speight G28" indicated that the variety was both a poor host and significantly resistant to the nematode.

Trial plots of repeated cultivations as a means of nematode control failed to produce positive results. The population densities of rootknot

nematodes were considerably low in all plots which had received either two or three preplant cultivations at different intervals (Table 7). Apparently the initial nematode infestations of all the plots were too low to facilitate the success of such an experiment. Otherwise, it has been reported that exposure of surface soil to solar desiccation through repeated cultivations could reduce the initial nematode inoculum in the soil substantially and hence save the next crop from heavy nematode damage (Milne, 1972; Taylor and Sasser, 1978).

Table 7. Rootknot nematodes population fluctuations and nematode damage on tobacco plots.

	Plots	Mean density* of Meloidogyne larvae/ 100 g of soil			Reproduction factor,	Mean Rootgall indices[2]	Percentage stunting	
		Pi[1]	Pm[1]	Pf[1]	Pf/Pi		Moderate	Severe
1.	Air-cured "Burley"							
	a. Farmers plots	10.2	62.9	225.7	22.1	1.8	3	1
	b. BAT (K) Ltd. variety trials plots	10.2	212.0	1360.0	133.3	2.4	14	6
2.	Fire-cured "Heavy Western"							
	a. Untreated plots	5.9	586.8	802.5	136.0	1.9	25	24
	b. Nemacur-treated plots	5.9	52.0	239.3	40.6	1.3	22	8
3.	Flue-cured "Speight G28"							
	a. Farmers plots	10.0	52.5	138.3	13.9	1.4	15	7
	b. BAT(K) Ltd. Repeated Cultivation trials plots	9.1	2.1	36.0	4.0	1.1	5	2

[1] Pi, Pm, Pf = Preplant, Midseason and postharvest nematode densities respectively.

[2] Rootgall indices: 0- no galls, 1- slight galling, 2- moderate galling, 3- severe galling.

4.4.4 Population densities of non-galling plant parasitic nematodes.

There were about six genera of the non-galling plant-parasitic nematodes which were found in soil around tobacco roots at every sampling time. They were Pratylenchus sp, Scutellonema sp, Rotylenchus sp, Helicotylenchus sp, Tylenchorhynchus sp and Hemicycliophora sp. However, their population densities at midseason and postharvest samplings were far too low relative to Meloidogyne sp. (Table 8). At postharvest sampling; they only constituted 15% of the total nematode population, while the remaining 85% were Meloidogyne sp. The most abundant of the six genera was Pratylenchus sp. (6%). The least abundant was Hemicycliophora sp. (0.4%) (Table 9).

Unlike the Meloidogyne spp. which occured in all the plots studied, these other genera were less frequent (Table 10). Both Scutellonema sp. and Helicotylenchus sp. were the most frequent in occurance (68%), and Hemicycliophora sp. the least frequent (21%).

The population densities of the non-galling plant-parasitic nematodes in all the plots were too low to be of economic importance. Olthof et al,

(1973) reported that for Pratylenchus sp. to be able to cause economic damage on tobacco, its preplant densities should be in excess of 200/100 g of soil. For the other ectoparasitic nematodes the critical densities should be even much higher (Milne, 1972).

Table 8. Population fluctuations of non-galling nematode genera on tobacco plots in Busia and Bungoma districts.

Plot description	Nematode genera and mean population/100 g of soil											
	Pratylenchus		Scutellonema		Rotylenchus		Helicotylenchus		Tylenchorhynchus		Hemicycliophora	
	Pi[1]	Pf[1]	Pi	Pf	Pi	Pf	Pi	Pf	Pi	Pf	Pi	Pf
1. Air-cured "Burley"												
a. Farmers plots	1.8	8.6	2.3	7.9	0.1	0	4.8	10.0	1.5	2.1	1.5	1.1
b. BAT (K) Ltd. variety trial plots	1.8	8.6	2.3	18.0	0.1	4.6	4.8	9.8	1.5	8.4	1.5	1.0
2. Fire-cured "Heavy Western"												
a. Nemacur-treated plots	1.3	7.9	2.4	7.1	1.4	0.5	2.9	11.1	1.5	3.0	1.6	0.0
b. Untreated plots	1.3	5.9	2.4	9.7	1.4	1.0	2.9	5.5	1.5	7.9	1.6	0.0
3. Flue-cured "Speight G28"												
a. Farmers plots	1.1	9.0	2.3	12.2	1.5	3.6	4.8	9.7	1.0	3.4	1.4	3.8
b. BAT (K) Ltd Repeated cultivation trial plots	0.0	2.0	1.9	5.9	0.9	1.3	6.3	4.7	1.9	3.4	0.8	0.5
Mean of total	1.2	7.3	2.3	10.1	0.9	1.8	4.4	8.5	1.5	4.7	1.4	1.0
Mean Reproduction factor Pf/Pi	6.1		4.4		2.0		2.0		3.1		0.8	

[1] Pi, Pf: Preplant and postharvest nematode densities, respectively.

Table 9. Relative density of nematode genera on tobacco plots in Busia and Bungoma districs.

	No of plots sampled	Nematode genera and relative density[1]						
		Meloid-ogyne	Pratyl-enchus	Scutell-onema	Rotyle-nchus	Helico-tylenc-hus	Tylenchorh-ynchus	Hemicycl-phora
1. Air-cured Burley								
a- Farmers plots	7	88	3	3	0	4	0.8	0.4
b. BAT (K)Ltd. variety trial plots	5	96	0.6	1	0.3	0.7	0.6	0
2. Fire-cured Heavy Western								
a. Nemacur-treated plots	8	94	0.7	1	0.1	0.6	0.9	0
b. Untreated plots	7	92	3	3	0.2	0.4	1	0
3. Flue-cured Speight G28								
a. Farmers plots	6	88	3	4	0.5	4	1	1
b. BAT (K) Ltd Repeated cultivation trial plots	27	50	28	8	2	6	5	0.7

[1] Relative density = $\left\{\frac{\text{(No of nematodes of a genus x100)}}{\text{(No of nematodes of all genera}}\right\}$, determined at postharvest sampling.

Table 10. Relative frequency of nematode genera on tobacco plots in Busia and Bungoma districts.

	No. of plots sampled	Nematode genera and relative frequency						
		Moloidogyne	Pratylenchus	Scutellonema	Rotylenchus	Helicotylenchus	Tylenchorhynchus	Hemicycliophora
1. Air-cured "Burley"								
a. Farmers plots	7	100	43	43	0	43	26	26
b. BAT (K) Ltd. variety trial plots	5	100	80	100	40	100	80	40
2. Fire-cured "Heavy Western"								
a. Nemacur-treated plots	8	100	38	63	13	63	63	0
b. Untreated plots	7	100	57	54	14	71	43	0
3. Flue-cured "Speight G28"								
a. Farmers plots	6	100	100	83	67	83	63	50
b. BAT(K) Ltd. Repeated cultivation trial plots	27	100	22	67	22	56	22	11
Mean of total	60	100	57	68	26	69	50	21

[1] Relative frequency = $\left(\frac{\text{No. of samples containing a genus x100}}{\text{No. of all samples collected}}\right)$, determined at postharvest sampling.

5. CONCLUSIONS

Rootknot nematodes, Meloidogyne spp. were found to be widely distributed in all tobacco growing areas covering 4,000 km^2 in Busia and Bungoma districts. Two hundred and thirty eight (238) preplant soil samples were collected over the area and 61% of them yielded the nematodes. Yet in 60 tobacco plots which were sampled at midseason and postharvest as well, Meloidogyne spp. occured in all; including 10 plots in which they were not detected at preplant.

Meloidogyne javanica was the predominant specie . It made up 85% of 100 specimens which were examined. M. incognita constituted the remaining 15%.

There were about 6 genera of the non-galling plant parasitic nematodes which were always found in soil around the tobacco roots. They were, in descending order of abundance, Pratylenchus sp., Tylenchorhynchus sp., Scutellonema sp., Helicotylenchus sp., Rotylenchus sp. and Hemicycliophora sp. Their total population densities were far below that of Meloidogyne spp. At postharvest sampling of 60 tobacco plots, they constituted 15% only of the total population of plant parasitic nematodes.

Meloidogyne spp. made up the remaining 85%.

Results of pot plants and field investigations on the response of commercial tobacco varieties to varying population densities of Meloidogyne javanica and M. incognita combined (6:1) showed that there was greater stunting and reduction of leaf yields on plants grown in sandy loam than in clay loam soils. On potted plants, the reduction of leaf yields was about 50% or more on sandy loam soils while plants in clay loam soil were apparently healthy even though the roots were often moderately galled by the nematodes. Similar trend was observed in the field too, but to a lesser extent.

The use of Nemacur (10 kg a.i./ha) to control nematodes in plots of fire-cured tobacco always gave significant response on sandy to gravel soils, while on clay loam soils; the crop performance between the treated and untreated paired plots were not significantly different.

Among the commercial tobacco varieties of air-cured "Burley 181", fire-cured "Heavy Western" and flue-cured "Speight G28", the last one was relatively tolerant to infection by Meloidogyne spp. "Speight G28" was always less galled and stunted at varying inoculum densities of the nematodes.

The early, dry-planted tobacco plants tended to tolerate nematode infection significantly, even though the roots were usually moderately to severely galled at postharvest sampling. Late planted and refil plants were often severely galled and stunted.

6. RECOMMENDATIONS

1. Due to the wide occurance of rootknot nematodes in the tobacco growing areas of Busia and Bungoma districts, the control of the nematodes should always be a major consideration in tobacco production, at least in the areas studied.

2. Since rootknot nematodes caused serious damage on tobacco which were grown in sandy or gravel soils and also, nematode control by Nemacur resulted in significant positive response in such soils than in the clay loam soils, the use of chemical nematicides is more necessary on sandy soils than in the clay loams.

3. There is need for further field experiments in order to elucidate the following:-

   a Nematode economic thresholds on tobacco under different soil textural compositions.

   b Evaluation of the cost-benefit ratios in the control of nematodes with nematicides on different soil textural compositions.

Appendix 1. Physical and chemical characteristics of soils used in potted tobacco plants experiment.

Characteristic	Soil preparation	
	Clay loam	Sandy loam
1. Ratio of red clay, sand and manure, respectively	3:2:1	1:1:0
2. Texture; % clay, sand and silt, respectively	60:31:9	25:69:6
3. pH in $CaCl_2$	6.5	6.2
4. Macroelements;* % C, N, P and K, respectively	3.8, 0.4, 2.5, 2.0	3.0, 0.3, 3.4, 0.2
5. Total CEC, me/100 g+	27.8	27.3

*C - Carbon, N - Nitrogen, P - Phosphorus, K - Potassium

+CEC - Cation Exchange Capacity measured in milli equivalents per 100 g

Appendix 2. Growth response of commercial tobacco varieties to increasing initial inoculum densities of Meloidogyne javanica and M. incognita concomitantly (6:1) in sandy and clay loam soils, of the pot plants experiment.

Tobacco variety	Nematode inoculum density, Pi*	Mean height in cm					
		Plants in clay loam soil			Plants in sandy loam soil		
		Days after transplanting			Days after transplanting		
		30	45	60	30	45	60
Burley 181	0	18	33	55	14	16	35
	5	20	40	63	11	13	26
	10	16	34	60	10	12	15
	20	16	33	50	8	9	13
	40	15	27	45	5	7	13
Heavy Western	0	18	35	48	19	28	45
	5	17	32	54	18	24	33
	10	14	28	45	10	14	17
	20	15	32	43	11	17	18
	40	15	30	40	10	15	18

Appendix 2. cont.

Tobacco variety	Nematode inoculum density, Pi*	Mean height in cm					
		Plants in clay loam soil			Plants in sandy loam soil		
		Days after transplanting			Days after transplanting		
		30	45	60	30	45	60
Speight G28	0	20	36	48	20	30	46
	5	25	35	50	20	28	45
	10	22	32	45	18	22	40
	20	25	37	48	13	18	24
	40	20	35	46	13	18	22

Pi = Initial density of M. javanica + M. incognita (6:1) larvae/100 g of soil

Appendix 3. Dry leaf yields of commercial tobacco varieties grown at increasing initial inoculum densities of *Meloidogyne javanica* and *M. incognita* concomitantly (6:1) in sandy and clay loam soils of pot plant experiment.

Tobacco variety	Nematode inoculum density, Pi*	Leaf yield, dry weight in g.	
		Clay loam soil	Sandy loam soil
Burley 181	0	64	54
	5	70	28
	10	66	11
	20	61	6
	40	54	6
Heavy Western	0	50	49
	5	52	38
	10	50	12
	20	50	11
	40	47	11
Speight G28	0	56	52
	5	59	40
	10	54	24
	20	56	19
	40	54	18

*Pi: Initial density of Meloidogyne sp./100 g of soil

Appendix 4a. Analysis of variance on the heights of potted tobacco plants at 30 days after inoculation.

Source	df	SS	MS	F cal
Total	119	2870.800		
Blocks (reps)	3	14.467	4.822	2.630^{NS}
Soil type(s)	1	760.033	760.033	414.571^{xx}
Error (a)$^{+}$	3	5.500	1.833^{+}	
Varieties (V)	2	885.800	442.900	119.884^{xx}
VS	2	141.867	70.933	19.200^{xx}
Error (b)$^{+}$	12	44.333	3.693^{+}	
Inoculum density (I)	4	543.050	135.762	88.699^{xx}
VI	8	102.700	12.837	8.387^{xx}
SI	4	178.050	44.512	29.082^{xx}
VSI	8	84.800	10.600	6.925^{xx}
Error (c)	72	110.200	1.531	

Appendix 4b. Analysis of variance on the heights of potted tobacco plants at 45 days after inoculation.

Source	df	SS	MS	F cal
Total	119	10496.367		
Blocks (reps)	3	2.967	0.989	1.556^{NS}
Soil type(s)	1	6900.833	6900.833	8056.490^{xx}
Error (a)	3	2.567	0.856	
Varieties (V)	2	919.217	459.608	352.028^{xx}
VS	2	721.117	360.558	276.163^{xx}
Error (b)	12	15.667	1.306	
Inoculum density (I)	4	1067.033	266.758	134.972^{xx}
VI	8	207.117	25.890	13.099^{xx}
SI	4	302.667	75.667	38.286^{xx}
VSI	8	214.883	26.860	13.591^{xx}
Error (c)	72	142.300	1.976	

Appendix 4c. Analysis of variance on the heights of potted tobacco plants at 60 days after inoculation.

Source	df	SS	MS	F cal
Total	119	25420.992		
Blocks (reps)	3	2.425	0.808	0.342^{NS}
Soil type(s)	1	14366.408	14366.408	6077.418^{xx}
Error (a)	3	7.092^{+}	2.364^{+}	
Varieties (V)	2	626.317	313.158	67.914^{xx}
VS	2	2567.817	1283.908	278.438^{xx}
Error (b)	12	55.333	4.611^{+}	
Inoculum density (I)	4	4862.200	1215.550	408.204^{xx}
VS	8	355.350	44.419	14.917^{xx}
VI	4	1569.133	392.283	131.736^{xx}
VSI	8	794.517	99.315	33.352^{xx}
Error (c)	72	214.400	2.978	

Appendix 4d. Analysis of variance on the leaf yields of potted tobacco plants.

Source	df	SS	MS	F cal
Total	119	50230.992		
Blocks(reps)	3	10.092	3.364	2.008^{NS}
Soil type(s)	1	29988.408	29988.408	17903.527^{xx}
Error (a)	3	5.025	1.675^{+}	
Varieties (V)	2	959.617	479.808	79.599^{xx}
VS	2	2209.517	1104.758	183.277^{xx}
Error (b)	12	72.333	.6.028^{+}	
Inoculum density(I)	4	9427.367	2356.842	468.380^{xx}
VI	8	428.883	53.610	10.654^{xx}
SI	4	6168.633	1542.158	306.476^{xx}
VSI	8	598.817	74.852	14.875^{xx}
Error(c)	72	362.300	5.032	

+ The fact that Error (a) is much less than Error (b) indicates a negative intraclass correlation between the soil types and varieties, it also indicates that there is greater variation between the main plots (soil types) than in subunits (varieties) and subsub-units (inoculum densities); which is discussed in Section 5.3.

^{xx}Strongly significant (P 0.01).

NS: Not Significant (P = 0.05)

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