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FACTORS INFLUENCING THE POPULATION DYNAMICS OF
MELOIDOGYNE KONAENSIS ON COFFEE IN HAWAII

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By

MARIO SERRACIN

TO MY FAMILY AND FRIENDS FOR SUPPORT ALONG THE TORTUOUS
PATH

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ABSTRACT

Experiments were conducted in the greenhouse, field and growth chambers to evaluate effects of soil type, soil moisture regimes, and porosity on selected aspects of the dynamics of the Kona coffee root-knot nematode, *Meloidogyne konaensis*. First, the reproduction and damage potential of *M. konaensis* on resistant and susceptible rootstocks of coffee in four soils under two moisture regimes representative of areas where coffee is grown in Hawaii were assessed in greenhouse experiments. *M. konaensis* suppressed growth of coffee in all four soils. Nematode reproduction occurred readily in all soil types. Reproduction was lowest in the Hydric Dystrandept soil where the nematode holotype was found. In contrast, root galling was greatest in this soil. Greater galling occurred under constant moisture (33kPa) than under fluctuating moisture conditions in this soil. A field experiment in Kainaliu, Hawaii was conducted to determine the influence of irrigation, plant age, cultivar and nematode on coffee growth and yield. The population densities of the nematode in the soil varied according to plant age and irrigation treatment. Soil populations under irrigated conditions were greater during the months of May to July which normally follows the greatest annual precipitation and a period of active plant growth. Nematode reproduction was greater on coffee transplanted as 6-month-old seedlings than on coffee transplanted at 12-month of age. Soil water tension varied by season and experimental treatment. Trees from 12-month-old transplants exhibited greater water tension fluctuation with greatest water tension occurring from January to April. Trees transplanted as 6-month-old seedlings into *M. konaensis* infested soil and irrigated yielded greater coffee fruit than the same aged trees treatment without irrigation. Crop loss and reduction of growth and yield were also more evident from 6-month-old seedlings without supplemental irrigation treatment. In contrast, yield from plots in treatments including irrigation, nematode and 12-

month-old transplants yielded poorly. Overall highest yields were obtained from trees free of nematode and with supplemental irrigation. Yield reductions from nematode-infected plants ranged from 30-60% which is economically significant. Penetration, development and reproduction of *M. konaensis* was determined on tomato as model plant at 0.77 and 0.65 porosity. The rate of root penetration and post-embryonic developmental rates occurred slightly faster the porosity treatment of 0.77 than in the more densely packed soil (porosity of 0.65). Development in the 0.65 porosity progressed slower than at 0.77. Even though the nematodes matured faster and began laying eggs sooner on plants growing at porosity of 0.77, much greater numbers of eggs were laid by 30 days after inoculation at the 0.65 porosity. treatment than those at the 0.77 porosity. The finding from this research illustrates the primary role of the Kona coffee root-knot nematode in the Coffee Decline. The soil environment and host suitability are conducive factors for the coffee decline disease. Proper soil moisture management combined with sources of genetic resistance could minimize the damage enabling the coffee industry to remain profitable.

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CHAPTER I

Dissertation Research Overview

FACTORS INFLUENCING THE POPULATION DYNAMICS OF *MELOIDOGYNE KONAENSIS* ON COFFEE IN HAWAII

Most plant-parasitic nematodes reside in the soil and thus must interact with their hosts, other organisms and the complex physical, chemical and biological components of the microenvironment. Essentially, all of these factors interact and influence each other. The continued existence of each species requires that individuals adapt to obtain sufficient food to meet basic life processes of consumption, metabolism, growth and reproduction (Yates, 1996).

Species in the genus *Meloidogyne* spp. are often associated with severe damage to coffee in coarse textured soils (Goldi, 1892, Campos, 1990). In contrast, in Central America, where some species are widespread, damage is frequently associated with fine-textured volcanic soils (Lopez and Salazar, 1989). Under optimal environmental conditions, the larger and more productive a plant root system, the larger the population of nematodes it can support (Yates, 1996). However, root-knot nematodes compete with the plant for nutrients and water (Hussey and Williamson, 1996). Changes in food, weather and soil physical, chemical and biological conditions generally affect the expression of the nematodes damage and their reproductive behavior (Norton, 1979; Simon, 1973).

The production and export of Arabica coffee (*Coffea arabica* L.) has provided important agricultural contributions to the state of Hawaii's economy since 1823 (Goto and Fukunaga, 1956). Hawaii's coffee production for the 2001-02 season was

3.64 million kg (parchment basis) harvested from approximately 2,550 hectares, adding total farm revenues estimated at US \$19.6 million (Statistics of Hawaiian Agriculture, 2003). Despite frequent drought and uneven rainfall that produce an early and relatively small harvest, irrigation is used only by growers that have large farms. Fortunately, coffee is affected by few diseases in Hawaii, but in the Kona district where coffee has been monoculture in developing volcanic soils with low pH and poor fertility since its original introduction, many orchards are infested with the Kona Coffee root-knot nematode, *Meloidogyne konaensis* (Eisenback, Bernard and Schmitt, 1994). The nematode is very damaging to coffee and it is also able to attack a wide number of plants (Zhang and Schmitt, 1994). *M. konaensis* reproduced readily and suppressed coffee growth when tested in four different soils from coffee regions in the Hawaiian Islands (Serracin and Schmitt, 2000; Chapter 2). However, its distribution is currently restricted to the Kona districts and a few isolated sites in the island of Hawaii. Feeding in the plant roots by the Kona coffee nematode causes direct loss of plant growth and yield (Zhang and Schmitt, 1995). The loss is increased by secondary infections. The disease that *M. konaensis* causes is known as coffee decline (Serracin et al, 1999; Chapter 4).

Coffee plants are sensitive to pests and can experience negative and irreversible effects due to moisture extremes (Nunes, 1976). Nematode-infected plants often wilt and display nutrient deficiencies that cannot be corrected by applications of water and fertilizer. In commercial plantations, the rate of decline of coffee plants infected with the nematode appeared to be related to the overall nutrient management and water. The symptoms of nematode-induced coffee decline

generally appear once the tree has reached its first peak of production, normally between 3-5 years after planting, and are more evident during the final stages of the fruiting cycle.

M. konaensis, a recently described new nematode pest on coffee in Kona, is being found in many new locations in Kona, presumably due to its rapid redistribution throughout the area. The rapid expansion of the coffee industry in recent years to new sites stimulated research regarding this plant pathogen, including concerns regarding the potential of the nematode to spread and to damage these new coffee growing regions. The soils across the Kona districts vary in parent material, degree of weathering, porosity and hydraulic properties (Ikawa, et al., 1985). A major question arose concerning this variation and the nematode's ability to damage trees under a range of edaphic and environmental conditions. If the nematode can spread readily within the Kona districts under a wide range of soil and soil water conditions, can it infest soils in other coffee producing areas of the state (topic of Chapter 3)? Is damage by the nematode influenced by the various conditions in which it has successfully established? Growers vary their production practices and use of cultivars or land races. Do these practices such as seedling age at transplanting have an effect (Topic of Chapter 3 and Appendix 1)? Based on these series of questions, a project goal was established to determine if certain specific factors of soil type (within Kona and across soil types in the state) and soil water influence on nematode reproduction and plant damage. Specific objectives to address this goal were: to assess the damage potential of *M. konaensis* in four soils used for

commercial coffee production; and to determine the influence of the nematode, seedling age and soil moisture on plant growth and yield.

The population densities of the nematode in the soil varied according to plant age and irrigation treatment. Soil nematode populations under irrigated conditions were greater during the months of May to July which normally follows the greatest annual precipitation and a period of active plant growth (Schmitt, *et al* 2002). This event is important to know for monitoring or diagnosing nematode problems (see Chapter 4). The overall nematode reproduction was greater in 6-month-old seedlings treatments than on 12-month-old seedlings treatments, suggesting plants are less tolerant to the nematode attack if they are transplanted too young. If irrigated, 6-month-old seedlings infected with *M. konaensis* yielded more coffee beans than those of unirrigated seedlings transplanted at 12-months of age, thus indicating a positive, but short-term benefit of the irrigation; in contrast, irrigated and nematode infected coffee transplanted as 12-month-old seedlings yielded the least. Yield reduction from nematode infected plants ranged from 30-60% which is economically important on a per hectare basis depending on the irrigation regime. It is thereafter, recommended that supplemental irrigation be reduced on nematode infested farms to reduce the rate of coffee decline.

Variation in soil formation and compaction in the field seemed to be a confounding factor for understanding the impact of soil moisture on nematode. Soil compaction is defined as the increase of soil bulk density by reduction of soil pore space (Brady, 1996). It is believed (Wallace, 1987) that pore size and pore geometry are directly related to the migration of the nematode due to inflow of oxygen and

moisture as well as to outflow of carbon dioxide from immediate surrounding roots. Nematodes migrate toward roots by following traces of carbon dioxide emitted by roots (Robinson, 1995). Thus, an experiment was performed in a growth chamber using tomato as a model to assess root penetration and nematode development and reproduction. Total porosity of the soil was calculated as:

$$\text{Total porosity} = 1 - (\text{dry bulk density} / \text{particle density}).$$

The dry bulk density was estimated by an oven dry procedure (Brady, 1996) and particle density for this soil was derived to be 2.6 (Brady, 1996). To obtain two soil porosity levels, the soil bulk density was adjusted to 0.6 and 0.9 g/cm³. The volumetric water content was lower in the least dense soil porosity of 0.77 (bulk density of 0.6 g/cm³). The rate of root penetration and post-embryonic developmental rates occurred slightly faster in the 0.77 porosity treatment than in the more densely packed soil (porosity of 0.65 and bulk density=0.9 g/cm³). Even though the nematode appeared to mature faster and began laying eggs sooner in plants growing at porosity of 0.77, egg laying stopped at 18 days after inoculation. In contrast, the slower developing nematodes at a porosity of 0.65 produced much greater numbers of eggs by day 30 days than those at a porosity of 0.77.

Major questions still exist on the nematodes interaction with the host plant and how these interactions are affected by components of the soil physical environment. Soil water management is important in this host-parasite relationship, but will not substitute as an effective management tool of the nematode. This interaction is complex and needs to be critically evaluated in terms of effects on birth and death rates of the nematode, pathogenicity and cellular host responses.

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Chapter II

***MELOIDOGYNE KONAENSIS* AND COFFEE ROOTSTOCK INTERACTIONS AT TWO MOISTURE REGIMES IN FOUR SOILS**

Abstract

Serracin, M. and D. P. Schmitt. *Meloidogyne konaensis* and coffee rootstock interactions at two moisture regimes in four soils.

Three experiments were conducted to evaluate the reproduction and damage potential of *Meloidogyne konaensis* on resistant and susceptible rootstocks of coffee in four soils under two moisture regimes representative of areas where coffee is grown in Hawaii. Reproduction of *M. konaensis* occurred readily in all soil types, but lowest in the Hydric Dystrandept soil where the holotype was first founded. Root galling, however, was greatest in this soil. *M. konaensis* suppressed growth of coffee in all four soils. Greater galling occurred under constant moisture (33kPa) than under fluctuating moisture (33-1500 kPa). Fifty percent more eggs were produced at constant moisture than under fluctuating moisture conditions. Development of *M. konaensis* was complete in *C. arabica*, as expected for a good host. *Coffea liberica* var. *dewevrei* was resistant to *M. konaensis* and *C. arabica* was susceptible. *C. liberica* var. *dewevrei* allowed development of a few adult females and males.

Key words: coffee, *Coffea arabica*, *Coffea liberica* var. *dewevrei*, Hawaii, irrigation, Kona coffee root-knot nematode, *Meloidogyne konaensis*, resistance, rootstocks.

INTRODUCTION

Plant responses to soil physical properties are determined by the plant genetic constitution (Flor, 1946; Vanderplank, 1963) and by pulses in the environment. Although the genotype determines its potential for growth and yield, environmental conditions, pests or human intervention can modify the extent to which that potential is realized (Wallace, 1989). A complex array of interactions occur in the soil environment that influence the growth potential of plants and the population densities of soil inhabitants, including plant-parasitic nematodes.

Coffee production in Hawaii occurs in a wide variety of soils and with different moisture characteristics and water management practices. Coffee yield potentials are not realized in Hawaii for numerous reasons but draught, improper management and damage by nematode are among the primary causes. Most of the fields on the island of Hawaii (1000 ha) are planted on Andisols and Inceptisols composed of fragments of 'Pahoehoe' lava rock and volcanic ash (Powers, 1932). On Kauai, 2,000 ha of coffee are planted on weathered Oxisols. The coffee estates on Molokai (200 ha) are established largely on Mollisols and Oxisols. This situation presents a wide range of growing conditions, different coffee management practices, and edaphic environments for nematodes.

Rainfall patterns are different across the islands. Coffee growers who irrigate use different systems and quality of water. In the Kona districts of the island of Hawaii, the Kona coffee root-knot nematode, *Meloidogyne konaensis* (Eisenback et al., 1994), is the most important organism causing damage to coffee (Zhang and

Schmitt, 1995a). Under optimal nematode control and production practices, values for the 1998 coffee crop was estimated at US \$ 30 million. However, actual revenues for the 1999 crop was US \$ 21 million (Statistics of Hawaiian Agriculture, 1998). Presently, *M. konaensis* is known to occur only in the Kona area on the island of Hawaii (Schmitt and Serracin, unpublished data, 2001). Surveys conducted in 1999-2000 indicate that the nematode was spread through planting of infected seedlings. An important concern is the potential of *M. konaensis* to spread to more coffee plantations throughout the state.

Associations between soil physical properties and nematodes damaging coffee have been documented worldwide (Sasser, 1954; Campos, 1994). At least some species of nematodes have soil type preferences. In Brazil, *M. paranaensis* and *M. exigua* occur primarily in sandy soils (Jaehn and Rebel, 1984; Campos *et al.*, 1990). Conversely, in Central America, *M. arabicida* (Lopez and Salazar, 1989), *M. exigua* (Schieber, 1974), and *M. incognita* (Chitwood and Berger, 1960) are widespread, and are considered very damaging to coffee cultivated on fine-textured volcanic soils. The broad category of soil types may not adequately explain environmental or resistance factors that influence nematode behavior .

The amount and frequency of precipitation and irrigation has a major effect on coffee growth and nematode activity (Schmitt *et al.* 2001). The seasonal rainfall in the Kona region of Hawaii is greatest from April to August and least from December to March. This is opposite to that of other areas of the islands where the greatest precipitation occurs from November to February. Nematode population densities are normally greatest in July and lowest from November through February (Schmitt *et*

al, 2001). Nematode infected coffee plants in Hawaii express symptoms primarily between July and November (Serracin , *et al*, 1999). Irrigation has been used to reduce symptoms of stress (wilting) associated with *M. konaensis* infection. However, lack of oxygen alone due to excessive moisture often leads to the detrimental effects to coffee roots (Wrigley, 1988).

Because *M. konaensis* is established in soils in the Kona district and on the island of Hawaii, it is important to determine its potential to spread, establish and cause damage to coffee in other areas. The role of soil moisture on nematode reproduction and coffee growth, and the response of *C. liberica* var. *dewevrei* to nematode infection are also equally important to ascertain. Zhang and Schmitt (1995a) demonstrated in greenhouse studies that *M. konaensis* is very damaging to coffee grown in sand, and that *C. arabica* cultivars vary in their reaction to the nematode. The use of nematode resistant rootstocks has been the most effective management tactic to increase vigor and productivity (Reyna, 1949; Campos, 1990; Schmitt et al, 2001). Rootstocks have been used since 1870 to manage nematodes in coffee (Cramer, 1934). In Brazil and Guatemala, scions of *C. arabica* cultivars ‘Mundo Novo’ and ‘Red Catuai’ are often grafted onto *C. canephora* rootstock as a management strategy against *M. exigua* and *Pratylenchus* spp. (Schieber, 1968). *C. liberica* W. Bull ex Hiern var. *dewevrei* appears resistant to *M. exigua* (Curi ., 1970, Fazuoli and Lordello, 1976. In Hawaii, scions of *C. arabica* ‘Typica’ land race Guatemalan were grafted onto *C. liberica* W. Bull ex Hiern var. *dewevrei* and later evaluated for cup quality; the coffee retained the high coffee quality grade expected from *C. arabica* coffee (Cavaletto, pers. comm.).

This research was undertaken to determine the reproductive and damage potential of *M. konaensis*: 1) on coffee in four soils with different chemical and physical properties representative of conditions where coffee is grown in the state of Hawaii, 2) under two moisture regimes, and 3) on two coffee species.

MATERIAL AND METHODS

Experiments: Three experiments were conducted in a greenhouse at Whitmore, Oahu, Hawaii, elevation 420 meters with diurnal temperatures fluctuating from 18-28 °C. The first experiment examined the effects of four soil types on reproduction and damage potential of *M. konaensis* on coffee. The second experiment tested the effects of two extreme irrigation regimes (constant 33kiloPascals (kPa), and fluctuating between 33-1500 kPa) on nematode reproduction in a Hydric Dystrandepts. The third experiment was designed to determine the resistance response of *C. arabica* and *C. liberica* var. *deweyrei* to *M. konaensis*. Soil in the pots was managed to keep its content near 33 kPa for 7 days before adding the nematodes. The duration of all the experiments was 120 days.

Plants: Seeds of *Coffea arabica* cv. Typica land race 'Guatemala' and *C. liberica* var. *deweyrei* were collected at the Kona Experiment Station, Kainaliu, Hawaii, and scarified to accelerate germination. Seeds were sown in Sunshine[®] mix and placed on greenhouse benches under 30% shade cloth to provide optimum conditions for coffee growth. Seedlings at the cotyledon stage were transplanted into 10-cm-diam tubes filled with Sunshine[®] mix. Immediately after transplanting some scions of *C. arabica* were grafted onto *C. liberica* var. *deweyrei* rootstocks (Reyna, 1966; Ito, 1989, unpublished). Grafted and non-grafted *C. arabica* were

grown in the greenhouse for an additional 3 months before being inoculated with the nematode.

Soils and irrigation regimes: Soils were collected from 4 of the primary coffee production regions of Hawaii (Table 2.1). The soils were steam treated at 76°C for 5 hours. Analysis of soil pH, salinity, N, P, K, B, Mg, and % organic carbon was performed on all soil (Table 2.1) before heat treatment by the University of Hawaii soil testing laboratory. All soils were used for Experiment 1. For Experiments 2 and 3, the Hydric Dystrandepts from Kona was selected.

An automated drip irrigation system for experiments 1 and 3 was programmed to maintain the soil water potential around 33kPa and to prevent water stress. This drip system delivered approximately 300 ml of water/day and gradually increased to 750 ml/day as the plants grew older in order to maintain the soil water potential near 33kPa. For experiment 2, irrigation goals were: 1) 33 Kpa and 2) allowing the soil to dry until the water potential reached 1500 Kpa, then watering to 33 Kpa. All plants were fertilized once a week with 100 ml of a soluble 20-20-20 (N-P-K) fertilizer (Rapid Grow[®], Chevron Chemical Company, San Ramon, CA).

Nematode treatments: Two nematode infestations levels were used: 0 and 2,500 J2/pot (1 liter of soil). *M. konaensis*, collected from galled coffee roots at the Kona Research Station, was cultured in the greenhouse on tomato (*Lycopersicon esculentum* (L). cv. Pixie. Eggs of *M. konaensis* were released from the gelatinous matrix with NaOCl (Hussey and Barker, 1973) and placed on Baermann funnels for hatching. Second stage juveniles (J2) that emerged during the first 24 hours were

discarded and those that emerged during the following 24 hours were used as inoculum. Control treatments were inoculated with 10 ml of water.

Data: Seedling height and number of new leaf pairs were determined at monthly intervals. At the termination of the experiments, plants were weighed and root galling evaluated. A modified galling index (Carneiro, 1995) was used: 0= No galls (all roots normal in appearance and quantity); 1= small galls visible in secondary roots and root tips; 2= swelling and discoloration of primary roots with few secondary roots present; 3= swelling of tap root, with cracking and corkiness of primary roots; 4= necrosis and cracking of most roots; and 5= intense corkiness and complete necrosis of tap root, no secondary roots present. Nematode reproduction factors (RF) were calculated for each experiment using $RF = Pf/Pi$, where $Pi = 2500$ and Pf = the population density at the termination of the experiment. Nematodes were recovered from 250 ml of soil by a combination of elutriation (Byrd., 1976) and centrifugal flotation (Jenkins, 1964). The roots were divided into two portions: one portion was placed in the mist chamber for 5 days (Seinhorst, 1956) to assay nematode root populations; eggs were collected from the remaining portion of the root system using a NaOCl method (Hussey and Barker, 1973). After the extraction process was completed, the roots were dried at 70 C for 1 week and weighed. In addition, for Experiment 3, nematodes within the roots were stained with acid fuchsin (Daykin and Hussey, 1985) to facilitate characterization of life stages.

Experimental design and data analysis: Experimental units were completely randomized with 8 replications per treatment. Nematode quantitative data were normalized by transforming to $\log_{10}(x+1)$ values before performing an analysis of

variance using a Statistical Analysis System (SAS Institute, Carey, NC). The Waller-Duncan K-ratio T-test was used as a multiple range test for comparing treatment means. For Experiment 1, differences in soil effects on galling indices, number of eggs per plant and root fresh weights were tested using orthogonal contrasts. The comparisons were 1) Hydric Dystrandepts (HD) vs. other soils, 2) Oxidic Haplustoll (OH) vs. Aridic Haplustoll (AH) and Vertic Haplustoll (VH), contrast 3) Aridic Haplustoll vs. Vertic Haplustoll.

RESULTS

Experiment 1: (Effect of soil type). Physico-chemical parameters of four soils representatives of the major coffee producing areas in the Hawaiian Islands are summarized (Table 2.1). Plant and root growth were both affected by soil (Table 2.2) with root growth being affected by soil and *M. konaensis*. Plant height was greatest in the Aridic Haplustoll (AH) and least in the Hydric Dystrandept (DH). Plants were 33% shorter in the Vertic Haplustoll (VH) soil, 24% shorter in the Hydric Dystrandept (HD), and 3% shorter in the Oxidic Haplustoll (OH) than they were in the Aridic Haplustoll (AH) if nematodes were present. *M. konaensis* suppressed root development in all soil types (Fig. 2.1). Root weight was 29%, 46% and 5% less, in the Aridic Haplustoll than in the Oxidic Haplustoll, Vertic Haplustoll, and Hydric Dystrandept respectively. The nematode suppressed root biomass development by 39-50% depending on the soil.

Reproduction occurred readily in all soils tested (Table. 2.2). The Hydric Dystrandept, the soil collected from the naturally infested areas of Kona, was the least suitable soil for egg production compared to other soils tested. The number of eggs

Table 2.1. Physico-chemical parameters of four soils representatives of the major coffee producing areas in the Hawaiian Islands.

Parameters	Source of Soil			
	Kualapuu, Molokai	Eleele, Kauai	Waimanalo, Oahu	Kainaliu, Hawaii
Depth (cm)	0-20	0-18	0-18	0-25
Water content at:				
1500 kPa	21.6	24.4	27.5	58.8
33 kPa	29.2	36.8	dna	84.8
Field Capacity	dna*	dna	dna	82
Bulk Density (g/cc)	1.26	1.09	1.18	0.48-0.72
Chemical Analysis				
pH (in water)	6.20	7.4	6.8	5.70
Salinity (EC)	0.98	0.44	0.80	0.28
P (ppm)	175	99	395	26.0
K	540	620	600	340
Ca	2200	3800	5800	2200
Mg	280	500	1700	360
B	0.84	108	0.40	0.60
OC (%)	1.22	2.44	1.31	7.54
Soil Series	Hoolehua silty clay	Makaweli Silty Clay Loam	Waialua Clay Variant	Honuaulu, Silty Clay Loam Hydric
Classification (Great group)	Aridic Haplustoll, Mollisol	Oxic Haplustoll, Oxisol	Vertic Haplustoll, Mollisol	Dystrandepet, Inceptisol Thixotropic over fragmental,
Minerology	Fine kaolinitic, Isohyperthermic	Fine kaolinitic, isohyperthermic	Very fine, kaolinitic, isohyperthermic	Isothermic

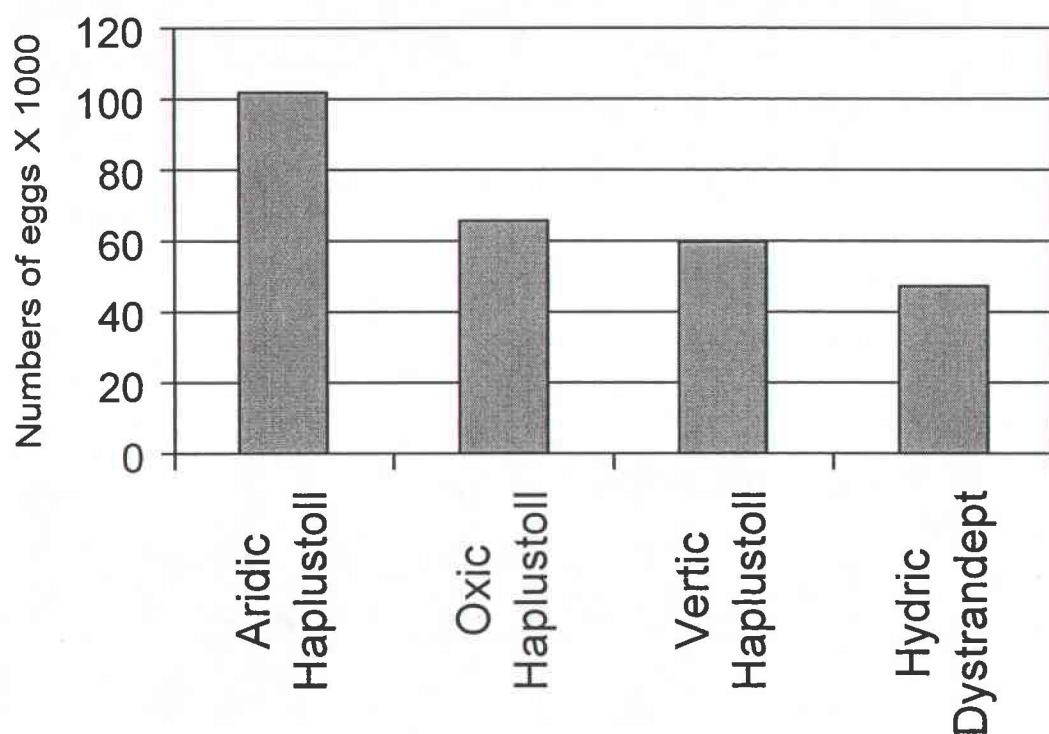
* dna= data not available.

Table 2.2. Coffee (*Coffea arabica* cv. Typica selection Guatemala) growth response to soils and *Meloidogyne konaensis*.

Soil	Nematode Pi (J2/soil)	Height (cm)	Shoot Dry Weight (g)	Root Dry Weight (g)
Aridic Haplustoll	0	29.0 a	15.5 a	11.9 a
	2500	27.5 a	14.5 a	6.0 a*
Oxic Haplustoll	0	24.3 b	14.0 a	8.4 b
	2500	26.7a	15.3 a	4.8 b*
Vertic Haplustoll	0	22.7 ab	9.8 b	6.4 b
	2500	18.5 c *	7.7 b	3.4 c*
Hydric Dystrandepts	0	21.3 ab	8.2 b	5.1 c
	2500	21.0 b	7.7 b	3.1c*

Data are means of 6 replicates. Means of soil effects (combined data for inoculated and control treatments) were compared and separated at $P < 0.05$ level according to Waller-Duncan k ratio t-test. Similar letters within each column do not differ significantly from each other.

Asterisk (*) indicates significant ($P < 0.05$) difference from non inoculated controls.



Orthogonal Contrasts

Hydric Dystrandepts vs Others

Oxidic Haplustoll vs Aridic Haplustoll and Vertic Haplustoll

Aridic Haplustoll vs. Vertic Haplustoll

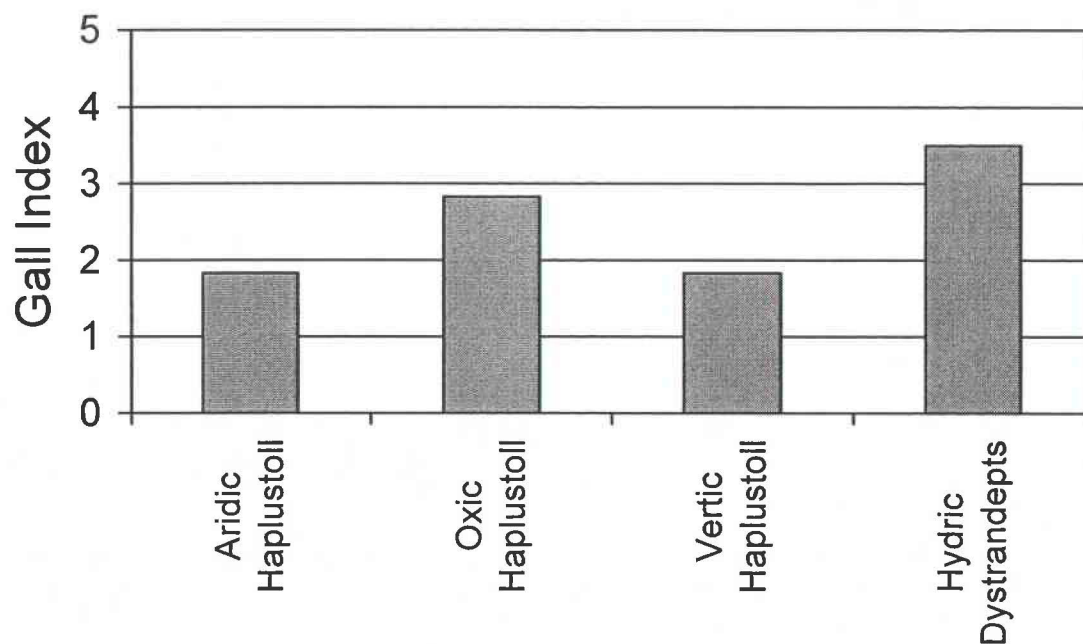
Pr >F

0.05

0.81

0.25

Fig. 2. 1. Number of eggs of *Meloidogyne konaensis* per plant (*Coffea arabica* Typica land race Guatemalan) grown in four different soils for 120 days.



Orthogonal Contrasts

Hydric Dystrandepts vs Others

Pr > F

0.02

Oxic Haplustoll vs Aridic Haplustoll and Vertic Haplustoll

0.19

Aridic Haplustoll vs. Vertic Haplustoll

0.25

Fig. 2. 2. Root-gall indices on coffee infected with *Meloidogyne konaensis* in four different soils. Gall index was modified : 0= No galls. all roots are normal in appearance and quantity; 1= small galls visible in secondary roots and root tips; 2= Swelling and discoloration of primary roots with few secondary roots; 3= swelling of tap root, cracking and corkiness of primary roots; 4= necrosis and cracking of most roots; and 5= Complete corkiness and necrosis of tap root, no primary or secondary roots present.

produced were 2.15, 1.70 and 1.55 times greater ($P=0.05$) in the Aridic Haplustoll than in the Hydric Dystrandept, Vertic Haplustoll and Oxic Haplustoll, respectively (Fig. 2.1). In contrast to egg production, more galling and necrosis ($P=0.02$) occurred in the Hydric Dystrandepts than in other soils (Fig. 2.2). Galling was intermediate in the Oxic Haplustoll and least in the Aridic and Vertic Haplustoll

Experiment 2: (Influence of moisture extremes). Shoot and root weights were only slightly affected ($P>0.05$) by the irrigation regime in the presence of nematodes (Table 2.3). Irrigation also affected coffee overall growth; at the end of the experimental period, nematode infected plants had greater total biomass than controls plants. Less galling ($P=0.04$) occurred under fluctuating moisture than under constant moisture of 33 kPa. Nematode fecundity was also affected by irrigation. The number of eggs produced was 1.5 times greater at constant moisture than under fluctuating moisture conditions. The total number of nematodes per pot was greater at constant 33 kPa than in the fluctuating of soil moisture treatment ($P=0.03$).

Table 2.3. Effects of two extreme soil moisture levels on *M. konaensis* and coffee growth.

Irrigation	<u>Dry Weights (g)</u>		Galling	Eggs/Pot
	Shoot	Root		
<i>Fluctuating (33-1500 kPa)</i>				
Nematode +	1.90	0.55	1.8*	1620*
Nematode -	3.58	0.71	0	0
<i>Constant (33 kPa)</i>				
Nematode +	0.90	0.64	2.0**	2222*
Nematode -	3.22	0.82	0.0	0

Data are the means of five replications.

Asterisks (*) indicate significance ($P < 0.05$) between +, - nematode treatment.

¹Initial population P_i = of 2500 juveniles per 1 liter pot.

Experiment 3: M. konaensis reproduces poorly in *C. liberica* var. *dewevrei* rootstock indicating moderate to high resistance (Table 2.4). When *C. arabica* was infected with *M. konaensis* its shoot fresh weight and root dry weight were less (but not statistically significant, $P=0.17$ and $P=0.23$, respectively) than those grafted onto *C. liberica* var. *dewevrei* seedlings. Differences in plant height were also evident ($P=0.05$) among rootstocks. Susceptible *C. arabica* cv. Typica landrace 'Guatemalan' plants infected with *M. konaensis* were approximately 45% shorter than *C. liberica* var. *dewevrei*. Gallings were greater on *C. arabica* ($P<0.0001$) in contrast to *C. liberica* var. *dewevrei* which showed little galling (Table 2.4). Nematode reproductive rates also differed among rootstocks ($P=0.0019$). The RF was 1.96 on *C. arabica* 'Typica' and 0.51 on *C. liberica* var. *dewevrei*. After one generation (approximately 90 days), *Meloidogyne konaensis*-infected *C. arabica* plants had seven times more eggs of than did *C. liberica* var. *dewevrei*. The results indicate that development of *M. konaensis* on *C. arabica* was typical of that previously observed on a good host, whereas juveniles in *C. liberica* var. *dewevrei* failed to develop beyond the fourth stage and males were present. No males were observed in *C. arabica* rootstocks.

Table 2.4. Interactive effects of *Meloidogyne konaensis* and *Coffea arabica* cv. Typica landrace Guatemalan and *C. liberica* var. *dewevrei* on plant and nematode response.

Parameters measured	Rootstocks			
	<i>C. arabica</i> cv. <i>Typica</i>		<i>C. liberica</i> var. <i>dewevrei</i>	
	Nematode <i>Pi</i>		Nematode <i>Pi</i>	
	2500	0	2500	0
Shoot Dry Weight (g)	0.41	0.53	0.61	0.65
Root Dry Weight (g)	0.19	0.35	0.29	0.27
Height (cm)	15.2	16.0	17.3	16.9
Gall Index (1-5)	3.9	0	1.0	0
Rep. Factor	2.0	0	0.50	0
Eggs/pot	30,266	0	4,245	0
Males	0	0	9	0

Values are the means of 6 replications. Significant differences were found among rootstocks in shoot dry weight ($P = 0.1680$), root dry weight ($P = 0.2306$); height ($P = 0.0057$), galling index ($P = 0.0001$), eggs ($P = 0.0001$), males ($P = 0.0705$), and reproductive factor ($P = 0.0019$).

DISCUSSION

Damage to coffee plants (*C. arabica*) by *M. konaensis* is usually more severe under low moisture conditions. This association of damage to coffee from root-knot nematodes in sandy soils has been well documented in Brazil (Jaehn and Rebel, 1984). In Hawaii, Zhang and Schmitt (1994) determined that even at the low inoculum level of 150 eggs/plant, *M. konaensis* causes considerable root damage to coffee grown in a mixture of sterilized sand and clay soil. A similar level of sensitivity was observed in our experiments with different soils.

Seinhorst and Kozłowska (1977) first related the response and tolerance threshold of plants infected by nematodes to changes in nematode density per root volume or length. In our experiments, soils that support the best root growth provide the most and best feeding sites for nematode. *M. konaensis* caused more damage to coffee roots in the Hydric Dystrandept, a well drained silty clay loam that formed in volcanic ash. This is the soil in which the Kona coffee root-knot nematode naturally is found. Due to mineralogical and physical properties, particle formation from fine volcanic ash have variable water retention capacity and often aggregate to form particles with sand-like behavior, thus explaining the greatest damage in this soil.

The Aridic Haplustoll (AH) supported the largest root system and the greatest number of egg production by *M. konaensis*. This clay soil also had the lowest percentage of organic carbon and it has been under cultivation for many years. The lack of organic carbon may affect the activity of soil-inhabiting microorganisms that would naturally suppress nematode as shown by Lindford . (1938) in a similar Hawaiian clay soil undergoing decomposition of organic matter. The Aridic

Haplustoll also had the lowest water holding capacity at 33 and 1500 kPa and the greatest bulk density. Therefore, it probably had a favorable soil pore and air ratio for root development. Pore size distribution regulates soil moisture and air relationships in soil microbial communities (Hassink, ., 1993) including nematodes (Wallace, 1956).

Water is one of the most important constituents of the coffee soil environment. The relationship of soil moisture to plant damage induced by nematodes has resulted in conflicting data, thus, it is difficult to separate effects because the soil phases are complex and interrelated with the corresponding soil moisture characteristics (Simons, 1973). In addition, coffee plants are sensitive to moisture extremes and experience irreversible symptoms of stress if waterlogged or exposed to sudden moisture deficit (Nuñez, 1976).

In the present experiment *M. konaensis* population development was greater when soil water conditions were constant (33 kPa) than when they fluctuated. This relationship between *M. konaensis* population density and irrigation regimes was similar to that previously reported for *M. hapla* by Couch and Bloom (1960). However, according to Dropkin and Martin (1957) and Barker (1982), egg production by *M. incognita* was slightly favored by low moisture. Egg production may also be affected by different soil type, initial population density and by changes in the physical environment inside the pots in which the experiment was conducted. The growth in the nematode infected coffee plants could be attributed to an initial response of plants to nematode infection. Inherent chemical properties of each soil may also be involved. However, once a nematode generation is developing, stress

conditions would reduce root and plant growth.

Host-plant resistance in *C. liberica* var. *dewevrei* was effective in suppressing *M. konaensis* development and reproduction. It is known that Robusta type coffee genotypes (such as *C. liberica* var. *dewevrei* and *C. canephora*) exhibit broad horizontal resistance to multiple pests and pathogens compared to *C. arabica* (Campos, 1990). In previous field work, *C. liberica* var. *dewevrei* showed moderate field resistance (Zhang and Schmitt, 1995a), but expressed high resistance in the present work. The difference in response between the two tests may be due in part to genetic segregation of the resistance component since this is characteristic for species of coffee which are cross-pollinated (Anthony, 1999). Greater galling indices, reproductive factors and numbers of eggs of *M. konaensis* indicated that *C. arabica* was a better host to the nematode than *C. liberica* var. *dewevrei*. Similar results were found in Brazil (Curi, 1970, Fazuoli and Lordello, 1976) for resistance response of *C. dewevrei* (De Wild.) to *M. exigua*.

Little is known about the mechanism of root-knot nematode resistance in coffee. The presence of males and arrested development of fourth stage juveniles suggests the inhibition of giant cell formation or some other event that adversely affects the nematode development and egg production. Mazzafera., (1990) determined that peroxidases, and polyphenoloxidase in a nematode-resistant coffee rootstock were detectable in *C. canephora* soon after infection by *M. incognita*. These substances are deposited in the cell wall and can impair development of pathogens (Agrios, 1997). Another component of the resistance mechanism in 'Robusta' type coffee was proposed by Goncalves *et al.*, (1995). A comparison of the

mineral and organic content between selection 'Robusta C2258' and *M. incognita* susceptible *C. arabica* cv. Mundo Novo indicated that nematode infection altered the absorption and translocation of essential nutrients within the plant. Potassium and zinc concentrations were high in leaves of nematode-resistant *C. canephora*, whereas phosphorus, magnesium, iron, boron and calcium concentrations were less (Goncalves, *et al.* 1995). Further characterization of the cellular response of resistant rootstocks when infected to *M. konaensis* infection is required.

Genetic resistance can provide a practical way to manage *M. konaensis* as demonstrated by the resistant rootstock *C. liberica* var. *dewevrei*. Furthermore, other rootstocks and scion combinations should be evaluated in an effort to deploy multiples sources levels of resistance as a means of managing the Kona coffee root-knot nematode. The interaction among soil environment, host and nematode also must be recognized when managing *M. konaensis* in the field. Another important conclusion is the relative insensitivity of reproduction to soil types. Avoid the movement of infested soil and plants to prevent further spread of this important coffee pathogen.

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CHAPTER III

GROWTH AND YIELD OF COFFEE AS AFFECTED BY IRRIGATION AND *MELOIDOGYNE KONAENSIS*

ABSTRACT

A field experiment in Kona, Hawaii was conducted to determine the effects of irrigation, plant age and cultivar on the potential of the Kona coffee root-knot nematode to affect coffee growth and yield. The population densities of the nematode in the soil varied according to plant age and irrigation treatment. Soil populations under irrigated conditions were greater during the months of May to July, which normally follows the greatest annual precipitation and a period of active plant growth. Nematode reproduction was greater on plants grown from in 6-month-old seedlings than those transplanted at 12 months of age. Soil water tension fluctuated by season, cultivar and plant age. Older plant treatments exhibited greater water tension fluctuation with greatest water tension occurring in from January to April. Coffee grown from six-month-old transplant coffee seedlings under *M. konaensis* infested soil yielded greater coffee than seedlings transplanted at 6-months of age and non-irrigated. Yield reduction and slower rate of growth were also more evident in this plant age treatment without irrigation; in contrast, irrigated and nematode infected plants grown from transplanted 12-mo-old seedling yielded lower compared to non-irrigated treatments. Overall, yield reduction from nematode infected plants ranged from 30-60% and is economically important.

INTRODUCTION

The Kona coffee industry in Hawaii is known for its coffee. Most of the orchards are located on Andisols, which characteristically are formed by a thin cover of volcanic ash over relatively young lava flows, have a low pH, poor fertility and variable depth and moisture relations. These factors, along with an inadequate supply of water and heavy infestations of *Meloidogyne konaensis*, limit coffee yield. The nematode is present on 85% of the hectarage in the Kona district and suppress yields by 40-60% (Serracin, *et al.* unpubl.).

The Andisol soils in the Kona area are derived from volcanic rock that, upon drying and aggregation, have poor water retention properties comparable to sand (Beaumont and Fukunaga, 1958). Thus, with relatively poor water holding capacity and the heat intensity from the sun, the plants need frequent irrigation. This water management is confounded by infection of the coffee roots by *M. konaensis*. Severe damage by the nematode alters the physiological aspects of coffee growth and development (Zhang and Schmitt, 1995, Wallace, H. R., 1987). The common response of trees infected with the nematode is decline due to wilt, symptoms of nutritional stress, and low yields (Serracin .et al, 1999). Some trees die suddenly, especially if they were infected as seedlings. A standard approach used by growers to solve the problem is arbitrary irrigation and fertilization, a practice that actually exacerbates the damage (Serracin and Schmitt, unpublished).

Soil moisture has a major influence on the population size of nematodes (Simons, 1973). *M. konaensis* population densities are generally greatest in July following the period of the highest annual precipitation (Zhang and Schmitt, 1995),

and decline to their lowest population densities during the winter (November through February), the driest months of the year (Schmitt., 2001). It has been already determined that coffee shoot and root growth is affected by *M. konaensis* (Zhang and Schmitt, 1995). Presumably, the majority of the root damage occurs during periods when the nematode populations are largest. This assumption is in agreement with coffee grower testimony that coffee decline is most evident in irrigated orchards.

The quantity of roots and the numbers of nematodes infecting those roots are related to the amount of early damage to the root system (galling and necrosis) and the subsequent potential of the plant to grow and yield (Zhang and Schmitt, 1995). For coffee, the amount of root development prior to exposure to the nematode is related to seedling age. Even at a low inoculum density of 150 eggs per plant, *M. konaensis* cause substantial galling and root necrosis on coffee grown in the greenhouse (Zhang and Schmitt, 1995). Planting young volunteer coffee seedlings has been previously recommended as a practice to coffee growers in Kona (Goto and Fukunaga, 1956). The rationale of this practice is to reduce the high cost of a nursery production and to quickly replant a declining field. Volunteer seedlings are believed to be best planting material since seedlings can be found almost at any time under mature coffee trees, particularly in nematode infested orchards that suffer severe defoliation and fruit drop. In cases where nursery transplanting of healthy plants is delayed, the plants seem to be more tolerant of infection by *M. konaensis* if transplanted as 6-months or older seedling. Thus, in those specific sites where the growers knowledge of nematode levels and heterogeneous soil properties are important, age of transplant to delay nematode infection would appear to be an

important factor to consider in a overall management program. The objectives of this research were to determine the effects of seedling age at transplant and irrigation on the temporal population fluctuations of *M. konaensis* and plant growth and yield under field conditions over time.

MATERIALS AND METHODS

The plots for this 4-year project were established in January 1997 in a field at the Kona Experiment Station in Kainaliu, Hawaii previously cropped with guava for 20 years. The elevation of the site is 420 m above sea level. Annual precipitation is highly variable, averaging 1240 mm per year (Fig. 3.1); the heaviest rainfall comes from May to September and air temperatures vary from minimum of 15 °C in the winter to maximum 32 °C in the summer (Cline, 1955). The soil is in the Pawaina series and is a hydrous-skeletal, ferrihydritic isothermic typic hydrudands (R. Gavenda, pers. comm.).

Plot preparation involved removal of existing vegetation, soil tillage, and establishment of the nematode. The 20-year-old guava trees were excavated and then the soil was chisel plowed to a depth of 30-cm. Plots were established with rows spaced 2.3-m apart and 10-m long, accommodating 6 coffee trees spaced 2-m apart. Hilograss, *Paspalum conjugatum* was planted between rows to reduce soil erosion and nematode dispersal, and to facilitate weed management. Plots designated for infestation with *M. konaensis* were planted with tomato infected plants (*Lycopersicum esculentum* cv. Rutgers). Nematodes were initially collected from infected coffee trees in an adjacent field at the Kona Experiment Station. The plots were sampled to monitor population development. These tomatoes plants were

grown for 3-months to allow sufficient time for the nematode to become established. The number of eggs and juveniles in each plot at this time was designated as the “initial” population density for the experiment. Nematodes were extracted from the soil using a combination of elutriation (Byrd , 1976) and centrifugal flotation (Jenkins, 1964). In July 1997, coffee seedlings were transplanted and irrigation treatments established.

The experiment was designed as a split plot with three factors arranged in blocks. The treatments were two levels of irrigation (with irrigation or natural rainfall), two levels of nematode infestation (0, and 490-560 second-stage juveniles/250 cm³ soil), and 3 levels of tree treatments (6-month-old seedlings of *C. arabica* cv. Typica selection Guatemala; 12-month old seedlings of *C. arabica* cv. Typica landrace Guatemalan; 6-month old seedlings of *C. arabica* cv. Catuai). Water for the irrigated plots was delivered through drip tubes twice a week at 15-l/tree/irrigation and increased to 26-l/tree/irrigation as the tree grew over the next two years. Tensiometers were placed at a depth of 25 cm and approximately 3 m between plots throughout the experimental site to monitor approximate soil moisture tension across treatments. Irrigation was applied to the natural rainfall treatment when the tensiometers indicated that water tension was approaching the wilting point (around 1500 kPa).

Nematode populations were assayed at 4-month intervals between July 1997 and June 1999. Two 2.5-cm-diameter X 30-cm deep cores were collected from the drip line of each tree per plot and composited. Nematodes were extracted from a 250-cm³ subsample of soil by a combination of elutriation (Byrd ., 1976) and

centrifugation (Jenkins, 1964). Roots were placed in a Seinhorst mist chamber to extract motile J2. Soil moisture was determined by a gravimetric procedure (Brady, 1996). Plant height was also determined at nematode sampling date. Fruits were hand-harvested in 1999 and 2000 and weighed. Soil and foliar nutrient analyses were performed at the initiation and termination of the experiment.

In February 18, 1999 and June 15, 1999, representative trees from each treatment were selected arbitrarily for sampling to describe the vertical and horizontal pattern of roots and nematodes. Soil blocks of approximately 500 cm³ were collected at 0-20, 20-40, and 40-60 cm laterally from the base of the trees and at depth intervals of 0-20, 20-40, and 40-60 cm. Roots were separated from soil by sieving through a screen with 2-mm openings and washed to dislodge any adhering soil. The amount of roots collected varied (Appendix 1, Table 1). Roots were placed in a Seinhorst mist chamber for 6 days to extract J2, removed and dried for 24 hours at 70 °C.

Nematode population dynamics data from soil was analyzed by calculating the area under the curve (nematode-dates) for each treatment and submitted to analysis of variance and the treatment means were compared by orthogonal contrast. Analysis of tree growth was done with repeated measurement analysis including two factors (irrigation and nematodes) with dates of measurement taken as repeated measures, and the slopes compared by regression analysis. Yield data was analyzed by individual harvest-year and by combining harvests of 1999 and 2000 by analysis of variance. Software used for the analyses was developed by the SAS Institute, Inc, Cary, NC.

RESULTS

The rainfall pattern for 1998 and 1999 differed from the historical patterns (Fig. 3.1). March was the wettest month of the year in 1998 and 1999. No precipitation occurred in August 1998 when several population peaks for *M. konaensis* J2 occurred, although the rain events in July totaled about 100 mm. Considerably more precipitation fell in 1999 than in 1998 (Fig.3.1). Tree age and irrigation influenced the pattern of population change of *M. konaensis* juveniles population in the soil (Fig. 3.2), but the response was variable. Irrigation did not affect nematode population density in the soil in seedlings transplanted at 12- month-old ($P=0.22$). However, seedlings transplanted at 6-months of age and irrigated had a lower nematode population density ($P=0.0004$) in the soil than same age transplants without irrigation (Fig 3. A). Irrigation did not influence the population development of the nematode in cultivar 'Catuai' ($P=0.85$). No differences in the pattern for the first 4 months of the experiment was observed, during this time, the populations decreased in all treatments from 500 J2/250 cm³ soil to less than 100. The numbers continued to decrease in plots without irrigation until February 1998, but began to increase in November 1997 in irrigated treatments. Thereafter, populations fluctuated throughout the duration of the experiment.

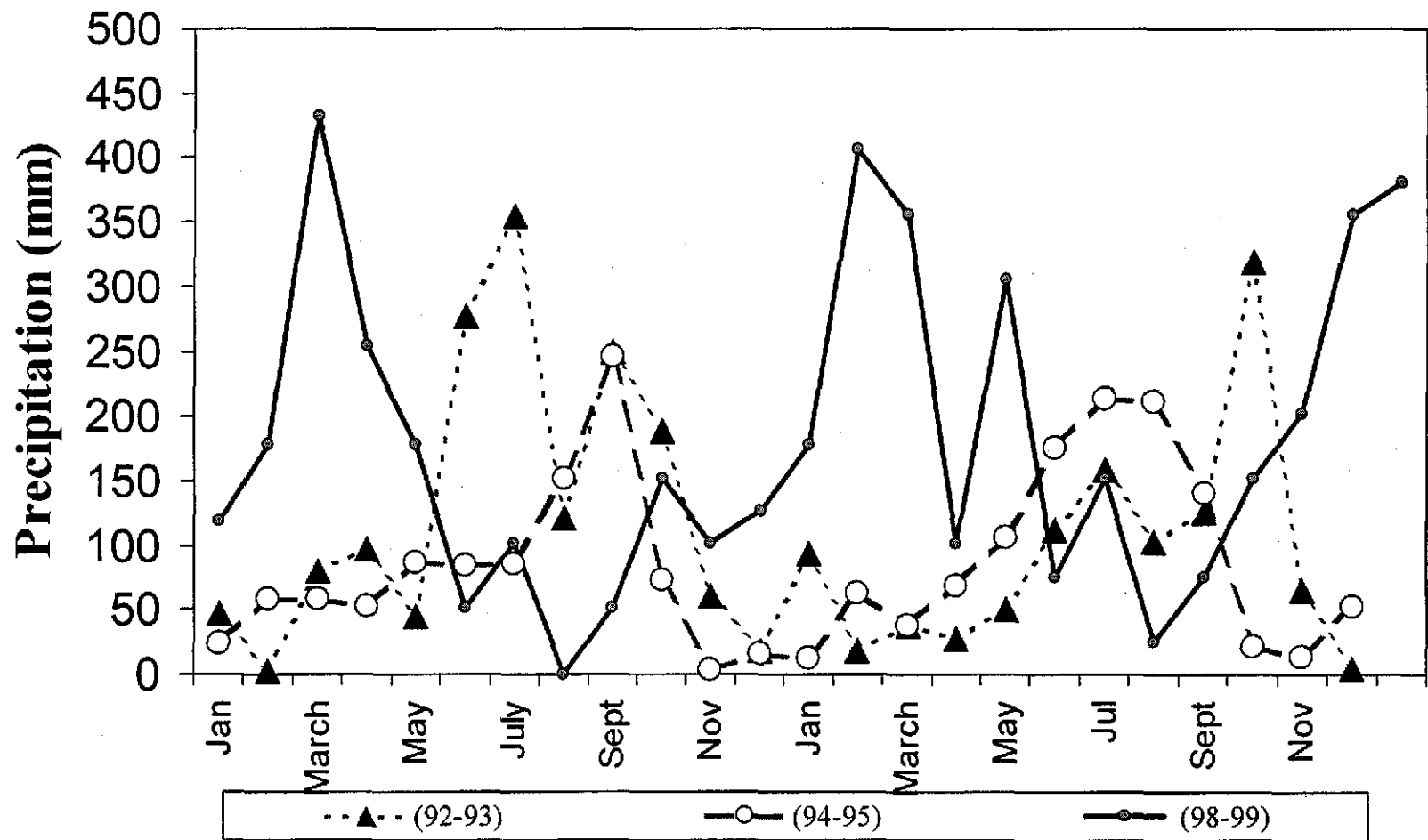


Fig 3.1. Comparison of frequency and amount of precipitation at the Kona Experiment Station, Kainaliu, Hawaii.

Figure 3.2. Coffee tree age and irrigation regime influences on the population dynamics of *M. konaensis* juveniles in the soil . Treatments are: A) 12-month-old plants without irrigation and nematode infected vs. 12-month-old plants with irrigation and nematode infected. B) 6-month-old plants without irrigation and nematode infested vs. 6-month-old plants without irrigation and nematode free C) Catuai without irrigation and nematode infected vs. Catuai plants with irrigation and nematode infected. Sampling times were:1= July 1997,,2=November 1997; 3=February 1998;4=April 1998;5=August 1998;6=November 1998; 7=February, 1998; 8=June 1999.

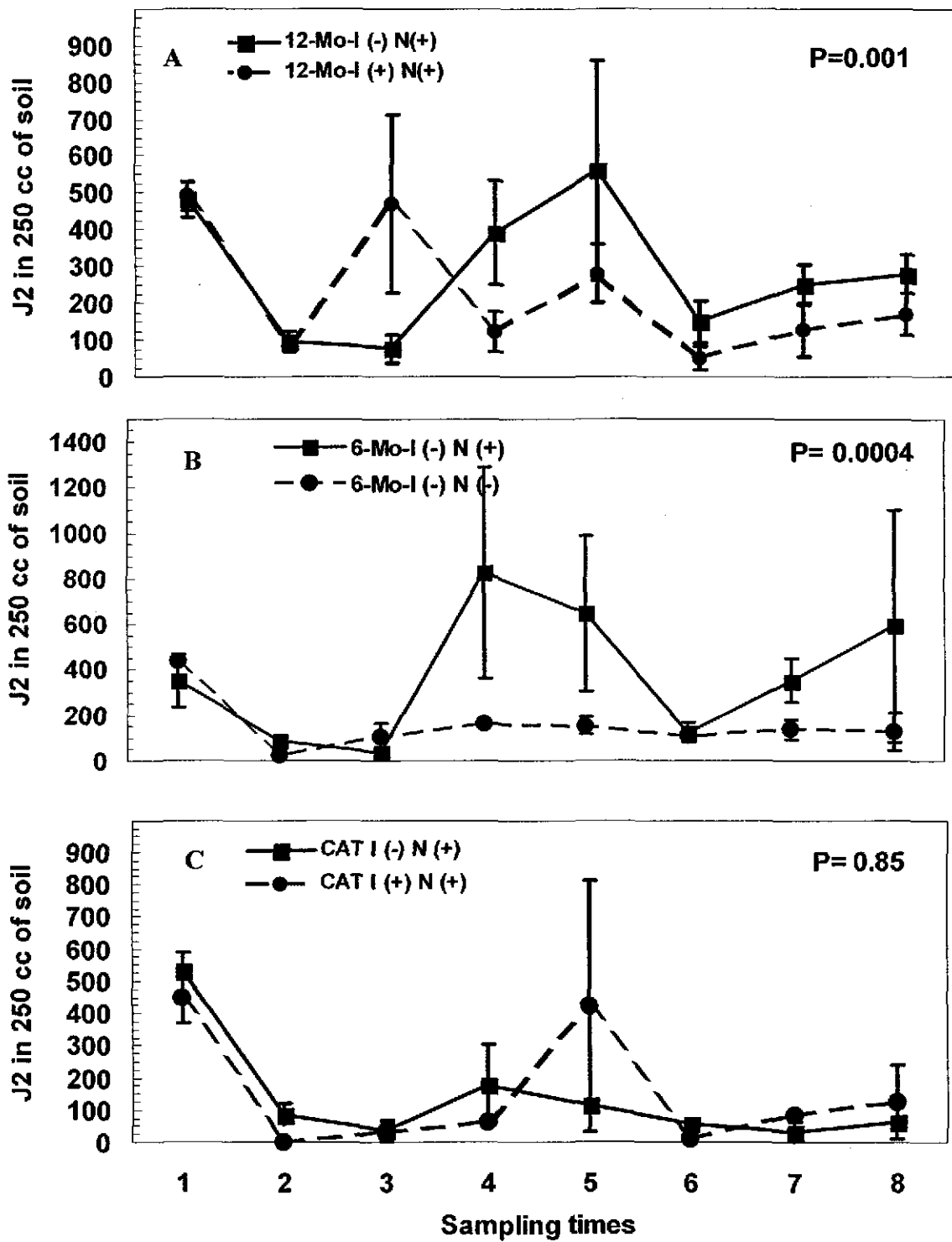
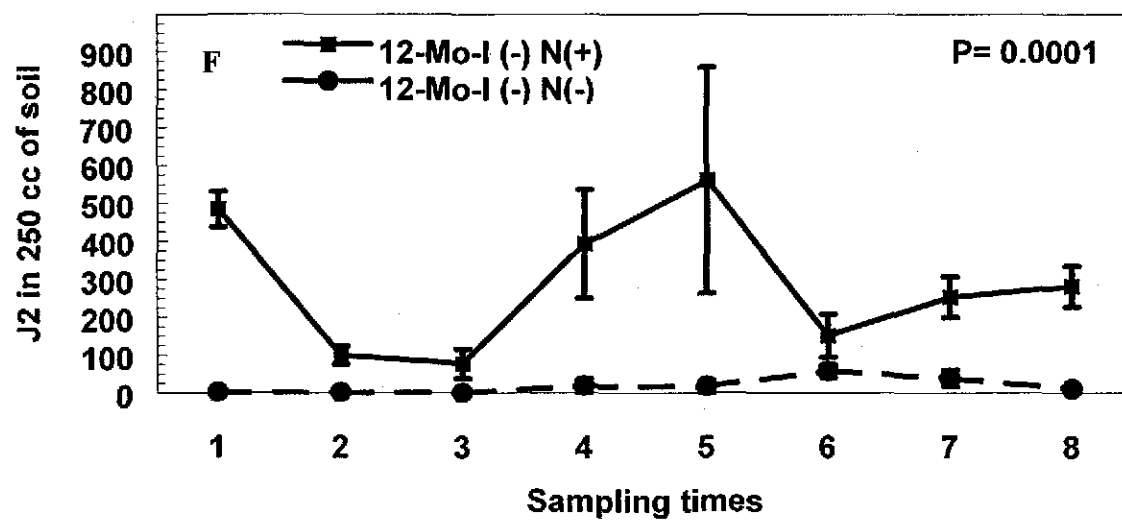
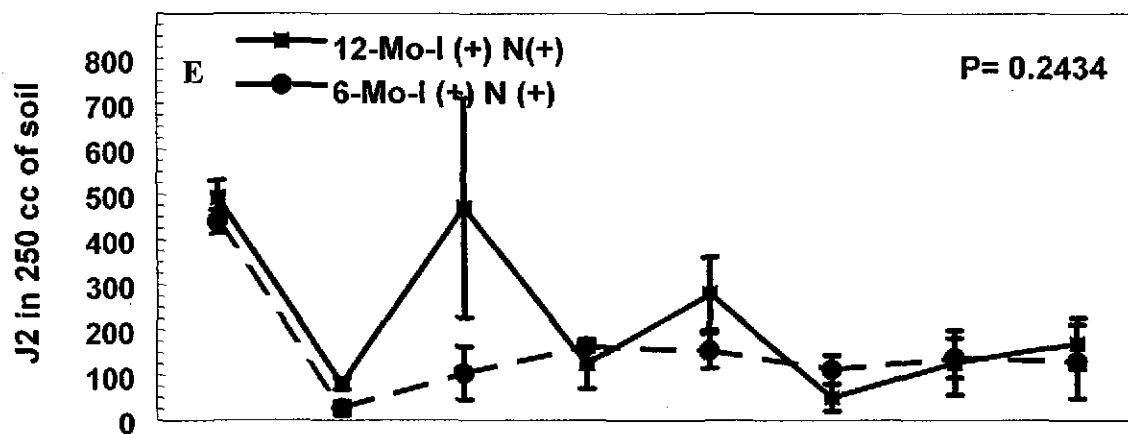
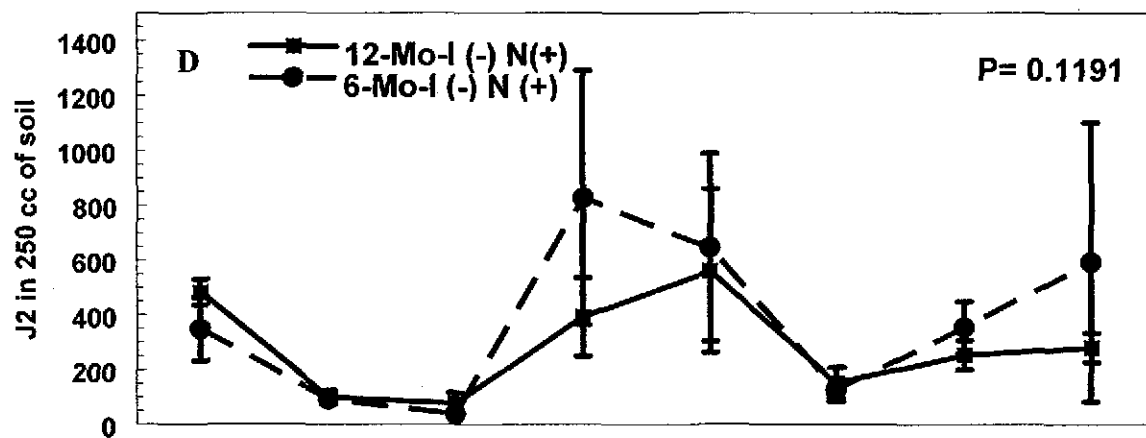


Figure 3.2 (cont). Coffee tree age and irrigation regime influences on the population dynamics of *M. konaensis* juveniles in the soil . Treatments are: D) 12-month-old plants without irrigation and nematode infected vs. 6-month-old plants without irrigation and nematode infected. E) 12-month-old plants with irrigation and nematode infested vs. 6-month-old plants with irrigation and nematode infected F) 12--month-old plants without irrigation and nematode infested vs. 12-month-old plants without irrigation and nematode free. Sampling times were: 1= July 1997,;2=November 1997; 3=February 1998;4=April 1998;5=August 1998;6=November 1998; 7=February, 1998; 8=June 1999.



On *C. arabica* cv. Typica land race 'Guatemalan' transplanted at 12-months-old, the nematode population density was not affected in trees without irrigation ($P=0.85$) or irrigated ($P=0.11$); however, greater nematode counts were found overall on the nonirrigated trees throughout most of the experiment (Fig. 3.2 A-F). The populations had peaks on this selection in August of 1998 in both irrigation treatments with 12-month old transplants. This event coincided with the period of lowest precipitation (Fig. 3.2 A). The numbers declined again in these treatments to around 100 J2 by November 1998 and remained at that level for the remainder of the experiment. Two peaks occurred on the nonirrigated treatment with 6-month old transplants: May 1998 and June 1999. On *C. arabica* 'Catuai', there was a trend for numbers of J2 to be greater in the winter on the nonirrigated trees and greater in the summer on the irrigated trees (Fig. 3.2 C). The population density of J2 was equivalent to the initial population only in August 1998; it was much lower on these trees at all other sampling times. The greatest difference in nematode population density on the soil was found in nematode infested seedlings transplanted at 12-months of age ($P=0.0001$).

Soil temperature was not very different during the season (Fig. 3.2). The highest soil temperature during the year was 25 °C and the lowest soil temperatures are between 20-23 °C during most of the year. The mean maximum soil temperatures were greatest during the last 6 months of the year. The mean minimum soil temperatures usually occurred in April. Water tension in the soil fluctuated according to irrigation, rainfall and plant age (Fig.3.3). In general, the greatest water tension occurred in 12-months-old seedling treatments of the 'Typica' selection.

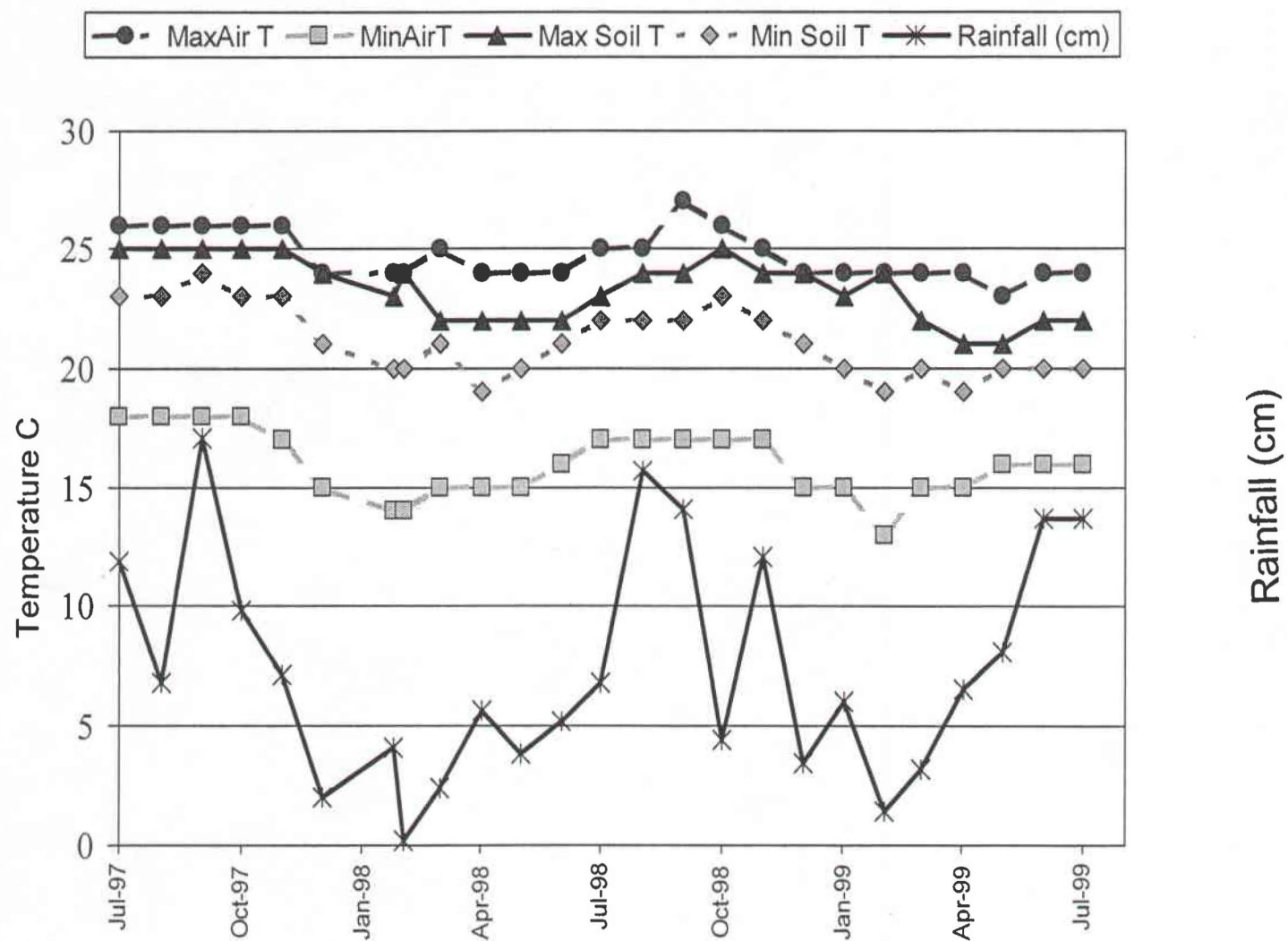


Fig 3.3 . Minimum and maximum air and soil Temperatures during experimental period at the Kona Experiment Station, Kainaliu, Hawaii.

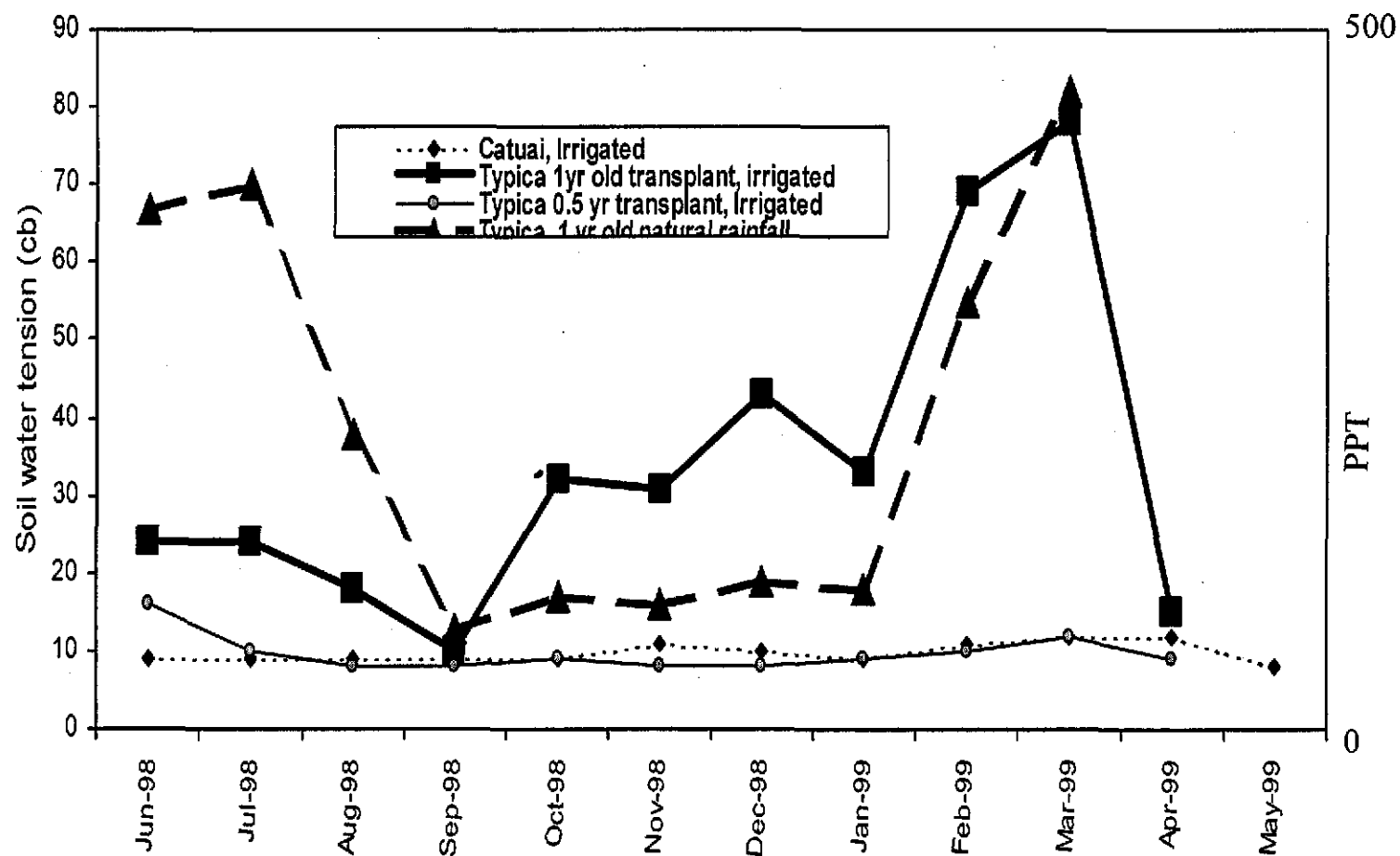


Fig 3.4 . Fluctuations in soil water potential in soil being cropped with *C. arabica* cv. Typica and 'Catuai' with or without irrigation or nematodes.

The nematode, irrigation and seedling age treatments influenced root development of coffee. Considering only main effects, irrigation resulted in nearly a doubling of the root biomass and *Meloidogyne konaensis* suppressed root weight by about 48% (Fig. 3.4). Considering the 12-month-old seedling treatment separately, irrigation enhanced root growth by 16% and the nematode suppressed root growth by 22% (Fig. 3.4). Six-month-old 'Catuai' seedlings of the cultivar responded similarly to the 6-month-old 'Typica' (Fig. 3.4).

The distribution of roots in the soil was relatively consistent across treatments. The greatest biomass was usually in the soil nearest the tree trunk (0-20 cm) and in the surface 20-cm of the soil profile (Fig 3.5 A, B). Two exceptions were in the nonirrigated, *M. konaensis* infested treatments involving 12-month-old seedling transplants of 'Guatemala' and 'Catuai' (Figs. 3.5 and 3.6). The other exception was the nematode-free, irrigated treatment on Catuai on which the greatest root biomass occurred in the 20-40 cm vertical slice (Fig. 3.6 E). Overall root growth from the 12-month-old transplants of the landrace 'Guatemalan' was greater than that of the 6-month-old seedlings (Figs. 3.7 and 3.8).

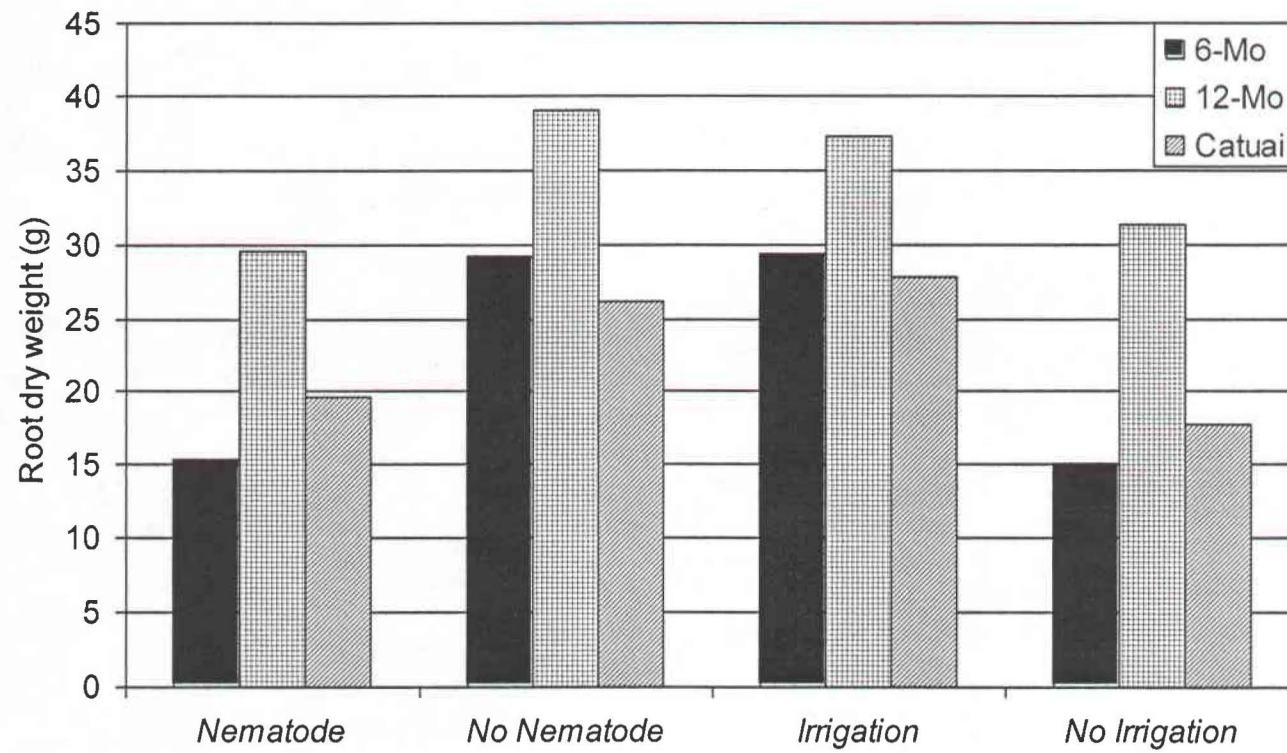


Figure 3.4. Total roots biomass of coffee growing under two levels of nematodes and irrigation regimes in Kona, Hawaii.

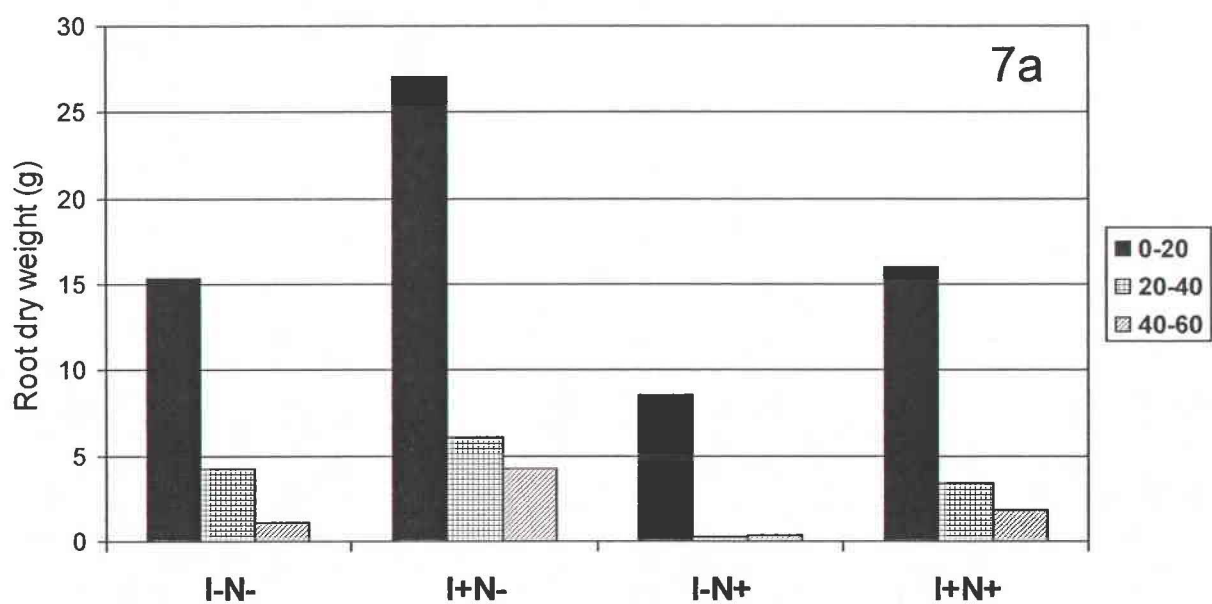
Fig. 3.6. Total number of *M. konaensis* per gram of dry root two years after inoculation with (+) or without irrigation (-). 6-month= Coffee seedlings of *C. arabica* cv. Typica of 6-months and 12-month

Y = 1 year old at transplant CAT= *C. arabica* cv. Catuai.

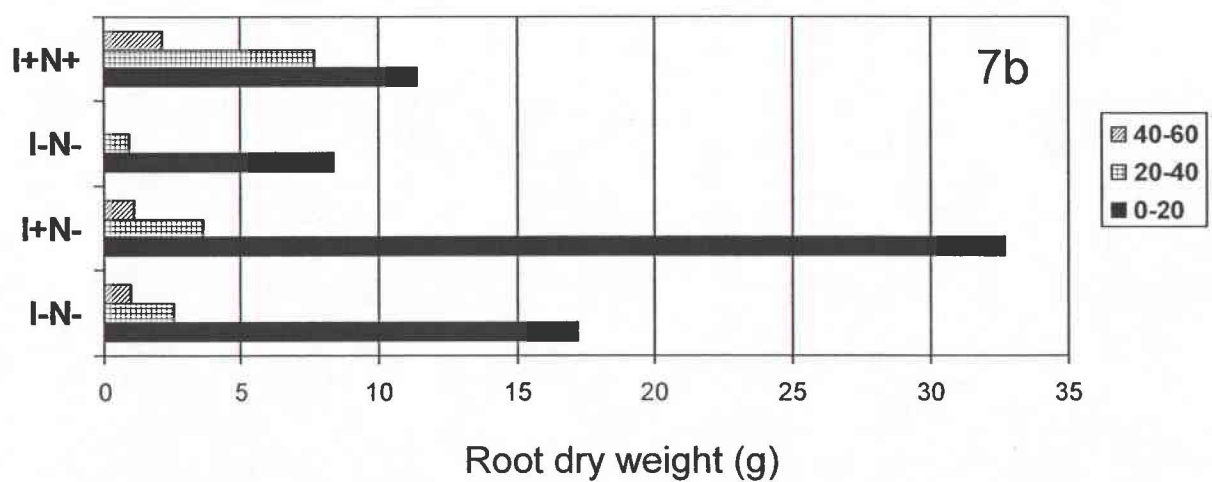
A) Vertical distribution. Depth sampled were 0-20 cm, 20-40 cm and 40-60 cm from soil surface.

B) Horizontal distribution. Lateral sampled was 0-20 cm, 20-40 cm and 40-60 cm from the trunk

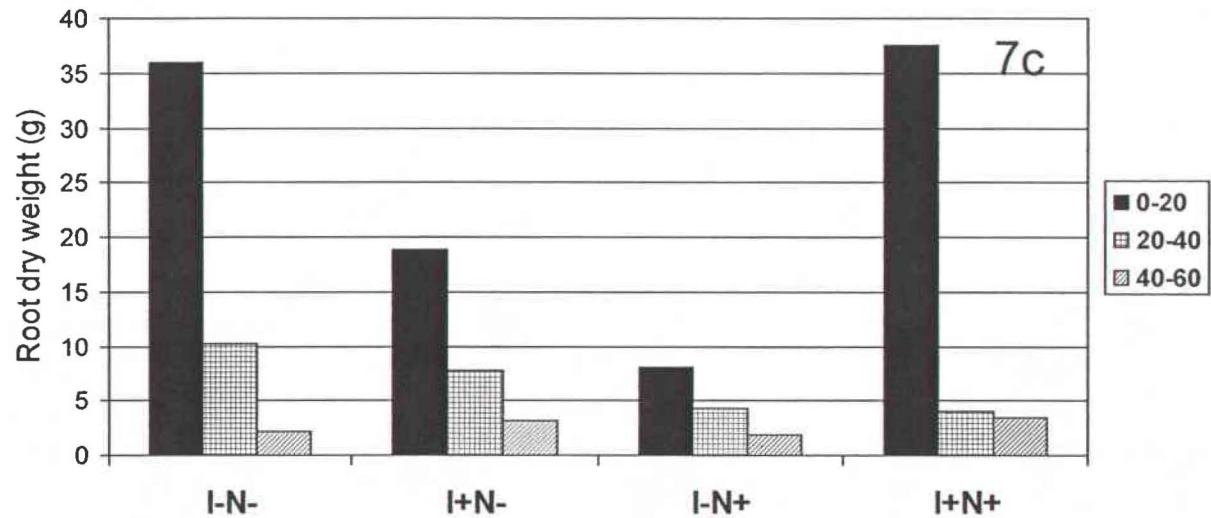
Fig. 3.6 Total root biomass (Vertical distribution) of 6-mo-old plants under different irrigation and nematode regimes



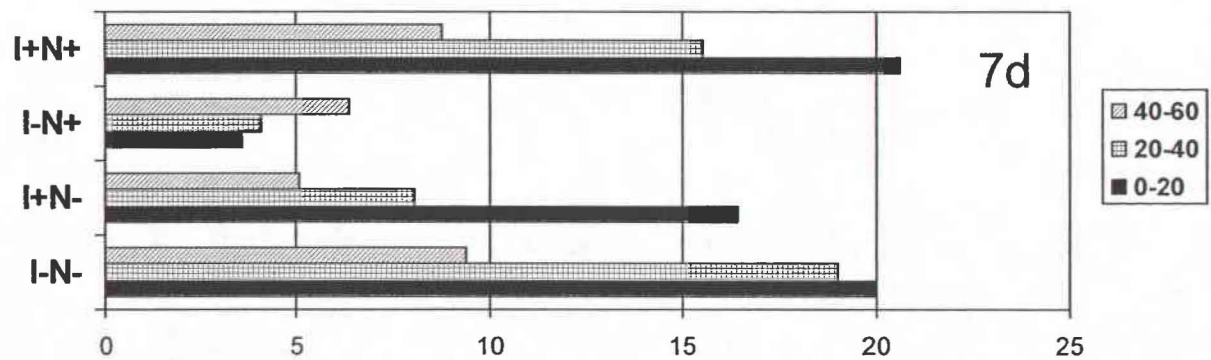
Total root biomass (Horizontal distribution 6-mo-old under different irrigation and nematode regime)



**Total root biomass
(Vertical distribution) of 12-mo-old under different irrigation and
nematode regimes**

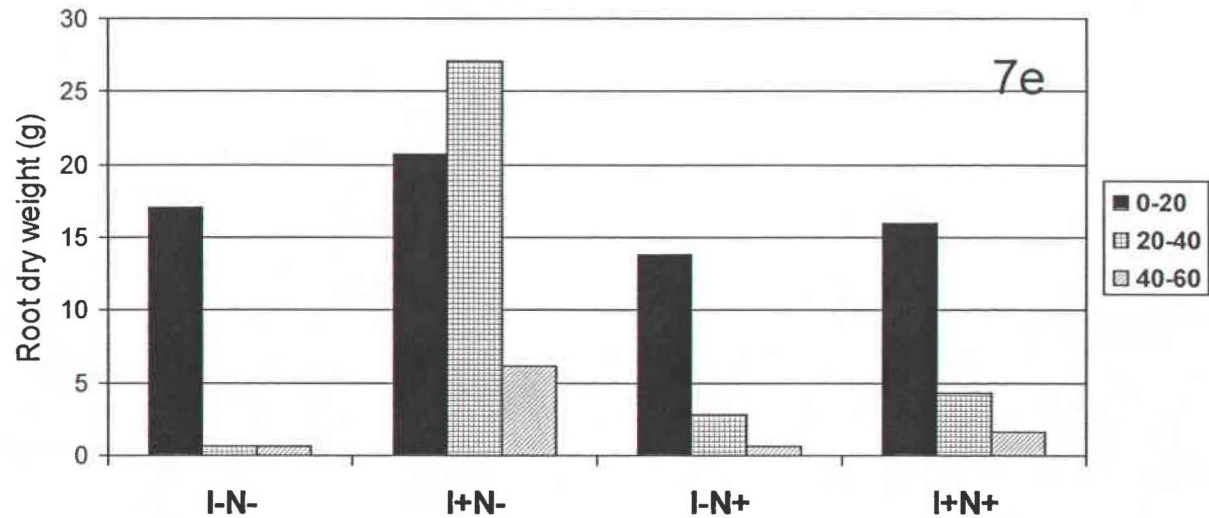


**Total root biomass (Horizontal distribution) of roots of *C. arabica* of
1Y of age under different nematodes and irrigation regimes**

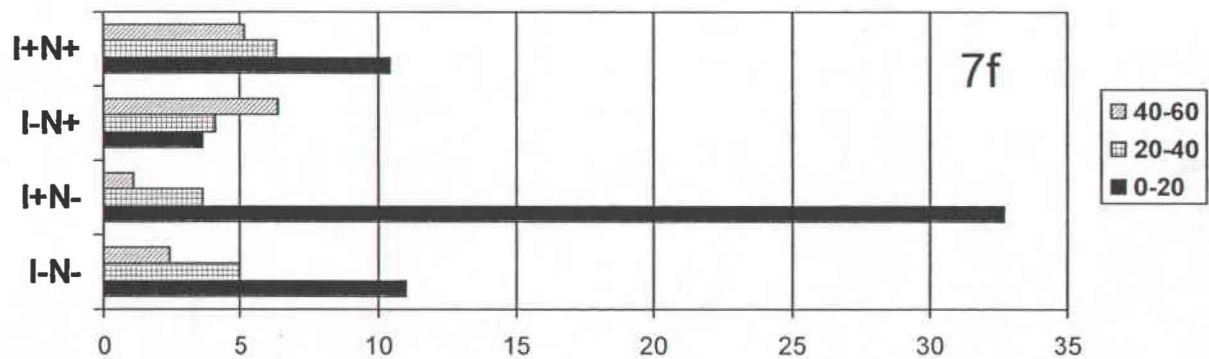


Root dry weight (g)

Total root biomass
(Vertical distribution) of 0.5 Y Catuai plants under different
irrigation and nematode regimes



Total root biomass (Horizontal distribution) of roots of Catuai
coffee under different nematodes and irrigation regimes



Root dry weight (g)

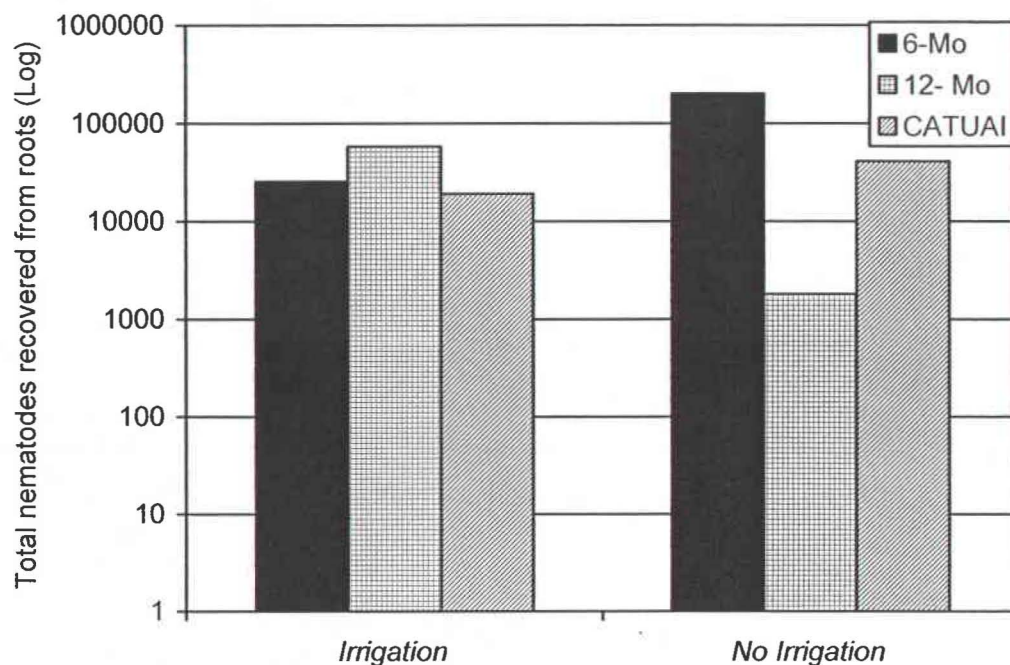


Fig. 3.5. Total nematodes recovered from roots excavated at different depth in coffee plants of two transplanted at 6 or 12 month and under two irrigation regimes in Kona, Hawaii.

Irrigation influenced the total population density of *M. konaensis* in coffee roots, but this effect was altered by time of sampling, seedling age at transplanting and coffee selection (Appendix 1. Table 1). Comparing the two times of assessments of February and June of 1999 there were not significant differences in the number of J2 ($P=0.1604$) or males ($P=0.4748$) but significant differences were detected in egg ($P=0.0064$) and total nematode population ($P=0.0051$). Differences were also detected among the treatments. The number of juveniles ($P=0.0161$), eggs ($P=0.0003$) and total number of nematodes juveniles recovered from excavated coffee roots was

greatest in June (Figure 3.9.) and in trees transplanted as 12-month and 6-month-old seedling without irrigation ($P=0.0161$). Conversely, the nematode population density was lower on the trees transplanted as 12-month-old seedlings. The difference was small on Catuai (Appendix 1, Table 1)

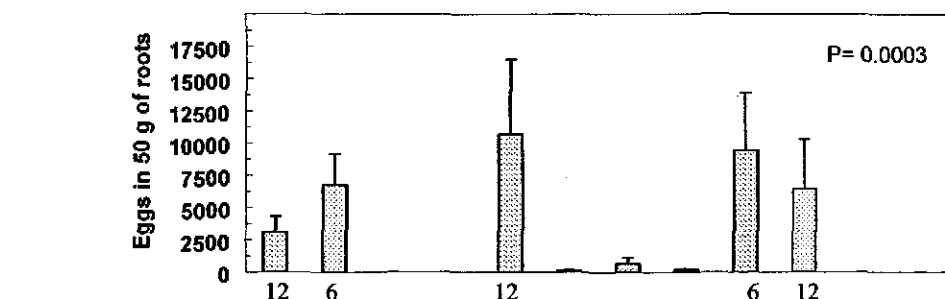
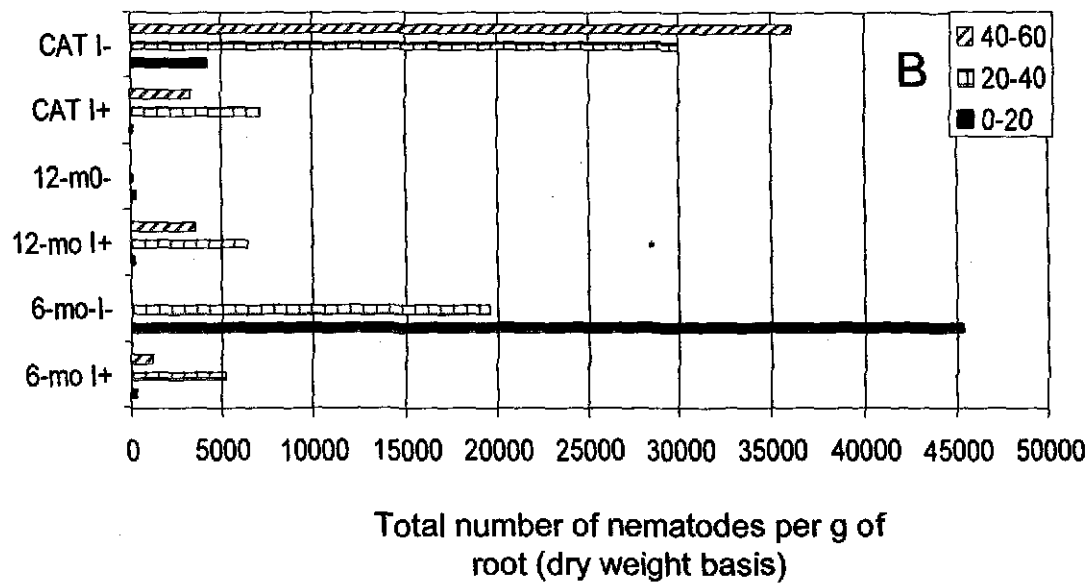
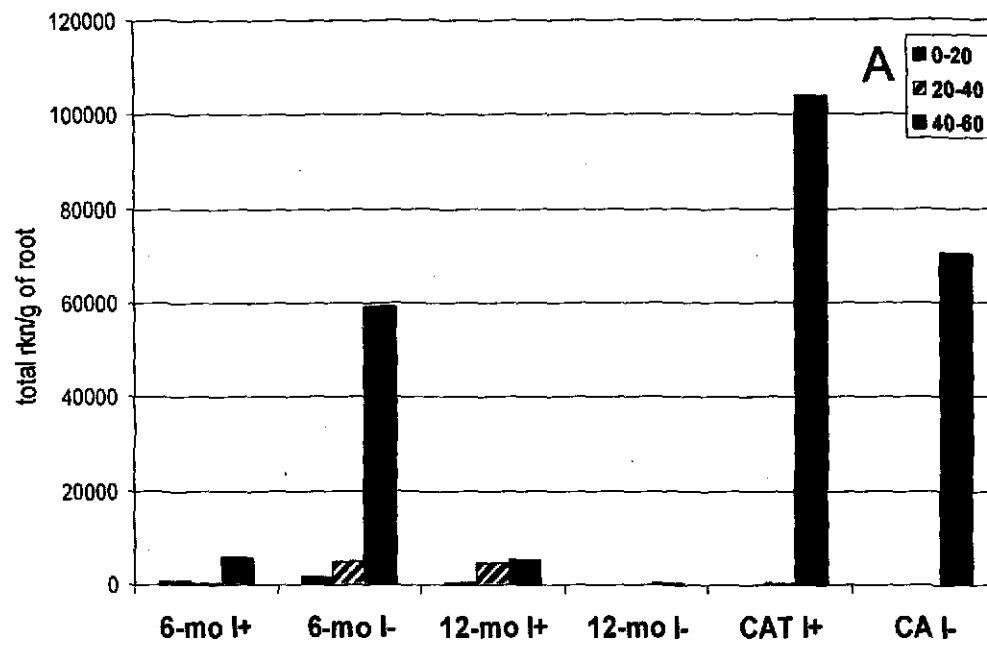


Figure 3.8. Number of eggs produced by *M. konaensis* in coffee roots in 12- and six month old seedling transplants sampled in Feb and June 1999..

The spatial pattern of *M. konaensis* varied by treatment (tree age, irrigation, and cultivar) in the excavated plots. 'Guatemalan' planted as 6-month-old seedlings, the greatest population density was in the 40-60-cm vertical zone (drip line) of the nonirrigated trees which (Fig. 3.9A). These nematodes were recovered only from the top 40-cm of soil with their numbers being about 2-fold greater at the 0-20 cm zone of the soil profile than from 20-40 cm deep (Fig. 3.9B). Irrigation resulted in fewer total nematodes/tree with the largest population occurring in the 20-40-cm depth in the 0-20-cm vertical zone. Few nematodes were recovered from the 20-40 vertical zone. Nematode population densities on trees of this selection planted as 12-month-old seedlings were low, especially on the nonirrigated trees (Fig. 3.9).



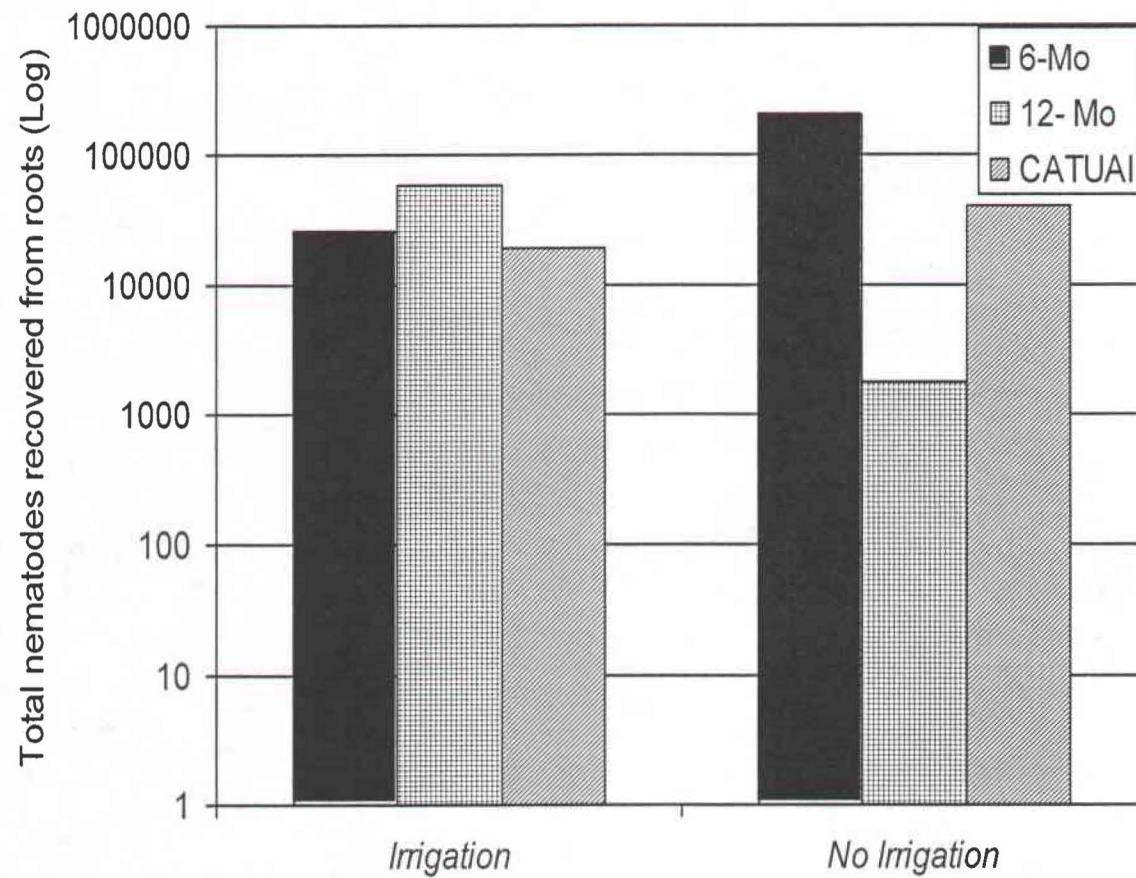


Fig. 3.7. Total nematodes recovered from roots excavated at different depth in coffee plants of two transplanted at 6 or 12 month and under two irrigation regimes in Kona, Hawaii.

M. konaensis occurred primarily at the two lower depths in the drip line zone on the Catuai (Appendix 1, Table 1).

Coffee shoot growth is relatively constant in the Kainaliu, Hawaii region (Fig. 3.10). Although it is not very evident in the figures, there is some reduction in the rate late in the fruiting cycle; the lack of clear influence of the nematode in plant growth does suggest that shoot vertical growth alone may not be a useful indicator of nematode damage. On the landrace 'Guatemalan' trees transplanted as 6-month-old seedlings, the slope of the regression equation was lowest for the nematode infected-irrigated treatment (slope=0.27) and the largest for the non-irrigated nematode free (slope=0.31). Similarly, the 'Guatemalan' trees transplanted as 12-month-old seedlings, the slope of the regression equation was largest for the nematode free-irrigated treatment (slope=0.34) and the smallest for the nematode infected-irrigated and nematode free-nonirrigated treatments (slope=0.31). In cultivar Catuai, the greatest slope (0.22) occurred in nematode free-irrigated plants and the lowest (0.16) in irrigated plants infected with the nematode. Significant effects of the nematode in coffee growth were found for the 6-month-old seedling transplants ($P=0.0001$) and time ($P=0.0001$). The effect of the irrigation and time was significant in Catuai ($P=0.0006$) and 12 month old seedlings ($P=0.0001$) but not for younger seedlings ($P=0.8487$). Growth reduction by the interaction of nematodes and time were evident in the 6-month-old seedlings ($P=0.0001$).

Fig. 3.10. Growth Rate of *M. konaensis* infested and non-infested coffee growing under two irrigation regimes in Kona, Hawaii. Plants were 6-month-old and 12-month old at trasnsplant then infested.

Legend:

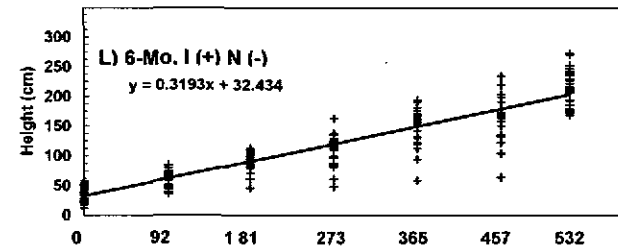
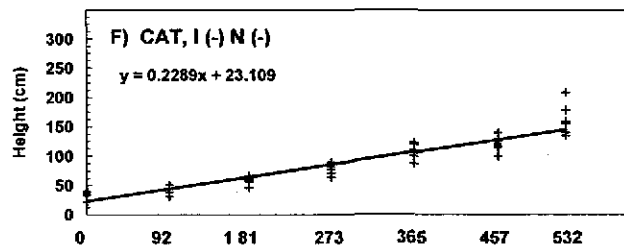
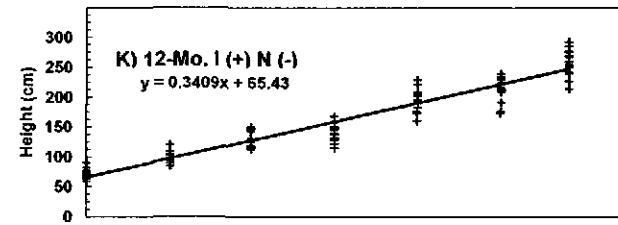
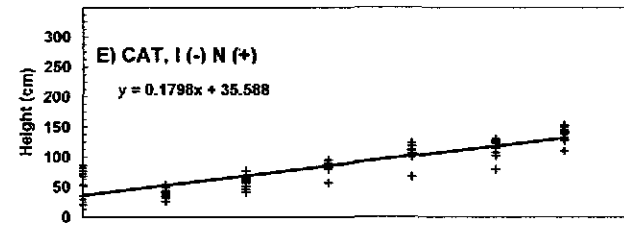
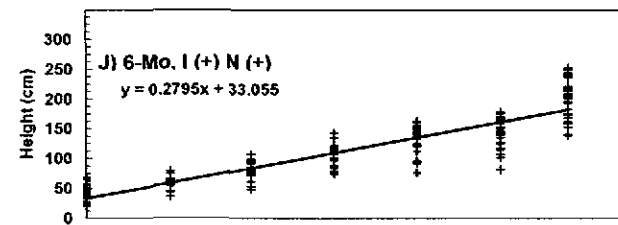
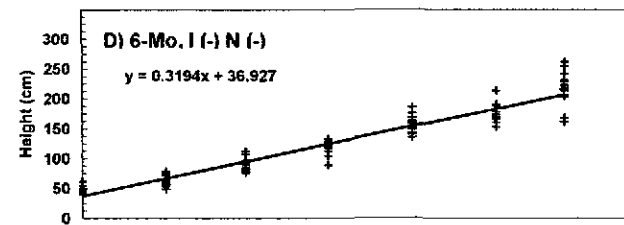
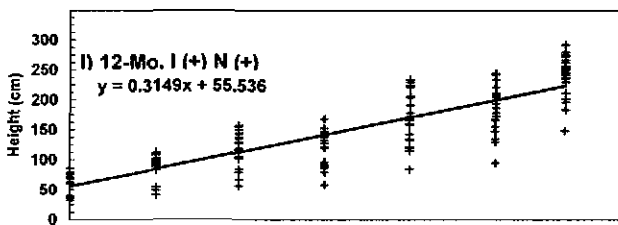
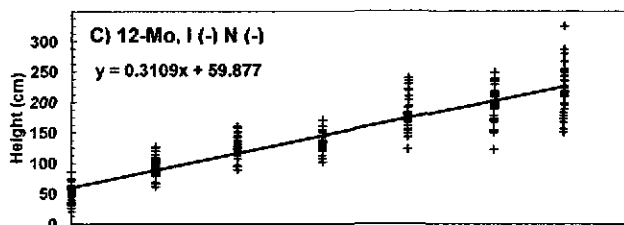
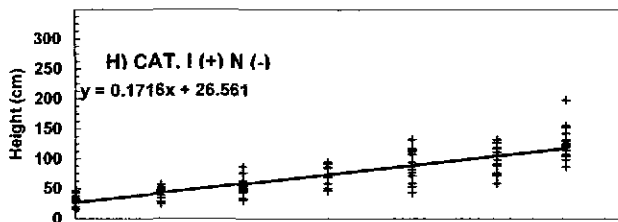
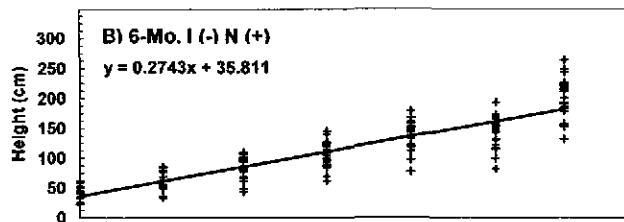
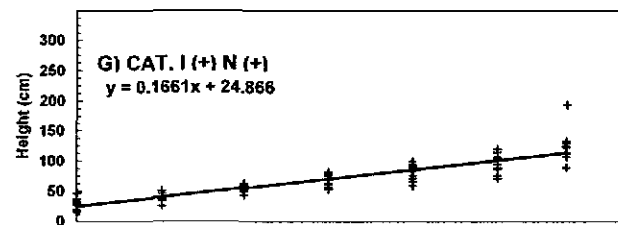
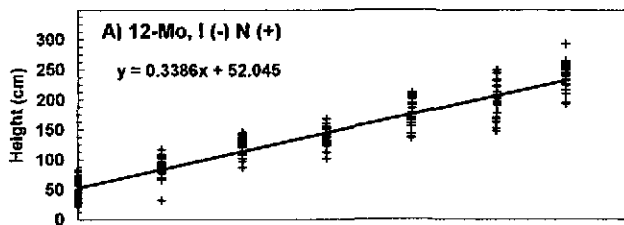
N= Nematode

I= Irrigation

(+)= with

(-)= without

CAT=Catuai



M. konaensis and irrigation affected the concentration of nutrients in the leaves (Table 3.1). Nitrogen was lower in irrigation treatments than in nonirrigated treatments except for selection Guatemala transplanted as 6-month-old seedlings. The concentration of iron was reduced by nematode infection and irrigation. This micronutrient occurred in highest quantities in landrace 'Guatemalan' transplanted as 12-month-old seedlings.

Table 3.1. Foliar nutrient analysis of coffee trees (*Coffea arabica* cv. Typica) and *Coffea arabica* cv. Catuai of two different ages as seedling transplants, with or without nematodes, and two years after transplanted at the Kona Experiment Station, Kainaliu, Hawaii. Nutrient analysis performed in June, 1999.

Table 3.2. Foliar nutrient analysis of *Coffea arabica* cv. Typica, cv. Catuai, and *C. arabica* Typica scion grafted into *C. dewevrei* rootstock at the Kona Experiment Station, Kainaliu, Hawaii, June 15, 1999.

<u>Coffee</u>	<u>Age at</u> <u>Transplant</u>	<u>Nematode</u> ¹	<u>Irrigation</u> ²	<u>MACRONUTRIENTS (%)</u>					<u>Micronutrients (ppm)</u>				
				<u>N</u>	<u>P</u>	<u>K</u>	<u>Ca</u>	<u>Mg</u>	<u>Mn</u>	<u>Fe</u>	<u>Cu</u>	<u>Zn</u>	<u>B</u>
Typica	12-months	-	-	2.43	0.13	1.80	0.81	0.36	57	115	12	7	33
Typica	12-month	-	+	1.37	0.16	2.23	0.62	0.27	55	127	11	4	56
Typica	12-months	+	-	2.35	0.14	1.85	0.58	0.30	43	84	12	6	32
Typica	12-months	+	+	1.52	0.19	1.97	0.77	0.34	48	92	12	6	62
Typica	6-months	-	-	1.54	0.12	1.99	0.49	0.23	44	74	12	6	29
Typica	6-months	-	+	2.13	0.14	2.09	0.66	0.31	53	94	11	5	52
Typica	6-months	+	-	1.75	0.13	2.02	0.63	0.26	49	111	8	7	31
Typica	6-months	+	+	1.21	0.17	1.77	0.71	0.34	68	109	12	5	63

Table 2 (Cont.). Foliar nutrient analysis of *Coffea arabica* cv. Typica, cv. Catuai, and *C. arabica* Typica scion grafted into *C. dewevrei* rootstock at the Kona Experiment Station, Kainaliu, Hawaii, June 15, 1999.

<u>Coffee</u>	<u>Age at</u> <u>Transplant</u>	<u>Nematode</u> ¹	<u>Irrigation</u> ²	Macronutrients (%)					Micronutrients (ppm)				
				<u>N</u>	<u>P</u>	<u>K</u>	<u>Ca</u>	<u>Mg</u>	<u>Mn</u>	<u>Fe</u>	<u>Cu</u>	<u>Zn</u>	<u>B</u>
Catuai	6-months	-	-	2.07	0.13	1.76	0.58	0.27	72	100	17	6	33
Catuai	6-months	-	+	1.96	0.15	1.47	0.84	0.35	68	98	14	7	40
Catuai	6-months	+	-	2.06	0.12	1.61	0.70	0.26	81	88	18	7	34
Catuai	6-months	+	+	1.68	0.15	1.78	0.66	0.26	46	77	10	6	43
<i>C. dewevrei</i>	12-months	-	-	1.90	0.12	1.72	0.84	0.27	22	80	10	6	20
<i>C. dewevrei</i>	12-months	+	-	2.08	0.11	1.68	0.84	0.25	22	80	10	6	20
<i>C. dewevrei</i>	12-months	+	+	1.23	0.13	1.46	1.32	0.38	46	100	11	6	43

Adequate nutrient levels for coffee (*C. arabica*) leaves according to Carvajal, 1984. *Culture and Fertilization of Coffee*

Phosphorus (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Manganese (Mn)	Zinc (Zn)	Boron (B)
0.15-0.30	2-3	0.8-1.6	0.3-0.5	50-500	15-150	25-75

The treatment effects on yield were evaluated by analyzing the variance of each harvest; low variance between harvest allowed combining the harvest of 1999 and 2000. Treatments influenced yields of coffee fruit differently by cultivar and plant age. Irrigation did not influence ($P=0.66$) yields in 12-month-old transplants infested with nematodes (Fig. 3.12.1); the highest yielding trees were from nematode-free, nonirrigated 12-month-old plots, however, the difference of 6 kg of cherry more per plot were not significant ($P=0.12$, Fig. 3.12.2). *M. konaensis* had a major negative impact on yield of trees transplanted as 6-month-old seedlings ($P=0.01$, Fig. 3.12.4) without irrigation. Yields were not influenced by seedling age in the absence of *M. konaensis* ($P=0.47$, Fig 3.12.5). On the average, non-irrigated 12-month-old plants produced 17 kg more coffee than 6-month-old without irrigation. The nematode effect upon yield was striking. The nematode suppressed yields in the irrigation treatments and on the trees transplanted as 6-month-old seedlings; in the nonirrigated treatment yields were reduced by 5 kg of coffee cherry ($P=0.06$). The effect of *M. konaensis* on yield of trees from the older seedlings without irrigation were slight ($P=0.09$, Fig 3.12.6). Based on nematode reproduction, growth and yield data, the cultivar Catuai appeared tolerant to the nematode (Appendix , Table 1) but yields appeared affected by irrigation (Appendix 1, Table 1).

In terms of gross economic returns per area to the grower (Table 3.2), losses due to nematode infestation and the cost of irrigation may prevent coffee cultivation from continuing to be an economically feasible activity. Irrigated coffee had the lowest return per area when infected with nematode regardless of plant age. Under irrigated conditions, nematode infested coffee returns were 43 and 31% less than in

nematode free plants. However, the greatest benefit of irrigation was observed in 12-mo-old-plants during the second harvest if kept nematode free and supplemented with irrigation. Under-non irrigated conditions, the yield of seedlings of 6 mo-old transplant coffee was reduced 64% by the nematode.

Figures 3. 10. Yield of coffee trees transplanted as seedlings of two ages (6 and 12-months) with Irrigation (I+) or without (I-) and nematode infested (N+) or nematode free (N-) in Kona, Hawaii.

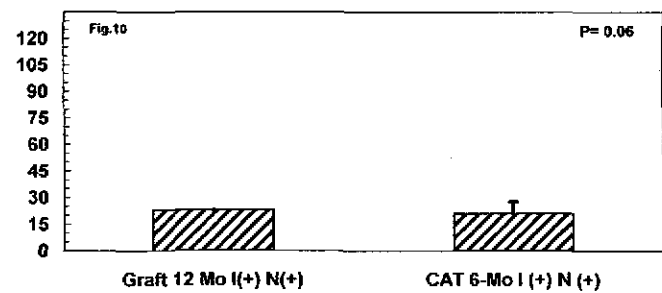
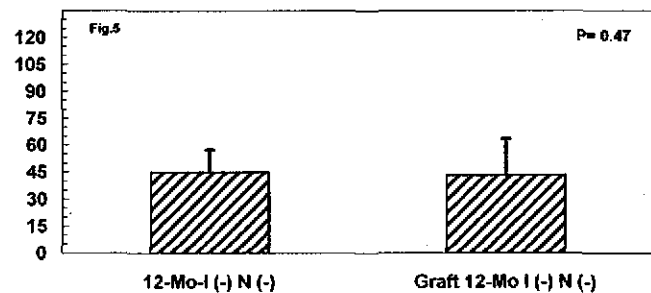
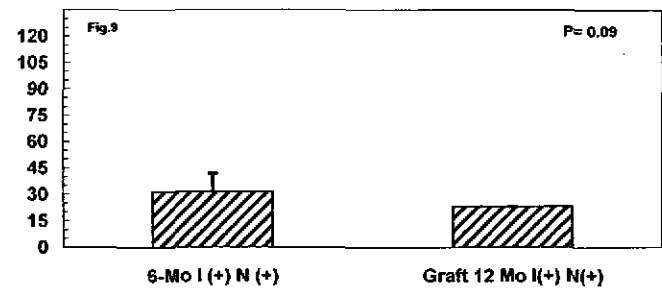
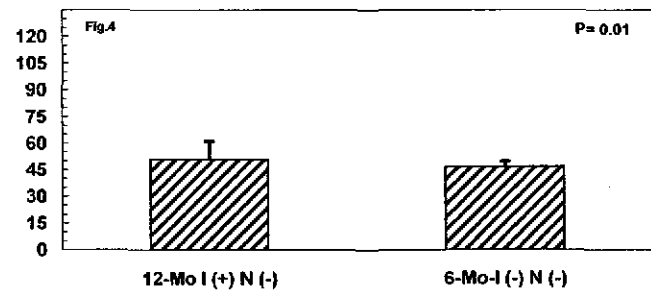
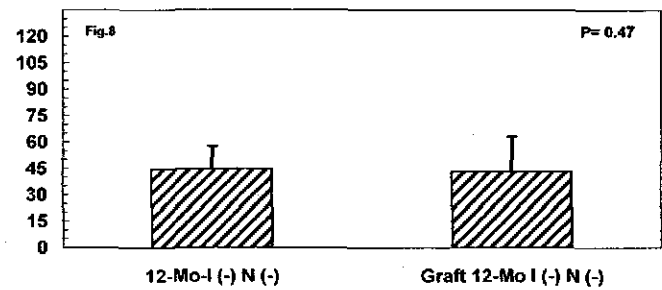
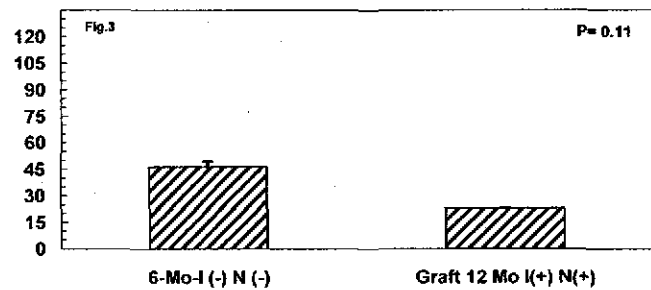
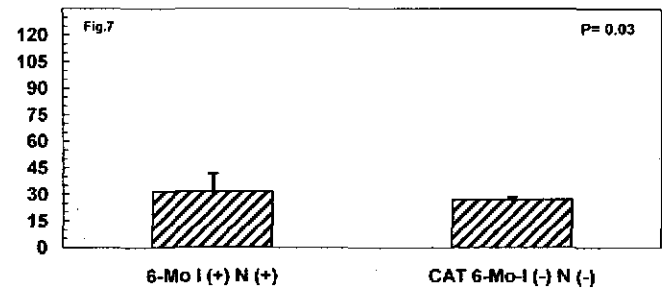
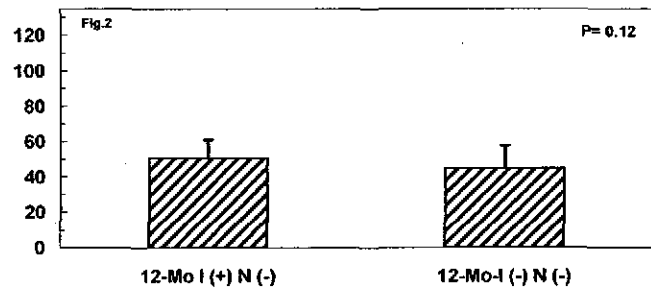
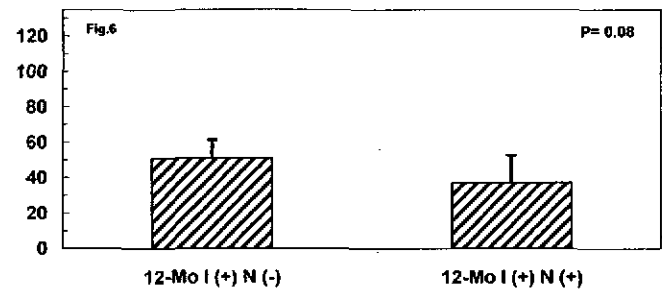
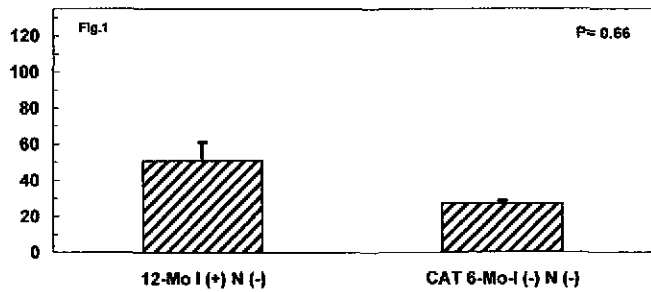


Table 3. 2. Gross economic return* (in US\$ in kg of green coffee (GC) per tree and per hectare) from the irrigation (+, -) and nematode (+, -) treatments on coffee yields in Kona, Hawaii. Coffee trees were of two different ages at planting in July 1997.

DISCUSSION

The dynamics of coffee tree growth and yield are influenced by many factors of which only three were tested and monitored in this experiment. Optimizing plant growth requires a holistic approach to system management (Schmitt, et al. 2003). *Meloidogyne konaensis* is so damaging to coffee that it must be controlled. Irrigation has a major impact on the tree and movement of nutrients and ultimately on the population dynamics of the nematode. It can have positive and negative affects and will require careful monitoring to optimize its utilization and maximize plant production. Seedling age at transplant has an effect for at least two years and may influence the plant system for many years (unpubl).

M.konaensis can greatly influence the growth and yield of coffee. The damage expression would depend of available root biomass and the temperature and moisture conditions in the surface or deeper. This is a difficult variable to estimate considering variability of the soil profile and relationships to *M. konaensis*. The present work suggests that overall vertical and horizontal distribution of the coffee roots and nematode population density on inversely related. Root biomass is an important indicator of plant health, therefore, it was surprising to find that seedlings transplanted at six-months-of age were unable to sustain a large nematode population without significant yield loss. The importance of excluding the nematode from the production system cannot be overemphasized. *M. konaensis* causes severe symptoms of nutrient deficiencies and water stress that adversely affect coffee growth and yield over time. Root biomass is an important variable that must be better understood in a model of time and nematode population changes when the resources are limiting to

Table 3.2. Gross economic return* (in US\$ in kg of green coffee (GC) per tree and per hectare) from the irrigation (+, -) and nematode (+, -) treatments on coffee yields in Kona, Hawaii. Coffee trees were of two different ages at planting in July 1997.

Harvest of 1999												
Irrigated							Non Irrigated					
Nematode +			Nematode -				Nematode +			Nematode -		
GC-kg/tree	\$/tree	\$/ha	GC-kg/tree	\$/tree	\$/ha		GC-kg/tree	\$/tree	\$/ha	GC-kg/tree	\$/tree	\$/ha
Coffee												
6-mo-old	1.35	4.8	8,578	2.2	7.8	13,980	1.45	5.1	9,680	2.7	9.6	17,757
12-mo-old	2.85	10.2	18,110	2.9	10.5	18,428	2.95	10.5	18,746	3.9	13.9	24,782

*Calculations are based on transforming green coffee at 20% of cherry weight and a market value of \$ 3.57/kg per kilogram of green coffee. Planting density equivalent to 1870 trees per hectare.

Table 3.2 (cont). Gross economic return* (in US\$ in kg of green coffee (GC) per tree and per hectare) from the irrigation (+, -) and nematode (+, -) treatments on coffee yields in Kona, Hawaii. Coffee trees were of two different ages at planting in July 1997.

Harvest of 2000												
Irrigated							Non Irrigated					
Nematode +			Nematode -				Nematode +			Nematode -		
GC-kg/tree	\$/tree	\$/ha	GC-kg/tree	\$/tree	\$/ha		GC-kg/tree	\$/tree	\$/ha	GC-kg/tree	\$/tree	\$/ha
Coffee												
6-mo-old	3.8	13.7	24,465	5.6	20.1	35,903	1.8	6.4	11,968	5.0	17.85	33,379
12-mo-old	3.3	11.7	20,970	5.8	20.7	36,856	3.6	13.0	24,310	4.0	14.10	26,180

*Calculations are based on transforming green coffee at 20% of cherry weight and a market value of \$ 3.57/kg per kilogram of green coffee. Planting density equivalent to 1870 trees per hectare.

sustain the nematode population. Larger plants infected at a later age can withstand nematode attack for a few cycles, but frequent irrigation to alleviate the problem would actually worsen the situation as the nematode population has already increased above damaging levels. In the case with younger seedlings, irrigation has a temporary positive effect for the plant to grow and yield. However, it only delays the inevitable decline of the coffee plant.

The association of environment, host and pathogen can lead to plant disease (Agrios, 1982). Our present discussion focus on the reaction of multiple independent factors observed in which *M. konaensis* modifies coffee root biomass. A restriction of root growth results in poor nutrient and water uptake despite provision of adequate levels. Mineral deficiencies and lack of moisture lead to loss in crop production. This reaction is more evident in seedlings transplanted at six month of age than in plants that were transplanted as 12-month-old seedlings. Decline symptoms appeared to be delayed in *C. arabica* cv. Catuai has some tolerance to the nematode. However, despite lack of severe symptom expression, this cultivar should not be used as a rootstock due to its good host status.

A yield increase in *M. konaensis*-infested coffee transplanted as 6-month-old seedling affected by irrigation should be interpreted with caution since the population densities of the nematode have already increased. Unfortunately, there are many aspects of the relationship of the soil components to plant nematodes that are poorly understood.

The marked seasonal variation of the nematodes population in the soil suggests that the nematodes are in synchrony with plant development. Evapotranspiration and water inflow from neighboring soil layers causes soil moisture and tension to fluctuate considerably in the course of the day. As the evaporative stress on the shoots vary diurnally, water films in the roots change. *Meloidogyne konaensis* is able to survive in the roots of coffee for an undetermined length of time. Migration and penetration to new feeding sites may occur during periods of time in which the juveniles are capable of migrating in a soil when it become adequately moist. Seasonal fluctuation in soil temperature may not be as important affecting nematode population as variations of soil moisture. This finding is in agreement with reported literature which states that nematode habitat in the films of moisture that cover the soil particles is important, thus soil factors that favor plants are also optimal for plant nematodes (Steiner, G. 1952).

Management of the entire system including the plant, the nematode and the soil is important to optimize coffee production. Moisture is a crucial factor for conditioning the activities of plant nematodes in the soil (Steiner, 1952). Supplemental irrigation and seasonal rains cause coffee roots to flush and root biomass to increase. Thus, conditions are then suitable for the nematode to feed and reproduce. *M. konaensis* and coffee decline have also been associated to nutritional imbalances. Further understanding of the role of nitrogen, irrigation to coffee phenology and *M. konaensis* population development is necessary.

The detrimental effects that *M. konaensis* causes to coffee production cannot be circumvented by irrigation. Planting older seedling such as 12-month-old seedling

can delay the ultimate need to replant; nevertheless, the plant will decline and may die.

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Chapter IV

COFFEE DECLINE CAUSED BY THE KONA COFFEE ROOT-KNOT NEMATODE

Coffee trees with yellowish leaves and thin canopies have been noted for almost 100 years in the district of Kona. (See Figure 4.1). Coffee decline has been locally associated with several causes including “replanting problems”, “transplanting decline”, “fertilizer problems” and “Kona wilt”. The first report of coffee decline in Hawaii nematodes attacking crops was recorded in a 1907 publication by the Hawaii Agricultural Experiment Station. Although the cause of the problem was not known at that time, the author mentioned nematodes as a possible cause. In fact, the cause was not identified until 1990 with the description of the causal nematode being published in 1994. The disease, “coffee decline”, is caused by the Kona coffee root knot- nematode (*Meloidogyne konaensis*). The economic losses due to this disease can be large. In orchards with heavy infestations, yields often are so low that production is not economical. Even low levels of nematodes can cause losses of 10-40% .



Figure 4.1. Foliar symptoms on coffee trees from damage caused by the Kona coffee root-knot nematode. Diseased tree showing early yellowing and final coffee decline (foreground). Healthy coffee trees (background).



Characteristics of the Coffee Decline disease

Symptoms of infection by the Kona coffee root-knot nematode are root galling and the reduction in the number of fibrous roots (See Fig. 4.2 and 4.3). Shoot symptoms are manifested in numerous ways. The trees may look normal, but yield lower than expected. Commonly, a tree will not grow as fast as a healthy tree and may exhibit symptoms of nutrient and/or water deficiency.

Figure 4.2. Typical symptoms of root galling caused by nematode infection. Note the stubby nature of the roots and the lack of fibrous roots.

A few years after seedlings become infected, symptoms of water stress and nutrient deficiencies appear gradually in clusters throughout the coffee field (Fig. 4.1). These plant responses are due to nematode entry, feeding and reproduction within roots that disrupts plant growth processes. A heavy bearing tree increases the plant nutrient demands, thus applying large amounts of fertilizers to coffee is a relatively common practice. The idea behind this practice is to assure adequate nutrients for a heavy bearing tree and/or to rejuvenate unproductive trees. On trees that have been damaged by nematodes, root injury due to high salinity burns roots. Fertilizers, especially excessive amounts are often wasted.

How the Kona Coffee root-knot nematode cause coffee decline

The cause of coffee decline is the Kona coffee root-knot nematode, a microscopic parasitic round worm that uses a needle-like piercing device to penetrate and feed within the roots of plants. It attacks coffee, many vegetables and weeds. It is found primarily in the Kona area, but occurs in a few isolated fields outside the Kona districts on the island of Hawaii.

The adult females live deep inside the root tissue where they lay eggs. Upon hatching from their egg, the freshly hatched juvenile nematodes seek root tips and penetrate into the root and establish permanent feeding sites. This feeding activity results in the formation of specialized cells in the root called giant cells. This activity causes the roots to swell into galls within a few days after feeding has begun. The nematode grows, molts and develops into a reproductive female. With favorable moisture and food conditions, eggs produced by the female will hatch and start their cycle again.

Plants suffering from continuous nematode infection and reproduction lose their roots and eventually die. The length of time from root entry to reproduction varies among different plants, but it is known to occur in coffee and tomato in approximately 50 and 30 days, respectively.



Figure 4.3. Damage to roots of a large tree caused by the Kona Coffee root-knot nematode.

In the absence of nematodes, irrigation

can result in nearly doubling the root biomass and improved yields; but nematode-damaged roots may not be very efficient in water uptake. Therefore, wilt is likely to occur even though soil moisture is adequate. Additional water applied to compensate for the poor uptake often creates a root environment that is low in oxygen. This situation causes further root damage and may speed the death of the plant. The decline can be worsened by improper utilization of otherwise important cultural practices. Prompt replanting, where trees have died, with young seedlings from a nursery or with volunteer seedlings is common. Immediate replanting after removal of infected trees allows the parasite to quickly re-enter new host roots and further continue its life cycle. Herbicide use, even when properly applied according to the label, may enhance the nematode damage and accelerate coffee decline. Insects also might be more attracted to a weakened tree and could further speed the decline of the tree. Most certainly, secondary infections by bacteria and fungi negatively affect plant health after the nematode has established in the roots.

Importance of Proper Disease Diagnosis.

The selection of an appropriate management practice can only be done when the cause of the disease is known. For this nematode, an accurate diagnosis can be achieved only with an assay.

A recommendation for sampling for the nematode is described in the following section entitled “Assaying for the Causal Nematode”. However, a preliminary diagnosis of the disease can be made in the field. Coffee trees infected with the Kona coffee root-knot nematode are often stunted and have a smaller trunk diameter than normal. Leaves may be yellow and exhibit various types of nutrient deficiencies. These trees tend to wilt



even with an adequate supply of water. These symptoms are also associated with other problems, but if the roots have small to large swellings (galls) on them along with a “corky” appearance of the galled tissue then the cause is most likely the Kona coffee root-knot nematode

Figure 4.4. “Corky” appearance of coffee roots infected by *M. konaensis*.

The roots are often in various stages of decomposition. The feeder roots which normally would appear white and in large quantities in healthy plants are fewer and exhibit a brown discoloration.

Assaying for the Kona Coffee Root-knot Nematode

Assays should be conducted at times of the year when the probability of detection is the greatest. Thus, some knowledge of the nematode’s biology is helpful in deciding when to sample. The number of nematodes in soil and roots fluctuates according to weather and stage of crop development, but is generally greatest following the periods of highest annual rainfall from June to September and decline to lowest counts in the soil during the dry season. It is important to understand, therefore, such significant environmental relationships.

The steps, timing and techniques for collecting soil and root samples for diagnosing Kona Coffee decline may vary by orchard and nursery conditions. Since a coffee nursery can be a source of the nematode and could be responsible for infesting many acres of coffee land, all nursery media should be tested for nematodes if synthetic

mixes with soil are used. Nursery plants should be sampled for nematodes at least twice before transplantation into the field. A standard sampling approach is to systematically collect soil and roots from at least 20 plants and send them for nematode assay and identification to a nematode diagnostic laboratory such as the Agricultural Diagnosis Service Center (ADSC) at the University of Hawaii, College of Tropical Agriculture (CTAHR). Contact your local county agent for further information.

In established plantations, samples of soil from where roots are found should be collected systematically. The proper way to sample for nematode assay is to dig around the tree canopy drip line of several trees for soil and roots with a shovel, pick or soil coring device. The soil and roots must be collected from the soil zone of 6-12 inches-deep for a reliable assay. It is best to sample a few weeks after periods of rainfall and while the soil is still moist. Sample from trees that exhibit early symptoms or from healthy appearing trees that are immediately adjacent to severely affected trees. To assay the infestation of nematodes in a large field, subsamples of soil and roots should be collected from around twenty trees per acre and mixed together. Separate roots from soil, then remove a volume of approximately one pint of soil and place it into a plastic bag, seal the bag and immediately place it into an insulated container. However, it is best to sample and send it immediately. Each soil sample should include the corresponding coffee roots. Proper identification of each sample includes date of sampling, origin, grower's contact information and any other information that will be useful. Prompt shipping to the laboratory is necessary.

Prevention and Suppression of Kona Coffee Decline.

The ideal approach to managing the nematode is to prevent its introduction to new areas. For farms that are known to be free of the Kona coffee root-knot nematode, only seedlings assayed and determined nematode-free should be planted. These farms should then be sampled at regular intervals (e.g., every 2-3 years) for nematode detection. Diverting runoff from up slope fields is important to prevent movement of large volume of soil that could potentially harbor the nematode and be a source of infestation.

In nematode-infested farms, management of the disease is more complex. Once assessment has been made as to the level of infestation and length of time that the farm has been in decline, replanting may be necessary. The goal of nematode management is to reduce population densities in the field and this can be accomplished by removing all plants and fallowing the fields. During the fallow period, weeds that may host *M. konaensis* should be eliminated and nematodes should be monitored by periodic soil sampling or a bioassay. A bioassay consists of planting nematode-free tomato seedlings randomly throughout the field, allowing them to grow for 18 days, then carefully removing them from soil and washing the roots in order to easily observe if root swelling and galling are evident.

It is safe to replant after the nematode population density in the field is reduced to non-detectable levels. The length of the fallow period required to reduce nematodes varies according to location and size of nematode population and weed management. It has been determined that the Kona coffee root-knot nematode has a broad host range, including common weed found in coffee farms. Weeds such as Amaranth ('Pigweed', 'Pakai') and Hilo grass should be promptly removed from fields. Replant only with

seedlings grafted onto *Coffea liberica* var. *dewevrei* 'Fukunaga', a CTAHR-released nematode resistant rootstock. If a fallow period has not been implemented, delaying the transplanting age to no more than 12-months-old may be helpful in order to increase their tolerance to nematode infection. The seedling should not be held too long in the nursery, as they may develop 'J' root. A 'J' root is a taproot that bends on an angle; as the root matures, the bend prevents proper growth. It is very important that the roots of the seedlings are not removed since the young roots provide for the uptake of nutrients and water. Of major importance also is the anchoring function of the roots, especially the tap root.

Before replanting, amending the soil with quantities of animal manure, mulch or coffee by-products and lime (if indicated from a soil test) may be helpful. Organic amendments are beneficial for the plant and soil environment. These amendments do not eliminate the nematode, but may be helpful in reducing some of its damage.

APPENDIX 1.

APPENDIX 1. DEVELOPMENT AND REPRODUCTION OF *MELOIDOGYNE KONAENSIS* AS AFFECTED BY SOIL POROSITY.

ABSTRACT

The impact of porosity (bulk density) treatments on nematode reproduction in a tropical Andisol soil was measured by assessing penetration, development and reproduction of *M. konaensis* at days 3, 12, 18 and 30 after inoculating tomato plants grown in polyvinyl chloride cylinders at 20 °C. The volumetric water content was lower in the least dense soil treatment of 0.6 g/cm³. Second-stage juveniles traveled at least 20-cm in 3-days to penetrate roots. In the 0.6 bulk density treatment, 74% percent of second-stage juveniles were recovered in the bottom half of the cylinder. The rate of root penetration and post-embryonic developmental rates occurred slightly faster at 0.6 g/cm³ bulk density treatment than in the more densely packed soil material (bulk density=0.9 g/cm³). By the 12th day, developmental stages varied from vermiform J2 to J3 in the soil material adjusted to bulk density of 0.6 gm/cm³. The range in the bulk density of 0.9 gm/cm³ was from vermiform J2 to J4. Development in the 0.9 gm/cm³ bulk density progressed slower than at 0.6 gm/cm³. At 18 days after inoculation, 69% of the developing juveniles in the roots were in the two middle sections of soil at the 0.6 bulk density treatment and females were laying eggs. Even though the nematode matured faster and began laying egg sooner on plants growing at bulk density of 0.6 g/cm³, egg laying stopped at 18-days after inoculation. In contrast, the slower developing nematodes on tomato growing at bulk density of 0.9 gm/cm³ produced much greater numbers of eggs by 30 days than those at the 0.6

gm/cm³ bulk density. The increase in penetration, movement and reproduction by *M. konaensis* with an increase of bulk density could be due to changes the physical environment in the soil; compacting the soil material could have resulted in pore space more favorable for migration and penetration to feeding sites, hence better development and egg production.

Key words: Bulk density, development, *Meloidogyne konaensis*, penetration, reproduction, root-knot nematode, soil moisture, soil texture.

INTRODUCTION

Nematode activity greatly depends on the adaptation to a heterogeneous environment, soil structure and nematode foraging strategy (Anderson, et al., 1996). Soil is a complex medium and any effect on soil inhabiting nematodes involves an array of interacting physical and biological factors. Whenever one component of the soil environment is altered, other aspects of the soil are impacted changing the environment for all associated organisms. Soil compaction, pore space size, oxygen content and soil water content are modified in farming, resulting in a different edaphic environment for nematodes.

Since population growth is determined by the rate of reproduction and rate of mortality, an important question to address is the impact of specific soil physical factors on reproduction. Soil physical factors vary considerably within fields and among fields. All of these physical factors influence the behavior of the organisms striving to grow and survive in that media. The consequence to an organism such as a plant is its ability to grow and reproduce. Directly and indirectly, the soil and the plant affect microorganisms such as plant-parasitic nematodes by way of imposing

resistance factors that regulates the nematodes population size by limiting reproduction. In turn, plant damage and symptom expression are related to all of these interacting factors.

The Kona coffee root-knot nematode (*Meloidogyne konaensis*) is primarily found in the Kona districts on the Island of Hawaii. It resides in a wide range of soils within Kona that differ in environment and age of soil parent material. Temperature, water, available food and plant nutrient are among the key factors regulating populations of *M. konaensis* (Zhang and Schmitt, 1994; Schmitt et al., 2002). The nutritional status of the crop also varies widely across the districts. The populations of this nematode respond to all of these variables. Their numbers increase during the rainy season and tend to decline in the drier winter months. Hatching, penetration and post-embryonic development occur rapidly after the first rains in the spring and early summer when the soil temperature is also optimal for the nematode (Zhang and Schmitt, 1994; Schmitt et al., 2002). Egg production, an indicator of the host-parasite relationship to the total environment, was 2-fold greater at a constant moisture of 33 kPa than under conditions of soil moisture fluctuation from wet to dry (Serracin and Schmitt, 2000). Plant vigor is also a determinant for the nematodes vigor. This relationship was evident in a field near Kealahou, Hawaii in which the clustered population densities of *M. konaensis* were inversely correlated to concentrations of soil nutrients (Schmitt et al., 2002). These nematode damaged roots were not able to take up the nutrients in areas of the field with high numbers of the nematode whereas roots growing in soil free of the nematode or in areas of low population density were vigorous and active in nutrient uptake.

Plant damage from nematodes tends to be greater in coarse-textured soils than in finer textured soils (Norton, 1969; Wallace, 1958); however, this response varies according to nematode species (Portillo, et al. 1999) and ecological adaptations to particle density of the parent material (Yates, 1996). In surveys of natural populations of insect predatory nematodes in Tennessee nursery soils, recovery of the nematodes was more frequent in soil samples with the bulk density averaging 1.4 gm/cm³ (Rueda et al., 1993). The activity and survival of the second-stage juveniles of root-knot nematode (*Meloidogyne* pp.) in the soil depends greatly on moisture content and soil porosity, which are related to aeration and water conductivity within soil aggregates and voids, and the presence of a food source, i.e. plant roots (Norton, 1978). Based on surveys of the Kona districts, number of *M. konaensis* were generally greater in coffee fields less than ten years of age planted in areas where ancient lava flows have weathered forming continuous layers of rich soil than in areas with irregular patches of minimally weathered rock (Schmitt and Serracin, unpublished 2002 data).

The relationship of the three dynamic phases in soil consisting of solid, liquid and gaseous phases is certainly important for nematode movement, thus is crucial for survival (Simmon, 1973). Any factor, such as bulk density, will affect the ability of the nematode to move from the point of birth to its infection site. Portillo-Aguilar et al., (1999) demonstrated that bulk density affected movement and survival of three species of entomopathogenic nematodes. At high soil porosity, movement was enhanced; when porosity was reduced (high bulk density), few nematodes moved, and the rate of development was drastically reduced (Portillo-Aguilar et al., 1999).

This response indicate a relationship between nematode size to soil porosity for optimal nematode movement. For example, movement of *Heterodera schachtii* and *Ditylenchus dipsaci* increased when the average diameter of the soil pores approached the diameter of the nematode (Wallace 1958b). This movement through pores was modified by soil moisture. A water film thickness of 2-5 μm around peds and particles was optimal for nematode movement (Wallace, 1958a). Soil pore neck diameter was also a critical physical parameter limiting or allowing movement of nematodes (Jones et al., 1976). Once an infection is established, it is speculative to judge if soil porosity would also affect the development and reproduction of *M. konaensis* as it affected reproduction of microbivorous nematodes (Ingham et al., 1985).

Coffee in Kona, Hawaii is grown in volcanic soils with distinctive properties (Ikeda and Takehiro, 1958). The parent material of these soils have a small particle size, low bulk density, small pore spaces, and they have high water content at field capacity and at the wilting point. Changes in hydraulic properties such water re-absorption of these volcanic soils after drying are irreversible. This poorly understood phenomena may be due to their genesis, mineralogy and aggregation (Maeda et al., 1977). These porous volcanic ashes, ideal for coffee production in the absence of *M. konaensis*, are very conducive for damage by the nematode. Their structure varies with cultivation, compaction and soil moisture. These factors relate to the amount of inhabitable pore space for food, water and air that are important for nematode population development (Jones, 1976). Just how much the soil physical structure, specifically bulk density, is operative as an environmental resistance factor

for *M. konaensis* is difficult to judge. Since nematicide effectiveness is related to the distribution of space in the soil (Bernard and Hussey, 1977; Schmitt, et al., 2001), naturally occurring soil chemicals may be retained that could influence the behavior and functioning of the nematode. Behavior of the nematode is most likely influenced by an array of interacting factors that are difficult to separate. Nevertheless, insights can be gained by adjusting one factor and evaluating the behavior of the nematode in response to that factor. Thus, the objective of this investigation was to determine the impact of soil bulk density on the development and reproduction of *M. konaensis*.

MATERIALS AND METHODS

This research was conducted in a growth chamber on the University of Hawaii-Manoa campus. The soil material for the experiment was collected from a coffee field plot at the Kona Experiment Station in Kainaliu, Hawaii. The soil at this location, a hydrous-skeletal, ferrihydritic isothermic typic hydruclands, is in the Pawaina series with a bulk density of 0.48 g/cm^3 (Ikeda, 1958; R. Gavenda, Pers. Comm.). It is derived from volcanic ash underlying a'a flows (Ikawa et al., 1985). The physical properties of this soil are described in a publication by Serracin and Schmitt (2000).

About 70-kilograms of the collected soil was processed for this experiment. The processing began by air drying. When it was dry, the soil was crushed, then sieved through a screen with 2-mm square openings. The soil was dispensed in increments of 100 grams into each of 16 25-cm tall X 8.5-cm diameter polyvinyl chloride cylinders. Each increment was moistened with a mist of water until the soil was at or near 33kPa. Each increment or layer was compressed by applying pressure

until the predetermined desired bulk densities of 0.6 and 0.9 g/cm³ and a matric potential of approximately field capacity (33 kPa) were achieved (Brady and Weil, 1996). This process was continued until the cylinders were full of soil. Each cylinder weighed between 1.0 to 1.2 kg depending of the bulk density treatment.

After the soil was adjusted to bulk densities of either 0.6 or 0.9 g/cm³, one tomato seedling were transplanted into each cylinder followed by an equilibration period prior to infestation of the soil with eggs of *M. konaensis*. One tomato cv. Pixie seedling was transplanted per column. The columns were placed in the vertical position in a 20°C growth chamber for two weeks to allow the system to equilibrate before infestation with eggs of *M. konaensis*. When the system was determined to be stable, egg masses were collected from cultures of *M. konaensis* reared on tomato. The eggs were released from eggs masses by treatment with NaOCl (Hussey and Barker, 1973) and placed on Baermann funnels for hatching. Second-stage juveniles that emerged during the first 24-hours were discarded and those that emerged during the following 24-hours were used as inoculum. Approximately 1,000 juveniles in 10 ml of water were introduced at the bottom of each soil column and the base covered with a 546 mesh screen (12 µm opening). The experimental design was a randomized complete block with 8 replications per run and the experiment was run twice.

Two replications were harvested at 3, 12, 18, and 30 days after infestation in both runs of the experiment. The 25-cm long columns were cut into four equal sections of 6.25-cm. The roots were removed and then the soil in each section was divided into two portions. From one portion, the gravimetric water content was determined. This soil portion was weighed and then oven dried for about 12-hours at

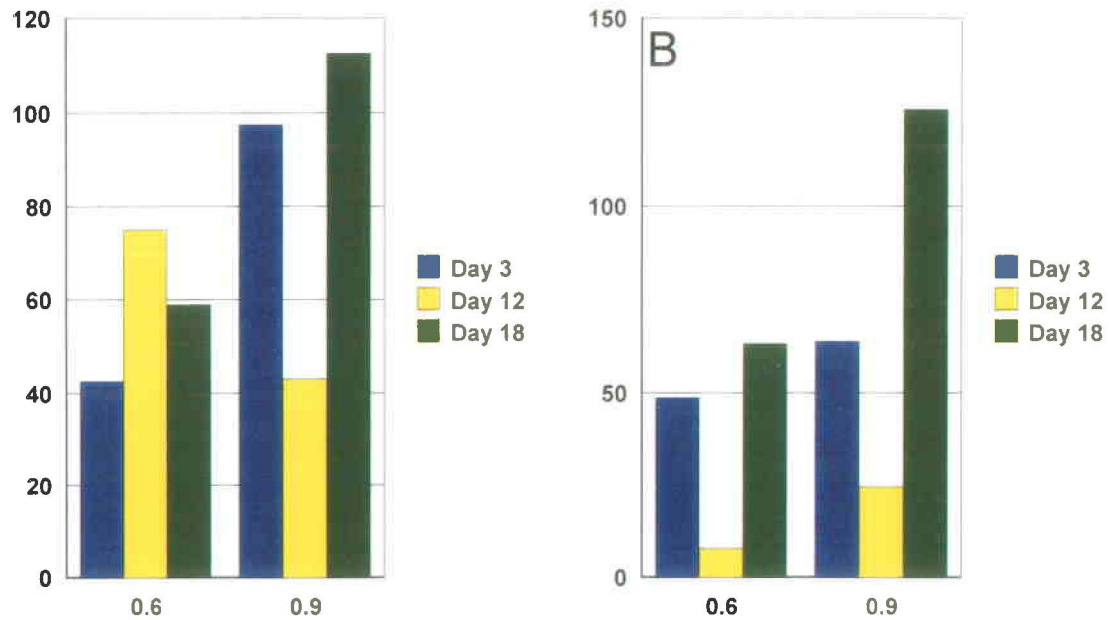
105°C. The difference between the moist and dry soil was the gravimeter water content. The volumetric water content was calculated by multiplying the gravimetric water content times the corresponding bulk density of each treatment. From the second portion of soil, the soil section was gently mixed. Second-stage juveniles were extracted from 100-cm³ subsamples of each section with a semi-automatic elutriator (Byrd et al., 1976) followed by centrifugal flotation (Jenkins, 1964). For the root assays at 3 day and 12 day harvests, roots were stained with acid fuchsin for visual observations of nematodes (Daykin and Hussey, 1985). On days 18 and 30, the roots were divided into two portions. One portion was stained with acid fuchsin as for days 3 and 12; the second portion was treated with NaOCl to remove eggs from egg matrices (Hussey and Barker, 1973).

Data were analyzed by examining treatment response over time and means were compared by T-tests. Nematode quantitative data was not transformed.

RESULTS AND DISCUSSION

Bulk density affected penetration, developmental and reproductive behavior of *M. konaensis*. Differences in root penetration by the nematode were more subtle than differences in nematode development and reproduction. Only about 5% of the second-stage juveniles added to the soil were recovered from the roots at 3 days after inoculation (Fig. Appendix 1.). Root penetration was variable across the replications within treatments, but still showed a trend toward more penetration in the soil material adjusted to a bulk density of 0.9 than 0.6 (Appendix Fig. 1B). Roots in most soil sections were invaded indicating that the nematode traveled at least 20-cm and penetrated the roots within 3-days. In the 0.6 g/cm³ bulk density treatment, 74%

percent of second-stage juveniles



Appendix 1. Fig. 1. Average number of *Meloidogyne konaensis* in 100 cm³ soil and coffee roots at selected number of days after infestation of soil adjusted to a bulk density of either 0.6 g/cm³ or 0.9 g/cm³: A) Second-stage juveniles in the soil, B) all stages in the root.

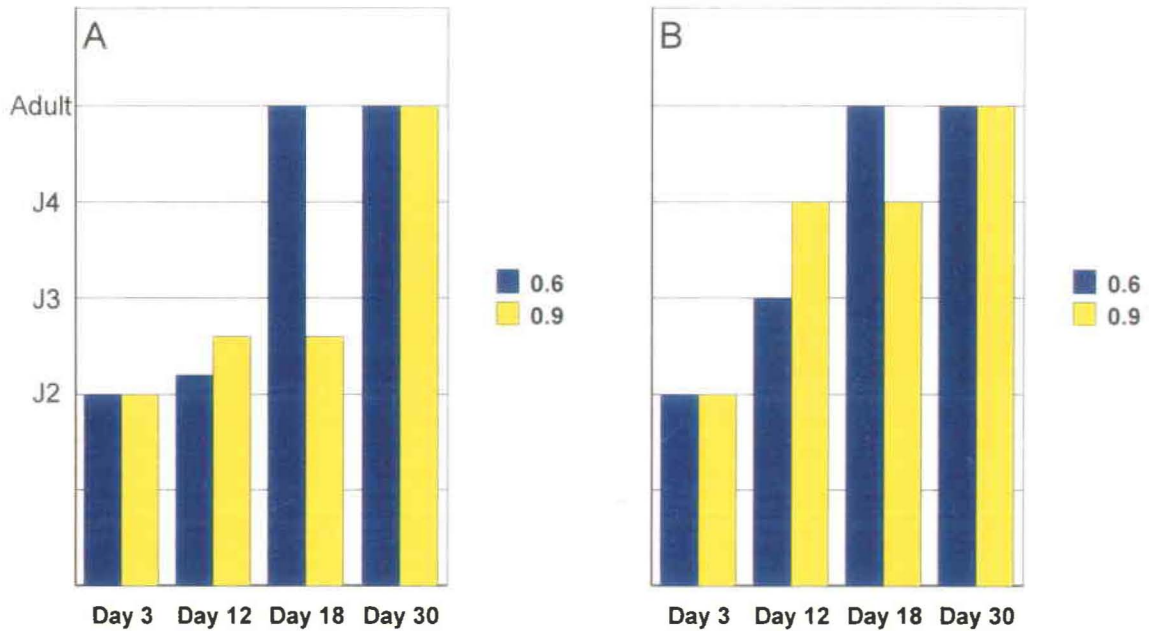
were recovered in the bottom half of the cylinder. In contrast, only 25% of the infections of tomato roots growing in 0.9 g/cm³ soil occurred in the bottom 50% of the cylinder.

Investigations with fungal pathogens illustrated that rhizosphere inhabiting organisms can interact with bulk density to influence their dispersal and pathogenicity (Bhatti and Kraft, 1991; Otten, et al., 2001). This response to bulk density by nematodes varies among nematode species, size and parasitic strategy (Portillo-Aguilar, 1999; Schmitt, 1971). Movement of the migratory endoparasite *Pratylenchus penetrans* was more restricted in silty loam soil than in loamy sand soil (Townshend and Webber, 1971). An increase in bulk density and a reduction in aggregate size reduced the fraction of the micropores and colonization efficiency of

the pathogen *Rhizoctonia solanii* (Otten et al., 2001). It is important to note that behavioral responses measured by movement vary and may be due to nematode size and stimulus (Portillo-Aguilar, 1999; Robinson, 1995). The duration of nematode activity depends on the amount of energy stored in their bodies in relation to changes in soil physical variables and food availability, but the behavioral responses in relation to compaction still needs further study. The gravitational pull associated with the downward movement of the soil water was not considered, but is probably affecting the ability of juveniles to move to their preferred feeding site. Apparently due to the diffusion of attractants in the soil profile, large numbers of bacterial feeding nematodes remained distal to their attractant bait (*Escherichia coli*) (Young et al., 1998). Similarly, *M. incognita* and four other nematode species responded to carbon dioxide gradients whose diffusion was affected by the physical condition of the sand medium (Robinson, 1995).

Post-embryonic developmental rates differed in the two bulk density treatments (Appendix 1, Fig. 2). At 3 days after infestation of the soil, no differences in development were obvious. By the 12th day, developmental stages varied from vermiform J2 to J3 in the soil material adjusted to bulk density of 0.6. The range in the bulk density of 0.9 was from vermiform J2 to J4. The progress in development by this date was slightly faster at the more dense soil treatment. However, development in the 0.9 bulk density did not progress during the next 6 days, yet the rate increased in the 0.6 gm/cm³ soil. In fact, egg laying females were present in this treatment on day 18 (Fig 2-3). Even though the nematode matured faster and began laying egg sooner on plants growing at bulk density of 0.6, egg laying stopped. In contrast, the

slower developing nematodes on tomato growing at bulk density of 0.9 produced much greater numbers of eggs by 30 days than those at the 0.6 bulk density. At 18 days after inoculation, 69% of the developing juveniles in the roots were in the two middle sections (of four sections) of soil at the 0.6



Appendix 1. Fig. 2. Post-embryonic development of *Meloidogyne konaensis* on tomato growing in soils at bulk densities of 0.6 and 0.9. A) Mean stage of development, B) most advanced stage of development.

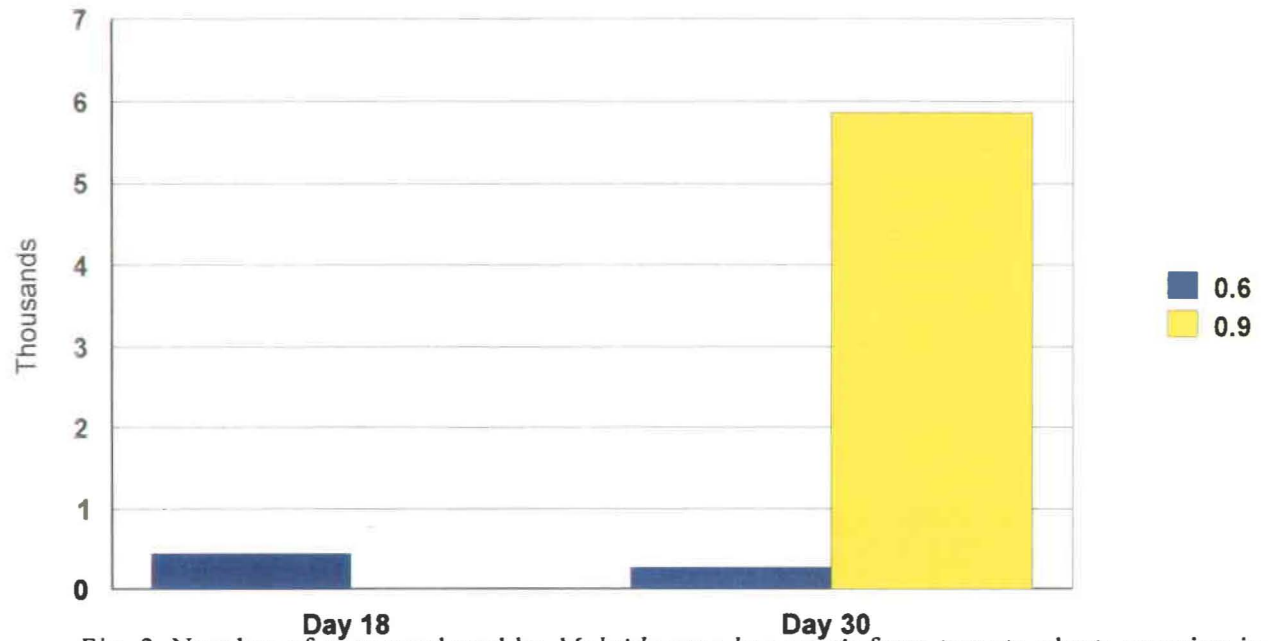
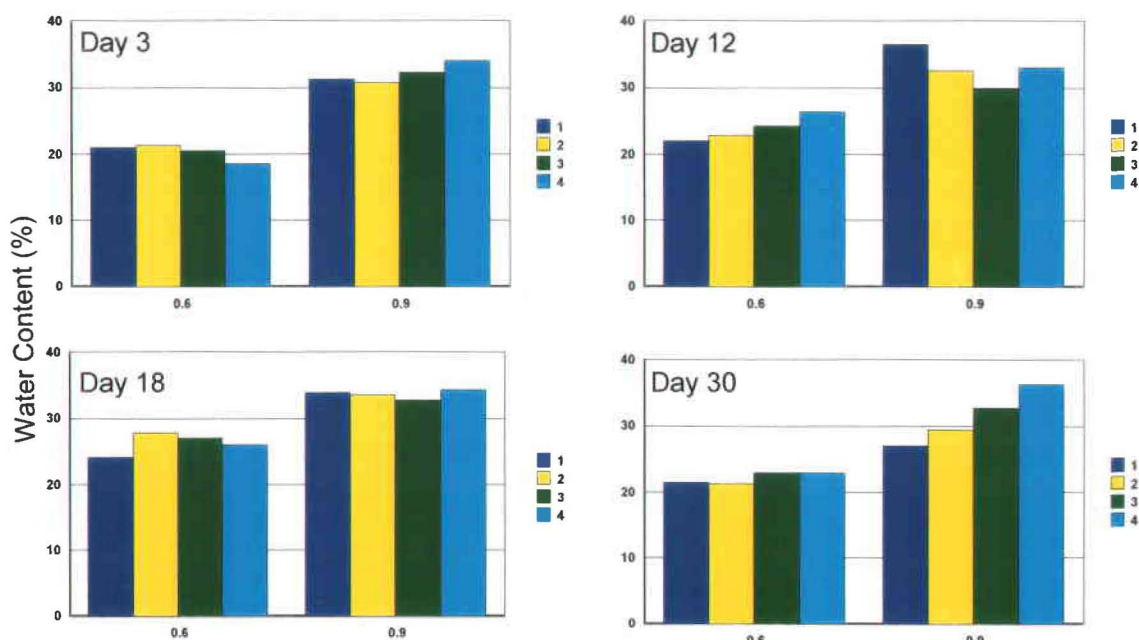


Fig. 3. Number of eggs produced by *Meloidogyne konaensis* from tomato plants growing in soil at bulk densities of 0.6 and 0.9.

bulk density treatment. This same percentage of the developing juveniles within a cylinder was present in the bottom 6.25-cm of soil in the 0.9 g/cm³ treatment.

The rating of the roots indicated better root development in the soil adjusted to 0.9 g/cm³. The plant growth was also more vigorous in this more densely packed soil material. As indicated in the introduction, a change in one factor will alter the entire system. In this case, the volumetric water content was lower in the least dense soil (Appendix 1, Fig. 4). As with any soil experiments, the complex of interaction

factors makes it difficult to identify the primary factor, but bulk



Appendix 1, Fig. 4. Volumetric water content of soil surface material collected from the Pawaina series at Kainaliu, Hawaii. 1=surface 0-6.25 cm of soil column, 2=6.25-12.5 cm, 3=12.5-19.5 cm, 4=19.5-25.0 cm.

density certainly had an affect on the nematodes developmental and reproductive behavior on pixie tomato. It is also important to indicate that root growth physically modifies the soil, which will modify the behavior of the nematode.

The increase in penetration, movement and reproduction by *M. konaensis* with an increase of bulk density could not be explained in terms of an apparent effect due to changes the physical environment in the soil; compacting the soil could have resulted in closer pore space that would allow better migration and penetration to feeding sites, hence better development and egg production. This is also illustrated by the difference in volumetric water content in the soil which was optimal for root development at the greater bulk density. It is plausible that changes in bulk density could cause oxygen levels to fluctuate that would affect the nematode activity, primarily penetration.

These results are also in agreement with research of Zhang and Schmitt (1994) where they recovered more *M. konaensis* in deeper areas of the soil profile where soil bulk density is greater (Serracin and Schmitt, 2000). In the coffee fields of Kona, formation of voids and aggregates when the soil is drying may influence the nematodes developmental and reproductive behavior such that in some areas of a field, reproduction may be high and in other areas, it may be low. This would effectively create patchiness in the population densities of the nematode. The important impact would be on the plant as observed with cotton, cabbage, and pineapple. On these three crops, lowest numbers of nematodes were recovered in areas where a subsequent crop grew least vigorously (Schmitt, Unpublished). It is apparent from this research that the reproduction of *M. konaensis* is affected by conditions which affect the host plant. We previously reported that the nematode reproduced better in soils from Molokai which have greater particle size and bulk density (Serracin and Schmitt, 2000). This work also shows the importance of understanding site specific variables such as bulk density for characterization of nematode activity (McSorley and Frederick, 1995).

**PROPOSED RESEARCH PLAN FOR NEW EXPERIMENTS IN POROSITY
AND
BULK DENSITY for *Meloidogyne konaensis***

Question #1: How is penetration of roots by *M. konaensis* affected by bulk density?

Premise: Movement to root will be optimal through soil pores in the size range of the body diameter of the J2.

Premise: Pores smaller than the body diameter of the J2 will restrict movement.

Premise: Pores larger than the body diameter of the J2 will be too large to provide leverage for the nematode.

Question #2: Does bulk density of soil affect post-embryonic development of *M. konaensis*?

Premise: Plant growth will be affected initially by soil bulk density.

Premise: Bulk density will regulate the soil matric potential.

Premise: Soil matric potential will influence plant growth.

Premise: Plant growth will be directly related to soil matric potential.

Premise: Nematode development is directly related to plant growth.

Question #3: Is reproduction of *M. konaensis* affected by soil bulk density?

Premise: Plant root growth through the soil media will begin to alter the soil bulk density.

Premise: Residual effect of initial bulk density will still be evident on plant vigor.

Premise: Soil matric potential will still be different between initial bulk density treatments by the end of the nematodes first generation.

Premise: Plant vigor will be related to the impact of the first three weeks of growing through soil adjusted to specific bulk densities.

Premise: Reproduction of the nematode will reflect the plants growth history.

SUGGESTED PROCEDURE

Methods for question #1:

Collect and sterilize soil from KES

Adjust soil bulk density to 0.6 and 0.9 g/cm³

Place soil in 6.25-cm high X 8-cm diameter columns

Sow cucumber seeds in soil

Two weeks after emergence, hatch nematode and add 3,000 J2/column

Three days after inoculation, determine bulk density and soil moisture from selected replications; remove roots from the soil of the remaining replications and stain; extract nematodes from the soil

Set up 3 replications for bulk density and soil moisture data and 6 replications for nematode data.

Methods for question #2:

Sow cucumber seeds in fine sand

After seedling emergence, infest sand with 10,000 eggs of MK
Collect and sterilize soil from KES
Adjust soil bulk density to 0.6 and 0.9 g/cm³
Place soil in 25-cm high X 8-cm diameter columns
Place under grow lights in lab
Three days after infestation of sand, remove plants from the media and wash all adhering material from the roots.
Transplant seedlings into soil columns
Harvest roots at 3-day intervals and stain
At each harvest: Identify stages of development of the first 20 specimens observed.
Determine bulk density and soil moisture from selected replications
Set up 10 replications for bulk density and soil moisture data and 30 replications for nematode data for each run and run twice.

Methods for question #3:

Collect and sterilize soil from KES
Adjust soil bulk density to 0.6 and 0.9 g/cm³
Place soil in 25-cm high X 8-cm diameter columns
Sow seeds of cucumber in soil columns
Place under grow lights in lab
Two weeks after emergence, infested soil with 10,000 MK eggs/column
At 30 days after inoculation

- Count soil J2, root J2, and eggs of MK.
- Determine bulk density and soil moisture from selected replications

Set up 3 replications for bulk density and soil moisture data and 8 replications for nematode data for each run and run twice.

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**Appendix 2. Tables. Dry weight of coffee roots (g) and nematodes numbers (J2)
in a 3-years- old coffee tree excavated in a Tropical Andisol, Kona, Hawaii.**

Table 1. Appendix. Dry weight of coffee roots (g) and nematodes numbers (J2) in a 3-years- old coffee tree excavated in a Tropical Andisol, Kona, Hawaii.

<i>C. dewevrei</i> (Non-irrigated)			
Depth (cm)	Lateral distance from tree base (cm)		
	0 - 20.3	20.3-40.6	40.6-61.0
0-20.3	1.8 (20)	1.0 (736)	0.3 (12)
20.3-40.6	5.5 (0)	5.4 (0)	0.1 (0)
40.6-61.0	1.5 (48)	0 (0)	0 (0)

<i>C. dewevrei</i> (Irrigated)			
Depth (cm)	Lateral distance from tree base (cm)		
	0 - 20.3	20.3-40.6	40.6-61.0
0-20.3	50 (0)	7.3 (6)	2.7 (14)
20.3-40.6	5.9 (0)	0.3 (0)	0.1 (1474)
40.6-61.0	1.1 (20)	0.4 (6)	0.6 (4)

Table 1. Appendix. Dry weight of coffee roots (g) and nematodes numbers (J2) in a 3-years- old coffee tree excavated in a Tropical Andisol, Kona, Hawaii.

***C. arabica* transplanted at 6-month- old (Non-irrigated)**

Depth (cm)	Lateral distance from tree base (cm)		
	0 - 20.3	20.3-40.6	40.6-61.0
0-20.3	8.2 (17,600)	0.1 (1,064)	0.1 (12,328)
20.3-40.6	0.4 (1,448)	0.2 (852)	0.3 (16,330)
40.6-61.0	0 (0)	0 (0)	0 (0)

***C. arabica* transplanted at 6-months-old (Irrigated)**

Depth (cm)	Lateral distance from tree base (cm)		
	0 - 20.3	20.3-40.6	40.6-61.0
0-20.3	8.2 (1,653)	2.3 (0)	0.9 (768)
20.3-40.6	6.3 (14,036)	0.8 (566)	0.6 (7,620)
40.6-61.0	1.5 (0)	0.3 (12)	0.3 (1,048)

Table 1. Appendix. Dry weight of coffee roots (g) and nematodes numbers (J2) in a 3-years- old coffee tree excavated in a Tropical Andisol, Kona, Hawaii.

***C. arabica* transplanted at 12- month- old (Non-irrigated)**

Depth (cm)	Lateral distance from tree base (cm)		
	0 - 20.3	20.3-40.6	40.6-61.0
0-20.3	1.5 (34)	0.9 (30)	1.2 (846)
20.3-40.6	2.3 (272)	1.3 (8)	0.5 (184)
40.6-61.0	4.2 (58)	2.1 (76)	0.1 (26)

***C. arabica* transplanted 12-months-old (Irrigated)**

Depth (cm)	Lateral distance from tree base (cm)		
	0 - 20.3	20.3-40.6	40.6-61.0
0-20.3	18.2 (6,604)	1.0 (218)	1.4 (0)
20.3-40.6	11.9 (10,442)	1.9 (18,720)	1.7 (14,400)
40.6-61.0	7.4 (6,720)	1.1 (3,408)	0.3 (1,958)

Table 1. Appendix. Dry weight of coffee roots (g) and nematodes numbers (J2) in a 3-years- old coffee tree excavated in a Tropical Andisol, Kona, Hawaii.

***C. arabica* "Catuai" transplanted 6-months-old (Non-irrigated)**

Depth (cm)	Lateral distance from tree base (cm)		
	0 - 20.3	20.3-40.6	40.6-61.0
0-20.3	10 (552)	1.4 (76)	0.2 (2,508)
20.3-40.6	2.5 (22)	0.7 (26)	0.3 (26,998)
40.6-61.0	1.3 (8)	0.7 (200)	0.1 (10,800)

***C. arabica* "Catuai" transplanted 6-mo-old (Irrigated)**

Depth (cm)	Distance from tree base (cm)		
	0 - 20.3	20.3-40.6	40.6-61.0
0-20.3	7.6 (2)	2.1 (852)	0.7 (20)
20.3-40.6	3.8 (34)	1.7 (116)	0.8 (17,064)
40.6-61.0	4.6 (10)	0.5 (28)	0.1 (984)

Table 2. Appendix. Soil characterization of experimental site, Kainaliu, Hawaii

1. Location. Island of Hawaii, Hawaii County. Kona Experiment Station, Kainaliu.
Soil Serie; Honuaulu Silty Clay Loam.

Order: Inceptisol Hydric Dystrandept, thixotropic over
fragmental, isothermic.

Parent Material: Volcanic Ash.

Drainage: Well drained.

Color: Very dark brown if wet; ash grey when dried.

Sticky when wet; dry from aggregates or peds, many pores, dehydrates
irreversibly.

Particle Size Analysis: Data unavailable at this time.

Mineralogy: Visible olivine fragments, quartz.

Chemical Analysis (performed at the University of Hawaii Soil Diagnostic
laboratory).

**2. Soil nutrient analysis of coffee infested to *M. konaensis* under irrigation
regimes in Kona, Hawaii.**

Date	Treatment	Nematode	pH (mmhos/cm)	Macronutrients (%)			
				P	K	Ca	Mg
04-15-96	Fallow	No	5.7	26	340	2200	360
07-26-99	Non irrigated	No	5.9	58	616	3038	734
07-26-99	Non Irrigated	Yes	5.1	125	802	1638	398
07-26-99	Irrigated	No	5.9	60	390	2446	536
07-26-99	Irrigated	Yes	5.9	46	452	2242	496
Optimum*	Non Irrigated	No	5.5-6.5	10-30	0.3	4-20	1-10

**Moisture data: Ikeda and Takeshiro, 1958. Soil Survey Investigation
Report No. 29. Hawaii.
Water Content (% of whole soil)**

Depth	Field Moisture	1/3 Bar	15 Bar	% OC	Bulk Density g/cm ³ (Field Moist)	
0-25 cm	82	84.8	58.4	13.06	0.52	30 kPa
25-45	156	157.1	124.7	7.19		
						PWP (1500 kPa)
45-80	192	184.7	149.6	5.28	0.32	58.4
80-100	155	191.8	125.9	2.82		

265-288	→	C3	C4	C2	33	C4	13	I+
241-264	←	C2	C1	C3	55	C1 ●	71	I-
217-240	→	C1	C1	C4 ●	84	C3	37	I-
193-216	←	C3	C4	C1	71 ●	C1	49	I+
169-192	→	C1	C4 ●	C4	49	C3	50	I-
145-168	←	C3	C4 ● ●	C1	66	C4	41	I+
121-144	→	C4	C1	C1	63	C4	92	I-
97-120	→	C4	C1	C4	114	C1	121	I+
73-96	→	C1	C4	C1	110	C4	30	I-
49-72	→	C4	C4	C1	106	C1	67	I+
25-48	→	C3 Catuai	C4 = 6-mo-old	C3	37	C4 ●	26	I+
1-24	→	C2	C1	C2 Grafted		C1 12-mo-old		I-