OCCURRENCE AND DISTRIBUTION OF THE PHYTONEMATODE *Tylenchulus semipenetrans* IN CITRUS GROVES IN JORDAN

BY

MOHAMMED SAUD K. AL-QASIM

SUPERVISOR

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Prof. WALID I. ABU-GHARBIEH

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The Examining committee considers this thesis satisfactory and acceptable for the award of Master of Science Degree in Plant Protection, Faculty of Agriculture, University of Jordan :

Walid Abu-Gharbieh W. I. Abu Gharbieh

(Professor, Dept. of Plant Protection)

(Chairman of the committee)

Anwar Battikhi A. M. Battikhi

(Professor, Dept. of Soils and Irrigation)

(Co-Advisor, Member of the committee)

Hifzi Abu-Blan H. Abu-Blan

(Associate Professor, Dept. of Plant Protection)

(Member of the committee)

Ahmad Al-Moumany A. AL-Momany

(Associate Professor, Dept. of Plant Protection)

(Member of the committee)

Mazen Akkawi M. Akkawi

(Assistant Professor, Dept. of Plant Protection)

(Member of the committee)

To My Mother

With...

Love

بسم الله الرحمن الرحيم

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SUMMARY

The present research was conducted to study the occurrence and distribution of the citrus nematode (Tylenchulus semipenetrans, cobb) in the major areas planted to citrus in Jordan. Also, the population densities as affected by natural field conditions as well as the degree of resistance in some rootstocks were studied.

One hundred and sixty two composite soil samples were taken during October, 1990 and March, 1991 from various locations of major citrus growing, namely : Northern Jordan Valley, Central Jordan Valley, Southern Jordan Valley, Southern Ghors, Jerash and Wadi Shueib. Soil chemical and physical analysis was performed on soil samples taken from Lemon orchards.

Field sampling revealed that the nematode was distributed widely all over the citrus orchards of Jordan. The problem becomes to be of large magnitude in the older plantations, as the source of inoculum was thought to originate from the few governmental nurseries infected with the nematode.

Nematode population densities were related to some soil and climatic conditions in that the nematode numbers were high in lighter sandy - loam soils (30.8% - 69.8% sand). Moreover, soils containing lower organic matter content sustained higher numbers of the citrus nematode than soils with relatively high organic matter. Soil reaction (pH) and soil salinity (EC) did

not seem to be of significant importance because of the narrow ranges of values obtained for both parameters.

Six 15-years old rootstocks, namely "Cleopetra" mandarin, sour orange, volkameriana, Keen sour orange, macrophylla (= alemow) and Barazilian sour orange, planted in Deir Alla Agricultural Experiment Station in the Central Jordan Valley and grafted with Shamooti, Washington Navel and Valencia orange, mandarin, Algerian tangerine, grapefruit (marsh), Eureka and Lesbon lemon, were investigated for susceptibility of rootstocks and their interaction with the graft as well as the population densities of the citrus nematode during 4 periods; November 1990, February, May and August 1991. All rootstocks were nearly similarly susceptible to the citrus nematode. However, volkameriana and macrophylla were suspected to be more susceptible especially when grafted with Lesbon lemon or Washington Navel orange.

Common citrus species grafted on sour orange in Jordan were found to be moderately to severely infected with the citrus nematode. However, relatively low numbers of second stage juveniles were associated with pomelo trees in the Northern Jordan Valley.

The population densities of the nematode in the Central Jordan Valley indicated higher numbers of the second stage juveniles in November and February, but with slightly more numbers occurring in the latter. Juvenile populations fell drastically during the May sampling and reached its lowest numbers during August. Decrease of juvenile populations was negatively correlated with temperature in the Summer.

Results illustrated significant tendency of higher nematode population build-up during March 1991 than those during October 1990 in three locations, namely Central Jordan Valley, Southern Jordan Valley and Southern Ghors. However, there were no significant differences between the two dates of sampling in Northern Jordan Valley 1, Northern Jordan Valley 2, Wadi Shueib and Jerash.

Histologically, females on infected citrus roots were seen to penetrate through the epidermis deeply inside the cortex and apparently feed from surrounding nurse cells making ubnormal modifications in these cells which possessed thick walls and dense cytoplasm with large nuclei and nucleoli.

الملخص العربي

توزيع نيماتودا الحمضيات (Tylenchulus semipenetrans) على اشجار الحمضيات في الاردن

(محمد القاسم)

تهدف هذه الدراسة الى معرفة حدوث و توزيع نيماتودا الحمضيات (T. semipenetarns) في مناطق زراعة الحمضيات في الاردن كما تهدف الى دراسة تأثر اعداد مجاميع يرقات النيماتودا بالظروف البيئية الحقلية، بالاضافة الى دراسة درجة المقاومة في بعض اصول الحمضيات.

تم جمع ١٦٢ عينة تربة خلال شهر تشرين الاول ١٩٩٠ و ١٦٢ عينة اخرى في شهر آذار ١٩٩١ من بيارات الحمضيات في المناطق التالية : غور الأردن الشمالي، غور الأردن الأوسط، غور الأردن الجنوبي، الاغوار الجنوبية، جرش و وادي شعيب. بالاضافة الى ذلك فقد تم اختيار عينات مأخوذة من تربة اشجار الليمون لاجراء تحاليل التربة الفيزيائية و الكيميائية عليها.

تشير النتائج الى ان نيماتودا الحمضيات تنتشر في جميع المناطق المزروعة باصناف الحمضيات حيث بلغت نسبة وجودها في العينات الى حوالي ٩٩% ويبدو ان مصدر العدوى يأتي من المشاتل المصابة بهذا المرض. وقد تباينت اعداد اليرقات بين المناطق المختلفة حيث وجدت أعداد عالية في الزراعات القديمة في غور الأردن الشمالي ٢.

وقد تمت مقارنة كثافة مجاميع النيماتودا مع بعض صفات التربة و المناخ، حيث تبين ان اعدادا عالية من يرقات نيماتودا الحمضيات تتواجد في التربة الخفيفة شبه الرملية (٨ر٣٠ % - ٨ر٦٩% رمل) و كذلك

في التربة التي تحتوي على نسبة قليلة من المادة العضوية . بينما لم يلاحظ تأثير كبير لحموضة او ملوحة التربة على اعداد اليرقات بسبب محدودية مدى القراءات المأخوذة .

دلت النتائج على ان اصناف الحمضيات المنتشرة في الاردن و المطعمة على الخشخاش هي متوسطة الى شديدة الاصابة بنيماتودا الحمضيات و بدرجات متقاربة ، الا ان اعداد اليرقات في تربة اشجار البوملي المزروعة في الاغوار الشمالية كانت اقل نسبيا من الاصناف الاخرى.

اجريت تجربة ثانية في ببارة للحمضيات في محطة دير علا للتجارب الزراعية لمعرفة تذبذب اعداد مجاميع يرقات نيماتودا الحمضيات خلال اربعة اوقات : تشرين الثاني ١٩٩٠، شباط ، ايار و اب ١٩٩١ . وكذلك لدراسة درجة المقاومة في ٦ اصول هي :

Cleopetra mandarin, sour orange, volkameriana, Keen sour orange, macrophylla (=alemow) and Barazilian sour orange .

مطعمة بثمانية انواع هي :

Shamooti, Washington Navel and Valencia orange, mandarin Algerian tangarin, grapefruit (marsh), Eureka and Lesbon lemon .

دلت النتائج على ان جميع الاصول الموجودة في محطة دير علا مصابة بدرجات متقاربة ، ولكن الاصل Volkameriana و الاصل macrophylla قد تكون الاكثر قابلية للاصابة بنيماتودا الحمضيات و خاصة اذا طعمت بـ Lebson lemon او Washigton Navel orange .

كما دلت نتائج تجربة دير علا على ان اعداد عالية من يرقات نيماتودا الحمضيات تتواجد خلال شهر تشرين الثاني و تزداد في شهر شباط ، ولكن الاعداد تهبط تدريجيا خلال شهر ايار الى ان تصل الى ادنى حد لها في شهر آب حيث يكون لدرجات الحرارة العالية في الصيف اثر سلبي على اعداد النيماتودا في غور الاردن الاوسط .

تشير النتائج في التجربة الاولى الى تواجد مجاميع يرقات نيماتودا الحمضيات باعداد اعلى خلال شهر آذار ١٩٩١ منها خلال شهر تشرين الاول ١٩٩٠ في ثلاث مناطق هي : غور الاردن الاوسط و غور الاردن الجنوبي و الاغوار الجنوبية ، بينما لا يوجد فرق معنوي بين اعداد المجاميع خلال الوقتين في كل من غور الاردن الشمالي ١ و غور الاردن الشمالي ٢ و وادي شعيب و جرش .

كشفت الدراسة السيتولوجية عن طبيعة تطفل النيماتودا ، حيث شوهدت اناث النيماتودا في مقطع عرضي لجذر مصاب وقد اخترق الجزء الامامي من الجسم عدة طبقات من خلايا القشرة (Cortex) ، واظهرت الصور ان النيماتودا تتغذى بواسطة خلايا تغذية خاصة (Nurse Cells) ازدادت فيها كثافة السيتوبلازم و حجم الانوية و النويات .

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1. INTRODUCTION

Citrus crops (Citrus spp. L.) are considered to be the most important fruit trees in Jordan. In 1990, the total area planted to all species of bearing citrus trees reached 5,289 hectares producing about 118.98 thousand tons which represents about 66% of fruit trees production in Jordan (Ministry of Agriculture, 1990).

A very high percentage of planted area (about 94% of total area planted to citrus) is concentrated in the Jordan Rift Valley, and only 6% of the planted area is distributed in other locations, mainly in Jerash and Wadi Shueib.

The citrus nematode (Tylenchulus semipenetrans cobb, 1914, Criconematoidea : Tylenchulidae) is the primary nematode pathogen of citrus. It is sedentary semi-endoparasite and causes a serious problem in all citrus growing areas of the world. Heavily infected citrus trees show decline symptoms that appear slowly and affect trees vigor resulting in a loss in fruit production which might exceed 50% (Van Gundy,1986 and Philis, 1989).

Soils of Jordan planted to citrus and climatic conditions prevalent in the majority of citrus groves are, suspected to be particularly unsuitable neither to the host nor to the pathogen; since an ideal soil environment for citrus root development and nematode invasion is a light-textured and well drained soil with moderate temperature (25-31 ^{o}C), (Marsh, 1973 and Reuther, 1973).

In Jordan, information on the biology of the citrus nematode, particularly on the distribution and effect of some local soil characteristics are meager or even lacking. Such information is necessary for performing better and successful control measures.

Hence, this research was initiated and conducted in the major areas planted to citrus to study the following :

1-The occurrence and distribution of the citrus nematode in the soils of citrus groves in Jordan.

2-The population densities of this nematode in the Central Jordan Valley.

3-The influence of some soil and climatic factors on the citrus nematode population densities.

4-The possible presence of resistance in some citrus rootstocks and species.

2. REVIEW OF LITERATURE

2.1 MORPHOLOGY AND BIOTYPES :

Siddiqi (1974) described the morphological characteristics of Tylenchulus semipenetrans cobb as follows : Immature female has vermiform body with lip region conoid-rounded, smooth and continues with body; labial framework moderately sclerotized; strong spear with well developed rounded basal knobs; metacorpus (median bulb) strongly muscular and with large oval cuticular thickening in center; basal bulb saccate containing esophageal glands with esophago-intestinal junction at the base; vulva is near posterior end with thick labia and has a single ovary outstretched anteriorly; the distinct excretory duct open through a prominent pore close in front of the vulva.

Mature female's body behind neck is swollen. Neck region is irregularly distorted. Ovary is coiled and uterus has single egg. Intestine syncytia filling most of the body cavity. Excretory pore is 17 μ in front of vulva.

Body of second stage larvae is straight or arcuate; lip region, spear and esophagus are as in immature female; median bulb with spindle-shaped cuticular thickening in center; excretory pore is behind middle of body; genital primordium 2- to 4-celled, posterior to excretory pore; male larvae are slightly shorter, more slender and have a more pointed tail end than the female larvae.

Baines *et al*. (1958) found differences in the pathogenicity of the citrus nematode on trifoliate orange and sweet orange roots. Later on, Stokes (1969) described a colony of *T. semipenetrans* (collected and reared on a grass, *Andropogon rhizomatus*) that failed to parasitize on four citrus rootstocks. He suggested existence of a distinct strain of *T. semipenetrans* named as "the grass strain" of the citrus nematode.

Inserra *et al*. (1980) indicated existence of 4 biotypes of *T. semipenetrans* distributed world-wide. The "Poncirus biotype" = biotype California C3, reproduces actively on *Citrus* spp., *Poncirus trifoliata*, their hybrids and on grape, but not on olive. The "Citrus biotype" = biotype California C1, C2 and C4, reproduces very poorly on *P. trifoliata* but infects *Citrus* spp., Carrizo and Troyer Citrange, olive, grape and persimmon. The "Mediterranean biotype" = biotype Italy C5, which is very close to the "Citrus biotype" and has the same host range, except for olive. The "Grass biotype" = biotype Florida C6, reproduces only on *Andropogon rhizomatus* found in Florida. Moreover, Inserra *et al*. reported in 1988, that the genus *Tylenchulus* cobb 1913, contains two species, *T. semipenetrans* cobb 1913 and *T. furcus* Van Den Berg & Spaull 1982. *T. semipenetrans* races attack dicotyledons, particularly rutaceous species like sour orange and sweet lemon, while *T. furcus* infects monocotyledons, especially *Saccharum* species as sugar cane.

2.2 HISTORY AND DISTRIBUTION :

The Citrus Nematode, T. semipenetrans, was first discovered in California on orange tree in 1912 by J.R. Hodges (Chitwood and Berchfield, 1957), and subsequently described in 1914 by Cobb. He gave attention to the anatomy, life history and distribution of this species and showed that it was found in all of the major citrus producing areas of the world (Chitwood and Birchfield, 1957 and Van Gundy, 1986). The nematode was then found in several other countries of Asia, Africa, America and Europe. In 1957, Chitwood and Birchfield showed that several citrus groves in Florida appear to be in a poor physiological condition due to T. semipenetrans. Alvira (1974) showed the importance of T. semipenetrans on citrus production in Spain by studying its distribution in several locations of citrus growing areas. Husain et al. (1981) studied vertical distribution of citrus nematode in Iraq and found that it is correlated with plant age, conditions of root system and soil factors. In Iraq also, Mohammad et al. (1982) stated that all orchards and nurseries under study were found infested with the citrus nematode and the percentage frequency of occurrence of this nematode in Iraq was substantially 100%. El-Shoura et al. (1985) carried a survey study in Egypt to determine the occurrence and distribution of citrus nematode T. semipenetrans during 1980-1983. The results indicated presence of the citrus nematode in 94.9% of the collected samples.

Bel-Kadhi (1986) studied nematodes associated with fruit trees nurseries in Tunisia and gave a particular attention to the problem caused by T. semipenetrans on citrus fruit trees. He stated that the distribution of nematode species on fruit trees is connected to both plant species and soil characteristics or prevailing climate.

Mamluk et al. (1984) and Yousef (1988) enlisted several nematode species found associated with citrus trees in Jordan. They indicated that the citrus nematode, T. semipenetrans, is present in the Jordan Valley associated with citrus trees.

2.3 HOST PLANTS :

2.3.1 Hosts :

A citrus nematode biotype was considered native to Florida soil. In 1957, Chitwood and Berchfield were able to recover females, males and juveniles of a nematode agreeing morphologically with T. semipenetrans from root of climbing hampweed, Mikania batatifolia, at a spot from which no records of any cultivation was known. Hannon et al. (1963) stated that there is a strain of T. semipenetrans parasitic on Mikania and indicated that T. semipenetrans populations living on citrus do not infect Mikania. They recorded some other hosts of the citrus nematode in addition to Citrus spp., namely grapes (Vitis vinifera L.), lilac (Buddlea alternifolia), persimmon (Diospyros spp.) and olive (Olae europea). Taha and Sultan

(1983) also stated that the citrus nematode could be parasitic to grape vine and olive .

2.3.2 Susceptibility of citrus rootstocks and hybrids :

The presence or absence of resistance to the citrus nematode T. semipenetrans in most species and varieties of citrus and other rutaceous crops have been investigated by several workers (Baines *et al.*, 1948, Van Gundy and Kirkpatrick, 1964, Kaplan, 1981 and Reddy *et al.*, 1987). In 1914 Cobb reported that trifoliate orange (*Poncirus trifoliata*), grapefruit, sweet orange and sour orange were susceptible to the citrus nematode in the United States (Baines *et al.*, 1960). However, Baines *et al.* (1948) reported that the trifoliate orange was highly resistant to citrus nematode. They found that certain selections or varieties of trifoliate orange and some other plants, botanically close to rus , were highly resistant or immune to the citrus ode. They also mentioned that the resistant plants might l for breeding of nematode resistant rootstocks for Cameron *et al.* (1954) reported that F1 hybrid seedlings from crosses with nematode resistant trifoliate orange and five susceptible citrus species showed marked resistance to the citrus nematode. Similarly, many variants and varieties of citrus and other rutaceous plants botanically close to *Citrus* were also recognized.

Baines *et al.*(1960) reported 23 *Citrus* species which are moderately or severely infected with the citrus nematode. Of these : Lime (*C. aurantifolia*), Sour Orange (*C. aurantium* L.), pomelo (*C. grandis* L.). *C. iambhiri* Lush., Citron (*C. medica* L.), Calamondin (*C. mitis* Blanco), grapefruit (*C. paradisi* Macfad), mandarin (*C. reticulata* Blanco), Sweet Orange (*C. sinensis* L.) and 12 varieties or selection of *Poncirus trifoliata*, except one, all of them were highly resistant to the citrus nematode.

2.4 BIOLOGY AND HISTOPATHOLOGY :

2.4.1 Life cycle :

Van Gundy (1958) studied the life cycle of *T. semipenetrans* and found that the nature of feeding on citrus roots varied with the developmental stage of the female citrus nematode (Schneider and Baines, 1964). A female generally lays 75 to 100 eggs in a gelatinous matrix, and the development cycle from egg to egg take 6-8 weeks at temperature of 25 ^{o}C. Laid eggs hatch in 12-14 days at about 24 ^{o}C (Giudice, 1985), the male larvae undergo three more molts over 7-10 days to attain vermiform adulthood. The second stage female juveniles molt once and may persist in soil for many months without feeding. They must feed on a susceptible hosts for further development at which the second stage juveniles molt three times to become young females (Schneider and Baines, 1964).

2.4.2 Feeding :

Cohn (1965) studied the penetration of the citrus nematode into the growing roots of its host in vitro. He indicated that juveniles were observed in contact with the roots for 19 days before actual penetration. Schneider and Baines (1964) stated that Van Gundy reported female juveniles feed on epidermal cells and do not penetrate into cortex. Also, they indicated that the female citrus nematode, in all of its life stages, feed on cortical cells. Moreover, juveniles occur free in the soil, free on the surface of roots, with anterior portion of their bodies in the epidermal and hypodermal cells.

During the period of feeding, female juveniles molt three times and become young adults. The young adult female penetrate midway deeper into the cortex by breaking into cortical cells and then become sedentary while heads move about within the last penetrated cell (Cohn, 1972 and Siddiqi, 1974).

The females attach themselves to the radical and secrete from their excretory pore a mucilaginous substance (gelatinous matrix) to which particles of soil become attached. This substance act as a protective shield against predators and other natural enemies, and also, used by the female to lay eggs from which individual nematodes of both sexes emerge.

2.4.3 Histology :

The cells that fed upon by the female nematodes do not die, but become greatly altered. The cytoplasm thicken and fill the cells, and the nucleus and nucleolus enlarge greatly, these cells are then called "nurse cells". (Schneider and Baines, 1964, Giudice, 1985 and B'Chir, 1987).

Himmelhoch et al. (1979) stated that the electron microscope studies of citrus roots infected with T. semipenetrans showed two distinctive symptoms associated with pathogensis. First, was the presence of para crystalline arrays within plastids. Second, was the formation of enlarged, lobed nuclei. Taha and Sultan (1983), studied the cellular responses of grape roots to the invasion of T. semipenetrans which was found to affect a range of cells extending from epidermal to endodermal and pericycle cells in newly emerged fine roots forming a ring of disorganized (giant) cells. B'Chir (1987) showed that the nematode-induced changes in the cortical tissue of citrus roots were formed by five to ten cells that have undergone profound modifications. The transformed cells lose an important part of their potential and become very sensitive to any metabolic disequilibrium .

Van Gundy and Kirkpatrick (1963, 1964) studied the histological relationship of resistance. They examined Severinia buxifolia (immune), Poncirus trifoliata (resistant) and Citrus jambhiri (susceptible) and illustrated that in case of immune and resistant hosts, entry and feeding of the juveniles into the hypodermis and cortical parenchyma produced a hypersensitive reaction in the protoplasm of the parasitized cells and a wound periderm was formed. That's why no females were found in the roots of resistant or immune seedlings.

Kaplan (1981) related incompatibility between T. semipenetrans populations and various rootstocks to the hypersensitive type responses to infection in the hypodermis, cavity formation within the cortex, cell wall thickening of invaded cells and timely vaculation of nurse cell cytoplasm, and also, wound periderm formation was observed.

The reproductive potential of T. semipenetrans was reported the highest on Sour Orange and Japanese lemon (Yousif, 1984), while a minimal rates of nematode reproduction occurred on Troyer Citrange and Cleopetra Mandarin. Reddy et al. (1987) selected the rootstock Poncirus trifoliata and hybrids between Citrus limonia and P. trifoliata to be rated resistant according to the reproduction factor of T. semipenetrans .

2.4.4 Symptoms :

The disease caused by the citrus nematode has been named "slow decline" since a great decrease in the rate of growth and yield was observed in heavily infected young citrus trees (Baines et al., 1978).

Symptoms of the nematode infestation are difficult to diagnose. On the under ground parts, roots infected with the nematode appear encrusted due to soil particles that adhere to the mucus excreted by the female. Heavily infected roots are discolored due to numerous lesions which develop from the invasion of large numbers of parasites. These root system generally lack vigor and smaller than uninfected ones (Baines *et al*., 1978 and Jenkins and Tylor, 1967).

Citrus affected by "slow decline" exhibits above ground symptoms related to the reduced ability of roots to maintain normal growth. The typical disease syndrome consisting of the death of terminal buds, chlorosis and dying of leaves, early wilting under moisture stress and twig die back. Fruit production in reduced in both quantity and quality and trees become uneconomic to maintain (Jenkins and Tylor, 1967).

Disease severity is much influenced by soil conditions. Citrus trees suffer more severe decline from *T. semipenetrans* in soils with 5-15% clay (Van Gundy and Kirkpatrick, 1964). The nutritional status of the trees also affects symptom expression. Van Gundy and Martin (1961) reported greatest reduction in growth of citrus seedlings from the presence of *T. semipenetrans* in soils maintained at levels of calcium, sodium or potassium that are borderline or unfavorable for citrus, and so crop loss is accentuated under conditions of stress.

2.5 ECOLOGY OF THE NEMATODE :

2.5.1 Soil Characteristics :

The Citrus nematode performs its life cycle in the rhizosphere of the citrus roots, so that, its reproduction and viability are directly affected by the physical conditions of the soil. T. semipenetrans can reproduce in a wide range of soil types but prefers lighter soils. Van Gundy and Martin (1962) reported that a neutral loam-textured soil (15% clay, 35% silt, 50% sand) contained 4 times as many citrus nematode juveniles, as a neutral sandy loam. Van Gundy et al. (1964) found that the highest rate of reproduction of the citrus nematode and the greatest plant growth reduction was in soils of 10% and 15% clay.On the other hand, Baines (1974) in his study on the effect of soil type on movement and infection rate of T. semipenetrans juveniles indicated that sandy loam soil (14.5% clay, 11.4% silt, 74% sand) was favorable for development of sweet orange seedlings and that the second-stage juveniles had moved readily into and through this soil.

Yousef et al. (1989) in Egypt, however, published a report on reproduction of T. semipenetrans on sour orange seedlings cultivated in different soil types. He noticed the maximum numbers of nematode juveniles after 40 days of inoculation was on seedlings cultivated in clay soil.

Temperature also affects the activity of T. semipenetrans. Soil temperature between 25 and 30 °C are most suitable for infestation, growth and reproduction of the citrus nematode while at 20 and 35 °C infestation was reduced (Kirkpatrick et al., 1965 and Giudice, 1985). Kirkpatrick et al. (1965) stated that the controlled studies of Van Gundy showed that citrus nematode reproduction began at 21-22 °C, reached its maximum between 28-31 °C but virtually nonexistence at more than 31 °C.

Effect of the soil water content differs from one soil type to another. In general, T. semipenetrans is very sensitive to drought conditions of the soil. The optimum moisture levels for development of the citrus nematode, as found by Van Gundy and Martin (1962), were at 30 and 10 millibars in the loam and loamy sand (5% clay, 29% silt, 75% sand) soil, respectively. However, Van Gundy and Kirkpatrick (1964) reported greater seedlings growth reduction, due to citrus nematode, in wet soils than in dry soils while nematode reproduction was favored by dry conditions in fine textured soil.

Aeration is correlated with ground humidity. Roots of citrus plants suffer from reduced oxygenation, and also the reproduction of T. semipenetrans slows down under poorly aerated soil conditions. Giudice (1985) reported that the development of citrus nematode eggs were reduced in soil with low oxygen availability .

O'Bannon (1967) stated that the organic matter is favorable for an early increase of the citrus nematode, but is not favorable for ultimate high density of the nematode juveniles Soil containing high levels of organic substances promotes infestation and rapid growth. But the addition of large quantities of organic substances in the form of peat (2:1 by volume) inhibits reproduction probably as an indirect result of a reduction in the pH which reached 4.0 in sand-peat (2:1 v/v), (Baines, 1974).

pH value was found to affect *T. semipenetrans* populations. Optimum pH values varied from 6 - 7.5. Below pH 5 and above pH 8, there was a constant reduction in the nematode reproduction (Giudice, 1985). Van Gundy and Martin (1962) stated that at neutral pH the soil had contained more juveniles than acidic. They, also, found that below pH 6 the number of juveniles was a function of soil acidity but above pH 6 the number was related principally to soil temperature. Moreover, Van Gundy and Kirkpatrick (1964) reported greater total populations at pH 7.5 than at pH 6 soils.

Soil salinity also has a great impact on citrus nematode reproduction. Yousef *et al*. (1989) reported few numbers of citrus nematode juveniles invaded sour orange in saline soil (EC = 2.4 mmhos/cm). In a pot experiment carried out to determine the effect of soil salinity on citrus nematode and sour orange seedlings growth, Sweelam and El-Gindi (1989)in Egypt showed that increased salinity cause plant growth decline. Moreover, addition of nematodes to salt-stressed seedlings caused further reduction in shoot and root weight of seedlings. They also concluded that 500 ppm salinity level was the optimum level for citrus nematode activity and sour orange seedling growth, and both were sharply affected as salinity increased over 5000 ppm level. Youssef *et al*. (1989) reported that juveniles infectivity after 25 days from inoculation was considerably suppressed on sour orange seedlings irrigated with chloride salts ($MgCl_2$, $CaCl_2$ and NaCl) using different concentrations, while maximum invasion was observed with $CaCl_2$

at 1000 ppm and intermediate rate was at 1000 and 3000 ppm of NaCl.

2.5.2 Population densities :

Seasonal changes of the citrus nematode populations were observed by several workers (1964, O'Bannon et al., 1972, Yousif, 1985, Stephan, 1988 and Stephan et al., 1990). A marked seasonal fluctuation in citrus nematode populations is due to temperature changes, amount of rainfall, and abundance of natural enemies. Observations from different citrus growing areas around the world indicated the capability of the citrus nematode for living under a wide range of field conditions and soil types.

O'Bannon et al. (1972) studied population fluctuation of T. semipenetrans on citrus in Florida. They found that high levels occur in April-May and November-December, and low levels in February-March and August-September. The nematode population increased over about 4 - 5 weeks after the spring and fall flush of root growth. Salem in Egypt (1980) reported that the population of T. semipenetrans were highest between March-May and between October-November While the lowest densities were obtained from June-September and from November-February. These fluctuations are presumably due to fluctuations in soil temperature which showed increase in the first period of high and low and a decrease in the second of high and low populations. In Iraq, Stephan et al.(1990) found high population levels of citrus nematode occurring during

March-July and September-November which were associated with the major root flushes during these periods. He also found that soil temperature had influenced nematode infectivity; where at 12 °C nematode infectivity started to increase but reached optimum at temperatures ranged between 20 - 25 °C. In the Jordan Valley, Yousef (1988) found that the maximum numbers of the citrus nematode were during March-May while minimum numbers were found during the September-January period.

Population dynamics and number of generations of the citrus nematode in the soil was studied in Egypt by Salem et al. (1984). They could calculate 4 generations of the citrus nematode per year in the rhizosphere of Citrus sinensis and Citrus ansanlifolia; 3 or 4 generations in the rhizosphere of Citrus reticulata and 4 or 5 generations per year in the rhizosphere of Citrus aurantifoliata.

2.6 OTHER NEMATODES ASSOCIATED WITH CITRUS :

The number of nematode species known to be associated with citrus plants were 28 in 1959 and 189 in 1968 belonging to 39 genera (Giudice,1985). In 1986 this number has increased to 200 species belonging to 44 different genera of which only 20 species have been shown to cause pathogenic injury to citrus plants (Van Gundy, 1986). The various types of nematodes which attack citrus plants differ in their damage, distribution and economic impact. Chitwood and Berchfield

(1957) stated that a survey in the United States was conducted and showed that several nematode species other than T. semipenetrans were found on citrus. These species included : Pratylenchus; Rotylenchus; Helicotylenchus; Hoplolaimus; Xiphinema; Criconemoides; Hemicycliophora and Tylenchorhynchus. Mohammed et al. (1982) studied citrus orchards in Iraq and found 23 different nematode genera, 13 species of which are pathogens of various plants including citrus. Most frequent nematode genera found associated with citrus other than T. semipenetrans were Tylenchus; Aphelenchus; Aphelenchoides spp.; Ditylenchus; Tylenchorhynchus and Helicotylenchus (Mohammed et al., 1982).

Reis (1982) obtained 18 genera from citrus orchards in Mozambique, of these nematodes one can list the following : T. semipenetrans; Xiphinema Trichodorus; Helicotylenchus; Tylenchorhynchus; Tylenchus; Meloidogyne; Aphelenchus and Hoplolaimus . Also, Khan et al.(1990) conducted a survey on nematodes associated with citrus nurseries in Pakistan. They found Hoplolaimus; Globodera rostochiensis and T. semipenetrans, other nematodes included Helicotylenchus ; Longidorus and Tylenchorhynchus .

Several investigations also showed that apart from T. semipenetrans , four migratory endoparasites, one sedentary endoparasite and eight migratory ectoparasitic nematodes are pathogenic to citrus plants. Pratylenchus brachyurus was found in Florida on citrus plants in 90% of citrus groves and frequently together with populations of Radopholus similis .

The physical properties of the soil or other environmental factors have no effect on P. brachyurus and it survives as migratory endoparasite even under adverse environmental conditions (O'Bannon et al., 1972). Moreover O'Bannon et al. (1972) and Kaplan and Timmer (1982) reported that P. coffeae was more pathogenic to citrus than P. brachyurus. On the other hand, R. similis was found on citrus trees in Florida where it causes a disease known as "spreading decline" (Kaplan, 1986).

Several species of migratory ectoparasitic nematodes, recognized as pathogens for citrus, such as Belonolaimus longicaudatus and Trichodorus christici have been found in Florida in the rhizosphere of decayed citrus plants (Kaplan, 1985). Hemicycliophora arenaria is known to damage citrus plants in California, other ectoparasites include Paratrichodorus and Xiphinema Among the sedentary endoparasitic nematodes, species of Meloidogyne have been found to affect citrus plants. (Giudice, 1985).

Mamluk et al. (1984) reported 6 frequent plant parasitic nematodes, apart from T. semipenetrans, associated with citrus trees in Jordan, they included Helicotylenchus; Longidorus; Pratylenchus; Tylenchorhynchus; Paratylenchus and Xiphinema. Yousef (1988) reported another 32 nematode genera associated with citrus trees in Jordan. He claimed that the most important plant-parasitic nematode was T. semipenetrans, while the other genera were low in numbers , and so, they have no great economic importance .

3. MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 SOIL SAMPLING :

3.1.1 First experiment :

This part deals with the occurrence and distribution of the citrus nematode in the different areas of citrus growing in Jordan. In addition, it includes determination of the nematode juvenile populations in the soils planted to different Citrus species during two periods of presumably high juvenile population levels (Yousef, 1988). This research also include study of the effect of certain soil characteristics on juvenile populations in the soils planted with lemon.

For this purpose, 162 soil samples were collected from seven different areas representing the various citrus growing regions in Jordan. Locations 1, 2, 3, 4 and 5 (Table 1, 2 and Fig. 1) represent citrus groves included in the study from northern to southern Jordan Rift Valley. While areas 6 and 7, represent the elevated areas of citrus growing in Jerash and Wadi Shueib, respectively.

The number of samples taken from all locations was determined depending on area planted to citrus species found in each particular locality (Table 2, 14). The areas were selected as based on the Annual Statistics Data, Ministry of Agriculture, 1990. Each sample was a composite of five sub samples taken at a depth of 5-20 cm from within the drip line

Table 1 : Locations and sites assessed for occurrence of the citrus nematode.

Locations	Sites
Northern Jordan Valley 1 (N.J.V.1)	Addasiya, Baqura, Manshiya, Waqqas, Tal-Arbaien, Mashare'.
Northern Jordan Valley 2 (N.J.V.2)	Wadi El-Yabis, Abu-Habeel, Rayhaniya.
Central Jordan Valley (C.J.V.)	Deir Alla, Al-A'rda, Al-Wadi Al-Abyad.
Sothern Jordan Valley (S.J.V.)	Kaffren.
Sothern Ghors (S.G.)	Ghor El-Safi, Ghor El-Hadethah.
Jerash (J.)	El-Jbarat.
Wadi Shueib (W.S.)	Wadi Shueib

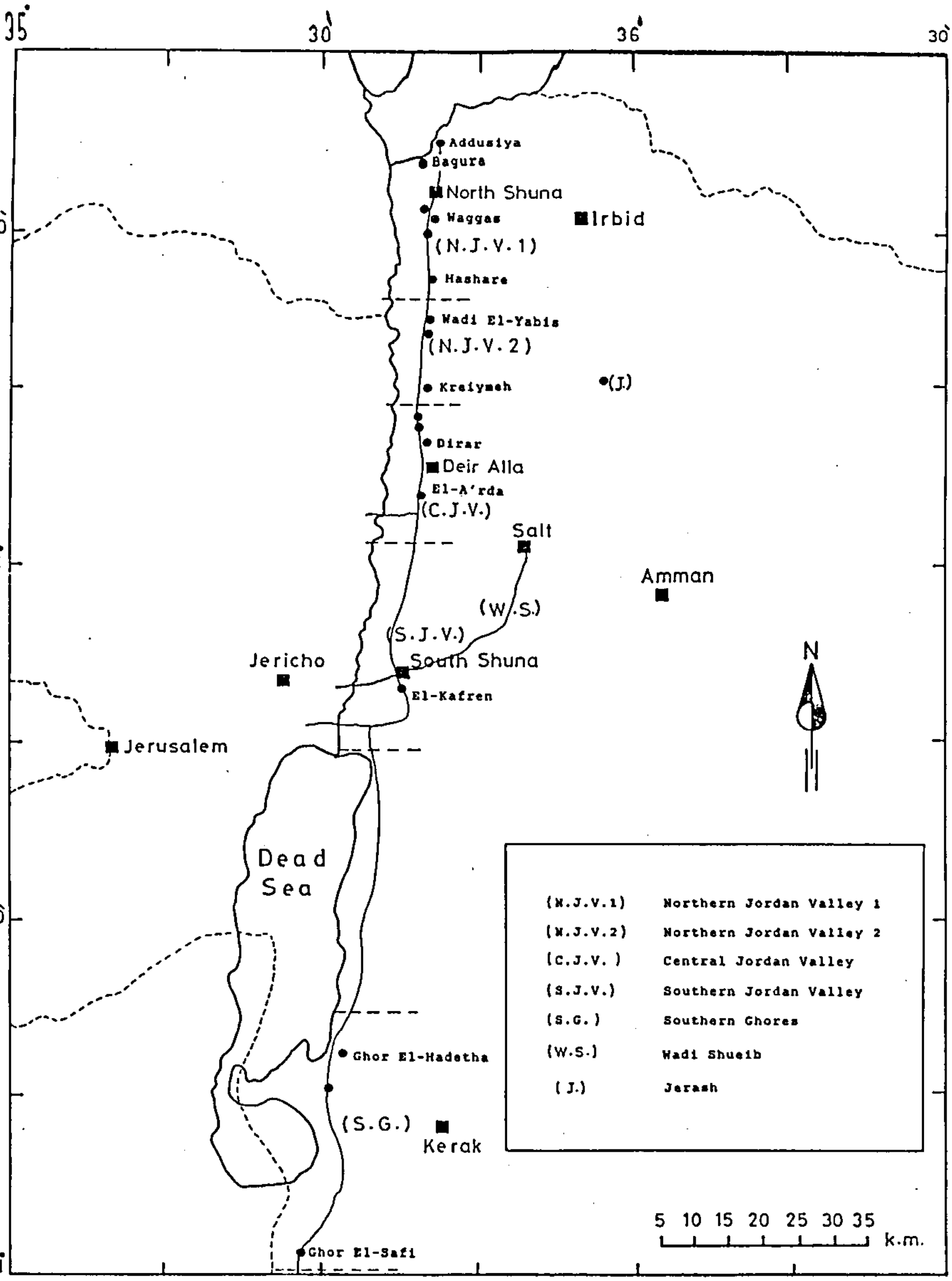

Fig.1: Areas and Locations of Citrus Growing in Jordan Included in the Study.

Table 2 : Number of samples taken from the different locations under study (1).

Locations	Lemon	Orange	Mandarin	Tangerine	Grapefruit	Pomelo	Total
Northern Jordan Valley 1	11 (2)	9	10	14	5	5	54
Northern Jordan Valley 2	8	7	8	10	5	7	45
Central Jordan Valley	5	6	4	5	2	3	25
Southern Jordan Valley	2	2	2	2	2	2	12
Southern Ghors	4	2	2	2	2	---	12
Jerash	2	2	2	2	---	2	10
Wadi Shueib	2	2	---	---	---	---	4
Total	34	30	28	35	16	19	162

(1) Number and distribution of samples was taken according to the planted areas and citrus species.

(2) One composite soil sample represents approximately 50 ha. of the entire area. From areas less than 50 ha., two composite soil samples were taken.

of each tree.

About 700 cc of soil and 5 gm of citrus fine roots were taken from the rhizosphere of each bearing, over 15 years old, citrus tree and placed in a plastic polyethylene bag. The bags were closed tightly and labeled. Immediately after sampling, the bags were placed in an ice box and taken to the laboratory for analysis.

3.1.2 Second experiment :

This part of the research was conducted in a 15-years old citrus orchard located at Deir Alla Agricultural Experiment Station. The orchard was previously designed for the study of various combinations of citrus rootstocks and species or cultivars. The orchard was planted in a split plot design using 6 Citrus rootstocks planted in main plots and eight types of Citrus species and/or cultivars planted in sub-plots and replicated 3 times. The rootstocks included "Cleopatra" mandarin (C. reticulata cv. 'Cleopatra'), volkameriana (lemon X mandarin hybrid), macrophylla (=alemow) (C. macrophylla), sour orange (C. aurantium), Keen sour orange (C. aurantium cv. Keen) and Barazilian sour orange (C. aurantium cv. Barazilia-n), and the citrus types included C. sinensis cv. Shamooti, Washington Navel and Valencia, Mandarin (C. reticulata), C. reticulata cv. Algerian Tangerine, grapefruit (C. paradisi cv. Marsh), lemon (C. lemon cv. Eureka and Lesbon).

The main objective was to study the effect of interaction between the different citrus root-stocks and species on the citrus nematode incidence. In addition, a study of the population density of the citrus nematode was made during 4 periods of the year.

Each soil and root sample was a composite of 4 cores taken from 5 - 20 cm soil depth from within the drip line of each tree. Then, all samples were treated as described in the first experiment.

3.2 NEMATODE EXTRACTION :

3.2.1 First experiment :

Soil samples were thoroughly mixed in the laboratory. About 250 cc soil aliquot was processed for nematode extraction using Cobb sieving followed by the Modified Baermann funnel method for further cleaning (Ayoub,1977). After 48 hours, the extracted nematodes were concentrated in 10 ml of tap water inside glass vials. Nematodes were then killed by gentle heat and preserved in a 3% formaldehyde solution which is formed by adding 10 ml of 6% formaldehyde solution over the 10 ml of the heat-killed nematode-suspension. The vials were closed tightly, labeled and placed in a refrigerator at 5 ^{o}C for further examination.

3.2.2 Second experiment :

About 500 cc of mixed soil samples were processed for nematode extraction using Cobb sieving method followed by Centrifugal - Flotation technique in which a sugar solution of

1.18 specific gravity was used (Ayoub,1977). Immediately, the extracted nematodes were concentrated in 10 ml of tap water in a small vial, killed by gentle heat and preserved in 3% formaldehyde solution. The glass vials were then placed in a refrigerator for further examination.

3.3 NEMATODE ASSESSMENT :

Extracted nematodes found in each vial (20 ml of 3% formaldehyde solution) of both experiments were assayed by counting second stage juveniles.One half milliliter suspension was taken from each vial and placed in a 0.5 ml capacity grooved slide. Nematodes were then counted under a binocular microscope. The average of five sub samples from each vial was determined and subsequently number of nematodes per vial was calculated.

3.4 SOIL ANALYSIS :

Soil samples collected from all orchards planted to lemon trees in the various locations were analyzed mechanically and chemically.

3.4.1 Soil texture :

Particle size distribution was determined by Bouyoucos method (Black et al., 1965).The results were expressed as the percentage of sand, silt and clay in less than 2 mm size fraction, at 25 C corrected air temperature.

3.4.2 Soil pH and electrical conductivity (EC) :

Hydrogen ion concentration and electrical conductivity were measured in saturated paste extract which was filtrated by a vacuum pump. pH and EC were measured using a conductivity bridge and glass electrode, respectively, standardized at 25^{o}C air temperature (Black et al., 1965).

3.4.3 Soil soluble Na^{+}, K^{+}, Ca^{++} and Mg^{++} :

Na^{+} and K^{+} were determined in the saturated paste extract using Flame Photometer Atomic Emission method. Readings in ppm of both ions were carried out by graphic resolution of the relation between ppm of standard concentration and their respective readings. Ca^{++} and Mg^{++} were determined in the saturated paste extract directly using the Atomic Absorption Spectrophotometer (Black et al., 1965).

3.4.4 Soil organic matter :

Percentage of organic matter was calculated from the organic carbon percentage which was determined by the Walkley - Black method (Black et al., 1965).

3.5 STAINING AND HISTOLOGICAL WORK :

The methods of microtechnique in nematology were followed as described by Daykin and Hussey (Barker et al.,1985).

Selected small roots were first stained by acid fuschin (Ayoub,1977) and then fixed in formalin-aceto-alcohol (FAA); composed of 90 ml of 50% ethanol, 5 ml of glacial acidic acid and 5 ml of 37% formaldehyde (Barker et al.,1985). The fixed tissues were dehydrated following the Tertiary butyl alcohol Dehydration Schedule method (Table 3). Alcohol in the tissues was replaced by a pure solution of liquid paraffin.

Sectioning was done on a rotary Microtome. Sections of 15 - 20 μm were stained according to Johansen's quadruple stain Schedule, (Table 4).

3.6 STATISTICAL ANALYSIS :

Data analysis in both experiments comprised the following :

1- Analysis of variance according to one way analysis (completely randomized design) with unequal observations. LSD and mean separation were used to identify the significant differences between means for each season, each location and each citrus species.

2- Regression analysis was made to describe the relationship between number of nematode juvenile population and certain soil characteristics.

3- The means and the confidence intervals were calculated to describe nematode occurrence and distribution in each location on each citrus species.

4- For the second experiment, the folowing was made :

a) The principles of the split-plot design analysis were followed at each sampling period, where the main plots were allocated by the different rootstocks while the sub-plots were allocated by the 8 citrus species.

b) A split-split plot in time design was followed to analyze nematode densities during all periods of sampling.

Table 3 : Tertiary butyl alcohol dehydration schedule (Barker et al., 1985).

Step	% Alcohol	Time
1	50	2 hr or more
2	70	overnight
3	85	1-2 hr
4	95	1-2 hr
5	100	1-3 hr
6	100	1-3 hr
7	100	1-3 hr
8	100	1-3 hr

Table 4 : Sass safranin and fast green stain (Barker et al., 1985).

Step	Solution	Time
1	xylene	5 min
2	absolute ethanol	5 min
3	95% ethanol	5 min
4	70% ethanol	5 min
5	50% ethanol	5 min
6	30% ethanol	5 min
7	1% aqueous safranin O	1-12 hr
	rinse in tap water	
8	30% ethanol	3 min
9	50% ethanol	3 min
10	70% ethanol	3 min
11	95% ethanol	3 min
12	0.1% fast green FCF in 95% ethanol	5-30 sec
13	absolute ethanol	15 sec
15	absolute ethanol	3 min
16	xylene-absolute ethanol (1:1)	5 min
17	xylene	5 min
18	xylene	5 min or longer

4. RESULTS

Table 5 : Occurrence of citrus nematode in soil samples taken from seven locations in Jordan during October 1990 and March 1991.

LOCATIONS	Number of samples (1)	Samples of October,1990		Samples of March,1991	
		Number of +ve samples	% occurrence	Number of +ve samples	% occurrence
Northern Jordan Valley 1	54	54	100	54	100
Northern Jordan Valley 2	45	45	100	44	97.8
Central Jordan Valley	25	25	100	25	100
Southern Jordan Valley	12	12	100	12	100
Southern Ghors	12	12	100	12	100
Jerash	10	10	100	10	100
Wadi Shueib	4	4	100	4	100
Total	162	162	100	161	99.4

(1) 162 soil samples were taken twice : in October 1990 and March 1991.

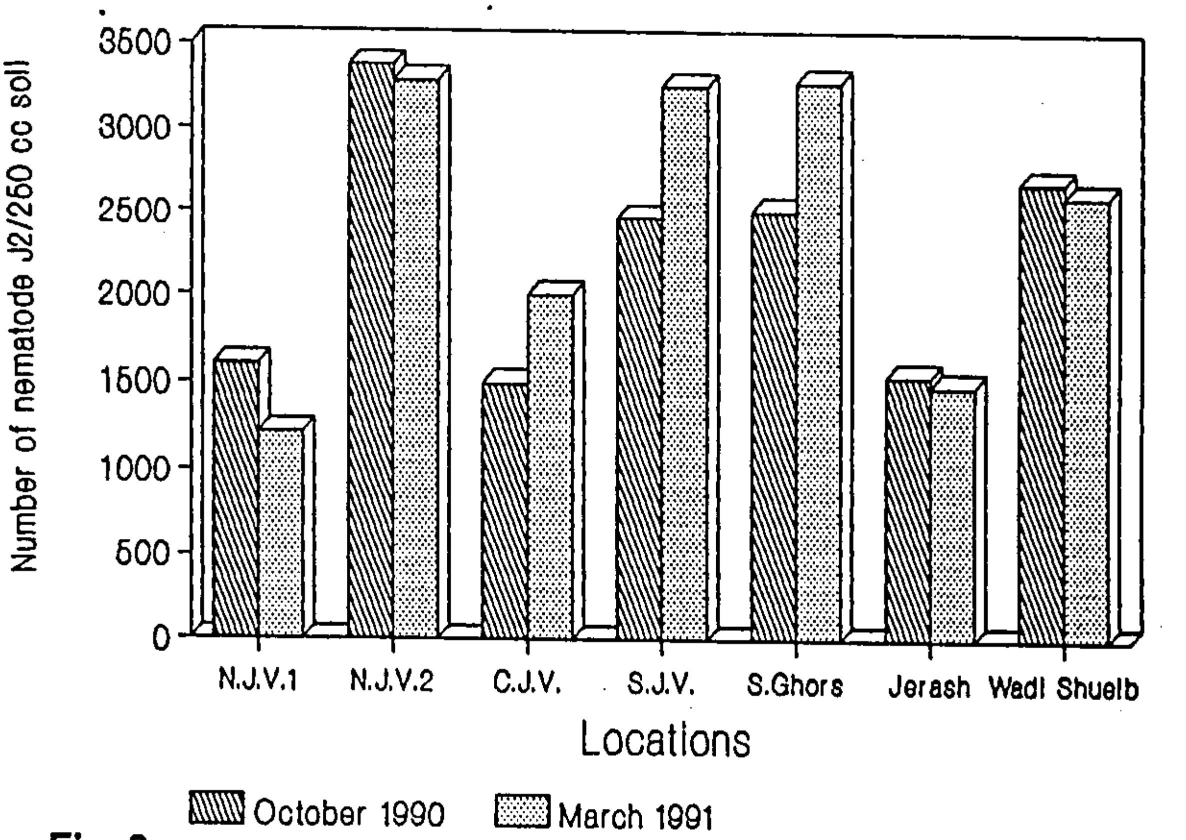

Fig. 2 : Average number of second stage juveniles (J2) populations of *T. semipenetrans* in all areas in October,1990 and March, 1991.

Table 6 : Average number of citrus nematode second stage juveniles isolated from the soils of all locations of citrus growing in Jordan.

Citrus type	Date (1)	Northern J.V.1	Northern J.V.2	Central J.V.	Southern J.V.	Southern Ghors	Jerash	Wadi shueib
Lemon	Oct.1990	1064(2)	4540	1296	980	1852	1200	2180
	Mar.1991	692	3144	1504	2560	2520	1440	2200
Orange	Oct.1990	1956	2324	2032	2560	5380	2060	3180
	Mar.1991	1736	2972	2920	3640	5500	1480	3000
Mandarin	Oct.1990	2040	3836	1490	3040	2000	1340	---
	Mar.1991	1576	3576	2130	3660	2560	1480	---
Tangerine	Oct.1990	2080	3328	280	2200	2820	500	---
	Mar.1991	1432	3372	840	2620	4220	1180	---
Grapefruit	Oct.1990	976	3352	800	3500	1160	---	---
	Mar.1991	872	4384	2020	4480	2380	---	---
Pomelo	Oct.1990	488	2684	3240	2540	---	2600	---
	Mar.1991	488	2548	2748	2600	---	1860	---
AVERAGE	Oct.1990	1596	3381	1494	2470	2510	1540	2680
	Mar.1991	1219	3288	2002	3260	3283	1488	2600

(1) Soil samples were taken in October 1990 and March 1991.
(2) Number of juveniles / 250 cc soil.
--- Data not available.

population build-up during March 1991 in three locations, namely Central Jordan Valley, Southern Jordan Valley and Southern Ghores with an average second stage juvenile populations of 2002, 3260 and 3283 per 250 cc soil, respectively (Table 6). In October 1990, however, nematode assessment showed higher populations in Northern Jordan Valley 1 than those encountered during March of 1991 with an average numbers of nematode populations of 1596. While there were no real differences in numbers of the second stage juvenile populations between the two dates at Northern Jordan Valley 2, Wadi Shueib and Jerash where population counts had an average of 3381, 2680 and 1540 during October 1990 and 3288, 2600 and 1488 per 250 cc soil, during March 1991, respectively. Statistically, when transformed data obtained from Central Jordan Valley, Southern Jordan Valley and Southern Ghores (Table 1) were analyzed together, numbers of nematode populations were significantly higher during March 1991 than those during October 1990. However, there were no significant differences between the two dates of sampling in Northern Jordan Valley 1, Northern Jordan Valley 2 Wadi Shueib and Jerash (Table 6).

4. RESULTS

4.1 Occurrence and Distribution of T. semipenetrans in Citrus Orchards of Jordan :

Examination of collected soil samples revealed the occurrence of the citrus nematode, T. semipenetrans, almost in all citrus growing areas in Jordan and the frequency of occurrence was nearly 100% (Table 5).

The average number of extracted citrus nematodes varied according to locations and citrus species (Fig.2 and Table 6). There were differences in the degrees of infestations in the different locations. Statistical analysis of transformed data showed that nematode numbers in Northern Jordan Valley 2 were significantly higher than Northern Jordan Valley 1, Central Jordan Valley and Jerash at 0.01 confidence level with an average of 3381 and 3288 per 250 cc soil during October 1990 and March 1991, respectively. In the Northern Jordan Valley 1, Central Jordan Valley and Jerash numbers were relatively low with averages of 1596, 1494 and 1540 during October 1990, and 1219, 2002 and 1488 during March 1991, respectively. However, the infestation was intermediate in Wadi Shueib, Southern Jordan Valley and Southern Ghors with an average of 2680, 2470 and 2510 during October 1990, and 2600, 3260 and 3283 during March 1991, respectively (Table 6).

Results illustrated tendency of higher nematode

Table 5 : Occurrence of citrus nematode in soil samples taken from seven locations in Jordan during October 1990 and March 1991.

LOCATIONS	Number of samples (1)	Samples of October, 1990		Samples of March, 1991	
		Number of +ve samples	% occurrence	Number of +ve samples	% occurrence
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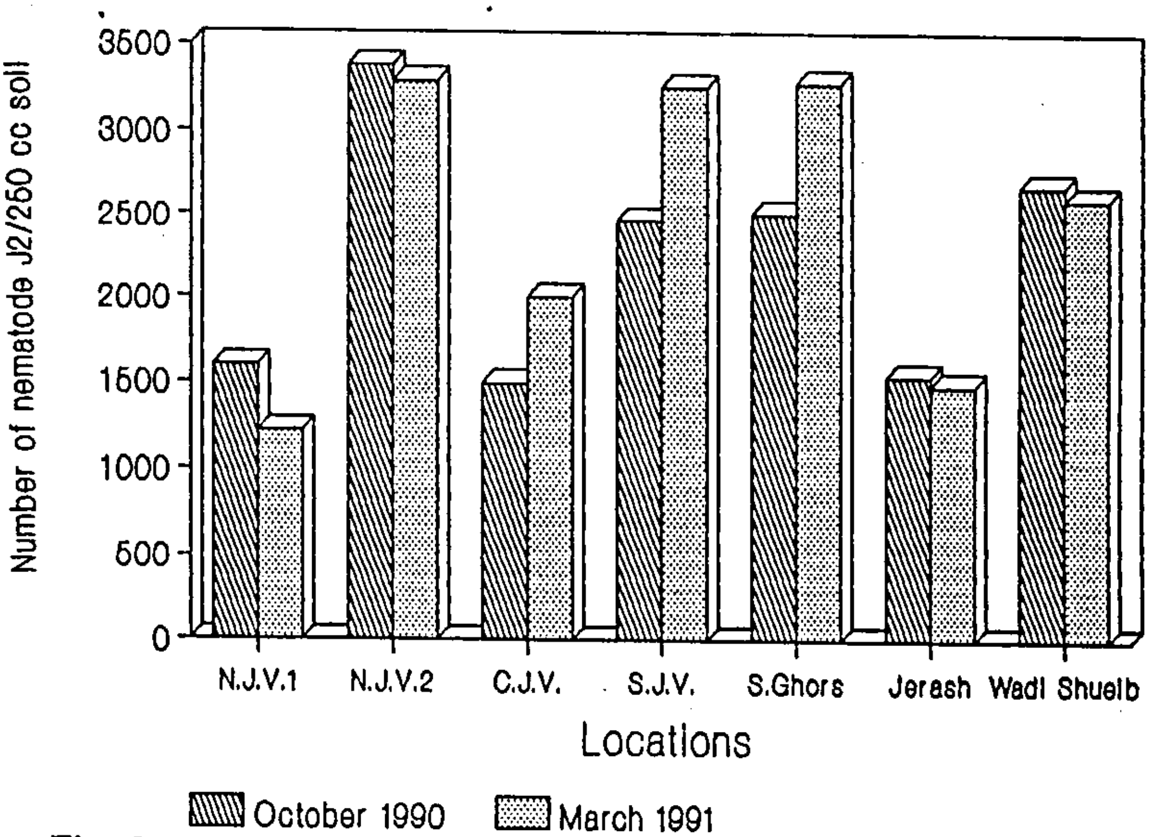

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4.2 Population Build-up of The Citrus Nematode in The Soils of Citrus Species Grafted on Sour Orange :

Almost all citrus species in Jordan are grafted on sour orange rootstock. However, in a small area and only in older plantations in the Northern Jordan Valley 2, lemon and orange were grafted on sweet lemon rootstock.

Nematode populations found in the rhizosphere of citrus roots varied according to citrus species (Table 6). During October of 1990, populations in the Northern Jordan Valley 1 on all citrus species were relatively close, except for Pomelo which showed significantly lower nematode counts with an average number of 488 juveniles per 250 cc soil. However, Tangerine, Mandarin and Orange attained high nematode counts with an average of 2080, 2040 and 1956 juveniles, respectively, while Lemon and Grapefruit attained intermediate nematode counts with an average of 1064 and 976 juveniles, respectively. In March 1991, Pomelo also attained relatively low nematode population build-up. Orange, Mandarin and Tangerine, however, reached significantly higher nematode populations than Lemon and Pomelo with an average number of 1736, 1576 and 1432 nematode juveniles per 250 cc soil. Nematode populations on Lemon and Grapefruit were intermediate (Table 6).

Statistical analysis of juvenile counts in the Northern Jordan Valley 2 revealed no differences among numbers of second stage juveniles in all citrus species. Concerning other areas, no comparison was made between nematode populations and citrus species because replications were not enough for statistical analysis.

4.3 Effect of Some Soil Characteristics on Nematode Populations :

Statistical analysis of numbers of T. semipenetrans second stage juveniles collected from Lemon trees indicated presence of a significant relationship between soil texture and the nematode population levels (Figs. 3,4 and Table 7). The results showed that whenever sand contents in the soil increase, the number of nematode juveniles also increase (Fig. 4), but whenever clay contents increase, there is a decline in juvenile numbers (Fig 3).

Results also showed significant relationship between soil organic matter content, and number of nematode second stage juvenile population (Fig. 5). With every increase of organic matter in the soil there was a decrease in nematode second stage juvenile population.

Soil pH in the orchards under study did not seem to influence numbers of nematode juveniles (Table 7). Also, soil electrical conductivity (EC) did not indicate any significant effect on the nematode populations.

Soluble soil minerals did not show significant effect, although there was a general trend in which nematode second

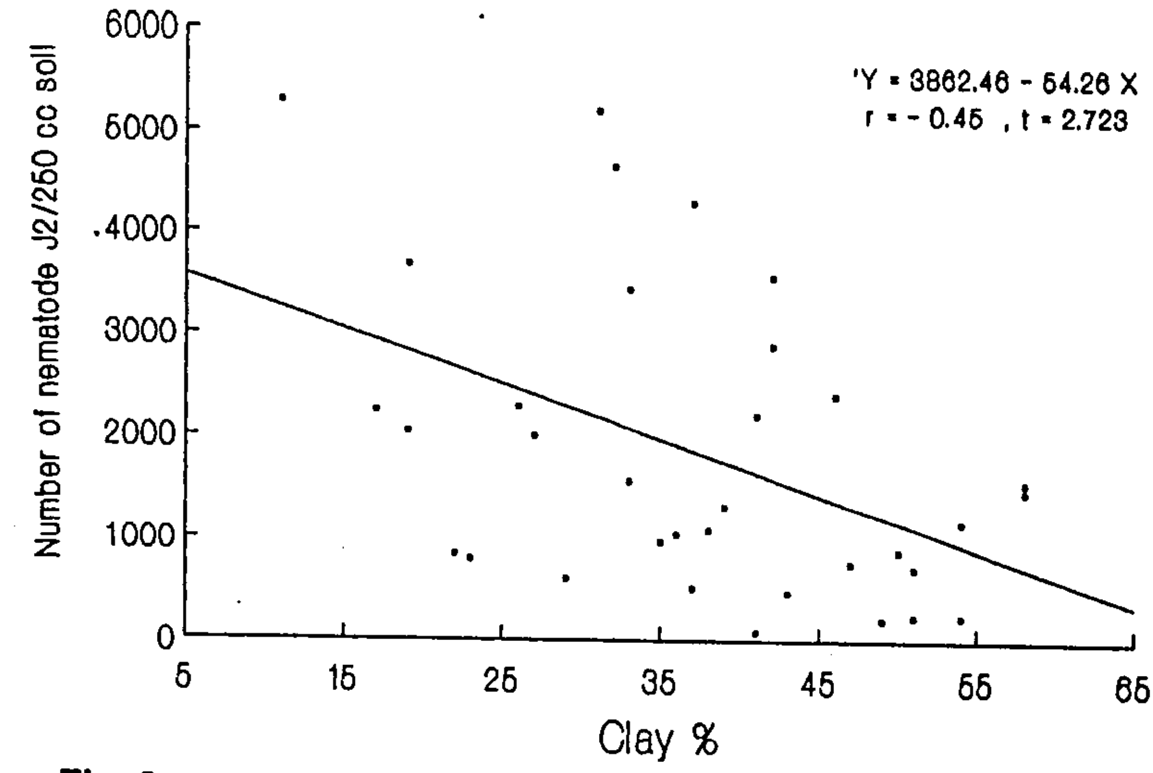

Fig. 3 : Effect of clay content on the populations of second stage juveniles (J2) of the citrus nematode in Lemon orchards of Jordan.

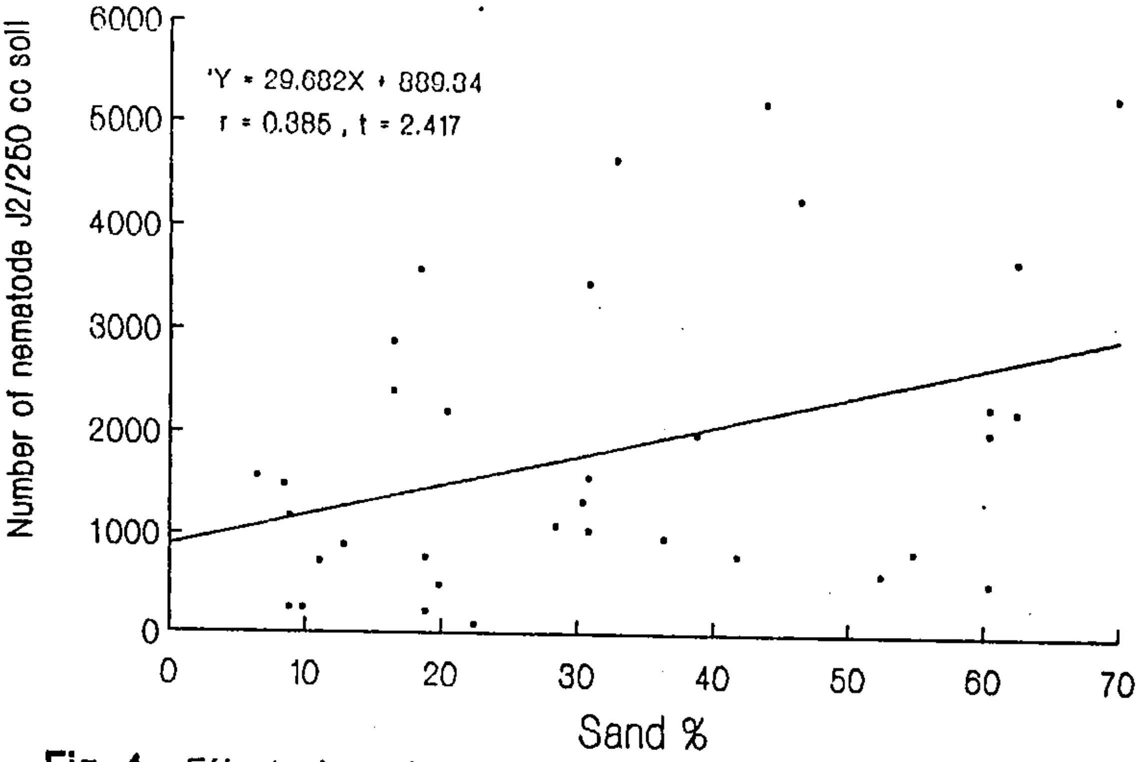

Fig. 4 : Effect of sand content on the populations of second stage juveniles (J2) of the citrus nematode in Lemon orchadrs in Jordan.

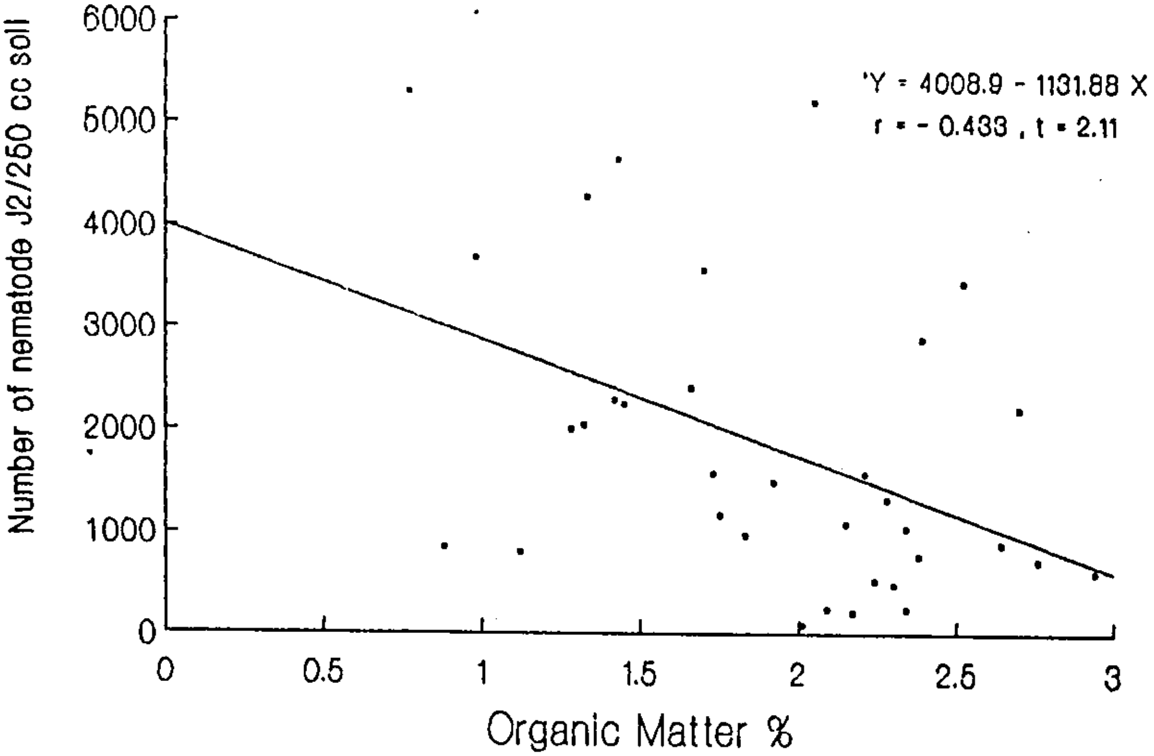

Fig. 5 : Effect of organic matter on populations of the second stage juveniles (J2) of the citrus nematode in Lemon orchards in Jordan.

Table 7a: Average number of citrus nematode second stage juveniles (per 250 cc soil) collected from lemon orchards with the corresponding soil analysis.

Locations	Sites	Nematode Numbers	Clay %	Silt %	Sand %	Organic Matter
Northern Jordan Valley 1	Adasiya	480	43.2	37.0	19.8	2.30
	Adasiya	880	49.6	37.6	12.8	2.64
	Adasiya	720	51.0	38.0	11.0	2.76
	Baqura	240	54.2	36.0	9.8	2.09
	Baqura	200	49.2	32.0	18.8	2.17
	Baqura	1320	38.6	31.0	30.4	2.28
	Waggas	800	23.2	35.0	41.8	1.12
	Waggas	240	51.2	40.0	8.8	2.34
	Mashare'	1160	53.7	38.0	8.8	1.75
	Mashare'	80	40.6	37.0	22.4	2.01
	Mashare'	1480	57.6	34.0	8.4	1.92
Northern Jordan Valley 2	Wadi El-Yabis	1560	57.6	36.0	6.4	2.21
	Wadi El-Yabis	5200	30.6	25.6	43.8	2.05
	Wadi El-Yabis	4640	32.2	35.0	32.8	1.43
	Rayhaniya	960	34.6	29.0	36.4	1.83
	Kreiymeh	2880	41.6	42.0	16.4	2.39
	Kreiymeh	2200	40.6	39.0	20.4	2.70
	Kreiymeh	3440	33.2	36.0	30.8	2.52
Central Jordan Valley	Dirar	4280	36.6	17.0	46.4	1.33
	Deir Alla	760	47.2	34.0	18.8	2.38
	Al-Wadi Al-Abyad	3680	18.6	19.0	62.4	0.98
	Al-Wadi Al-Abyad	840	22.2	23.0	54.8	0.88
	El-A'rda	1080	37.6	34.0	28.4	2.15
	El-A'rda	1040	36.2	33.0	30.8	2.34
Southern Jordan Valleh	El-Kafren	3560	41.6	40.0	18.4	1.70
	El-Kafren	1560	33.2	36.0	30.8	1.73
Southern Ghors	Ghor El-Hadetha	2240	16.6	21.0	62.4	1.45
	Ghor El-Hadetha	520	36.6	3.0	60.4	2.24
	Ghor El-Safi	5280	11.2	19.0	69.8	0.77
	Ghor El-Safi	2040	18.6	21.0	60.4	1.32
Wadi Shueib	Wadi Shueib	2000	27.4	33.8	38.8	1.28
	Wadi Shueib	2400	45.6	38.0	16.4	1.66
Jerash	Jerash	600	28.6	19.0	52.4	2.94
	Jerash	2280	25.6	14.0	60.4	1.42

Table 7b : Average number of citrus nematode second stage juveniles (per 250 cc soil) collected from lemon orchards with the corresponding soil analysis.

Locations	Sites	Nematode Numbers	pH	EC (dS/m)	K (ppm)	Na (ppm)	Ca (ppm)	Mg (ppm)
Northern Jordan Valley (1)	Adasiya	480	8.8	1.0	27	107	93	29
	Adasiya	880	8.8	1.0	19	82	82	32
	Adasiya	720	8.8	1.0	28	53	96	34
	Baqura	240	9.0	0.7	10	70	70	22
	Baqura	200	8.6	1.0	20	40	470	63
	Baqura	1320	8.9	1.4	65	118	180	73
	Waggas	800	8.7	1.3	20	82	94	50
	Waggas	240	9.1	0.8	22	58	74	23
	Mashare'	1160	9.1	0.2	19	70	96	33
	Mashare'	80	8.5	2.9	60	182	364	189
	Mashare'	1480	8.8	0.7	13	57	64	26
Northern Jordan Valley (2)	Wadi El-Yabis	1560	8.7	2.2	61	102	225	112
	Wadi El-Yabis	5200	9.0	1.2	24	85	87	36
	Wadi El-Yabis	4640	9.2	0.8	22	64	75	28
	Rayhaniya	960	8.9	1.0	28	135	64	37
	Kreiymeh	2880	8.7	1.4	38	76	91	62
	Kreiymeh	2200	8.8	1.2	37	66	132	48
	Kreiymeh	3440	8.9	2.0	17	162	130	51
Central Jordan Valley	Dirar	4280	8.6	2.0	35	85	213	72
	Deir Alla	760	8.6	1.2	28	134	78	30
	Al-Wadi Al-Abyad	3680	8.6	1.5	48	148	119	48
	Al-Wadi Al-Abyad	840	8.8	2.5	87	299	127	52
	El-A'rda	1080	9.1	1.3	36	128	97	41
	El-A'rda	1040	8.8	1.9	39	196	151	56
Southern Jordan Valleh	El-Kaffren	3560	8.9	1.9	28	178	107	66
	El-Kaffren	1560	9.1	1.7	36	130	205	78
Southern Ghors	Ghor El-Hadetha	2240	8.7	1.6	29	124	137	56
	Ghor El-Hadetha	520	8.9	1.9	32	140	154	79
	Ghor El-Safi	5280	8.8	0.7	16	49	91	33
	Ghor El-Safi	2040	8.7	1.1	24	85	129	37
Wadi Shueib	Wadi Shueib	2000	9.0	1.3	37	26	220	76
	Wadi Shueib	2400	8.7	1.1	16	34	279	48
Jerash	Jerash	600	8.8	1.0	24	111	60	23
	Jerash	2280	8.6	1.4	32	46	91	36

stage juvenile populations decrease as soil soluble Na^{+}, K^{+}, Ca^{++} and Mg^{++} increase (Table 7).

4.4 Deir Alla Experiment :

4.4.1 Relative Susceptibility of Different Rootstocks :

Statistical analysis of the data obtained from Deir Alla Agriculture Experiment Station during November of 1990, February, May and August of 1991 (Tables 8, 9, 10 and 11) indicated no significant differences among the tested rootstocks in the first three periods of sampling. However, in the August sampling date (Table 11), Macrophylla attained significantly higher nematode population build-up than other rootstocks with an average of 722 juveniles per 500 cc soil, while volkameriana, Cleopetra mandarin, Barazilian sour orange, Keen sour orange and sour orange attained 375, 288, 252, 247 and 172 juveniles per 500 cc soil, respectively, but without significant differences among them. Statistical analysis of the data in the first, second and fourth sampling period illustrated significant interaction relationships between the tested citrus rootstocks and species or cultivars where volkameriana-Lesbon lemon interaction showed the highest juvenile populations build-up with an average of 3337 during November of 1990 and 4000 per 500 cc soil during February of 1991. While during August of 1991, macrophylla-Washington

Table 8 : Average number of second stage juveniles of the citrus nematode isolated in November, 1990 from the soils of various citrus root stocks and species at Dier Alla Station. (1)

ROOT STOCK	Citrus spp.(2)								Average
	Sh.	W.N.	V.	M.	A.T.	G.	E.L.	L.L.	
Cleopetra Mandarin	733	453	800	547	333	200	4307	1080	1057
Sour Orange	1267	587	1520	3333	480	1893	773	680	1317
Volka Meriana	2280	413	453	960	1387	507	3387	6827	2027
Keen Sour Orange	1267	1080	1787	3693	427	1213	1560	893	1490
Macrophylla	933	3560	1133	533	560	733	667	400	1065
Barazilian Sour Orange	2307	1813	1373	493	1533	1227	1147	1120	1377

(1) Numbers(larvae per 500 soil aliqut) are averages of 3 replicates.

(2) Sh=Shamooti, W.N.=Washington Navel, V=Valencia, M=Mandarin, A.T.=Algerian Tangerine, G.=Grapefruit (marsh), E.L.=Eurica Lemon, L.L.=Lisbon Lemon.

Table 9 : Average number of second stage juveniles of the citrus nematode isolated in February, 1991 from the soils of various citrus root stocks and species at Dier Alla Station. (1)

ROOT STOCK	Citrus spp.(2)								Average
	Sh.	W.N.	V.	M.	A.T.	G.	E.L.	L.L.	
Cleopetra Mandarin	973	827	1173	960	453	680	1280	973	915
Sour Orange	560	1480	560	4120	1067	1213	573	1867	1430
Volka Meriana	2107	240	1053	3787	3973	1360	1520	4000	2255
Keen Sour Orange	573	1267	1053	1573	1440	1733	2227	613	1310
Macrophylla	1453	3347	1720	1320	1800	2053	1720	2667	2010
Barazilian Sour Orange	1093	1427	627	800	1013	1387	1733	2400	1310

(1) Numbers(larvae per 500 soil aliqut) are averages of 3 replicates.

(2) Sh=Shamooti, W.N.=Washington Navel, V=Valencia, M=Mandarin, A.T.=Algerian Tangerine, G.=Grapefruit (marsh), E.L.=Eurica Lemon, L.L.=Lisbon Lemon.

Table 10 : Average number of second stage juveniles of the citrus nematode isolated in May , 1991 from the soils of various citrus root stocks and species at Dier Alla Station. (1)

ROOT STOCK	Citrus spp.(2)								Average
	Sh.	W.N.	V.	M.	A.T.	G.	E.L.	L.L.	
Cleopetra Mandarin	520	1187	1653	813	613	93	253	2867	1000
Sour Orange	1027	1653	493	1667	440	533	347	640	850
Volka Meriana	2773	1653	680	773	613	1027	400	1987	1238
Keen Sour Orange	573	413	387	680	493	227	627	1360	595
Macrophylla	1533	813	320	880	960	2440	1613	1573	1267
Barazilian Sour Orange	307	560	453	373	987	1160	1053	867	720

(1) Numbers(larvae per 500 soil aliqut) are averages of 3 replicates.

(2) Sh=Shamooti, W.N.=Washington Navel, V=Valencia, M=Mandarin, A.T.=Algerian Tangerine, G.=Grapefruit (marsh), E.L.=Eurica Lemon, L.L.=Lisbon Lemon.

Table 11 : Average number of second stage juveniles of the citrus nematode isolated in Augusty, 1991 from the soils of various citrus root stocks and species at Dier Alla Station. (1)

ROOT STOCK	Citrus spp.(2)								Average
	Sh.	W.N.	V.	M.	A.T.	G.	E.L.	L.L.	
Cleopetra Mandarin	267	80	160	267	120	80	1173	160	288
Sour Orange	120	40	147	213	227	107	253	267	172
Volka Meriana	307	53	120	1027	493	240	227	533	375
Keen Sour Orange	427	307	107	147	93	333	227	333	247
Macrophylla	253	2253	80	333	240	387	360	1866	722
Barazilian Sour Orange	120	346	200	53	320	240	253	480	252

(1) Numbers(larvae per 500 soil aliqut) are averages of 3 replicates.

(2) Sh=Shamooti, W.N.=Washington Navel, V=Valencia, M=Mandarin, A.T.=Algerian Tangerine, G.=Grapefruit (marsh), E.L.=Eurica Lemon, L.L.=Lisbon Lemon.

Navel and macrophylla-lesborn lemon interactions attained the highest counts with an average of 2253 and 1867 juveniles , respectively. Considering the highest 13 readings during November of 1990, the percentage of frequency appearance of volkameriana and Lesbon lemon were 36.5% and 30.8% . Also, the highest 10 readings during February of 1991 were 40% and 30%, respectively. On the other hand, the percentage of frequency appearance of the highest 4 readings during August of 1991 was 50% for macrophylla and 25% for volkameriana, Washington Navel and Lesbon lemon. While the percentage of frequency appearance of the other citrus rootstocks and species were less than 25% in all sampling dates.

Analysis of data showed significant differences among citrus species or cultivars in all four periods. Such differences appeared also among the various citrus rootstock-species interactions and between sampling date-citrus rootstock-citrus species interactions.

Nematode juvenile populations were highest in Lesbon lemon and Mandarin, with an average juveniles counts of 1519 and 1223 per 500 cc soil, respectively. They were intermediate in Eureka lemon, Washington Navel, Shamooti, grapefruit and Algerian tangerine with an average juvenile counts of 1153, 1077, 991, 878 and 836 per 500 cc soil, respectively While lowest counts were found in Valencia with an average juveniles of 752 per 500 cc soil irrespective of the sampling date.

The interaction between volkameriana and Lesbon lemon attained the highest juvenile counts with an average of 3337 ,

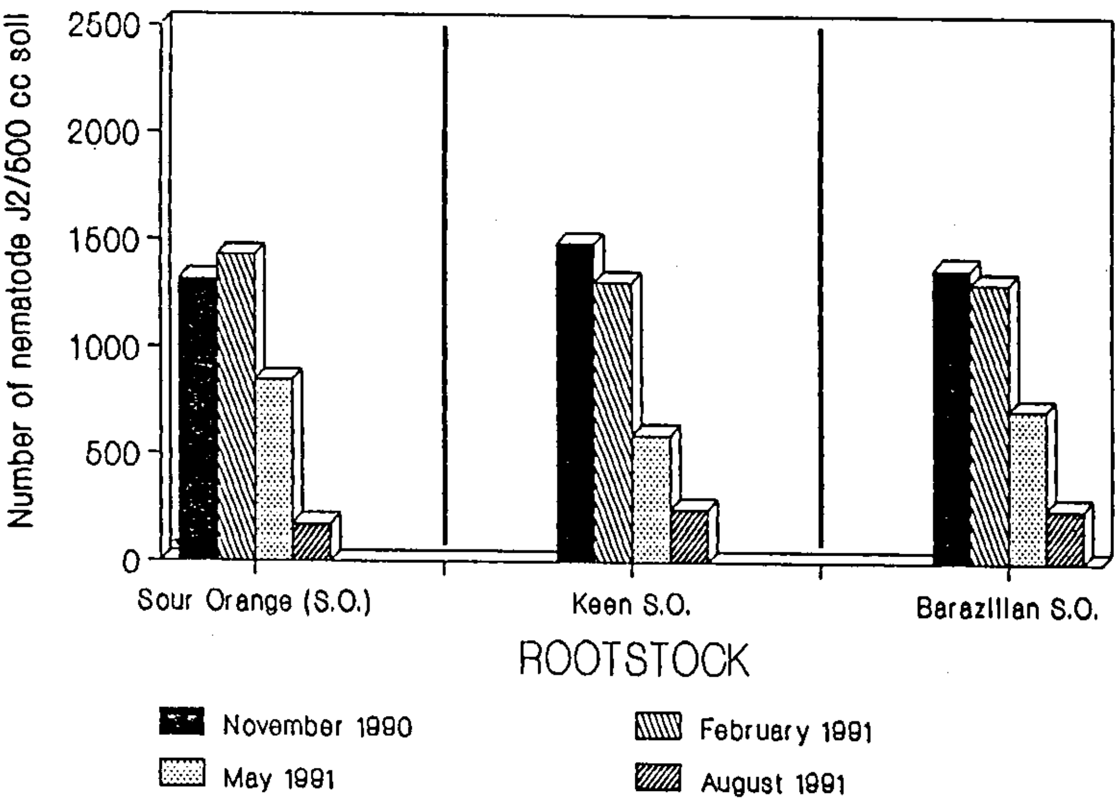

Fig. 6 : Population densities of the citrus nematode second stage juveniles (J2) at Deir Alla Agricultural Experiment Station on different rootstocks.

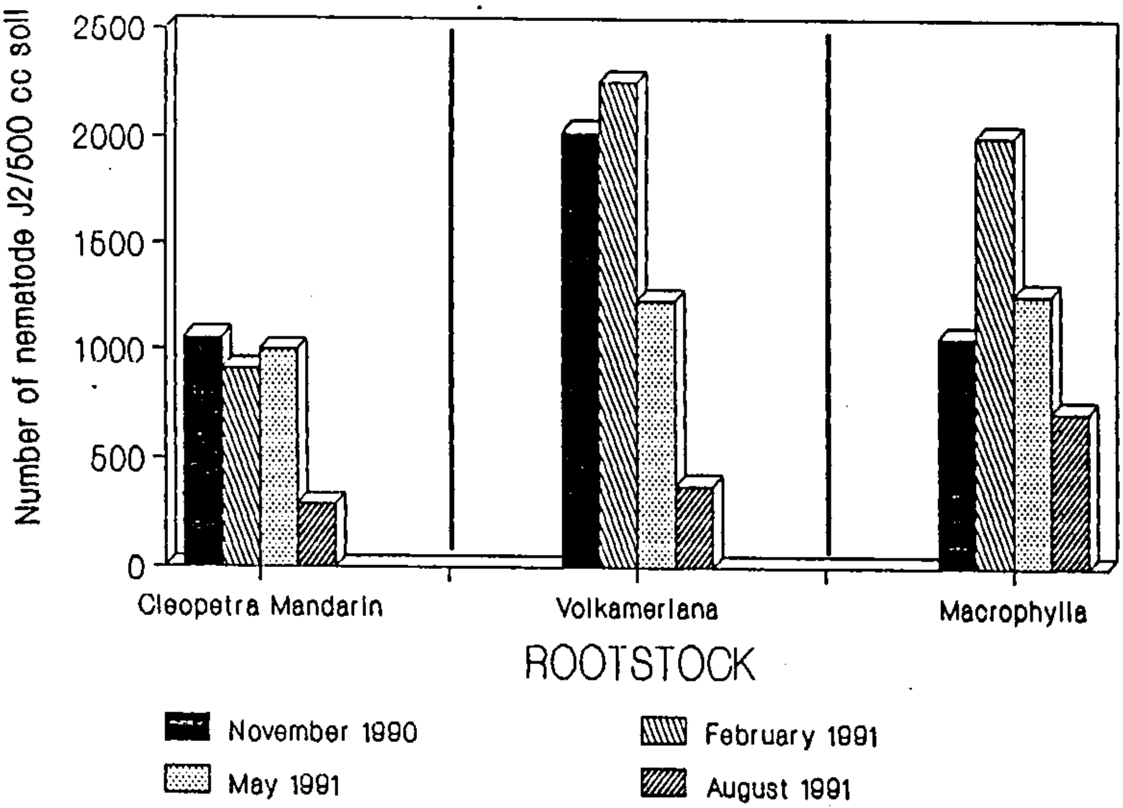

Fig. 6 (Con.) : Population densities of the citrus nematode second stage juveniles (J2) at Deir Alla Agricultural Experiment Station on different rootstocks.

while the lowest counts were in the interaction between Cleopatra mandarin and grapefruit with an average of 263 juveniles irrespective of the sampling date. Concerning the sampling date, the combination between volkameriana and Lesbon lemon showed the highest juvenile counts in November of 1990 with an average of 6827 per 500 cc soil compared to other combinations and sampling dates (Table 8).

4.4.2 Studying Population Densities :

Data obtained from Deir Alla Agriculture Experiment Station in all four periods of sampling (Tables 8, 9, 10 and 11) indicated that numbers of nematode juveniles were high in the soil of all citrus rootstocks during November and February with an average of 1389 and 1538 juveniles per 500 cc soil, respectively (Table 12). However juvenile populations decreased significantly to reach 945 juveniles during May and only 342 juveniles during August which was significantly the lowest average of nematode counts in all rootstocks tested (Fig. 6 and 7).

4.5 Histopathology of Infected Citrus Roots :

Macroscopic examinations indicated that the fibrous rootlets were most damaged by the nematode. The stained infected fine roots showed nematode pattern of attachment in which the red-stained mature females were seen with their

Table 12 : Population densities of the citrus nematode second stage juveniles in the soil of different citrus rootstocks at Deir Alla Station.

ROOTSTOCK	Date			
	Nov.1990	Feb.1991	May 1991	Aug.1991
Cleopetra Mandarin	1057(1)	915	1000	288
Sour Orange	1316	1430	850	172
Volkameriana	2027	2255	1238	375
Keen Sour Orange	1490	1310	595	247
Macrophylla	1065	2010	1267	722
Barazilian Sour Orange	1377	1310	720	252
Average (2)	1389 A	1538 A	945 B	342 C

(1) Number of second stage larvae / 500 cc soil; average of 24 soil samples.

(2) Mean separation significant at 1% level of confidance.

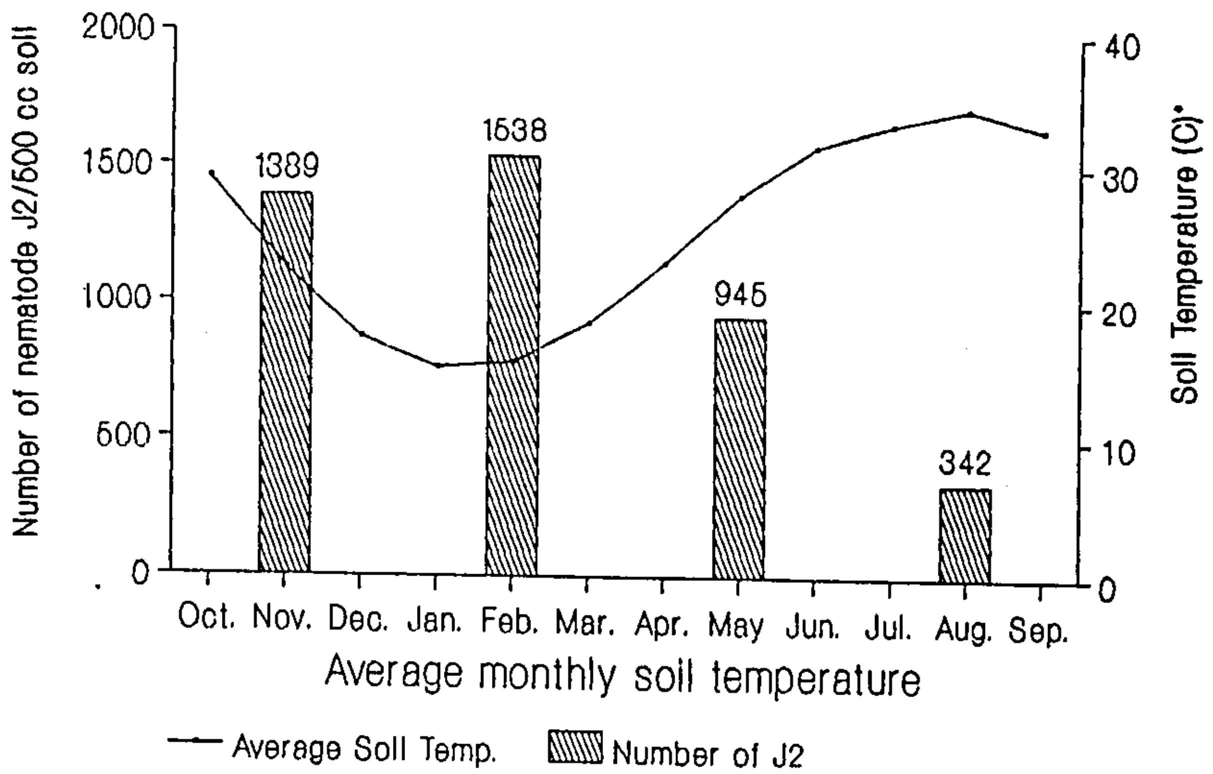

Fig. 7 : Population dynamics of the citrus nematode second stage juveniles (J2) at Deir Alla on all rootstocks.

* Temperatures at 20 cm depth.

heads imbedded in the root and the rest of their bodies remaining outside. One or several females could be seen in the same infection site and a single gelatinous matrix excreted from 1 female, or a large one (covered with fine soil particles) excreted from several females was seen containing several hundreds of eggs in various stages of embryonic development (Fig. 8, 9, 10, and 13).

Microscopic examination of infected root sections showed the adult females penetrating deeply into the cortex where their heads became surrounded by a feeding site consisting of 6 - 10 altered cortical cells, these "nurse cells" showed dense cytoplasm with no vacuole and a much inlarged nucleus and nucleolus (Figs. 10 and 13). The altered cells were normal in size but with abnormally thick walls, while the immediately adjacent cells showed no departures from their normal morphology.

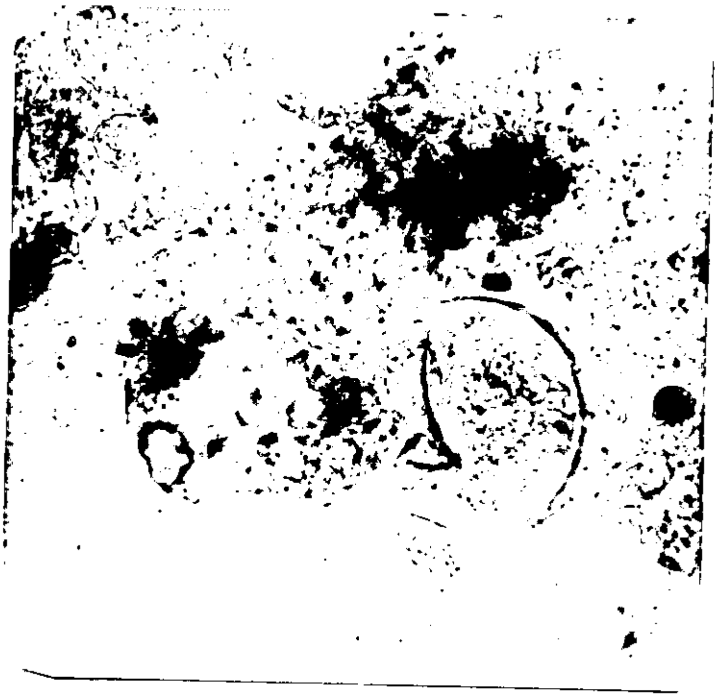

Fig. 8 : Mature female of *T. semipenetrans* with several eggs embeded in gelatinous matrix .

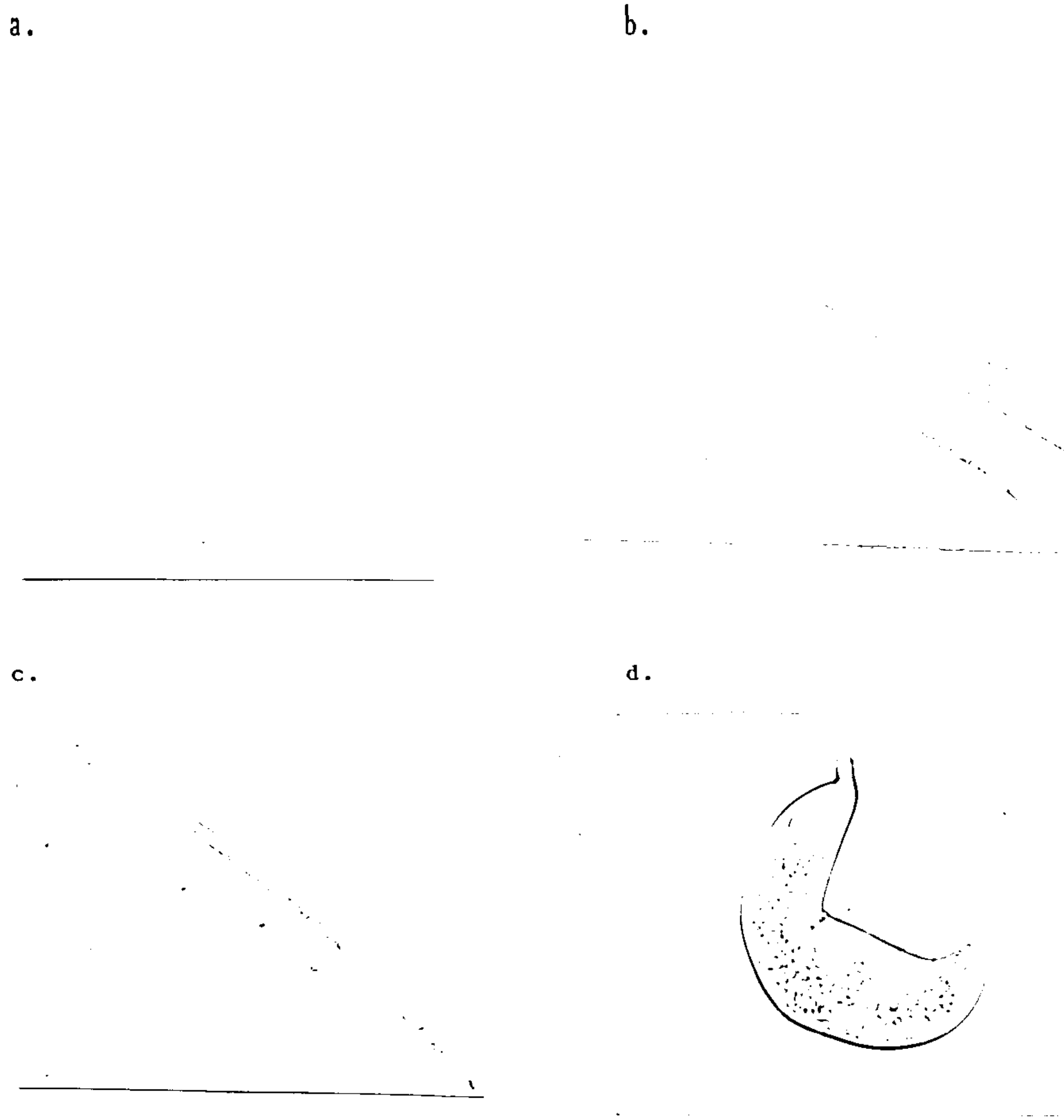

Fig. 9 : Different stages in the life cycle of _T. semipenetrans_.

a. Embryonic development (second stage juvenile inside the egg), b. Second stage juvenile, c. Mature male, d. Mature female.

Fig. 10 : Cross section in a heavily infected fine citrus root.

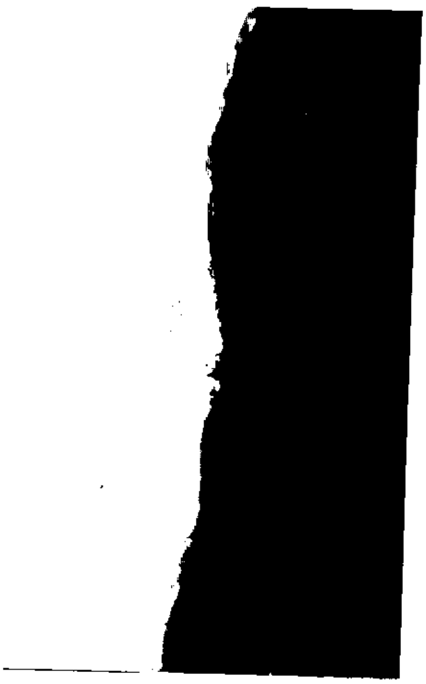

Fig. 11 : Mature female of T. semipenetrans attached to a citrus root.

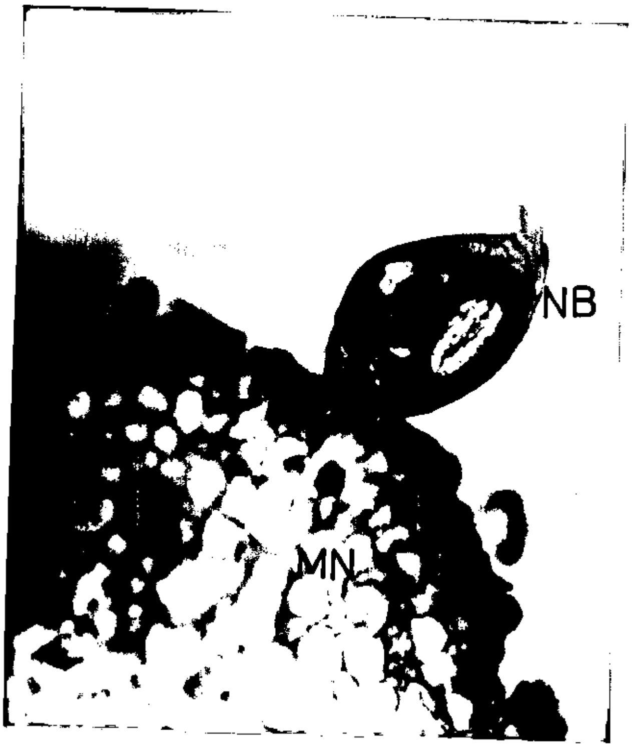

Fig. 12: Pattern of attachment of mature T. semipenetrans female with the anterior part penetrating cortex.

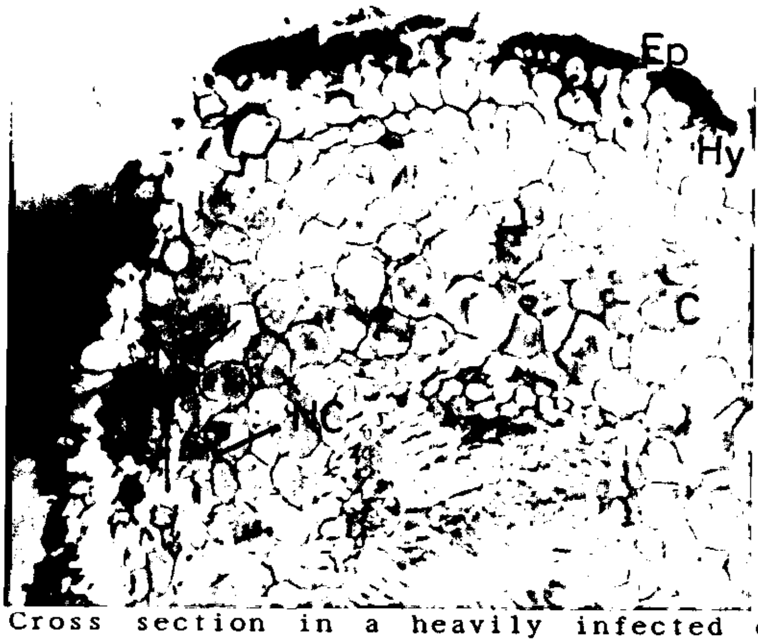

Fig. 13 : Cross section in a heavily infected citrus root showing several nurse cells. (Abbreviations for Fig. 12 and 13 : Ep=Epidermis, Hy=Hypodermis, C=Cortex, NB=Nematode Body, NN=Nematode Neck, NH=Nematode Head, NC=Nurse Cells).

5. DISCUSSION

The Citrus Nematode, T. semipenetrans, was found to be distributed in all countries and under various climatic conditions wherever citrus is grown. And the majority of this problem is suspected to be due to the consequence of a previous infection of plants in the nurseries (Bel-Kadhi, 1986). The importance of production of seedlings in nurseries with infested soil contribute to the wide distribution of this nematode within a country where citrus is planted. Soils in these nurseries must be sterilized by fumigation or by using other sanitary measures that help in producing nematode-free seedlings. Otherwise, very high infestation might occur and may adversely affect health of the trees and decrease their fruit production.

Major nurseries of citrus seedlings production in Jordan are located in three areas, namely Wadi Shueib, Deir Alla and Baqura. Soil samples collected from these areas were found to be infested with the citrus nematode (Table 6).

The degree of soil infestation in all studied locations in Jordan was only near 20% of density levels usually encountered in coastal areas that may reach 10,000 juveniles per 100 cc soil (Philis, 1989). Results of this study disagreed with Yousef (1988) who reported that the citrus nematode juvenile densities in Central and Southern Jordan Valley were close to the economic threshold of 10,000 juveniles. An ideal citrus soil for citrus growing and nematode development requires certain soil characteristics such as : light texture, good drainage and low salt's concentration (Jones and Embleton, 1973); but since such favorable conditions are not prevalent in some areas of Jordan Valley, where citrus trees and the nematode are under stress, lower nematode numbers are expected to be found.

Although the nematode was found to occur in all sampled areas, there were different levels of nematode juvenile populations in the various geographical areas of citrus growing in Jordan. The distribution of the citrus nematode populations was high in Northern Jordan Valley 2. This could have been due to the fact that citrus trees in this area are very old, and so, the long period of infestation enables the citrus nematode to grow in populations to reach that high level, even though it prefers better climatic and soil conditions that are not prevalent in this area. Nematode populations were low in the Northern Jordan Valley 1 and started to increase from Central Jordan Valley to the south.

Concerning soil factors, the most suitable soil temperature is between 25-30 °C (Kirkpatrick et al., 1965 and Giudice, 1985). In Northern Jordan Valley 1, mean soil temperature during March, the second date of sample collection, decreases down to 13.6 C which may not be suitable for nematode reproduction. On the other hand, mean soil temperature in Southern Ghors increases to reach 21.6 °C during March, which may explain the relatively low population densities found in both of Northern Jordan Valley 1 and 2 during March, while higher population densities occurred during March in Central and Southern Jordan Valley and Southern Ghors. Lower densities in Northern Jordan Valley 1 can also be explained according to soil characteristics; the citrus nematode reproduction, as found in this study, is favored by dry conditions in lighter soil with low organic matter. While the average yearly rain fall at Northern Jordan Valley 1 is relatively high (380.3 mm) compared with only 71.2 mm in the Southern Ghors (Table 13). This has a negative effect on juvenile populations in these areas. Soil mechanical analysis indicated that clay percentage decreased from North to South Jordan Valley with a decrease in the soil organic matter percentage occurs in Southern parts of Jordan Valley which led to high populations there. These also were thought to have a direct effect on the distribution of the citrus nematode juvenile populations in citrus soils of Jordan Valley.

The situation of this problem in the uplands is that the infestation in Wadi Shueib appeared to be more than in Jerash and since soil analysis were relatively close, the main factors influences the citrus nematode in these areas are climatic conditions. Climatic observations indicates that Jerash is, in fact, a cooler area at which low temperatures might have a negative effect on nematode populations. On the other hand, one of the two farms, which had the low counts in

Table 13 : Effect of certain environmental weather conditions on population levels of *T. semipenetrans* juveniles in March 1991 (average of 20 years).

Locations	No. of Samples	Average No. of Nematode	Total Rain Fall	Mean Readings of March :			
				Soil Temperature	R.H. %	Min. Air Temperature	Max. Air Temperature
Northern Jordan Valley 1 (Baqura) *	11	692	380.3	17.4	65	10.0	22.9
Northern Jordan Valley 2 (Wadi EL-Yabis)	8	3144	286.0	18.1	68	9.3	23.7
Central Jordan Valley (Deir Alla)	5	1504	277.4	18.4	58	12.4	23.7
Southern Jordan Valley (South Shuna)	2	2560	(161.1)	---	(55)	(14.3)	(23.8)
Southern Ghors (Ghor El-Safi)	4	2520	71.2	21.6	50	14.6	25.6
Jerash (Faisal Husbandry)	2	1440	(350.0)	---	(60)	(9.0)	(19.0)
Wadi Shueib (W.S. Husbandry)	2	2200	(390.0)	---	---	(19.3)	(28.8)

* Meteorological Station.
( ) Number between brackets are approximate.
--- No Data Available.

Jerash, used the sewage water of Zarqa River for irrigation. This water contains high levels of organic matter which might have reduced juvenile populations of the citrus nematode and led to lower juvenile counts in Jerash area.

A clear picture can be taken from the first experiment in which some soil characteristics were correlated with the citrus nematode populations in lemon trees under study. Data on the nature and composition of Jordan soils planted to citrus revealed that they generally contain high percentage of clay content (Table 7). This gives an indication about the status of citrus roots that might be suffering from poor drainage, and thus, accumulation of free water in the root zone results in poor aeration and injury to the roots which might increase their susceptibility to infection (Marsh, 1973). Salinity problems are of little effect since the soil electrical conductivity ranged between 0.18 - 2.88 dS/m , which lies within the acceptable range. Citrus trees are particularly sensitive to high concentrations of salts (Jones and Embleton, 1973). A very good yield potential occurs in soils with electrical conductivity of 1.7 dS/m whereas at more than 2.3 dS/m , citrus trees begin to suffer from salinity (FAO, 1985).

Field observations reported by several workers indicated that the citrus nematode is capable of reproducing and living under a wide range of soil and climatic conditions (Baines et al., 1978 and Van Gundy, 1986) but obviously larger population densities are expected to be found wherever more favorable conditions prevail. Among the various soil characteristics tested, only soil texture and soil organic matter were found to significantly influence the citrus nematode larval populations.

Soil texture influences the nematode directly and/or indirectly. Directly by affecting aeration and percentage of moisture content which play an important role in determining nematode movement and availability of oxygen; and indirectly by affecting distribution of citrus roots which influences nematode reproduction.

Soil texture results demonstrated that the citrus nematode can and does occur in soils differing greatly in texture (11.2% - 57.6% clay). However, in soil containing low clay percentage, larval populations were relatively high, which indicates that sandy soils are favored by the citrus nematode movement and reproduction (Baines, 1974).

Soil moisture is correlated with soil texture since sandy soils can hold less amounts of water with good aeration. While water in heavy clay soils fills the small spaces and reduces gaseous exchange. And thus, the citrus nematode was found to prefer low levels of soil moisture found in light soils (Van Gundy and Martin, 1962 and Giudice, 1963). This might be another factor which limit chances for the nematode to build-up very high populations in Jordanian heavy soils.

Soil organic matter had a negative effect on population levels of the citrus nematode juveniles (O'Bannon, 1967). The direct effect probably through its influence on soil mesophauna that contains several species of parasitic and predator micro-organisms such as mites (e.g. Macrocheles sp.), beetles (e.g. Philonthus sp.) (Osman et al., 1988) and bacteria (e.g. Pasteuria penetrans) (Fattah et al., 1989). On the other hand, high organic matter in the soil result in a reduction of the soil pH which in turn affects the larval population of citrus nematode since this nematode favors neutral to alkaline soils (Baines, 1974). But this is not expected to apply on the soils in Jordan since the lowest pH detected was 8.5 at which there is a continuous reduction in nematode reproduction (Giudice, 1985), and the available percentage of organic matter is not expected to reduce the soil pH significantly.

Other soil characteristics tested (pH, EC and major soil soluble cations) did not show clear effect on the population densities of the citrus nematode. This may be attributed to the narrow ranges of values obtained from soils in the Jordan Valley, but it is reported that citrus nematode is favored by pH varied from 6-7.8. Below pH 5 and above pH 8, there is a constant reduction in the nematode reproduction (Van Gundy, and Martin, 1962), While citrus soils in Jordan are calcareous (Table 7). In case of the major soil soluble cations, there were general negative effects of the concentrations of Na^{+}, K^{+}, Ca^{++} and Mg^{++} salts, where at low concentrations larval populations increased. This indicates that saline soil has a negative impact on the citrus nematode juvenile populations (Sweelam and El-Gindi, 1989; and Youssef et al., 1989).

All citrus species were found to be infected with the citrus nematode but to varying degrees (Cameron et al., 1954, Baines et al., 1948 and 1960), and with no indication of resistance. However, some species harboring high number of nematode juveniles may be able to provide a suitable soil micro climate for the citrus nematode populations to build-up more than other species. Apparently, Pomelo trees planted in the Northern Jordan Valley 1 are less favored by the citrus nematode since it showed the lowest juvenile counts in both of October and March sampling dates (Table 6).

It is reported that there are variations in susceptibility between citrus rootstocks. Poncirus trifoliata is highly resistant to citrus nematode infection (Cameron et al. , 1954, Baines et al. , 1984, Van Gundy and Kirkpatrick, 1964 and 1963). Unfortunately, rootstocks belonging to this genus are not common in Jordan and a very high percentage of citrus species are grafted on sour orange (C. aurantium) which has varieties moderately-severely susceptible to the citrus nematode infection.

None of the tested rootstocks showed resistance to the nematode, so that, it is thought that long period of nematode infection helps juveniles accumulation in relatively same moderate numbers on the different rootstock tested. However, volkameriana and macrophylla are suspected to be highly susceptible to the citrus nematode specially when grafted with Lesborn lemon or Washington Navel.

Interaction between the graft and the rootstock may give citrus tree the ability to be more or less infected with the citrus nematode (Yousif, 1984). The results of this experiment proved the importance of this interaction in determining populations build-up of the citrus nematode, but the complexity of different interactions makes it difficult to assess all interactions, however Lesborn lemon grafted on volkameriana rootstock is the most susceptible to the citrus nematode infection since high levels of nematode juvenile populations were detected, while the least susceptible interaction is grapefruit grafted on "Cleopetra" mandarin rootstock (Yousif, 1984).

Monitoring population densities of the citrus nematode in Central Jordan Valley during one season revealed that high numbers of juveniles occur during Winter and Spring where soil temperatures (17.3 and 22.9 °C as lowest winter and highest spring temperatures, respectively) are quite suitable for nematode reproduction and also for new citrus root growth (Reuther, 1973). Juvenile populations then decline, as they are affected by the high summer soil temperature which may reach 34.5 °C, to be in its lowest level at Autumn where soil temperature reaches 32.9 °C. Then populations start to increase as temperature decreases. Apparently, nematode population densities increased following the spring and fall flush of roots (O'Bannon et al., 1972 and Stephan et al., 1990).

The description of populations trends illustrated that there is a great tendency in the citrus nematode to be affected mainly by soil temperature which also affects root growth and considers a limiting factor for its reproduction (Kirkpatrick et al., 1965).

6. CONCLUSIONS

From the results of this study the following conclusions could be drawn :

1- The citrus nematode was distributed widely almost in all citrus orchards of Jordan but generally occur in relatively moderate levels.

2- The nematode problem becomes of large magnitude in older plantations.

3- The citrus nematode may exist in a great diversity of local climatic and soil factors, but it prefers moderate climate and lighter soil with low percentage of organic matter.

4- There was no direct effect of pH or soil salinity on nematode juvenile populations. This was probably due to the narrow range of values obtained.

5- All citrus species were susceptible, and moderately to severely infected with the citrus nematode.

6- All commercially used rootstocks in Deir Alla Agricultural Experiment Station have nearly similar susceptibility to the citrus nematode but volkameriana and macrophylla appeared to be more susceptible.

7- Nematode juvenile populations were inversely affected by soil temperature at Deir Alla Station. Higher juvenile populations occurred in November and February. However, populations decreased in May and reached minimum levels in August.

Appendex 1: Area planted to Citrus species in Jordan (in hectares)*.

Locations	Lemon	Orange	Mandarin	Tangerine	Grapefruit	Pomelo	Total
Northern Jordan Valley **	974.0	748.4	674.8	1195.0	42.7	84.3	3719.2
Central Jordan Valley	224.6	249.4	137.4	253.4	5.2	17.3	887.3
Southern Jordan Valley	79.0	50.5	47.3	57.2	6.6	14.7	255.3
Southern Ghors	58.0	20.4	5.8	11.0	5.7	---	100.9
Jerash	68.0	75.0	10.5	22.5	---	2.5	178.5
Wadi Shueib	15.5	6.0	---	---	---	---	51.2
Total	1419.1	1149.7	875.8	1539.1	60.2	118.8	5162.7

* Source : Annual statistical report of fruit trees in Jordan, 1990. Ministry of Agriculture.

** Northern Jordan Valley was devided into 2 sampling areas, namely 1 and 2, since it occupies nearly 72% of citrus planted area in Jordan.

Appendex 2 : Locations with their altitudes and some climatic characters (readings are average of 1967-1987). Meteorological Dept., 1988.

Locations	Altitude (m) *	Mean air temp.	Max. air temp.(1)	Min. air temp.(2)	Annual rain fall (mm)
Northern Jordan Valley 1 & 2	-170	30.3	37.1	8.5	380
Central Jordan Valley	-224	31.0	38.7	10.6	277
Sothern Jordan Valley	-230	(24.6)	(29.5)	(19.6)	161
Sothern Ghors	-350	33.0	39.1	10.8	71
Jerash	280	17.2	23.3	11.1	350
Wadi Shueib	300	(19.6)	(24.5)	(14.6)	390

* Negative signe means below sea level.
(1) Temperature (C) in August, the hottest month of the year.
(2) Temperature (C) in January, the coldest month of the year.
( ) Data between brachets are approximate.

Appendex 3: Means and confidance intervals of the citrus nematode populations on citrus species planted at N.J.V.1 during October 1990 and March 1991.

Citrus species	October 1990	March 1991
Lemon	(1064 + 484)*	(692 + 324)
Orange	(1956 + 1136)	(1736 + 820)
Tangarine	(2080 + 1016)	(1432 + 828)
Mandarin	(2040 + 968)	(1576 + 616)
Grapefruit	(976 + 484)	(872 + 800)
Pomelo	(488 + 404)	(488 + 408)

* Intervals at 95% level of confidance.

Appendex 4: Means and confidance intervals of the citrus nematode populations on citrus species planted at N.J.V.2 during October 1990 and March 1991.

Citrus species	October 1990	March 1991
Lemon	(4540 + 1164)	(3144 + 1268)
Orange	(2324 + 856)	(2972 + 1960)
Tangarine	(3328 + 1932)	(3372 + 1208)
Mandarin	(3836 + 1468)	(3576 + 1636)
Grapefruit	(3352 + 1936)	(4384 + 2048)
Pomelo	(2686 + 2192)	(2584 + 1552)

* Intervals at 95% level of confidance.

Appendex 5: Analysis of variance for nematode numbers in different locations included in the study during October 1990.

Source	df	SS	MS	F
Locations	6	240.75	40.13	5.72 **
Error	155	1087.13	7.01	
Total	161	1372.88		

** Significant at 1% level of confidance

Mean separation :

N.J.V.2	3381	A
W.S.	2680	AB
S.G.	2510	ABC
S.J.V.	2470	ABC
N.J.V.1	1596	BC
J.	1540	BC
C.J.V.	1494	C

Appendex 6: Analysis of variance for nematode numbers in different locations included in the study during March 1991.

Source	df	SS	MS	F
Locations	6	393.24	65.54	11.05 **
Error	155	918.97	5.95	
Total	161	1312.21		

** Significant at 1% level of confidance

Mean separation :

N.J.V.2	3288	A
S.G.	3283	A
S.J.V.	3260	ABC
W.S.	2600	ABC
C.J.V.	2002	BC
J.	1488	BC
N.J.V.1	1219	C

Appendex 7: Analysis of variance between nematode densities at October 1990 and at March 1991 in three locations, C.J.V, S.J.V. and S.G.

Source	df	SS	MS	F
Date	1	30.11	30.11	4.16 **
Error	100	724.24	7.27	
Total	101	754.35		

** Significant at 5% level of confidance

Mean separation :

March, 1991	2823	A
October, 1990	2137	B

Appendex 8: Analysis of variance between nematode densitiies on six citrus species in N.J.V.1 during October 1990.

Source	df	SS	MS	F
Citrus sp.	5	70.41	14.08	2.61 **
Error	48	259.28	5.40	
Total	53	329.69		

** Significant at 5% level of confidance

Mean separation :

Tagerine	2080	A
Mandarin	2040	A
Orange	1956	A
Lemon	1064	AB
Grapefruit	976	AB
Pomelo	488	B

Appendex 9: Analysis of variance between nematode densities on six citrus species in N.J.V.1 during March 1991.

Source	df	SS	MS	F
Citrus sp.	5	58.13	11.63	2.76 **
Error	48	202.40	4.22	
Total	53	260.53		

** Significant at 5% level of confidance

Mean separation :

Orange	1736	A
Mandarin	1576	A
Tangerine	1432	AB
Grapefruit	872	ABC
Lemon	692	BC
Pomelo	488	C

Appendex 10: Analysis of variance for second stege juvenile counts at Deir Alla according to split-split design (second experiment).

Source	df	SS	F
Main (Rootstock)	5	19845.72	2.02
Error (a)	10	19687.12	2.92
Sub (citrus types)	7	19288.69	3.07 *
No. Replicates	2	30100.46	7.64
Main*Sub	35	79843.22	2.54 *
Error (b)	84	75428.58	
Sub-sub (Time)	3	77819.04	38.42 *
Main*Sub-sub	15	10864.12	1.67
Sub*Sub-sub	21	14960.01	1.06
Main*Sub*Sub-sub	105	122657.82	1.73 *
Error (c)	288	194438.50	
Total	575	670933.30	

* Significant at 1% level of confidance

Mean separation :

1- Sub-plots :

Lesborn Lemon	1519	A
Mandarin	1223	AB
Erica Lemon	1153	ABC
Washington Navel	1077	BC
Shamooti	990	BC
Grapefruit	878	BC
Algerian Tangerine	836	BC
Valencia	752	C

2- Sub Sub-plots :

February, 1991	1538	A
November, 1990	1389	A
May, 1991	945	B
August, 1991	342	C

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