

THE EFFECT OF VARIOUS FACTORS ON THE EXPRESSION OF GENETIC
RESISTANCE TO ROOT-KNOT NEMATODE (MELOIDOGYNE INCOGNITA
(KOFOID AND WHITE) CHITWOOD) IN SNAP-BEAN (PHASEOLUS
VULGARIS L.), TOMATO (LYCOPERSICON ESCULENTUM MILL.),
SOYBEAN (GLYCINE MAX MERR.), AND LIMA BEAN
(PHASEOLUS LUNATUS L.)

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ABSTRACT

Cultivars of vegetables resistant and tolerant to root-knot nematode (Meloidogyne incognita Kofoid and White, Chitwood) bred in Hawaii were tested to determine the factors which lower the effectiveness of the resistance. Emphasis was placed on possible formation of pathogenic races of M. incognita which could result from continuous cropping of resistant cultivars, and the effect of high soil temperature on expression of resistance. Cultivars tested were 'Manoa Wonder' snap-bean (Phaseolus vulgaris L.), 'Healani' tomato (Lycopersicon esculentum Mill.), 'Kailua' soybean (Glycine max Merr.), and 'White Ventura N' lima bean (Phaseolus lunatus L.).

Field tests were conducted in 3 root-knot nematode infested fields (fields P-1, P-2, and Q-1) at the Poamoho Experimental Farm where galling of the resistant cultivars had occurred. Tests were conducted throughout the year to obtain seasonal effects on the performance of the cultivars.

Root-knot nematode populations established in the greenhouse on a susceptible tomato cultivar from galled roots of both resistant and susceptible cultivars from fields P-2 and Q-1. These were used in tests to investigate the formation of a more virulent race of root-knot nematode, the effect of soil temperature and level of inoculum on the rate of galling, and the development of a root-knot nematode population which can parasitize resistant cultivars of more than one crop. Data used to measure the resistance were the gall formation and egg-mass production of the root-knot nematode on the plant roots.

'Manoa Wonder' snap-bean showed considerably increased galling when exposed to the field P-2 M. incognita population. This population showed an increase in pathogenicity on 'Manoa Wonder' under continuous planting. No increase of pathogenicity was observed in the field Q-1 population. Effectiveness of the genetic resistance was reduced when the soil temperature was kept at $29^{\circ} \pm 1^{\circ}\text{C}$, but was effective under fluctuating soil temperatures ($21^{\circ} - 33^{\circ}\text{C}$), although scattered galls were sometimes found.

The resistant tomato cultivar 'Healani' was galled heavily by a more virulent race of M. incognita established in field Q-1. It was not galled by the field P-2 M. incognita population. The level of resistance was significantly lowered at a soil temperature of $29^{\circ} \pm 1^{\circ}\text{C}$, and, at the higher inoculum level it was completely ineffective. However, the resistance was effective under fluctuating soil temperature ($21^{\circ} - 33^{\circ}\text{C}$). It was not possible to induce the formation of a pathogenic race of the root-knot nematode population from field P-2 five times the basic rate, which indicates that the genetic resistance of 'Healani' is strong.

Soybean cultivar 'Kailua' was observed to have a stable, strong genetic resistance. Repeated inoculation with root-knot nematodes did not result in any increased pathogenicity. Under fluctuating soil temperature ($21^{\circ} - 33^{\circ}\text{C}$) and increased, continuous soil temperatures up to $34^{\circ} \pm 1^{\circ}\text{C}$, with the inoculum levels tested, the resistance proved to be excellent.

The tolerant lima bean cultivar 'White Ventura N' was partially resistant with its degree of galling dependent upon the

level of inoculum available. Increased soil temperature also increased the ability of M. incognita to cause galling and produce egg-masses on the roots.

The attempt to induce a root-knot nematode population which is able to parasitize more than one resistant cultivar was not successful.

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I. INTRODUCTION

Annual crop losses due to nematodes were estimated at over \$300 million in the United States in 1964, of which more than \$47 million was in vegetables (Le Clerg, 1964). By 1972 however, the annual loss of vegetables alone caused by nematodes was estimated as high as \$250 million (Jensen, 1972). The 5 vegetable species which suffered the largest yield loss were tomatoes, potatoes, beans, cantaloupes, and lima beans.

Some of the most important species of plant parasitic nematodes are the root-knot nematodes of the genus Meloidogyne. They damage the root system, weaken the plants, and reduce the yields. Root-knot nematodes have a large host range which include over 2,000 plant species of almost all plant families (Jenkins and Taylor, 1967). Control measures in root-knot nematode infested fields are difficult and expensive. In the United States, these nematodes are considered the most serious nematode pests of vegetable crops, particularly in regions south of the 40th parallel (Lownsberry and Thomason, 1959).

Development of root-knot nematode resistant cultivars is considered the most efficient method of control. This method does not require additional seed cost to the grower, the control is long lasting, and the grower is not limited in the crops he can cultivate.

A resistant cultivar grown in a root-knot nematode infested field may be free from or has light galling and grows normally as compared to a susceptible cultivar which will be galled heavily and suffer a impediment of root function.

Root-knot nematode resistant or tolerant cultivars have been developed in Hawaii for 4 vegetable species: 'Manoa Wonder' snap-bean (Phaseolus vulgaris L.), 'Healani' tomato (Lycopersicon esculentum Mill.), 'Kailua' soybean (Glycine max Merr.), and 'White Ventura N' lima bean (Phaseolus lunatus L.). A lack of resistance at certain times in certain places has been observed for these resistant cultivars. This study was therefore made to investigate the possible causes of heavy galling of the resistant cultivars.

The objective of this thesis is to determine the factors which reduce the effectiveness of the resistance. The resistant cultivars will be subjected to tests on the possible formation of a more virulent race of root-knot nematodes and the lowered resistance due to increased soil temperatures. The ineffectiveness of resistance due to these factors were previously reported (Holtzmann and Gilbert, 1969).

II. LITERATURE REVIEW

A. Taxonomy, host range and geographical distribution of root-knot nematodes

Root-knot nematodes were recorded for the first time in England in 1855 on cucumber roots (Franklin, 1957). In 1875 they were observed in roots of Sempervivum tectorum, and in 1878 they were reported attacking coffee in Brazil. The first proposed name was Anguillula marioni by Cornu in 1879. Goeldi (1887) proposed another generic name Meloidogyne exigua (Chitwood, 1949). During the next decade root-knot nematodes were reported in different parts of the world and given various names, Heterodera radicola by Muller, Heterodera javanica by Treub, Anguillula arenaria by Neal, Anguillula vialae by Lavergne, Caconema sp. by Cobb, and Ditylenchus by Filipjev (Whitehead, 1968). Goodey (1932) concluded that root-knot nematodes should belong to the genus Heterodera due to resemblance to other Heteroderas, and, since Cornu was the first person who gave the specific name marioni, he named them Heterodera marioni. This generic name was used quite frequently until the revision by Chitwood in 1949.

Chitwood (1949) separated the cyst nematodes, Heterodera, and the root-knot nematodes, which he designated Meloidogyne after Goeldi. He described 5 Meloidogyne species and 1 sub-species: M. javanica (Treub, 1885), M. exigua Goeldi, 1887, M. incognita (Kofoid & White, 1919), M. incognita var. acrita, M. hapla, and M. arenaria (Neal, 1889).

Chitwood (1949) considered the most important species character for identification to be the perineal pattern of the adult female. These cuticular striae around the tail region have a typical pattern although there may be some variation within a species. Due to this variation, several patterns are needed for species determination. The validity of using the perineal pattern was questioned by Allen (1952) due to its considerable variation, but supported by Dropkin (1953) who suggested that perineal pattern is hereditary and stable. In 1955 Taylor, Dropkin, and Martin composed a key based on perineal pattern to identify 6 root-knot nematode species and 2 sub-species. As more species were observed and described, Franklin (1965) designed a perineal pattern key which included 13 root-knot nematode species and 3 sub-species. She noted that M. incognita and M. incognita acrita were often lumped together as M. incognita due to frequent intermediate perineal pattern types and no other morphological distinctions. Triantaphyllou and Sasser (1960) and Whitehead (1968) observed similar phenomena and agreed to include M. incognita acrita in M. incognita. Holtzmann concurred (personal communication) that M. incognita acrita and M. incognita, previously identified by him from Hawaii, should be lumped together as M. incognita. Hereafter references to M. incognita acrita will be identified by brackets to signify that the nematodes in those studies would now be considered M. incognita.

Nematodes belong to Class Nematoda (Steiner, 1964). Goodey (1963) divided the Class Nematoda into 11 Orders. Root-knot

nematodes are in the Order Tylenchida which has as diagnostic features stoma armed with a protrusile spear or stylet, spear aperture always ventrally located, median esophageal bulb usually present, generally with valvular apparatus, basal portion of esophagus forming a bulb, without valvular apparatus, or lobe-like (Thorne, 1961). The Order Tylenchida is divided into 2 Superfamilies. Root-knot nematodes are in the Superfamily Tylenchoidea, which has a dorsal esophageal gland opening into the lumen of the esophagus near the base of the stylet; bursa, when present, not supported by ribs. Within the Superfamily Tylenchoidea are included 5 Families. The root-knot nematodes (Meloidogyne) are included in the Family Heteroderidae, along with the cyst nematodes (Heterodera) and the cystoid nematodes (Meloidodera). The Family Heteroderidae is characterized by a strongly developed spear and head skeleton, and sexual dimorphism in which the females become saccate and the males are vermiform.

In Meloidogyne females do not become a cyst after depositing their eggs into a gelatinous egg sac, and remain colorless or pearly white; in Heterodera females form a cyst enclosing the eggs, and may acquire a yellow to brown protective layer. In Meloidodera the female body wall is tough, white, and there is no distinct cyst stage, although the eggs are retained in the body. Nematodes of genus Meloidogyne will usually cause gall formation on the roots they parasitize while those of Heterodera and Meloidodera do not produce root galls (Franklin, 1965). Franklin (1971) included 2 other genera, Meloidoderita and Cryphodera in

the Family Heteroderidae. These 2 genera differ from Meloidogyne in their cuticle being tough, annulated, or having a netlike pattern. Wouts (1972) proposed a revision for the Family Heteroderidae, in which he separated Meloidogyne and Heterodera into 2 different Families, and included 2 new genera, Sarisodera and Atalodera which were described by Wouts & Sher (1971). His reclassification is listed as follows:

1. Family Meloidogynidae, includes genera Meloidogyne and Meloidoderita (genus inquirenda).
2. Family Heteroderidae is divided into 3 Sub-families:
 - a. Sub-family Heteroderinae, includes genera Heterodera and Sarisodera.
 - b. Sub-family Meloidoderinae, includes genera Meloidodera and Cryphodera.
 - c. Sub-family Ataloderinae, includes genus Atalodera.

The Family Meloidogynidae can be distinguished by the anterior position of the excretory pore of the female, while the Family Heteroderidae has a more posterior position of the excretory pore of the female, a robust stylet in the second stage larvae, and a stylet still present in the third and fourth stage larvae.

The genus Meloidogyne is presently divided into 30 species (Thorne, 1961, Franklin, 1965, 1971, Whitehead, 1968). The 5 most important species and 1 sub-species described by Chitwood (1949) are listed below with the authority, the common name if available, host range, geographical distribution, and the availability of the pathotype.

1. M. incognita (Kofoid & White, 1919) Chitwood, 1949.

- Type host: wide range, includes the plant families Leguminosae, Compositae, Solanaceae, Bromeliaceae, Crucifereae, Gramineae, Chenopodiaceae, Rosaceae, Musaceae, Vitaceae, Convolvulaceae, Caryophyllaceae, and Euphorbiaceae. Type locality: El Paso, Texas, USA., also cosmopolitan. Common name: Southern root-knot nematode. Pathotypes are present.
2. (M. incognita acrita) Chitwood, 1949. Type host: wide range, includes vegetables. Distribution: cosmopolitan.
 3. M. javanica (Treub, 1885) Chitwood, 1949. Type host: sugarcane, also 400 other plant species from the families Leguminosae, Compositae, Gramineae, Solanaceae, Cucurbitaceae, Rosaceae, Crucifereae, Caryophyllaceae, Convolvulaceae, Linaceae, Moraceae, Musaceae, and Passifloraceae. Included are many vegetable crops, cereals, and other cultivated plants. Type locality: Java, Indonesia, also cosmopolitan. Common name: Javanese root-knot nematode. Pathotypes are present.
 4. M. arenaria (Neal, 1889) Chitwood, 1949. Type host: peanut, also other 150 plant species, i.e. corn, vines, watermelon, wheat, barley, Lycopersicon peruvianum. Type locality: Florida, USA., also cosmopolitan. Common name: peanut root-knot nematode. Pathotypes are present.
 5. M. exigua Goeldi, 1887. Type host: Coffee, also found on tea, pepper, watermelon. Type locality: province of

Rio de Janeiro, Brazil. Also observed in Peru, Martinique, and New York Botanical Garden. Common name: coffee root-knot nematode. Pathotypes are present.

6. M. hapla Chitwood, 1949. Type host: potato, also 350 species from the families Leguminosae, Compositae, Solanaceae, Bromeliaceae, Cruciferaeae, Gramineae, Cannabiaceae, Chenopodiaceae, and Rosaceae. Type locality: Bridgehampton, New York, USA. Also cosmopolitan. Common name: Northern root-knot nematode. Pathotypes are present.

Most root-knot nematodes are native to tropical and semi-tropical regions and reproduce best at soil temperatures from 25^o to 32^oC (Thomason and Lear, 1961). Taylor and Buhner (1958) reported that M. incognita, (M. incognita acrita), M. hapla, M. arenaria, M. thamesi, and M. javanica are found in the United States. The most common species reported in Hawaii have been M. incognita and (M. incognita acrita) (Barham & Winstead, 1957, McGuire & Allard, 1958, Gilbert, 1959, McGuire, Allard and Harding, 1961, Hartmann, 1971). Holtzmann (1968) reported M. arenaria from the island of Hawaii, M. incognita from Oahu, Hawaii, Maui, Kauai, and Lanai, M. hapla from Oahu and Maui, and M. javanica from Oahu and Hawaii.

B. Root-knot nematode parasitism on plant roots

1. Symptoms of parasitism of root-knot nematode on plants

The most typical symptoms of root-knot nematode infestation are the galls on the roots. Sometimes the root also ceases growth and becomes swollen. Above ground symptoms may include

chlorosis, defoliation, partial wilting, symptoms of mineral deficiency, decreased vigor, retardation of growth or stunting, and reduced yields. The quality of the crop may also be lowered. When the soil infestation is low to moderate at the early stage, the above ground symptoms may not be easily detected, except that decreased yields are almost always found. However, a very light infection may result in a stimulation of top growth and yield (Jenkins & Taylor, 1967). A severe infestation may cause a complete crop failure, the plants becoming stunted and unfruitful, and sometimes dying.

The nature of root-knot nematode damage to the plant was studied on tomatoes with M. incognita (Bergeson, 1968) and on lettuce with M. hapla (Wong & Mai, 1973). Plant growth reduction was severe when the infection occurred at an early growing stage of the plant. A higher inoculum level was necessary to induce a similar degree of growth reduction when the plants were older. A mineral analysis of the plant indicated that the infected tomato plant did not suffer any lack of minerals or water, but rather a general reduction of growth. However, in the field infected roots may lose some effective surface area which could cause mineral or water deficiency.

Mayol & Bergeson (1970) indicated the important role of secondary infection by soil microorganisms on the damage of an infected plant. When soil microorganisms were present with root-knot nematodes the infected plant weight reduction was higher (75%) than when only root-knot nematodes were

present (37%). Bacteria are the most important secondary infective agents, followed by fungi (Trichoderma sp., Fusarium sp., and Rhizoctonia solani).

2. Development of root galls or knots

Root-knot nematodes are endoparasites which feed on roots of susceptible plants. The infective stage is the second stage larvae which are newly hatched from the developed eggs which hatch when temperature and moisture are favorable. The first stage forms in the egg and moults before the egg hatches. The slender second stage (350 to 450 microns long) larvae are attracted by actively growing roots, often feed on the root's surface, enter the roots through the epidermis usually near the root cap, and then migrate intercellularly in the cortex (Krusberg, 1963) to the feeding site, either the cortical or vascular parenchyma cells. The larvae puncture the cell wall with their stylets, inject esophageal gland secretions while sucking out a portion of the cell contents, and thus stimulate the formation of giant cells or syncytia (Thorne, 1961). In these cells the nuclei enlarge, become polyploid, and undergo synchronous mitosis. Further, the cytoplasm become granular and new cells are incorporated by cell wall dissolution. The wall of the syncytium thickens and the cells of the pericycle divide repeatedly. In short, the formation of what is observed as galls or knots are syncytia of root-knot nematodes, consisting of discrete units of thick-walled giant cells which are the result of extensive pericycle hyperplasia and cortical hypertrophy (Dropkin, 1969a).

3. Development of the root-knot nematode

Once the larva enters the root and becomes established at the feeding site, it starts to grow slowly. In the first 8 days, Bird (1959) observed a doubling of the cross-sectional area in M. javanica and M. hapla on tomato roots. Castillo et al. (1973) reported that M. hapla larvae remained vermiform until the 5th day, started enlarging on the 6th day, and on the 8th day became hemispherical with a spike tail.

Triantaphyllou and Hirschmann (1960) reported that sex of the larvae can be determined at the late second stage in M. incognita on tomato roots. Under 29°C the earliest second moult occurred on the 11th day (the stylet and the spike tail were lost), followed by the third moult a few hours later, and the final moult was observed 2 to 4 days later. After the stylet was reformed and the digestive and reproductive organs were developed within 2 to 3 days, the first adult female appeared on the 15th day, and eggs were deposited 6 days later.

The young adult females are pear shaped, and further grow to a saccate or spherical (pyriform) with an elongated neck. Their size may vary from .5 to 1.0 mm long and .3 to .6 mm. wide, and they are usually pearly white with a shiny cuticle. The adult males are vermiform, can reach .88 to 2.0 mm. long, and are usually found much less frequently than the females. Sexual reproduction can occur although parthenogenesis is the usual means of reproduction in Meloidogyne spp.

Eggs are produced in masses surrounded by a gelatinous material which functions as a protective shield which prevents dryness. Usually the head of the female is imbedded inside the root tissue while the posterior is somewhat closer to the root epidermis so the egg sacs and eggs are deposited outward. In deeper infestation the female and its eggs are located within the root. The average number of eggs produced per female ranges from 200 to 500 (Jenkins & Taylor, 1967). The female will remain at its feeding site until death. The eggs will hatch after embryonic development and the first moult. Tyler (1933) found at least 9 days were required at 27°C for the embryonic development from egg laying to the first moult and hatching. At 24.5°C and 16.5°C the time required were 15-17 days and 31 days, respectively. The species of the root-knot nematode in Tyler's work was not listed.

4. Environmental factors which affect root-knot nematode infestation

Root-knot nematode populations fluctuate from season to season depending upon the availability of host plants and other factors. Occasionally, in a certain period of the year or area, the population may drop to a very low level in the presence of a suitable host in continuous crop cultivation. Sayre (1971) reported several biotic factors in the soil that can affect root-knot nematode life. Decomposition of organic matter can have a nematicidal effect and reduce the root-knot nematode population. Other factors which may reduce the population include predacious fungi, nematodes and protozoa.

In addition, parasitic viruses and protozoa have been reported to attack M. incognita and M. javanica (Loewenberg et al., 1959, Prasad & Mankau, 1969).

Abiotic soil factors also affect root-knot nematodes. An investigation by Sleeth & Reynolds (1955) indicated that soil texture influenced the infection. Plants grown in a coarser-textured soil such as loamy sand showed much higher gall indices than in a finer-textured clay-loam soil. They mentioned the possibility of predicting root-knot nematode problems based on soil maps. According to Wallace (1971), the pores in soils with finer particles may somewhat inhibit nematode movement. Discussing the effect of soil water per se, he indicated that, as the water-soil interface gets thinner, the surface tension increases, the nematode tends to adhere to the soil, and therefore movement or invasion is restricted. Moisture conditions from field capacity to permanent wilting point do not inhibit M. hapla hatching but movement and invasion are better at field capacity. An excess of soil water will affect root-knot nematodes adversely due to a lack of aeration and oxygen. Nematodes become inactive or quiescent in a low O₂ level. M. javanica has been observed to continue to grow at a 3.5% O₂ level. Galling was reduced at the 5.5% O₂ level compared to 10% O₂. In general, an O₂ level of 10% or above at field capacity is considered necessary for development, hatching and movement.

Unhatched eggs under dry conditions can survive for 3 - 4 years. However, the eggs alone at 3 - 4% moisture cannot

survive, they have to be protected by plant material (Peacock, 1957). Persistence of eggs under high soil moisture content is short due to hatching. Daulton and Nusbaum (1962) found a faster decrease in the viability of M. javanica eggs at 20.4% than 3.4% soil moisture. With the atmospheric relative humidity at 100%, unprotected eggs in direct contact with air could survive 20 days; but at a relative humidity of 0, 20, 90, and 93%, eggs were viable only for three to six days. This condition might be comparable to the soil condition between field capacity and permanent wilting point. The fact that Meloidogyne can survive under dry fallow condition for periods ranging from several months to several years might be attributed to unhatched eggs protected by plant material. Another survival mechanism is the ability of the eggs not to hatch and to remain viable when surrounded by a higher osmotic pressure. One experiment showed that eggs of M. javanica remained viable after being kept in a shallow solution of 2% NaCl for 429 days (Whellan, 1962). Since soil contains various salts to some degree, osmotic pressure may contribute to the root-knot survival under moisture stress.

Soil temperatures are often a limiting factor in nematode survival. As was mentioned earlier, most root-knot nematode species are found in tropical, sub-tropical and mild temperate regions, but some species are found in temperate zones. Eggs of M. hapla, a temperate zone species, were able to overwinter and could survive in frozen soil for 3 months with the temperature down to -11°C , but those of tropical species such as

M. incognita could not (Sayre, 1963). Bergeson (1959) indicated that in the absence of a host, 10°C was the best survival temperature for larvae and eggs of (M. incognita acrita), which remained infective for 1 year. At 0°C and 4.4°C the larvae were still infective for 8 to 12 days, while the eggs slowly lost viability up to 6 months when they were completely dead. At temperatures of 32° to 37.7°C the larvae succumbed fast during several days and the eggs lost their viability after a slightly longer period. Bergeson (1959) observed that, at 0°C M. hapla larvae survived for 28 days and eggs survived for 90 days. Daulton and Nusbaum (1961) found that M. hapla eggs survived better at 2°C and -2°C than M. javanica eggs, and also that survival was higher in dry soil (1.4% moisture) than in wet soil (6.1% moisture). At 33°C and 36°C, the eggs died rapidly in dry soil, but in wet soil some viability remained up to 24 days when all the eggs succumbed. The rate of decline in viability was slightly higher for M. hapla than for M. javanica. At 40°C the eggs lost their viability rapidly, even in wet soil, and M. hapla was the most intolerant.

Development of root-knot nematodes was studied by several investigators. Bird and Wallace (1965) reported that for M. hapla the optimum temperatures were 25°C for hatching, 20°C for movement, 15° - 20°C for invasion, and 20°C for growth. For M. javanica the optimum temperatures were 30°C for hatching, 25°C for movement, 15° - 35°C for invasion and 25° - 30°C for

growth. The best hatching for M. incognita was at a temperature range of 15.5°C to 26.5°C (Bergeson, 1959). Thomason & Lear (1961) found that the reproduction rate for M. hapla on tomato roots expressed as the number of eggmasses produced at 35 days after inoculation was highest at 25°C, slightly lower at 20°C and 30°C, and very much lower at 35°C. The reproduction rate for M. javanica, (M. incognita acrita) and M. arenaria was highest at 30°C, lower at 25°C and 20°C, and at 15.6°C was very low. Egg production was decreased at 32.2°C to 35°C. On the host plant Sesbania exaltata, the reproduction threshold of M. hapla and M. arenaria were 32.6°C and 35.3°C, respectively. M. javanica and M. incognita could reproduce up to 36°C but only very poorly at 15.6°C.

In summary, the tropical Meloidogyne species such as M. incognita and M. javanica thrive at a soil temperature range from 20 to 32°C; at 35°C - 40°C soil infestation would be very light and above 40°C they cannot survive. The degree of infection would be low in areas with soil temperatures below 20°C, and with freezing temperatures during the winter these root-knot species won't be a disease problem. However, the more temperate species such as M. hapla, which can overwinter and grow best around 20° - 25°C, are capable of becoming a disease problem in temperate zones. In regions where the soil temperature constantly stays around 30°C, it would probably not be found.

Another soil property which can influence nematodes is pH (Wallace; 1971). The effect of pH is mostly an indirect

effect through the host plant.

C. Economics of root-knot nematode control

The annual loss due to nematodes in vegetables was estimated to be more than \$47 million (Le Clerg, 1964) between 1950 and 1960 with tomatoes over \$20 million, followed by potatoes (over \$18 million), green snap-bean (over \$4 million), cantaloupes (almost \$3 million), and green lima beans (almost \$1 million). Soybean yield losses were estimated at more than \$20 million per year. The average crop damage due to nematodes in the United States was estimated at \$132.57 per hectare for vegetable crops in 1971, of which tomato, bean, cucumber, cantaloup, and carrot suffered the largest losses (Anon., 1971). Damage in bean, brussels sprouts, carrots, cucumbers, and melons were 20% of the total crop, while in green peppers and tomatoes the damage was 15%.

The presence of root-knot nematodes increases the cost of vegetable production either by the increased expenses of control measures or by a reduction in value of the crop. Besides reducing the yields of many vegetable crops, nematode infection may also lower the quality of tuber and root crops, i.e. potatoes, carrots, and sweet potatoes. The size and form of the tubers or roots may be distorted so that they are not marketable. In addition, root-knot nematodes can also cause cracking on the tuber or root surface.

Grainger (1964) noted that the amount of money which can be used to control nematodes is often limited. As an example, for greenhouse tomatoes with a value of \$31,250 per hectare a grower can afford to spend 10% or \$3,125 for nematode control. For potatoes, which are valued at only \$1,250 per hectare, the

amount which can be used for nematode control is only about \$175. Control measures must not only be effective against the nematodes, but their cost must be within the limitation of the crop value. Grainger noted that an inorganic mercury compound can be applied at the time of land preparation at a cost of \$62.50 to \$75 per hectare. However, this compound does not have a high degree of control in reducing the viable nematodes. A combination of D-D fumigation with solubilised Xylenol drenching can effectively control Meloidogyne at a cost of \$625 per hectare.

Oostenbrink (1972) found a linear relationship ($r = -0.94$) for pea seed yield and M. hapla infestation. When there was no infestation, about 8 kg/16 m² of pea seed was obtained, while with 1,000 larvae/100 ml of soil the yield was reduced to 3 kg/16 m². The range of root-knot nematode infestation in the field, expressed as the number of larvae available, was given for which moderate crop damage could be expected. Higher infestation rates would probably be too risky for vegetable crop production. For potato and carrot, the range was 20-200 and 10-100 larvae of M. hapla per 100 ml of soil, respectively. For tomato the range was 20-50 larvae of M. incognita per 100 ml of soil. The degree of susceptibility of vegetable species to root-knot nematode infection sometimes does not correspond to the degree of the nematode's reproduction rate, i.e. onion was heavily damaged by M. hapla but only light reproduction was found while potatoes were only moderately damaged but there was a high reproduction rate (Oostenbrink, 1972).

Hartmann (1968) compared the yield of the resistant snap-bean cultivar 'Manoa Wonder' with the susceptible cultivar 'Hawaiian Wonder' in Hawaii. Where the soil was heavily infested with root-knot nematodes, 'Hawaiian Wonder' had symptoms of poor growth, defoliation and severe root galling while 'Manoa Wonder' grew normally and was free from galling. The yield of 'Manoa Wonder' was higher than that of 'Hawaiian Wonder'. In another trial the relative effectiveness of two methods of control, fumigation and genetic resistance, was studied under conditions where root-knot is an important factor in determining the yield of a susceptible crop (Hartmann, 1968). With fumigation (with D-D, a mixture of dichloropropene and dichloropropane), there was no difference between the yield of 'Manoa Wonder' and 'Hawaiian Wonder'. However, there was a difference without fumigation, 20,793 kg/hectare for 'Manoa Wonder' compared to 7,868 kg/hectare for 'Hawaiian Wonder'. Fumigation increased the yield of 'Hawaiian Wonder' to 17,576 kg/hectare, a highly significant increase. Nettles (1954) also reported snap-bean yield reduction due to root-knot nematodes. The average yields of non-fumigated plots during 3 successive years were 12,107, 10,395 and 6,292 kg/hectare, respectively, while the average of the best fumigated plots was 17,710, 11,362 and 11,252 kg/hectare, respectively. The author attributed the difference to the root-knot nematode infestation.

Holtzmann and Ishii (1963) studied the effect of fumigation on lima bean yields in a root-knot nematode infested field. The root-knot nematode counts (before and after planting) and the gall indices were generally found to be inversely proportional to the

yields. As the count and gall index increased meaning more severe root-knot attack, the yields decreased. The lowest yield in the summer planting was obtained from the untreated check, 3.3 kg/3 m row, while the yields from the nematicide-treated plots ranged from 3.5 to 4.3 kg/3 m row. In the winter planting however, there was no difference between fumigated and non-fumigated plots. This was attributed to higher soil moisture levels and lower temperatures in the winter, with less stress on the plants.

In tomato, root-knot nematode infestation has also caused great reductions in yield, especially in warmer regions where the build-up of the nematode population can be so rapid that, after fumigation, the population level can be restored in a single crop season. A heavy infestation can result in such fast and heavy galling that sometimes the plant cannot tolerate the impediment to normal root functions and is more susceptible to fungal parasites, and will be seriously weakened or killed (Gilbert, 1952). In a root-knot nematode infested field more than 22,650 kg/hectare was obtained from a resistant line, but the susceptible lines were severely galled, stunted, and unfruitful. Furthermore, there was no difference between the resistant and susceptible lines when grown free from root-knot nematodes (Gilbert and McGuire, 1952). Fumigation has also been used to obtain an increase in tomato yields. (Good and Steele, 1958).

Soybeans are also susceptible to root-knot nematodes. The yield loss due to nematodes in the United States was over \$20 million (Le Clerg, 1964). Yields have been increased by fumigation

up to 126% in a root-knot infested field (Anonymous, 1968).

Investigators have also been searching for and developing resistant cultivars (Taylor, 1942, Holston and Crittenden, 1951, Crittenden, 1955, Gilbert, Chinn & Tanaka, 1970). In tropical areas where root-knot nematodes have been established, they are probably a major obstacle in crop production as has been noted in Hawaii (Gilbert, Chinn and Tanaka, 1970).

D. Control measures

1. Cultural

In general, control measures consist of either prevention of establishment of a nematode population in new areas or stabilization of the nematode population below a damaging level. Prevention of establishment is accomplished by quarantine regulation or, eradication if the pest is found only in a few spots of a large area (Anonymous, 1968).

When nematodes are already well established, efforts must be made to control the population level. Presently, the most effective way is by chemical soil fumigation with nematicidal compounds, i.e. halogenated hydrocarbons. Usually the root-knot nematode population can be reduced to a non-damaging level, with no visible galls observed on the roots of susceptible plants. However, if the crop grown is a susceptible species on which the nematode can reproduce well, by the end of the season the nematode population may reach the initial damaging level again.

Other methods of control include sanitation, land management, and cultural practices. One example of a sanitation

measure is seed or propagation-material treatment. Land management measures include fallowing, flooding, heat treatment, etc. Fallowing combined with drying out the soil for several months can reduce the nematode population, but it may be impractical due to long idling of the land. Furthermore, in tropical countries, fallow land usually cannot be kept free from weed species which may be reservoir hosts. Flooding and heat treatment are restricted to certain localities where facilities are available or conditions permit.

Biological control by using sporozoans, fungi, or bacteria is still in the experimental phase, although some effective results have been observed (Oostenbrink, 1972).

For most efficient control, chemical fumigation followed by crop rotation is recommended. However, chemical control may be relatively expensive compared to the value of the crop and frequently nematicides are not available to the grower. In these cases, the crop grown must be limited to one which is not susceptible or one in which genetically resistant cultivars are available (Oostenbrink, 1972).

2. Genetic resistance

a. Definition of resistance

According to Steiner (1925) "resistant plants are those that actually resist the entry of nematode larvae by some mechanical or chemical means; host immunity is the ability of certain plants in nematode-infested soil to show few signs of suffering in spite of the presence or

the absence of the root-knot galls". The term immunity included phenomena where the nematode was not attracted by the roots, host indifference, host repellancy, and actual host resistance. Barrons (1939) defined root-knot resistance as any perceptible ability on the part of the plant to grow in nematode-infested soil without the formation of root-knot galls, while root-knot immunity was defined as a complete resistance to root-knot. Sasser (1954) defined an immune plant as one which, under conditions favorable for infection, is not invaded by larvae of the root-knot nematode. Resistance to infection would be expressed as a reduction in the degree of larval invasion. Sasser also distinguished between a host and non-host plant of a root-knot nematode species. Infection can take place on both plants, but reproduction of the parasite can occur only in a host plant. After infection occurs, the plant may be susceptible with the nematode reproducing freely, or resistant with the nematode's growth and reproduction inhibited.

Christie (1959) suggested that the mechanism of resistance is either in the process of infection (invasion of larvae) or in the parasite development within the plant. He considered plants susceptible if they had the quality of suitable hosts and resistant if they had the quality of unsuitable hosts.

In a broad sense, Rohde (1964) defined resistance as a set of characteristics of the host plants which acts

more or less to the detriment of the parasite. The degree of the resistance, which can vary from light to complete, would only be based on the ability of the parasite to survive, and would not always be directly related to the plant growth conditions.

Peacock (1959) used the following terms:

- (1) Absolute resistance = no larvae gain entry to the host.
- (2) Resistance = few or many larvae enter, but few or none develop.
- (3) Susceptibility = many larvae enter and many develop.

Dropkin and Nelson (1960) viewed the host parasite relationship from both the host and parasite aspects.

They differentiated as follows:

		Host growth	
		Good	Poor
Parasite growth	Good	Tolerant	Susceptible
	Poor	Resistant	Intolerant

In plant breeding programs the term resistance has been used to mean a reduction in symptoms of nematode infection in comparison to susceptible plants when grown in root-knot nematode infested soil (Gilbert, 1952, 1959). Resistant plants thus would show no or little galling compared to heavily galled, susceptible plants. In addition, a reduction in nematode reproduction rate or

egg-mass production has also been used as a resistance criterion by plant breeders.

b. Development of resistant cultivars

Efforts began in the 1940's to incorporate root-knot nematode resistance in tomato (Lycopersicon esculentum L.) cultivars. The source of resistance was found in a wild relative of the tomato, L. peruvianum (Ellis, 1943). Once F-1 plants were obtained from a cross of L. esculentum X L. peruvianum P. I. 128,657 by Smith using embryo culture (Smith, 1944), followed by the successful backcross of the hybrid to L. esculentum by Watts (Gilbert, 1952), the program of producing root-knot nematode resistant cultivars progressed rapidly. Homozygous resistance combined with good horticultural qualities was obtained. The resistance was effective against (M. incognita acrita), M. arenaria, and M. javanica (Barham & Windstead, 1957). Resistance against M. hapla was also observed in different lines of L. peruvianum, but it was probably governed by a different gene or genes (Dropkin, Davis, and Webb, 1967). Many cultivars have been released with good horticultural characters and true breeding root-knot nematode resistance. In Hawaii, the first root-knot nematode resistant cultivar was 'Anahu' (Gilbert, 1952). Others are 'Kalohi', 'Puunui', and 'Healani'. In addition, hybrids have also been released with root-knot and other pest and disease resistance combined with uniformity, vigor, and high yield (Gilbert et al., 1961).

Root-knot resistance in snap-bean (Phaseolus vulgaris L.) was first reported by Isbell (1931). Blazey et al. (1964) reported that root-knot resistance was found in cultivars 'Alabama #1', 'Alabama #18', 'Alabama #19', 'Coffee Wonder', 'Isbell's nematode resistant', 'Spring-water Half Runner', and 'Wingard Wonder'. The resistance was confined to M. incognita. They were susceptible to M. hapla, M. arenaria, M. thamesi, and M. javanica. In 1968 Hartmann released the pole snap-bean cultivar 'Manoa Wonder' which is resistant to M. incognita. This cultivar was derived from a cross between cv. 'Alabama #1' X cv. 'Hawaiian Wonder' (local susceptible cultivar). Looking for another source of resistance among bean plant introductions, Fassuliotis et al. (1970) reported greater resistance to M. incognita than the resistance of 'Alabama #1' in 2 accessions, P. I. 165,426 and P. I. 165,425 from Mexico.

Taylor (1942) tested soybean cultivars for resistance against root-knot nematodes, but none of the tested cultivars were reliably resistant. Smith and Taylor (1947) found that cultivar 'Laredo' was the most resistant soybean out of five cultivars tested. Another test was conducted by Holston and Crittenden (1951) using 8 soybean cultivars. As in the previous test (Smith & Taylor, 1947), 2 cultivars showed relatively high resistance as indicated by lower root-knot (gall) indices, the others were intermediate or susceptible. However, nematode infestation

and egg-mass production were found in all cultivars. They concluded the low gall indices merely indicated tolerance. A more extensive search for root-knot nematode resistance was conducted in 1955 by Crittenden. He found 10 cultivars highly resistant to (M. incognita acrita) but susceptible to M. hapla. In addition he found there was a difference in time required for gall formation and egg-mass production on resistant or susceptible cultivars. On the resistant cultivar of soybean the root-knot nematode growth was delayed (Crittenden, 1959).

The soybean breeding program for root-knot nematode resistance in Hawaii has been concerned primarily with vegetable-type soybeans which are consumed in the green pod stage. The local cultivar 'Bansei' is susceptible to root-knot nematodes. Resistance was obtained from line UD-288 from the University of Delaware (Crittenden) and the U.S. Regional Soybean Laboratory, Urbana, Illinois. The resistance is good against (M. incognita acrita). The original cross of 'Bansei' (susceptible) X UD-288 (resistant) and the performance of the F-2 and F-3 were reported by Chang (1963). Further selections were conducted by Gilbert et al. (1970) and 4 root-knot resistant, vegetable type soybean cultivars ('Kailua', 'Kaikoo', 'Kahala', and 'Mokapu') were released.

Resistance to root-knot nematodes has been incorporated in 2 lima bean cultivars which were released in 1935 and 1946, 'Hopi 5989' and 'Westan'. Both are dry, edible,

baby lima beans (Allard, 1948). Some resistant strains among cultivars grown in California were later also noted (Allard, 1954). However, the effort to transfer resistance from these baby lima beans to the large seeded type has not been successful due to difficulty in recovering resistant types in the progeny of the hybrids. Another resistant strain, L-76, was considered to have a simpler genetic control and therefore possibly be a better donor parent.

Since testing under field conditions sometimes gave erratic results, Wester (1950) conducted an experiment to compare greenhouse and field tests. Greenhouse results were higher in gall indices and had faster root-knot nematode infestation although treated with lower inoculum than in the field, probably due to the fact that soil in the greenhouse was kept at 32.2°C constantly. Modifying the method, i.e. lowering the soil temperature to 20° - 23.9°C, Allard (1954) also tested the resistant materials in the greenhouse against (M. incognita acrita). Besides lima bean strain L-76, he found 12 other strains had good resistance and many have different genetic controls. Allard and McGuire (1958) reported obtaining dependable field test results in Hawaii where the resistant and susceptible lima beans were planted in a field infested with (M. incognita acrita), and soil temperature ranged from 15.6° to 29.4°C.

In a study using more than one species of root-knot nematode, Pattimore and Allard (1962) found different degrees of galling as well as reproduction rates in different lima bean cultivars and lines. Relatively resistant against (M. incognita acrita), the cv. 'Westan' was attacked considerably by M. javanica. Some resistant plant introduction accessions showed good resistance against (M. incognita acrita), M. hapla, and M. thamesi, having either no galls or small ones. Slightly higher gall indices were obtained when these accessions were exposed to M. javanica. All susceptible cultivars were heavily galled by (M. incognita acrita) and M. javanica, but had clean to moderately galled roots when inoculated with M. hapla and M. thamesi. They also mentioned the positive correlation between the gall indices and the nematodes' reproduction rates and yield reduction of the lima beans.

A breeding program using a large population and a backcross method with rigid selection was considered feasible. Using this method, Tucker (personal communication) attempted to develop a root-knot nematode resistant, large seeded, vine 'White Ventura' type lima bean cultivar. The donor parent was L-76 which is resistant to (M. incognita acrita), and the recurrent parents were the cultivars 'Ventura' and 'White Ventura 65'. Testing and selection were conducted at the Poamoho Experimental Farm, Hawaii, where the root-knot nematode species have been reported as

M. incognita and (M. incognita acrita). A root-knot nematode tolerant cultivar named 'White Ventura N' resulted from this breeding work.

c. The inheritance of root-knot nematode resistance

The inheritance of resistance in tomato has been studied by several investigators (Frazier & Dennet, 1949, Gilbert, 1952, Gilbert & McGuire, 1956, Barham & Winstead, 1957, Gilbert, 1959, Hernandez et al., 1965). Resistance was found to be dominant, and controlled by a small number of factors (Frazier & Dennet, 1949). Gilbert (1952, 1959) obtained a ratio of 3:1 in segregating F-2 progenies and postulated one major dominant gene, named Mi (abbreviation of Meloidogyne incognita). A single dominant gene governing resistance was also reported by Barham & Winstead (1959) and by Hernandez et al. (1965). However, when Sikora et al. (1973) tested several cultivars bearing the Mi gene against M. favanica, 2 cultivars, FVN-8 and FVN-368, were heavily galled while others such as 'Anahu' were clean. They suggested that one or more genes other than Mi may directly or indirectly affect root-knot resistance in tomatoes.

Barrons (1940) postulated the inheritance of the resistance of cv. 'Alabama #1' snap-bean was controlled by the interaction of 2 independent recessive genes. Blazey et al. (1964) tested root-knot nematode resistant cultivars 'Spring-water Half Runner' and 'Wingard Wonder' and concluded

that the resistance was also controlled by 2 independent recessive genes. More recently, Hartmann (1971) tested the inheritance of resistance which originated from cv. 'Alabama #1' and stated that the resistance was controlled by at least 3 pairs of recessive genes. He also found homozygous partially resistant lines.

The inheritance of root-knot nematode resistance in soybean was studied by Chang (1963) and he indicated that it was monofactorial and dominant, although some modifiers were also noted.

McGuire, Allard, and Harding (1961) studied the inheritance of root-knot nematode resistance in lima bean and reported a relatively low heritability and inconsistent progeny results and thus could postulate no simple Mendelian ratio. Since there were many F-2 segregants with performance equal to the parents, they suggested that a few major genes govern the resistance. Although different major genes may be found in different resistant strains, the existence of one or more common resistant genes with each contributing a low level of resistance was not ruled out.

E. Factors affecting genetic resistance

The development of root-knot resistant cultivars of tomatoes, snap-beans, soybeans, and lima beans has nearly eliminated one of the major obstacles to successful production of these crops in areas where root-knot nematodes are widely distributed. In

addition to the reduction or removal of the fumigation cost, the level of the parasite infestation is also reduced enough that other susceptible crops may be grown in a rotation program. The practical value of a disease resistance breeding program is increased if the resistant cultivar has an equal or better yield, even in the absence of the plant pathogen.

However, the availability of a resistant cultivar does not eliminate all nematode disease problems. No cultivar is resistant to all nematodes at all times. Two major factors which influence resistance are the species or races of the root-knot nematode and the soil temperature.

Each species of nematode has its own host range and ability to infect cultivated plants. Some resistant cultivars are effective against only one species of root-knot nematodes, i.e. the snap-bean cultivar 'Alabama #1' is resistant only to M. incognita. Continuous cultivation of such a resistant cultivar in fields infested with a mixture of root-knot nematodes should have a selective effect favoring certain species or the better adapted individuals of the population and a loss in effectiveness of the resistance.

Other resistant cultivars are effective against most of the important root-knot nematode species such as the tomato cultivar 'Anahu' which is resistant to M. incognita, (M. incognita acrita), M. arenaria, and M. javanica. Growing this type of resistant cultivar continuously will suppress those root-knot nematode species if they are present in the field, but theoretically, a prolonged starving due to unavailability of a compatible host can

induce the selection of better adapted individuals (Kehr, 1966). Steiner (1925) reported that starving will force the nematodes to parasitize plants which they usually do not attack. Specialization of root-knot nematodes to certain host species was observed by Sasser & Nusbaum (1955). They found a population of (M. incognita acrita) which was able to attack cotton and not tobacco and another population of the same species which was able to attack both cotton and tobacco.

Riggs and Winstead (1959) showed that better adapted individuals of root-knot nematodes were able to survive when exposed continuously to a resistant tomato line. This led to the formation of a new pathogenic biotype which was able to parasitize the resistant tomatoes. The new capacity for parasitism was observed to be stable (Winstead & Riggs, 1963). This new root-knot nematode population was called 'B biotype'.

Other investigators who have observed evidence of root-knot nematode races are Martin (1954), Dropkin (1959), Gophen, Stanford and Allen (1959), Triantaphyllou and Sasser (1959, 1960), and Minton (1963).

A pathogenic biotype of root-knot nematode species has also been called a physiological race, a race, or a pathotype. The races or pathotypes within a species usually cannot be distinguished morphologically, but have a different pathogenic ability to parasitize plants.

Most new race formation in Meloidogyne has been associated with a prolonged starvation in which compatible host plants were

eliminated either artificially or naturally. The experiment (Riggs & Winstead, 1959) which resulted in a new pathogenic biotype was conducted in pots (no weeds) with an incompatible host (resistant plant). Four repeated inoculations on incompatible hosts could be interpreted as prolonged starvation of the nematodes which increases the selection pressure and formation of a new pathogenically distinct race of nematodes. Kehr (1966) suggested that these conditions increase the mutation rate of the root-knot nematodes which leads toward specialization in race formation.

However, only rarely has a new physiological race been reported in a grower's field even though resistant tomato cultivars have been planted extensively (Holtzmann & Gilbert, 1969, Gilbert, personal communication, 1973). This indicates that the resistance is effective, even though it is controlled by a single dominant gene.

Based on the stable performance of the resistant tomato line when planted in rotation with susceptible plants in an experiment by Giles & Hutton (1958), Van der Plank (1968) concluded that resistance to Meloidogyne in tomato is strong, which means it is not easily overcome by formation of a new race.

A root-knot nematode race capable of attacking resistant tomatoes in the field was reported in Australia (Sauer & Giles, 1959) where the majority of the root-knot nematode is M. javanica and in Hawaii (Gilbert, 1959, Holtzmann & Gilbert, 1969), where the nematode species is M. incognita. In Hawaii the new race was

restricted to the field where selected resistant tomato seedlings (which after inoculation in the nursery, proved to be free from galls or had only small galls) were grown repeatedly for years. In essence, this is similar to the inoculation of a non-host plant reported by Riggs & Winstead (1959), and indicates that the selection of plants and nematodes occurred simultaneously.

The second factor which influences the expression of resistance is the soil temperature. In general, a higher temperature will tend to make the plant more susceptible to root-knot nematode infection. Infection of resistant plants due to increased soil temperature has been reported by Holtzmann (1965) with tomatoes, by Fassuliotis et al. (1970) with snap-beans, and by Wester (1950) with lima beans. A survey of 18 known resistant plant species and cultivars showed that resistance to Meloidogyne is related to soil temperature in some plants and not in others (Dropkin, 1969b). Resistance which did not show a reversed effect due to elevated temperature included 3 classes of reaction. The first class was characterized by constant cell necrosis and little or no change in the nematode's growth at higher temperature. This class was represented by Crotalaria spectabilis Roth.; Desmodium intortum (Mill.) Urb. cv. 'Greenleaf'; Desmodium tortuosum (Sw.) DC. cv. 'Tifton 61-27'; Glycine javanica G. wightii, cv. 'Verdcourt', 'Clarence'; and Medicago sativa L. cv. 'African' and 'Moapa'. The second class had none or very light cell necrosis with inhibited root-knot nematode development at 16° to 28°C. The plants included in this class were Aeschynomene americana L.;

Carthamus tinctorius L.; and Lespedeza cuneata. The third class showed an increase in resistance to M. incognita when the temperature was increased from 16° to 28°C. This was observed on Cucumis metuliferus.

The effect of temperature on the expression of resistance was noted by Walker (1957). He mentioned that monogenic resistance was effective up to 26°C, while polygenic resistance was effective only up to 22°C. Holtzmann and Gilbert (1969) reported that monogenic tomato resistance which was effective at 20°C and 25°C, but reduced at 30°C and 34.5°C supported Walker's hypothesis.

Root-knot nematode resistance in plants is seldom expressed as a complete freedom from infection, but often small galls and a few females may develop on roots of resistant plants and result in slight reproduction. This phenomenon has been observed in tomatoes (Gilbert, 1952, 1959), snap-beans (Barrons, 1940, Hartmann, 1968), soybeans (Smith & Taylor, 1947, Holston & Crittenden, 1951), and lima beans (Allard, 1954, Pattimore & Allard, 1962). Possible causes are a high level of root-knot nematode inoculum, a mixed root-knot nematode population, or soil temperatures.

III. MATERIALS AND METHODS

Materials:

A. Host plants

Four species of vegetable crops, for which resistant and susceptible cultivars were available, were used:

1. Snap-bean (Phaseolus vulgaris L.):
 - a. P.I. No. 165,426 (resistant, abbreviated as r., Fassuliotis et al., 1970)
 - b. 'Manoa Wonder' (r.)
 - c. 'Hawaiian Wonder' (susceptible, abbreviated as s.)
2. Tomato (Lycopersicon esculentum Mill.):
 - a. 'Healani' (r.)
 - b. 'Yellow Plum' (s.)
3. Soybean (Glycine max Merr.):
 - a. 'Kailua' (r.)
 - b. 'Bansei' (s.)
4. Lima bean (Phaseolus lunatus L.):
 - a. 'White Ventura N.' (tolerant, Tucker, personal communication)
 - b. 'White Ventura 65' (s.)
 - c. L-136 (r., Pattimore & Allard, 1962)

Although it is not related to the cultivar 'Healani', the cultivar 'Yellow Plum' was used as the susceptible tomato due to its easily distinguishable plant characters. In addition, there is no susceptible cultivar similar to 'Healani'. Snap-bean P.I. No. 165,426 and lima bean line L-136 were included in the experiment since they were reported to have different sources

of genetic resistance than the resistant cultivars used. Moreover, the snap-bean P.I. No. 165,426 was observed to have a better resistance to root-knot nematodes than the cultivar 'Alabama No. 1', which was the resistant parent of the cultivar 'Manoa Wonder'.

B. Source of root-knot nematode inoculum

Root-knot nematodes for the inoculation test were obtained from the fields at the Poamoho Experimental Farm (located at 200 m above sea level with Wahiawa silty clay soil type) of the University of Hawaii, in which the resistant cultivars had been observed to perform differently. In field Q-1 the resistant tomato cultivars were usually galled, apparently because of a new physiological race of the nematodes (Holtzmann and Gilbert, 1969). Field P-1 was infested with root-knot nematodes but no ineffectiveness of resistance in tomato and snap-bean has ever been observed. In field P-2 the resistant snap-bean cultivar 'Manoa Wonder' has sometimes been heavily galled (Hartmann, personal communication). The root-knot nematode populations from these fields and from individual cultivars in these fields were established on 'Yellow Plum' tomato grown in pots in the greenhouse. The populations were maintained by reinoculating new seedlings with the galled roots from the old plants every 3 months.

C. Medium

The medium consisted of a mixture of equal amount of sterilized soil (93.3°C for 3 hours) and new Vermiculite No. 2. For the temperature controlled experiment, the medium was sterilized silica sand.

Methods:A. Gall & egg-mass rating

When sufficient time had elapsed for gall development, the plants were dug up carefully and the gall formation was rated according to a modified scale of Holtzmann (1963):

1 = clean, no visible gall (No galling)

2 = few small galls (Light galling)

3 = numerous small galls (Moderate galling)

4 = numerous small and few large compound galls (Heavy galling)

5 = numerous small and large compound galls (Severe galling)

Gall indices 1 and 2 are considered an indication of resistance, while 3 to 5 are susceptible.

In the field tests, 2 to 3 months were allowed before the plants were dug up for observation. The objective here was to study the plant's resistance during a period similar to the normal crop cycle. In addition it was anticipated that a possible unequal distribution of root-knot nematode inoculum in the soil would be equalized by having more than one generation to attack all plants. In the greenhouse tests with controlled inoculation, 35 days were determined adequate for gall development and reproduction of the root-knot nematodes. However, in the experiments to investigate the possible formation of a new root-knot nematode population able to attack the resistant plants, 2 months (2 root-knot nematode life cycles) were allowed before the plants were checked. When egg-mass inoculation was used,

2 months were also allowed before the plants were dug up to allow the second generation of the root-knot nematode to infect the plants.

The frequency of egg-mass formation was rated as follows:

1 = no egg-masses

2 = few egg-masses

3 = 10 to 50 egg-masses

4 = numerous, more than 50 egg-masses

To obtain the data on egg-masses, the roots were observed under the dissecting microscope (14 X). Egg-mass indices 1 and 2 are considered an indication of plant resistance, while 3 and 4 are plant susceptibility.

B. Root-knot nematode inoculum preparation

The test plants were inoculated with root-knot nematodes in 3 ways:

1. Inoculation with galled roots. The inoculum was prepared from galled roots of infected plants. The galled roots were washed carefully, and cut into small pieces. Inoculation was made by placing the chopped galled roots approximately 2.5 cm under the transplanted tomato seedlings or planted bean seeds.

2. Inoculation with a single egg-mass. Single egg-masses were collected from galled roots under the dissecting microscope, and were placed in distilled water in a small watch glass. Inoculation was accomplished by placing the egg-mass near the tip of a lateral root of a seedling plant, covering it gently with medium, and watering the pot moderately.

3. Inoculation with larvae. Galled roots from infected plants were collected and carefully washed. They were chopped into small pieces and the larvae were extracted with a Baermann funnel. To increase the amount of larvae extracted, the host plants were given minimum amount of water (sometimes causing partial wilting) for 10 days prior to larvae extraction as recommended by Dropkin (1959). The viable larvae obtained were counted and diluted to a concentration of 20 larvae per ml of water. Twenty-five or 125 ml of this larval suspension (containing approximately 500 or 2,500 larvae) per plant was poured into a 1 to 2 cm deep furrow circling the base of the stem. The furrow was covered lightly with the medium, and the pot was watered moderately.

C. Identification of root-knot nematode species

1. Host range test

A host range test following the method of Sasser (1954) was conducted to identify the species of the root-knot nematodes. This test can identify four species and one subspecies of root-knot nematodes, M. arenaria, M. javanica, M. hapla, M. incognita, and (M. incognita acrita). These are the most important, commonly-found world-wide species, which have also been reported in Hawaii (Holtzmann, 1968).

The cultivars used in Sasser's test are:

- a. Corn: cv. 'Golden Cross Bantam'
- b. Watermelon: cv. 'Dixie Queen'
- c. Peanut: cv. 'Spanish'
- d. Pepper: cv. 'California Wonder'
- e. Lycopersicon peruvianum: P.I. No. 126,441

Key of the Sasser's host range test for root-knot nematode species identification is given below:

<u>Meloidogyne</u> species	Plant species			
	Peanuts	Watermelon, Wheat, Barley or corn	Pepper	<u>L. peruvianum</u>
<u>M. hapla</u>	+ ^a	-	+	+
<u>M. arenaria</u>	+	+	+	+
<u>M. javanica</u>	-	+	-	+
<u>M. incognita</u>	-	+	+	-
(<u>M. incognita</u> <u>acrita</u>)	-	+	+	+

^a Plus signs = susceptibility, minus signs = resistance or immunity

Seeds of the host plant for the test were planted in Jiffy-7 peat pots and transplanted at the age of 3 weeks into 1 gallon pots containing equal amounts of sterilized soil and Vermiculite No. 2. Three to four days later each plant was inoculated with 500 larvae obtained from galled roots of 'Yellow Plum' tomato on which had been established the different populations of nematodes. Thirty-five days after inoculation the plants were dug up carefully, and their roots were washed and rated for gall formation.

The host range test was first run on the root-knot nematode populations established on 'Yellow Plum' tomato by inoculation with galled roots from three fields, Q-1, P-1, and P-2. The test was run two times, each with three replications.

The host range test was also run on 15 populations established on 'Yellow Plum' tomato by single egg-mass inoculation. These populations identified by the cultivar and field from which they were obtained, were:

- a. 'Yellow Plum' Q-1
- b. 'Yellow Plum' P-2
- c. 'Healani' Q-1
- d. 'Bansei' Q-1
- e. 'Bansei' P-2
- f. 'Kailua' P-2
- g. 'Hawaiian Wonder' Q-1
- h. 'Hawaiian Wonder' P-2
- i. 'Manoa Wonder' Q-1
- j. 'Manoa Wonder' P-2
- k. P.I. 165,426 P-2
- l. 'White Ventura 65' Q-1
- m. 'White Ventura 65' P-2
- n. 'White Ventura N' Q-1
- o. 'White Ventura N' P-2

No single egg-mass populations were included from field P-1 because the nematodes in this field had been identified as the M. incognita group and no ineffectiveness of resistance had been observed in this field. Populations 'Healani' P-2, 'Kailua' Q-1, and P.I. 165,426 Q-1 could not be established because the degree of galling in the field was very low and the egg-masses were very scarce and not fully developed. This test was run once with 3 replications.

2. Perineal pattern studies

The root-knot nematode species were also identified morphologically on the basis of perineal pattern or posterior cuticular pattern of adult, egg-laying females. Fresh adult females were picked carefully from infected roots, and transferred into hot lactophenol in a small watch glass. After at least 48 hours the nematode was cut in half under a dissecting microscope with a sharp scalpel. The posterior portion was cleaned from the adhering soft parts by teasing it with a fine tip of the mid rib of a feather. The perineal pattern or tail section can be located from the thickened cuticle spot which contains an area of cuticular striae surrounding the tail tip, phasmids, anus, and vulva. After the spot is located, the posterior half is trimmed to leave only the small part of the tail section. This part is mounted on a slide with a drop of lactophenol. Approximately 10 perineal patterns were mounted from each root-knot nematode population to be identified.

Identification was done under a microscope with 1,000 X magnification based on the key and specimen pictures from Taylor, Dropkin, and Martin (1955), Franklin (1965), and Whitehead (1968).

D. Host plant and root-knot nematode performance tests

1. Field tests

Field tests were conducted to observe the performance of resistant and susceptible cultivars in the root-knot nematode infested fields throughout the year.

Nine resistant and susceptible cultivars were planted at monthly intervals throughout the year in the three fields, Q-1, P-1, and P-2 of the Poamoho Experimental Farm of the University of Hawaii. Two replications of each cultivar were planted in each field in a completely randomized design.

Soybeans, snap-beans and lima beans were seeded directly in the field. Tomatoes were transplanted at an age of 3 to 4 weeks. The tomatoes were germinated in a metal tray with Vermiculite No. 2 in the greenhouse.

Each replication consisted of a 6 m row for the tomatoes and lima beans, and a 4.5 m row for the soybeans and snap-beans. Tomatoes and lima beans were spaced 60 cm apart, while the soybeans and snap-beans were 45 cm apart, with 10 plants in each plot. Snap-beans and lima beans were allowed to climb on either nylon net or individual poles.

In fields Q-1 and P-2, soil temperatures in the rhizospheres were recorded with soil thermographs. Sensors or probes were placed horizontally under the growing plants at a depth of 15 cm, measured from the surface of the bed.

2. Resistance to known inoculum

This experiment was intended to test performance of resistant vegetable cultivars under continuous nematode infestation, or, in other words, the stability of root-knot nematode pathogenicity on resistant hosts.

For this test all plants were grown in the mixture of equal amounts of sterilized soil and Vermiculite No. 2.

The snap-beans, soybeans, and lima beans were direct seeded and the tomatoes were transplanted using 3 week old seedlings. After the medium was placed in the pots, 50 of inoculum per plant in a gallon of medium was placed 2.5 cm below the seeds or seedlings.

The inoculum was obtained from galled roots of 'Yellow Plum' tomato on which the nematode populations from fields Q-1 and P-2 had been established. The nine resistant and susceptible cultivars were included in the test. The test was repeated five times in succession to coincide with the work of Riggs and Winstead (1959). For the second and later plantings, inoculum was obtained from the galled roots of the previous planting of the same cultivar. If a resistant cultivar did not produce sufficient galled roots, the inoculum was obtained from galled roots of the susceptible cultivar of the same species. The first two tests were replicated eight times, and the last three tests six times. Two months after inoculation the plants were dug up carefully, the roots were washed, and the galls were rated.

One test with three replications was run with the 15 root-knot nematode populations established from single egg-masses. One population for each source was used, except for populations 'Healani' Q-1, 'Kailua' P-2, 'Manoa Wonder' P-2, and 'White Ventura N' P-2 for which four populations per source were established (established with four single egg-masses on four 'Yellow Plum' tomato). The reason for establishing more than one population per source for these

resistant cultivars was that they had sometimes been observed to be partially or completely susceptible in the fields from which they came. Population 'Manoa Wonder' Q-1 or P-2 was used to inoculate cv. 'Manoa Wonder', population 'Healani' Q-1 was used to inoculate cv. 'Healani', etc.

3. Competition test

This test was conducted to examine root-knot nematode preference when resistant and susceptible hosts are available at the same time. Two cultivars, one resistant, one susceptible, were grown together in one pot to allow the roots to grow close together, overlapping each other.

Pairs of resistant and susceptible cultivars were direct-seeded in pots. At an age of 3 weeks for the tomatoes and 1 week for the snap-beans, soybeans and lima beans, 10 single egg-masses were inoculated onto the roots of the susceptible cultivar to insure that infestation would take place.

The snap-bean, soybean, and lima bean tests were replicated four times, and the tomato test was replicated eight times. All pots were arranged in a completely randomized design. Pairs of resistant and susceptible cultivars used were:

- a. 'Healani' and 'Yellow Plum' tomato
- b. 'Kailua' and 'Bansei' soybean
- c. P.I. 165,426 and 'Hawaiian Wonder' snap-bean
- d. P.I. 165,426 and 'Manoa Wonder' snap-bean
- e. 'Manoa Wonder' and 'Hawaiian Wonder' snap-bean
- f. L-136 and 'White Ventura N' lima bean
- g. L-136 and 'White Ventura 65' lima bean
- h. 'White Ventura N' and 'White Ventura 65' lima bean

Single egg-masses used for inoculation were obtained from the single egg-mass root-knot nematode populations established on 'Yellow Plum' tomato. Only the populations from fields Q-1 and P-2 were used. Populations 'Healani' Q-1 and 'Yellow Plum' P-2 were used for tomato pairs, populations 'Kailua' P-2 and 'Bansei' Q-1 for soybean pairs, populations 'Manoa Wonder' Q-1 and P-2 for snap-bean pairs, and populations 'White Ventura N' Q-1 and P-2 for lima bean pairs.

Two months after inoculation the pairs were dug up carefully, the roots were washed and separated, and their galls and egg-masses were rated.

4. Selection of a root-knot nematode race capable of parasitizing more than one resistant cultivar

The test was intended to observe whether a super root-knot nematode population, able to attack resistant cultivars of more than one vegetable species, can be induced.

Root-knot nematode populations 'Healani' Q-1 and 'Manoa Wonder' P-2 were able to cause gall formation on the resistant cultivars of tomatoes and snap-beans to a degree, respectively. An attempt to develop a race that could infect resistant cultivars of both crops was made by inoculating 'Healani' tomato with root-knot nematode population 'Manoa Wonder' P-2 and 'Manoa Wonder' snap-bean with root-knot nematode population 'Healani' Q-1. An inoculum rate of 200 g of galled roots derived from the last sequential inoculation per gallon

medium was used to create an effect of a mass attack of root-knot nematode larvae on the roots. The test was conducted two times in succession, and replicated four times in a completely randomized design.

Serial inoculation tests had indicated a possible increase of pathogenicity of root-knot nematode populations 'White Ventura N' Q-1 and P-2. These root-knot nematode populations were used to inoculate 'Healani' tomato; and 'White Ventura N' was inoculated with population 'Healani' Q-1. This test was run two times and replicated four times for each trial.

Thirty-five days after inoculation the plants were dug up and the root galls were rated.

5. Effect of environment and root-knot nematode sources on resistant and susceptible cultivars

This experiment was conducted to study effects of soil temperature, source of root-knot nematode population, level of inoculum, and the resistant cultivars.

Plants were direct seeded in cans filled with sand and placed in a circulating waterbath equipped with a thermostat that could heat and cool. Temperature was controlled at 3 levels, $T_1 = 24.5^\circ \pm 1^\circ\text{C}$, $T_2 = 29^\circ \pm 1^\circ\text{C}$, and $T_3 = 34^\circ \pm 1^\circ\text{C}$. In addition, there was a temperature level T_0 where the cans were placed at ambient (air) temperature which ranged from 21° to 33°C .

Two sources of inoculum from single egg-mass cultures established from population 'Healani' Q-1 and 'Manoa Wonder'

P-2 were used. Only these 2 populations were used because previous experiments indicated the possibility of the presence of new races in them, and not in others. The plants were inoculated with a larval suspension at two levels, $L_1 = 500$ larvae/plant, and $L_2 = 2,500$ larvae/plant. Tomatoes were inoculated 17 days after seeding, the snap-beans, soybeans, and lima beans 7 days after seeding.

The cultivars used were 'Yellow Plum' and 'Healani' tomato, 'Bansei' and 'Kailua' soybean, 'Hawaiian Wonder', 'Manoa Wonder', and P.I. 165,426 snap-bean, and 'White Ventura 65', 'White Ventura N', and L-136 lima bean.

In all experiments plants were arranged in a completely randomized design with three replications.

Each vegetable species thus had treatments as follows:

- a. Four levels of temperature
- b. Two sources of inoculum
- c. Two levels of inoculum

Thirty-five days after inoculation the plants were dug up, roots were washed, and gall formation and egg-mass production were rated.

IV. RESULTS AND DISCUSSION

A. Root-knot nematode species identification

All root-knot nematode populations established from single egg-masses from fields P-2 and Q-1 were identified as the Meloidogyne incognita group. These are the same species reported by previous investigators (Barham & Winstead, 1957, McGuire & Allard, 1958, Gilbert, 1959, McGuire, Allard & Harding, 1961 and Hartmann, 1971).

However, in root-knot nematode populations established from galled roots, M. javanica was also identified in one replication of the Q-1 population in the host range test, and observed in the perineal pattern studies of the P-2 population which was corroborated by Holtzmann (personal communication). Nevertheless, since most of the identifications were of the M. incognita group, it was concluded that the M. incognita group is the predominant species and M. javanica is only a small, insignificant fraction of the population. Additional perineal pattern studies on the root-knot nematodes from fields P-2 and Q-1 parasitizing resistant cultivars after the 3rd sequential inoculation confirmed the predominance of the M. incognita group.

B. Host plant and root-knot nematode performance tests

1. Snap-bean

a. Field test

In field Q-1 the susceptible cv. 'Hawaiian Wonder' had gall indices above 2.0, except when planted in January and December (Fig. 1). During these two months the soil

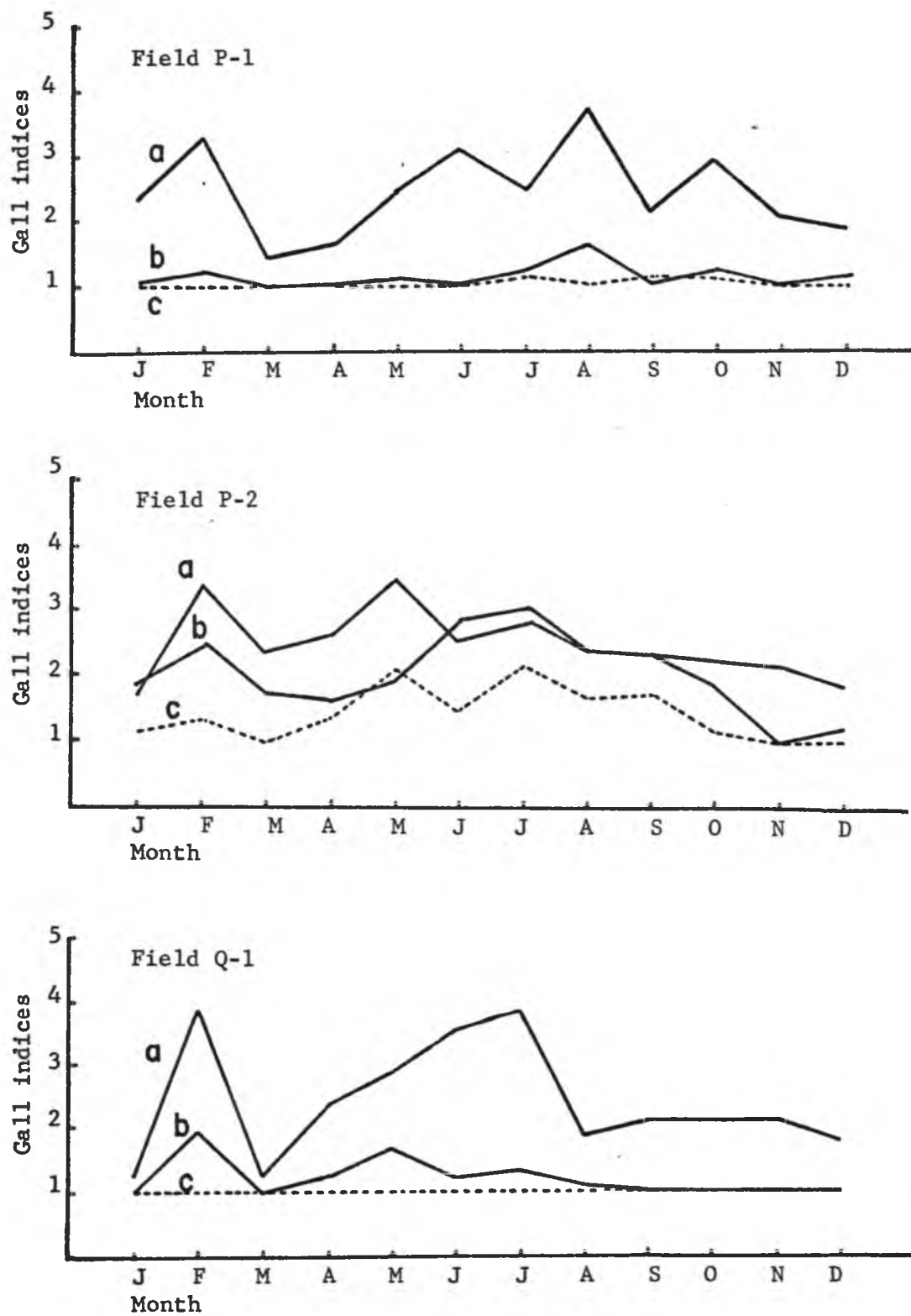


Fig. 1. Gall indices of snap-bean cultivars: (a) 'Hawaiian Wonder' (s), (b) 'Manoa Wonder' (r), and (c) P.I. 165,426 (r) planted monthly in 3 fields of Poamoho Experimental Farm

temperature was always below 26.7°C (Fig. 2) and the rainfall was 67.5 and 36.3 inches (the highest monthly rainfall for the year; Fig. 3). These conditions probably caused a reduction in the lower inoculum level and slower parasite growth with the result there was less gall development. The resistance of 'Manoa Wonder' was expressed well throughout the year in field Q-1 with gall indices always below 2.0. 'Manoa Wonder', like its parent, 'Alabama #1', is not always free from small, scattered galls, particularly when the inoculum is high (Blazey et al., 1964). Although M. javanica was identified in a Q-1 population, and 'Manoa Wonder' is not resistant to it, there was no indication of heavy infestation of 'Manoa Wonder' by M. javanica in the field. Perhaps this was due to a very low number of M. javanica present. P.I. 165,426 proved to be an excellent resistant line in field Q-1. It was free from galling in all months throughout the year. The 3 snap-bean cultivars performed similarly in field P-1.

In field P-2 the gall indices of the susceptible 'Hawaiian Wonder' fluctuated around 2.0 and 3.0 (Fig. 1). The resistant 'Manoa Wonder' and P.I. 165,426 had different gall indices during different months of the year. The 'Manoa Wonder' gall indices followed the pattern of 'Hawaiian Wonder' at lower rates, and were below 2.0 until May, except in February when the root-knot nematode inoculum was apparently relatively high, as indicated by an increase in the gall index of the

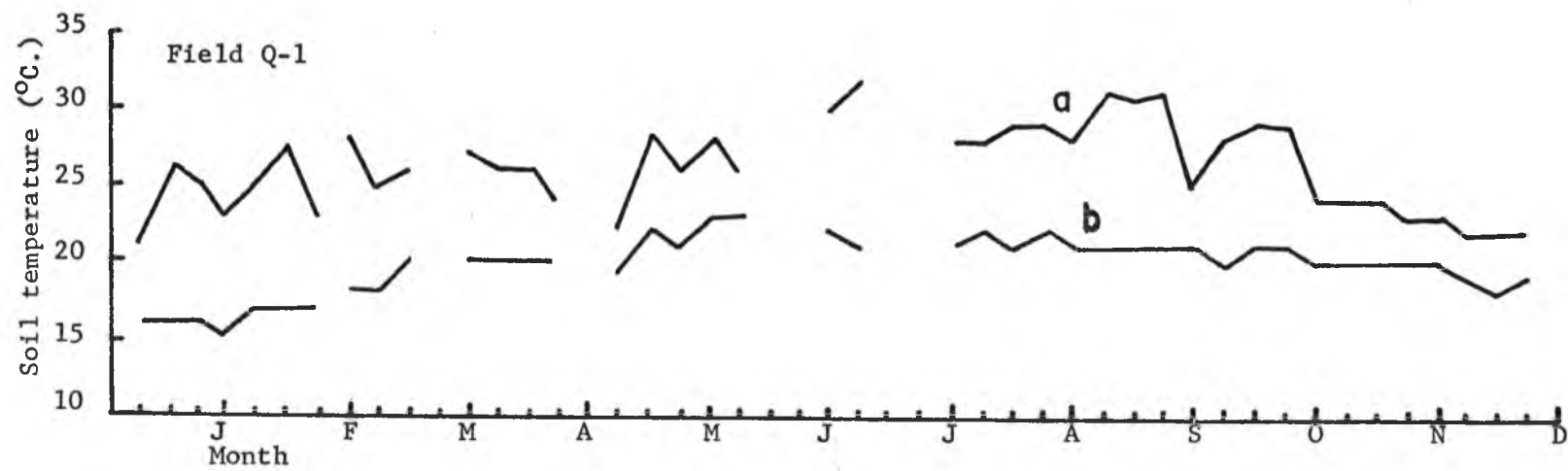
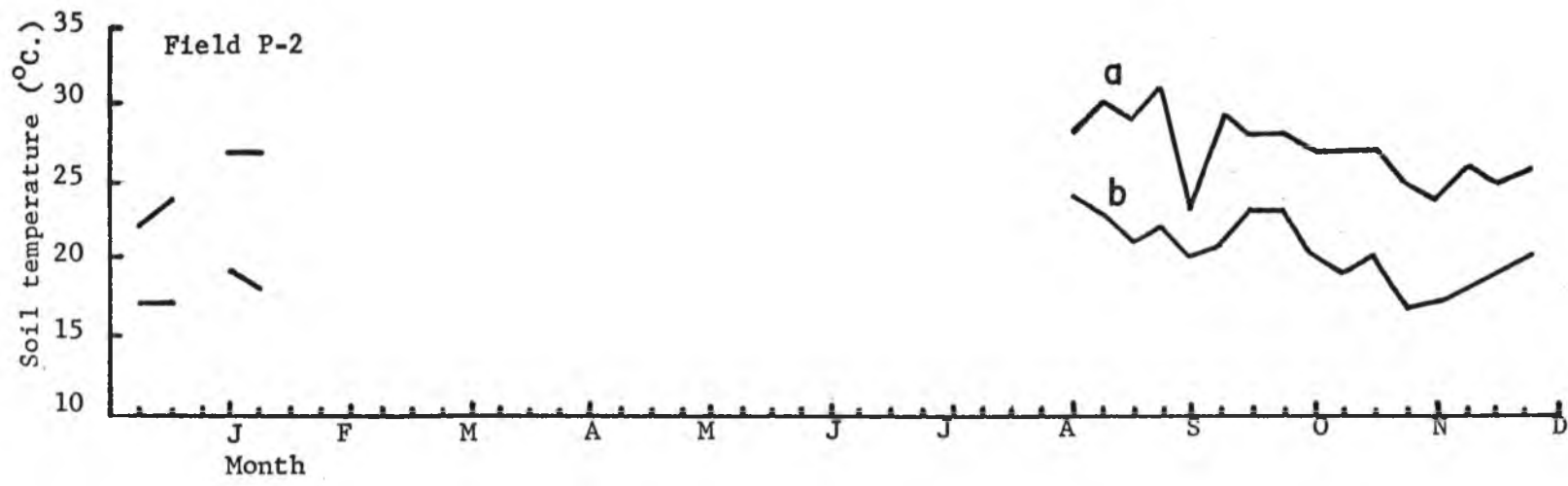


Fig. 2. Maximum (a) and minimum (b) soil temperatures at a depth of 6 inches of 2 fields of Poamoho Experimental Farm throughout the year of the experiments

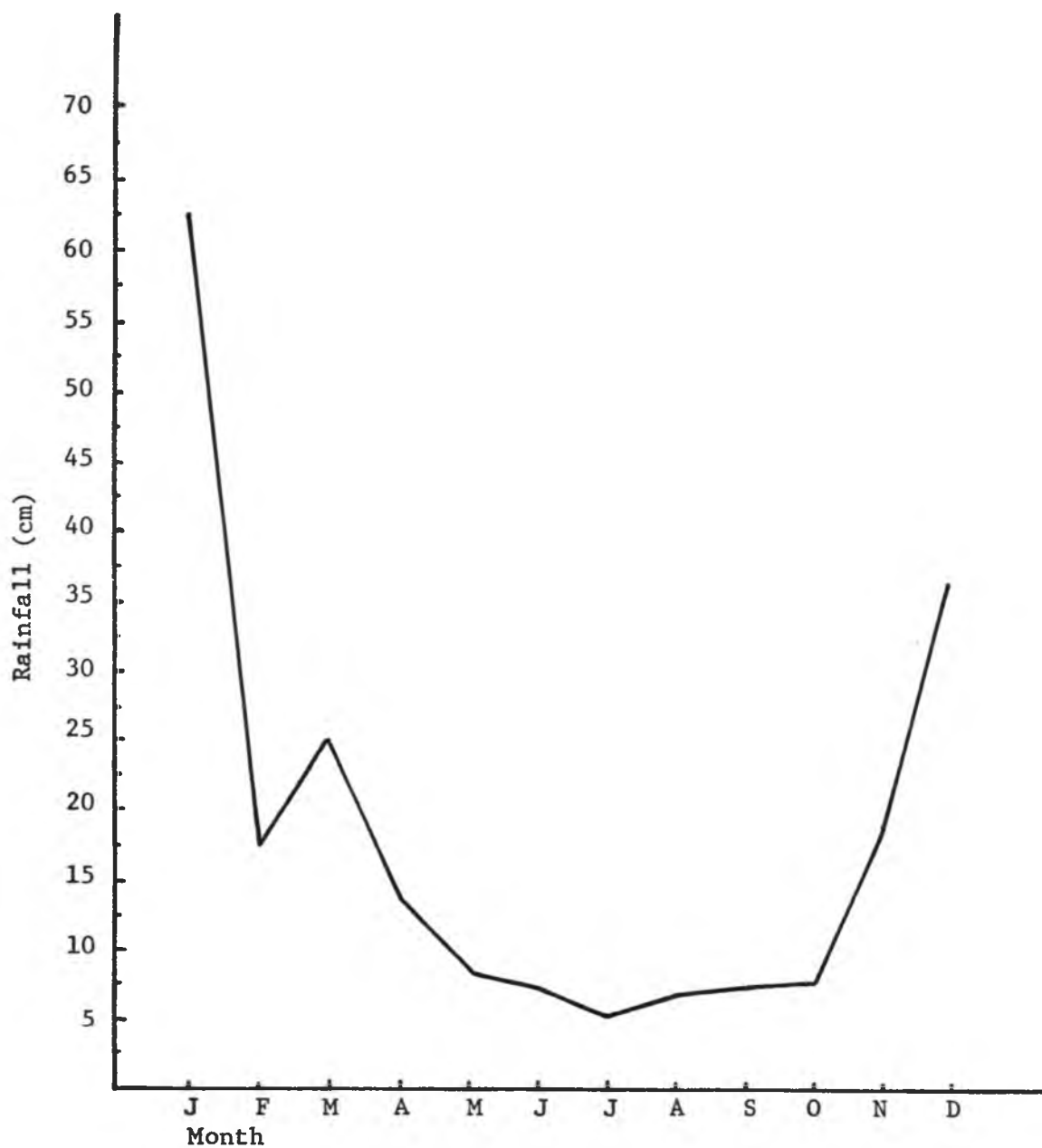


Fig. 3. Monthly rainfall (cm) recorded throughout the year of the experiments

susceptible 'Hawaiian Wonder'. Between June and September, however, the resistant 'Manoa Wonder' showed similar gall indices than the susceptible 'Hawaiian Wonder' which can be interpreted that the resistance was not effective. A possible explanation is increased soil temperature during the summer which would reduce the resistance of 'Manoa Wonder' or P.I. 165,426. In field Q-1, the maximum soil temperatures recorded in the summer were between 26.7° and 32.2°C . Fassuliotis et al. (1970) showed that the effectiveness of the resistance of P.I. 165,426 against M. incognita was reduced at 28°C . The gall indices of P.I. 165,426 during this period indicate a possibility of such a loss of resistance. However, a higher soil temperature may not only increase the susceptibility of the resistant cultivars, but also increase the root-knot nematode infection and growth in the susceptible cultivar (Fassuliotis, 1970) which did not occur. Still another possible explanation for the high galling of 'Manoa Wonder' is an increase in virulence of M. incognita which enables it to attack the resistant cultivars.

An inverse correlation was found between the monthly gall indices in field P-2 and the bimonthly rainfall. However, the correlation coefficients for 'Manoa Wonder' and P.I. 165,426 were not significant. Perhaps the higher rainfall reduced the inoculum available due to more hatching (Daulton & Nusbaum, 1962) and lack of aeration in the soil (Wallace, 1971).

b. Resistance to known inoculum

(1) Sequential inoculation with populations established from galled roots

Results of the sequential inoculations (Fig. 4) showed some increase in the ability of the root-knot nematode to attack the resistant snap-bean cultivars. Initial gall indices were rated 4.0 at inoculation 0, this was the galling rate of 'Yellow Plum' tomato from which the initial inoculum was obtained.

An increase of root-knot nematode virulence toward the resistant cultivar 'Manoa Wonder' was obtained when inoculum from field P-2 was used. There was no significant difference in the gall indices of 'Manoa Wonder' from the 3rd to the 5th inoculation (Table 1). However, these last three inoculations had higher gall indices than the 2nd and 1st. Comparing the last 3 inoculations (indices 3.5 to 3.8) with the 1st inoculation which had a gall index of 2.4, the increase is highly significant. After the 3rd inoculation, apparently there was no increase in the root-knot nematode virulence, and at this point the 'Manoa Wonder' and 'Hawaiian Wonder' gall indices were similar (Fig. 4). These results would support the hypothesis that field P-2 M. incognita has a potential of increasing its virulence on 'Manoa Wonder'.

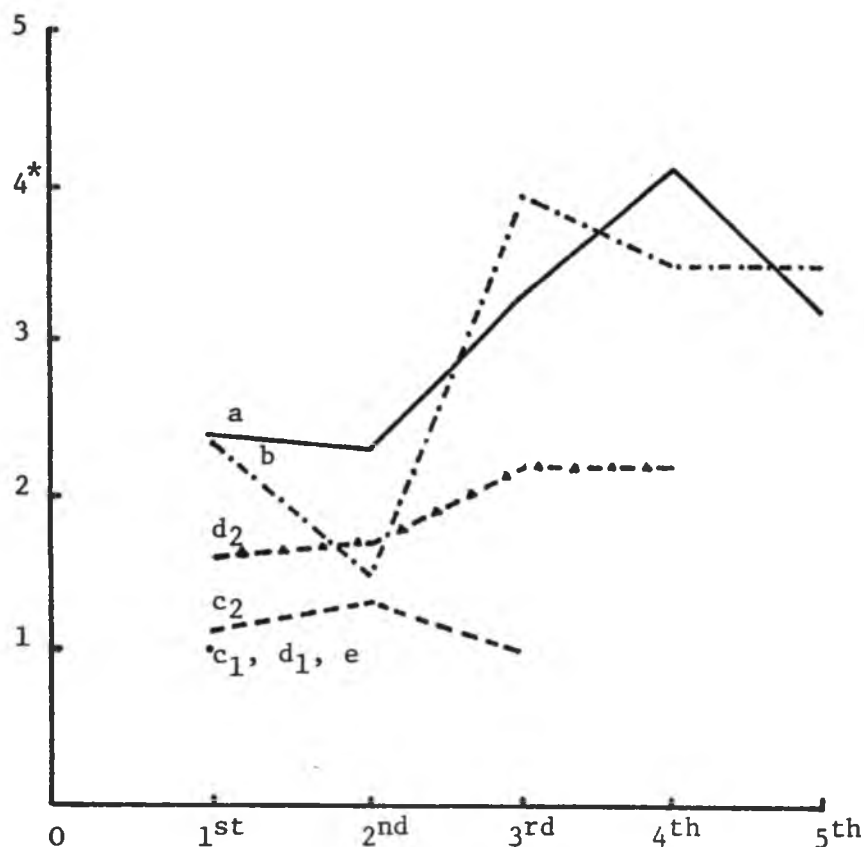


Fig. 4. Gall indices of snap-bean cultivars in the sequential inoculation

- * = Gall rate on 'Yellow Plum' stock plant
- a = Cultivar 'Hawaiian Wonder' inoculated with root-knot nematodes from fields Q-1 and P-2 (Average)
- b = Cultivar 'Manoa Wonder' inoculated with root-knot nematodes from field P-2
- c₁ = First attempt at inoculating cultivar 'Manoa Wonder' with root-knot nematodes from field Q-1, two inoculations resulted in no galling
- c₂ = Second attempt at inoculating cultivar 'Manoa Wonder' with root-knot nematodes from field Q-1
- d₁ = First attempt at inoculating P.I. 165,426 with root-knot nematodes from field P-2, one inoculation resulted in no galling
- d₂ = Second attempt at inoculating P.I. 165,426 with root-knot nematodes from field P-2
- e = P.I. 165,426 inoculated with root-knot nematodes from field Q-1, all five inoculations resulted in no galling

Table 1. -- Gall indices of resistant and susceptible snap-bean cultivars inoculated with root-knot nematodes from fields Q-1 and P-2 of Poamoho Experimental Farm, in five inoculations in succession

Inoculation sequence	Snap-bean cultivar				
	Manoa Wonder Inoculum		P.I. 165,426 Inoculum		Hawaiian Wonder Inoculum
	Q-1 ^a	P-2	Q-1 ^b	P-2 ^c	Average Q-1 & P-2
1 st	1.1	2.4	1.0	1.6	2.4
2 nd	1.3	1.5		1.7	2.3
3 rd	1.0	3.8		2.2	3.3
4 th		3.5		2.2	4.1
5 th		3.5			3.2
LSD 5%	.4	.6		.5	
1%	.5	.8		.6	

^a Two inoculations resulted in no galling

^b No galling from all inoculations

^c One inoculation resulted in no galling

When inoculum from field Q-1 was used, there was no increase in the gall indices from one inoculation to the next. The first 2 inoculations did not cause any galling. The 3rd and 4th attempt resulted in only slight galling ranging from 1.1 to 1.3, but the last inoculation showed no galling at all.

It can be concluded that the potential of increasing pathogenicity on 'Manoa Wonder' was confined only to M. incognita from field P-2.

The performance of the resistant P.I. 165,426 was similar to that of 'Manoa Wonder', but at a greater level of resistance. The inoculum from field P-2 produced no galling from the 1st inoculation, but the 2nd inoculation resulted in slight galling (1.6) and the gall indices gradually increased in the following inoculations. A significant increase in the gall index was obtained from the 3rd inoculation. The last gall index obtained from this serial planting was 2.2, which under practical conditions is not considered a serious infection. However, there is a possibility of increasing the virulence of the nematode toward P.I. 165,426.

All attempts at inoculating P.I. 165,426 with the inoculum from field Q-1 resulted in no galling. This population of root-knot nematode could not develop a compatible relationship with P.I. 165,426.

(2) Inoculation with populations established from single egg-masses

Gall indices of 'Manoa Wonder' inoculated with the nematode populations established from single egg-masses 'Manoa Wonder' Q-1 and 'Manoa Wonder' P-2.1-4 ranged from 1.7 to 3.0 with no significant differences (Table 2). The gall rates of 1.7, 2.0 and 2.0 for population 'Manoa Wonder' P-2 and 2.3 for population 'Manoa Wonder' Q-1 would probably be the normal expression of the resistant 'Manoa Wonder', since it often has small, scattered galls (Barrons, 1940, Hartmann, 1968). The highest gall index, 3.0, obtained from the 'Manoa Wonder' P-2.1 inoculum, although not significantly different from the other gall indices, may indicate that this population of root-knot nematode was more virulent on 'Manoa Wonder' than the others.

P.I. 165,426 was free from galling with 'Hawaiian Wonder' Q-1 inoculum and only formed a few small galls with P.I. 165,426 P-2 inoculum. These results agree with the first inoculation of the previous test, where the inoculum used came from root-knot nematode populations established from galled roots.

c. Competition test

Pairs of cultivars 'Hawaiian Wonder' & 'Manoa Wonder' and 'Hawaiian Wonder' & P.I. 165,426 clearly showed differences in performance of resistant versus susceptible cultivars when they were grown closely together. While

Table 2. -- Gall indices of resistant and susceptible snap-bean cultivars inoculated with root-knot nematode population established from single egg-masses from fields Q-1 and P-2 of Poamoho Experimental Farm

Inoculum	Snap-bean cultivar		
	Manoa Wonder	P.I. 165,426	Hawaiian Wonder
H. Wonder Q-1		1.0	3.0
H. Wonder P-2			3.7
M. Wonder Q-1	2.3		
M. Wonder P-2.1	3.0		
M. Wonder P-2.2	2.3		
M. Wonder P-2.3	1.7		
M. Wonder P-2.4	2.0		
P.I. 165,426 P-2		1.7	
LSD 5%	1.4		
1%	2.0		

'H. Wonder' = 'Hawaiian Wonder', 'M. Wonder' = 'Manoa Wonder'

the resistant plants were free from galling, the susceptible were galled profusely, regardless of whether the inoculum came from field Q-1 or P-2 (Table 3). This indicates that the root-knot nematodes did not attack resistant 'Manoa Wonder' and susceptible 'Hawaiian Wonder' indiscriminately, which can be interpreted that there was no distinct M. incognita race which is pathogenic to 'Manoa Wonder'. Similar results were obtained for egg-mass production. A possible practical value of this phenomenon is that alternating resistant and susceptible cultivars, mixed in the same field at the same time, may prolong the stability of the resistant cultivars.

Table 3. -- Gall and egg-mass indices of snap-bean cultivar pairs inoculated with root-knot nematodes established from single egg-masses from fields Q-1 and P-2 of Poamoho Experimental Farm

Snap-bean pair	Inoculum			
	Manoa Wonder Q-1		Manoa Wonder P-2	
	Gall indices	Egg-mass indices	Gall indices	Egg-mass indices
Hawaiian Wonder	4.3	4.0	4.5	4.0
Manoa Wonder	1.0	1.0	1.0	1.0
Hawaiian Wonder	4.5	4.0	4.8	4.0
P.I. 165,426	1.0	1.0	1.0	1.0
Manoa Wonder	1.0	1.0	1.0	1.3
P.I. 165,426	1.0	1.0	1.0	1.0

The pair of 'Manoa Wonder' and P.I. 165,426 showed no galling at all. Failure of the inoculum to attack the plants may have been due to the low level of inoculum, which was 10 egg-masses per pair (as compared to 50 galled roots/plant in the previous tests), in addition to the resistance of these cultivars.

d. Effect of environment and root-knot nematode sources on the resistant and susceptible cultivars

(1) 'Hawaiian Wonder'

Root-knot nematodes were able to attack the susceptible cultivar 'Hawaiian Wonder' at all soil temperatures (Table 4). Under similar inoculum levels, there was no significant difference of gall indices among different soil temperatures, except under the 2,500 larvae/plant inoculum level when the soil temperature was increased from $29^{\circ} \pm 1^{\circ}\text{C}$ to $34^{\circ} \pm 1^{\circ}\text{C}$. In this case the gall indices decreased significantly, probably due to the higher than optimum temperature for the nematode's growth and reproduction (Thomason & Lear, 1961). The gall and egg-mass indices were indicative of the susceptibility of 'Hawaiian Wonder'.

The gall index and reproduction generally increased at the higher level of inoculum. However, the differences in gall and egg-mass indices between the two sources of inoculum (fields Q-1 and P-2) were not significant. These results indicate that for the susceptible cultivar, temperature is important

Table 4. -- Gall and egg-mass indices of snap-bean cultivars inoculated with 2 sources of root-knot nematodes, at 2 inoculum levels, and at 4 soil temperatures

Source of inoculum	Level of inoculum	Soil temperature	H. Wonder		M. Wonder		P.I. 165,426	
			Gall ind.	Egg-mass ind.	Gall ind.	Egg-mass ind.	Gall ind.	Egg-mass ind.
Field Q-1	500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	2.3	2.7	1.0	1.0	1.0	1.0
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	2.7	2.3	1.0	1.0	1.0	1.0
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	2.7	2.3	2.7	3.7	1.0	1.0
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	2.7	2.7	2.0	3.3	1.0	1.0
	2,500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	3.3	3.0	1.7	2.0	1.0	1.0
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	3.7	3.3	1.0	1.0	1.0	1.0
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	4.0	4.0	3.3	4.0	1.3	2.3
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	2.7	4.0	2.7	3.7	2.0	2.7
Field P-2	500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	2.7	2.3	1.0	1.0	1.0	1.0
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	2.3	3.0	1.0	1.0	1.0	1.0
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	3.3	3.0	2.7	4.0	1.3	1.0
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	2.7	2.7	2.3	2.3	2.0	1.0
	2,500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	3.7	2.3	1.7	1.3	1.0	1.0
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	3.7	3.7	1.7	1.7	1.0	1.0
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	4.0	4.0	3.3	4.0	1.7	1.0
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	3.0	3.0	2.3	3.7	2.0	1.0
LSD 5%			.9	.8	.7	.8	.4	.3
1%			1.3	1.1	1.0	1.0	.5	.5

'H. Wonder' = 'Hawaiian Wonder', 'M. Wonder' = 'Manoa Wonder', Gall ind. = Gall indices, Egg-mass ind. = Egg-mass indices

only when above the optimum; the level of inoculum is quite important; and source of inoculum is not important.

(2) 'Manoa Wonder'

Under the fluctuating temperature ($21^{\circ} - 33^{\circ}\text{C}$) the cultivar 'Manoa Wonder' was galled lightly with the 2,500 larvae/plant inoculum rate. Both inoculum sources (Q-1 and P-2) resulted in light galling and poor egg-mass production. These conditions are perhaps somewhat similar to field conditions, since similar small, scattered galls have been observed in 'Manoa Wonder' (Hartmann, 1968). Fassuliotis (1970) also observed that the cultivar 'Alabama #1', the resistant parent of 'Manoa Wonder', was not free from root-knot nematode females developing in the roots in the field.

There was no galling and no reproduction when the soil temperature was $23.5^{\circ} \pm 1^{\circ}\text{C}$, except that the P-2 inoculum at the level of 2,500 larvae/plant produced light galling and very slight reproduction at levels which were not significant. When the temperature was increased to $29^{\circ} \pm 1^{\circ}\text{C}$, the nematodes from both sources at both levels were able to infect, grow, and reproduce. There was no significant difference between the two sources of inoculum. There was no difference also between the two levels

of inoculum at $29^{\circ} \pm 1^{\circ}\text{C}$. Blazey et al. (1964) also reported small galls and slight reproduction on the cultivar 'Alabama #1' when heavily inoculated at a soil temperature of 26.7°C .

At $34^{\circ} \pm 1^{\circ}\text{C}$ the nematodes did not grow and reproduce as well as they did at $29^{\circ} \pm 1^{\circ}\text{C}$. This was in accordance with what Thomason & Lear (1961) had observed. Gall indices ranged from 2.0 to 2.7, and did not differ significantly from one another. Reproduction rates ranged from 2.3 to 3.7, the only significant difference was between the low level P-2 inoculum and the other 3 levels and sources of inoculum.

Inoculum level is important only under the fluctuating temperature. The source of inoculum did not show any effect, which could indicate that there was no racial difference between root-knot nematode populations tested. 'Manoa Wonder' was completely resistant at a soil temperature of $23.5^{\circ} \pm 1^{\circ}\text{C}$, but increasing the temperature to $29^{\circ} \pm 1^{\circ}\text{C}$ produced a significant increase in gall indices as well as in egg-mass production which means a reduction in resistance. Apparently at this temperature the resistance was not effective.

The fact that the polygenic resistance of 'Manoa Wonder' was effective at $23.5^{\circ} \pm 1^{\circ}\text{C}$ does not agree with Walker's (1957) observation on polygenic

resistance to Cabbage Yellows, which was effective only up to 22°C.

(3) P.I. 165,426

P.I. 165,426 was never galled when the root-knot nematode inoculum from field Q-1 was used at the lower level. When the inoculum was from field P-2, light galling was obtained with soil temperatures of 29° ± 1°C and 34° ± 1°C with both sources of inoculum, but reproduction was obtained only with the P-2 inoculum.

The resistance of P.I. 165,426 has been reported to be better than that of 'Alabama #1' (Fassuliotis et al., 1970). In a controlled inoculation with 10,000 larvae/plant, P.I. 165,426 was galled lightly at soil temperatures of 21° and 28°C. Reproduction rates were traced to moderate. At 16°C no galling or reproduction was observed.

2. Tomato

a. Field test

The resistance of 'Healani' was effective throughout the year in fields P-1 and P-2 (Fig. 5). The gall index was 1.0 most of the time, and never reached 1.5 while the gall indices of the susceptible 'Yellow Plum' fluctuated around 3.0 and 4.0. In field Q-1, however, the gall indices of both cultivars fluctuated more. The 'Healani' gall indices followed the pattern of 'Yellow Plum' but at a slightly lower level. Thus, while 'Yellow Plum' was galled in almost all months in all 3 fields, 'Healani' was

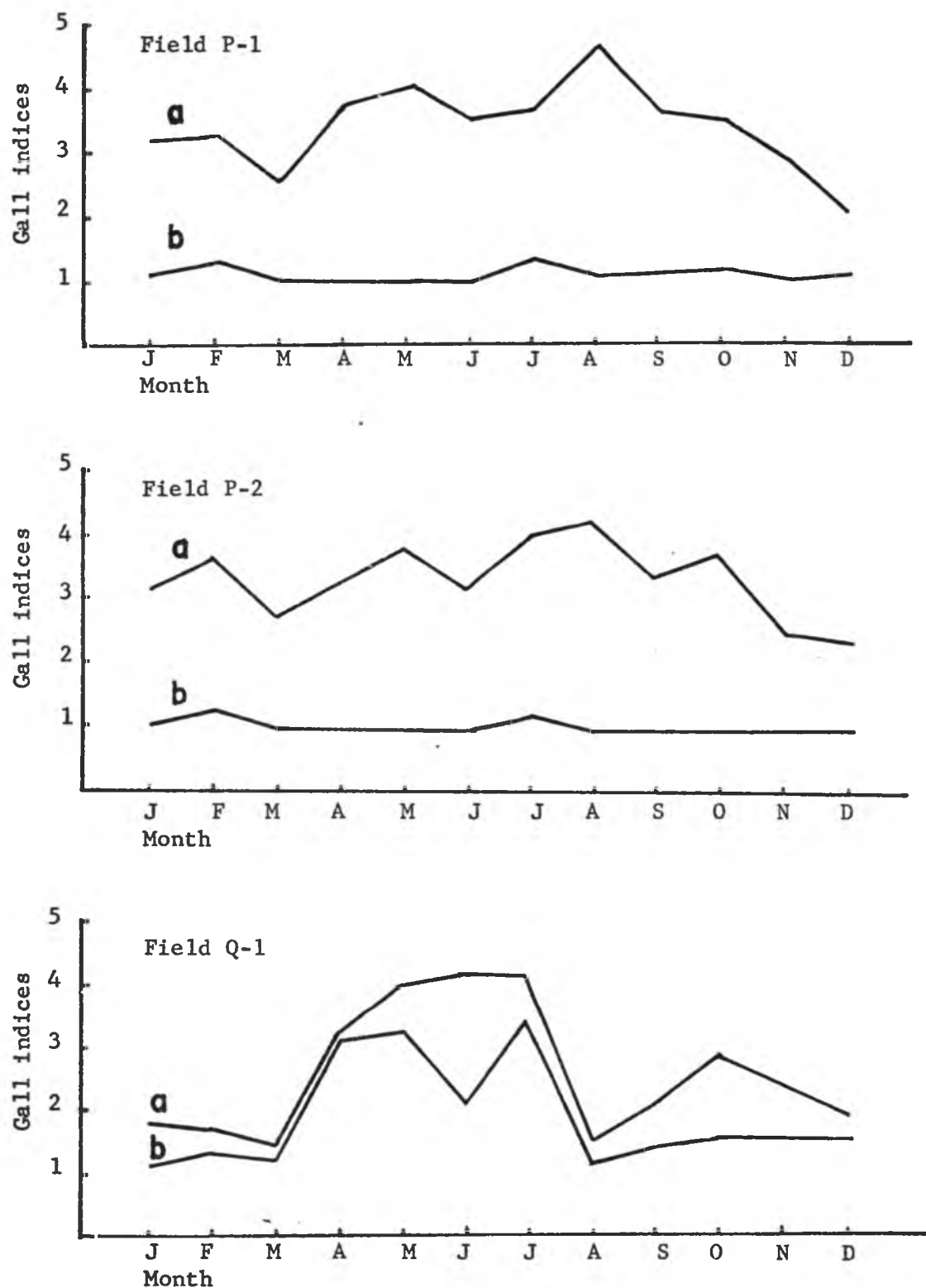


Fig. 5. Gall indices of tomato cultivars: (a) 'Yellow Plum' (s) and (b) 'Healani' (r) planted monthly in 3 fields of Poamoho Experimental Farm

galled only in field Q-1, particularly during the months of April through July. The 'Healani' gall indices showed significant differences among months, varying between nearly 1.0 to over 3.0. Higher rainfall tended to decrease the degree of galling in tomato, as it did in snap-bean. However, the different response of 'Healani' in Q-1 compared to P-1 and P-2 indicates that in Q-1 the galling on 'Healani' must be due to a different population of root-knot nematodes which is able to parasitize 'Healani'. Since 'Healani' is also resistant to M. javanica which was also found in field Q-1, the galling must be caused by a new race of root-knot nematode. Since all the single egg-mass cultures from field Q-1 have been identified as M. incognita, the new pathogenic race must be a race of M. incognita.

This race of M. incognita which is able to attack resistant tomato was first reported by Gilbert (1959). Continuous planting in the field with tomato seedlings with small galls that had been selected after severe root-knot testing, has resulted in formation of the new race (Holtzmann & Gilbert, 1969). A similar situation was observed by Sauer & Giles (1959) when testing resistant tomato cultivars in a field infested with M. javanica.

b. Resistance to known inoculum

(1) Sequential inoculation with populations established from galled roots

The difference between the ability of the Q-1 and P-2 populations of root-knot nematode to parasitize the

cultivar 'Healani' is clearly illustrated in Fig. 6. The Q-1 population was able to cause gall formation on 'Healani' as high as on 'Yellow Plum'. The first three inoculations had gall indices ranging from 3.1 to 3.5, which were not significant from each other (Table 5). The gall indices from the 4th and 5th inoculations were not significantly different also. The only significant increase of galling (5% level) was from the 3rd to the 4th inoculation which was from moderate to heavy galling. Riggs and Winstead (1959) obtained an increased virulence from light to heavy galling of M. incognita 'Biotype B' on a resistant tomato line after four sequential inoculations. Comparing these results it seems that the M. incognita which is more virulent on cv. 'Healani' was already established in the field Q-1, which confirmed the reports of Gilbert (1959) and Holtzmann & Gilbert (1969).

Inoculum obtained from field P-2, however, failed to parasitize 'Healani'. Only the fourth attempt resulted in slight gall development, but the subsequent inoculation was unsuccessful.

(2) Inoculation with populations established from single egg-masses

The results of this test (Table 6) corroborated the previous test (Table 5). The root-knot nematode populations 'Healani' Q-1.1-4 were able to attack 'Healani', while the population 'Yellow Plum' P-2 was not.

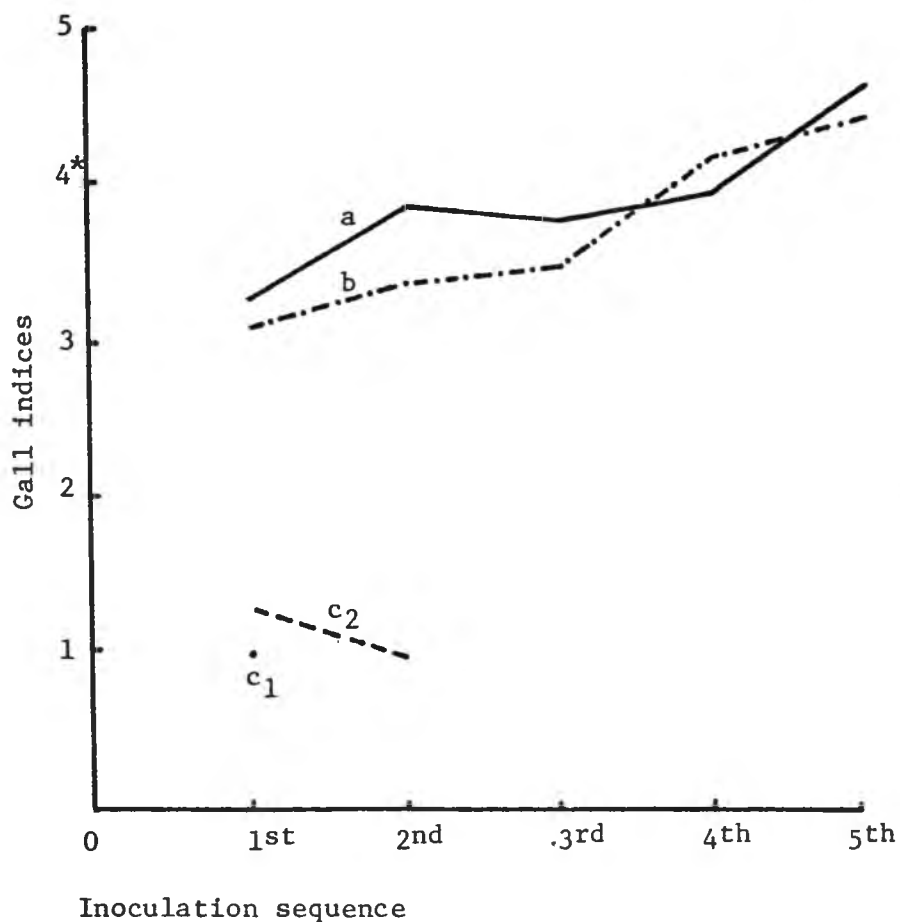


Fig. 6. Gall indices of tomato cultivars in the sequential inoculation

- * = Gall rate on 'Yellow Plum' stock plant
- a = Cultivar 'Yellow Plum' inoculated with root-knot nematodes from Q-1 and P-2 fields (Average)
- b = Cultivar 'Healani' inoculated with root-knot nematodes from field Q-1
- c₁ = First attempt at inoculating cultivar 'Healani' with root-knot nematodes from field P-2, three inoculations resulted in no galling
- c₂ = Second attempt at inoculating cultivar 'Healani' with root-knot nematodes from field P-2

Table 5. -- Gall indices of resistant and susceptible tomato cultivars inoculated with root-knot nematodes from fields Q-1 and P-2 of Poamoho Experimental Farm, in five inoculations in succession

Inoculation sequence	Tomato cultivar		
	Healani Inoculum		Yellow Plum Inoculum
	Q-1	P-2 ^a	Average Q-1 & P-2
1 st	3.1	1.4	3.3
2 nd	3.5	1.0	3.9
3 rd	3.4		3.8
4 th	4.2		4.0
5 th	4.5		4.7
LSD 5%	.7	.3	
1%	1.0	.4	

^aThree inoculations did not result in galling

There was no significant difference among the gall indices caused by the 'Healani' Q-1 root-knot nematode populations, but there was a significant difference between the gall indices caused by the 'Healani' Q-1 and 'Yellow Plum' P-2 root-knot nematode populations (Table 6). These two populations differ in the ability to attack 'Healani', and must be different races of M. incognita.

Table 6. -- Gall indices of resistant and susceptible tomato cultivars inoculated with root-knot nematode populations established from single egg-masses from fields Q-1 and P-2 of Poamoho Experimental Farm

Inoculum	Tomato cultivar	
	Healani	Yellow Plum
Yellow Plum Q-1		4.7
Yellow Plum P-2	1.0	4.3
Healani Q-1.1	4.7	
Healani Q-1.2	3.7	
Healani Q-1.3	4.3	
Healani Q-1.4	3.7	
LSD 5%	1.2	
1%	1.7	

c. Competition test

The pair of tomato cultivars 'Yellow Plum' and 'Healani' clearly showed differences in galling and egg-mass production when they were inoculated with the root-knot nematodes from field P-2, but no differences when Q-1 inoculum was used (Table 7). As in the previous tests, this result indicates a distinctive race of root-knot nematode in field Q-1 which attacks with the same pathogenicity both the resistant and susceptible cultivars. However, the

Table 7. -- Gall and egg-mass indices of tomato cultivar pairs inoculated with root-knot nematodes established from single egg-masses from fields Q-1 and P-2 of Poamoho Experimental Farm

Tomato pair	Inoculum			
	Healani Q-1		Yellow Plum P-2	
	Gall indices	Egg-mass indices	Gall indices	Egg-mass indices
Yellow Plum	3.9	3.9	3.6	4.0
Healani	3.8	4.0	1.0	1.0

root-knot nematodes from field P-2 attack only the susceptible cultivar. If most M. incognita populations were similar to that in field P-2, alternating a resistant with a susceptible cultivar in a rotation program could be a way to keep the resistance effective. Sauer & Giles (1959) found that a resistant tomato cultivar was stable against M. javanica when a susceptible cultivar was included in a rotation, but resistance was ineffective after 5 continuous croppings with the resistant tomato when a more pathogenic

M. javanica population was formed.

d. Effect of environment and root-knot nematode sources on the resistant and susceptible cultivars

(1) 'Yellow Plum'

The gall and egg-mass indices clearly indicate the susceptibility of 'Yellow Plum' (Table 8). There were no differences between the levels of inoculum, the sources of inoculum, or the temperatures.

(2) 'Healani'

There were differences in gall and egg-mass indices when the two sources of root-knot nematodes were compared. This again showed that the cultivar 'Healani' behaves differently toward these sources of inoculum. With inoculum from field Q-1, the response of the cultivar 'Healani' to the nematodes was the same as the cultivar 'Yellow Plum', which indicates susceptibility. However, when field P-2 inoculum was used, to which 'Healani' was resistant, the effects of soil temperature and inoculum level on galling could also be observed. At soil temperatures of $21^{\circ} - 33^{\circ}\text{C}$ and $23.5^{\circ} \pm 1^{\circ}\text{C}$ no galling was observed. At soil temperatures of $29^{\circ} \pm 1^{\circ}\text{C}$ and $34^{\circ} \pm 1^{\circ}\text{C}$ the inoculum level of 500 larvae/plant caused small galls, but with the inoculum level of 2,500 larvae/plant, moderate galling was observed. This rate of galling was not significantly different from that obtained when field Q-1

Table 8. -- Gall and egg-mass indices of resistant and susceptible tomato cultivars inoculated with 2 sources of root-knot nematodes, at 2 inoculum levels, and at 4 soil temperatures

Source of inoculum	Level of inoculum	Soil temperature	Y. Plum		Healani	
			Gall indices	Egg-mass indices	Gall indices	Egg-mass indices
Field Q-1	500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	3.0	2.7	3.0	3.3
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	3.0	2.3	2.7	2.3
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	3.0	2.7	3.3	2.7
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	2.7	2.0	2.7	2.3
	2,500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	4.0	3.3	3.3	3.3
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	3.3	2.7	3.3	2.7
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	4.0	3.7	3.7	3.7
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	3.3	2.7	3.0	2.0
Field P-2	500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	2.7	2.3	1.0	1.0
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	2.7	1.7	1.0	1.0
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	2.7	2.3	2.0	1.3
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	2.0	2.0	2.0	1.3
	2,500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	3.7	2.7	1.0	1.0
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	3.7	2.7	1.0	1.0
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	3.7	3.7	3.0	1.7
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	2.7	2.0	3.0	1.3
LSD 5%			1.2	1.0	.8	.8
1%			1.6	1.4	1.1	1.0

'Y. Plum'='Yellow Plum'

inoculum was used. It can be concluded that, with 2,500 larvae/plant and soil temperature $29^{\circ} \pm 1^{\circ}\text{C}$ to $34^{\circ} \pm 1^{\circ}\text{C}$, the resistance was overcome to the same degree that it was by the root-knot nematode population from field Q-1. The threshold of soil temperature where the resistant performance was lower was also investigated by Holtzmann (1965). He found that the percentage of root-knot nematode (*M. incognita*) penetrance and mature females in the roots of the resistant tomato began to increase considerably at 30°C , and increased even more at 34.5°C . The ability of the root-knot nematode to grow inside the resistant tomato roots was also found to increase as the temperature was elevated from 28° to 33°C (Dropkin, 1969b).

The monogenic tomato resistance was effective at $23.5^{\circ} \pm 1^{\circ}\text{C}$ but declined at $29^{\circ} \pm 1^{\circ}\text{C}$. This agrees with Holtzmann's (1965) results on tomato resistance and Walker's (1957) observation on Cabbage Yellows resistance.

3. Soybean

a. Field test

Gall indices of the susceptible cultivar 'Bansei' were relatively lower than expected throughout the experiment. In all three fields, they ranged from 2.0 to 3.0 in most months (Fig. 7). An assumption can be made that the inoculum requirement to induce high gall indices on soybean is higher than what was available in these fields. Gall

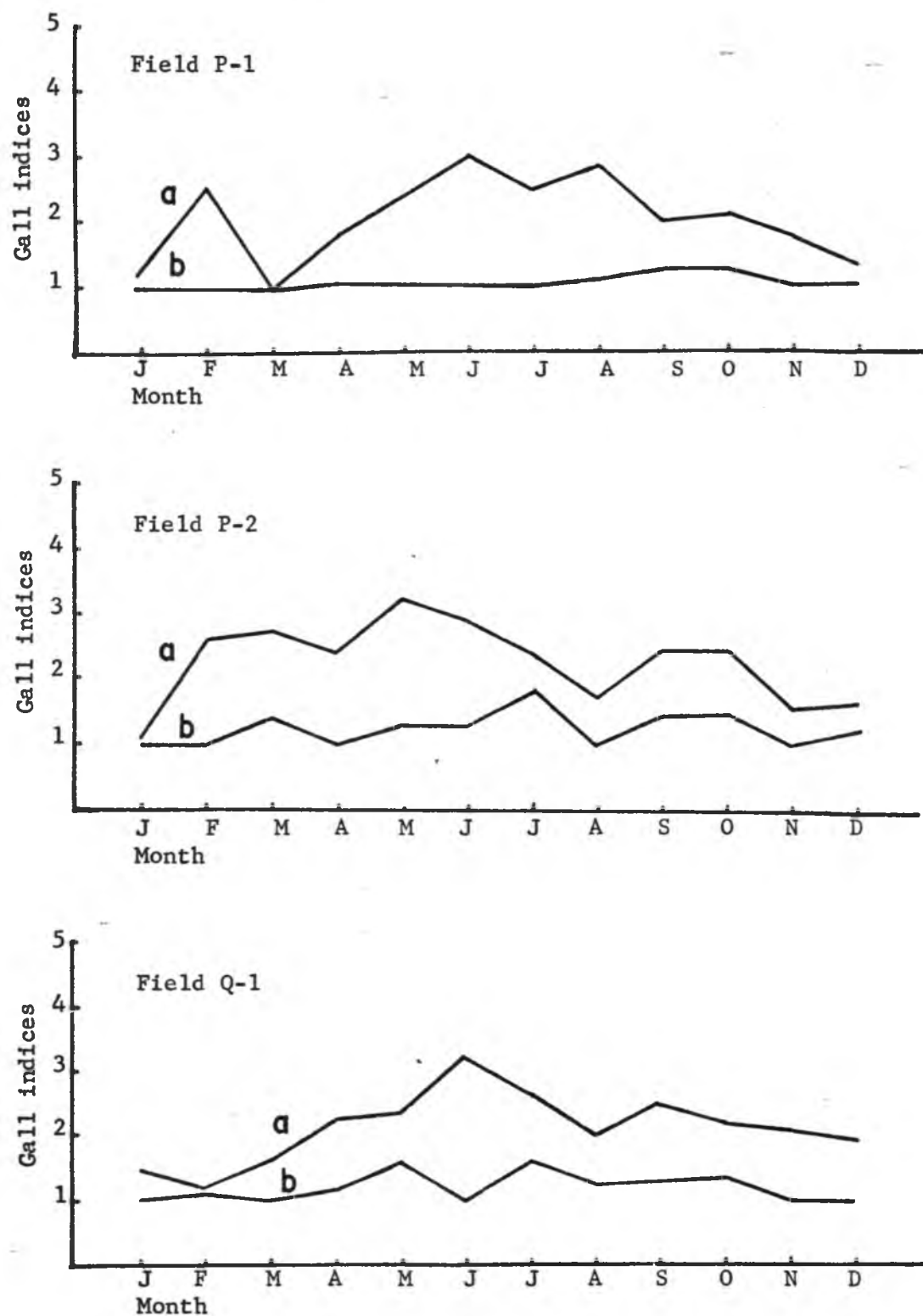


Fig. 7. Gall indices of soybean cultivars (a) 'Bansei' (s) and (b) 'Kailua' (r) planted monthly in 3 fields of Poamoho Experimental Farm

indices of resistant cultivar 'Kailua' never reached 2.0 in fields Q-1 and P-2, and were even lower in field P-1.

b. Resistance to known inoculum

(1) Sequential inoculation with populations established from galled roots

The root-knot nematode population from field P-2 was able to cause gall formation on the resistant 'Kailua' in the first two inoculations, but the subsequent inoculation failed (Fig. 8). The gall indices from the first two inoculations were 2.9 and 2.4, significantly higher than for the third inoculation which had 1.0 gall index. The next inoculation, which had to be reinoculated from 'Bansei' galled roots, had a gall index of 1.8, but the following inoculation again had a gall index of 1.0 (Table 9). Comparing the results with the gall indices of the susceptible 'Bansei' which were stable around 2.4 and 2.9, it may be concluded that the first two inoculations of 'Kailua' which resulted in some degree of galling were probably the result of mass action of root-knot nematode larvae rather than a more virulent M. incognita.

The inoculation of 'Kailua' with the nematode population from field Q-1 produced no galling at all. This is similar to the results with the tomato cultivar 'Healani' inoculated with the root-knot nematode population from field P-2.

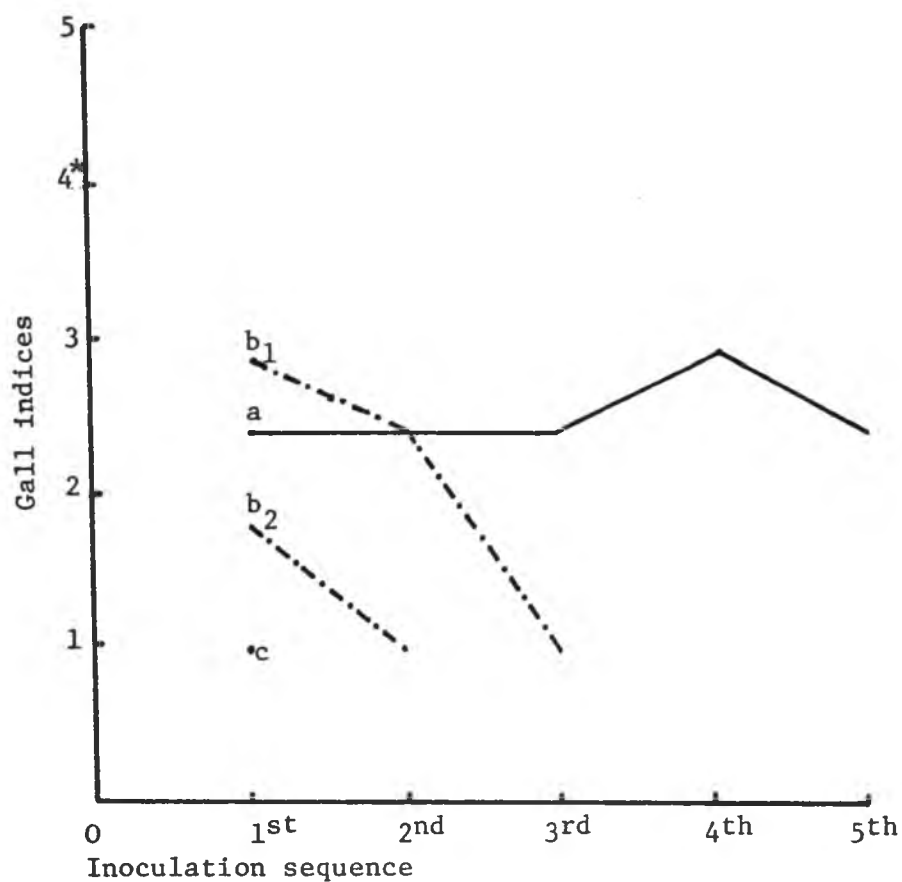


Fig. 8. Gall indices of soybean cultivars in the sequential inoculation

* = Gall rate on 'Yellow Plum' stock plant

a = Cultivar 'Bansei' inoculated with root-knot nematodes from Q-1 and P-2 fields (Average)

b₁ = First attempt at inoculating cultivar 'Kailua' with root-knot nematodes from field P-2

b₂ = Second attempt at inoculating cultivar 'Kailua' with root-knot nematodes from field P-2

c = Cultivar 'Kailua' inoculated with root-knot nematodes from field Q-1, all five inoculations resulted in no galling

Table 9. -- Gall indices of resistant and susceptible soybean cultivars inoculated with root-knot nematodes from fields Q-1 and P-2 of Poamoho Experimental Farm, in five inoculations in succession

Inoculation sequence	Soybean cultivar			
	Kailua		Bansei	
	Inoculum		Inoculum	
	Q-1 ^a	P-2 ^b	Average Q-1 & P-2	
1 st	1.1	2.9	1.8	2.4
2 nd	1.0	2.4	1.2	2.4
3 rd		1.0		2.4
4 th				2.9
5 th				2.4
LSD 5%	.2	.7		
1%	.8	.9		

^aThree inoculations resulted in no galling

^bThe third inoculation resulted in no galling, the fourth inoculation was restarted from susceptible 'Bansei'.

(2) Inoculation with populations established from single egg-masses

The resistant 'Kailua' inoculated with the root-knot nematode populations from fields Q-1 and P-2 either formed no galls (index of 1.0) or a few (index of 1.7) (Table 10). These small galls, as in the case of snap-bean cultivar 'Manoa Wonder', may be considered the normal expression of the resistant cultivar. This agreed with the previous test (Table 9) in which the resistance of 'Kailua' maintained its effectiveness.

Table 10. -- Gall indices of resistant and susceptible soybean cultivars inoculated with root-knot nematode populations established from single egg-masses from fields Q-1 and P-2 of Poamoho Experimental Farm

Inoculum	Soybean cultivar	
	Kailua	Bansei
Bansei Q-1	1.0	2.3
Bansei P-2		2.0
Kailua P-2.1	1.7	
Kailua P-2.2	1.0	
Kailua P-2.3	1.7	
Kailua P-2.4	1.0	
LSD 5%	.7	
1%	.9	

c. Competition test

Inoculation of the soybean pairs produced moderate galling on the susceptible cultivar and no galling on the resistant cultivar (Table 11). Egg-mass indices also were moderate on 'Bansei' and none on 'Kailua'. There was no racial difference between the Q-1 and P-2 root-knot nematode populations as far as responses of these soybean cultivars were concerned.

Table 11. -- Gall and egg-mass indices of soybean cultivar pairs inoculated with root-knot nematodes established from single egg-masses from fields Q-1 and P-2 of Poamoho Experimental Farm

Soybean pair	Inoculum			
	Bansei Q-1		Kailua P-2	
	Gall indices	Egg-mass indices	Gall indices	Egg-mass indices
Bansei	3.3	3.3	3.0	2.3
Kailua	1.0	1.0	1.0	1.0

d. Effect of environment and root-knot nematode sources on the resistant and susceptible cultivars

(1) 'Bansei'

Light to moderate galling and low reproduction were obtained from the roots of the cultivar 'Bansei' when it was inoculated with 500 larvae/plant (Table 12). With the higher inoculum level, moderate galling and egg-mass production were obtained. Apparently an even higher inoculum level is required to obtain heavy galling.

The effect of temperature was mostly not significant, but the soil temperature of $29^{\circ} \pm 1^{\circ}\text{C}$ showed the highest indices. When the temperature was raised to $34^{\circ} \pm 1^{\circ}\text{C}$, the galling and reproduction often declined, probably due to a decline in the nematode's ability to grow and reproduce. There was no effect from the source of inoculum, which indicated there was no nematode racial difference.

(2) 'Kailua'

All treatments were unsuccessful in inducing galling and reproduction on the cultivar 'Kailua', except the 2,500 larvae/plant inoculum level from field Q-1 at the soil temperature of $34^{\circ} \pm 1^{\circ}\text{C}$, which produced only a few small galls and very light reproduction. The resistance of the cultivar 'Kailua' was effective regardless of soil temperature, inoculum level, or source of inoculum (no racial difference).

Table 12. -- Gall and egg-mass indices of resistant and susceptible soybean cultivars inoculated with 2 sources of root-knot nematodes, at 2 inoculum levels, and at 4 soil temperatures

Source of inoculum	Level of inoculum		Bansei		Kailua	
			Gall indices	Egg-mass indices	Gall indices	Egg-mass indices
Field Q-1	500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	1.7	1.0	1.0	1.0
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	2.3	2.0	1.0	1.0
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	3.0	2.0	1.0	1.0
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	2.0	2.0	1.0	1.0
	2,500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	3.0	1.0	1.0	1.0
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	3.3	2.0	1.0	1.0
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	3.7	3.3	1.0	1.0
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	3.0	2.3	2.0	2.0
Field P-2	500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	2.0	1.0	1.0	1.0
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	2.0	1.3	1.0	1.0
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	2.7	1.7	1.0	1.0
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	1.7	2.0	1.0	1.0
	2,500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	3.0	1.0	1.0	1.0
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	2.3	1.3	1.0	1.0
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	3.0	2.0	1.0	1.0
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	2.0	2.3	1.0	1.0
LSD 5%			.7	.7	-	-
1%			1.0	1.0	-	-

4. Lima bean

a. Field test

In field Q-1 (Fig. 9), the susceptible 'White Ventura 65' showed little galling with gall indices around 2.0 to 3.0 in most months. Tolerant 'White Ventura N' was relatively free from galling in most plantings. In field P-1, however, 'White Ventura 65' showed gall indices between 3.0 and 4.0 which indicated susceptibility, while 'White Ventura N' gall indices were mostly below 2.0, except in August, September, and October, when they were 2.2, 2.5, and 2.8, respectively. Considering these results and those of Q-1, it seems that the tolerance in cultivar 'White Ventura N' is expressed most when the susceptible 'White Ventura 65' was not galled heavily, which perhaps was due to the level of inoculum. The seasonal effect on the gall indices of 'White Ventura 65' was not pronounced, but on the gall indices of 'White Ventura N' there was an increased galling trend from July to October. A correlation test between monthly gall indices and bimonthly rainfall was found to have a correlation coefficient of +.17, which was not significant.

Gall indices in field P-2 of 'White Ventura 65' were similar to those in field P-1. However, 'White Ventura N' showed gall indices around 2.0 to 3.0 in most months, which were higher than those in field P-1. Since the root-knot nematode species in both fields was M. incognita, the population in field P-2 might be more able to attack

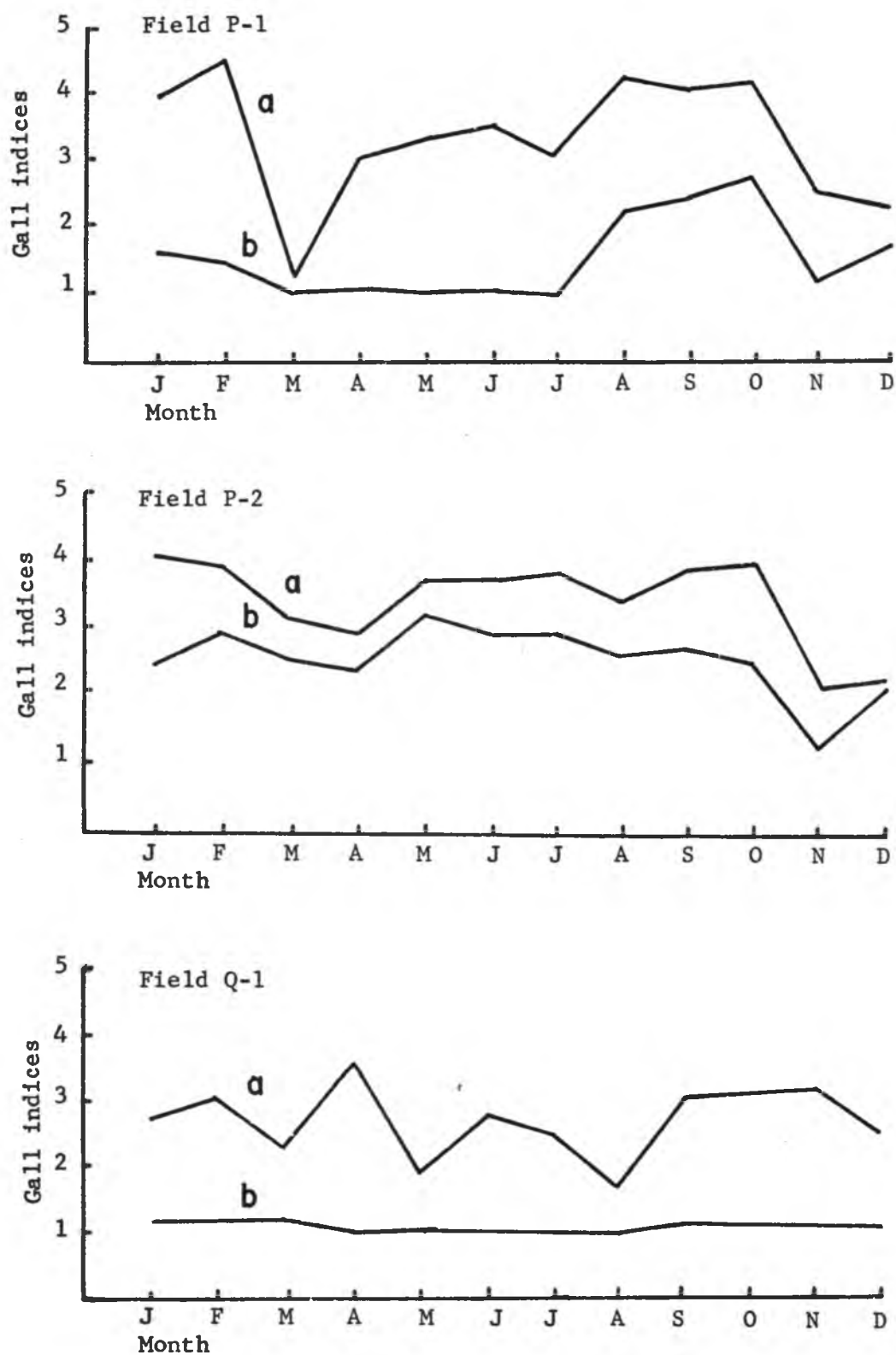


Fig. 9. Gall indices of lima bean cultivars (a) 'White Ventura 65'(s) and (b) 'White Ventura N'(tolerant) planted monthly in 3 fields of Poamoho Experimental Farm

'White Ventura N' than the population in field P-1. Monthly gall indices of 'White Ventura N' grown in field P-2 showed significant differences, the highest was 3.3 for the May planting, the lowest were 1.3 and 2.2 for the November and December plantings, and the rest ranged from 2.4 to 3.0. A test to measure the degree of correlation between the gall indices and the bimonthly rainfall during which the plants were grown showed a correlation coefficient of $-.23$. Soil temperature effect during the summer was not pronounced with little difference in gall index from January through October.

b. Resistance to inoculum

(1) Sequential inoculation with populations established from galled roots

The tolerant cultivar 'White Ventura N' could be galled by the root-knot nematode population from field P-2 (Fig. 10, Table 13). The gall indices from the first and second inoculations were 3.9 and 3.8, 2.8 from the third inoculation, and 5.0 and 4.3 from the fourth and fifth inoculation. With the inoculum from field Q-1, it took three inoculations before a significant increase of virulence (from 1.5 to 3.2 to 4.7) of root-knot nematode on 'White Ventura N' was observed. There was no difference between the gall indices of 'White Ventura N' and 'White Ventura 65' from the 5th inoculation. The tolerance in the cultivar 'White Ventura N' could result in lower gall development,

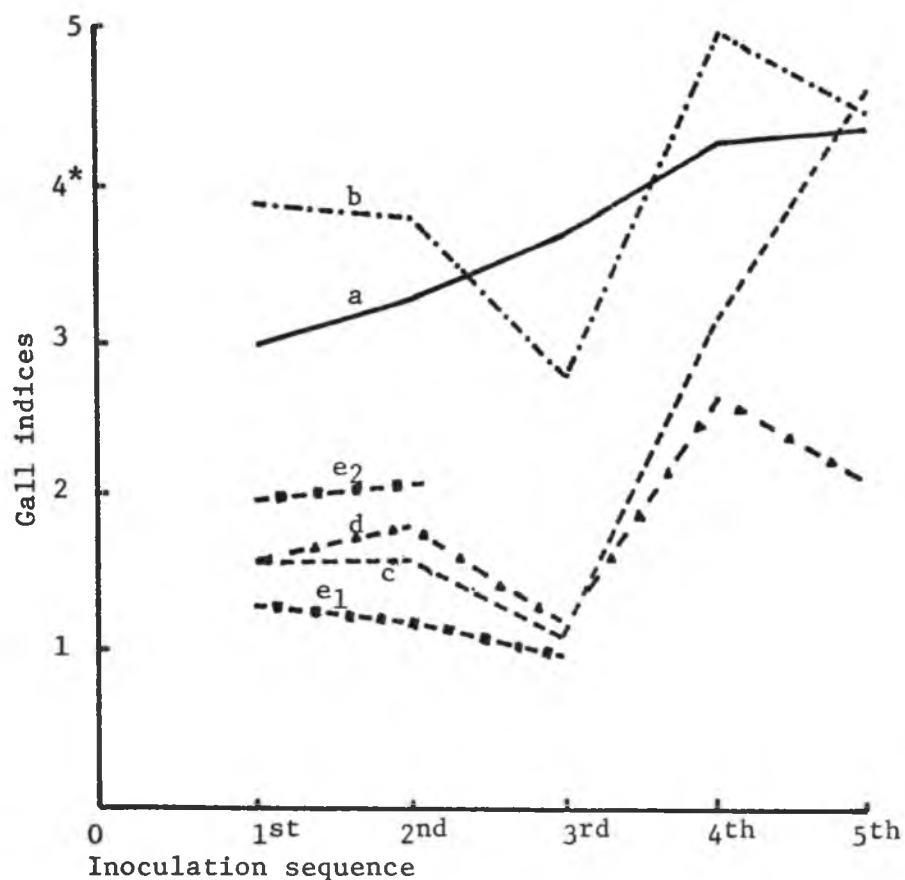


Fig. 10. Gall indices of lima bean cultivars in the sequential inoculation

- * = Gall rate on 'Yellow Plum' stock plant
- a = Cultivar 'White Ventura 65' inoculated with root-knot nematodes from fields Q-1 and P-2 (Average)
- b = Cultivar 'White Ventura N' inoculated with root-knot nematodes from field P-2
- c = Cultivar 'White Ventura N' inoculated with root-knot nematodes from field Q-1
- d = L-136 inoculated with root-knot nematodes from field P-2
- e₁ = First attempt at inoculating L-136 with root-knot nematodes from field Q-1, the 3rd inoculation resulted in no galling
- e₂ = Second attempt at inoculating with root-knot nematodes from field Q-1

Table 13. -- Gall indices of resistant and susceptible lima bean cultivars inoculated with root-knot nematodes from fields Q-1 and P-2 of Poamoho Experimental Farm, in five inoculations in succession

Inoculation sequence	Lima bean cultivar					
	White Ventura N Inoculum		L-136 Inoculum		White Ventura 65 Inoculum	
	Q-1	P-2	Q-1 ^a	P-2	Average Q-1 & P-2	
1 st	1.6	3.9	1.4	2.0	1.6	3.1
2 nd	1.6	3.8	1.2	2.2	1.8	3.3
3 rd	1.5	2.8	1.0		1.7	3.7
4 th	3.2	5.0			2.7	4.3
5 th	4.7	4.3			2.2	4.4
LSD 5%	.6	.5	.5	.5		
1%	.8	.6	.7	.7		

^aThe third inoculation resulted in no galling, the fourth inoculation was restarted from susceptible 'White Ventura 65'.

but, given adequate time, the nematode can adjust to the cultivar and parasitize it equally. This was seen with the root-knot nematode population from field Q-1, while the population from field P-2 seemed to be able to infect 'White Ventura N' readily.

The results with line L-136 inoculated with the population from field P-2 were similar to those of 'White Ventura N' except that the gall indices were not as high. Indices from the first three inoculations were about 1.6, increased significantly to 2.7, and then decreased non significantly to 2.2 (Table 13). Further continuous inoculation could perhaps induce a nematode population more able to parasitize this line.

However, when line L-136 was inoculated with the field Q-1 population, the first three attempts produced no galls. A fourth attempt resulted in a gall index of 2.0 which increased to 2.2 in the next inoculation. Apparently the Q-1 root-knot nematode population was not able to attack the line L-136 like the population of P-2.

(2) Inoculation with populations established from single egg-masses

All of the root-knot nematode populations from fields Q-1 and P-2 were able to cause galling on 'White Ventura N' (Table 14). The gall indices were above 3.0, except one from P-2.1 inoculum which was 2.0 and significantly different from all the rest. The gall indices of the susceptible 'White Ventura 65' were 3.7 and 4.0. It can be concluded that the cultivar 'White Ventura N' was readily attacked by most of the root-knot nematodes from both fields. The same results were obtained in the previous test (Table 13).

Inoculated with root-knot nematode populations from fields Q-1 and P-2, the resistant line L-136 showed no or little galling. These results were comparable to the first inoculation of the previous test (Table 13).

Steiner (1925) observed that nematodes would feed on the same plants as their ancestors, but when the compatible host was not available, the nematodes then were forced by starvation to feed on other plants.

Table 14. -- Gall indices of resistant and susceptible lima bean cultivars inoculated with root-knot nematode populations established from single egg-masses from fields Q-1 and P-2 of Poamoho Experimental Farm

Inoculum	Lima bean cultivar		
	W. Ventura N	L-136	W. Ventura 65
W. Ventura 65 Q-1		1.0	3.7
W. Ventura 65 P-2		1.3	4.0
W. Ventura N Q-1	3.3		
W. Ventura N P-2.1	2.0		
W. Ventura N P-2.2	3.3		
W. Ventura N P-2.3	3.7		
W. Ventura N P-2.4	4.7		
LSD 5%	1.1		
1%	1.6		

'W. Ventura 65' = 'White Ventura 65', 'W. Ventura N' = 'White Ventura N'

Only a small fraction of nematodes probably would survive on the new host, or perhaps none would survive. The surviving nematodes, however, if they were able to reproduce, could form a new population with a different ability to parasitize plants. Resistant and susceptible cultivars can be considered as the new and old host for the root-knot nematode, respectively. When the old host on which the root-knot nematode used to feed is unavailable, the resistant cultivar may be attacked.

c. Competition test

Tolerant cultivar, 'White Ventura N' and the resistant line L-136 were galled and egg-masses were produced on the roots, even in the presence of the susceptible cultivar 'White Ventura 65', when inoculum from either Q-1 or P-2 was used (Table 15). However, gall and egg-mass indices were, except in one case, lower on the tolerant cultivar and the resistant line than on the susceptible cultivar.

Comparing 'White Ventura N' with and without susceptible cv. 'White Ventura 65', there was no difference in galling and reproduction, except for the egg-mass index when inoculated with Q-1 root-knot nematode. L-136 showed similar results.

From this experiment it can be concluded that 'White Ventura N' and L-136 are partially resistant, and there was no racial difference of M. incognita tested.

Table 15. -- Gall and egg-mass indices of lima bean cultivar pairs inoculated with root-knot nematodes established from single egg-masses from fields Q-1 and P-2 of Poamoho Experimental Farm

Lima bean pair	Inoculum			
	White Ventura N Q-1		White Ventura N P-2	
	gall indices	egg-mass indices	gall indices	egg-mass indices
White Ventura 65	4.0	4.0	3.7	4.0
White Ventura N	2.3	3.0	2.7	3.7
White Ventura 65	3.7	4.0	3.7	4.0
L-136	2.0	3.0	2.0	2.7
White Ventura N	3.0	4.0	3.3	4.0
L-136	2.7	4.0	2.7	4.0
LSD 5%	.8	.8	.9	.6
1%	1.0	1.0	1.3	.8

d. Effect of environment and root-knot nematode sources on the resistant and susceptible cultivars

(1) 'White Ventura 65'

Similar to the other susceptible cultivars, the cultivar 'White Ventura 65' was always galled and root-knot nematode reproduction always took place (Table 16). The effect of temperature was not pronounced, except that gall and egg-mass indices were lower at the soil temperature of $34^{\circ} \pm 1^{\circ}\text{C}$ than at $29^{\circ} \pm 1^{\circ}\text{C}$, probably due to slower root-knot nematode development. An increase of inoculum level usually increased the indices of galling and reproduction. There was no difference between the 2 sources of inoculum.

Table 16. -- Gall and egg-mass indices of resistant and susceptible lima bean cultivars inoculated with two sources of root-knot nematodes, at two inoculum levels, and at four soil temperatures

Source of inoculum	Level of inoculum	Soil temperature	WV.65		WV.N.		L-136	
			Gall ind.	Egg-mass rates	Gall ind.	Egg-mass ind.	Gall ind.	Egg-mass indi.
Field Q-1	500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	3.0	2.7	1.0	1.0	2.0	1.7
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	3.0	2.3	1.0	1.0	1.7	1.3
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	3.0	2.7	1.0	1.0	1.7	1.7
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	2.7	2.0	1.0	1.0	1.7	1.3
	2,500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	3.3	3.3	2.3	2.0	1.7	1.3
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	3.3	2.7	1.0	1.0	2.3	1.7
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	4.0	3.7	2.0	3.3	2.3	3.3
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	3.3	2.7	1.0	1.0	2.7	2.7
Field P-2	500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	2.7	2.3	1.0	1.3	1.3	1.0
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	2.7	1.7	1.0	1.0	1.7	1.3
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	2.7	2.3	1.0	1.0	1.7	1.7
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	2.0	2.0	1.0	1.0	1.7	1.7
	2,500 larvae per plant	$T_0=21^{\circ}-33^{\circ}\text{C}$	3.7	2.7	1.7	1.3	2.0	2.7
		$T_1=23.5^{\circ}\pm 1^{\circ}\text{C}$	3.7	2.0	1.0	1.0	2.0	3.3
		$T_2=29^{\circ}\pm 1^{\circ}\text{C}$	3.7	3.7	2.7	2.3	2.7	3.7
		$T_3=34^{\circ}\pm 1^{\circ}\text{C}$	2.7	2.0	1.7	1.3	2.7	3.3
LSD 5%			1.2	1.0	.5	.8	.9	.9
1%			1.6	1.4	.6	1.0	1.2	1.2

'WV.65' = 'White Ventura 65', 'WV.N.' = 'White Ventura N.', Gall ind. = Gall indices, Egg-mass ind. = Egg-mass indices

(2) 'White Ventura N'

The inoculum level had a significant effect on the galling and egg-mass indices. Most of the 500 larvae/plant inoculum level produced 1.0 indices. At the 2500 larvae/plant inoculum level the gall and egg-mass indices were light to moderate at soil temperatures of $21^{\circ} - 33^{\circ}\text{C}$ and $29^{\circ} \pm 1^{\circ}\text{C}$, and were higher than at $23.5^{\circ} \pm 1^{\circ}\text{C}$ and $34^{\circ} \pm 1^{\circ}\text{C}$. Based on these results, it can be concluded that the tolerance of the cultivar 'White Ventura N' is due to an increase in the inoculum level required to induce galling and reproduction. In addition, the cultivar 'White Ventura N' showed lower gall and egg-mass indices than 'White Ventura 65', and thus may be considered partially resistant. The effect of the source of inoculum was not pronounced which indicates there is no difference in root-knot nematode virulence.

(3) L-136

When L-136 was compared to the cultivar 'White Ventura 65', it showed some resistance. There was no difference in response of L-136 to the different sources of inoculum which can be interpreted that there were no different races in the sources of inoculum. The effect of soil temperature on the gall indices within the same inoculum level was not significant, and the reproduction rate increased significantly only at $29^{\circ} \pm 1^{\circ}\text{C}$ and $34^{\circ} \pm 1^{\circ}\text{C}$ with inoculum from field Q-1 at 2,500 larvae/plant. An

increase of infestation was also found when the inoculum level was raised from 500 to 2,500 larvae/plant, but this occurred mostly at soil temperatures of $29^{\circ} \pm 1^{\circ} \text{C}$ and $34^{\circ} \pm 1^{\circ} \text{C}$. In general, L-136 was partially resistant, but responded to the soil temperature, inoculum source, and inoculum level like the susceptible 'White Ventura 65'.

C. Selection of root-knot nematode race which is capable of parasitizing resistant cultivars of more than one vegetable species

The attempt to develop a root-knot nematode population which is able to attack both the resistant tomato cultivar 'Healani' and the resistant snap-bean cultivar 'Manoa Wonder' was not successful. Despite the application of a higher inoculum level, the highest gall index obtained from cultivar 'Healani' inoculated with root-knot nematode population 'Manoa Wonder' P-2 was 1.5 (Table 17). Small galls like these are often observed when the resistant tomato is grown in a root-knot nematode infested field. The cultivar 'Manoa Wonder' inoculated with root-knot nematode population

Table 17. -- Gall indices of resistant cultivars of snap-bean, tomato, and lima bean inoculated with root-knot nematode populations which are able to attack one resistant cultivar

Inoculum	Cultivar inoculated	Gall indices	
		1 st test	2 nd test
Healani Q-1	Manoa Wonder	1.0	1.0
Manoa Wonder P-2	Healani	1.3	1.5
Healani Q-1	White Ventura N	3.3	3.3
White Ventura N Q-1	Healani	2.8	3.3
White Ventura N P-2	Healani	1.5	1.0

'Healani' Q-1 also did not produce galls (index of 1.0). Apparently these root-knot nematode populations are far apart in their specialization for resistant tomato and snap-bean cultivars. Riggs & Winstead (1959) reported similar results with 'Biotype B' of M. incognita, which could attack resistant tomato but not resistant snap-bean cv. 'Alabama #1'.

With reciprocal inoculation of the cultivars 'Healani' and 'White Ventura N', the root-knot nematode population 'Healani' Q-1 could cause considerable galling on 'White Ventura N', and the root-knot nematode population 'White Ventura N' Q-1 could do the same on 'Healani' (Table 17). The cultivar 'White Ventura N' performed like a susceptible cultivar under a heavy inoculum level (200 g/gallon of soil) as indicated by its gall indices of 3.3 in both tests. This confirmed that galling of 'White Ventura N' is dependent upon inoculum level. The root-knot nematode population 'White Ventura N' Q-1 was the same M. incognita population from field Q-1 which was able to cause galling on cultivar 'Healani', although the 1st test produced only a 2.8 gall index. The following test produced a 3.3 gall index which indicated a positive host parasite compatibility relationship. However, the attempt to induce gall formation on the cultivar 'Healani' by inoculating with the root-knot nematode population 'White Ventura N' P-2 failed. This root-knot nematode population of M. incognita was similar to others from field P-2 which could not gall the resistant 'Healani'.

V. CONCLUSIONS

A. Species of root-knot nematode

The species of root-knot nematode in this study was Meloidogyne incognita. Although M. javanica was also observed in the field population, evidence was that it was insignificant.

B. Races of M. incognita

A pathogenic race of M. incognita from field Q-1 which is able to parasitize resistant tomato cultivars as previously observed by Gilbert (1959) and Holtzmann and Gilbert (1969) was confirmed. The root-knot nematode population from field P-2 which has been observed (Hartmann, personal communication) to attack resistant snap-bean was found to have an ability to increase in virulence, but evidence did not show a distinctive race in the field. Racial differences were not observed in root-knot nematode populations inoculated on resistant soybean and tolerant lima bean cultivars.

C. Relationship between the inheritance of resistance and the effect of temperature

The performance of monogenic tomato resistance which was effective at $23.5^{\circ} \pm 1^{\circ}\text{C}$ but not at $29^{\circ} \pm 1^{\circ}\text{C}$ agreed with the results of Holtzmann (1965) with nematode-resistant tomato and Walker (1957) with monogenically-controlled resistance to Cabbage Yellows. However, polygenically-controlled resistance in snap-bean was effective at $23.5^{\circ} \pm 1^{\circ}\text{C}$ and thus did not agree with Walker's (1957) observation that polygenically-controlled resistance, as in Cabbage Yellows', was effective only up to 22°C . In addition,

the tentatively determined monogenic resistance of soybean was effective up to $29^{\circ} \pm 1^{\circ}\text{C}$, higher than was to be expected.

- D. The attempt to obtain a root-knot nematode population which is able to parasitize resistant cultivars of more than one crop was not successful.
- E. The factors which cause galling or ineffectiveness of resistant cultivars

1. Snap-bean

Resistant cv. 'Manoa Wonder' was galled moderately in field P-2 and not in fields Q-1 and P-1 which indicated a more virulent P-2 strain of M. incognita. The sequential inoculations (five times) which resulted in an increase of virulence of P-2 M. incognita on 'Manoa Wonder', confirms that P-2 M. incognita has a potential to increase its virulence and thus decrease the effectiveness of 'Manoa Wonder' resistance. Since the initial inoculation with P-2 inoculum resulted in only light to moderate galling, as did the inoculation with the single egg-mass culture from this field, the conclusion is that P-2 M. incognita has not yet reached a stage of becoming a distinct, pathogenic race to 'Manoa Wonder', but does have the potential to do so.

At the 500 and 2,500 larvae/plant inoculum levels, P-2 M. incognita did not decrease the effectiveness of 'Manoa Wonder' resistance, probably due to a relatively low inoculum level as compared to 50 grams of galled roots/gallon of potting medium.

Elevated soil temperature of $29^{\circ} \pm 1^{\circ}\text{C}$ reduced the resistance of 'Manoa Wonder' significantly. Similar reduction of resistance in snap-bean was observed at 28°C by Fassuliotis (1970). However, under fluctuating soil temperature the resistance of 'Manoa Wonder' was effective, although light galling sometimes occurred.

The performance of P.I. 165,426 was similar to that of 'Manoa Wonder', except that in most cases this line performed better and showed a more stable resistance to M. incognita. Therefore it should be good resistant breeding material, although it is also subject to attack by the more virulent P-2 strain of the root-knot nematode.

2. Tomato

The previously observed severe galling of resistant tomato cultivars in field Q-1 of Poamoho Experimental Farm (Gilbert, 1959, Holtzmann & Gilbert, 1969) was confirmed. All results lead to the conclusion that the galling was caused by a new, more virulent race of M. incognita which was already established in the field. The ability of the Q-1 race of nematodes to parasitize 'Healani' was observed in the field test, the sequential inoculation, the inoculation in the presence of a susceptible cultivar, and the inoculation at different soil temperatures. This race has similar virulence to the 'Biotype B' M. incognita of Riggs and Winstead (1959). In the field this race showed a stable infection in resistant cv. 'Healani', although the degree of galling depended on the natural fluctuating population level.

In addition, a significantly reduced level of resistance was also observed at a soil temperature of $29^{\circ} \pm 1^{\circ}\text{C}$. However, under fluctuating soil temperature (in the field or the greenhouse) the resistance of 'Healani' was effective.

3. Soybean

Since resistant cultivar 'Kailua' was released in 1970, there has been no report of a reduction of the effectiveness of its resistance. Results of this study confirmed the effectiveness of the resistance of 'Kailua'. Cultivar 'Kailua' was almost free from root-knot nematode galling and reproduction in the field test. Furthermore, the attempt to induce the formation of a more virulent M. incognita in the sequential inoculation was not successful, which indicated that the genetic resistance was strong. The resistance was also effective at both fluctuating temperature ($21^{\circ} - 33^{\circ}\text{C}$) and continuous elevated soil temperature up to $34^{\circ} \pm 1^{\circ}\text{C}$ at the inoculum levels tested. At higher inoculum levels, the effectiveness of resistance may still need further investigation.

4. Lima bean

The galling of the tolerant cv. 'White Ventura N' in the field occurred only when the inoculum level was high which was indicated by heavy galling of susceptible 'White Ventura 65'. The effect of inoculum level on galling of 'White Ventura N' was corroborated in the greenhouse when the inoculum level was increased from 500 to 2,500 larvae/plant. Therefore, 'White Ventura N' is partially resistant and the galling in

the field at some times was dependent upon the level of inoculum.

The sequential inoculation proved that there was no racial difference in Q-1 and P-2 M. incognita populations inoculated on 'White Ventura N', except that Q-1 M. incognita needed some time to adapt in parasitizing the cultivar.

At elevated soil temperature ($29^{\circ} \pm 1^{\circ}\text{C}$) the galling of 'White Ventura N' was increased with 2,500 larvae/plant inoculum level. Higher inoculum level could possibly cause a susceptible response as in the sequential inoculation.

The resistant line L-136 proved also partially resistant, but it was stable with little or no change in galling and nematode reproduction at increased soil temperature, or at different levels and sources of inoculum. This line could possibly contribute its partial resistance in a breeding program.

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