Infectivity of Two Biotypes of the Citrus Nematode on Citrus and on Some Other Hosts

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Abstract: The infectivity and development of two biotypes of citrus nematode (*Tylenchulus semipenetrans*) were compared on highly resistant *Poncirus trifoliata* selection 'Pomeroy,' moderately susceptible 'Troyer' citrange, and highly susceptible sweet orange selection 'Homosassa' small seedlings in a glasshouse. Biotype-1 was more infective on the above hosts and developed faster on sweet orange and on 'Troyer' citrange than Biotype-2. The differences in infectivity were interpreted to reflect differences in the ability of the nematodes to penetrate the epidermis and hypodermis and become established in host roots. *Poncirus* selections 'Pomeroy,' 'Webber-Fawcett,' and 'Rubidoux' seedlings were highly resistant to the citrus nematode in California, but seedlings of 'Pomeroy' and 'Rubidoux' were only moderately resistant in Japan. These differences in degree of infection may indicate different biotypes of the nematode. Host range tests with California Biotype-1 indicate that it differs from those occurring in Israel.

Large differences in infectivity of the citrus nematode (*Tylenchulus semipenetrans* Cobb), on 'Troyer' citrange (a hybrid of navel orange pollinated with *Poncirus trifoliata* Raf.) in orchards in California, were briefly reported by Baines *et al.* (1, 2). These differences seemed to represent different populations or biotypes of the nematode. Another biotype of the citrus nematode may exist in Israel. Cohn (5) observed grape and olive to be free of the citrus nematode in Israel and considered their population to be different from those found in Australia or California. Grape (14) and olive (3) are hosts in California, and grape (16) is a host in Australia.

Biotypes are common in other species of plant parasitic nematodes. Biotypes of *Ditylenchus dipsaci* (Kuhn) Filipjev were reported by Hodson (9), Seinhorst (18), Smith (19), and Southey (20). Seinhorst suggested differential hosts for distinguishing 11 biotypes. Sturhan (21) and Webster (24) demonstrated the ability of biotypes of *D. dipsaci* to infect and multiply on a host to be a heritable character. Cotten (6) reported resistant and susceptible genotypes of barley were invaded equally by biotypes of *Heterodera avenae* (Mortenson *et al.*) Filipjev, but nematode development in resistant genotypes was retarded after invasion. The occurrence of biotypes of *H. rostochiensis* Woll. and their importance in the development of resistant varieties of potatoes were reviewed by Jones (10). He reported biotypes of the golden nematode in the British Isles differing in their ability to reproduce on some varieties and clones of potatoes. Martin (11) reported isolates of *Meloidogyne incognita* (Kofoid and White) Chitwood and of *M. incognita acrita* Chitwood that differed in their ability to develop on certain varieties of cotton. Goplen *et al.* (8) found three biotypes of *M. incognita acrita*, two of *M. javanica javanica* and two of *M. hapla* differing in ability to reproduce in five alfalfa varieties or selections. Riggs and Winstead (15) developed highly pathogenic biotypes of the root-knot nematode *M. incognita*, *M. incognita acrita* and *M. arenaria* (Neal) Chitwood by repeated transfers to resistant tomatoes. Two biotypes of the burrowing nematode, *Radopholus similis* (Cobb) Thorne, were described by DuCharme and Birchfield (7). Olthof (13)

Received for publication 2 October 1968.

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reported two *Pratylenchus penetrans* Cobb biotypes differed in reproductive potential on tobacco.

Data on the infectivity and rate of development of larvae of two populations of the citrus nematode on roots of 'Troyer' citrange and sweet orange (*Citrus sinensis* Osbeck), and their infectivity on grape (*Vitis vinifera* L.), two species of pear (*Pyrus communis* L. and *P. serotina* Rehd.), cape chestnut (*Calodendrum capense* Thurb.) and 'trifoliate' orange (*P. trifoliata*), roots are presented herein.

METHODS

To obtain citrus nematode inoculum, infected roots were gently washed to remove adhering soil and placed in aerated water. After 24 hr the nematodes, mainly second-stage larvae, were collected on coarse filter paper in a funnel, and then were transferred onto coarse filter paper supported on a wire grid in a large dish. Nematodes that migrated through the paper were disinfested in 40 ppm copper sulfate for 1 hr, filtered, and again washed onto filter paper. Nematodes that had passed through the paper after 24 hr were used.

To determine the degree of infection on small seedlings, randomly selected 1-cm sections of roots were stained in hot acid-fuchsin-lactophenol, rinsed in water, and the nematodes counted at 30 magnifications. Roots of large plants were washed gently to remove adhering soil and usually were stored in 3.7% formaldehyde. Two-gram samples of moist-blotted feeder roots were selected, stained in hot acid-fuchsin-lactophenol, then comminuted in water in a blender for successive periods of 10, 15, 15, and 20 sec. After each period the suspension of roots was poured onto nested 0.147 mm and 0.043 mm pore-size sieves. Material retained on the 0.147 mm-pore sieve was returned to the blender for further treatment after each period. Female nematodes collected on the 0.043 mm-pore sieve were counted at 30 magnifications. The average number of nematodes/cm of root was calculated after determining cm/g of roots.

One gram of fresh, field-grown *Poncirus trifoliata* or 'Troyer' citrange roots was equivalent to 475 and 375 cm of roots, respectively. One gram of fresh feeder-roots of greenhouse-grown grape, sweet orange, 'Pomeroy' *Poncirus* and 'Troyer' citrange was equivalent to approximately 240, 345, 495, and 425 cm of roots, respectively.

RESULTS

INFECTION OF 'TROYER' CITRANGE IN SOUTHERN CALIFORNIA ORCHARDS: 'Troyer' citrange and sweet orange feeder roots (rootstocks of three-year-old trees with navel orange scions) were collected in five orchards, and the mature female citrus nematodes on twenty or more 1-cm sections of roots were counted. An occasional mature female citrus nematode (trace classification) was found on the 'Troyer' citrange roots from two orchards, and an average of 1.2, 2.7 and 4.1 females/cm of root were found on those from the other three orchards. The differences in density of infection did not appear to be related to differences in soils, since 15 to 30 females/cm of sweet orange root occurred in the same orchards.

The infectivity of two populations of the citrus nematode was tested on sweet orange and on 'Troyer' citrange seedlings in a glasshouse. Small 'Troyer' citrange or 'Homosassa' sweet orange seedlings were planted in similar sandy loams in 3-liter metal pots. Soil-1 was obtained from around sweet orange roots heavily infected with the citrus nematode; nearby 'Troyer' citrange roots were infected lightly. It contained approximately 1,500 larvae/100 cc of soil. Soil-2 was obtained from around

sweet orange roots infected heavily with the citrus nematode, but adjacent 'Troyer' citrange trees were either nematode-free or infected very lightly. It contained 1,800 larvae/100 cc of soil. Six pots of each of the infested soils were planted with a small sweet orange or 'Troyer' citrange seedling, one per pot. A similar number of seedlings were planted in pasteurized soil. After four months the roots of each seedling were carefully freed of soil, and the number of mature female nematodes on two 1-g samples of roots were determined.

Average infection on the 'Troyer' citrange roots in soil-1 was 0.86 females/cm and in soil-2, 0.14 females/cm of root. Sweet orange roots in soil-1 had 2.94 females/cm and in soil-2 had 3.43 females/cm of root. Differences between the number of female nematodes on 'Troyer' citrange roots from the two soils were significant at the 0.01 level, but the differences between the number of female nematodes on the sweet orange roots were not significant at the 0.05 level. The population in soil-1 is called Biotype-1 and that in soil-2, Biotype-2.

INFECTIVITY OF THE CITRUS NEMATODE ON PONCIRUS: Low populations of citrus nematode were observed on highly resistant *Poncirus* roots of orange trees in five orchards in California. The infectivity of the nematode larvae obtained from *Poncirus* roots in three of the orchards was compared with that of larvae of Biotype-1 from heavily infected sweet orange roots at Riverside. Small nucellar seedlings produced from seed from self-pollinated flowers of 'Pomeroy' and 'Webber-Fawcett' *Poncirus*, and from open-pollinated flowers of sour orange, 'Standard' selection, were inoculated with the different populations of citrus nematode. Each treatment consisted of six pots containing four or five seedlings each. Approximately 20,000 larvae that has passed through a coarse filter paper were put into the sandy loam soil of each pot when the seedlings were 5–6 cm tall. After eight months in a glasshouse at 21 C to 27 C the average number of female citrus nematodes/cm of root on each seedling was determined. Infection classes were: none, 0; light, 1 to 5; moderate, 6–10; and heavy, more than 10 females/cm of root.

The larvae obtained from *Poncirus* roots produced light infection on 91% and moderate infection on 9% of the *Poncirus* seedlings (Table 1). There was no significant difference between the infectivity of the nematodes obtained from Poncirus roots in the three orchards, nor in the susceptibility of the 'Pomeroy' and 'Webber-Fawcett' *Poncirus*. Larvae from sweet orange roots infected 1% of the *Poncirus* trees lightly and 99% were free. Inoculum from all four orchards infected 100% of the sour orange seedlings moderately or severely.

TABLE 1. Percent of *Poncirus trifoliata* and standard sour orange seedlings infected by citrus nematode larvae from *Poncirus* or sweet orange roots.

Source of inoculum	Seedlings inoculated	Number	Infection class[a]			
			None	Light	Moderate	Heavy
			%	%	%	%
Poncirus[b]	*Poncirus*[c]	183	0	91	9	0
Sweet orange	*Poncirus*[c]	68	99	1	0	0
Sweet orange	Sour orange	29	0	0	10	90
Poncirus[b]	Sour orange	78	0	1	28	71

[a] Infection classes: none, 0; light, 1 to 5 females/cm of root; moderate, 6 to 10 females; heavy, more than 10.
[b] Larvae from Poncirus trees in three orchards were used separately.
[c] Pomeroy and Webber-Fawcett selections.

INFECTIVITY OF NEMATODE POPULATIONS IN CALIFORNIA AND IN JAPAN: *Poncirus* roots from 25-year-old satsuma mandarin trees near Tokushima, Japan had an average of 1.37 mature female citrus nematodes per centimeter of root. Since this was considerably higher than the numbers usually found on this host in California, the susceptibility of five selections of *Poncirus* grown in Japan and of three selections used for rootstocks in California was determined. Seed produced by open-pollinated flowers were used. Six small trees of each selection of *Poncirus* and of 'Troyer' citrange were planted in sandy loam field soil infested with the citrus nematode near Riverside, California. Trees of each selection were randomized in six blocks, and the degree of infection was determined after four years. Roots in the 10 to 30 cm depth of soil on opposite sides of the trunks of the trees were collected, and the number of female nematodes on duplicate 2-g samples of roots were determined.

The 'Taiyo,' 'Shoyo,' 'Japanese common,' 'Hiryo,' 'Japanese tetraploid,' 'Rubidoux,' 'Pomeroy,' and 'Webber-Fawcett' *Poncirus* and the 'Troyer' citrange had an average of 0.25, 0.19, 0.02, 0.01, 0.001, 0.01, 0, 0, and 4.1 females/cm of root, respectively. Infection on 'Troyer' citrange and on most of the *Poncirus* trees was similar to that expected for Biotype-1. The degree of infection on 'Taiyo' and 'Shoyo' *Poncirus* was significantly different from that on the other six *Poncirus* selections, and the infection on 'Troyer' citrange was different from that on the '*Poncirus*' selections at the 0.01 level by Duncan's Multiple Range Test.

In another test, small seedlings of 'Pomeroy,' 'Rubidoux,' and 'Texas' *Poncirus*, 'Troyer' citrange, and 'Homosassa' sweet orange were exposed to infection by Biotype-1 of the citrus nematode, and to a nematode population at Tokushima, Japan.

TABLE 2. Infectivity of the citrus nematode on three selections of *Poncirus trifoliata* in pots in California and in Tokushima, Japan.

Host	Avg no. of female nematodes/cm of root	
	California	Tokushima
Poncirus, 'Pomeroy'	0.0	2.3
Poncirus, 'Rubidoux'	0.0	9.1
Poncirus, 'Texas'	0.0	6.8
Sweet orange, 'Homosassa'	4.8	3.2
Citrange, 'Troyer'	3.6	4.2

Similar lots of seed were used at both locations. Ten seedlings of each selection were planted, one per 15-cm diameter pot, in a sandy loam. Five pots of each variety were inoculated with 50,000 to 60,000 larvae from sweet orange roots, and five were left uninfested as controls at each location. The larvae had passed through a coarse filter paper before inoculation. After one year, 20 one-centimeter sections of roots were examined for young or mature females (Table 2).

No mature female nematodes were found on the roots of 'Pomeroy,' 'Rubidoux,' and 'Texas' selections inoculated with Biotype-1, while 2.3, 9.1, and 6.8 females/cm of root occurred on the trees inoculated with the Tokushima population. 'Troyer' citrange seedlings inoculated with Biotype-1 contained 3.6 females/cm and those inoculated with the population at Tokushima 4.2 females/cm of root. These data suggest that the population of the citrus nematode at Tokushima is more infective on *Poncirus* than Biotype-1.

SUSCEPTIBILITY OF OTHER HOSTS: The infectivity of Biotype-1 of the citrus nematode on Cape chestnut (*Calodendrum capense*), grape 'Thompson' seedless, two species of pear (*Pyrus communis, P. serotina*), 'Homosassa' sweet orange, and 'Troyer' citrange was determined. Rooted grape cuttings and seedlings of the trees were

planted in 3-liter metal pots in a sandy loam that contained 9,400 larvae/100 cc of soil. Four plants of each species were planted one per pot in the infested soil, and four were planted in steam-pasteurized soil. The metal pots were placed in 8-liter pots and the space filled with vermiculite for insulation. Soil temperatures ranged between 21 and 29 C in the glasshouse. In a second test, seedlings of *P. communis, P. serotina*, sweet orange, and 'Troyer' citrange were planted in a steam-pasteurized sandy loam in clay pots 25 cm in diameter. One month after planting, four pots of each kind of seedling were each inoculated with 183,000 larvae of Biotype-1 that had been surface disinfected in copper sulfate solution. Four pots of each of the test plants were kept uninoculated. The number of female nematodes per gram of fresh feeder roots was determined four months after inoculation.

The inoculated grape, *P. communis, P. serotina*, sweet orange, and 'Troyer' citrange plants contained 195, 1.4, 0.5, 275, and 354 mature female nematodes/g of feeder roots, respectively, or approximately one female nematode/1.25 cm of grape, sweet orange, and 'Troyer' citrange root (average of two tests except one of grape). The infection on grape was light and that on *P. communis* and *P. serotina* trace. Three female citrus nematodes with eggs were found on *P. serotina* roots which proves the nematode can complete it's life cycle on this highly resistant host. No young mature female citrus nematodes were found on inoculated *Calodendrum capense* roots or on the non-inoculated trees.

EFFECT OF HOST ON INFECTIVITY: Changes of infectivity of Biotype-1 induced by hosting on *Poncirus trifoliata* for three years were determined. Outdoor microplots, 2 × 2 m, were planted with four 'Pomeroy' *Poncirus* or 'Homosassa' sweet orange trees. Four replicated plots of each host were used. Samples taken at 10 to 30 cm depth from the *Poncirus* plots yielded 234 larvae/100 cc of soil and roots after two years, and 82 larvae after three years. Similar samples taken from the sweet orange plots yielded 2160 and 3238 larvae/100 cc of soil and roots after 2 and 3 years respectively. Since the *Poncirus* samples contained low numbers of larvae after 2 years, sufficient soil and roots were taken after 26 months to fill four 11-liter glazed pots. Each pot was planted with two sweet orange trees and maintained in a glasshouse. After 10 months the soil with roots contained 11,560 larvae/100 cc of soil and after mixing with steam pasteurized sandy loam it contained 2914 larvae/100 cc of soil. The infectivity and rate of reproduction of nematodes from these three sources (*Poncirus* or sweet orange for three years, and *Poncirus* for 26 months and then sweet orange for 10 months) were

TABLE 3. Effect of host on infectivity and rate of reproduction of *Tylenchulus semipenetrans*.

Innoculum		No. mature females/cm of root		
History	No. larvae/ 100 cc soil	sweet orange	Troyer citrange	*Poncirus trifoliata*
Six weeks after inoculation				
sweet orange	3238	.40	.08	0.0
Poncirus trifoliata 3 years	82	.04	.13	0.02
P. trifoliata 26 months then sweet orange for 10 months	2914	.51	.33	0.0
Six months after inoculation				
sweet orange	3238	3.31	1.18	0.0
P. trifoliata 3 years	82	6.14	7.34	0.43
P. trifoliata 26 months then sweet orange for 10 months	2914	2.13	1.95	0.03

tested on 'Pomeroy' *Poncirus*, 'Homosassa' sweet orange, and on 'Troyer' citrange. A seedling 10 to 12 cm tall was planted in a 3-liter metal pot in sandy loam soil containing eggs and larvae of one of the above populations of the citrus nematode. Eight pots of each host were planted with each inoculum treatment and in noninfested soil. The degree of infection on the roots of four trees was determined after six weeks and six months. At six weeks the number of female nematodes on 20 1-cm pieces of mature feeder roots selected from each tree, and at six months the number of females on four 2-g samples of feeder roots from each tree was determined.

Sweet orange and 'Troyer' citrange seedlings inoculated with larvae from sweet orange averaged 0.40 and 0.08 females/cm of root, respectively, six weeks after planting (Table 3). Those inoculated with larvae obtained after hosting for 26 months on *Poncirus* and then 10 months on sweet orange contained 0.51 and 0.33 females/cm of root, respectively (Table 3). Sweet orange and 'Troyer' citrange seedlings inoculated with larvae from *Poncirus* contained 0.04 and 0.13 females/cm of root. A higher percentage of the larvae from *Poncirus* infected sweet orange and 'Troyer' citrange roots than those of the other two sources; since the infested soil from *Poncirus* trees contained only 2.5% and 2.8%, as many larvae as that from the two other sources.

Sweet orange, 'Troyer' citrange, or *Poncirus* seedlings contained 6.14, 7.34, and 0.43 females/cm root, respectively, six months (approximately four generations) after inoculation with larvae from *Poncirus*, and those that were inoculated with larvae from sweet orange contained 3.31, 1.18 and 0 females/cm of root, respectively. Fewer nematodes developed on the sweet orange, 'Troyer' citrange and the *Poncirus* trees (2.13, 1.95 and 0.03 females/cm of root, respectively) that were inoculated with larvae produced during 26 months on *Poncirus* followed by 10 months on sweet orange than on those inoculated with larvae produced during 36 months on *Poncirus*. These data indicate larvae from *Poncirus* roots are more infective and reproduce more rapidly on sweet orange, 'Troyer' citrange, and *Poncirus* than those from sweet orange roots; and that hosting on sweet orange may cause a decrease in the ability of subsequent generations of larvae to infect 'Troyer' citrange.

RATE OF DEVELOPMENT OF LARVAE OF TWO BIOTYPES: The infectivity and rate of development of larvae of Biotypes-1 and -2 on 'Homosassa' sweet orange and on 'Troyer' citrange was determined. A seed of one of the test hosts was planted 15 mm deep in loamy sand 150 mm deep in 23 × 200 mm glass test tubes and supported in a water bath at 27 C in a glasshouse. Later, tubes that contained a seedling or seedlings 4–5 cm tall were selected for testing and fertilized (Hoagland's nutrient solution). Larvae of Biotypes-1 and -2 were obtained from infected sweet orange roots in two orchards near Riverside. Approximately 81,000 surface-disinfested larvae and males (15%) were placed in each tube by means of a syringe with a 10-cm steel cannula. Eight tubes containing orange and eight containing citrange seedlings were inoculated with each biotype. Seedlings in two tubes of each treatment were removed after three weeks, and those from three tubes after four and five weeks. Their roots were immediately placed in water at 50 C for twenty minutes and then preserved in F.A.A. The developmental stages of the nematodes were determined at 90 magnifications after staining in hot acid-fuschin-lactophenol (22). Between 439 and 2749 nematodes attached to roots were examined per treatment at

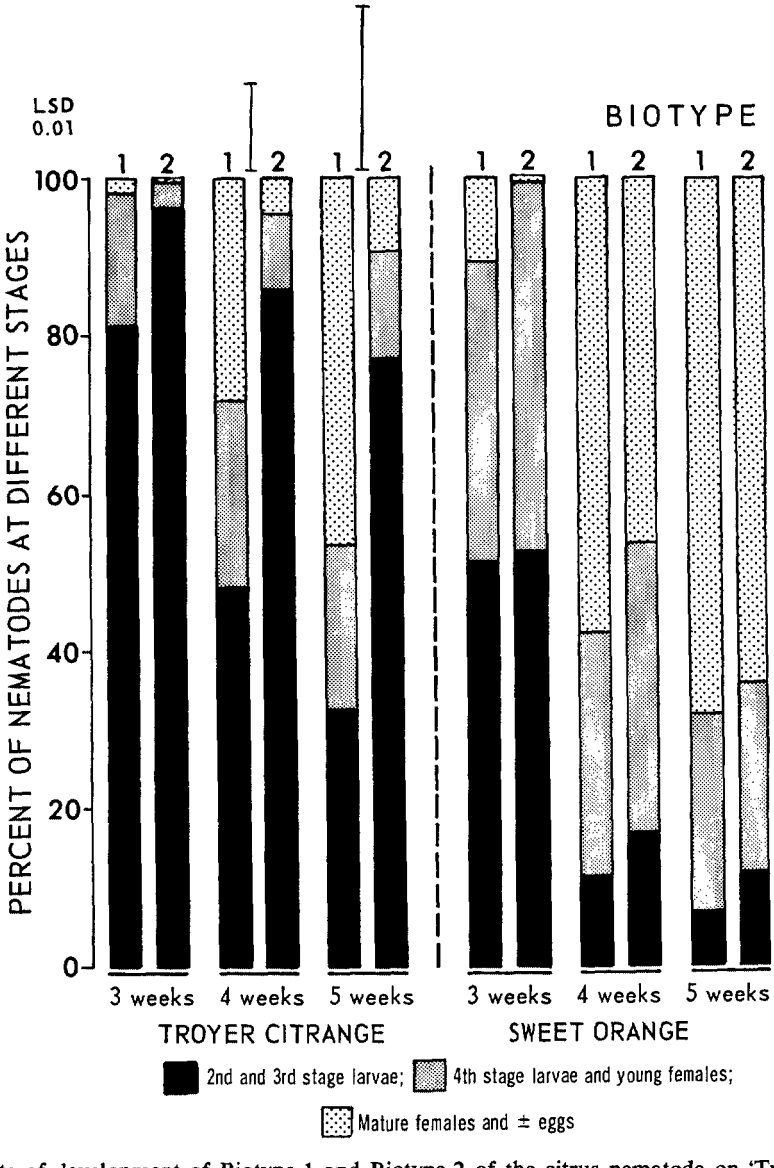

FIG. 1. Rate of development of Biotype-1 and Biotype-2 of the citrus nematode on 'Troyer' citrange and on sweet orange roots.

each harvest. The nematodes in the rinse water and those in the sand were collected on a 128-mesh sieve, fixed in warm 5% formalin, and their stage of development determined.

Three weeks after inoculation of the 'Troyer' citrange seedlings with larvae of Biotype-1, 50.7%, 30.5% and 10.9% of the nematodes found on the root surface or partially within the roots were second-, third-, and fourth-stage larvae, respectively, and 7.8% were young or mature females

(Fig. 1). Similar 'Troyer' citrange seedlings inoculated with larvae of Biotype-2 contained 81.1%, 15.7%, and 1.4% second-, third-, and fourth-stage larvae, respectively, and 1.8% young and mature females. A higher percentage of nematodes in advanced stages of development also occurred on the 'Troyer' citrange seedlings four weeks after inoculation with larvae of Biotype-1, than on those inoculated with larvae of Biotype-2. 'Troyer' citrange seedlings five weeks after inoculation with larvae of Biotype-1 contained 12.4%, 20.2% and 9.7% second-, third-, and fourth-stage larvae, and 11.5% young and 46.2% mature females; those inoculated with Biotype-2 larvae, contained 42.3%, 34.5% and 8.0% second-, third-, and fourth-stage larvae, and 6.4% young and 8.8% mature females, respectively. At five weeks after inoculation 17.7% and 0.6% of the females of Biotypes-1 and -2 had produced eggs, respectively.

Larvae of Biotype-1 also were slightly more infective and matured more rapidly on highly-susceptible sweet orange seedlings than those of Biotype-2. Five weeks after inoculation with larvae of Biotypes-1 or -2, 68.0% and 64.2% of the nematodes in the roots were mature females, respectively. Differences greater than 17.6% between stages of development of Biotypes-1 and -2 on sweet orange and on 'Troyer' citrange at five weeks are significant at the 0.05 level.

Mainly second- and third-stage larvae and males were found free of the roots in the sand. Second- and third-stage larvae also were observed free on the root surface or with their heads within the root. Fourth-stage larvae, young and mature females occurred with their anterior portions well within the roots (17). An average of 0.87% of the larvae of Biotype-1 and 0.62% of those of Biotype-2 infected sweet orange roots, and 0.44% of the larvae of Biotype-1 and 0.21% of those of Biotype-2 infected 'Troyer' citrange roots.

DISCUSSION

Resistance of 'Troyer' citrange and *Poncirus* roots to penetration and establishment of successful feeding sites by the citrus nematode was reported to be associated to a hypersensitive reaction, the production of wound gum and formation of a periderm in the cortex that walls-off the nematode (23). The higher percentage of third- and fourth-stage larvae that developed on sweet orange roots as compared to 'Troyer' roots three weeks after inoculation, and the higher percent of the larvae that infected sweet orange support this mode of resistance. Differences in infectivity and rate of development between Biotypes-1 and -2 apparently are due to differences in the ability of the nematodes to penetrate into the cortex and establish a satisfactory feeding site.

An increase in the infectivity of the citrus nematode during hosting on *Poncirus*, a resistant host, may result from selection and reproduction of aggressive and/or nutritionally-adapted individuals from a mixed population or from genetical changes. Biotypes that are highly infective on *Poncirus* did not develop during reproduction for many generations on this host in microplots, nor in five orchards in California. This indicates a high level of resistance occurs in some *Poncirus* selections and that the infectivity of some populations of the citrus nematode may change readily only within certain limits. The high level of resistance to the citrus nematode occurring in some *Poncirus* selections is inherited and has been utilized to develop resistant rootstocks for commercial types of *Citrus* (4). It is important, especially in rootstock-breeding programs, to test the susceptibility of citrus rootstocks and progeny from hybridization to highly infective biotypes of the citrus nematode.

Nishino *et al.* (12) reported the number of female citrus nematodes ranged from 0.11 to 0.63, averaging 0.25/cm of *Poncirus* roots in seven orchards of satsuma mandarin near Shizuoka, Japan. In the present study, Miyakawa reported slightly higher levels of infection on *Poncirus* roots near Tokushima, which suggests the presence of different biotypes of the citrus nematode in Japan.

Cohn (5) reported *Calodendrum capense* susceptible to the citrus nematode in Israel. In California, however, Biotype-1 did not infect this host.

Pear (*P. serotina*) was infected slightly by the citrus nematode in Japan according to Yoshida (25). An occasional mature female citrus nematode developed on *P. communis* and on *P. serotina* roots inoculated with Biotype-1 in California, but mature females with eggs were found only on *P. serotina.* These two species of pear, therefore, are considered very poor hosts for the citrus nematode in California and in Japan (correspondence with T. Yoshida).

Slightly-susceptible 'Troyer' citrange was a better host for differentiation of California Biotypes-1 and -2 than sweet orange. Other plants such as Cape chestnut, grape, olive, and *Poncirus* are likely to be superior to 'Troyer' citrange for differentiation of other biotypes of the citrus nematode.

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