Development and Selection of Rootstocks Resistant to the Citrus Nematode, *Tylenchulus semipenetrans*

J. H. O'Bannon and D. J. Hutchison

Summary

The citrus nematode, *Tylenchulus semipenetrans* Cobb, which causes a disease of citrus called "slow decline", attacks most citrus rootstocks used commercially. In studies to evaluate citrus relatives, hybrids, and varieties for reaction to this nematode, about 300 different varieties and species have been tested in Florida. Most *Citrus* species and *Citrus* X *Poncirus* hybrid populations are susceptible. *Poncirus trifoliata* (L.) Raf. selections have consistently shown a high degree of resistance. Of these, Argentine trifoliate orange appears promising.

Most citrus rootstocks used commercially are attacked by the citrus nematode, *T. semipenetrans*. The nematode causes a disease of citrus called "slow decline". Because there are no obvious root symptoms to indicate the presence of this pest on nursery stock, it often is not noticed, and in this manner has been inadvertently distributed to all citrus-growing areas of the world. It has been estimated that 70-80% of the citrus trees throughout the world are infested with *T. semipenetrans.*

Studies on the biology and control of the citrus nematode, *T. semipenetrans,* have been in progress for many years since it was first discovered in California in 1912. Cobb (5) reported several hosts of the citrus nematode, including trifoliate orange, *Poncirus trifoliata* (L.) Raf.; grapefruit, *Citrus paradisi* Macf.; sweet orange, *C. sinensis* (L.) Osbeck; and sour orange, *C. aurantium* L.

DuCharme (6) found that the trifoliate orange in field plantings near Concordia, Entre Rios, Argentina, was highly resistant to the citrus nematode. Baines and coworkers (1) found that certain selections or varieties of the trifoliate orange, and some other plants taxonomically related to citrus, are highly resistant or immune to the citrus nematode, and a number of citrus species are susceptible. Feder (7) found several *P. trifoliata* strains resistant to the citrus nematode, but he also found variation in susceptibility among strains of *Poncirus.* Cameron and coworkers (4) reported that first-generation hybrid seedlings from crosses of nematode-resistant trifoliate orange and 5 susceptible citrus species were resistant to the citrus nematode. Swingle (11) developed 2 commercially acceptable hybrids ('Troyer' and 'Carrizo' citranges) from crosses of *P. trifoliata* with *C. sinensis.*

Van Gundy and Kirkpatrick (12), studying host resistance, identified 3 reactions in varieties resistant to the citrus nematode as: a hypersensitive cell reaction to the feeding of the nematode, a formation of wound periderm in the root cortex, and a toxic factor in the root juice. Later Baines and coworkers (3) showed different field populations of the citrus nematode to show different host preference, and demonstrated the existence of biotypes on citrus. Thus, the variability of *P. trifoliata* in their tolerance to *T. semipenetrans* is further complicated by the existence of several citrus biotypes of the nematode. This was first discovered by differences in degree of infection of the citrus nematode on 'Troyer' citrange in California in 1969. 'Troyer' citrange was previously found to be moderately resistant to the citrus nematode and has been used for this purpose. Florida greenhouse studies have shown 'Troyer' and 'Carrizo' citrange to be susceptible to a Florida population of *T. semipenetrans* in several tests. In field trials in Australia, 'Troyer' citrange also supported moderate populations of *T. semipenetrans* (10).

In Florida our objectives are to find rootstocks that are readily propagated, horticulturally acceptable, and resistant to or tolerant of the important citrus diseases as well as the citrus nematode and the other nematodes attacking citrus, such as *Radopholus similis* (Cobb) Thorne and *Pratylenchus coffeae* (Zimmerman), Filipjev and Schuurmans Stekhoven.

The nematode-resistant rootstock improvement program in Florida is conducted in several phases. One phase evaluates selected citrus rootstocks that have shown resistance to or tolerance of *Phytophthora,* another evaluates other promising citrus species or citrus relatives for resistance to *T. semipenetrans,* a third evaluates promising candidates for resistance to *R. similis* and *P. coffeae,* a fourth and most recent phase evaluates candidate rootstocks for resistance to citrus-nematode populations from Arizona, California, and Texas, as well as Florida, under the same conditions.

Citrus nematodes from each citrus-growing state have been introduced into Florida, where they are maintained in isolation under quarantine. The final phase has been an exchange of seeds for testing in respective areas.

Our program includes identification of plants with resistance for use as parents in hybridization. We now have about 200 hybrid seedlings that have citrus-nematode resistance in their parentage. In addition, hybridization of 10 to 15 specific combinations involving selections with burrowing and citrus nematode resistance are made each year. These pollinations are designed to produce about 300 hybrids a year as candidates for our screening program.

In the screening program, all seeds are germinated in flats containing a mixture of Astatula fine sand and peat moss as a planting medium. The seedlings are grown in the flats until they are 2 to 6 months old, receiving routine fertilization, watering, and other care. When ready for transplanting, seedlings are carefully removed and transplanted directly into citrus-nematode-infested soil contained in large above-ground growth bins constructed of concrete block. The soil in these bins is a mixture containing equal parts of Astatula fine sand and sphagnum peat moss. High organic-matter content, provided by the peat moss in the sand, favors maximum root infection by the citrus nematode.

All clones are randomized and replicated within soil bins. Plants are grown 6 to 12 months. At harvest, roots are washed, root samples are weighed, and linear measurements are made to determine the length of roots in cm per gram of root. To extract larvae, feeder roots are incubated in pint jars as described by Young (13). After 3 or 4 days, the roots in each jar are rinsed with 50 cc of water, a 5-cc sample is withdrawn and pipetted into a Cooper dish, and the number of nematodes is counted with the aid of a dissecting microscope. The moist roots are weighed, and the nematode larvae per gram of root are recorded. To determine the number of adult females, the roots are minced in a blender for two successive 15-sec. intervals. After each period, the blended material is poured into 60- and 325-mesh sieves. The material remaining on the 325 sieve, which includes the adult citrus nematode females, is rinsed into 50-cc beakers and brought to volume. A 5-cc sample is examined with the aid of a dissecting microscope, and the number of adult females is counted. From these data the numbers of adult female *T. semipenetrans* per gram or cm of root are calculated. Nematode data are reported as either number of citrus nematode larvae per gram of root, or number of citrus nematode females per gram or cm of root.

Resistance ratings have been prepared, based on statistical ranges or natural groupings. In this paper, plant ratings are based on the following conditions: (i) immune -- no nematodes, the nematode neither penetrates nor feeds on host tissue and does not reproduce; (ii) resistant -- the host may be invaded by nematodes, but growth is not retarded, and nematode population diminishes to a low level or completely disappears; (iii) tolerant -- nematodes will invade the host, reproduce, and maintain a population that causes moderate to severe growth reduction in the host.

The first citrus-nematode studies at the USDA Horticultural Research Laboratory on differential susceptibility of selections of *P. trifoliata* were conducted by Feder (7). The selections tested were grouped into 2 categories, based on numbers of adult female nematodes per gram of root. Of those tested, none were immune, 22 were highly resistant, and 16 susceptible, Table 1. As shown by these and other data (2), there is variation in the susceptibility of *P. trifoliata* strains to attack by the citrus nematode.

Later studies by Hutchison and O'Bannon (9), evaluating the reaction of certain citrus and non-citrus selections to the citrus nematode, support Feder's findings of variable response of *P. trifoliata* strains to the Florida biotype of *T. semipenetrans,* Table 2. Of the several *Citrus* X *Poncirus* hybrid populations studied under greenhouse conditions, all have been susceptible, Table 3. Only highly resistant strains of *P. trifoliata* should be considered for breeding parents.

Seedlings of 80 commercially available citrus rootstock selections provided by the Florida Division of Plant Industry were evaluated for their reaction to *Phytophthora parasitica* Dastur, *R. similis*, and *T. semipenetrans* (9). All *P. trifoliata* selections and 1 trifoliate orange hybrid *(C. paradisi* X *P. trifoliata)* showed resistance to *P. parasitica* and *T. semipenetrans.* 'Carrizo', 'Rusk', and 'Troyer' citranges were susceptible to *T. semipenetrans.*

Studies with newer hybrids by J. W. Cameron and R. C. Baines (personal communication) have shown several of these hybrids to be highly resistant to *T. semipenetrans.* Dr. Cameron recently provided the authors with seeds of 4 of these promising hybrids for tests with the Florida population of *T. semipenetrans.* Comparative results of greenhouse studies with these selected hybrids conducted by Baines in California with 2 biotypes and O'Bannon in Florida with 1 biotype are shown in Table 4. These hybrids look promising for continued work in Florida as well as in California.

A simultaneous study was conducted in Arizona and Florida to compare *Citrus* X *Poncirus* hybrids with a *T. semipenetrans*-resistant *P. trifoliata* rootstocks that has shown promise in Florida, Table 5. Argentine *P. trifoliata,* which has shown resistance

Table 1. Strains and hybrids of *Poncirus trifoliata* resistant or susceptible to attack by the citrus nematode, *Tylenchulus semipenetrans**

Strains	No. of females/g feeder root	Resistance rating**	Strains	No. of females/g feeder root	Resistance rating
C63-25 Benecke 56-3	1.4	R	C63-29 Rubidoux 56-6	138	S
C63-30 Rubidoux Sdlg 55-123	1.3	R	C63-32 *P. trifoliata* Swingle	123	S
C63-31 Rubidoux Sdlg 55-124	2.2	R	C63-34 Davis A 58-87	57	S
C63-33 Yamaguchi 56-7	1.3	R	C63-46 Rich 5-2	145	S
C63-44 Town F	0.8	R	C63-65 Rich 22-2	71	S
C63-66 Kryder 8-5	3.3	R	C63-68 English small	28	S
C63-70 Argentina	0.22	R	HF63-7-4-16 *P. trifoliata* small flowered	196	S
C63-71 Marks 54-96-11	0.87	R	C63-82 Sacaton citradia IF63-49 CPB 50097	169	S
C63-74 Marks 54-96-13	5.5	R	C63-63 Rich 16-6	204	S
C63-75 Kryder 55-1	1.4	R	C63-24 *P. trifoliata* Sdlg 3F-14	122	S
C63-80 Kryder 43-3	3.5	R	C63-27 Jacobson 56-5	238	S
C63-26 Christian 56-4	1.5	R	C63-28 English large	238	S
C63-45 Rich 5-2	2.0	R	C63-69 Ronnse	40	S
C63-47 Rich 7-5	1.7	R	C63-77 Kryder 25-4	16.8	S
C63-62 Marks small 54-96-4	1.7	R	C63-79 Kryder 60-2	5.9	S
C63-67 Town G	3.3	R	IF63-64 *P. trifoliata* Chambers	82.7	S
C63-73 Marks 54-96-13	3.7	R	C63-83 Yuma citrange 56-24 hybrid	94.5	S
C63-76 Kryder medium	1.8	R	HF63-7-14-14 Thomasville citrangequat hybrid	383	S
C63-78 Kryder 60-2	2.3	R	Seville sour orange (Control)	258	S
C63-81 Kryder 15-5	3.3	R			
IF63-62 *P. trifoliata* Gainesville	2.2	R			
HF63-7-3-16 *P. trifoliata* large flowered Orlando	2.6	R			

*From Feder 1968.
**R = resistant, S = susceptible (see text for explanation of ratings.)

Table 2. Reaction of *Poncirus trifoliata* strains to attack by the citrus nematode, *Tylenchulus semipenetrans*--Greenhouse test

Selection	No. of larvae/g feeder root	No. of females/cm feeder root	Resistance rating*
English Dwarf FF1-52-105	24	0.18	R
Argentine FF1-53-95, 96	24	0.02	R
Christiansen FF1-53-99	25	0.06	R
Kryder 5-5 FF1-53-105	27	0.02	R
Rich 7-5 FF1-53-103, 104	27	0.03	R
Flying Dragon C66-40	32	0.16	R
Marks 54-96-11	53	0.07	R
Argentine C57-217	130	0.15	MR
Rubidoux HF7-20-13	160	1.00	MR
Towne FF1-53-101, 102	184	0.07	MR
Small Flower HF7-4-16	184	1.20	MR
Rich 5-2 FF1-53-94	224	1.00	MR
Florida rough lemon	3,056	4.70	S

*R = resistant, MR = moderately resistant, S = susceptible.

Table 3. Reaction of *Citrus* X *Poncirus* hybrid strains in the greenhouse to attack by the citrus nematode, *Tylenchulus semipenetrans*

Selection	No. of larvae/g feeder root	No. of females/cm feeder root	Resistance rating*
Rusk citrange *(C. sinensis* X *P. trif.)*	395	2.0	S
Citrime-81 *(C. limonia* X *P. trif.)*	779	4.3	S
Citrange-9 *(C. sinensis* X *P. trif.)*	848	5.5	S
Sacaton citrumelo *(C. paradisi* X *P. trif.)*	858	10.4	S
Citrime-82 *(C. limonia* X *P. trif.)*	960	2.3	S
Citrange-19 (*C. sinensis* X *P. trif.)*	1,408	7.4	S
Citrandarin-100 *(C. reticulata* X *P. trif.)*	1,876	4.3	S
Troyer citrange *(C. sinensis* X *P. trif.)*	1,886	5.4	S
Citrandarin-15 *(C. reticulata* X *P. trif.)*	2,080	7.1	S
Citrandarin-58 *(C. reticulata* X *P. trif.)*	2,872	6.5	S
Citrime-8 *(C. limonia* X *P. trif.)*	4,136	7.9	S
Citrumelo-4481 *(C. paradisi* X *P. trif.)*	4,117	4.7	S

*R = resistant, S = susceptible.

Table 4. Reaction of *Citrus* X *Poncirus* hybrid strains to attack by 2 *T. semipenetrans* biotypes from California* and 1 from Florida

	Citrus nematode biotypes resistance rating**		
	California		Florida
Selection	1	3	
CRC13-7 *(C. sinensis* X *P. trifoliata)*	R	S	R
CRC14-7 " "	R	S	R
CRC15-7 " "	R	MR	R
CRC15-16 " "	R	S	R

*Data supplied by R. C. Baines, University of California, Riverside.

**R = resistant, MR = moderately resistant, S = susceptible

Table 5. Comparison of several rootstocks to attack by *T. semipenetrans* biotypes from Arizona and Florida

Selections	Resistance rating* Florida	Arizona
Argentine FF1-53-95 *(P. trifoliata)*	*R*	*R*
Carrizo HF 7-18-17 *(C. sinensis* X *P. trifoliata)*	*S*	*MR*
Troyer HF 7-16-17 *(C. sinensis* X *P. trifoliata)*	*S*	*MR*
Yuma FF 1-10-5 *(C. sinensis* X *P. trifoliata)*	*S*	*MR*
Florida rough lemon *(C. limon)*	*S*	*S*
Seville sour orange *(C. aurantium)*	*S*	*S*

*R = resistant, MR = moderately resistant, S = susceptible.

Table 6. Reaction of several rootstocks to *Radopholus similis* and *Tylenchulus semipenetrans*

Selection	Resistance rating* *R. similis*	*T. semipenetrans*
Algerian navel *(C. sinensis, R. similis,* resistant)	R	S
Milam lemon *(Citrus* sp., *R. similis,* resistant)	R	S
Ridge Pineapple *(C. sinensis, R. similis,* resistant)	R	S
Carrizo citrange *(C. sinensis* X *P. trif., R. similis,* tolerant)	MR	S
Estes rough lemon *(C. limon, R. similis,* tolerant)	S	S
Argentine *(P. trifoliata, R. similis,* susceptible)	S	R
Troyer citrange *(C. sinensis* X *P. trif.,* susceptible)	S	S
Yuma citrange *(C. sinensis* X *P. trif.,* susceptible)	S	S
Smooth flat Seville sour orange *(C. aurantium,* susceptible)	S	S
Florida rough lemon *(C. limon,* susceptible)	S	S

R = resistant, MR - moderately resistant, S = susceptible.

Table 7. The reaction of commercially available rootstocks in Florida to foot rot, tristeza, and citrus nematode

Rootstock	Foot rot	Tristeza	Citrus nematode
Rough lemon	S	T	S
Sour orange	T	S	S
Cleopatra mandarin	T	T	S
Carrizo citrange	T	T	S
Sweet orange	S	T	S
Milam lemon	S	T	T
P. trifoliata	R	R	R
Rangpur lime	T	T	S

R = resistant; T = tolerant; S = susceptible.

to *Phytophthora* and is highly resistant to the Florida population of *T. semipenetrans*, was found to be free of the Arizona citrus nematode a year after exposure to the nematode in the Arizona test. This indicates that Argentine is also highly resistant to the Arizona citrus nematode population.

The burrowing nematode, *R. similis*, causes a serious disease of citrus in Florida called "spreading decline". Several rootstocks have been classified as either resistant or tolerant to *R. similis*. Five of these rootstocks and 5 *R. similis*-susceptible rootstocks were separately infected with *R. similis* or *T. semipenetrans* and grown for study of individual nematode response and behavior under comparable conditions. Plants were grown in the greenhouse for 1 year. Nematode-resistance ratings are shown in Table 6. The 3 *R. similis*-resistant varieties were attacked by *T. semipenetrans*. Argentine trifoliate orange showed high resistance to *T. semipenetrans* and was susceptible to *R. similis*. None of the *R. similis*-resistant or -tolerant rootstocks were resistant to *T. semipenetrans*.

The initial citrus nematode rootstock program has been of an exploratory nature in Florida. While the importance of the citrus nematode in citrus production has been partially recognized for many years, researchers have only recently realized that this pest causes important crop damage in Florida.

At present, there is no available rootstock that possesses all of the desirable traits needed by the citrus industry. Most commercially available rootstocks have more than 1 serious weakness. Table 7 shows the reactions of commercially available rootstocks to 3 important citrus diseases. Only *P. trifoliata* has resistance to all 3 problems. With the continued development of the citrus-rootstock improvement program, our goals are to develop superior rootstock varieties for the citrus industry.

To date, about 300 different citrus varieties and species have been tested in Florida. In general, these plants show certain characteristics that may be classified as follows: 1) resistant-plants lightly infected, good root and top growth, low nematode recovery; 2) moderately resistant-plants light to moderately infected, but good root and top growth, low nematode recovery; 3) tolerant-plants moderately to heavily infected, good root and top growth, high nematode recovery; and 4) susceptible-plants heavily infected, severe root damage, poor growth, high nematode recovery. The ultimate would be: 5) plants not infected, or if infected, no nematodes are recovered, good root and top growth. As a result of these studies, we have a better understanding of the overall citrus-rootstock program and have been able to develop an effective, large-scale program for screening citrus for resistance to the citrus nematode as well as to the burrowing and lesion nematodes.

LITERATURE CITED

1. Baines, R. C., W. P. Bitters, and O. F. Clarke. 1960. Susceptibility of some species and varieties of citrus and some other rutaceous plants to the citrus nematode. *Plant Dis. Rep.* 44:281-285.

2. Baines, R. C., T. Miyakawa, and R. H. Small. 1967. Biotypes of the citrus nematode *(Tylenchulus semipenetrans)* and their effect on resistant rootstock. *Nematologica* 13:137.

3. Baines, R. C., T. Miyakawa, J. W. Cameron, and R. H. Small. 1969. Infectivity of two biotypes of the citrus nematode on citrus and on some other hosts. *J. Nematol.* 1:150-159.

4. Cameron, J. W., R. C. Baines, and O. F. Clarke. 1954. Resistance of hybrid seedlings of the trifoliate orange to infestation by the citrus nematode. *Phytopathology* 44:456-458.

5. Cobb, N. A. 1914. Citrus-root nematode. J. Agr. Res. 2:217-230.

6. DuCharme, E. P. 1948. Resistance of *Poncirus trifoliata* rootstock to nematode infestation in Argentina. *Citrus Ind.* 29:9,15.

7. Feder, W. A. 1968. Differential susceptibility of selections of *Poncirus trifoliata* to attack by the citrus nematode, *Tylenchulus semipenetrans. Israel Jour. Agr. Res.* 18:175-179.

8. Hutchison, D. J., and J. H. O'Bannon. 1972. Evaluating the reaction of citrus selections to *Tylenchulus semipenetrans. Plant Dis. Rep.* 56:747-751.

9. Hutchison, D. J., J. H. O'Bannon, G. R. Grimm, and G. D. Bridges. 1972. Reaction of selected citrus rootstocks to foot rot, burrowing and citrus nematodes. *Proc. Fla. State Hort. Soc.* 85:39-43.

10. Meagher, J. W. 1969. Nematodes as a factor in citrus production in Australia. *Proc. First Intern. Citrus Symp.,* Riverside, Calif. 2:999-1006.

11. Swingle, W. T. 1946. The botany of citrus and its wild relatives of the orange subfamily. *In The Citrus Industry,* H. J. Webber and L. D. Batchelor, ed. Univ. California Press, Berkeley, Calif. Vol. I, p. 129-474.

12. Van Gundy, S. D., and J. D. Kirkpatrick. 1964. Nature of resistance in certain rootstocks to citrus nematode. *Phytopathology* 54:419-427.

13. Young, T. W. 1954. An incubation method for collecting migratory emdoparasitic nematodes. *Plant Dis. Rep.* 38:794-795.