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Variation in *Radopholus citrophilus* Population Densities in the Citrus Rootstock Carrizo Citrange

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Abstract: Seedlings of the hybrid citrus rootstock, Carrizo citrange (*Citrus sinensis* × *Poncirus trifoliata*) do not uniformly limit development of the citrus burrowing nematode, *Radopholus citrophilus*. Variation in nematode population densities in roots of seedlings germinating from the same seed suggests that factors responsible for nematode incompatibility are not functional or are not inherited uniformly among progeny. Seeds which produced a single seedling were more likely to produce plants which suppressed citrus burrowing nematode population increase than were seeds which produced two or three seedlings.
Key words: resistance, tolerance, nematode, polyembryony, citrus burrowing nematode.

Spreading decline of citrus (14) caused by the citrus burrowing nematode (CBN) *Radopholus citrophilus* (5) had been managed in Florida for more than 25 years through the integration of several control strategies. These included the "push and treat" program to reduce nematode population densities to nondetectable levels prior to replanting (12), physical barriers such as chemically treated fallow buffers between infested and noninfested groves to prevent nematode dispersal, and resistant or tolerant rootstocks. The recent ban on ethylene dibromide (EDB) and the current lack of a registered, effective, and economic substitute for EDB has essentially terminated both the push and treat and chemical barrier programs.

Future programs to manage spreading decline will continue to use certified CBN-free citrus nursery stock to prevent spread of CBN into noninfested groves, nematode-resistant or tolerant rootstocks to limit nematode reproduction and (or) damage in infested sites, sound cultural practices designed to minimize stress in CBN-infected trees, and environmentally safe postplant nematicides to reduce CBN population densities to nondamaging levels.

Economic and environmental considerations will probably increase grower reliance on rootstocks to minimize CBN-related losses. Rootstocks currently available for CBN management generally harbor low CBN population densities which persist in roots of these rootstocks for prolonged periods of time (9; Kaplan, unpubl.; Ford and O'Bannon, unpubl.). The persistence of CBN in roots of resistant rootstocks may have contributed to the development of a resistance-breaking biotype (7) isolated from a sweet orange (*Citrus sinensis* (L.) Osbeck) on Milam (a putative hybrid of rough lemon, *C. limon* (L.) Burm. f.) planting with spreading decline symptoms. Decline symptoms were observed in isolated plantings of sweet orange on Milam lemon and Carrizo citrange (*C. sinensis* × *Poncirus trifoliata* (L.) Raf.), rootstocks previously reported to be resistant or tolerant to *R. citrophilus* (2,3,10,11).

The purpose of this study was to determine if Carrizo citrange seed sources and (or) seedlings germinated from the same seed vary in their ability to suppress development of CBN populations.

Materials and Methods

Comparison of different Carrizo and Troyer citrange seed sources on R. citrophilus reproduction: Seed was collected from 25 commercial seed sources of Carrizo citrange, two sources of 'Troyer' citrange, and one source of rough lemon. Ten 5-month-old plants from each seed source were planted in 20-cm-d pots containing steam sterilized Astatula fine sand (hyperthermic, uncoated typic quartzipsamments) and randomized on a greenhouse bench. Plants were selected for uniform vigor and phenotype. Two months later, the soil in each pot was infested with 100 nematodes (mixed life cycle stages) from a population previously designated as *R. citrophilus* biotype 1 (7). Inoculum was derived from monoxenic carrot disc cultures (8). Plants were maintained in a greenhouse with soil tempera-

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TABLE 1. *Radopholus citrophilus* biotype 1 population densities in fibrous roots of 25 Carrizo and 2 Troyer citrange sources and rough lemon (10 plants per seed source).

Source	Nemas/g root dry weight*	Standard deviation	Percent†
01-21	949	2,048	40
78-355	792	1,182	50
78-437	642	1,134	50
01-20	397	836	20
78-334	370	662	70
Rough lemon	360	104	80
F52-5 (Troyer)	298	446	60
F17-20 (Troyer)	197	204	50
01-9	194	488	30
01-19	180	569	10
78-333	175	236	70
01-25	170	537	10
01-26	161	410	20
78-332	154	162	60
78-438	139	169	50
01-12	131	413	10
78-331	81	91	50
01-16	59	127	20
01-17	54	170	10
01-7	51	162	10
01-13	40	83	30
01-8	25	80	10
01-22	23	50	20
01-24	19	59	10
01-10	0	0	0
01-18	0	0	0

* Column means not significantly different according to Duncan's multiple-range test log(x + 1) ($P = 0.05$).

† Percentage of test plants where *R. citrophilus* was detected in roots.

ture averaging 24 ± 5 C. Twelve months after infestation, nematodes were extracted from all fibrous roots from each plant for 7 days at 25 ± 1 C (15). Roots were oven-dried for 48 hours at 76 C and weighed. Data were expressed as nematodes per gram root dry weight.

CBN population densities in plants germinated from Carrizo citrange seeds: Seventeen seedlings from seed that produced single seedlings, 19 sets of twin plants from 19 seeds, and 9 sets of triplet plants from 9 seeds, all collected from one seed source (78-438), were individually planted in Astatula fine sand as before. Soil around each plant was infested with 100 *R. citrophilus* derived from monoxenic cultures when plants were 7 months old. Plants were randomized and maintained in a greenhouse for 15 months. At harvest, stem diameter was determined, nematodes extracted from fibrous roots, and root weights determined as described in the previous paragraph.

TABLE 2. *Radopholus citrophilus* population densities in single, twin, and triplet seedlings arising from individual Carrizo citrange seeds.

Single plants	Twin plants		Triplet plants		
1	1	2	1	2	3
847	7,506	0	3,571	796	699
476	5,665	5,521	2,631	2,558	1,010
469	2,820	141	2,207	1,702	0
430	3,705	442	2,160	766	75
270	2,352	0	1,566	202	73
79	1,653	201	587	98	0
72	1,606	20	560	34	0
9	844	0	283	247	0
0	715	0	282	138	0
0	666	0			
0	591	0			
0	441	0			
0	405	0			
0	347	141			
0	192	0			
0	192	0			
0	86	59			
	53	34			
	16	10			

Data were analyzed to determine if nematode population density was correlated with stem diameter or total fibrous root weight. Nematode population densities were expressed as nematodes per gram root dry weight.

RESULTS

The large standard deviations in Table 1 show the variation in CBN population densities associated with plants of Carrizo citrange from different seed sources. In nine seed sources, 50–70% of the plants tested supported large CBN population densities; in 14 seed sources, 10–40% of plants tested supported large CBN populations; and plants selected from only two seed sources appeared to uniformly suppress development of large CBN populations. Consequently, seed sources supporting mean populations as high as 949 nematodes per gram root dry weight could not be distinguished from sources with no detectable nematodes in their roots by Duncan's multiple-range test ($P = 0.05$).

Subsequent study of single, twin, and triplet Carrizo citrange plants indicated that even though two or three plants may germinate from the same seed, they often differ in their suitability for CBN population increases (Table 2). In this experiment, CBN was not detected in roots of

52, 23, and 19% of the plants that germinated from seeds producing one, two, or three seedlings, respectively. The likelihood of selecting Carrizo citrange plants that suppress development of CBN populations was negatively correlated with the number of seedlings germinating from a single seed ($r = -0.91$).

Stem diameter and fibrous root weight were suppressed in single ($r = -0.35$, -0.50), twin ($r = -0.50$, -0.37), and triplet ($r = -0.39$, -0.41) plants, respectively, as population densities of CBN increased.

Discussion

Some Carrizo citrange seedlings were distinctly incompatible with CBN, whereas others supported large numbers of nematodes. No obvious phenotypic character (e.g., leaf shape or growth habit) was associated with incompatibility to CBN. Some Carrizo citrange seedlings are primarily nucellar, but some may be tetraploid or zygotic. Tetraploid and zygotic seedlings can be readily identified by their coarse roots, thick leaves, or growth habit. Neither tetraploid nor zygotic seedlings were included in this study. Variation in seedling vigor was apparent among Carrizo citrange plants. In the first study, the most vigorous and uniform plants from each of 25 Carrizo and 2 Troyer citrange seed sources were evaluated for their ability to suppress CBN population densities. On evaluation of the data from the first experiment, the second experiment was initiated to better document variation toward CBN within Carrizo citrange seed sources. Variability in vigor is common among Carrizo citrange seedlings, but plants used as rootstocks are selected for both uniformity and vigor. In the experiment where plants were selected on the basis of the number of seedlings arising from each seed, vigor was disregarded. However, plants with atypical leaves or abnormal growth habit were avoided. Some seedlings that would never be used commercially were used experimentally. Nonetheless, the experiment clearly demonstrated that seedlings arising from the same seed may differ in their suitability to suppress CBN populations.

Variation in Carrizo and Troyer citranges has been studied previously (3,13). Carrizo citrange was first designated as a rootstock resistant to CBN (3), then as tolerant (2), and again as resistant (11). Variation among seedlings of Carrizo citrange regarding their influence on CBN population densities may explain why designation of this rootstock as CBN resistant has been difficult.

Carrizo citrange is a nucellar plant. Nucellar embryony is unique to *Citrus* and its close relatives (4). Nucellar embryos develop asexually from nucellus cells by mitotic division, and nucellar seedlings are considered genetically identical to their seed parents (1,4). Nucellar seedlings may vary from their parent if somatic mutations (rare) occur or if chromosomes or parts of chromosomes are inherited cytoplasmically (frequency unknown). Pollination is usually essential to nucellar embryo development; however, the relationship of fecundation to embryo development is unclear (1). Although Carrizo citrange is considered nucellar (13), the ability to suppress CBN population increase is not expressed uniformly. Plants that suppress CBN may be cloned vegetatively through cuttings to ascertain the planting of a rootstock that will suppress CBN. At present, Carrizo citrange should not be considered CBN resistant (6).

Occurrence of spreading decline symptoms in Carrizo citrange rootstock plantings may reflect the inability of some Carrizo citrange rootstocks to suppress CBN population development. However, occurrence of CBN populations that differ in virulence (7) may also play a role when spreading decline symptoms appear in plantings of Carrizo citrange rootstocks.

Literature Cited

1. Cameron, J. W., and H. B. Frost. 1968. Genetics, breeding, and nucellar embryony. Pp. 325–371 *in* S. Reuther, L. D. Batchelor, and H. J. Webber, eds. The citrus industry, vol. 2. Richmond: University of California Press.
2. Ford, H. W. 1970. Nematode status of 'Carrizo' citrange. Proceedings of the Florida State Horticultural Society 83:87–88.
3. Ford, H. W., and W. A. Feder. 1962. Laboratory evaluation of certain citrus rootstock selections for resistance to the burrowing nematode. Journal of the Rio Grande Valley Horticultural Society 10:35–39.
4. Frost, H. B., and R. K. Soost. 1968. Seed reproduction: Development of gametes and embryos. Pp. 290–324 *in* W. Reuther, L. D. Batchelor, and H. J. Webber, eds. The citrus industry, vol. 2. Richmond: University of California Press.
5. Huettel, R. N., D. W. Dickson, and D. T. Kaplan. 1984. *Radopholus citrophilus* sp. n. (Nematoda),

a sibling species of *Radopholus similis.* Proceedings of the Helminthological Society of Washington 51:32–35.

6. Kaplan, D. T., and N. T. Keen. 1980. Mechanisms conferring plant incompatibility to nematodes. Revue de Nematologie 3:123–134.

7. Kaplan, D. T., and J. H. O'Bannon. 1985. The occurrence of biotypes in *Radopholus citrophilus.* Journal of Nematology 17:158–162.

8. Moody, E. H., B. F. Lownsbery, and J. M. Ahmed. 1973. Culture of the root lesion nematode, *Pratylenchus vulnus,* on carrot discs. Journal of Nematology 5:225–226.

9. O'Bannon, J. H., and H. W. Ford. 1979. Possible *Radopholus similis* biotypes reproducing and migrating on resistant citrus rootstocks. Nematropica 9: 104 (Abstr.).

10. O'Bannon, J. H., and H. W. Ford. 1977. Resistance in citrus rootstocks to *Radopholus similis* and *Tylenchulus semipenetrans* (Nematoda). Proceedings of the International Society of Citriculture 2:544–549.

11. O'Bannon, J. H., and H. W. Ford. 1976. An evaluation of several *Radopholus similis*-resistant or -tolerant citrus rootstocks. Plant Disease Reporter 60: 620–624.

12. Poucher, C., R. F. Suit, H. W. Ford, and E. P. DuCharme. 1967. Burrowing nematode in citrus. Florida Department of Plant Industry Bulletin No. 7.

13. Savage, E. M., and F. E. Gardner. 1965. The origin and history of Troyer and Carrizo citranges. The Citrus Industry 26:5.

14. Suit, R. F., and E. P. DuCharme. 1953. The burrowing nematode and other plant parasitic nematodes in relation to spreading decline of citrus. Plant Disease Reporter 37:379–383.

15. Young, T. W. 1954. An incubation method for collecting migratory endo-parasitic nematodes. Plant Disease Reporter 38:794–795.