Freezing Resistance of Progeny from Open-pollinated Pummelo × Trifoliolate Orange Citrus Hybrids

G. Yelenosky, D. Hutchison¹, and H. Barrett

U.S. Department of Agriculture, Agricultural Research Service, Horticultural Research Laboratory, 2120 Camden Road, Orlando, FL 32803

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Abstract. Ten-month-old seedlings, grown from seed extracted from 22 individual pummelo [*Citrus grandis* (L.) Osbeck] × trifoliolate orange [*Poncirus trifoliata* (L.) Raf.] citrus hybrid trees that survived -15°C freezes near Monticello, Fla., were cold-acclimated in controlled-environment rooms and freeze-tested at -6.7°C for 4 h. Freeze damage to open-pollinated progeny was ranked by the number of uninjured seedlings and percentage of leaves killed and wood dieback. Morphological segregation was not associated with differences in freeze survival, and the dominant trifoliolate gene was readily evident. Progeny from one tree, identified as 98-71, are considered the most likely candidates for further study in developing cold-hardy citrus trees.

In continuing searches for cold-hardy citrus types in Florida to support genetic initiatives in citrus freeze survival, open-pollinated seedling progeny from twenty-two 15-year-old pummelo × trifoliolate orange citrus hybrid trees were screened for cold hardiness. [Original cross was made by A.P. Pieringer (retired), Univ. of Florida, Institute of Food and Agricultural Sciences, Lake Alfred.] Trees consistently survived severe freezes without visible injury when grown near Monticello, Fla., which is in hardiness zone 8 [-6.6 to -12.3°C average annual minimum; U.S. Dept. of Agriculture (USDA) *Plant Hardiness Zone Map*]. On site, temperatures were as low as -15.6°C in Jan. 1985 (P.C. Anderson, personal communication). Pummelo often expresses a high ratio of zygotic progeny, hybrid vigor, and fruitfulness with few unfavorable traits (Cameron and Soost, 1974). These characteristics complement the superior cold hardiness of *Poncirus*, which has a dominant trifoliolate leaf gene. In a numerical taxonomic study of affinity relationships, pummelo is considered a true biological species, and trifoliolate orange is connected to *Citrus* through *C. aurantium* L., an apomictically perpetuated biotype of hybrid origin (Barrett and Rhodes, 1976). Open-pollinated pummelo × trifoliolate orange progeny have not been screened for freeze survival potential.

Seedlings were grown from seed extracted from 22 individual hybrid trees at Monticello,

and each tree lot was germinated in peat/vermiculite in separate trays under greenhouse conditions. Polyembryonic seed were suspect in five of the 22 tree seed lots that are identified in Table 1. Presumed nucellar seedlings (based on leaf morphology) were excluded from freeze survival performance. Three-month-old seedlings in each tray were separated into different foliate groups based on leaf morphology to

determine if differences in leaf morphology were associated with freeze performance of zygotic seedlings. There is no evidence to suggest such an association, which apparently has not been addressed in citrus cold-hardiness studies. Seedlings were transplanted to 3-liter plastic pots (one per pot) containing "Pro-Mix" [shredded sphagnum peat with equal parts of vermiculite and perlite with macro- and micronutrients added (Premier Brands, New Rochelle, N.Y.)]. Seedlings were maintained in a shaded greenhouse under natural daylight at 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ maximum photosynthetic photon flux (PPF). The air ranged from 32°C day maximums to 15°C night minimums, and relative humidity (RH) ranged from 35% day lows to 99% night highs. Seedlings were watered every 2 days and fertilized monthly with 250 ml of 12N-2.6P-5K that included micronutrients.

Seedlings, ≈ 10 months old, were cold-acclimated by consecutively cooler day/night temperatures in controlled-environment rooms described by Yelenosky (1979). Cold-hardening conditions were 21°C, 12-h day (500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF), 10°C night, and 50% $\pm 5\%$ RH for 2 weeks immediately followed by 2 weeks of 15°C days and 4°C nights. Freeze tests, which immediately followed 4 weeks of cold acclimation, were done in darkness in a separate room adjacent to acclimation rooms. Freeze tests included 1-h equilibration at 2°C followed by a 1°C/h decrease to -6.7°C, a 4-h holding period at -6.7°C, and a 1°C/h thaw to 5°C. Thawed seedlings were returned to a greenhouse and

Table 1. Freeze² injury and leaf and stem survival of cold-acclimated potted seedlings that were grown from open-pollinated seed of 22 pummelo × trifoliolate orange hybrid citrus trees.

Tree ID ^y	Seedlings tested (no.)	Type of injury/seedling (%)			Extent of injury/seedling	
		None	Leaf	Leaf and stem	Leaves killed/seedling (%)	Stem dieback/seedling (%)
98-71 ^x	68	97	3	0	6 $\pm$ 4 ^w	0
98-88	15	87	13	0	5 $\pm$ 2	3 $\pm$ 1
98-43 ^x	98	81	7	12	11 $\pm$ 6	3 $\pm$ 2
98-53 ^x	64	76	11	13	6 $\pm$ 3	1 $\pm$ 1
98-45	74	66	4	30	10 $\pm$ 5	5 $\pm$ 2
98-62	171	65	13	22	18 $\pm$ 8	8 $\pm$ 3
98-74	100	65	2	33	28 $\pm$ 10	20 $\pm$ 8
98-70	67	58	15	27	14 $\pm$ 7	9 $\pm$ 5
98-69	67	57	3	40	33 $\pm$ 10	24 $\pm$ 8
98-61	201	56	12	32	27 $\pm$ 6	18 $\pm$ 5
98-85	151	56	3	41	27 $\pm$ 8	15 $\pm$ 5
98-39	51	55	10	35	25 $\pm$ 6	10 $\pm$ 5
98-59	114	55	14	31	46 $\pm$ 9	17 $\pm$ 11
98-73	132	55	2	43	33 $\pm$ 11	24 $\pm$ 8
98-79	123	54	7	39	27 $\pm$ 6	18 $\pm$ 4
98-84 ^x	64	52	11	37	30 $\pm$ 6	15 $\pm$ 4
98-67	87	47	1	52	31 $\pm$ 9	25 $\pm$ 8
98-68	129	47	4	49	27 $\pm$ 10	13 $\pm$ 7
98-80	109	44	6	50	43 $\pm$ 11	32 $\pm$ 9
98-72	43	37	23	40	13 $\pm$ 6	2 $\pm$ 1
98-54	39	36	18	46	17 $\pm$ 5	4 $\pm$ 2
98-83	147	33	28	39	22 $\pm$ 6	2 $\pm$ 1
Total	1950	59 $\pm$ 15	9 $\pm$ 7	32 $\pm$ 14	---	---

¹Freeze defined as -6.7°C for 4 h.

²Listed in order of largest percentage of seedlings that had no visible damage during 5 weeks of postfreeze observations.

^wMean $\pm$ SD.

^xTrees whose seed produced some presumed nucellar seedlings (based on leaf morphology) that were excluded from data in Table 2.

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¹Retired.

Table 2. Morphological differences^a and freeze^b survival of cold-acclimated seedlings developed from seed of 22 pummelo x trifoliate orange citrus hybrid trees.

Seed origin	Leaf shape	Seedling tested (no.)	Type of injury/seedling (%)			Extent of injury/seedling	
			None	Leaf	Leaf and stem	Leaves killed/seedlings (%)	Stem dieback/seedlings (%)
Nucellar	Trifoliate	115	80	16	4	6 ± 4 ^x	1 ± 1
Zygotic	Trifoliate, small	98	68	10	22	14 ± 6	6 ± 6
	Trifoliate, broad/round	514	57	11	32	20 ± 12	11 ± 8
	Trifoliate, pointed	1068	55	8	37	22 ± 10	12 ± 8
	Mixed (tri-, bi-, uni-)	311	54	10	36	28 ± 11	19 ± 6
	Unifoliate	82	54	19	27	24 ± 12	13 ± 10

^aBased on leaf shape relative to "normal" nucellar trifoliate.

^bFreeze defined as -6.7C for 4 h.

^xMean ± SD.

rated for damage during 5 weeks of postfreeze observations. Trees were ranked from 1 to 22 based on the percentage of uninjured seedlings, and freeze damage was based on the percentage of leaves lost relative to the total number originally on a seedling and stem dieback relative to stem height above soil level. Statistical analyses, other than numerical ranking and listing comparison of means and standard deviations, were largely precluded because of nonreplication of seed lots. Means included all seedlings per tree seed lot, except nucellars, which were excluded in Table 1.

Results of freeze tests targeted tree 98-71 as the most likely candidate for inclusion in the

USDA citrus germplasm pool for genetic initiatives in citrus freeze survival (Table 1). More seedlings (97%) from tree 98-71 were uninjured during 4 h of -6.7C than any of the other 21 trees included in this study (ranging from 33% to 87% uninjured). *Citrus* × *Poncirus* hybrids are noted for their multifoliate leaf expressions, and a relatively low percentage of unifoliate progeny are characteristic from open-pollinated highly zygotic trifoliate orange hybrids (Cameron and Soost, 1980). Trifoliate, unifoliate, and a mix of all three foliate types segregated as expected. The impact of environment, known to influence leaf type, was not discernible in our screening results.

There were no indications that morphological differences were associated with the high rate of freeze survival (Table 2). None of the five morphological groups classified in this study was associated with increased freeze survival over presumed nucellar seedlings, which were 7% of the 98-71 population. Progeny of 98-71 are candidates for the USDA citrus rootstock/scion development program (C.J. Hearn and K.D. Bowman, personal communication). This program progressively moves research material from germplasm acquisition through various horticultural evaluations leading to commercial citrus releases and includes germplasm evaluation for genetic transformation of desired traits. Comparison tests with commercial rootstock remain to be done.

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