Freezing Tolerance and Growth Characteristics of USDA Intergeneric Citrus Hybrids US 119 and Selection 17-11

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Additional index words. cold hardiness, electrolyte leakage, field trials

Abstract. Two USDA intergeneric, hybrid citrus scions, US 119 [*Citrus paradisi* Mac. 'Duncan' x *Poncirus trifoliata* (L.) Raf.] x *C. sinensis* Osb. 'Succory' and selection 17-11 [*C. grandis* US 145 x [*Citrus paradisi* Mac. 'Duncan' x *P. trifoliata* (L.) Raf.]] on 'Swingle' citrumelo (*C. paradisi* x *P. trifoliata*) rootstocks were examined for freeze hardiness traits (4 years) and general growth characteristics (2 years). Hardiness was compared with that of 'Hamlin' orange [*C. sinensis* (L.) Osb.] and satsuma mandarin (*C. unshiu* Marc) from Fall 1993 to Spring 1997. As expected, US 119 and 17-11 were both harder than 'Hamlin' orange as determined by leaf disc electrolyte leakage (EL). Both showed freezing tolerance similar to that of satsuma mandarin, but 17-11 was significantly harder than satsuma or US 119 at several times during the 4-year study. Trunk diameter and tree height were similar for US 119 and selection 17-11.

The Florida citrus industry lost more than \$1 billion U.S. in tree and crop damage due to freezes in the 1980s alone. In some of these freezes, losses were reduced by using freeze protection methods such as petroleum heaters, soil banking, tree wraps, and microsprinkler irrigation (Davies et al., 1984; Jackson et al., 1983; Parsons et al., 1982). All these methods are successful in preventing some damage to young citrus trees during a freeze, especially microsprinkler irrigation in conjunction with tree wraps (Rieger et al., 1985, 1986), but water availability and cost may limit microsprinkler use in the future.

Another avenue for decreasing citrus tree loss due to freezes is breeding for greater hardiness, which could provide long-term, reliable, and economical freeze protection (Yelenosky, 1985). The goal of such a program is not necessarily to move citrus planting northward in the United States, but rather to provide harder trees for current growing locations (Yelenosky, 1985).

A U.S. Dept. of Agriculture (USDA) breeding program in Orlando, Fla., has been developing more freeze-hardy citrus scions and rootstocks for many years (Barrett and Young, 1982). *Poncirus trifoliata*, in particular, has been used to introduce hardiness and winter dormancy traits into breeding lines (Barrett, 1990, 1994). US 119 and selection 17-11 are

both products of this breeding program. US 119 was the result of an intergeneric cross of a selection of (*C. paradisi* 'Duncan' x *P. trifoliata*) x *C. sinensis* 'Succory' (Fig. 1A). US 119 was released primarily because of its resistance to citrus tristeza virus (CTV) (Garnsey et al., 1981, 1987) and lack of unpalatable fruit often associated with hybrids having *P. trifoliata* as a parent (Barrett, 1990). There was also anecdotal evidence that US

119 stems had survived a freeze of -12.2 °C (Barrett, 1990), suggesting that it was substantially more hardy than any commercial citrus cultivar in the United States.

USDA selection 17-11 was chosen from individuals produced following the cross of *C. grandis* US 145 x (*C. paradisi* 'Duncan' x *P. trifoliata*) (Fig. 1B) (W.B. Sherman and H.C. Barrett, unpublished data). Nothing has been reported about the hardiness of 17-11.

The primary objective of this study was to determine the leaf hardiness of both intergeneric hybrids in comparison with that of commercial cultivars such as 'Hamlin', the most widely planted orange cultivar in Florida, and satsuma mandarin, the hardest commercially used citrus in the world. Growth characteristics of each hybrid and their relationship to hardiness were also examined.

Materials and Methods

Plant material and cultural practices. Budwood collected from existing stock plants of US 119 and selection 17-11 (provided by H. Barrett, USDA, Orlando, Fla.) and satsuma mandarin was budded onto commercially propagated 'Swingle' citrumelo liners. Following 3 weeks of growth in the greenhouse, 10 individuals of each scion-rootstock combination were planted at the Fifield Horticultural Research farm in Gainesville, Fla., in May 1993. On 3 May 1995, 10 additional trees of each scion-rootstock combination were also planted at the Univ. of Florida Horticultural Research Unit in Gainesville.

Fertilizer (8N-2P-8K-2Mg; Seminole Fer-

Fig. 1. Parentage and leaf morphology of (A) US 119 and (B) selection 17-11.

Received for publication 21 Aug. 1997. Accepted for publication 13 Dec. 1997. Florida Agricultural Experiment Station Journal Series No. R-05912. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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tizer Corp., Bartow, Fla.) was applied about every 5 weeks (for a total of six applications) at 0.10, 0.14, 0.22, and 0.33 kg N/tree/year in 1993, 1994, 1995, and 1996, respectively, based on recommendations for young citrus trees by Koo et al. (1984). Trees were irrigated when soil water depletion exceeded 30% (Marler and Davies, 1990) as determined by a Troxler model 4300 neutron probe (Research Triangle Park, N.C.). Weeds were controlled using glyphosate (N-phosphonomethyl glycine) as needed. Neither trunk wraps nor microsprinkler irrigation were used for freeze protection.

Plant growth measurements. All trees were examined weekly to determine the presence or absence of a growth flush. For the tree to be scored as flushing, rapidly elongating vegetative buds or expanding leaves had to be present on at least three different branches, thus avoiding recording isolated budbreak, which occurs occasionally on individual shoots throughout the growing season. A flush was considered finished when all leaves were fully expanded.

Tree height was measured monthly from the soil to the highest apical meristem. Scion and rootstock trunk diameters were measured monthly at the graft interface, which was typically 20–25 cm above the soil line, using a caliper oriented north–south. Scion and rootstock measurement locations were marked with permanent ink to ensure accuracy in recording growth.

Leaf N analysis. Ten fully expanded leaves from the spring flush, free of obvious damage or infestation, were sampled in late summer from each scion–rootstock combination. Leaves were washed in distilled water and placed in a drying oven at 70 °C for 72 h. Leaf tissue was then ground and stored in scintillation vials. A 250-mg subsample from each vial was placed in a Pyrex digestion tube (Corning Labware and Equipment, Big Flats, N.Y.), and total N was determined using the micro Kjeldahl procedure (Wolf, 1982). Concentrated H₂SO₄ (2.5 mL) was added and samples digested for 24 h, followed by repeated rounds of neutralizing with H₂O₂ and heating on a 375 °C digestion block until samples were clear. Samples were then cooled and volume brought to 25 mL with distilled water. The solution was then passed through filter paper and stored at 4 °C in scintillation vials until samples were analyzed by the Univ. of Florida Analytical Research Laboratory in Gainesville.

Hardiness determination. Hardiness of scion–rootstock combinations was determined using the leaf electrolyte leakage method as previously described (Tignor et al., 1997). Leaves from each cultivar being tested were collected monthly in 1993–94, weekly in 1994–95 and 1995–96, and at 3-week intervals in 1996–97. No more than five leaves were selected from any individual tree on each collection date. Collection took place before 0800 hr and leaves were transported in a cooler to the laboratory. Level of hardiness was determined based on a method developed by Flint et al. (1967). All leaves were washed in three successive baths of distilled deionized (dd) water to remove dust and other field debris. Three

leaf discs were removed from each collected leaf and placed in a single 16 × 100 mm tube, and 100 µL of dd water was added. Intergeneric hybrids US 119 and selection 17-11 show variable leaf morphology. Unifoliate, bifoliate, and trifoliate leaves develop on both selections, but unifoliate leaves predominate (Fig. 1 A and B). Disks were removed from only the large, center leaflet of any bifoliate or trifoliate leaves for consistency among the leaf disk samples. Six (1994) replicate tubes (six leaves) or 10 (1993, 1995, 1996) replicate tubes (10 leaves) for each genotype at four test temperatures were used. Tubes were racked and immersed in a precooled (–1.0 °C) model 2425 circulating glycol bath (Forma Scientific, Marietta, Ohio) and allowed to equilibrate for 30 min. One subsample of all treatments was removed as a nonfrozen control. Water in the bottom of the remaining tubes was then nucleated homogeneously in all tubes by adding a small cloud of ice crystals. Temperatures in the bath were lowered at 0.1 °C/min until –3, –6, or –9 °C was reached. Samples were held at each test temperature for 1 h and a subsample was removed. Frozen samples were allowed to thaw overnight at 1 °C to eliminate injury caused by rapid warming. Following the thaw, tubes were removed from the incubator, allowed to reach 20 °C, and initial electrolyte leakage (EL) values were taken with a model CDM3 conductivity meter (Radiometer, Copenhagen, Denmark). Samples were autoclaved for 25 min to simulate 100% damage, then EL was measured again to determine maximum EL for each sample. Percent injury (adjusted EL) for each

sample was determined using the equation developed by Flint et al. (1967). This equation compensates for differences in EL due to leaf disk excision or inherent variation among leaves.

Statistical analysis. A completely randomized design was used and analysis of variance (ANOVA) was performed using a general linear model procedure (GLM). For growth flush and EL data, ANOVA tables were generated for each date and significant differences among means determined using Duncan's multiple range test. Data for trunk diameter and plant height were evaluated using an analysis of covariance to standardize any initial differences in tree size and generate adjusted least squared means, as well as regression analysis. All statistical calculations were completed using Statistical Analysis Software (SAS Institute, Cary, N.C.).

Results and Discussion

Growth and development. In 1994, US 119 had four distinct growth flushes (Fig. 2A). USDA selection 17-11 also had four growth flushes, but continued growing later in this season than did US 119. During the second growth flush of 1994, the percentage of trees flushing was significantly greater for US 119 than for 17-11 for 3 weeks (as denoted by filled symbols in Fig. 2A), although growth began later. The reverse was true during the beginning of the third growth flush when US 119 flushed one week later than 17-11. During the 2 weeks prior to the end of its third growth flush, US 119 showed more growth flush

Fig. 2. Growth flush patterns of trees of USDA selection 17-11 and US 119 in 1994 (A) and 1995 (B) at the Horticultural Research Unit in Gainesville, Fla. Growth was measured weekly during the growing season. Solid symbols indicate significant differences between genotypes on a given date using Duncan's multiple range test, $P \leq 0.05$, $n = 10$.

activity than 17-11. In addition, 17-11 grew very little from 12 Oct. to 6 Nov 1994.

In 1995, the only significant difference in growth flush patterns came during the first flush when US 119 began flushing earlier than 17-11 (Fig. 2B). Otherwise both selections had three distinct growth flushes followed by a fourth period of low-level flushing activity from 23 Aug. to 2 Oct. Each flush in 1995 occurred 30 to 60 d earlier than corresponding flushes in 1994. In general, the percentage of US 119 trees flushing was 20% lower in 1995 than in 1994 for flushes two, three, and four.

There were no significant differences in trunk diameters between 17-11 and US 119 at any date in 1994 (Fig. 3A) or 1995 (Fig. 3B), and slopes for their regression lines were similar. Similarly, neither trunk diameter nor tree height of the 'Swingle' citrumelo rootstock was affected by the scion (US 119 vs. 17-11) at any date of measurement in 1994 and 1995 (data not shown).

Leaf N concentration was not significantly different between US 119 and 17-11 in 1994 or 1995 (data not shown). In 1994 and 1995 mean percent foliar N for all plants was 4.0 and 2.7, respectively.

In summary, US 119 and 17-11 grow in a similar manner, as determined by scion and rootstock trunk diameter and tree height. Selection 17-11 tended to have more upright growth and was sparsely branching with numerous large thorns. It was also extremely susceptible to rust mite [*Phyllocoptruta oleivora* (Ashmead)] damage during the 4 years of this study. In comparison, US 119 was slightly more compact, with fewer thorns, and was more densely foliated. General visual observations for US 119 were in agreement with those of Barrett (1990).

In 1994, which was warmer and drier than 1995, US 119 flushed later than selection 17-11 and growth continued into early winter (Fig. 2A). This would have increased its susceptibility to early freezes, especially for new shoots and young leaves, in comparison with selection 17-11. In 1994, growth flush patterns of selection 17-11 were similar to those of 'Hamlin' orange located at the same site, but flushing stopped sooner. This suggests that 17-11 ceased growth, and possibly freeze acclimation began sooner than in either 'Hamlin' orange or US 119, in this relatively dry year. Growth cessation *per se* does not increase citrus hardiness (Young, 1970), but lack of growth later in the year is generally associated with increases in hardiness (Cooper et al., 1963). Both US 119 and 17-11 stopped growing actively ≈ 2 weeks sooner than 'Hamlin' in 1995 (data not shown).

Freeze acclimation and hardiness development. In 1993, leaf hardiness of US 119, 17-11, and satsuma mandarin was examined monthly to determine if either of the intergeneric hybrids was more hardy than satsuma mandarin, which is the hardiest commercially grown citrus (Yelenosky, 1985). No significant differences in leaf disk EL among genotypes were found at -4 , -8 , or -12 °C at any of the dates (data not shown). All selections were hardy to -4 °C, showing negligible damage by

Fig. 3. Trunk diameters of USDA selections 17-11 and US 119 in 1994 (A) and 1995 (B) at the Horticultural Research Unit in Gainesville, Fla. Slopes of regression lines were not significantly different in 1994 or 1995. Adjusted LS means are shown, $n = 10$.

Fig. 4. Evaluation of freeze acclimation and hardiness of USDA selections 17-11, US 119, and 'Hamlin' orange in 1994 and 1995 at -3 (A), -6 (B), and -9 °C (C). Leaves were sampled from trees planted at the Horticultural Research Unit in Gainesville, Fla. Solid symbols represent means that differ significantly from all other genotypes on a given date using Duncan's multiple range test, $P \leq 0.05$, $n = 10$.

21 Oct. EL decreased to $<25\%$ for all selections by 27 Dec.

Samples for the second freeze acclimation study were taken weekly from Nov. 1994 through Mar. 1995, to determine if there were more frequent fluctuations in leaf hardiness. 'Hamlin' leaves were compared with those of

selection 17-11 and US 119. During early acclimation (21 Nov. to 19 Dec. at -3 °C) US 119 was significantly more hardy than either selection 17-11 or 'Hamlin' (Fig. 4). Both 17-11 and US 119 had significantly less EL at -3 °C than did 'Hamlin' orange from 5 to 24 Jan., but the two hybrids were not significantly

different from one another (Fig. 4A). 'Hamlin' again had significantly more EL than either hybrid from 21 Feb. to 7 Mar. (Fig. 4A). Leaf discs of both 17-11 and US 119 exposed to -6°C showed significantly less EL from 21 Nov. 1994 to 21 Feb. 1995 (Fig. 4B) than did those of 'Hamlin' orange leaf disks. Differences between selection 17-11 and US 119 were not significant except on 5, 12, and 19 Dec. when 17-11 had between a 100% and 66% reduction in EL compared with US 119 (Fig. 4B). US 119, 17-11, and 'Hamlin' orange had similar amounts of EL at -6°C on 28 Feb. and 7 Mar. With the exception of three dates (21 Nov., 12 Dec., and 7 Mar.), selection 17-11 and US 119 discs had significantly less EL than 'Hamlin' orange leaf disks frozen to -9°C .

In 1995, 'Hamlin' orange and satsuma mandarin were compared with 17-11 and US 119 (data not shown). As expected at -3°C on 11 Sept., EL was significantly less for US 119, 17-11, and satsuma than for 'Hamlin,' and US 119 had significantly less EL than either 17-11 or satsuma on this date. A month later (11 Oct.) EL was significantly different among cultivars with relative hardness as follows: 17-11 > US 119 > satsuma > 'Hamlin'. On 10 Nov. and 10 Dec., 17-11, US 119, and satsuma all had significantly less EL than 'Hamlin.' At both -6 and -9°C , selection 17-11 had significantly less EL than satsuma and US 119, all of which had significantly less EL than 'Hamlin,' but differences among 17-11, US 119, and satsuma were small. There were no significant differences in EL on 11 Sept. and 11 Oct. at -9°C .

From Sept. 1996 until Mar. 1997, all four citrus genotypes were tested every 3 weeks to examine hardness trends for a fourth year. At -3°C there were no significant differences in EL among genotypes (Fig. 5A). At -6°C 'Hamlin' had significantly more EL than 17-11 or US 119 on 2 Nov., 20 Nov., 21 Dec., and 17 Feb. On 2 Nov., 20 Nov., 21 Dec., and 17 Feb., US 119, 17-11, and satsuma had less EL than 'Hamlin', but were not significantly different from each other, except on 7 Mar. when 'Hamlin' and US 119 had similar amounts of EL following exposure to -6°C (Fig. 5B). Large significant differences arose in EL on 21 Dec. 1996 and 4 Jan. 1997 when US 119, 17-11, and satsuma leaves had significantly less EL than 'Hamlin' following exposure to -9°C (Fig. 5C). In addition, on 25 Jan. and 17 Feb., selection 17-11 had significantly less EL than US 119, satsuma, and 'Hamlin'.

Electrolyte leakage is an indirect measure of freeze hardness (Flint et al., 1967). Although data vary by year and date, there are several important trends. Selection 17-11 was generally most hardy, followed by US 119, satsuma mandarin, and 'Hamlin' orange. However, in 1993 there were few significant differences in leaf EL among selection 17-11, US 119, and satsuma, probably because of too few sampling dates. When significant differences were observed, 17-11 was harder than US 119 and satsuma at least once during the time period tested.

In years where EL was determined more often, 'Hamlin' orange acclimated later and

deacclimated faster than the other selections. Selection 17-11 seemed to follow more closely the same general acclimation pattern as 'Hamlin' (Fig. 4A, Fig. 5B). However, leaf EL values were consistently higher for the first three freeze tests at -3°C than at -6°C . On rare occasions singular aberrant values appear for trees being tested, but these values for selection 17-11 for 3 consecutive weeks are unexplained and were not observed during any other time period during 4 years of freeze tests.

In Winter 1994-95 and 1996-97, 'Hamlin' EL averaged 23% and 18%, respectively, when leaf discs were exposed to -6°C . Based on our comparison of acclimated vs. nonacclimated 'Hamlin' on 'Swingle' citrumelo trees, whole trees with these EL values would have experienced no stem damage and little or no leaf damage. This is in agreement with Yelenosky's data (1990). Our results help to establish that this technique has merit in determining freeze hardness and acclimation rates over time. US 119 survived freezes with -12.2°C minimum air temperature in 1981 (Barrett, 1990). The trees were completely defoliated at this temperature, but bloomed and set fruit during the following year. We observed similar defoliation in both US 119 and 17-11 following two freezes when air temperature reached -10°C in 1996. In addition, defoliation occurred to a lesser extent when air temperatures reached -9°C in 1995. In both years defoliation oc-

curred via abscission at the petiole-leaf interface with the petiole remaining on the tree until the spring flush occurred. This indicates less severe freezing injury than dead leaves persisting on a tree following a severe freeze.

In summary, these freeze tests indicated that US 119 was harder than all commercially produced citrus cultivars in the United States, except satsuma mandarin. In combination with its apparent resistance to CTV, US 119 should be a useful source for parent selection for breeding commercial citrus scion cultivars. Selection 17-11, in addition to being harder than 'Hamlin' orange, may be potentially harder than US 119 and satsuma, and therefore is also a potential parent for citrus breeding programs. US 119 and 17-11 also show different patterns of growth and different levels of freeze hardness on the same dates, suggesting different responses to temperature, although subsequent experiments are needed to establish this.

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Fig. 5. Evaluation of freeze hardness and acclimation of U.S.D.A. selections 17-11, US 119, satsuma mandarin, and 'Hamlin' orange in 1996-97 at -3°C (A), -6°C (B), and -9°C (C). Leaves were sampled from trees planted at the Fifiield Horticultural Research Farm in Gainesville, Fla. On a given date, transition from solid symbols to hollow symbols represents a change in groups of significantly different means as determined by Duncan's multiple range test, $P \leq 0.05$, $n = 10$. Shaded symbols between solid and hollow symbols denote values that do not differ significantly from adjacent means.

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