

Freeze Response of *Citrus* and *Citrus*-related Genotypes in a Florida Field Planting

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Abstract. A test population consisting of progenies of 92 seed-source genotypes (hereafter called “parent genotypes”) of *Citrus* and *Citrus* relatives in the field in east-central Florida was assessed after natural freeze events in the winters of 2010 and 2011. Eight seedlings per parent genotype were planted in a randomized complete block design; however, as a result of mortality, the number of plants assessed in some genotype groups was reduced at some or all sampling dates. The citrus diseases huanglongbing and citrus canker were endemic in the planting and may have influenced tree response to cold temperatures. Unusually low temperatures (near -4°C each winter) for east-central Florida were experienced during the trial period. Defoliation and dieback were significantly greater in the winter of 2011 than in the winter of 2010. The winter in 2011 was preceded by a period of extraordinarily low temperatures in mid-December with no period of cool temperatures to allow trees to acclimate. In 2010 the average defoliation was $53\% \pm 28\%$ and less than 13% of the trees exhibited any noticeable dieback, whereas in 2011, the average defoliation and dieback were $93\% \pm 17\%$ and $51\% \pm 35\%$, respectively. Within the genus *Citrus*, several progenies were identified that had 16% to 24% dieback in 2011 and these were from parent genotypes *C. reticulata* (CRC 2590) (23%), *C. sinensis* (CRC 3858) (24%), *C. maxima* (CRC 3945) (16%), *C. hassaku* (CRC 3907 and 3942) (16% and 17%), *C. aurantium* (CRC 628 and 2717) (18% and 7%), *C. taiwanica* (CRC 2588) (21%), and *C. neo-aurantium* (*C. obovoidea* + *C. unshiu* graft chimera) (CRC 3816) (23%). Within other genera in the Aurantiaceae, *Poncirus trifoliata* (CRCs 301, 3957, 3549, and 4007), *Severinia buxifolia* (CRC 1497), *Bergera koenigii* (CRC 3165), and *Glycosmis pentaphylla* (CRC 3285) had the least amount of dieback, all at less than 23%. The two species within the Toddaliaceae subfamily of the Rutaceae (*Casimiroa edulis* and *Zanthoxylum ailanthoides*) had among the least amount of dieback (1% and 8%, respectively). When considered by groups, the Citrons and Australian natives had the greatest amount of dieback in 2011, 68% and 65%, respectively. The trifoliates (*Poncirus* and hybrids) had the least dieback, ranging from 4% to 40%. The information from this study may be useful in germplasm enhancement and *Citrus* breeding targeting greater cold tolerance.

Citrus is the most economically important fruit tree crop in Florida with an estimated economic value of \$1.35 billion annually (National Agricultural Statistics Services, 2012). Low temperatures are among the greatest risk factors for U.S. citrus production. Most commercial citrus cultivars are cold-sensitive (Soost and Roose, 1996) and damage will often result when exposed to

temperatures below -2.2°C (Yelenosky, 1985). In the 1980s there were multiple severe freeze events in Florida (1981, 1982, 1983, 1985, and 1989) that resulted in crop and yield losses in excess of \$1 billion U.S. (Tignor et al., 1998). After these extreme freezes, citrus production shifted to the warmer southern parts of the state (Miller, 1991; Yelenosky, 1996). Citrus growers take preventative measures to minimize crop losses from freezes such as use of trunk wraps and water applied through elevated micro-sprinklers (Bourgeois et al., 1990; Davies et al., 1984; Jackson et al., 1983). However, the effectiveness of these techniques is dependent on wind conditions, types of freezes (advective vs. radiation), water availability,

and tree and grove size (Bourgeois et al., 1990; Ebel et al., 2005). Protection using micro-sprinklers is compromised by high wind speeds (Nisbitt et al., 2000). Developing more cold-tolerant citrus varieties through breeding and selection has long been considered the most effective long-term solution (Grosser et al., 2000; Yelenosky, 1985).

Citrus and *Citrus* relatives are members of the family Rutaceae. The subtribe Citrinae is composed of *Citrus* (mandarins, oranges, pummelos, grapefruits, papedas, limes, lemons, citrons, and sour oranges); *Poncirus* (deciduous trifoliolate oranges); *Fortunella* (kumquats); *Microcitrus* and *Eremocitrus* (both Australian natives); and *Clymenia* (Penjor et al., 2013). There is considerable morphological and ecological variation within this group. With *Citrus*, cold-hardiness ranges from cold-tolerant to cold-sensitive (Soost and Roose, 1996). *Poncirus* and *Fortunella* are considered the most cold-tolerant genera that are cross-compatible with *Citrus*. *Poncirus trifoliata* reportedly can withstand temperatures as low as -30°C with proper acclimation (Lang et al., 2005) and is used as a cold-tolerant rootstock for some commercial citrus production (Ebel et al., 2008; Tignor et al., 1998). The most cold-tolerant commercial *Citrus* variety is considered *C. unshiu* (Satsuma mandarin), whereas *C. aurantifolia* Swing. (Mexican Lime), *C. limon* L. Burm. f. (Lemon), and *C. medica* L. (Citron) are considered the most susceptible to freeze damage (Davies and Albrigo, 1994; Grosser et al., 1998).

A period of acclimation is important for inducing cold tolerance in *Citrus*. Cold tolerance in many plant species is not constitutively expressed; it is induced in response to reduction in daylength and the exposure to non-freezing chilling temperatures. Periods of warm weather before a freeze event will make citrus plants less cold-acclimated and thus more susceptible to the freezing conditions (Davies and Albrigo, 1994). Gene expression differences during acclimation have been associated with freeze tolerance in *Citrus* (He et al., 2012; Lang et al., 2005; Zhang et al., 2005). Genes for cold tolerance have been identified in *P. trifoliata*, *C. grandis* (= *C. maxima*), *C. paradisi*, *C. sinensis*, and *C. jambhiri* (Champ et al., 2007; He et al., 2012; Long et al., 2012; Sahin-Çevik and Moore, 2006; Webber et al., 2003). Use of cold-tolerant rootstocks such as *Poncirus* can increase the expression of cold tolerance associated genes in cold-tolerant scions (Ebel et al., 2005; Huang et al., 2011; Yelenosky and Vu; 1992).

Non-cultivated *Citrus* and *Citrus* relatives have economically important traits that could be extremely valuable in the development of new cultivars (Grosser and Gmitter, 1990). Breeding within the *Citrus* genepool is complicated by many factors including prolonged juvenility, self- and cross-incompatibility, polyembryony (apomixis), heterozygosity, and inbreeding depression (Grosser and Gmitter, 1990; Soost and Cameron, 1975; Soost and Roose, 1996). However, genetic transformation techniques such as protoplast

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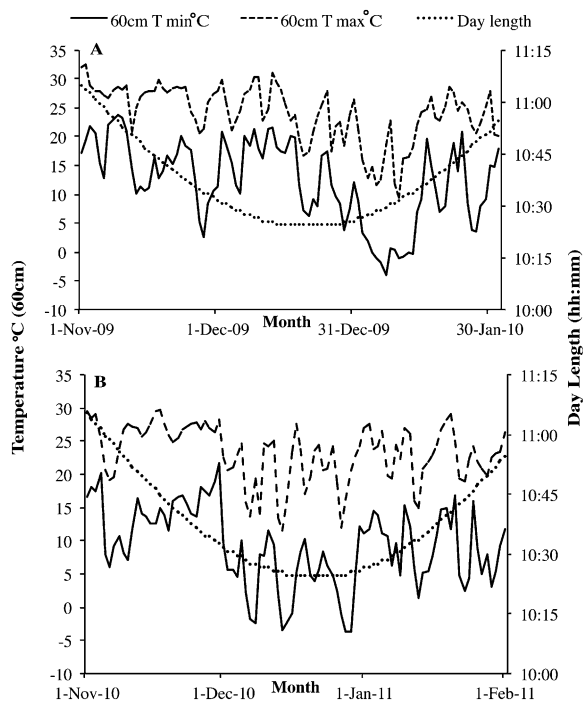


Fig. 1. Day length (hh:mm) and minimum and maximum temperatures (°C) for Fort Pierce, FL, in (A) Winter 2010 (1 Nov. 2009 to 30 Jan. 2010) and (B) Winter 2011 (1 Nov. 2010 to 30 Jan. 2011).

transformation (Fleming et al., 2000; Grosser and Gmitter, 1990), particle bombardment (Yao et al., 1996), and *Agrobacterium*-mediated transformation (Luth and Moore, 1999; Moore et al., 1992) may be used to develop genotypes with cold resistance genes from distant relatives but with desired fruit quality.

The objective of this study was to survey genotypes within the Rutaceae for freeze damage as an indication of cold tolerance. Seedlings from 92 highly diverse seed-source genotypes were exposed to natural freeze events in east-central Florida. Defoliation and dieback were assessed in the winters of 2010 and 2011.

Materials and Methods

Seeds from *Citrus* and *Citrus* hybrids were obtained from the USDA-ARS National Clonal Germplasm Repository for Citrus and Dates (NCGRCD) at the University of California at Riverside (UCR), Riverside, CA (<<http://www.citrusvariety.ucr.edu>>). This collection contains over 1100 accessions, each one identified by a unique Citrus Research Center (CRC) number. The names and species designations reported here are those used by the NCGRCD at the time of publication. In this study, seeds from 124 accessions were initially acquired representing ≈85% of the genetic diversity within the UCR collection (Barkley, 2003). The phylogenetic relationships of the parent genotypes used are described in Barkley (2003) and Bayer et al. (2009). Seeds of *Afraegle paniculata* (Schum.) Engl. and *Aegle marmelos* (L.) Corr (subfamily Aurantiaceae), and *Casimiroa edulis* (Llave et Lex, subfamily Toddaliaceae) were obtained from the Fruit

and Spice Park (Miami/Dade County, FL). *Zanthoxylum ailanthoides* (L.) (subfamily Toddaliaceae) was obtained from the University of Georgia. Within the Rutaceae, nucellar embryony varies greatly (Frost and Soost, 1968); therefore, the progeny included in this study were either genetically identical to the maternal parent or half-sib hybrids with a known seed parent and an undetermined pollen parent.

Under greenhouse conditions at the USDA-ARS U.S. Horticultural Research Laboratory in Fort Pierce, FL, seeds of 92 of the 124 seed-source accessions were successfully propagated in individual plastic cells (3.8 × 21 cm) (SC-10 super cell Cone-tainers; Stuewe and Sons, Corvallis, OR) containing sterile potting mix. After 4 to 7 months, the seedlings were transferred to 3.7-L containers, which were watered daily and fertilized on a weekly basis. The maximum and minimum diurnal temperatures of the greenhouse were 35 and 23 °C in the summer and 32 and 20 °C in the winter. In June and July 2009, the 6- to 9-month-old seedlings were planted 0.6 m apart in three rows (3.5 m apart) at the USDA-ARS, Fort Pierce, FL, farm. The planting was established as a randomized complete block design.

Two weeks after freeze events in Jan. 2010, the percentage defoliation (1% to 100%) was visually estimated on each tree and presence or absence of dieback (0 or 1) was recorded. In 2011, plants were rated for percentage defoliation (1% to 100%) in February. Percentage of canopy dieback (1% to 100%) was visually estimated on each plant in May. Weather data for 2010 and 2011 were collected using the Florida Automated Weather Network (FAWN) for the Ft.

Pierce site, which was 3.2 km from the test plot. A weather station on the farm site indicated temperatures within 0.5 °C of the FAWN data on each freeze night. Micro-sprinklers were run during the freeze events but were at ground level as used for standard irrigation.

Statistical analyses were performed with JMP® Version 11 (SAS Institute Inc., 2013). The data were not normally distributed and did not meet the assumptions for analysis of variance. Therefore, nonparametric statistical methods were used to analyze the data. Means were calculated for defoliation and dieback for seedlings of each of the parent genotypes and of each of the genotypic groups. Genotypic groups were assigned based on pedigree of 50% or more of a non-*Citrus* genus or distant *Citrus* relative, and within *Citrus* only by a pedigree of 60% or more of the indicated species group. Designation of pummelo/mandarin hybrid was chosen for parent genotypes, which were predominately comprised of equal portions of *C. maxima* (pummelo) and *C. reticulata* (mandarin), except that sour oranges (generally 50% pummelo and 50% mandarin) were maintained as a group based on their distinct phenotype. Differences in percentage defoliation and dieback within years were compared using the Wilcoxon/Kruskal-Wallis test (rank sums) and χ^2 test. Spearman correlations were calculated to determine if defoliation and dieback were correlated within individual trees in each of the years.

Results

Winter of 2010. In the winter of 2010, freezing temperatures were recorded between 5 and 12 Jan. with the lowest temperature of -4 °C on 7 Jan. (Fig. 1A). After these freeze events, average defoliation was 53% ± 28% and ranged from 14% to 100% (n = 522 plants). *P. trifoliata* and its hybrids were recorded as having the greatest amount of defoliation (83%), but *P. trifoliata* is fully deciduous and its hybrids are partially deciduous in most winters. All other parent genotypes/groups assessed in this study are fully non-deciduous (evergreen). Even with the *P. trifoliata* and its hybrids removed, there were significant differences in defoliation among parent genotypes ($\chi^2 = 298$, df = 69, $P < 0.0001$). As a group, the Citrons had the lowest average level of defoliation (18%) (Fig. 2A). Within *Citrus*, *C. medica* (CRCs 3546 and 661) had the lowest defoliation at 14%. Seven other *Citrus* genotypes had less than 35% defoliation and included *C. reticulata* (CRC 3260), *C. medica* (CRC 3523), *C. excelsa* (CRC 2317), *C. macrophylla* (CRC 3842), *C. limonia* (CRC 712), *C. volkameriana* × *C. limonia* (CRC 3050), and ×*Citrofortunella* (CRC 3172) (Table 1). Among the genera in the Aurantiaceae that are not cross-compatible with *Citrus* (hereinafter named the “other Aurantiaceae” group), *Murraya paniculata* (CRC 1637) had the lowest amount of defoliation, 18% (Table 1). Other genera of relatives with less than 35% defoliation

Table 1. Average defoliation (%) and dieback (%) of progenies of 92 *Citrus* and *Citrus* relative seed source genotypes exposed to freezing temperatures in the field in Fort Pierce, FL, in the winter of 2010 (1 Nov. 2009 to 30 Jan. 2010) and the Winter 2011 (1 Nov. 2010 to 30 Jan. 2011).^z

Botanical name of seed parent ^y (CRC) ^x	Common name	Primary group	Winter 2010		Winter 2011		
			No.	Defoliation (%) ^w	No.	Defoliation (%) ^w	Dieback (%) ^w
<i>Clausena harmandiana</i> (Pierre)	Clausena	Other Aurantiodeae	—	—	8	100 ^A	99 ^A
Guillaumin (4034)							
<i>Afraegle paniculata</i> (b)	Nigerian powder flask	Other Aurantiodeae	—	—	8	54 ^F	98 ^{AB}
<i>Clausena excavata</i> Burm. f. (3166)	Pink wampee	Other Aurantiodeae	—	—	8	100 ^A	94 ^{ABC}
<i>C. maderaspatana</i> Tan. (3225)	Kitchli sour orange	Sour orange	—	—	7	100 ^A	94 ^{ABC}
<i>Microcitrus inodora</i> (F.M. Bail)	Large leaf Australian wild lime	Australian	8	88 ^{AB}	8	100 ^A	93 ^{ABC}
Swing. (3785)							
<i>Aeglopsis chevalieri</i> (2878)	Chevalieri's aeglopsis	Other Aurantiodeae	2	100 ^{A-E}	2	100 ^{A-E}	93 ^{A-R}
<i>C. reticulata</i> Blanco (3022)	Frua mandarin	Mandarin	8	79 ^{ABC}	8	100 ^A	91 ^{ABCD}
<i>C. aurantiifolia</i> (Christm.) Swing (3822)	Mexican lime type	Lime	8	50 ^{B-L}	8	100 ^A	90 ^{A-E}
<i>C. nobilis</i> Lour. (3845)	King tangor	Mandarin	8	86 ^{AB}	8	100 ^A	90 ^{ABCD}
<i>Microcitrus</i> hybrid (<i>M. australis</i> × <i>M. australasica</i>) (1485)	Sydney Hybrid	Australian	7	26 ^{IJKL}	8	76 ^{DE}	82 ^{A-F}
<i>C. reticulata</i> hybrid ('Clementine' × 'Orlando') (3851)	Lee mandarin	Mandarin	8	58 ^{A-K}	8	96 ^{ABCD}	81 ^{A-F}
<i>C. davaoensis</i> (Wester) Tan. (2427)	Davao lemon (Papeda)	Papeda	—	—	8	100 ^A	80 ^{A-F}
<i>C. benikoji</i> hort. ex Tan. (3149)	C. benikoji	Papeda	—	—	8	99 ^A	79 ^{A-G}
<i>C. medica</i> L. (661)	Indian citron hybrid	Citron	8	14 ^L	8	100 ^A	78 ^{A-H}
<i>C. limon</i> (L.) Burm.f (3593)	Interdonato lemon	Lemon	8	30 ^{HL-L}	8	95 ^{ABCD}	78 ^{A-I}
<i>Citropsis daweanae</i> Swingle & Kellerm (3294)	Mozambique cherry-orange	Other Aurantiodeae	6	73 ^{A-H}	5	100 ^{ABC}	77 ^{A-M}
<i>Aegle marmelos</i> (L.) Corr. (3140)	Indian Bael fruit	Other Aurantiodeae	8	23 ^{JKL}	7	100 ^A	75 ^{A-M}
<i>Balsamocitrus dawei</i> Stapf (3514)	Uganda Powder-flask	Other Aurantiodeae	7	50 ^{B-L}	8	98 ^{AB}	75 ^{A-J}
<i>Microcitrus australis</i> (Planch.) Swing. (3673)	Australian round lime	Australian	8	25 ^{JKL}	8	76 ^{CDE}	74 ^{A-J}
<i>C. halmii</i> B.C. Stone (3780)	Citron hybrid	Kumquat	—	—	8	99 ^{AB}	74 ^{A-J}
<i>C. hybrid</i> (53-1-16 'Clem' × 'Hamlin') × Chinotto F1 (3715)	Sour orange hybrid ex-India	Sour orange	8	58 ^{A-K}	8	100 ^A	74 ^{A-J}
<i>C. limonia</i> (L.) Osbeck (3919)	Lamas rangpur lime (not rangpur)	Lemon	8	54 ^{A-L}	8	98 ^{AB}	73 ^{A-L}
<i>C. excelsa</i> Wester (2317)	Limon Real	Papeda	8	28 ^{IJKL}	8	100 ^A	72 ^{A-L}
<i>C. reticulata</i> Blanco (3363)	Belady mandarin	Mandarin	8	79 ^{ABC}	8	100 ^A	69 ^{A-N}
<i>C. lycopersiciformis</i> hort ex Tan. (3564)	Monkey orange	Papeda	—	—	8	100 ^A	69 ^{A-N}
<i>C. maxima</i> (Burm.) Merr. (3959)	Egami pummelo	Pommelo	8	60 ^{A-J}	8	100 ^A	69 ^{A-N}
<i>C. reticulata</i> Blanco (300)	Parson's Special	Mandarin	8	46 ^{C-L}	8	100 ^A	68 ^{A-O}
<i>C. medica</i> L. (3523)	Diamante citron	Citron	8	25 ^{JKL}	8	92 ^{A-E}	66 ^{A-P}
<i>C. limettioides</i> Tan. (1482)	Palestine sweet lime	Lime	8	49 ^{B-L}	8	100 ^A	66 ^{A-O}
<i>C. medica</i> L. (3546)	South Coast Field Station citron	Citron	8	14 ^L	8	94 ^{ABCD}	65 ^{A-P}
<i>C. reticulata</i> Blanco (3326)	Scarlet Emperor mandarin	Mandarin	8	79 ^{ABC}	8	100 ^A	65 ^{A-P}
<i>C. reticulata</i> Blanco (3752)	Som Keowan mandarin	Mandarin	8	55 ^{A-L}	8	97 ^{AB}	65 ^{A-P}
<i>C. limon</i> (L.) Burm.f. (3176)	Frost nucellar Lisbon	Lemon	8	63 ^{A-J}	8	98 ^{AB}	64 ^{A-Q}
<i>C. limon</i> (L.) Burm.f (3892)	Mesero lemon	Lemon	8	69 ^{A-I}	8	99 ^{AB}	64 ^{A-Q}
<i>Hesperethusa crenulata</i> (Roxb.) Nicolson (2879)	Eelelai maram	Other Aurantiodeae	—	—	8	99 ^{AB}	62 ^{A-R}
<i>C. reticulata</i> Blanco (3558)	Fremont mandarin	Mandarin	8	61 ^{A-J}	8	99 ^A	61 ^{A-R}
<i>C. reticulata</i> Blanco (3018)	Dweet tangor	Mandarin	—	—	8	100 ^A	60 ^{A-R}
<i>C. sunki</i> hort. ex Tan. (3143)	Sunki mandarin	Mandarin	—	—	8	100 ^A	58 ^{A-S}
<i>C. reticulata</i> hybrid ('Clementine' × 'Orlando') (3850)	Robinson mandarin	Mandarin	8	48 ^{B-L}	8	100 ^A	56 ^{A-S}
<i>C. latipes</i> (Swing.) Tan. (3052)	Khasi papeda	Papeda	8	70 ^{A-H}	8	97 ^A	56 ^{A-Q}
<i>C. maxima</i> (Burm.) Merr. (3859)	Reinking pummelo	Pommelo	8	64 ^{A-J}	8	99 ^A	56 ^{A-T}
<i>C. paradisi</i> Macf. (3781)	Tahitian pummelo × Star Ruby grapefruit	Pommelo	8	63 ^{A-J}	8	99 ^A	56 ^{A-S}
<i>Murraya paniculata</i> (L.) Jack (1637)	Orange Jessamine	Other Aurantiodeae	8	33 ^{F-L}	8	79 ^{A-E}	56 ^{A-S}
<i>C. webberi</i> Wester (1455)	Kalpi papeda	Papeda	8	78 ^{ABCD}	8	99 ^A	54 ^{A-T}
<i>C. reticulata</i> Blanco (3958)	Koster mandarin	Mandarin	8	58 ^{A-L}	8	99 ^{AB}	53 ^{A-T}
<i>C. longispina</i> Wester (2320)	Talamisan	Papeda	—	—	8	100 ^A	53 ^{A-T}
<i>Microcitrus australasica</i> (F.J. Muell.) Swing. (1484)	Australian finger lime var. Sanguinea	Australian	7	61 ^{A-J}	8	88 ^{A-E}	51 ^{A-S}
× <i>Citrofortunella</i> sp. (3172)	Tavares limequat	Kumquat	8	24 ^{JKL}	8	95 ^{ABCD}	51 ^{A-T}
<i>C. volkameriana</i> / <i>C. limonia</i> Osbeck (3050)	Volkamer lemon hybrid	Lemon	8	35 ^{F-L}	8	99 ^A	51 ^{A-T}
<i>C. aurantium</i> L. (3289)	Yama-milan sour orange	Sour orange	5	64 ^{A-L}	4	100 ^{ABCD}	51 ^{A-L}
<i>C. jambhiri</i> Lush. (400)	Florida rough lemon	Lemon	—	—	8	100 ^A	50 ^{A-T}
× <i>Microcitronella</i> sp. (<i>M. australasica</i> × <i>Calamondin</i>) (1466)	Fastrimedid	Australian	—	—	8	79 ^{A-E}	49 ^{A-T}
<i>C. maxima</i> (Burm.) Merr. (2248)	Kao Panne pummelo	Pommelo	8	50 ^{B-L}	8	100 ^A	48 ^{A-T}
<i>Severinia buxifolia</i> (Poiret) Tan. (1491)	Chinese box orange	Other Aurantiodeae	—	—	8	47 ^F	48 ^{A-T}
<i>Murraya paniculata</i> L. (3171)	var. ovatifoliolata	Other Aurantiodeae	8	18 ^{KL}	8	71 ^{EF}	48 ^{A-T}
<i>C. limon</i> L. Burm.f. (3005)	Frost nucellar Eureka lemon	Lemon	8	48 ^{B-L}	8	98 ^{AB}	46 ^{A-T}
<i>C. limonia</i> (L.) Osbeck (712)	Santa Barbara red lime	Lime	8	23 ^{JKL}	8	96 ^{ABCD}	46 ^{A-T}
<i>C. aurantifolia</i> (Christm.) Swing. (2450)	India lime	Lime	8	36 ^{D-L}	8	93 ^{ABCD}	46 ^{A-T}
<i>C. amblycarpa</i> Och. (2485)	Nasharan mandarin	Mandarin	8	60 ^{A-J}	8	94 ^{ABCD}	46 ^{A-T}
<i>C. macrophylla</i> Wester (3842)	Alemow	Papeda	8	33 ^{F-L}	8	100 ^A	46 ^{A-T}

(Continued on next page)

Table 1. (Continued) Average defoliation (%) and dieback (%) of progenies of 92 *Citrus* and *Citrus* relative seed source genotypes exposed to freezing temperatures in the field in Fort Pierce, FL, in the winter of 2010 (1 Nov. 2009 to 30 Jan. 2010) and the Winter 2011 (1 Nov. 2010 to 30 Jan. 2011).^z

Botanical name of seed parent ^y (CRC) ^x	Common name	Primary group	No.	Winter 2010		Winter 2011	
				Defoliation (%) ^w	No.	Defoliation (%) ^w	Dieback (%) ^w
<i>C. limon</i> (L.) Burm. f. (3885)	Local variety from Iran	Lemon	8	36 ^{D-L}	8	97 ^{ABC}	44 ^{A-T}
<i>Eremocitrus glauca</i> (Lindley) Swing. Hybrid (4105)	Australian desert lime hybrid	Australian	8	58 ^{A-K}	8	83 ^{A-E}	43 ^{B-T}
<i>C. reticulata</i> Blanco (4003)	Sun Chu Sha mandarin	Mandarin	—	—	8	98 ^{AB}	41 ^{B-T}
<i>C. neo-aurantium</i> Tan. (3611)	Konejime sour orange hybrid	Sour orange	8	43 ^{C-L}	8	96 ^{ABCD}	40 ^{C-T}
× <i>Citroncirus</i> sp. (3552)	S-281 Citrangelo	Trifoliolate	8	88 ^{AB}	8	100 ^A	40 ^{C-T}
<i>C. aurantium</i> L. (3929)	Gou Tou Cheng	Sour orange	8	63 ^{A-J}	8	98 ^{AB}	39 ^{C-T}
<i>C. maxima</i> (Burm.) Merr. (4026)	Pomelit	Pommelo/mandarin	8	64 ^{A-J}	8	99 ^A	36 ^{D-T}
<i>C. reticulata</i> Blanco (3812)	Unnamed mandarin	Mandarin	—	—	8	93 ^{ABCD}	35 ^{E-T}
<i>C. leiocarpa</i> hort ex Tan. (3147)	Koji mandarin	Papeda	—	—	8	91 ^{A-E}	34 ^{E-T}
<i>C. reticulata</i> Blanco (3260)	Soh niamtra mandarin	Mandarin	8	31 ^{G-L}	8	95 ^{ABCD}	33 ^{F-T}
<i>C. maxima</i> (Burm.) Merr. (2242)	Kao Pan pummelo	Pommelo	8	56 ^{A-K}	8	97 ^{ABC}	33 ^{F-T}
<i>C. aurantium</i> L. (3930)	Zhuluan sour orange hybrid	Sour orange	8	76 ^{A-E}	8	91 ^{A-E}	33 ^{F-T}
<i>C. intermedia</i> hort ex Tan. (3474)	Konejime sour orange hybrid	Sour orange	8	59 ^{A-K}	8	99 ^{AB}	32 ^{F-T}
<i>C. sinensis</i> (L.) Osbeck (3858)	Pineapple sweet orange	Mandarin	8	59 ^{A-K}	8	99 ^A	24 ^{G-T}
<i>C. reticulata</i> Blanco (2590)	Tien Chieh mandarin	Mandarin	8	59 ^{A-K}	8	91 ^{A-E}	23 ^{I-T}
<i>Bergera koenigii</i> L. (3165)	Curry leaf	Other Aurantiodeae	8	36 ^{D-L}	8	100 ^A	23 ^{I-T}
<i>C. neo-aurantium</i> (<i>C. obovoidea</i> + <i>C. unshiu</i> graft chimera) (3816)	Kinkoji Unshiu graft chimera	Sour orange	—	—	8	94 ^{ABCD}	23 ^{H-T}
<i>C. taiwanica</i> Tan. & Shimada (2588)	Nansho Daidai sour orange	Sour orange	8	74 ^{A-F}	8	97 ^{ABCD}	21 ^{J-T}
<i>C. aurantium</i> L. (628)	Standard sour orange	Sour orange	—	—	7	98 ^{AB}	18 ^{J-T}
<i>C. hassaku</i> hort ex Tan. (3907)	Hassaku pummelo hybrid	Pommelo/mandarin	8	76 ^{A-E}	8	89 ^{A-E}	17 ^{M-T}
<i>Glycosmis pentaphylla</i> (Retz.) Corr. (3285)	Orange berry	Other Aurantiodeae	8	31 ^{G-L}	8	53 ^F	17 ^{M-T}
<i>C. maxima</i> (Burm.) Merr. (3945)	Mato Buntan pummelo	Pommelo	—	—	7	85 ^{A-E}	16 ^{K-T}
<i>C. hassaku</i> hort ex Tan. (3942)	Hassaku pummelo hybrid	Pommelo/mandarin	8	59 ^{A-K}	8	100 ^A	16 ^{N-T}
<i>Poncirus trifoliata</i> L. (4007)	“Little-leaf” trifoliolate	Trifoliolate	8	94 ^A	8	99 ^A	14 ^{O-T}
<i>Severinia buxifolia</i> (Poirlet) Tan. (1497)	Chinese box orange (brachytic form)	Other Aurantiodeae	—	—	8	7 ^E	11 ^{P-T}
<i>Poncirus trifoliata</i> L. (3549)	Simmons trifoliolate	Trifoliolate	—	—	8	100 ^A	8 ^{RST}
<i>C. aurantium</i> L. (2717)	Olivelands sour orange	Sour orange	8	39 ^{C-L}	8	93 ^{ABCD}	7 ^{RST}
<i>Zanthoxylum ailanthoides</i> L. (a)	Japanese prickly-ash	Toddalioideae	8	100 ^A	8	100 ^B	7 ^{QRST}
× <i>Citroncirus</i> sp. (<i>C. paradisi</i> ‘Duncan’ × <i>P. trifoliata</i>) (3771)	Swingle citrumelo	Trifoliolate	8	73 ^{A-G}	8	96 ^{ABCD}	5 ST
× <i>Citroncirus</i> sp. (301)	Rusk citrange trifoliolate hybrid	Trifoliolate	8	78 ^{ABCD}	8	96 ^{ABCD}	4 ST
× <i>Citroncirus</i> sp. (‘Cleopatra’ mandarin × trifoliolate) (3957)	Trifoliolate	Trifoliolate	8	94 ^A	8	93 ^{ABCD}	4 ^{RST}
<i>Casimiroa edulis</i> Llave & Lex (<i>e</i>)	White Sapote	Toddalioideae	8	18 ^{KL}	8	54 ^{CD}	1 ^T

^zThe table is sorted based on dieback observed during Winter 2011.

^yBotanical and common names specified by Citrus Variety Collection, Riverside, CA (<<http://www.citrusvariety.ucr.edu>>).

^xAccession number assigned by Citrus Research Center and now maintained by the UC Riverside Citrus Variety Collection.

^wAverage defoliation and dieback were compared using nonparametric Wilcoxon/Kruskal Wallis test. Values followed by the same letter within a column are not significantly different at $P = 0.05$.

included *Microcitrus australis* (CRC 3673), *Microcitrus* hybrid (*M. australis* × *M. australis*) (CRC 1485), *Hesperethusa crenulata* (CRC 2879), *Murraya paniculata* (CRC 3171), and *Glycosmis pentaphylla* (CRC 3285). *Aeglopsis chevalier* (CRC 2878) had the greatest amount of defoliation, 100% (Table 1). In 2010, dieback was also different among parent genotypes ($\chi^2 = 96$, $df = 91$, $P = 0.014$). Less than 13% of the total plants exhibit signs of dieback (based on present or absent). The Australian natives had the greatest amount of dieback; 21% of the plants in this group showed some dieback (Fig. 2A). All of the plants in 2010 recovered (data not shown).

Winter of 2011. Preceding the winter of 2011, on 8 Dec. 2010, the temperature rapidly dropped to -2.5 °C followed by two more freeze events below -2.5 °C on 14 and 28 Dec. with the lowest temperature recorded at -3.7 °C (Fig. 1B). Average defoliation was significantly higher in 2011 than in 2010 and ranged from 100% to 7% with an average of $93\% \pm 17\%$ ($n = 719$). There were significant differences among progenies of parent genotypes in defoliation ($\chi^2 = 374$, $df = 91$, $P <$

0.0001) and dieback ($\chi^2 = 383$, $df = 91$, $P <$ 0.0001). Analyzed as groups, the “true” citrus had the greatest amount of defoliation, ranging from 95% to 98%. The “other Aurantiodeae” showed the least amount of defoliation at 80% (Fig. 2B), but this was largely the result of the low defoliation in *Severinia* with seedlings of accessions *Severinia buxifolia* (CRC 1497) showing significantly less defoliation (7%) than all others in the trial. The other *Citrus*-related genera (including the other Aurantiodeae, Toddalioideae, and Australian natives) with less than 80% defoliation included *Microcitrus australis* (CRC 8673), *Murraya paniculata* (CRCs1637 and 3171), *Glycosmis pentaphylla* (3285), and *Casimiroa edulis* (Table 1). In these groups, progeny of the *Microcitrus* hybrids CRCs 1485 and 1466 had the least amount of defoliation, 76% and 78%, respectively (Table 1).

There was much greater dieback in 2011 than in 2010. In 2011, average dieback was 49% and ranged from 99% to 1% (Table 1). *Casimiroa edulis* and *Zanthoxylum ailanthoides*, within the Toddalioideae subfamily, had very low amounts of dieback at 1%

and 7%. Within the Aurantiodeae, *Poncirus trifoliata* and hybrids were lowest ranging from 4% to 40% dieback. Within the groups of the genus *Citrus*, several parent genotypes had less than 25% dieback and included *C. reticulata* (CRC 2590), *C. sinensis* (CRC 3858), *C. maxima* (CRC 3945), *C. hassaku* (CRC 3907 and 3942), *C. aurantium* (CRC 628 and 2717), *C. taiwanica* (CRC 2588), and *C. neo-aurantium* (CRC 3816). Within the “other Aurantiodeae” group, *Severinia buxifolia* (CRC 1497), *Bergera koenigii* (CRC 3165), and *Glycosmis pentaphylla* (CRC 3285) had less than 25% dieback (Table 1). Analyzed as groups, the Citrons and Australian natives had the greatest amount of dieback, 68% and 66%, respectively. Pummelo/mandarin hybrids, trifoliates, and Toddalioideae had the least amount of dieback, 16%, 13%, and 4%, respectively (Fig. 2B). Defoliation and dieback within individual trees were positively correlated in the winter of 2011 (Spearman’s $\rho = 0.4591$, $P <$ 0.0001). Mortality resulting from freeze events in 2011 resulted in loss of 8.1% of the trees.

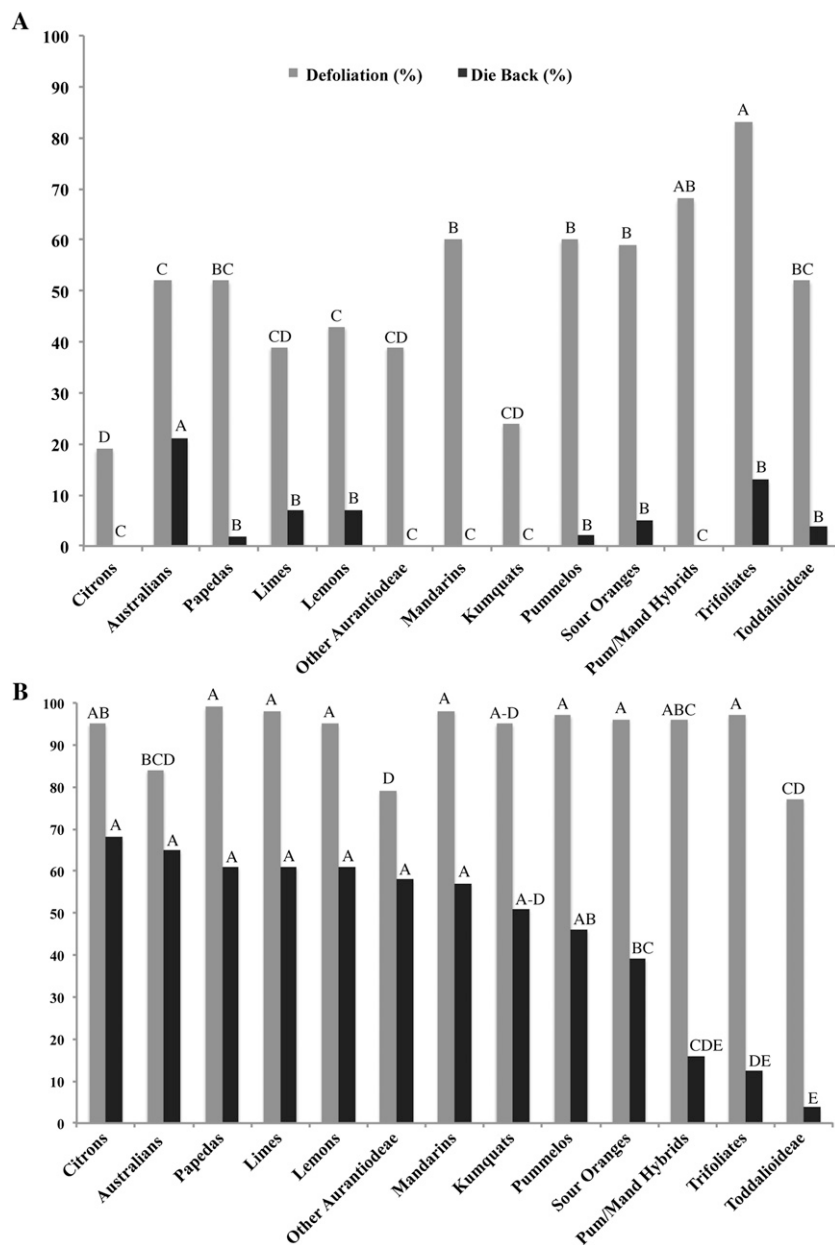


Fig. 2. Average defoliation (%) and dieback (%) of citrus taxonomic groups for progenies of 94 *Citrus* and *Citrus* relative seed source genotypes exposed to freezing temperatures in the field in Fort Pierce, FL, in (A) Winter 2010 and (B) Winter 2011. Means within years were compared using the Wilcoxon/Kruskal-Wallis non-parametric means separation test.

Discussion

In this study, defoliation and dieback were used to determine the response to cold temperatures in *Citrus* and *Citrus* relatives when exposed to natural freeze events in Florida. However, one of the problems with using defoliation as a measure of cold tolerance is that some of the varieties/species used in this study, including *Poncirus trifoliata* and associated hybrids, are deciduous or semi-deciduous. Also, it can be a healthy citrus response to drop leaves when exposed to freezing temperatures that damage leaves but not stems, and failure to drop leaves after such conditions reflects more serious damage (Ketchie, 1969). Stem damage is generally

a better measure of cold tolerance (Cooper et al., 1955; Ketchie, 1969). Stems tend to be the more cold-tolerant than leaves or fruit (Hendershott, 1962). A measure of electrolyte leakage is considered the best measure (Yelenosky, 1975). However, this method is not feasible in large-scale field trials.

In the Florida citrus community, there is a widely accepted view that “no two freezes are ever the same.” Tree responses are affected by differences in factors such as acclimation conditions (Yelenosky and Vu, 1992), rate of temperature change before and after the hard freeze, wind conditions, and time of occurrence within the seasonal span of tree quiescence. Damage in commercial Florida citrus trees is typically expected after

4 or more hours below $-2.2\text{ }^{\circ}\text{C}$ (Yelenosky, 1996). It is noteworthy that citrus in California is much more regularly exposed to freezing temperatures than citrus in Florida, and usually there is little tree damage, likely as a result of sustained cool temperatures in the fall and winter that maintain tree quiescence. The Lindsay, CA, weather station (typical of Central Valley, CA, citrus production) indicates 13 of 16 years and 309 total days with sub-freezing temperatures in 1998–2013 (UC IPM Online, 2014), and in the same period, there were 9 of 16 years and 96 d with temperatures below the $-2.2\text{ }^{\circ}\text{C}$ Florida citrus damage threshold. In the Lindsay, CA, 1998–2013 weather data set, the December median low is $1.7\text{ }^{\circ}\text{C}$ and average low is $1.8\text{ }^{\circ}\text{C}$; January median low is $2.8\text{ }^{\circ}\text{C}$ and average low is $2.5\text{ }^{\circ}\text{C}$ (Table 2).

In contrast, for the Ft. Pierce, FL, 1998–2013 FAWN weather data set, the December median low is $13.5\text{ }^{\circ}\text{C}$ and average low is $12.5\text{ }^{\circ}\text{C}$; January median low is $10.7\text{ }^{\circ}\text{C}$ and average low is $10.0\text{ }^{\circ}\text{C}$. In Fort Pierce data for this 16-year period, freezing temperatures have been recorded in seven of the 16 winters, for a total of 35 d. Temperatures below the $-2.2\text{ }^{\circ}\text{C}$ thresholds have been observed in only three of 16 winters, and two coincided with the years of this study, in Jan. 2010 and Dec. 2010 (Table 2). The 2010 freeze events were the only December freezes recorded at the Fort Pierce FAWN station in 16 years of operation. The low temperature averaged $8.5\text{ }^{\circ}\text{C}$ for the week before the first freeze and averaged $16.4\text{ }^{\circ}\text{C}$ for the last week of November, making it highly likely that the freeze events occurred before the plants were acclimated. This might have contributed to the marked defoliation and dieback in 2011. The age and size of trees will affect the degree to which the plants are acclimated, and younger trees are generally less cold-tolerant than older trees (Yelenosky, 1996). The seedlings in this study were only 1 to 2 years old when exposed to natural freeze events. Therefore, they were likely more susceptible to cold damage than they would have been if they were more mature at the times of exposure.

As temperatures approach a critical threshold, even slight differences in temperature may have a large effect on resulting tree damage. Therefore, effects such as trapping of warmer air within the canopy, differences in stem diameter that affect temperature change, and microclimate differences may influence damage. These differences likely contributed to the variability leading to modest statistical mean separation within this experiment so that approximately half of the parental genotypes were not statistically different ($\alpha = 0.05$) from the one with lowest dieback and approximately half were not statistically different from the one with highest dieback.

There have been a number of studies on cold-hardiness of citrus. Although there were some notable differences, in many cases, their reports were consistent with our observations. Trifoliates are generally reported to

Table 2. Freezing temperature-related data for 1998–2013 for the location of this study in Ft. Pierce, FL, and a typical California citrus area in Lindsay, CA.

	Ft. Pierce, FL ^z	Lindsay, CA ^y
December average low temp. (°C)	12.5	1.8
December median low temp. (°C)	13.5	1.7
Jan. median low temp. (°C)	10	2.5
Jan. average low temp. (°C)	10.7	2.8
Years below 0 °C (no.)	7	13
Years below -2.2 °C (no.)	3	9
Days below 0 °C (no.)	35	309
Days below -2.2 °C (no.)	8	96

^zData are from the Florida Automated Weather Network (FAWN, 2014).

^yData are from UC IPM Online (2014).

be the most cold-tolerant followed by kumquats and Satsuma mandarins. Sour oranges, tangerines, tangelos, sweet oranges, and grapefruit are generally intermediate in cold-hardiness. Lemons, limes, and citrons are generally the least cold-tolerant (Rieger et al., 2003). In our study, *P. trifoliata* and its hybrids were some of the most cold-tolerant seed parents as assessed by dieback in 2011. By all measures in this study, within the *Citrus* gene pool, the kumquat group was always in the least cold-damaged statistical grouping. Within the groups of genus *Citrus*, the pummelo/mandarin hybrids and sour oranges (which are also pummelo/mandarin hybrids) were in the statistical group with the least dieback. Contrary to the low cold tolerance typically reported, the citron group in 2010 had the least amount of defoliation; however, in 2011, the citrons had the greatest amount of dieback and defoliation. The freeze events in 2010 were less severe than in 2011, which may account for the differences in the response to cold temperatures.

One of the limitations of broad-scale screening for cold-tolerant genes among *Citrus* relatives is the small number of accessions in genera related to *Citrus* (Barkley, 2003; Bayer et al., 2009). Many of the distant *Citrus* relatives used in this study, including *Severinia*, *Glycosmis*, and *Casimiroa*, were among the most cold-tolerant. These species do not readily cross with *Citrus*. Cross-compatibility tends to decrease as distance increases among taxa as a result of pre-zygotic and post-zygotic barriers to hybridization (Ladizinsky, 1992). There has been very little successful intergeneric sexual hybridization between the Aurantioideae and Toddaliodeae. However, use of biotechnology has generated intergeneric somatic hybrids of some sexual-incompatible taxa such as *C. senensis* ‘Hamlin’ combined with *Severinia disticha* and *Citropsis gillenti*, *Citrus* + *Citropsis*, and *Citrus* + *Severinia* (Grosser et al., 1990, 1998; Grosser and Gmitter, 1990; Medina-Filho et al., 1998). These approaches may permit integration of genes for cold-hardiness.

It is noteworthy that the lowest dieback in the *Citrus* gene pool in 2011 was observed for seedlings with parental genotypes including *Poncirus trifoliata* or hybrids of pummelo × mandarin (including sour orange types). Only one parental genotype with *Poncirus* in its pedigree (CRC3552) showed more than 14% dieback, and two parental genotypes that were *Poncirus* × cultivated *Citrus* displayed

only 4% dieback. *Poncirus* and *Citrus* are sexually and graft-compatible (Gmitter, 1994; Grosser and Gmitter, 1990; Sanghera et al., 2011). *Poncirus* has been used in many intergeneric crosses to increase cold tolerance, although the fruit of *Poncirus* itself is inedible (Barrett, 1990; Gmitter, 1994; Tignor et al., 1998). The USDA citrus breeding program has been using *Poncirus* in some hybridizations for many years, specifically to enhance cold-hardiness (Barrett and Young, 1982; Tignor et al., 1998) and many advanced selections now include *Poncirus* in their pedigree, although so far none has been released as cultivars.

Six parental genotypes that were in the pummelo × mandarin hybrid or sour orange group had less than 25% dieback, but four pummelo × mandarin hybrids and sour orange parental types and many pummelos and mandarins showed substantial dieback. Somewhat surprising in this study was the minimal cold damage to seedlings of Mato Buntan pummelo, because pummelos are generally considered more “tropical” than mandarins and sweet oranges. However, Mato Buntan is the seed parent of Tanikawa Butan, which is relatively more cold-tolerant than most pummelos (Hodgson, 1967), and pummelos are completely monoembryonic; therefore, each of the seedlings in our test were unique hybrids. These results suggest that judicious breeding through selection of appropriate parents may confer greater cold-hardiness even through conventional citrus breeding, because pummelo and mandarin provide the primary genetic contribution to most commercial citrus scions.

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