Screening Citrus Rootstocks and Related Selections in Soil and Solution Culture for Tolerance to Low-iron Stress

William S. Castle¹ and James Nunnallee

University of Florida, IFAS, Horticultural Sciences Department, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850

John A. Manthey

U.S. Citrus and Subtropical Products Laboratory, USDA, ARS, 600 Avenue S, NW, Winter Haven, FL 33881

Additional index words. calcareous, citrus relatives, Fe reduction, leaf greenness, sample size

Abstract. A broad range of plant selections across the orange subfamily *Aurantioidae* were screened in solution and soil culture for their tolerance to low iron (Fe) stress. Young seedlings grown in soil were transferred to tubs of +Fe nutrient solution, which was later replaced after a brief period with a –Fe solution. Over several trials, ≈20 white root tips were harvested periodically from the plants in each tub and assayed for their ability to reduce Fe³⁺. The procedure was miniaturized to determine if a fewer number of root tips could be assayed to screen individual plants and to estimate the required sample size. For solution screening, seven root tips were estimated to be adequate for representing a single plant. Seedlings of a few selections were also grown in small containers of soil amended with 0% to 5.9% CaCO₃. The results in solution and soil culture were consistent with each other and with previous assessments of the various selections. Based on a summary of the solution and soil responses, the citrus selections were grouped in descending order of Fe³⁺ reduction rates as Volkamer lemon/Rangpur/sour orange selections/*Citrus macrophylla* > mandarins and mandarin hybrids > citranges > citrumelos > trifoliolate orange. Of the citrus relatives tested in solution culture only, those in the genera *Glycosmis*, *Citropsis*, *Clausena*, and *Murraya* had high Fe reduction rates with good seedling growth and new leaves developed a light yellow color or showed no loss of greenness. Other citrus relatives in the genera *Severinia*, *Atalantia*, and *Fortunella* and most somatic hybrids had low seedling vigor and produced too few root tips to be properly assessed. The results are useful because of the breadth of selections screened, the identification of various citrus relatives as potential sources of low-Fe stress tolerance in breeding new rootstocks, and the apparent positive relationship between the Fe³⁺ reduction responses, soil screening responses, and field experiences with carbonate-induced Fe chlorosis responses.

Citrus trees on many commercial rootstocks do not perform well in high-carbonate soils (Campbell, 1991; Castle, 1987; Castle et al., 2004; Cooper and Peynado, 1953, 1954; Ferguson et al., 1990; Hamze et al., 1986; Hodgson, 1967; Sagee et al., 1992; Sudahono and Rouse, 1994; Wutscher, 1979). Such rootstocks are limited by their inability to sufficiently extract micronutrients, including iron (Fe), that are rendered largely unavailable in these kinds of soils (Korcak, 1987; Manthey et al., 1994a). This limitation particularly applies to *Poncirus trifoliata* and its hybrids, which include some of the world's most popular rootstocks like Troyer and Carrizo citranges and Swingle

citrumelo (Castle, 1987; Wutscher, 1979). The international significance of this problem is evident in the continued reporting of trials and germplasm releases concerning rootstocks specifically investigated for tolerance to high-carbonate soils (Tagliavini and Rombolá, 2001; Wei et al., 1994).

In the United States, citrange and citrumelo rootstocks are the mainstays of the Florida and California citrus industries, but both states also have areas of high-carbonate soils (Castle et al., 1993; Ferguson et al., 1990). Trees can be grown in those questionable sites if the grower is willing to choose other rootstocks with less desirable traits (Castle et al., 1992). Two examples are sour orange and rough lemon rootstocks, which are both well adapted to calcareous soils. Trees on sour orange produce excellent quality fruit, but are susceptible to citrus tristeza virus; those on rough lemon are high-yielding, but produce poor-quality fruit and are susceptible to citrus blight. Other management options

for high-carbonate soils such as application of chelates are often expensive, so there is a strong incentive to develop new rootstocks (Castle et al., 2004; Grosser et al., 2004).

We began a screening project to search for superior citrus rootstocks suitable for calcareous sites based on the measurement of root Fe³⁺ reduction rates (Castle and Manthey, 1998). This method was selected because of its potential to minimize various interfering conditions that can occur in other screening methods mostly related to Fe chemistry (Cooper and Peynado, 1954; Hamze et al., 1986; Korcak, 1987; Manthey et al., 1993, 1994a; Pestana et al., 2005; Sagee et al., 1992; Sudahono and Rouse, 1994). For example, when using soil, pH affects the chemical state of Fe, and both pH and air-drying influence bicarbonate content, and the buffering system in solution studies affect Fe availability and uptake (Korcak, 1987).

Plants can respond to low-Fe stress through several inducible mechanisms, including, among others, electron release at the root surface (Briat and Lobréaux, 1997; Manthey et al., 1994b). By measuring a fundamental plant response, Fe reduction rate, in the simple test environment of a complete nutrient solution minus Fe, other possibly complicating factors could be minimized and the results would be more broadly applicable.

Our first screening project involved primarily common rootstocks and other citrus selections (Castle and Manthey, 1998). The selections ranked in terms of Fe³⁺ reduction rates in the same general order as their rankings developed from field-based and other screening trials, i.e., the lemon-type rootstocks such as Rangpur and Volkamer and rough lemon had the highest Fe reduction rates, the mandarins had intermediate rates, and the citranges and citrumelos along with trifoliolate orange had the lowest rates. However, some selections ranked well below their expected ranking from field observation; also, measurements of Fe³⁺ reduction rates along with growth and leaf chlorosis suggested that the selections fell into several categories based on a composite of the three variables that improved the ranking procedure.

Two questions remaining after our first efforts were: 1) could the procedure be miniaturized so that individual plants might be rapidly and efficiently evaluated, thus enabling high-throughput screening for citrus breeders, a clear advantage in plant breeding; and 2) how do the solution culture and soil screening methods compare given that soil is a more natural environment and may produce results more closely linked to field experience? Thus, our objectives were to expand the range of material screened for tolerance to low-Fe stress to include additional rootstocks, citrus relatives, and other selections to confirm our previous results, develop a miniaturized procedure, and compare solution culture with soil screening.

Materials and Methods

Plant material. All plants were either seedlings or cuttings grown in containers

Received for publication 29 Dec. 2008. Accepted for publication 24 Mar. 2009.

The technical assistance of James Baldwin is greatly appreciated.

¹To whom reprint requests should be addressed; e-mail bcastle@ufl.edu.

with a peat-based medium before their use in these experiments (Table 1). The seedlings were grown in the spring, except as noted, in a temperature-controlled greenhouse with natural light and were fertilized regularly with a tap water mix of a water-soluble 20N–20P–20K plus micronutrients fertilizer.

Nutrient solution screening, Summer and Fall 1998. Uniform seedlings ≈10 cm tall

with four to six leaves were removed from their containers and washed free of the medium. They were transferred to 11-L plastic tubs filled with a nutrient solution of 1.3 mM Ca(NO₃)₂, 1.0 mM KNO₃, 0.8 mM MgSO₄, 0.1 mM K₂HPO₄, 0.56 μM ZnSO₄, 6.7 μM MnSO₄, 0.24 μM CuSO₄, 0.2 μM Na₂MoO₄, 33.0 μM boric acid, H₃BO₃, and 35 μM FeHEDTA. The solutions were prepared with

tap water containing less than 0.15 mg·L⁻¹ (2.7 μM) Fe. There were two tubs (replications) of each selection and each tub held 28 plants suspended in the lid in 3-cm diameter, equally spaced holes and kept in place with a soft foam stopper. Some screening runs included single 22-L tubs holding 54 plants of one selection. Plants were grown in the +FeHEDTA solution for ≈10 d and then the solution was replaced

Table 1. Plant materials for low-iron stress screening.

Common name	Scientific name	Source ^z	Trials ^y						
			Summer 98 soln.	Fall 98 soln.	1999 soil	Soln. 1999	Mintube 1999	Soln. 2001	Mintube 2001
Benton citrange	<i>C. sinensis</i> (L.) Osb. × <i>Poncirus trifoliata</i> (L.) Raf.	USNGR	x						
C-32 citrange	<i>C. sinensis</i> Ruby × <i>P. trifoliata</i>	USNGR	x						
C-35 citrange	<i>C. sinensis</i> Ruby × <i>P. trifoliata</i>	USNGR						x	
Calamondin	<i>C. madurensis</i> Lour.	DPI	x						
Carrizo citrange	<i>C. sinensis</i> × <i>P. trifoliata</i>	Reed Bros.	x						x
Ceylon Atalantia	<i>Atalantia ceylanica</i> (Arn.) Oliv.	USNGR		One tub	x	x	x		
Chinese box orange	<i>Severinia buxifolia</i> (Poir.) Ten.	DPI	x						
Chinese glycosmis	<i>Glycosmis pentaphylla</i> Auct.	DPI	x						
Chinese wampee	<i>Clausena lansium</i> (Lour.) Skeels	USNGR	x						
Chinotto sour orange	<i>C. myrtifolia</i> Raf.	DPI	x						
Cleo + FDT	<i>C. reticulata</i> Blanco Cleopatra + <i>P. trifoliata</i> Flying Dragon	JG	x						
Cleo × TF (52)	<i>C. reticulata</i> × <i>P. trifoliata</i>	Spain	x						
Cleo × TF (51)	<i>C. reticulata</i> × <i>P. trifoliata</i>	Spain	x			x			
Cleo × TF (50)	<i>C. reticulata</i> × <i>P. trifoliata</i>	Spain	One tub			x			
Cleopatra mandarin	<i>C. reticulata</i>	DPI	x		x				
F80-8 citrumelo	<i>C. paradisi</i> × <i>P. trifoliata</i>	DPI	x						
Gillet's cherry-orange	<i>Citropsis gilletiana</i> Swing. & M. Kell.	USNGR		x					
Hamlin + rough lemon	<i>C. sinensis</i> + <i>C. jambhiri</i> Lush.	JG	x						
Indian bael fruit	<i>Aegle marmelos</i> (L.) Corr.	USNGR	x						
Kinkoji	<i>C. obovoidea</i> Hort. ex Tak.	DPI			x			x	
Kuharske citrange	<i>C. sinensis</i> × <i>P. trifoliata</i>	DPI	x						
Macrophylla	<i>C. macrophylla</i> Wester	DPI						x	
Marumi kumquat	<i>Fortunella japonica</i> (Thunb.) Swing.	DPI	x						
Meiwa kumquat	<i>Fortunella crassifolia</i> Swing.	DPI	x						
Milam + Kinkoji	<i>C. jambhiri</i> hybrid + <i>C. obovoidea</i>	JG	One tub						
Nova + HB pummelo	[<i>C. reticulata</i> × (<i>C. paradisi</i> × <i>C. reticulata</i>)] + <i>C. maxima</i> (Burm.) Merrill	JG		x	x				
Nova + Ichangensis	[<i>C. reticulata</i> × (<i>C. paradisi</i> × <i>C. reticulata</i>)] + <i>C. ichangensis</i> Swing.	JG		x					
Orange jessamine	<i>Murraya paniculata</i> (L.) Jack, Malay	DPI	x						
Procimequat	<i>Fortunella</i> hybrid	DPI	x						
Rangpur	<i>Citrus limonia</i> Osb.	DPI		x	x			x	
Smooth Flat Seville	<i>C. aurantium</i> hybrid	Reed Bros.			x				
Sour orange	<i>C. aurantium</i> (L.)	DPI		x	x	x	x		
Sour orange + Benton citrange	<i>C. aurantium</i> + (<i>C. sinensis</i> × <i>P. trifoliata</i>)	JG	x						
Sour orange + Carrizo	<i>C. aurantium</i> + (<i>C. sinensis</i> × <i>P. trifoliata</i>)	JG			x				x
Sour orange + Flying Dragon TF	<i>C. aurantium</i> + <i>P. trifoliata</i>	JG							x
Sour orange + PSL	<i>C. aurantium</i> + <i>C. limettoides</i> Tan.	JG		x				x	
Sour orange + Rangpur	<i>C. aurantium</i> + <i>C. limonia</i>	JG	x						
Sour orange + TF (50-7)	<i>C. aurantium</i> + <i>P. trifoliata</i>	JG		x					
Succari + <i>Atalantia ceylanica</i>	<i>C. sinensis</i> + <i>A. ceylanica</i>	JG		x					
Sunki × Benecke TF	<i>C. sunki</i> Hort. ex Tak. × <i>P. trifoliata</i> Benecke	DPI			x				
Swingle citrumelo	<i>C. paradisi</i> Macf. × <i>P. trifoliata</i>	DPI			x				x
Tachibana	<i>C. tachibana</i>	USDA	x						
Taiwanica	<i>C. taiwanica</i> Tan. & Shim.	DPI	x						
TF 50-7	<i>P. trifoliata</i>	DPI			x				
Troyer × mandarin	(<i>C. sinensis</i> × <i>P. trifoliata</i>) × <i>C. reticulata</i>	Spain 020418	One tub						
Uvalde citrange	<i>C. sinensis</i> (L.) Osb. × <i>Poncirus trifoliata</i>	USDA	One tub						
Volkamer lemon	<i>Citrus volkameriana</i> Ten. & Pasq.	DPI	x	x	x	x	x	x	x
West African cherry-orange	<i>Citropsis articulata</i> (Willd.) Swing. & M. Kell.	USNGR		x					
Willits citrange	<i>C. sinensis</i> × <i>P. trifoliata</i>	USDA	One tub						
x639	<i>C. reticulata</i> Cleopatra × <i>P. trifoliata</i> Rubidoux	DPI					x		

^zUSNGR = U.S. National Germplasm Repository for Citrus & Dates, Riverside, CA; DPI = Florida Dept. Agr. Consumer Services, Div. Plt. Industry, Winter Haven, FL; Reed Bros. = commercial seed supplier, Dundee, FL; JG = Dr. Jude Grosser, UF/IFAS, CREC, Lake Alfred; Spain = Dr. Juan Forner; USDA = Whitmore Foundation Farm, Leesburg, FL.

^ySee "Materials and Methods" for descriptions.

with one excluding Fe. The solution was changed approximately every 2 to 3 weeks at which time the initial nitrogen (N) level had declined not more than 20% as determined in previous studies. Each solution change was amended with 0.8 g (small tubs) or 1.6 g (large tubs) of Banrot[®] fungicide (Grace-Sierra Crop Protection, Milpas, CA). Solution pH was ≈ 8.0 and weekly monitoring showed that it varied less than 0.2 units during a run. A typical run was 6 to 10 weeks.

Screening runs using these standard procedures were conducted in the summer and fall, often in the same or a similar greenhouse as where the seedlings were grown. The tubs for all solution screenings were arranged in a completely randomized design on a bench and located to minimize any environmental effects within the greenhouse. One aquarium-style aeration stone was placed in each tub and connected to a small air compressor. Aeration was provided for 20 min every hour throughout the 24-h day. Clamps were used so that aeration was a gentle stream of bubbles in each tub.

Approximately 20 to 30 white root tips, each 1.0 to 1.5 cm long, were harvested weekly among all the plants in each tub. The same plants were not used at each harvest. The root tips were placed in 300-mL Fleaker beakers (Pyrex[®], Lowell, MA) containing a solution of 2-(4-morpholino)-ethane sulfonic acid buffer, $\text{Ca}(\text{NO}_3)_2$, KNO_3 , MgSO_4 , and 0.2 mM bathophenanthrolinedisulfonic acid (BPDS); 0.3 mM FeHEDTA was added to the solution and root Fe^{3+} reduction rates assayed in a darkened room at 33 °C in a water shaker bath (Castle and Manthey, 1998; Manthey et al., 1993). The amount of Fe^{2+} (BPDS)₃ formed was measured at 2-h intervals over a 6-h period on a spectrophotometer (Turner Model 340; Biomolecular, Inc., Reno, NV) at 536 nm. Compensation for microlocation-specific Fe^{3+} reduction by sources other than roots was accomplished by spectrophotometric use of a blank assay mixture attached to each Fleaker beaker. New roots generally appeared within the 10-d period when the plants were still in +Fe solution; thus, baseline Fe^{3+} reduction rates were measured when the plants were being transitioned from the +Fe to the -Fe solution. Assays continued until a peak value was identified. At the completion of each assay, the root tips were retrieved and oven-dried at 70 °C. Fe^{3+} reduction rates were expressed as $\mu\text{M Fe}^{3+}/\text{h}\cdot\text{g}^{-1}$ root dry weight.

Fresh weights of all plants were measured before placing them in the tubs and at the end of a run; also, two tubs each of Volkamer lemon, Cleopatra mandarin, and Swingle citrumelo seedlings in +Fe solution were included in the summer run to verify good growth when Fe was not limiting. The development of Fe deficiency symptoms was monitored by measuring changes in leaf greenness with a SPAD-502 chlorophyll meter (Minolta Camera Co., Osaka, Japan) (Monge and Bugbee, 1992). Five plants were randomly selected in each tub. One fully

expanded lower leaf selected at the beginning of a run and the most recent fully expanded upper leaf were measured biweekly on the same plants. All SPAD data were the mean of five readings/leaf.

Modified nutrient solution screening, summers of 1999 and 2001. A screening experiment was conducted in 1999 with eight tubs each of Volkamer lemon, Carrizo citrange, and x639 rootstocks. Four tubs (replicates) of each rootstock were assigned for measurement of Fe^{3+} reduction rates by the standard method described previously using 20 to 30 root tips at each sampling date. To assess the potential for scaling down measurements to individual plants, four tubs were used to collect 10 root tips from one plant in each tub. Each root tip was assayed in a 1.5-mL microcentrifuge tube. Adequate roots were generally produced from individual plants for multiple harvests, but occasionally another plant was selected, but the same length of root tip was collected at each harvest. The root tips from single plants were pooled when dried and their mean weight used for calculating Fe reduction rates. The outcomes of this trial were used to develop an estimate of sample size for the 2001 assays using the root tips of single plants.

The 1999 experiment was repeated in 2001 with Volkamer lemon, Carrizo citrange, and Swingle citrumelo with two tubs/treatment. The standard procedure was compared with the modified one; however, in the latter treatment, nine root tips were collected and assayed individually from each of four plants to examine among-plant variability within tubs.

Additional rootstock selections were screened in the 2001 trial using the standard procedure. The somatic hybrid selections in this trial were first grown in +Fe standard nutrient solution or one amended with 0.25 mM $(\text{NH}_4)_2\text{SO}_4$ to observe their root growth response to extra N and to potentially promote root acidification. There was no apparent effect resulting from N source and rate. Adequate root growth occurred only after 30 d at which time the somatic hybrid plants were transferred to -Fe solutions.

Soil screening, 1999. Immokalee fine sand (sandy, siliceous, hyperthermic Arenic Haplaquod) soil was collected at a depth of 0 to 15 cm from an uncultivated site and amended with CaCO_3 at the rates of none, 1.25%, 2.50%, 5%, or 10% by weight [rates suggested by T.A. Obreza based on previous field experience (Obreza, 1995)]. The soil was wetted to field capacity. The amended

Table 2. Iron (Fe) reduction rates ($n = 2$) for *Citrus* and related selections in solution culture.

Selection	1998 Fe^{3+} reduction rate ($\mu\text{mol Fe}^{3+}/\text{h}\cdot\text{g}^{-1}$)				
	Summer		Fall		
	Baseline ^a	Peak	Baseline	Peak	
Volkamer lemon	3.0	17.7	2.8	16.9	a
Chinese glycosmis	2.9	16.4			ab
Carrizo citrange	1.8	14.1			a-c
Orange jessamine	2.4	11.4			b-e
Cleo $\times$ TF (52)	2.3	10.2			c-f
Cleopatra mandarin	3.9	9.7			c-g
Uvalde citrange	3.6	7.8			d-i
Chinese wampee	3.0	6.9			d-i
Cleo $\times$ TF (50) ^{y,x}	3.3	6.3			
Taiwanica	3.5	5.9			d-i
C-32 citrange	2.4	5.5			e-h
Meiwa kumquat	4.2	5.5			e-h
Cleo $\times$ TF (51)	2.1	4.7			f-i
Sour orange + Benton	2.8	4.4			f-i
Indian bael fruit	2.9	4.3			f-i
Kuharske citrange	2.0	4.3			f-i
Tachibana	2.3	4.1			f-i
Benton citrange	1.7	4.0			g-i
Cleo + FDT ^y	2.0	3.8			
Chinotto sour orange	2.0	3.6			g-i
Marumi kumquat	3.4	3.4			hi
Calamondin	2.8	3.3			hi
Swingle citrumelo	1.6	3.3			hi
Sunki $\times$ Benecke TF	2.1	3.1			hi
F80-8 citrumelo	1.9	2.9			hi
Chinese box orange	2.3	2.8			i
Procimequat	1.8	2.7			i
Troyer $\times$ mandarin ^{y,x}	1.9	2.4			
TF 50-7	1.4	1.9			i
Gillet's cherry-orange			2.6	12.1	ab
Rangpur			2.0	2.3	c
Sour orange			1.7	4.5	c
West African cherry-orange			2.7	11.2	ab

^aBaseline rates are for the first new roots that appeared after the plants were transferred into -Fe solution.

^yThe peak values are probably valid, but inadequate root growth did not allow these selections to be screened for the normal duration. Data were excluded from the statistical analyses.

^xOnly one large, 52-plant tub included in the trial.

^yMean separation by Tukey's honestly significant difference test at $P \leq 0.05$.

Table 3. Leaf greenness (SPAD) readings (n = 2) among selections tested in the Summer and Fall 1998 solution culture trials.

Selection	Ratio, final:initial ^z				Selection	Final ratio, lower:upper leaf ^y	
	Lower leaf		Upper leaf				
Chinese glycosmis	0.7	cde ^x	0.2	h	Chinese wampee	12.7	a
Cleo + FDT	1.0	a–d	0.2	h	F80-8 citrumelo	11.3	ab
Procimequat	1.0	a–d	0.3	fgh	Cleo + FDT	11.2	ab
Swingle citrumelo	1.1	a–d	0.3	fgh	Chinese glycosmis	8.2	a–d
Taiwanica	1.1	a–d	0.3	gh	Swingle citrumelo	5.7	a–d
Benton citrange	1.2	a	0.4	d–h	TF 50-7	5.5	a–d
C-32 citrange	1.1	a–d	0.4	d–h	Taiwanica	4.8	a–d
Calamondin	1.3	ab	0.4	d–h	Orange jessamine	4.7	a–d
Chinese wampee	1.2	ab	0.4	d–h	Sour orange + Rangpur	4.7	a–d
Cleo × TF (51)	1.0	a–d	0.4	d–h	Procimequat	4.6	a–d
Cleopatra mandarin	1.2	abc	0.4	d–h	Sour orange	4.1	bcd
Orange jessamine	1.0	a–d	0.4	d–h	Chinotto sour orange	3.8	bcd
Sour orange	1.0	a–d	0.4	d–h	Sunki × Benecke TF	3.8	bcd
Sour orange + Rangpur	1.0	a–d	0.4	d–h	Calamondin	3.4	bcd
Sunki × Benecke TF	1.4	a	0.4	d–h	Tachibana	3.4	bcd
Tachibana	1.1	a–d	0.4	d–h	Benton citrange	2.8	bcd
Carrizo citrange	1.2	abc	0.5	d–h	Cleo × TF (51)	2.8	bcd
Chinotto sour orange	1.1	a–d	0.5	d–h	Uvalde citrange	2.7	cd
Kuharske citrange	1.0	a–d	0.5	d–h	Cleopatra mandarin	2.6	d
Chinese box orange	0.8	bcd	0.6	d–h	C–32 citrange	2.4	d
F80-8 citrumelo	1.1	a–d	0.6	c–h	Carrizo citrange	2.4	d
Hamlin + rough lemon	0.6	de	0.6	c–h	Chinese box orange	2.4	d
TF 50-7	1.0	a–d	0.6	c–h	Kuharske citrange	2.2	d
Marumi kumquat	0.9	bcd	0.7	b–g	Marumi kumquat	2.0	d
Meiwa kumquat	0.9	bcd	0.8	b–e	Meiwa kumquat	1.9	d
Uvalde citrange	1.1	abc	0.8	bcd	Hamlin + rough lemon	1.4	d
Volkamer lemon	1.0	a–d	0.8	bcd	Volkamer lemon	1.4	d
Cleo × TF (52)	0.9	bcd	1.0	abc	Indian bael fruit	1.0	d
Indian bael fruit	1.0	a–d	1.1	ab	Sour orange + Benton citrange	0.3	d
Sour orange + Benton citrange	0.3	e	1.4	a	Cleo × TF (52)	0.2	d

^zSorted on upper leaf data. Ratios 1.0 or less indicate that upper leaves decreased in leaf greenness during the trial period.

^yRatios 1.0 or greater indicate increasing differences between the lower and upper leaves generally reflecting the decreasing greenness of the upper leaf.

^xMean separation by Tukey's honestly significant difference test at $P \leq 0.05$.

soil was used to fill 2.5-L plastic pots. After a 30-d equilibration period, samples taken from a subset of pots were shown to contain 0.4%, 1.4%, 2.2%, 4.2%, and 5.9% CaCO_3 , respectively, using an acetic acid procedure (Loeppert et al., 1984). Additional sampling indicated that no changes occurred in soil CaCO_3 concentration during the screening run. Factorial treatments were formed from combinations of the five CaCO_3 rates and 12 rootstocks. After equilibration, six single-pot replications of each rootstock selection were planted and arranged in a completely randomized design on greenhouse benches in early summer. A row of buffer plants was placed at the edges of each bench. All plants were irrigated as needed and fertilized monthly with a 30.4N-30.4P-30.4K-6.1Mg-0.8Cu-0.1B-0.3Zn-.5Mn nutrient solution with $\text{Ca}(\text{NO}_3)_2$ as the N source and no Fe. Leaf greenness was measured as described previously along with plant fresh weights at the beginning and end of the run and biweekly plant heights.

Statistical procedures. The standard method nutrient solution experiments and the experiment in soil were conducted in a completely randomized design. Data were analyzed by SAS PROC GLM (SAS Institute, Inc., Cary, NC) and comparison of means was with Tukey's honestly significant difference test. The soil experiment treatments were factorially arranged and analyzed as such; no interactions occurred, thus mean separation was by Tukey's test. The sample size estimate was determining by assessing

root tip and plant-to-plant variability and calculating when they were approximately equal using PROC GLM and PROC MIXED analyses to estimate variance components.

Results and Discussion

Nutrient solution screening, Summer and Fall 1998. The baseline Fe^{3+} reduction rates for plants before their transfer to the -Fe solution were similar among all selections and to those reported in our previous work (Table 2; Castle and Manthey, 1998). After transfer to the -Fe solution, peak values in the summer and fall trials confirmed the consistently high Fe^{3+} reduction rate of Volkamer lemon, a commercial rootstock that was significantly different from all others in the summer trial except Chinese Glycosmis and Carrizo citrange. Other selections with relatively high Fe^{3+} reduction rates were Orange jessamine (*Murraya paniculata*), a citrus relative, and a new Cleopatra mandarin trifoliolate orange hybrid from Spain (Forner et al., 2000). Among those with the lowest reduction rates were Swingle and F80-8 citrumelos and Chinese box orange (*Severinia buxifolia*). Overall, selections tested previously repeated their general relationship of Fe reduction rates: Volkamer lemon and mandarin types > citranges > citrumelos > trifoliolate orange (Castle and Manthey, 1998). Selections not tested previously were mostly citranges. Their performance ranged from a high reduction rate (Carrizo) to intermediate (Uvalde, C-32, Kuharske, and Benton) sug-

gesting that expectations of poor response to low Fe stress because of their trifoliolate orange parent may not be justified depending on the particular citrange. We found a similar result for mandarin rootstocks (Castle and Manthey, 1998).

Among the citrus relatives tested, Chinese Glycosmis, Orange jessamine, a second species of *Citropsis*, and Chinese wampee had Fe^{3+} reduction rates from ≈ 7 to 16. Other citrus relatives, including the kumquat species, Indian bael fruit, calamondin, Chinese box orange, and Ceylon Atalantia, showed little response with Fe^{3+} reduction rates 5.0 or less (Table 2).

A large number of selections, in particular somatic hybrids, were dropped from the trial because of poor root production. Sour orange and Rangpur also had low Fe^{3+} reduction rates apparently because the buffered assay solution prevented plants of those selections from expressing their ability to acidify their growth solutions as reported previously (Castle and Manthey, 1998). Acidification is the mechanism that apparently explains the known tolerance of those rootstocks to low-Fe stress (Castle and Manthey, 1998; Manthey et al., 1994a).

1998 leaf greenness and plant growth. There were essentially no changes in leaf greenness of lower leaves present at the start of the trial regardless of Fe^{3+} reduction rate (Table 3). Changes in the color of young (upper) leaves, where Fe stress symptoms normally appear, were generally related to Fe^{3+} reduction rates, i.e., those selections

Table 4. Changes in plant fresh weight (n = 2) in the Summer and Fall 1998 iron (Fe) reduction trials conducted in -Fe solution culture except as noted.^z

Rootstock	Fresh wt (g)				Rel. growth rate (g·g ⁻¹)	
	Initial		Final			
Indian bael fruit	2.9	cde ^y	21.5	a	6.1	a
Volkamer lemon (-Fe)	2.2	d-h	16.0	b	6.1	a
Volkamer lemon (+Fe)	2.2	d-h	10.9	cde	3.9	b
Kuharske citrange	3.1	cd	13.3	c	3.3	bc
Carrizo citrange	2.4	d-h	10.4	def	3.2	bc
Calamondin	1.6	d-h	6.5	h-m	3.1	cd
Tachibana	1.3	e-h	5.0	l-s	2.8	cde
Sour orange	2.1	d-h	7.7	g-j	2.7	c-f
Swingle citrumelo (+Fe)	3.1	cd	10.7	cd	2.7	c-f
Cleopatra mandarin (+Fe)	1.8	d-h	6.4	h-n	2.4	d-h
Taiwanica	2.6	c-h	8.6	e-h	2.3	d-i
Uvalde citrange	1.4	e-h	4.6	m-s	2.3	d-i
C-32 citrange	1.8	d-h	5.9	i-p	2.2	e-j
Cleopatra mandarin (-Fe)	1.7	d-h	5.4	k-r	2.1	e-k
Cleo × TF (50)	1.0	h	3.1	rs	2.0	e-l
Cleo × TF (52)	1.8	d-h	5.3	k-r	2.0	f-l
Benton citrange	2.0	d-h	5.8	k-n	1.9	f-l
F80-8 citrumelo	1.4	e-f	4.1	m-s	1.9	g-l
Cleo × TF (51)	1.2	gh	3.2	qrs	1.8	h-l
TF 50-7	1.4	e-h	4.0	n-s	1.8	h-l
Chinese glycosmis	2.0	d-h	5.6	k-q	1.7	h-l
Chinotto sour orange	2.7	c-g	7.6	g-k	1.7	h-l
Meiwa kumquat	1.7	d-h	3.8	o-s	1.7	h-l
Swingle citrumelo (-Fe)	2.7	c-g	7.1	g-l	1.6	i-m
Orange jessamine	2.2	d-h	5.3	k-r	1.4	l-n
Sunki × Benecke TF	2.3	d-h	5.7	k-q	1.4	l-n
Chinese wampee	2.2	d-h	5.0	l-s	1.3	k-n
Cleo + FDT	4.1	cb	9.3	d-g	1.3	k-o
Procimequat	1.3	fgh	2.8	s	1.3	l-o
Chinese box orange	1.9	d-h	3.5	q-s	0.8	m-p
Sour orange + Rangpur	5.3	b	8.4	f-i	0.6	nop
Marumi kumquat	2.9	c-f	4.4	m-s	0.5	op
Sour orange + Benton citrange	14.9	a	21.1	a	0.4	op
Hamlin + rough lemon	5.2	b	6.1	i-o	0.3	p

^zSorted on relative growth rate.

^yMean separation by Tukey's honestly significant difference test at $P \leq 0.05$.

Table 5. Root tip iron (Fe) reduction rates in solution culture among citrus selections and methods.^z

Selection	Fe ³⁺ reduction rate (μmol Fe ³⁺ /h·g ⁻¹)		
	Control	Peak	
Volkamer lemon (individual)	2.8	10.1	a
Volkamer lemon (standard)	3.3	7.7	ab
Carrizo citrange (individual)	2.0	4.3	bc
Carrizo citrange (standard)	3.0	4.0	c
x639 (individual)	2.0	3.8	c
x639 (standard)	2.7	3.3	c
P value	<0.0001		

^zSee text for data collection and statistical details. Individual = root tips collected from separate plants; standard = root tips collected from a number of plants growing in a single tub.

Table 6. Analysis of variance and variance components for sample size estimate.

Source	df	Sum of squares	Mean square	Expected MS	F value	Pr > F
<i>Proc GLM</i>						
Model	11	1,237.37	112.49	s ² _{tip}	14.43	<0.0001
Error	108	841.95	7.79			
Rootstock	2	1,007.51	503.75		64.62	<0.0001
Tub(rtstk)	9	229.87	25.54	s ² _{tip} + 10 s ² _{plant}	3.28	0.0015
<i>Proc Mixed</i>						
Cov. parm.			Estimate			
Tub(rtstk)			1.77			
Residual			7.79			

with the highest Fe³⁺ reduction rates showed the least yellowing, but with some exceptions such as Chinese Glycosmis (Table 3). There were few statistical differences among the selections, but most values were less than 1 indicating some yellowing occurred in each

selection. The upper leaves of the Sour orange + Benton citrange, *Aegle marmelos* (Indian bael fruit), and Cleopatra mandarin × trifoliate orange became slightly greener as the trial progressed. The upper leaves of Chinese Glycosmis and a somatic hybrid,

Cleo + Flying Dragon TF, had the most change. Those results are supported by comparing the ratio of lower to upper leaves initially and at the end of the trial (Table 3). Like with the lower leaves, the initial lower:upper leaf greenness ratios among selections were not significantly different (data not given). At the end of the trial, plants of *Clausena lansium* (Wampee), F80-8 citrumelo, and Cleopatra mandarin + Flying Dragon trifoliate orange hybrid had high ratios because of considerable yellowing of the upper leaves, but overall, there were few differences among selections.

We did not examine the relationship of Fe reduction rate to leaf yellowing in these experiments in which plants were grown in solution culture with virtually no Fe. However, in conjunction with our first study (Castle and Manthey, 1998), we analyzed the nutrient composition of plant parts before and after growth in the -Fe solution. The unreported results suggested that most selections with high Fe reduction rates had higher initial leaf Fe contents than those with low rates and they showed some evidence of Fe redistribution while growing in the -Fe solution.

Plants of most selections weighed ≈1 to 3 g initially and grew at different rates (Table 4). Final fresh weights ranged from 2.8 to 21.5 g/plant. Differences in initial size were accommodated by calculating relative growth rates which ranged from less than 1 to 6.1. Indian bael fruit (*Aegle marmelos*) plants grew the most vigorously, although they were not significantly different from the Volkamer lemon plants in the -Fe solution. Plants of Volkamer lemon, Swingle citrumelo, and Cleopatra mandarin grew relatively well in the +Fe solution, thereby serving as a general indicator of satisfactory growth among all plants. The Volkamer plants had high Fe³⁺ reduction rates and grew vigorously in the -Fe solution but less so in the +Fe solution. That particular result was observed in some of our other unreported studies and is an unexplained response. Perhaps some selections are more sensitive to HEDTA than others. The similar growth of the Cleopatra mandarin seedlings in both solutions combined with its Fe³⁺ reduction responses supports its intermediate ranking in regard to low-Fe stress tolerance. The Swingle citrumelo plants, which had the lowest Fe³⁺ reduction rate, grew significantly better in the +Fe solution indicating its lower tolerance to Fe stress.

Modified nutrient solution screening, summers of 1999 and 2001. The Fe³⁺ reduction rates of pooled samples of 20 to 30 root tips (standard method) among three selections did not differ when compared with the mean of individual root tips harvested from single plants and assayed (Table 5). Analysis of variance components showed that among the selections, plant-to-plant variability exceeded within-plant variability (Table 6). Using the estimates of those two sources of variability and calculating when tip-to-tip and plant-to-plant variability were equal, a sample size of seven root tips was adequate

Table 7. Root tip iron (Fe) reduction rates (n = 2) among citrus selections and assay methods.

Rootstock	Assay method	Fe ³⁺ reduction rate (μmol Fe ³⁺ /h·g ⁻¹)			
		Control		Peak ^{z,y}	
Volkamer lemon	Individual	2.0	a	11.3	a
Volkamer lemon	Standard	2.1	a	11.5	a
Carrizo citrange	Individual	1.3	a	7.4	b
Carrizo citrange	Standard	1.3	a	6.2	b
Swingle citrumelo	Individual	1.2	a	2.5	c
Swingle citrumelo	Standard	1.5	a	1.7	c
Macrophylla	Standard	2.1	a	9.2	ab
C-35 citrange	Standard	2.0	a	5.0	bc
Kinkoji	Standard	2.2	a	2.5	c
Rangpur	Standard	1.6	a	3.6	c
Sour orange	Standard	1.3	a	2.8	c
Sour orange + Carrizo	Standard	0.5	b	1.5	c
Sour orange + Flying Dragon TF	Standard	0.7	ab	1.5	c
Sour orange + PSL	Standard	0.4	b	1.3	c

^zAll selections were part of a single trial, but there were two statistical comparisons: between-assay methods for Volkamer, Carrizo, and Swingle and among all selections assayed by the standard method. Indications of significant differences only apply within each comparison.

^yMean separation by Tukey's honestly significant difference test ($P \leq 0.05$).

Table 8. Plant growth and changes in leaf greenness (n = 6) among rootstocks in the Summer 1999 iron nutrition trial conducted in soil amended with CaCO₃.

Selection	Fresh wt (FW) (g)		FW rel. growth rate (g·g ⁻¹)		HT rel. growth rate (cm·cm ⁻¹)		Leaf greenness ratio ^z	
	Initial	Final						
Cleopatra mandarin	3.4 f ^y	87.2 de	24.4	a	6.7	a	1.1	c
Sour orange	5.8 cd	122.5 a	20.6	b	4.5	b	1.4	c
Sour orange + Carrizo citrange	6.5 bc	111.5 ab	16.5	c	3.7	bcd	1.4	c
Kinkoji	7.3 a	120.5 a	15.7	cd	4.5	b	1.0	c
Volkamer lemon	6.5 bc	105.9 abc	15.7	cd	4.4	b	1.4	c
Rangpur	5.8 cd	92.2 cde	15.0	cd	2.6	e	1.8	bc
Sunki × Benecke TF	5.1 d	78.4 e	14.5	cd	2.9	de	1.5	bc
Carrizo citrange	5.3 d	78.4 e	14.1	cd	3.0	cde	1.7	bc
Smooth Flat Seville	6.6 ab	95.5 bcd	13.8	cd	3.9	bc	1.7	bc
Cleo × Trifoliolate orange	4.2 e	56.6 f	12.5	de	3.9	bc	2.0	bc
Swingle citrumelo	5.2 d	51.6 f	9.1	e	2.4	e	5.8	a
TF 50-7	5.6 d	33.8 g	5.1	f	1.1	f	2.5	b
CaCO ₃ means								
0.4			15.6	a	3.8	ab	1.4	c
1.4			16.0	a	3.9	a	1.8	b
2.2			15.0	ab	3.7	ab	1.9	b
4.2			13.5	c	3.5	b	2.1	ab
5.9			13.9	bc	3.4	b	2.3	a
P values								
Rootstock	<0.0001	<0.0001	<0.0001		<0.0001		<0.000	
CaCO ₃	0.5244	<0.0001	<0.002		0.0135		0.001	
Interaction	0.3816	0.5243	0.5688		0.086		0.099	

^zReadings taken with a SPAD meter. Ratio of lower:upper leaves after 14 weeks. Ratios 1.0 or greater generally indicate decreasing greenness of the upper leaf relative to the lower leaf.

^yMean separation by Tukey's honestly significant difference test ($P \leq 0.05$).

to represent one plant. A second run of the modified procedure confirmed the first results. The differences in Fe³⁺ reduction rates among selections were as observed previously, but there was no difference between methods (Table 7).

Among the selections tested using the standard method, Volkamer lemon plants had the highest Fe³⁺ reduction rate; *C. macrophylla*, C-35, and Carrizo citranges were intermediate; and Swingle citrumelo, Kinkoji, Rangpur, sour orange, and the somatic hybrids had the lowest rates. The consistently low rates with the latter may be a physiological anomaly associated with being tetraploid. Somatic hybrids have not grown well in the nutrient solution used in these trials and have always had low Fe³⁺ reduction rates regardless of the parents. Those hybrids did not respond

to additional N or a change in N source. Their root production was generally weak.

Soil screening, 1999. Initial plant fresh weight was 3.4 to 7.3 g with some significant differences among selections, but not CaCO₃ treatments (Table 8). After 14 weeks, fresh weight relative growth rates (FW-RGR) varied among rootstocks and decreased with increasing CaCO₃ level in the soil, but with no interaction between those two treatment factors. Cleopatra mandarin and sour orange plants had the highest FW-RGR and Swingle citrumelo and trifoliolate orange plants had the lowest values. There were no statistical differences among virtually all the remaining selections, which had RGR values between 12.5 and 16.5. Plant relative growth rate expressed as height (HT-RGR) was highly correlated ($r = 0.89^{***}$) with FW-RGR and

HT-RGR likewise decreased as CaCO₃ soil content increased. Leaf greenness values of the lower and upper leaves were not different initially (data not given) and increased from 1.0 to 2.0 indicating the development of lighter green color in upper leaves of most selections after 14 weeks; however, the ratios of Swingle citrumelo and trifoliolate orange plants were significantly higher because the upper leaves turned yellow, especially at the higher CaCO₃ rates. Leaf greenness ratios increased as CaCO₃ soil content increased.

The difference in FW-RGR among selections was larger than it was among CaCO₃ rates. That result along with the absence of a plant × CaCO₃ interaction suggests that the range in CaCO₃ rates may have been too narrow to separate the selections for their tolerance to calcareous conditions (Obreza, 1995); and RGR differences among the selections were more a reflection of their inherent vigor rather than responses to CaCO₃.

A summary of the solution and soil culture results confirms and expands the general relationships among citrus types established in our previous study and in other studies (Table 9; Castle et al., 1993, 2004, 2006; Wutscher, 1979; Wutscher and Olsen, 1970). Volkamer lemon was very consistent in its tolerance to low-Fe stress in soil and solution culture, which matches its reported performance as a rootstock in the field (Castle, 1987; Wutscher, 1979). As such, it would be a reliable stress-tolerant control selection in screening trials. Also in the top group was *C. macrophylla*, another species likely to have citron in its genetic makeup as does Volkamer lemon (Barrett and Rhodes, 1976). Citron was a high performer in our earlier trials (Castle and Manthey, 1998). Mandarins and mandarin hybrids formed the next group in descending order. Cleopatra mandarin has also been a consistently good performer and is well known for its suitability for use in calcareous soils (Castle, 1987; Chapman, 1968; Wutscher, 1979). It appeared to confer tolerance to the Cleo × trifoliolate orange hybrids tested in this trial, including x639, but not to the somatic hybrid with Flying Dragon trifoliolate orange.

Citranges and citrumelos formed the middle group in that order. Six citranges were tested and did not differ from each other, but Carrizo in all trials displayed the best tolerance within the group with moderately high Fe reduction rates in solution and good growth in soil culture. Swingle and F80-8 citrumelos had similar Fe reduction rates as the citranges, but almost always exhibited greater leaf chlorosis. Trifoliolate orange is relatively intolerant of low-Fe stress as consistently demonstrated in our trials. Like Volkamer lemon, it would be useful as a control selection in screening trials, but as the poor tolerance representative.

The citrus relatives, and other species of *Citrus* not mentioned here, constituted a large portion of the selections reported here. Their results provided taxonomic insights and suggest the possibility of different mechanisms of plant response. In Swingle's classification, there are two tribes in the orange subfamily,

Table 9. Summary of relative plant selection responses.^z

Plant selection	Solution culture responses ^y			Response to soil CaCO ₃ ^x		Remarks
	Iron reduction	Growth	Leaf chlorosis	Growth	Leaf chlorosis	
Benton citrange	3	3	2	1	2	Consistently good responses in solution and soil culture Citrus relative with apparently low tolerance to Fe stress Citrus relative with excellent Fe reduction response in solution, but with leaf yellowing.
C-32 citrange	3	2	2			
C-35 citrange	3	3	3			
Calamondin	3	2	2			
Carrizo citrange	2	2	2			
Chinese box orange	3	4	2			
Chinese glycosmis	1	3	3			
Chinotto sour orange	3	3	2	1	1	New rootstock released from Spain for tolerance to calcareous soil New rootstock released from Spain for tolerance to calcareous soil New rootstock released from Spain for tolerance to calcareous soil Citrus relative with excellent Fe reduction response without leaf yellowing Low Fe reduction rate in solution, but excellent vigor and green leaf color Mediocre response in solution culture; excellent performance in soil
Cleo + FDT	3	3	4			
Cleo × TF (52)	2	3	2			
Cleo × TF (51)	3	3	2			
Cleo × TF (50)	2	3	2			
Cleopatra mandarin	2	2	2			
F80-8 citrumelo	3	3	4			
Gillet's cherry-orange	1	2	2	2	3	Citrus relative with excellent Fe reduction response in solution, but with leaf yellowing
Hamlin + rough lemon	4	4	1			
Indian bael fruit	3	1	1			
Kinkoji	3	3	2			
Kuharske citrange	3	2	2			
Macrophylla	1	2	2			
Marumi kumquat	3	4	2			
Meiwa kumquat	2	3	2	1	1	Low Fe reduction rate in solution; excellent performance in soil
Nova + HB pummelo						
Orange jessamine	1	3	3			
Procimequat	3	4	3			
Rangpur	4	3	1			
Smooth Flat Seville						
Sour orange	4	2	2			
Sour orange + Benton citrange	3	4	1	2	3	Low Fe reduction rate in solution; excellent performance in soil
Sour orange + Carrizo	4	4	3			
Sour orange + Flying Dragon TF	4	4	3			
Sour orange + PSL	4	4	3			
Sour orange + Rangpur	4	4	3			
Sour orange + TF (50-7)	4	4	3			
Succari + Atalantia ceylanica	4	4	3			
Sunki × Benecke	3	3	2	2	3	Consistently high Fe reduction rates in solution and excellent performance in soil Citrus relative with good Fe reduction rate, but with leaf yellowing and fair growth Citrus relative with overall good performance New rootstock (Cleo × TF) with responses similar to Cleo
Swingle citrumelo	3	3	3			
Tachibana	3	2	2			
Taiwanica	2	2	3			
TF 50-7	4	3	3			
Uvalde citrange	2	2	2			
Volkamer lemon	1	1	1			
Wampee	2	3	4	2	2	
West African cherry-orange	1	2	2			
x639	2	2	2			

^zAll rankings are based on the statistical outcomes of the experiments.^yCompilation of iron (Fe) reduction and relative growth rate responses from all trials: 1 = highest rate; 2 = intermediate; 3 = low; 4 = lowest. Leaf chlorosis rankings are based on changes in upper leaf color: 1 = no or little change; 2 = slight yellowing; 3 = moderate yellowing; 4 = substantial change in leaf color.^xRelative growth rate difference between 0 and 5.9% soil CaCO₃: 1 = little growth difference; 2 = moderate difference; 3 = large difference. Leaf chlorosis at 0% versus 4.2/5.9% soil CaCO₃: 1 = small; 2 = moderate; 3 = large difference.^wBlank spaces indicate no data.

Aurantioideae (Swingle and Reece, 1967). The subtribe, *Clauseninae*, has three genera, *Glycosmis*, *Clausena*, and *Murraya*. The species we tested from each of those genera had among the highest Fe³⁺ reduction rates in solution culture and should be tested in soil. In every instance, the species grew well, but leaf yellowing was common. They may be a

source of genetic material, including ferretin-type genes (Briat and Lobréaux, 1997; Briat et al., 1999; Goto and Yoshihara, 2001) that could be used to introduce low-Fe stress tolerance into commercial species and in breeding new selections. Indian bael fruit (*Aegle marmelos*) seedlings produced an opposite response. They had a relatively

low Fe³⁺ reduction rate, but grew vigorously and remained green. In the other tribe, *Citreae*, of the orange subfamily, several genera of subtribe 2, *Citrinae*, that includes *Citrus*, were tested in solution culture. Chinese box orange (*Severinia buxifolia*) seedlings hardly grew and had low Fe³⁺ reduction rates. We were unable to produce useable seedlings

of Ceylon *Atalantia*, but the two species of *Citropsis* (Gillet's cherry-orange and West African cherry-orange) grew well, had among the highest Fe^{3+} reduction rates, and showed little to no leaf yellowing. Thus, *Citropsis gilletiana* and *articulata* may also be good genetic sources of low-Fe stress tolerance, particularly because they are one of the most closely related species to *Citrus* among all selections evaluated.

Somatic hybrids (tetraploids) were overall poor performers despite having, in many instances, one parent known to be relatively tolerant to low-Fe stress. Most of those hybrids were slow-growing and did not produce enough roots in solution culture for screening; thus, it may be that their true potential was not accurately assessed. Likewise, the citrus relatives of the *Fortunella* genus (kumquat) and the Procimequat hybrid were of moderate to low vigor with generally low Fe^{3+} reduction rates.

Results from our previous study (Castle and Manthey, 1998) and those reported here show that among a broad range of *Citrus* selections, low-Fe stress tolerance (i.e., a combination of plant growth, expression of Fe deficiency symptoms, and solution versus soil culture responses) is generally best among selections with a *Citrus medica* (citron) base and sour orange and related selections. Good sources of tolerance also appear to exist within several genera of the citrus relatives. Mandarin selections and some citranges have relatively high tolerances. Low tolerance occurs among sweet oranges, citrumelos, and trifoliolate orange. In many instances, however, there are exceptions and so sweeping generalizations are not appropriate.

Observations made with Rangpur and sour orange suggest that these rootstocks may exhibit rhizosphere acidification as their primary response to Fe deficiency (Castle and Manthey, 1998; Manthey et al., 1994a; Treeby and Uren, 1993). Rangpur has been previously shown to express a high level of Fe deficiency-induced Fe^{3+} reduction (Manthey et al., 1994a); likewise, sour orange, at times, expressed similarly induced rates of Fe^{3+} reduction (Manthey, unpublished data). However, observations of plants grown in hydroponic solutions suggested that induced Fe^{3+} reduction for these two rootstocks did not simultaneously occur with root acidification, but rather, the two responses occurred separately. Whether this occurs with soil-grown roots is unknown. However, these abilities to acidify the root environment as well as to express an inducible Fe^{3+} reduction ability may explain why those selections are so well adapted to calcareous soils. Rhizosphere acidification increases $\text{Fe}^{3+}_{(\text{free})}$ solubility at the root surface 1000 times per decrease in pH unit, thus making Fe^{3+} reduction by either enzymatic or nonenzymatically catalyzed reactions far more rapid than at neutral or alkaline pH (Manthey et al., 1994b). Additionally, roots of a selection that is responsive to low Fe stress, growing in an acidified and reduction-rich microenvironment, may have sharply contrasting microbial and chemical

ecologies, than non-responding selections growing in high carbonate soils (Manthey et al., 1994b). These possibilities may explain the variability in plant Fe^{3+} reduction, growth, and leaf greenness responses we recorded for other selections, including those related to sour orange, e.g., Chinotto, Taiwanica, Kinkoji, and Smooth Flat Seville.

We conclude that measuring Fe^{3+} reduction rate is a useful screening tool because of the generally good relationship between those plant responses in solution culture and soil and their combined positive relationship to known carbonate-induced Fe chlorosis responses of plants grown in soil under field conditions. However, plant responses were not always consistent and may be an expression of one or more tolerance mechanisms. It is also critical to be aware that some important aspects of Fe tolerance are not captured using this method; thus, this technique could exclude important tolerant genotypes.

Literature Cited

- Barrett, H.C. and A.M. Rhodes. 1976. A numerical taxonomic study of affinity relationships in cultivated *Citrus* and its close relatives. *Syst. Bot.* 1:105–136.
- Briat, J.F. and S. Lobreux. 1997. Iron transport and storage in plants. *Trends Plant Sci.* 2:187–193.
- Briat, J.F., S. Lobreux, N. Grignon, and G. Vansuyt. 1999. Regulation of plant ferritin synthesis: How and why. *Cell. Mol. Life Sci.* 56:155–166.
- Campbell, C.W. 1991. Rootstocks for the Tahiti lime. *Proc. Fla. State Hort. Soc.* 104:28–30.
- Castle, W.S. 1987. Citrus rootstocks, p. 361–399. In: Rom, R.C. and R.F. Carlson (eds.). *Rootstocks for fruit crops*. John Wiley and Sons, New York, NY.
- Castle, W.S., K.D. Bowman, J.H. Graham Jr., and D.P.H. Tucker. 2006. Florida rootstock selection guide. *Univ. Fla. Coop. Ext. Pub.*, SP-248.
- Castle, W.S., J.W. Grosser, F.G. Gmitter, R.J. Schnell, T. Ayala-Silva, J.H. Crane, and K.D. Bowman. 2004. Evaluation of new citrus rootstocks for 'Tahiti' lime production in southern Florida. *Proc. Fla. State Hort. Soc.* 117:174–181.
- Castle, W.S. and J.A. Manthey. 1998. Screening citrus rootstocks for iron-deficiency tolerance. *Fruits* 53:375–381.
- Castle, W.S., R.R. Pelosi, C.O. Youtsey, F.G. Gmitter Jr., R.F. Lee, C.A. Powell, and X. Hu. 1992. Rootstocks similar to sour orange for Florida citrus trees. *Proc. Fla. State Hort. Soc.* 105:56–60.
- Castle, W.S., D.P.H. Tucker, A.H. Krezdorn, and C.O. Youtsey. 1993. Rootstocks for Florida citrus. 2nd Ed. *Univ. Fla. Coop. Ext. Pub.*, SP-42.
- Chapman, H.D. 1968. The mineral nutrition of citrus, p. 127–289. In: Reuther, W., L.D. Batchelor and H.J. Webber (eds.). *The citrus industry*, Vol. 2. Univ. Calif., Berkeley, CA.
- Cooper, W.C. and A. Peynado. 1953. A comparison of sour orange and Cleopatra mandarin seedlings on salty and calcareous soils. *Proc. Rio Grande Valley Hort. Soc.* 7:95–101.
- Cooper, W.C. and A. Peynado. 1954. Screening citrus seedlings for tolerance to calcareous soils. *J. Rio Grande Valley Hort. Soc.* 8:100–105.
- Ferguson, L., N. Sacovich, and M. Roose. 1990. California citrus rootstocks. *Univ. Calif. Pub.* 21477.
- Forner, J.B., M.A. Forner, A. Alcaide, S. Verdejo-Lucas, and F.J. Sorribas. 2000. New hybrid citrus rootstocks released in Spain. *Proc. Intl. Soc. Citricult.* 1:58–61.
- Goto, F. and T. Yoshihara. 2001. Improvement of micronutrient contents by genetic engineering—Development of high iron content crop. *Plant Biotechnol.* 18:7–15.
- Grosser, J.W., V. Medina-Urrutia, G. Ananthakrishnan, and P. Serrano. 2004. Building a replacement sour orange rootstock: Somatic hybridization of selected mandarin + pummelo combinations. *J. Amer. Soc. Hort. Sci.* 129:530–534.
- Hamze, M., J. Ryan, and N. Zaabout. 1986. Screening of citrus rootstocks for lime-induced chlorosis tolerance. *J. Plant Nutr.* 9:459–469.
- Hodgson, R.W. 1967. Horticultural varieties of citrus, p. 431–591. In: Reuther, W., H.J. Webber and L.D. Batchelor (eds.). *The citrus industry*, Vol. 1, Univ. Calif., Berkeley, CA.
- Korcak, R. 1987. Iron deficiency chlorosis. *Hort. Rev. (Amer. Soc. Hort. Sci.)* 9:133–186.
- Loeppert, R.H., C.T. Hallmark, and M.M. Koshy. 1984. Routine procedure for rapid determination of soil carbonates. *Soil Sci. Amer. J.* 48:1030–1033.
- Manthey, J.A., D.L. McCoy, and D.E. Crowley. 1993. Chelation effects on the iron reduction and uptake by low-Fe stress tolerant and intolerant citrus rootstocks. *J. Plant Nutr.* 16:881–893.
- Manthey, J.A., D.L. McCoy, and D.E. Crowley. 1994a. Stimulation of rhizosphere iron reduction and uptake in response to iron deficiency in citrus rootstocks. *Plant Physiol Biochem.* 32:211–215.
- Manthey, J.A., D.E. Crowley, and D.G. Luster. 1994b. *Biochemistry of metal micronutrients in the rhizosphere*. CRC Press, Boca Raton, FL.
- Monge, D.A. and B. Bugbee. 1992. Inherent limitations of non-destructive chlorophyll meters: A comparison. *HortScience* 27:69–71.
- Obreza, T.A. 1995. Soil CaCO_3 concentration affects growth of young grapefruit trees on Swingle citrumelo rootstock. *Proc. Fla. State Hort. Soc.* 108:147–150.
- Pestana, M., A. de Varennes, J. Abadia, and E.A. Faria. 2005. Differential tolerance to iron deficiency of citrus rootstocks grown in nutrient solution. *Sci. Hort.* 104:25–36.
- Sagee, O., D. Hasdai, M. Hamou, and A. Shaked. 1992. Screenhouse evaluation of new citrus rootstocks for tolerance to adverse soil conditions. *Proc. Intl. Soc. Citricult.* 1:299–303.
- Sudahono, D.H.B. and R.E. Rouse. 1994. Greenhouse screening of citrus rootstocks for tolerance to bicarbonate-induced iron chlorosis. *HortScience* 29:113–116.
- Swingle, W.T. and P.C. Reece. 1967. The botany of citrus and its wild relatives, p. 190–430. In: Reuther, W., H.J. Webber and L.D. Batchelor (eds.). *The citrus industry*, Vol. 1, Univ. Calif., Berkeley, CA.
- Tagliavini, M. and A.D. Rombolá. 2001. Iron deficiency and chlorosis in orchard and vineyard ecosystems. *Eur. J. Agron.* 15:71–92.
- Treeby, M. and N. Uren. 1993. Iron deficiency stress responses amongst citrus rootstock. *Z. Pflanzenenahr. Bodenk.* 56:75–81.
- Wei, L.C., W.R. Ocumpaugh, and R.H. Loeppert. 1994. Plant growth and nutrient uptake characteristics of Fe-deficiency chlorosis susceptible and resistant subclovers. *Plant Soil* 165:235–240.
- Wutscher, H.K. 1979. Citrus rootstocks. *Hort. Rev. (Amer. Soc. Hort. Sci.)* 1:237–269.
- Wutscher, H.K. and E.O. Olsen. 1970. Leaf nutrient levels, chlorosis, and growth of young grapefruit trees on 16 rootstocks grown on calcareous soil. *J. Amer. Soc. Hort. Sci.* 95:259–261.