HortScience 50(11):1731–1734. 2015.

Five New Citrus Rootstocks with Improved Tolerance to Huanglongbing

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Additional index words. orange, huanglongbing, cultivar, breeding, genetics

US-1279, US-1281, US-1282, US-1283, and US-1284 are new citrus rootstock cultivars released in Sept. 2014 by the Agricultural Research Service (ARS) of the U.S. Department of Agriculture (USDA) (Bowman and McCollum, 2014a, 2014b, 2014c, 2014d, 2014e). Compared with other common citrus rootstock cultivars used in the east coast region of Florida, when grafted with sweet orange scion, these rootstock cultivars produce trees that have superior production of good quality fruit after the trees are infected with *Candidatus* Liberibacter asiaticus (Las), the believed causal agent of huanglongbing disease (HLB). All five rootstocks, when grafted with common commercial citrus scions, produce medium-sized trees and appear adapted to production on the flatwoods soils of the Florida east coast. Seed is not available for these rootstocks, but they propagate readily by semihardwood cuttings on a mist bench.

Origin

All five rootstock selections originated from crosses made at the A. H. Whitmore Foundation Farm in 1995 by Kim Bowman of the USDA, ARS. 'US-1279' is the result of a cross of 'Changsha' mandarin (*Citrus reticulata* L. Blanco) × 'Gotha Road #6' trifoliate orange [*Poncirus trifoliata* (L.) Raf.], 'US-1281' and 'US-1282' are from a cross of 'Cleopatra' mandarin (*C. reticulata*) × 'Gotha Road #6' trifoliate orange, and 'US-1283' and 'US-1284' are from a cross of 'Ninkat' mandarin (*C. reticulata*) × 'Gotha Road #6' trifoliate orange. Selection, evaluation, and field testing of the rootstocks was planned and conducted by Dr. Kim Bowman, in collaboration with or support from industry partners, including Florida Citrus Research Foundation, Florida Citrus Production Research Advisory Council, Florida Citrus Research and Development Foundation, and Mid-Florida Citrus Foundation. Dr. Greg McCollum (also of USDA, ARS) collaborated in the evaluation of fruit quality from field trials.

Leaf descriptive data were collected from fully expanded leaves on young greenhouse-grown rooted cuttings ('US-1279', 'US-1281', 'US-1282', 'US-1283', and 'US-1284') or seedlings ('Swingle', 'Carrizo', and 'US-812') during 2015. The two field trials conducted as part of the performance documentation included here were established into the field sites in 2000 and 2002 as indicated in the respective tables (Tables 2–6), and data were collected using common methods. For all experiments, the data were tested by analysis of variance (ANOVA) using Statistica version 10 (StatSoft, Tulsa, OK). Duncan's multiple range test was used for mean comparison within columns when the F test was significant at $P < 0.05$.

Description

For the two trials reported here, rooted cuttings of each of the five new rootstock selections (and the standard rootstocks) were grafted with Hamlin scion in a nursery and subsequently transplanted into the field site at about 1 year of age. No source trees of the five new rootstock cultivars have yet fruited, so all field testing and other evaluations of these five new rootstocks were conducted with rooted cuttings from the vegetative source. However, based on parent characteristics, it is anticipated that fruit of these new rootstocks would yield seed of predominately apomictic origin, and uniform seed propagation would likely be possible once seed source trees begin fruiting. Rooted cuttings or in vitro micropropagation are recommended as the methods of propagation until seed becomes available.

Like most other first-generation hybrids of *Poncirus trifoliata* with *Citrus* spp., the five new rootstocks possess trifoliolate leaves with winged petioles. A comparison of leaf traits for these rootstocks with other common Florida rootstocks demonstrates that they can be distinguished from 'Carrizo' citrange [*Citrus sinensis* (L.) Osbeck × *P. trifoliata*], 'Swingle' citrumelo (*Citrus* ×*paradisi* Macf. × *P. trifoliata*), and 'US-812' (*C. reticulata* × *P. trifoliata*) by a combination of several measurements, especially petiole length (Table 1). Among the five new rootstocks, only 'US-1282' was readily distinguished from the other four (in having a larger leaf blade size). It can be expected that leaf measures would vary considerably by growing conditions and would further limit visual identification of the different hybrid rootstocks. Grafted trees of citrus cultivars on these five new rootstocks typically have some rootstock overgrowth of the scion (or benching) similar to that of many other trifoliate hybrid rootstocks, and are not distinctive in physical appearance.

Performance

Field performance of the five new rootstocks was evaluated through 15 years of age in a randomized replicated field trial in the Indian River production area, St. Lucie County, FL. In this trial, performance of 'US-1279', 'US-1281', 'US-1282', 'US-1283', and 'US-1284' was outstanding in comparison with the commercial rootstocks and other hybrids included, particularly in the years after the planting became infected with Las. Another replicated field planting in Lake County, FL, containing some of the new rootstocks was also evaluated and data were presented. Overall, field performance of the five new rootstocks was good to outstanding, as described below.

Yield. Field testing of the five new rootstocks has been primarily in two trials and with 'Hamlin' sweet orange scion, by far the most common early season orange in Florida. The longest field test involving these rootstocks is a replicated trial in St. Lucie County, FL, at the U.S. Horticultural Research Laboratory (USHRL) research farm in Ft. Pierce. The site is typical Florida flatwoods, with a soil characterized as Riviera fine sand, and trees were planted in double row beds at 3.0 m × 7.6 m spacing and irrigated by microjet. In this trial, seven 'Hamlin' sweet orange trees on each of the rootstocks were compared with a similar number of trees on 20 other rootstocks planted in July 2000, and using a randomized complete block statistical design. From 2004 to 2012, granular fertilizer was applied by broadcast spreader in three equal applications of 12–2–16 with magnesium (Mg), at a rate of 72 kg nitrogen (N) per acre per year. Beginning in 2012, the granular fertilizer application changed to twice per year (at the same rate of N per year), and was supplemented with a complete foliar nutritional to improve health of the HLB-affected trees (Spann and Schumann, 2010). The foliar nutritional was applied by low-volume sprayer 15 times per year beginning at bloom, and contained a commercial blend of 20–10–20 with minors. Trees in this trial were damaged by hurricanes Frances and Jeanne in 2004, and both HLB and citrus canker (*Xanthomonas citri* subsp. *citri*) began to affect the trees in 2007–08. Fruit production of 'Hamlin' in this trial was first measured in the 2006 harvest season, before HLB symptoms were noted in the block. During the 2006 season, fruit production on 'US-1284' was found to be significantly higher (Table 2) than production of trees on 'Swingle', 'Carrizo', and 'Flying Dragon' (a selection of *P. trifoliata*), while the other four new hybrids were similar to 'Swingle' and 'Carrizo' in yield.

Received for publication 10 July 2015. Accepted for publication 11 Sept. 2015.

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In the following years, Las spread rapidly throughout the trial, and overall tree health declined noticeably. From 2007 to 2012, cropping on all rootstocks declined from the pre-HLB yields, but cropping on 'Swingle' rootstock (the most common rootstock in Florida) suffered more dramatically than cropping on the five new rootstocks. The average fruit production during this period on the five new rootstocks was significantly higher than fruit production on 'Swingle', and measured 1.6 to 2.0 times the fruit production on 'Swingle'.

As a result of increased application of plant nutrients, overall tree health in the trial improved during 2012–14. In the 2013 harvest season, trees on the five new rootstocks produced an average of about 61 kg fruit per tree, or about the same as the 58 kg average annual crop produced on those trees during the 2006 season (before HLB impact). In comparison, trees on 'Swingle' rootstock in the 2013 harvest season continued a very low fruit production, yielding 19 kg fruit per tree, or about 40% the annual crop produced on those trees during the 2006 season. The pattern of superior performance for the five new rootstocks as compared with 'Swingle' was observed to continue in the 2014 season. The significantly higher fruit production of trees on the five new rootstocks than trees on 'Swingle' following tree infection with Las, and also following the implementation of an improved nutritional program, suggests a greater tolerance of these rootstocks to HLB disease. The performance of trees on 'Flying Dragon' rootstock was also very poor following Las infection, while performance of trees on 'Carrizo' was somewhat intermediate and significantly better than performance on 'Swingle'. 'Flying Dragon' was included in the trial because of its known dwarfing effect on the scion, so it is not especially surprising that yield per tree on this rootstock was low. However, even taking this size difference into account, 'Flying Dragon' performance was poor, and this is noteworthy, since *P. trifoliata* has been identified as a source of tolerance to HLB in other studies (Albrecht and Bowman, 2012; Folimonova et al., 2009).

A second field trial containing some of the new rootstocks was conducted at Water Conserv II, a cooperator field site in Lake County, FL, and managed by the Mid-Florida Citrus Foundation. The rootstocks 'US-1279', 'US-1283', and 'US-1284' were tested in a trial with 'Hamlin' scion planted in 2002. The trial contained 12 trees on each rootstock and was planted in a randomized complete block statistical design. This site is on soil characterized as Candler fine sand, and trees were planted at 4.5 m × 7.5 m, and irrigated with microjets. Crop yield measurements were taken from the trial between 2005 and 2014 (Table 3). By 2012, the effects of HLB and reduced tree care in this trial resulted in very poor yields for all rootstocks, and yield data were not collected in that year. In cumulative crop yields from 2005 to 2011, trees on 'US-1279', 'US-1283', and 'US-1284' were not significantly different from those on 'Swingle'. During this same period, it can be noted that 'Carrizo' and 'US-812' (Bowman and Wutscher, 2001; Bowman and Rouse, 2006) were significantly more productive than trees on 'Swingle', while trees on 'US-942' (Bowman, 2010; Wutscher and Bowman, 1999) were significantly more productive than trees on all other rootstocks. During the 2013 and 2014 harvest periods, tree health and yield were poor because of minimal tree care and there was little significant difference among the rootstocks in effect on yield.

The comparison of results from the two trials indicate that yield of sweet orange on 'US-1279, 'US-1281', 'US-1282', 'US-1283', and 'US-1284' were clearly superior to 'Swingle' rootstock on a Florida flatwoods site with HLB and receiving good management care, but yields of sweet orange on 'US-1279', 'US-1283', and 'US-1284' were similar to 'Swingle' at a Florida ridge site with HLB. It can be noted that all the rootstocks performed more poorly at the flatwoods site than the ridge site, although there was an especially large difference in performance of 'Swingle' between the two sites.

Fruit quality. Several measures of fruit quality were assessed with samples from the replicated trial in St. Lucie County just before harvest in 2005–08 and 2010–14. No data were available for 2009. There were no meaningful trends in the data by year, and fruit quality was analyzed using data from all years combined (Table 4). Average fruit

Table 1. Leaf and spine morphology of the five new rootstocks, compared with three common Florida rootstocks.

Trait	Center leaflet length (mm)	Center leaflet width (mm)	Side leaflet length (mm)	Side leaflet width (mm)	Petiole length (mm)	Petiole width (mm)	Spine length (mm)
Swingle	59 a	28 a	33 a	18 a	22 a	4 a	8 c
US-1282	58 a	25 bc	32 a	14 b	16 bc	3 b	13 b
US-812	54 ab	26 ab	28 b	13 bcd	21 a	4 ab	19 a
US-1281	51 bc	21 de	28 b	14 bc	18 b	4 a	11 c
US-1279	50 bc	20 de	24 c	10 ef	14 c	3 b	8 c
US-1284	49 c	20 e	27 b	12 cd	18 b	2 c	4 d
Carrizo	46 c	23 cd	24 c	12 de	21 a	4 a	10 c
US-1283	41 d	15 f	23 c	9 f	12 d	1 c	4 d

Mean separations for significant ANOVA within columns were by Duncan's multiple range test at $P < 0.05$.

Table 2. Yield of Hamlin trees on eight rootstocks in a St. Lucie County trial, planted 2000.

Rootstock	Yield 2006 (kg/tree)	Yield 2007–12 (kg/tree)	Yield 2013 (kg/tree)	Yield 2014 (kg/tree)	Yield 2013–14 (kg/tree)
US-1282	54 ab	206 a	63 a	51 a	114 a
US-1283	51 ab	188 a	67 a	42 a	109 a
US-1284	73 a	171 a	63 a	47 a	109 a
US-1279	49 ab	174 a	61 a	45 a	107 a
US-1281	64 ab	165 a	52 a	38 a	90 a
Carrizo	44 bc	166 a	45 a	29 ab	74 a
Flying Dragon	23 c	64 b	16 b	9 bc	26 b
Swingle	47 bc	100 b	19 b	7 c	26 b

Mean separations for significant ANOVA within columns were by Duncan's multiple range test at $P < 0.05$.

Table 3. Yield of Hamlin trees on seven rootstocks in Lake County trial, planted 2002.

Rootstock	Yield 2005–11 (kg/tree)	Yield 2013 (kg/tree)	Yield 2014 (kg/tree)	Yield 2013–14 (kg/tree)
US-942	512 a	85	77 ab	163
US-812	437 b	91	71 ab	163
Carrizo	434 b	87	77 ab	164
US-1283	430 bc	89	88 a	177
US-1284	389 bcd	54	52 b	106
Swingle	353 cd	71	82 ab	153
US-1279	346 d	65	76 ab	140

Mean separations for significant ANOVA within columns were by Duncan's multiple range test at $P < 0.05$.

Table 4. Fruit quality of Hamlin on eight rootstocks in a St. Lucie County trial.

Rootstock	Fruit wt (g)	TSS	TSS/acid	Juice color (CN)
US-1282	193.6 a	8.67 bc	14.22 abc	34.9
US-1283	189.5 ab	8.43 c	13.62 bcd	34.7
US-1284	185.2 ab	8.77 abc	13.36 cd	34.6
US-1279	192.7 a	8.80 abc	14.08 abcd	34.6
US-1281	190.0 ab	8.90 abc	14.40 ab	34.7
Carrizo	189.9 ab	9.06 ab	14.99 a	34.8
Flying Dragon	176.1 b	9.15 a	14.83 a	34.9
Swingle	159.8 c	8.51 c	13.19 d	34.9

TSS = total soluble solids; CN = color number.
Mean separations for significant ANOVA within columns were by Duncan's multiple range test at $P < 0.05$.

weight from trees on the five new hybrid rootstocks was 185.2 to 193.6 g and significantly higher than the 159.8 g fruit on 'Swingle'. Fruit size was also significantly larger for trees on 'US-1279' and 'US-1282' than trees on 'Flying Dragon', but fruit size on the new hybrids was similar to fruit size on 'Carrizo'. Total soluble solids (TSSs) were not significantly different for fruit on the new hybrids than fruit on 'Swingle', while TSS was significantly higher for fruit on 'Flying Dragon' than fruit on 'US-1282', 'US-1283', and 'Swingle'. TSS/acid ratio was significantly higher for fruit on 'US-1282', 'US-1282', 'Carrizo', and 'Flying Dragon' than fruit on 'Swingle'. Juice color was not observed to differ in any significant way by rootstock.

Tree size. Canopy size was measured on the trees in the replicated trial at the USHRL farm in St. Lucie County in June 2006 and 8 years later in Aug. 2014 (Table 5). At 6 years of age in 2006, and before Las infection, there were significant differences in tree size by rootstock, with 'US-1282' and 'US-1284' making the largest trees, 'Flying Dragon' making the smallest trees, and all the other rootstocks somewhat intermediate in canopy height and diameter. Between 2006 and 2014, average canopy volume decreased for trees on all rootstocks except 'Flying Dragon', with ≈33% reduction in canopy volume across all rootstocks (except 'Flying Dragon'). Canopy volume reduction between 2006 and 2014 was the result of generalized branch dieback due to HLB from 2007 to 2011, and tree topping completed in 2012 at a height of 2.5 m to remove dead wood and help rejuvenate the trees. The dwarfing rootstock 'Flying Dragon' produced trees that were by far the smallest in 2006, and these trees did not change in size between 2006 and 2014. When trees were measured in 2014, the rootstock 'US-1284' produced the largest (tallest and widest) trees, while trees on 'Flying Dragon' and 'Swingle' were the smallest.

Disease and pest resistance. Health, growth, and fruit production of trees on all the rootstocks in the two trials were affected negatively by infection with Las, and the ways in which rootstocks respond to Las infection have been previously described (Albrecht and Bowman, 2011, 2012; Albrecht et al., 2012). In the St. Lucie County trial, the local disease incidence and insect vector were especially severe, and trees became infected with Las soon after the Florida epidemic began in 2006. By 2014, all trees were infected and symptomatic (Table 6). It can be noted that all trees on all rootstocks, even the rootstock most affected by the infection ('Swingle'), remained alive and continued to bear some crop each year. While HLB reduces tree health and vigor, it appears that normally it will not be the cause of actual tree death. As reported here, the five new rootstocks exhibited significantly improved tolerance to Las under flatwoods conditions for many years after infection, as compared with 'Swingle', the most common rootstock used in Florida. Since trees on the new hybrid rootstocks became 100% infected with Las, and bacterial titer in trees on the new hybrid rootstocks appeared similar to the titer for trees on the sensitive standard 'Swingle', it is suggested that the Las reaction of the new rootstocks is best referred to as tolerance, rather than resistance. The large difference in relative performance of 'Swingle' at the two field trial sites affected by HLB and reported here, indicates a rather strong interaction between Las infection and other biotic and/or abiotic factors in field performance of rootstocks. Citrus tristeza virus, phytophthora, and Diaprepes weevil were present at the St. Lucie County site, and at least the first two were also present at the Water Conserv II site. Soils at both sites would be defined as relatively good for citrus production. Further testing at additional sites will be necessary to more fully define the resistance or tolerance of the new rootstocks to various biotic and abiotic factors, and to define where they are best suited for commercial use. After infection with Las, trees on the new rootstocks showed significantly less decline in fruit yield and quality at a flatwoods site than trees on the common rootstock 'Swingle', and appear to offer a useful new tool for the management of HLB disease.

Table 5. Size of Hamlin trees on eight rootstocks in St. Lucie County trial.

	Tree size 2006			Tree size 2014		
Rootstock	Canopy ht (m)	Canopy Diam (m)	Canopy volume (m^3)	Canopy ht (m)	Canopy diam (m)	Canopy volume (m^3)
US-1282	3.19 a	2.91 ab	6.81 a	2.56 ab	2.44 ab	3.91 ab
US-1283	3.02 a	2.58 bc	5.10 bc	2.63 ab	2.49 ab	4.13 ab
US-1284	3.27 a	2.99 a	7.33 a	2.71 a	2.76 a	5.28 a
US-1279	3.11 a	2.55 bc	5.16 bc	2.60 ab	2.43 ab	4.03 ab
US-1281	3.21 a	2.72 abc	6.03 ab	2.49 b	2.17 bc	3.00 bc
Carrizo	3.10 a	2.54 cd	5.08 bc	2.47 b	2.62 ab	4.33 ab
Flying Dragon	2.10 b	1.85 e	1.81 d	2.13 c	1.67 d	1.63 c
Swingle	3.04 a	2.39 d	4.36 c	2.44 b	1.85 cd	2.22 c

Mean separations for significant ANOVA within columns were by Duncan's multiple range test at $P < 0.05$.

Table 6. Las infection and copy number for Hamlin on eight rootstocks in St. Lucie County trial.

Rootstock	N	Las-infected trees (%)	CtLas	Las copy number/g
US-1282	7	100	24.7	2,143,900
US-1283	7	100	25.1	1,507,681
US-1284	7	100	24.5	2,432,189
US-1279	7	100	25.1	1,831,557
US-1281	7	100	24.7	2,016,472
Carrizo	7	100	24.8	2,071,116
Flying Dragon	6	100	25.1	1,482,230
Swingle	7	100	25.1	1,584,108

ANOVA within columns were not significant ($P > 0.8$).

Availability

Disease-indexed budwood to establish seed trees or make cuttings is being distributed by Florida Department of Agriculture (Division of Plant Industry, 3027 Lake Alfred Road, Winter Haven, FL 33881) to registered Florida citrus nurseries. Requests for budwood from other states or countries should be sent to the USDA-ARS National Clonal Germplasm Repository for Citrus (1060 Martin Luther King Boulevard, Riverside, CA 92507). Seed for commercial propagation is not available at this time. Liners of the rootstocks are being propagated by cuttings or in vitro micropropagation by Phil Rucks Citrus Nursery (Frostproof, FL) and Agromillora (North American Plants, Inc., McMinnville, OR), and should be available for future use.

Literature Cited

Albrecht, U. and K.D. Bowman. 2011. Tolerance of the trifoliate citrus hybrid US-897 (*Citrus reticulata* Blanco × *Poncirus trifoliata* L. Raf.) to Huanglongbing. HortScience 46: 16–22.

Albrecht, U. and K.D. Bowman. 2012. Tolerance of trifoliate citrus rootstock hybrids to *Candidatus Liberibacter asiaticus*. Sci. Hort. 147: 71–80.

Albrecht, U., G. McCollum, and K.D. Bowman. 2012. Influence of rootstock variety on Huanglongbing disease development in field-grown sweet orange (*Citrus sinensis* [L.] Osbeck) trees. Sci. Hort. 138:210–220.

Bowman, K.D. 2010. Notice to fruit growers and nurserymen relative to the naming and release of the US-942 citrus rootstock. U.S. Department of Agriculture, ARS, Washington, D.C.

Bowman, K.D. and G.M. McCollum. 2014a. Release of the US-1279, citrus rootstock. U.S. Dept. Agr., ARS, Washington, D.C.

Bowman, K.D. and G.M. McCollum. 2014b. Release of the US-1281, citrus rootstock. U.S. Dept. Agr., ARS, Washington, D.C.

Bowman, K.D. and G.M. McCollum. 2014c. Release of the US-1282, citrus rootstock. U.S. Dept. Agr., ARS, Washington, D.C.

Bowman, K.D. and G.M. McCollum. 2014d. Release of the US-1283, citrus rootstock. U.S. Dept. Agr., ARS, Washington, D.C.

Bowman, K.D. and G.M. McCollum. 2014e. Release of the US-1284, citrus rootstock. U.S. Dept. Agr., ARS, Washington, D.C.
Bowman, K.D. and H.K. Wutscher. 2001. Notice to fruit growers and nurserymen relative to the naming and release of the US-812 citrus rootstock. U.S. Dept. Agr., ARS, Washington, D.C.
Bowman, K.D. and R.E. Rouse. 2006. US-812 citrus rootstock. HortScience 41:832–836.
Folimonova, S.Y., C.J. Robertson, S.M. Garnsey, S. Gowda, and W.O. Dawson. 2009. Examination of the responses of different genotypes of citrus to huanglongbing (citrus greening) under different conditions. Phytopathology 99:1346–1354.
Spann, T.M. and A.W. Schumann. 2010. The role of plant nutrients in disease development with emphasis on citrus and huanglongbing. Proc. Fla. State Hort. Soc. 122:169–171.
Wutscher, H.K. and K.D. Bowman. 1999. Performance of 'Valencia' orange on 21 rootstocks. HortScience 34:622–624.