



Accelerating Implementation of Huanglongbing-tolerant Hybrids As New Commercial Cultivars for Fresh and Processed Citrus

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Citrus greening or huanglongbing (HLB) has reduced citrus and especially orange yields, resulting in closure of packing houses and processing plants in Florida. To maintain the remaining packing and processing infrastructure, a National Institute of Food and Agriculture (NIFA), Citrus Disease Research and Extension (CDRE) grant was obtained to identify hybrids in breeding programs with HLB tolerance and commercial quality for either the fresh fruit or processed juice industry. The proposed research would also determine HLB resistance or tolerance DNA markers for marker assisted breeding or use in transgenic programs. This paper will discuss the first season performance of some of these hybrids for their intrinsic flavor quality and similarity to either orange or mandarin, since both orange and mandarin are present in the hybrids' genetic backgrounds. Both sensory and chemical evaluations for flavor were conducted. Several commercial cultivars were also harvested and evaluated for comparison. These hybrids were harvested once or multiple times over the season. Out of the 20 hybrids evaluated, 7 exhibited orange similarity, and most scored relatively high for orange flavor in at least one harvest. One of these hybrids has since been released as 'U.S. SunDragon'. Seven hybrids exhibited similarity to mandarin and/or mandarin flavor, with three that also had similarity to orange. Overall, the trial showed that there is much diversity in the breeding programs for flavor, sugars, acids, and bitter limonoids. There is also potential for both HLB tolerance and commercial quality. Hybrids with HLB tolerance and good flavor would be candidates for citrus juice blends.

Huanglongbing (HLB) or citrus greening disease has plagued Florida citrus industry since 2005, and citrus production acreage has dropped from 762,000 acres in 1999–2000 to 400,000 acres in 2017–18 (USDA NASS, 2019). In spite of this shrinkage, Florida remains the number two supplier of orange juice in the world (USDA FAS, 2020), with production that has stabilized at around 300,000 metric tons/ year (65 °Brix) since 2015 (this not counting the drop following hurricane Irma in 2017). However, this number is far below the most productive years, and several processing plants have closed due to the reduction in fruit volume in Florida resulting from HLB. A National Institute of Food and Agriculture (NIFA) grant was obtained to identify HLB-tolerant scion cultivars and or genetic material from the U.S. Department of Agriculture (USDA), University of California, Riverside (UCR) and University of Florida (UF) breeding programs that can be quickly mobilized for use by the U.S. citrus industry. The project is identifying HLB resistant/tolerant orange mandarin and grapefruit like hybrids with good flavor quality, identifying DNA markers associated with tolerance to HLB and evaluating fruit and juice quality of HLB tolerant scions. This material can

then be used either as stand alone varieties, hybrid classification as "orange," "mandarin," or "grapefruit" and/or development of juice combinations to supplement the current processing stream. These results are continuously communicated to the industry to facilitate industry implementation of HLB tolerant scion cultivars.

This paper will present the first year flavor data for the HLB tolerant lines analyzed in the 2016–17 seasons for similarity to "orange" or "mandarin" and for overall flavor quality traits. We hope the research will result in a sustainable solution when used either alone or in combination with other disease mitigation methods. Hopefully, developing HLB resistant varieties with commercial quality will decrease production costs per acre, increase crop yields, enhance consumer satisfaction, and maintain production and processing infrastructure. In addition, analysis and comparison of sensory and chemical flavor analyses will improve understanding of citrus flavor.

Materials and Methods

Fruit from HLB tolerant trees from the USDA breeding program were harvested once or multiple times from November 2016 to February 2017 to determine optimal harvest dates, if not known, or at optimal harvest time if previously determined. Fruit were brought to the USDA laboratory, washed, juiced and frozen at –20 °C pending sensory and chemical analyses. For sensory analysis, juice was tested in comparison to orange and tangerine reference juices, using the polarized sensory positioning method (Teillet et al., 2010; Valentin et al., 2012). The reference juices were from fresh squeezed unpasteurized 'Valencia' oranges (Al's Family Farm, Fort Pierce, FL) and locally processed fresh

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tangerines “gourmet pasteurized” (Orchid Island Juice Co., Fort Pierce, FL). Six trained panelists, with three to 10 years of training and experience rating citrus juice products, rated the juice on a 0–15 scale for similarity to orange or tangerine references (0 = exactly the same flavor as reference, 15 = totally different from reference). Panelists then rated sweetness, sourness, bitterness, orange like flavor, mandarin like flavor and off flavor on a 0–15 scale, where lower and higher values indicate lower or higher intensity of perception for a flavor characteristic, respectively. Panelists were also asked to specify off flavor, when perceived, and were free to make any comment about the juice. Six to eight samples were tasted per day and within a day, samples were presented in a completely randomized order across panelists. Taste panels took place in isolated booths with red lighting to mask color differences among juices, and using Compusense® Cloud (West Guelph, ON, Canada) for data acquisition. Samples from the same juice were analyzed for pH and titratable acidity (TA) to a pH 8.1 endpoint using an autotitrator (Mettler Toledo DL50, Columbus, OH), soluble solids content (SSC) by refractometer (Atago PR 101, Tokyo, Japan), SSC/TA ratio, and for levels of the bitter compounds limonin and nomilin by chromatography as described in Raithore et al., (2020).

Sensory data were analyzed using SenpaQ v.5.01 (Qi Statistics, Berkshire, U.K.). Correlation tests and principal components analyses (PCA) were performed to compare instrument with sensory data using XLSTAT v. 2019 (Addinsoft, New York, NY).

Results and Discussion

An alternative to descriptive sensory analysis was tested in this project, where the samples were compared with either an orange or a tangerine juice reference (Valentin et al., 2012). Not knowing what the hybrids would taste like “a priori”, we hypothesized that this method would help in classifying the hybrids. A principal components analysis (PCA) showed that rating samples in comparison with a reference juice sample (“same as reference”), or simply rating the flavor by providing a conceptual reference standard gave similar results; indeed, correlations (Table 1) as well as the PCA graph (Fig. 1) showed that descriptors “same as orange” or “same as mandarin” were highly correlated with “orange like” and “mandarin like” flavors, respectively. However, in general, the similarity ratings were higher (up to 10.5 points on a 0–15 scale) than the absolute ratings

(up to 6.3 points) (Table 2). Furthermore, more samples had high similarity to orange than mandarin and also had relatively high orange flavor ratings, whereas samples that had high similarity with mandarin juice did not all have high mandarin flavor ratings. The latter can be explained by the fact that the mandarin juice used as a reference was pasteurized, and pasteurization, even at low temperatures, can change the flavor profile of a juice (Perez Cacho and Rouseff, 2008).

Table 2 presents average ratings for sensory descriptors as well as instrumental data. Color coded sensory means indicate relatively high values: orange color for “same as orange” and “orange flavor”, peach color for “same as tangerine” and “tangerine flavor”, yellow for high “sweet” ratings, and green, blue or grey for high “sour”, “bitter” or “off flavor” ratings, respectively. Samples with the highest ratings for “same as orange” (≥ 7) were ‘Bower’ (Dec.), ‘Fortune’ (Jan. Feb.), FTP 6-49-96 (Nov. Dec., newly released ‘U.S. SunDragon’, which also had high “same as mandarin” rating for Nov.), FF 1-8-96 (Dec.), FF 1-10-61 (Nov. Dec.), FF 1-74-14 (Dec.), FF 1-75-113 (Nov.), FF 1-85-109 (Nov. Jan.) and FF 1-85-119 (Jan. Feb., which also had high rating for “same as mandarin” for Feb.). Of these, all but ‘Bower’ and FF 1-74-14 (Dec.) also had relatively high (≥ 5) “orange flavor” ratings in at least one harvest. This includes ‘Fortune’ (Jan.), FF 1-8-96 (Dec.), FF 1-10-61 (Nov. Dec.), FF 1-75-113 (Nov.), FF 1-85-109 (Jan.) and FF 1-85-119 (Jan. Feb.) (Table 2). For “same as mandarin”, ‘Nova’ (Dec.), FTP 6-49-96 ‘U.S. SunDragon’ (Nov.), FF 1-10-1 (Jan.), FF 1-78-62 (Dec.), FF 1-85-119 (Feb.), FF 1-8-70 (Feb.) and FF 5-51-2 (Dec.) had relatively high ratings (≥ 7), and among them, only ‘Nova’ (Dec.), FF 1-78-62 (Dec.) and FF 5-51-2 (Dec.) had relatively high ratings for “mandarin flavor” in addition to FF 1-8-96 (Dec.) (Table 2). The sweetest samples tended to also be high in mandarin, orange, or both flavors (Table 2). High and significant correlations between sweet and “same as orange” and with mandarin flavor were found (Table 1), as well as with the SSC/TA ratio. A SSC/TA ratio greater than 12.0 generally resulted in a high sweetness rating, but there were exceptions. Volatile compounds also contribute to the perception of sweetness, as demonstrated by Bartoshuk et al. (2017) in strawberries, or in orange juice (unpublished data).

Some samples had an exceedingly high level of sourness, greater than 9.5 on the 0–15 scale: FF 1-85-82 (Nov.), FF 1-8-70 (both harvests), FTP 6-45-137 (Jan), FTP 6-46-15 (both harvests), FTP 6-47-13 (both harvests), and FTP 6-47-9 (all harvests),

Table 1. Pearson correlation coefficients between sensory and instrumental measurements (n = 41).

Variables	Orange	Same as	Mandarin				Off-	SSC	TA	SSC/TA	Limonin	Nomilin
	Flavor	Mandarin	Flavor	Sweet	Sour	Bitter	flavor	(°Brix)	(% Acid)			
Same as orange	0.797^a	0.360	0.432	0.665	-0.628	-0.500	-0.623	0.212	-0.371	0.258	-0.307	-0.352
Orange flavor		0.237	0.451	0.564	-0.424	-0.247	-0.595	0.401	-0.191	0.087	-0.170	-0.322
Same as mandarin			0.680	0.442	-0.495	-0.168	-0.387	0.323	-0.513	0.517	-0.007	0.010
Mandarin flavor				0.701	-0.689	-0.087	-0.445	0.304	-0.600	0.562	-0.089	-0.109
Sweet					-0.894	-0.222	-0.317	0.510	-0.647	0.657	-0.125	-0.242
Sour						0.318	0.357	-0.313	0.782	-0.773	0.233	0.283
Bitter							0.533	0.097	0.378	-0.286	0.692	0.401
Off-flavor								-0.018	0.346	-0.203	0.272	0.245
SSC (Brix)									-0.079	0.359	0.029	-0.077
TA (% Acid)										3	0.134	0.197
SSC/TA											-0.141	-0.194
Limonin												0.696

^aValues in bold are different from 0 with a significance level $\alpha < 0.0001$.

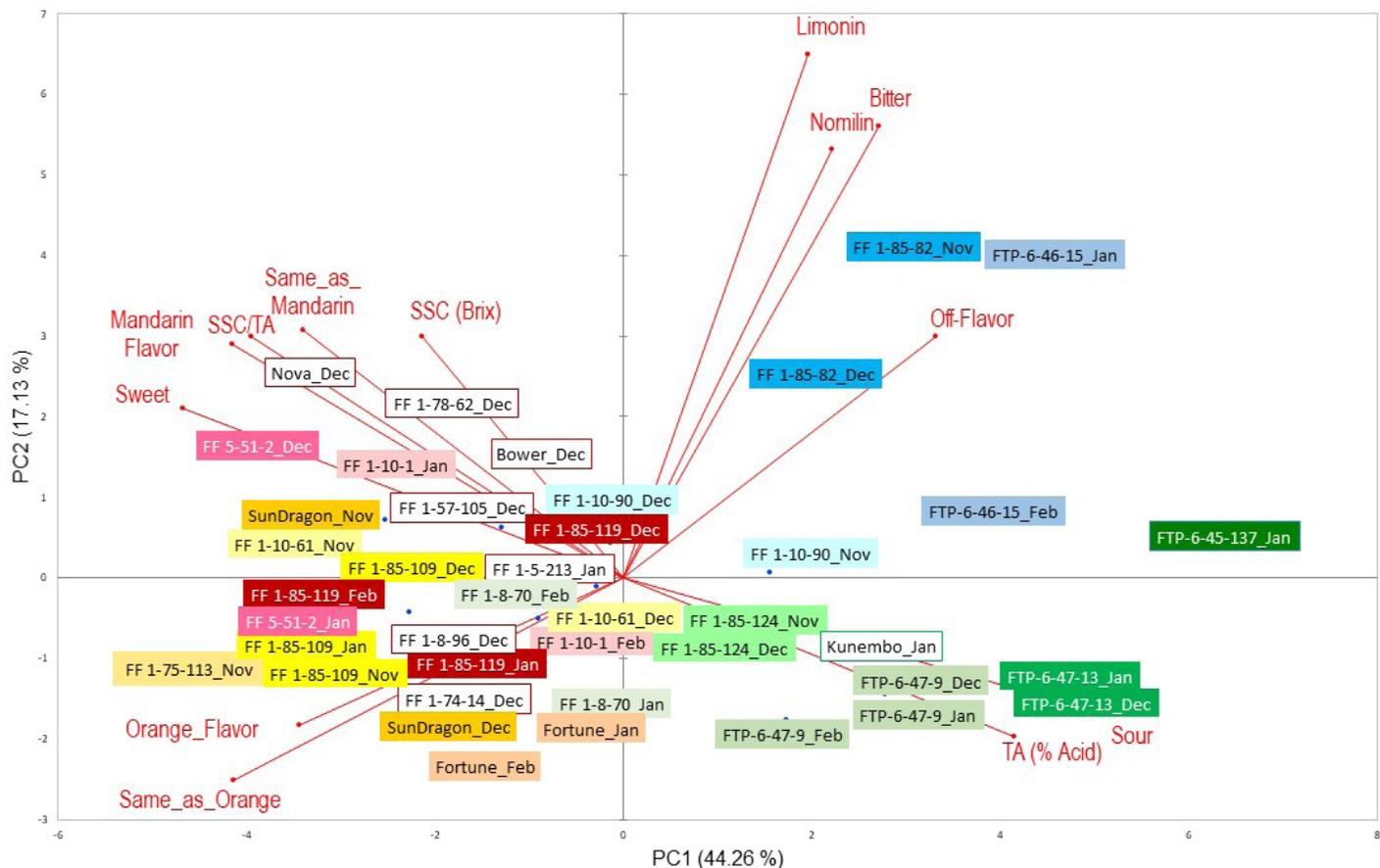


Fig. 1. Principal components analysis (PCA) of sensory and instrumental quality ratings of 24 citrus hybrids harvested multiple times.

associated with high TA levels (greater than 1.25% and up to 2.16%) and low SSC/TA ratio (less than 10.0) (Table 2). Some of these samples were additionally perceived as noticeably or highly bitter, depending on panelists, and had high limonin or limonin + nomilin levels: FF 1-10-90 (Nov.), FF 1-85-124 (Nov.), FF 1-85-82 (both harvests), FTP 6-45-137 (Jan.), FTP 6-46-15 (both harvests), and FF 6-47-13 (Jan.) (Table 2). Dea et al. (2013) found that in a complex matrix such as orange juice, limonin recognition threshold (i.e. concentration of limonin at which point it is perceived as bitter) was 4.7 mg·L⁻¹. The current data indicate that most of the time, limonin greater than 3.43 mg·L⁻¹ induced perception of bitterness, but some samples with lower levels of limonin were also perceived as bitter (2.34 and 2.16 mg·L⁻¹ for FTP 6-45-137 (Jan.) and FTP 6-47-13 (Jan.), respectively). Interestingly, bitterness was perceived in FF 1-10-61 (Nov.) and FF 1-78-62 (Dec.) which had relatively high limonin levels (3.96 and 3.77 mg·L⁻¹, respectively), but with high soluble solids content (15.1 and 13.7 °Brix, respectively) and high SSC/TA ratio (13.7 and 11.4, respectively), panelists gave those hybrids high sweetness ratings, as well as relatively high mandarin flavor (Table 2 and Fig. 1).

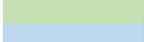
Excessive sourness and high bitterness also resulted in high off flavor ratings. Comments for FTP 6-45-137 (Jan.), which had sour and bitter ratings of 13.5 (the highest) and 4.8, respectively, were “so sour, it is difficult to perceive anything else”. FTP 6-47-13 (Dec. and Jan.) was also found too sour but not bitter, with additional notes of vinegar, woody, “green” and sulfury (data not shown). Off flavor in FF 1-85-82 (Nov. and Dec.) was

described as harsh, bitter, gasoline, soapy, woody, which might be reminiscent of off flavor that has been found in fruit from some Poncirus hybrids. Other samples in which panelists found poncirus like off flavor were FF 1-10-1 (also soapy, pumpkin like, musty), FF 6-46-15 (also bitter, harsh, metallic, soapy, sulfury), and FF 1-85-119 (also metallic, gasoline, sulfury, woody). Off flavor in ‘Bower’ was described as carrot/pumpkin, not unpleasant but not typical of orange juice, which is unsurprising for this largely mandarin cultivar. Finally, off flavor in ‘Kunembo’ was associated with high sourness, and it is possible it was harvested too early (Jan. 2017).

The PCA plot summarizes quality of these 24 samples harvested in 2016–17 (Fig. 1). The first two PCs accounted for 61.39% of the variability. All samples on the left side of the graph (negative PC1) had desirable attributes, sweet, mandarin like and orange flavor with high SSC and SSC/TA, while samples on the right side of the graph were highly sour with high TA (negative PC2) or highly bitter with high limonin and nomilin levels (positive PC2). This first year of observations allowed sorting out which hybrids produce fruit with negative attributes, in spite of having shown some HLB tolerance. FTP 6-45-137, FTP 6-46-15, FTP 6-47-13, and FF 1-85-82 were removed from the list of hybrids for future evaluation. FTP 6-47-9 was also a possible candidate for removal, but was given a second chance in 2018 2019 with later harvests (end of February and March) with the hope of decreasing sourness; however, sourness ratings were still quite high in 2018–19 (data not shown). FF 1-85-124 on the other hand, improved with a later harvest in 2019. Hybrids

Table 2. Sensory ratings (0–15 scale), soluble solids content (SSC), titratable acidity (TA), limonin (L) and nomilin (N) of 24 citrus hybrids harvested in the 2016–17 season.

Genotype	Harvest date	Same as Orange	Orange flavor	Same as mandarin	Mandarin Flavor	TA								
						Sweet	Sour	Bitter	Off flavor	SSC (°Brix)	(% citric acid)	SSC/TA	Limonin (mg L ⁻¹)	Nomilin (mg L ⁻¹)
Bower	13 Dec.	8.0	3.5	7.0	3.2	8.3	4.7	2.3	6.2	13.4	1.02	13.2	3.37	2.40
Kunembo	12 Jan.	3.5	2.7	4.2	3.3	4.2	9.3	2.8	5.7	11.6	1.49	7.8	0.86	0.91
Nova	13 Dec.	6.5	4.2	8.2	6.0	9.2	3.0	2.7	3.5	12.6	0.71	17.7	2.80	2.40
Fortune	12 Jan.	7.7	5.0	5.5	3.3	5.8	9.2	0.7	1.8	13.6	1.63	8.4	1.53	1.05
	15 Feb.	9.7	4.8	6.7	3.5	6.0	7.2	0.7	0.7	12.7	1.55	8.1	0.45	0.76
FTP 6-49-96 'SunDragon'	17 Nov.	7.7	4.2	9.7	4.7	5.8	5.7	1.8	1.8	12.0	0.79	15.3	2.90	1.19
	22 Dec.	8.7	4.7	5.0	3.8	5.8	5.3	0.7	1.2	11.0	0.93	11.8	1.18	1.73
FF 1-5-213	25 Jan.	4.8	3.5	6.2	4.2	6.0	8.5	2.7	3.0	12.4	1.06	11.8	1.33	1.01
FF 1-8-96	22 Dec.	8.0	5.2	6.5	5.3	7.7	5.8	2.2	2.0	11.3	1.03	10.9	0.97	1.27
FF 1-10-1	25 Jan.	5.3	3.8	7.5	4.5	7.7	5.0	2.5	4.2	15.2	0.80	19.0	1.29	0.86
	15 Feb.	7.2	4.5	6.5	3.7	6.0	5.2	2.8	5.2	10.8	1.51	7.1	1.30	1.24
FF 1-10-61	17 Nov.	9.3	6.3	5.3	4.2	9.2	5.5	3.0	1.0	15.1	1.11	13.7	3.96	0.86
	22 Dec.	8.5	5.2	5.7	2.8	6.2	8.5	2.3	3.3	15.2	1.55	9.8	2.85	1.06
FF 1-10-90	17 Nov.	6.8	3.8	4.8	3.8	5.2	8.7	3.3	1.8	6.7	1.23	5.6	6.14	2.39
	22 Dec.	6.0	4.3	5.8	3.5	6.2	6.8	3.3	2.0	12.3	1.13	10.9	4.75	1.82
FF 1-57-105	22 Dec.	4.5	1.5	5.3	3.3	8.3	2.8	1.0	5.0	12.0	0.66	18.3	1.49	1.33
FF 1-74-14	22 Dec.	10.0	4.8	4.7	4.2	7.7	5.7	2.2	4.3	11.5	1.27	9.2	0.96	0.97
FF 1-75-113	17 Nov.	10.5	6.3	5.8	4.8	9.0	4.0	1.5	2.0	9.8	0.84	11.6		
FF 1-78-62	22 Dec.	6.5	3.8	8.3	5.8	8.0	4.3	4.5	1.8	13.7	1.21	11.4	3.77	1.39
FF 1-85-109	17 Nov.	7.8	4.8	6.5	3.8	6.3	5.7	1.0	2.7	12.7	1.04	12.2	1.08	1.53
	22 Dec.	6.2	4.7	7.2	4.8	6.7	6.5	1.0	3.5	13.8	1.38	10.1	1.41	1.24
	25 Jan.	7.8	5.7	5.3	4.3	7.7	5.0	1.7	2.0	14.8	1.17	12.7	0.94	0.76
FF 1-85-119	22 Dec.	6.2	3.2	6.8	4.0	6.7	7.3	2.0	4.0	12.5	1.28	9.8	1.23	2.87
	25 Jan.	8.0	5.5	6.2	3.2	6.3	7.0	0.7	3.2	12.4	1.15	10.8	2.58	2.43
	15 Feb.	9.8	5.8	8.3	4.7	7.7	7.0	1.3	2.5	14.1	1.34	10.5	1.75	1.31
FF 1-85-124	17 Nov.	5.5	3.8	6.7	4.0	3.8	9.0	3.2	2.7	10.4	1.25	8.4	0.84	1.44
	22 Dec.	5.0	3.5	6.0	3.5	5.0	9.0	2.2	2.7	11.6	1.25	9.3	0.55	1.39
FF 1-85-82	17 Nov.	3.0	2.5	6.2	2.5	4.0	10.5	6.5	8.2	12.3	1.13	10.9	8.23	3.45
	22 Dec.	4.0	3.3	5.8	4.0	6.0	7.8	5.0	6.8	14.2	1.44	9.9	5.18	1.85
FF 1-8-70	25 Jan.	7.2	4.8	6.2	3.7	5.0	9.7	1.2	2.0	11.0	1.36	8.1	0.98	1.23
	15 Feb.	4.0	3.3	7.7	4.2	4.5	10.0	0.7	2.2	13.2	0.91	14.5	1.48	1.09
FF 5-51-2	22 Dec.	7.2	2.5	8.5	5.5	7.0	3.3	1.2	3.2	11.8	0.52	22.5	2.03	1.76
	25 Jan.	6.8	2.8	6.3	3.8	7.0	3.0	0.5	2.2	11.6	0.50	23.3	0.48	0.71
FTP 6-45-137	12 Jan.	1.0	0.8	3.7	2.2	3.0	13.5	4.8	10.7	12.2	2.16	5.6	2.34	1.58
FTP 6-46-15	12 Jan.	3.0	2.2	6.3	3.7	4.0	11.7	4.3	4.2	13.0	1.82	7.1	7.86	6.76
	15 Feb.	2.8	3.3	5.3	3.0	3.3	10.8	5.0	3.2	13.2	2.09	6.3	3.43	2.80
FTP 6-47-13	13 Dec.	3.0	2.0	2.3	1.8	3.0	11.5	1.2	5.7	9.2	1.47	6.3	1.92	2.47
	12 Jan.	3.8	3.2	3.0	2.2	3.3	11.8	4.0	5.8	10.6	2.07	5.1	2.16	0.82
FTP 6-47-9	13 Dec.	4.0	1.8	6.3	3.0	2.8	12.2	1.3	2.8	8.4	1.48	5.7	2.31	1.66
	12 Jan.	3.8	2.2	5.3	1.7	3.5	11.2	1.3	3.8	10.6	1.62	6.5	2.42	1.25
	15 Feb.	7.2	2.2	6.7	2.2	3.8	9.5	1.3	3.2	11.2	1.76	6.3	0.80	1.65

Color legend	
	Same as orange flavor (≥7.5)
	Orange flavor (≥5)
	Same as tangerine flavor (≥7.5)
	Tangerine flavor (≥5)
	High sweet (≥7)
	High sour (≥7)
	High bitter (≥3)
	High off flavor (≥3)

with positive or intermediate attributes will continue to be evaluated, both for tree performance and fruit quality. These include FF 5-51-2, FF 1-75-113, FF 1-85-109, FF 1-85-119, FF 1-5-213, FF 1-57-105, FF 1-78-62, FF 1-8-70, and FTP 6-49-96, which has since been released as 'U.S. SunDragon'.

In conclusion, there is great diversity among the breeding program hybrids, with some showing promise for commercialization along with HLB tolerance. 'U.S. SunDragon' has been released and shows much promise, but in addition, other hybrids from the breeding programs have potential. Optimal harvest dates need to be established for the new hybrids, as there is great variation over the harvest season and from season to season, necessitating multiple seasons of data. This is true not only for the flavor characteristics, but also for yield and HLB tolerance. The best performing hybrids and others have been sampled and analyzed in the 2017–18 and 2018–19 seasons, with plans to continue for the next few years.

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