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The Rediscovery of ‘Donaldson’ Sweet Orange, a Variety That Has Potential for Use in Orange Juice

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Abstract. **In Summer 2021, a comprehensive evaluation of germplasm for the US Department of Agriculture (USDA), Agricultural Research Service (ARS) citrus breeding program was conducted to assess all citrus scions that may have commercial potential for use in not-from-concentrate orange juice (NFCOJ) to support Florida’s citrus industry that has been ravaged by Huanglongbing (HLB), causing production losses of 90%. The ‘Donaldson’ sweet orange tree (*Citrus sinensis*) stood out as being exceptionally healthy compared with industry standards that were planted close by and were in decline or had died. The tree tested positive for the presence of *Candidatus* Liberibacter asiaticus (*C*Las), the presumed causal agent of HLB, indicating that the tree may have tolerance to HLB, as that tree was planted more than 30 years ago. The fruit was displayed at a field day in Dec 2021; the healthy-looking tree attracted growers’ attention and generated a records search for information. This is the only mature tree growing at the USDA, ARS Whitmore Foundation Farm. The results show that ‘Donaldson’ is an early season sweet orange variety that matures from December to January in HLB endemic conditions and has juice as a potential substitute for the early season orange variety ‘Hamlin’, contributing to NFCOJ. HLB tolerance is difficult to assess from a single tree and the HLB tolerance of ‘Donaldson’ will be confirmed in multiple replicated plantings.**

Sweet orange (*C. sinensis*) is the most cultivated fruit crop in the world as reported by total volume (Roussos 2016). There are hundreds of varieties of sweet orange that exist and have been characterized by organoleptic and morphological differences. There is now molecular evidence that all sweet oranges originated from one progenitor and the differences between varieties are the result of environmental, accumulated, or induced mutations resulting in a narrow genetic base for sweet oranges (Wu et al. 2018). The varieties of sweet orange were often selected to serve a particular niche and were traditionally selected by farmers, gardeners, and homeowners who noticed unique differences in growth habit, productivity, or flavor. As a result, there are now many hundreds of cultivars that are usually named after the person who found the mutant or the place where it was discovered. In the case of ‘Donladson’, this is most likely named after the grower who discovered the mutation or was cultivating a particular orange tree.

The mutations found in sweet orange have produced many cultivars that range in seasonality and end-use as a fresh or processed product. Navels are a mutant of sweet orange that produce a good eating quality fresh fruit but develop bitterness upon juicing and pasteurization (Raithore et al. 2016; Sandhu and Minhas 2006). “Delayed bitterness” is the result of the enzyme limonoid D-ring lactone hydrolase, which releases the bitter compound limonin when the vesicle cell wall is broken on juicing and was described in several sports of Navel (Brasili et al. 2017; Esteve and Frígola 2007; Maier et al. 1969). There are also oranges that are suitable for processing that have desirable organoleptic qualities such as orange flavor and have low levels of limonin after processing (Raithore et al. 2016). Oranges suitable for processing have dominated the Florida citrus industry for their positive organoleptic qualities and high yields. These include ‘Valencia’, ‘Hamlin’, ‘Midsweet’, ‘Pera’, ‘Pineapple’, etc. In addition to bitterness due to limonin, other off flavors can develop on processing, storage, and heat pasteurization and can negatively affect juice quality (Perez-Cacho and Rouseff 2008a; Sadecka et al. 2014). Some of these are related to peel bitterness (Chavan et al. 2018), peel oil (Ahmed et al. 1978a; Blair et al. 1952; Perez-Cacho and Rouseff 2008a), and seed (Siddiqui et al. 2013) components in the juice. Commercially there are methods to remove bitter compounds and off flavors to some extent, but this comes at an economic trade off (Ghanem 2012) and can remove other positive components of orange juice flavor (Fernández-Vázquez et al. 2013). The post processing flavor after storage is a commercially important trait for a new sweet orange variety to be able replace other industry standards as a component of NFCOJ.

A standard practice for juice production in Florida is to blend the predominant early season variety ‘Hamlin’ with the late season variety ‘Valencia’. ‘Hamlin’ is notorious for being highly productive but has a juice that is poorly colored; it provides a “base” to blend with a higher-quality late season ‘Valencia’ juice. However, the HLB epidemic in Florida has led to a greater decline of ‘Hamlin’ trees due to their increased susceptibility to HLB as compared with late season cultivar Valencia (Dala-Paula et al. 2018). The decline in early season orange production has caused problems in the supply chain forcing many processing plants to purchase juice from abroad (Mexico, Brazil) or start processing early season oranges later in the season (Singerman and Rogers 2020). Due to the HLB susceptibility of ‘Hamlin’ there is a need to look for an early season orange to sustain the current model of the Florida orange juice processing industry.

In that context, the ‘Donaldson’ tree was first observed in Summer 2021 at the USDA’s A.H. Whitmore Foundation Farm in Groveland, FL, USA, as a survivor tree with outstanding HLB tolerance on the farm’s variety block that was originally planted in 1986. Observations in Fall 2021 revealed that ‘Donaldson’ fruit had started to mature in November with fruit that resembled a typical seedy sweet orange. During an open house field day at the A.H. Whitmore Foundation Farm, growers became interested in the tree after tasting fresh fruit that were displayed and observing the healthy appearance of the tree in the variety collection surrounded by other trees that had succumbed to HLB pressure and freeze damage over the course of many years. The rediscovery of the ‘Donaldson’ tree that showed potential for HLB tolerance gives the Florida citrus industry hope that such varieties may be part of the solution to solving HLB in Florida. To quickly provide information to the citrus industry, a historical internal document search was conducted on the origin of this tree, and extensive data were taken from the single mature ‘Donaldson’, including field data to determine various characteristics of the tree, and sensory and chemical data to demonstrate juice quality for potential use in NFCOJ.

Materials and Methods

Historical record search

A literature search for ‘Donaldson’ in Fall 2021 returned few results for ‘Donaldson’ documented in published literature, only indicating that it was a sweet orange. In Winter 2022, an extensive and systematic search of the historical records were conducted that included the review of more than 1000 historical documents and breeding records that are a part of the USDA, ARS citrus breeding program's internal files.

Tree and fruit data collection

'Hamlin' and 'Donaldson' trees on 'Swingle' rootstock were planted in the same block between in 1987 and 1991 at the A.H. Whitmore Citrus Research Foundation Farm, Groveland, FL, USA (lat. 28.69°N, long. 81.89°W) grown under subtropical conditions with high summer rainfall. Tree and fruit data were collected in early Jan 2022 when fruit were at commercial mature stage. Tree height was measured at the highest point in the canopy, canopy diameter was the average value of the longest and shortest diameters, and canopy volume was calculated based on tree height and canopy diameter, according to the equation proposed by Mendel (1956). The rootstock diameter was the average value of the largest and smallest diameters determined at 10 cm below the graft union and the scion diameter was the measurement taken at 10 cm above the graft union. Canopy density measurements were obtained using CI-110 digital canopy analyzer (CID Bio-Science, Camas, WA, USA) as an unbiased indicator of tree health, and gap fraction profile, photosynthetically active radiation, and leaf area index were estimated. Fruit numbers were counted for each cultivar.

For the external fruit parameters, 10 fruit were harvested, and fruit size (diameter and height), peel thickness, and seed numbers were determined. The rootstock was also tested with SSR markers to verify identity as described in Bisi et al. (2020).

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Fruit processing for quality analysis. The 'Hamlin' tree had very few fruit, so for fruit processing quality tests, fruit from 'Hamlin' trees in a nearby block that had been planted between 2015 and 2017 were used for comparisons. In early Jan 2022, 160 'Hamlin' and 120 'Donaldson' fruit were harvested at commercial maturity stage. Fruit were hand washed (Fruit Cleaner 395; JBT FoodTech, Lakeland, FL, USA), sanitized with 100 ppm peroxyacetic acid (Jet-Oxide, Jet Harvest, Longwood, FL, USA), and hand-juiced with a Vinci Hand Free Juicer (Vinci Housewares, La Mirada, CA, USA). Fruit were juiced in four batches of 30 to 40 fruits to comprise four biological replications.

In Jan 2023 and 2024, ~280 fruits were harvested from the same trees as in 2022 and transported to JBT® FoodTech (Lakeland, FL, USA) to be processed following the industrial procedures. A JBT 391 Extractor with "Standard Industry Setting" was used for juice extraction in 2023. In 2024, a premium setting was used for 'Donaldson' to minimize the oil content after the juice was found to have too much oil in 2023. Juice was passed through a pressure filtration finisher with screen size 0.51 mm and then pasteurized at 90 °C for 10 s (1.2 L per min) and cooled to 10 °C using a pilot pasteurizer (UHT/HTST Laboratory 25EHV Hybrid; Microthermics, Inc., Raleigh, NC, USA). Juices were cold filled into 1.8-L plastic jugs and cooled further to 5 °C using an ice bath and frozen at −20 °C until analysis for chemical or sensorial characteristics (1 to 2 months).

Sub-samples of juice, 50 mL for sugars, acids and limonoid analysis, and 6 mL in 20-mL vials for volatile analysis were taken on each processing day. Sub-samples were taken in four replicates.

Chemical analysis. (1) Soluble solids contents, titratable acidity, and limonoids analysis: Juice samples were centrifuged at 10,000 g_n for 15 min and the soluble solids content (SSC) of the supernatant was measured with a refractometer (Atago RX-5000α, Tokyo, Japan). The titratable acidity (TA) of the juice samples was measured by titrating ~5 g of juice supernatant to pH 8.1 with 0.1 N NaOH using a titrator (Dosino model 800, Metrohm, Herisau, Switzerland). Four replicates were measured, and their averages were reported.

Limonin and nomilin concentration was measured via liquid chromatography tandem mass spectrometry (LC-MS/MS) from centrifuged and filtered juice (Jeffries et al. 2024). A 1290 Infinity II UPLC coupled with a 6470 triple quadrupole MS (Agilent, Santa Clara, CA, USA) was used. The separation column was ZORBAX RRHD Eclipse Plus C18 (2.1 × 50 mm, 1.8 μm, Agilent) with mobile phases consisting of water and 0.1% formic acid (A) and acetonitrile and 0.1% formic acid (B). The MS detector was operated in MRM mode and nomilin was detected with a m/z 515.3 precursor ion and a m/z 411.2 product ion with fragmentor and collision energy voltages set to 135 V and 14 V, respectively. Limonin was detected with a m/z 471.2 precursor ion and an m/z 425.2 product ion with fragmentor and collision energy voltages set to 135 V and 19 V, respectively. Limonoids were identified by comparing the MRM transitions and retention times with those of analytical standards. Quantification of limonoids was performed by integrating the area under the chromatographic peak and calculating the amount of each compound based on standard curves ($R^2 \geq 0.99$ with range 0.006 to 25 $mg \cdot L^{-1}$). MassHunter Quantitative Analysis software (Agilent) was used for data analysis.

(2) Volatile analysis: Volatile profiling was performed using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GCMS) (Bai et al. 2016; Fan et al. 2024). The GC-MS was Agilent 7890 GC equipped with a DB-5 column (60 m × 0.25 mm i.d., 1.00-μm film thickness, J&W Scientific, Folsom, CA, USA) and coupled with a 5975 MS (Agilent Technologies, Palo Alto, CA, USA). The volatile compounds were identified with NIST 14 (http://chemdata.nist.gov) and Adams (2017) spectral libraries. MassHunter Quantitative Analysis software (Agilent) was used for data analysis and peak areas are reported. Four replicates were measured, and their averages were reported. Juice samples along with authentic volatile compound standards were confirmed using a DB-Wax capillary column (60 m × 0.25 mm i.d., 0.5-μm film thickness; J&W Scientific).

Sensory evaluation. In 2022, juice was evaluated by a 10-member trained panel that rated juice as part of a larger project (Fan et al. 2024; Jeffries et al. 2024). Panelists evaluated four samples (35 mL) served in 118-mL cups along with reference standards, which were served as 18-mL samples in 30-mL cups (Solo® Cups Co., Urbana, IL, USA). Panelists rated intensities of orange, mandarin, and grapefruit flavor; sweetness; sourness; bitterness; off flavor; and aftertaste. A linear intensity scale with anchor points from 0 to 15 (none to high) was used and ratings were recorded using the Compusense® software (Compusense Inc., Guelph, ON, Canada).

In 2023 and 2024, 'Donaldson' or 'Hamlin' commercially processed juice was blended with 'Valencia' juice processed following the same procedure. Tests were performed to see whether untrained panelists could differentiate between 'Hamlin' and 'Donaldson' when mixed with 'Valencia' juice at a ratio of 40:60 as is customarily done commercially. A Tetrad difference test was performed (Ishii et al. 2014; Sanderson 2017). Panelists were presented with four sample cups, and they were instructed that the cups were two pairs of the same sample. Panelists were asked to group the paired samples together. If they chose the correct answer (i.e., they could discriminate between the two samples presented as two pairs), they were asked to select which pair they preferred, which was sweeter, and which had more orange flavor and then comment on their selection.

Statistical analysis

JMP Pro (version 17, SAS Institute, Gary, NC, USA) was used for analysis of the chemical data. Mean separation was determined by a

Fig. 1. Tree, fruit, and aerial canopy of ‘Donaldson’ and ‘Hamlin’ trees planted circa 1990 on Swingle rootstock. Data taken in Nov 2023.

paired samples *t* test (P = 5%). Sensory trained panel data were analyzed using SenpaQ version 5.01 (Qi Statistics, Reading, UK). A mixed model was used, with “panelist” as a random variable and where the main effects (panelist or sample) were tested against their interaction, with the two replications included in the error term (Lawless and Heymann 1999). Tetrad results were analyzed with the Compusense® software.

Results and Discussion

Historical document review. Working backward with records from the oldest living tree planted at the USDA’s A.H. Whitmore Foundation Farm (Groveland, FL, USA) between 1987 and 1991, the source of ‘Donaldson’ was identified as coming most recently from a collection of material at the Hiawassee farm (Orlando, FL, USA) as a nucellar open pollenated seedling that was grown from seed to eliminate *Citrus exocortis*, a viroid that causes tree stunting. The Hiwassee farm was closed in the mid-1960s triggering a move to a greenhouse at the Whitmore Foundation Farm, first documented in greenhouse records in 1978. The oldest documentation of ‘Donaldson’ is from 1943 at the Hiwassee farm, which show no plant introduction (PI) number associated with this accession, only the letters T.R.R. are found in the PI column. A search of the PI records confirm that ‘Donaldson’ was never entered into the national quarantine program suggesting that the variety ‘Donaldson’ was first selected and collected from a source in Florida.

The letters T.R.R. are the initials for T. Ralph Robinson who was the technician for Walter Tennyson Swingle, a USDA botanist and breeder who contributed greatly to the classification and introduction of citrus into North America. Although Robinson retired from the USDA in 1940, he continued Swingle’s work after he retired in 1941 as a

Table 1. Tree growth traits taken for ‘Donaldson’ and ‘Hamlin’ single trees in 2023 at the US Department of Agriculture’s A.H. Whitmore Foundation Farm, Groveland, FL, USA.

Tree	PAR	LAI	GFP	Fruit no.	Tree height (m)	Canopy diam (m)	Canopy volume (m^3)	Rootstock diam (cm)	Scion diam (cm)
Hamlin	34	5.20	1.21	98	3.96	5.03	1845.1	33.05	16.35
Donaldson	113	2.73	0.75	557	4.05	5.32	2046.7	35.40	20.20

PAR = photosynthetically active radiation; LAI = leaf area index; GFP = gap fraction profile.

Table 2. Average fruit characteristics from a 10-fruit sample for single ‘Donaldson’ and ‘Hamlin’ trees in 2023 at the A.H. Whitmore Foundation Farm Groveland, FL, USA.

Cultivar	Fruit diam (cm)	Fruit ht (cm)	Peel thickness (cm)	No. of seeds	Outside color (RHS color chart fifth edition)	Inside color	SSC (°Brix)
Hamlin	6.11 b[i]	6.15 b	0.29	6.5 b	Yellow-Green Group 153 D, Yellow-Green Group 153 C, Yellow Group 12 A	Yellow Group 13 B, Yellow Group 13 C, Yellow Group 12 B	8.08 b
Donaldson	7.22 a	6.87 a	0.31	11.7 a	Yellow-Green Group 153 C, Yellow Group 13 A, Yellow Group 12 A, Yellow Group 17 B, Yellow-Green Group 153 D	Yellow Group 15 B, Yellow Group 17 C, Yellow Group 15 C	10.02 a
n	10	10	10	10			4

[i] Values that are not followed by the same letter in the same column show significant difference at the 0.05 level using paired samples *t* test.
RHS = Royal Horticultural Society; SSC = soluble solids content.

Table 3. Juice taste related chemical attributes of ‘Donaldson’ and ‘Hamlin’ oranges harvested in the 2021–22, 2022–23, and 2023–24 seasons (n = 4).

	SSC (%)		TA (%)		SSC/TA		Limonin (μg/mL)		Nomilin (μg/mL)	
Harvest date	Donaldson	Hamlin	Donaldson	Hamlin	Donaldson	Hamlin	Donaldson	Hamlin	Donaldson	Hamlin
4 Jan 2022	10.11 a[i]	9.68 a	0.76 a	0.35 b	13.29 b	18.10 a	5.03 a	2.83 b	0.32 a	0.08 b
11 Jan 2023	10.10 a	11.16 a	0.64 a	0.43 b	15.92 b	26.24 a	5.15 a	5.22 a	0.35 a	0.25 a
10 Jan 2024	10.53 a	10.64 a	0.64 a	0.54 a	16.73 a	19.77 a	4.91 b	6.32 a	0.18 b	0.37 a

[i] Means followed by a different letter within a row are statistically different with the least significant difference test at $P < 0.05$ level.
SSC = soluble solids content; TA = titratable acidity.

volunteer until his death in 1967. The records indicate that from 1941 until the mid-1960s, Robinson had several populations of plants he was observing for use by the citrus industry that were accumulated from Swingle’s collection travels domestically and abroad. ‘Donaldson’, along with other citrus accessions, were evaluated on a 1 to 3 scale, based on the perceived breeding value of material, as the Hiawassee farm was scheduled to close and move the Whitmore Foundation Farm as an attempt to evaluate and downsize the accessions being moved. ‘Donaldson’ was ranked a “2,” which indicated it was of limited importance at the time of evaluation. Before 1943, there are no records that indicate the exact origin of ‘Donaldson’, where it came from, or why it was collected, which add to the mystic of this re-discovered cultivar. In published literature ‘Donaldson’ was listed as a sweet orange, but no additional information was found (Cottin et al. 1997).

Field data collection. ‘Donaldson’ was observed as a healthy individual with few HLB symptoms mostly present on lower leaves. Budwood was sent to the Florida Division of Plant Industry for shoot tip clean up and pathogen testing and confirming the presence of *C*Las, the pathogen that causes HLB, and a mild strain of *Citrus tristeza virus*. Comparisons made between ‘Donaldson’ and ‘Hamlin 4-4-1’ show differences in tree health and canopy density, while similarities between the fruit appearance and color were apparent (Fig. 1). The quantitative measures of tree health and growth showed that ‘Donaldson’ was a larger healthier tree with a thicker canopy and trunk diameter and larger quantity of fruit (Table 1). Statistics could not be conducted on the single trees and data are simply presented to quantify the visual difference between the accessions.

Fruit size, peel thickness, and seeds. The 10 fruit that were field measured and juiced show that ‘Donaldson’ fruit were statistically larger than ‘Hamlin’ with higher SSC and had more seeds per fruit than ‘Hamlin’. No

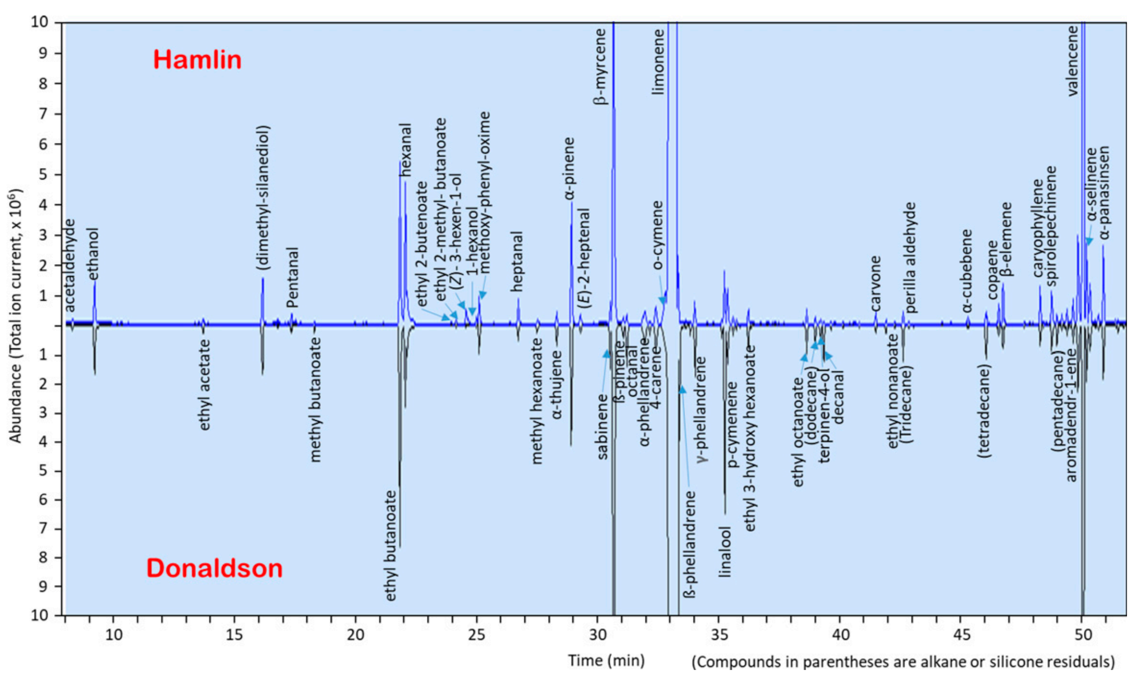

Fig. 2. Side-by-side chromatograms of headspace volatiles of juice samples extracted from ‘Hamlin’ (top) and ‘Donaldson’ (bottom) oranges. Compound names are labeled in the greater peak size side. Compound names in brackets indicate non-aromatic alkanes and silicone residuals.

Table 4. Comparison of the abundances (total ion current × 10^7) of volatiles in juices extracted from 'Donaldson' and 'Hamlin' over three harvest seasons.

		2022[i]		2023[i]		2024[i]	
Volatile compound	Class	Donaldson	Hamlin	Donaldson	Hamlin	Donaldson	Hamlin
Methyl acetate	Ester	0.08	0.02	0.04	0.00		
Ethyl acetate	Ester	0.98 a[ii]	0.48 b	1.14 a	0.52 b	0.32	0.24
Pentanal	Aldehyde	1.33	1.45	0.93	1.07	0.04 b	0.26 a
Ethyl propanoate	Ester	0.08	0.04	0.08	0.05		
Methyl butanoate	Ester	2.14 a	0.47 b	0.80 a	0.18 b	0.17	0.12
1-pentanol	Alcohol	0.14	0.20	0.03	0.09		
Ethyl butanoate	Ester	69.24 a	35.47 b	27.65 a	15.49 b	4.19	3.32
Hexanal	Aldehyde	16.65	24.30	8.31 b	16.04 a	3.76 b	11.64 a
Ethyl 2-methylbutanoate	Ester	0.59	0.61	0.22	0.34		
3-hexen-1-ol	Alcohol	0.37 b	1.95 a	0.87	1.69		
(E)-2-hexenal	Aldehyde	0.16 b	1.01 a			0.58 b	2.54 a
1-hexanol	Alcohol	0.73	1.54	0.82	0.55	0.03	0.14
Heptanal	Aldehyde	2.78	3.59	1.79	2.61	0.13 b	0.49 a
Methyl hexanoate	Ester	2.59 a	0.65 b	1.09 a	0.20 b	0.31	0.53
a-thujene	Monoterpene	4.64	3.18	0.00 b	1.06 a	1.91	3.78
α-pinene	Monoterpene	12.95	13.50	9.56	11.70	33.06	31.86
Sabinene	Monoterpene	0.16	0.10	0.00 b	1.97 a	4.42 b	10.67 a
β-myrcene	Monoterpene	109.39	103.78	46.41	45.27	124.90	111.72
Ethyl hexanoate	Ester	26.89 a	14.43 b	18.03 a	7.25 b		
β-pinene	Monoterpene	2.08	1.20	0.90	0.64	1.66	2.21
Octanal	Aldehyde	1.68	0.72	1.54	0.97	18.11 a	2.48 b
α-phellandrene	Monoterpene	1.49	1.39	2.30	2.35	6.33	4.64
δ-2-carene	Monoterpene	5.66	4.26	0.37	0.15	0.12	0.19
p-cymene	Monoterpene	22.50	22.68	9.36	5.85		
Limonene	Monoterpene	676.67	661.25	625.99	521.94	628.25	602.24
Octanol	Alcohol	0.29	0.05	0.40 a	0.00 b	0.50 a	0.08 b
γ-terpinene	Monoterpene	2.76	1.62	1.95	2.07	8.86	10.94
p-mentha-3,8-diene	Monoterpene	0.13	0.13	0.06	0.04	0.13	0.17
α-terpinolene	Monoterpene	17.02 a	7.47 b	7.43	5.16		
Linalool	Terpene alcohol	11.56 a	0.59 b	12.43 a	0.00 b	27.87	14.83
Nonanal	Aldehyde	0.37	0.31	0.65	0.72	3.81 a	0.00 b
Ethyl 3-hydroxyhexanoate	Ester	4.05 a	1.91 b	2.35	1.19	0.53	0.44
Allo-ocimene	Monoterpene	0.18	0.11			0.22	0.28
Ethyl octanoate	Ester	5.97	3.34	4.16 a	1.53 b	0.35	0.56
Terpinen-4-ol	Terpene alcohol	0.10	0.04	1.34 a	0.35 b	3.72	4.60
Decanal	Aldehyde	1.45 a	0.25 b	0.23	0.25	12.21 a	3.07 b
α-terpineol	Terpene alcohol	0.44 a	0.14 b	0.42 a	0.00 b	1.03	0.45
(*E*)-carveol	Terpene alcohol	0.04	0.03			0.00	0.28
Carvone	Terpene ketone	2.03	2.13	1.00	0.93	0.72 b	3.11 a
α-cubebene	Sesquiterpene	0.15 b	0.99 a	0.06 b	0.80 a	0.62 b	1.48 a
α-copaene	Sesquiterpene	0.53 b	2.62 a	0.48 b	2.56 a	2.40	2.32
β-elemene	Sesquiterpene	0.84	1.43	3.10	4.66	1.66	2.42
(*E*)-caryophyllene	Sesquiterpene	0.98	2.00	2.13	3.51	1.52	2.54
α-humulene	Sesquiterpene	0.26	0.54	0.47	0.75	0.31	0.56
valencene	Sesquiterpene	71.86	89.70	95.34	92.70	35.42	42.56
α-selinene	Sesquiterpene	3.37	4.95	6.94	7.05	2.06	1.89
δ-Cadinene	Sesquiterpene	1.87 b	4.20 a	4.80	4.49	1.99	2.55
Total		1088.25	1022.82	904.01	766.75	934.22	884.19
Esters		112.61 a	57.42 b	55.57 a	26.75 b	5.86	5.22
Monoterpenes/monoterpenoids		869.71	823.58	718.18	599.15	839.49	797.38
Sesquiterpenes		79.87 b	106.41 a	113.33	116.51	45.98	56.29

[i] Fruits were hand-juiced in 2022, processed using a JBT 391 extractor under "standard industry setting" in 2023 and under "premium setting" in 2024.
[ii] Values followed by a different letter within the same row and year show significant differences at the 0.05 level using paired samples *t* test (n = 4).

difference was observed for peel and segment thickness and internal and external color ratings were very similar (Table 2, Fig. 1). Descriptive color classifications were assessed with the Royal Society of Horticulture color chart and descriptions of internal and external color are provided in (Table 2).

Fruit internal quality. There was no significant difference in SSC between 'Hamlin' and 'Donaldson' in any of the years harvested (Table 3). However, 'Hamlin' had a much lower TA in 2022 and 2023, resulting in higher SSC/TA ratio. 'Hamlin' fruits were harvested from a rootstock trial in which trees were much younger than 'Donaldson' and had not shown any symptom of HLB yet. There were no significant differences in SSC or TA between 'Hamlin' and 'Donaldson' in 2024. The limonoid content was lower in 'Hamlin' fruit harvested from young trees in 2022, whereas there was no difference between 'Hamlin' and 'Donaldson' in 2023. In 2024, 'Hamlin' limonoids were greater than in 'Donaldson' fruit, suggesting that by that time, trees had become infected with *C*Las and likely showed greater limonoid concentration (Table 3).

Volatile profiles. A total of 47 volatiles were detected, including 10 esters, seven aldehydes, four alcohols, 13 monoterpenes, eight sesquiterpenes, four terpene alcohols and one terpene ketone (Fig. 2, Table 4). Esters were in greater abundance in 'Donaldson' than in 'Hamlin' in hand-squeezed juice (2022) and juice processed with the standard settings with the JBT extractor (2023). The greater abundance of esters is significant in terms of flavor aromatics, as esters have been shown to contribute to orange flavor (Fan et al. 2024; Wang et al. 2020). In particular, ethyl butanoate has long been recognized to be an important contributor of fruitiness in orange juice, having a very low odor threshold (Perez-Cacho and Rouseff 2008b; Plotto et al. 2008). Other esters also contribute to fruity flavor in orange juice, including ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, and ethyl hexanoate. The aldehydes octanal,

Table 5. Sensory evaluation of 'Hamlin' and 'Donaldson' juice using a trained panel. Ratings on a 0 to 15 linear scale.

	Orange	Tangerine	Grapefruit	Sweet	Sour	Bitter	Off flavor	Aftertaste
Donaldson	5.2 a[i]	2.5 a	1.8 a	5.2 a	5.8 a	2.1 a	2.4 a	1.4 a
Hamlin	6.2 a	2.7 a	0.7 b	5.9 a	4.4 b	0.6 b	0.9 a	1.0 a

[i]Means followed by a different letter within a column are statistically different with the Fisher's least significant difference at $P < 0.05$ level.
Fruit harvested on 4 Jan 2022.

nonanal, and decanal with characteristic citrus aroma (Perez-Cacho and Rouseff 2008b; Plotto et al. 2008) were in greater abundance in the 'Donaldson' juice processed in 2024 with premium settings than in 'Hamlin' processed with standard settings in 2023. Octanol, α-terpinolene, linalool, terpinene-4-ol, and α-terpineol were also in greater abundance in 'Donaldson' juice than in 'Hamlin', in 1 or 2 of the 3 years of the study (Table 4). Those alcohols, monoterpenes, or terpene alcohols contribute in various degree to orange flavor. On the other hand, the C-6/C7 aldehydes or alcohol, hexanal, 3-hexen-1-ol, €-2-hexenal, and heptanal were in greater abundance in 'Hamlin' juice in some of the 3 years of the study. Those aldehydes provide "green" fresh notes when present in concentration greater than their thresholds. Monoterpenes are the primary contributors to the aromatic profile of citrus fruits, including oranges. They are responsible for the fresh, vibrant citrus notes that are characteristically associated with orange juice (Bai et al. 2016; Perez-Cacho and Rouseff 2008b; Wang et al. 2020). The most abundant monoterpenes include limonene, which provides the classic citrus character, myrcene, α-pinene, and β-pinene, and their levels in orange juice depend on processing methods. Myrcene has been reported to have a negative contribution, earthy and metallic, if in too high concentration, whereas β-pinene contributes to a fresh piney odor to orange juice (Ahmed et al. 1978b). α-Pinene adds a hint of piney and resinous tones and contributes to the freshness of the aroma (Bai et al. 2016; Perez-Cacho and Rouseff 2008b; Wang et al. 2020). There were no significant differences between 'Hamlin' and 'Donaldson' in the monoterpenes, except for α-thujene and sabinene present in greater abundance in 'Hamlin' and α-terpinolene greater in 'Donaldson' in 2024 (Table 4). Sesquiterpenes are generally less volatile and present in smaller amounts in orange juice compared with monoterpenes, but they still play a role in adding depth to the fruit aroma (Bai et al. 2016). The most important sesquiterpene is valencene, which contributes a rich, sweet, and somewhat woody note to the orange flavor (Wang et al. 2020). There were no significant differences between 'Hamlin' and 'Donaldson' in valencene concentration; however, α-cubebene and α-copaene were in greater abundance in 'Hamlin' than in 'Donaldson'. The olfactory significance of these differences is likely minimal, as sesquiterpenes tend to have high odor thresholds. Overall, 'Hamlin' and 'Donaldson' had similar volatile profiles, and both showed typical orange flavor dominated by monoterpenes and esters. 'Donaldson' had significantly greater abundance of esters when hand-squeezed (2022) or extracted with the JBT extractor with standard settings (2023). This greater abundance of esters is relevant in the era of HLB where the disease affects juice quality including a decrease in esters (Dala-Paula et al. 2018).

Sensory evaluation. In 2022, a trained panel evaluated 'Hamlin' and 'Donaldson' that had been hand-juiced. There were no differences between 'Hamlin' and 'Donaldson' for sweetness or orange and mandarin flavor (Table 5). However, grapefruit flavor, sourness, bitterness, off flavor, and aftertaste were rated higher in 'Donaldson' than in 'Hamlin', explained by higher TA and limonoid content in 2022 (Table 3).

In 2023 and 2024, panels were conducted to see whether untrained consumers could perceive a difference if 'Hamlin' was to be replaced by 'Donaldson' in a commercially processed juice. The juice was prepared as standard commercial practices, in a 60:40 mix of 'Valencia' (60% juice), with either 'Hamlin' or 'Donaldson' (40% juice). In both years, panelists could detect the difference between juices prepared with either 'Donaldson' or 'Hamlin' (Table 6). In 2023, panelists who could detect the differences preferred the juice made with 'Hamlin' rather than with 'Donaldson' and generally found it sweeter (data not shown). However, those panelists found orange flavor to be very similar in both juices. Interestingly, the amount of peel oil in the 'Donaldson' juice was 0.033%, greater than in 'Hamlin' juice (0.022%), but those differences were not perceived in the blend with 'Valencia' juice.

In 2024, differences between the two juices were less striking (only 50% of panelists could detect the differences, Table 6), which could only be explained by a slightly greater but not significant SSC/TA ratio in 'Hamlin' and lower limonoids in 'Donaldson' (Table 3).

Overall, 'Donaldson' could replace 'Hamlin' in processed orange juice, providing the proper blending with other cultivars.

Table 6. Sensory comparison of 'Donaldson' and 'Hamlin' juice in a simulated commercial mix with 60% 'Valencia' juice.

Year	N	Correct	Incorrect	d'	*P* value	Significant
2023	38	24	14	1.5	0.00	YES
2024	36	18	18	1.0	0.03	YES

Conclusion

The rediscovery of the 'Donaldson' sweet orange offers a promising alternative for the Florida citrus industry, particularly in the context of the ongoing HLB crisis. This study demonstrated that the 'Donaldson' tree, despite being infected with the HLB pathogen, exhibited remarkable health and resilience compared with the declining 'Hamlin' trees, suggesting a potential tolerance to the disease. The 'Donaldson' orange not only produced larger fruit with desirable juice qualities but also showed a favorable volatile profile, particularly in its ester content, which is crucial for enhancing orange juice flavor. Sensory evaluations indicated that although 'Donaldson' juice has a slightly different taste profile compared with 'Hamlin', it still possesses the key attributes needed for commercial orange juice production. The ability of consumers to detect subtle differences between 'Donaldson' and 'Hamlin' in juice blends underscores its potential as a viable replacement in the market. Given its observed tolerance to HLB and positive juice characteristics, 'Donaldson' could play a crucial role in sustaining the Florida orange juice industry, although further research is needed to fully validate its long-term potential and optimize its use in commercial applications. Field screening of plantings of 'Donaldson' in multiple locations should be performed to ensure that the observed tolerance is robust under a diversity of conditions, particularly as this is a long-lived perennial tree.

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