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Fruit Size as an Indicator of Fruit Drop in Huanglongbing-affected 'Valencia'

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Abstract. **Increased preharvest fruit drop rates and reduced fruit sizes are common symptoms associated with Huanglongbing (HLB) in sweet orange. Small fruit may be more likely to drop during preharvest fruit drop. The objective of the current study was to determine whether fruit size could be used as an indicator of the preharvest fruit drop likelihood. Nearly 1400 fruit were surveyed over the following three timepoints across two years: 11 Mar 2022, 15 Apr 2022, and 22 Mar 2023. Each fruit was evaluated to determine the equator and peduncle diameter, fruit detachment force (FDF), type of detachment (mechanically broken or abscission), and lopsidedness. The total soluble solids content was determined for a subset of fruit. The FDF was consistently lower in abscising fruit than in mechanically broken fruit, suggesting that the physiological process of abscission had begun in these fruit and that they were more likely to drop. The fruit diameter was significantly smaller in abscising fruit than in mechanically broken fruit on 11 Mar 2022 and 22 Mar 2023, but not on 15 Apr 2022. Similarly, the fruit diameter and FDF were also significantly and positively correlated, but this relationship was weaker at time points late in the season (closer to harvest). These findings suggest that small fruit are indeed more likely to drop early in the preharvest period. The abscising fruit had total soluble solids contents similar to those of the mechanically broken fruit. Therefore, we hypothesized that the smaller fruit ceased growing and are able to respond to abscission signals earlier than the larger fruit. Therefore, any strategies to mitigate fruit drop, such as the use of plant growth regulators, should be applied early in the season when the fruit are still actively growing. When growth ceases, the fruit are vulnerable to drop.**

Currently, Huanglongbing (HLB), or citrus greening, is the most widespread and devastating citrus disease. This disease is associated with infection by the phloem-limited bacteria '*Candidatus* liberibacter asiaticus' (*C*Las) and transmitted by its vector the Asian citrus psyllid (*Diaphoriana citri*) (Ammar et al. 2020; Bové 2006; Inoue et al. 2009). Since its arrival in 2005, HLB has spread throughout all of Florida's citrus production areas (Graham et al. 2020). As a result, the Florida citrus industry has observed a continuous decline in production over the past 15 years (US Department of Agriculture, National Agricultural Services 2022). This decline is largely caused by the reduced productivity and slow decline of HLB-affected trees (Bové 2006).

This reduced productivity translates into lower yields at harvest in HLB-affected trees (Bassanezi et al. 2011; Graham et al. 2020; US Department of Agriculture, National Agricultural Services 2022). Although multiple HLB-related symptoms (e.g., canopy dieback, increased rates of preharvest fruit drop, poor fruit quality, etc.) contribute to a reduction in marketable yield (Albrigo and Stover 2015; Bassanezi et al. 2009; Levy et al. 2023; Tang et al. 2019, 2020), growers are concerned about the increased rates of preharvest fruit rates. The loss of mature fruit in the months leading up to commercial harvest represents a significant visual loss of their revenue. While altered cultural practices, such as altered irrigation schedules (Kadyampakeni and Morgan 2017), nutrient applications (Atta et al. 2023), and PGR applications (Albrigo and Stover 2015; Singh et al. 2022), can cause a general increase in the health of HLB-affected trees (Levy et al. 2023), the effects on preharvest fruit drop are variable.

Preharvest fruit drop begins approximately 3 months before commercial harvest and describes any mature fruit abscission during this time. In citrus, mature fruit abscise at abscission zone C (AZ-C), which is the junction between the calyx and the fruit (Iglesias et al. 2007). Although this preharvest fruit drop is a normal physiological process and some degree of fruit drop can be expected even in healthy trees, it is greatly exacerbated in HLB-affected citrus, and drop rates continue to increase as HLB severity worsens (Albrigo and Stover 2015; Tang et al. 2019, 2020). Unfortunately, why this drop is so much worse with HLB remains unclear. This study aimed to increase the understanding of preharvest fruit drop.

It is well-documented that HLB-affected trees have smaller fruit at harvest (Baldwin et al. 2018; Bassanezi et al. 2009; Liao and Burns 2012; Rosales and Burns 2011) compared with fruit from the pre-HLB era (Spann and Oswalt 2008). This reduction in fruit size is more pronounced in slow-developing cultivars, such as Valencia (Bassanezi et al. 2009). Within HLB-affected trees, fruit sizes continue to decline with worsening HLB severity (Tang et al. 2019, 2020). Recently, fruit dropped during preharvest fruit drop were found to be smaller than those retained on the tree (Tang et al. 2019, 2020). Therefore, the objective of the current study was to determine if the smaller fruit on the tree were more susceptible to preharvest fruit drop, and whether fruit size could be used as an indicator of drop. The secondary objective was to determine if other fruit characteristics, particularly those associated with HLB symptoms (e.g., lopsidedness), were also associated with the drop likelihood. Therefore, a large-scale survey consisting of nearly 1400 fruit over three time points was conducted. Several fruit characteristic parameters were compared between mechanically broken fruit and abscising fruit to identify which fruit characteristics are indicative of fruit drop during the preharvest period.

Materials and Methods

Plant materials, cultural practices, and experimental design. 'Valencia' sweet orange trees were used for this study. Fifteen-year-old trees on 'Swingle' rootstock located in Lake Alfred, FL, USA, were used in year 1 of this study (2022). Twenty-year-old 'Valencia' trees on 'Swingle' rootstock located in Fort Meade, FL, USA, were used in year 2 of this study (2023). These orchards were maintained by the respective grove crews following the recommendations of the University of Florida/Institute of Food and Agricultural Sciences. Trees that displayed moderate HLB symptoms were selected using the canopy density method described by Singh et al. (2022). Trees with canopies that intercepted more than 85% of photosynthetically active radiation were selected. In both years, fruit were treated as individual replicates with the tree serving as a blocking factor. In year 1, 100% of the fruit were removed from four trees on 11 Mar 2022 ($n = 648$) and 15 Apr 2022 ($n = 248$). In year 2, 75% to 80% fruit were removed from four trees on 22 Mar 2023 ($n = 511$).

Sample collection and fruit physiological measurements. At all timepoints, fruit still attached to the tree were clipped ∼10 to 15 cm above the calyx and transported to Lake Alfred, FL, USA, for analyses. All measurements were completed the same day as that of sample collection.

The fruit detachment force (FDF) was measured using a digital force gauge (Force

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One; Wagner Instruments, Greenwich, CT, USA) to determine the force needed to remove the fruit from the stem (Malladi and Burns 2008; Tang and Vashisth 2020; Tang et al. 2019). Individual fruit were secured to the gauge, and the stem was pulled straight down and away from the fruit. To obtain accurate FDF measurements, the attached stem must be straight (Fig. 1A) because the angle between the fruit and stem can affect the detachment force (Hield et al. 1967). Therefore, FDF measurements could only be performed for the subset of fruit with straight stems (year 1, 11 Mar 2022: n = 227; year 1, 15 Apr 2022: n = 190; year 2, 22 Mar 2023: n = 484).

Regardless of whether FDF could be measured, all fruit were removed from the stem and the detachment type was visually determined. Fruit that did not cleanly separate and broke above the calyx, along the stem, or caused the peel to rip were considered a mechanical break or not abscised (Fig. 1B) (Chen et al. 2016). Fruit that cleanly separated at AZ-C were considered abscised (Fig. 1C).

The diameters of the fruit and peduncles were obtained using digital calipers. The fruit diameter was measured at the widest part along the fruit equator. The peduncle diameter was measured equidistant between abscission zone A and AZ-C (Fig. 1A). In year 2, individual fruit weight was also obtained using a digital gram scale (Atago Co., Ltd., Tokyo, Japan) to confirm that the fruit diameter was representative of the fruit size.

Each fruit was visually scored as lopsided or nonlopsided (Fig. 1D and 1E, respectively). In year 2, a more rigorous scoring system was used in addition to the visual scoring system. Each fruit was bisected so that the lopsided and normal halves were visible in each cross-section (Fig. 1F). Photographs of each fruit were obtained and analyzed using ImageJ (Schneider et al. 2012). Fruit were assigned a score of 0 to 10 based on the angle of the columella and surface area of the two sides of the fruit. Zero to five points were assigned based on the deviation of the columella angle from 180° (Table 1, Fig. 1F). Another zero to five points were assigned based on the juice segment surface area ratio of the larger half to the smaller half (Table 1, Fig. 1F). An asymptomatic fruit with a straight columella (180°) and symmetrical shape (surface area ratio = 1) received a score of zero. The fruit depicted in Fig. 1F, which is a severely symptomatic fruit with a large columella angle deviation and highly asymmetrical shape, received a score of nine.

Juice was extracted from a subset of fruit at each timepoint, and the soluble solids content was determined using a citrus pocket Brix acidity meter (Atago Co., Ltd., Tokyo, Japan).

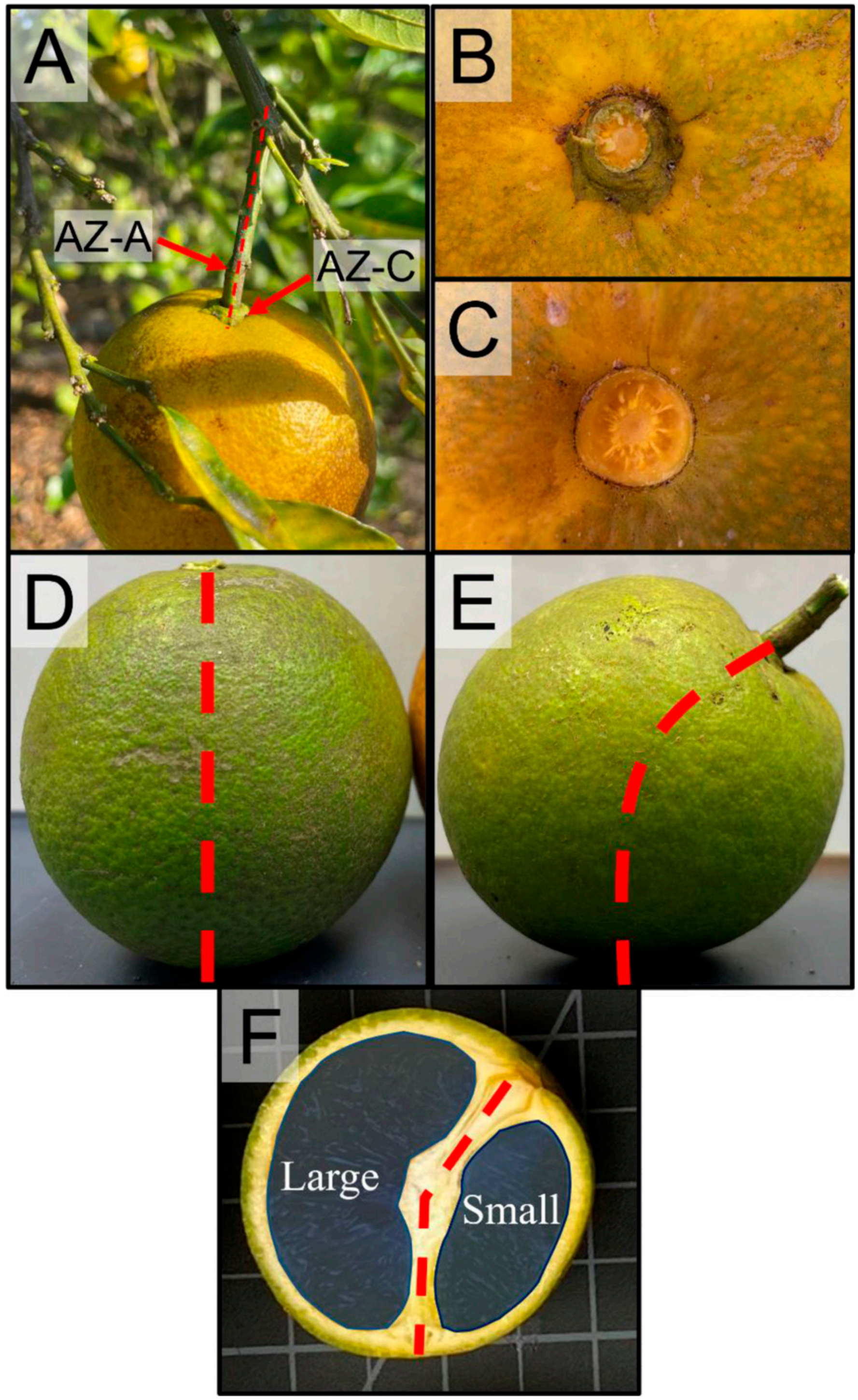

Fig. 1. Visual criteria used during sample collection to identify external (detachment type and fruit shape) and internal (columella deformation and juice segment surface area) characteristics of fruit of mature Huanglongbing (HLB)-affected ‘Valencia’ fruit. (**A**) A fruit used for the fruit detachment force (FDF) measurements. The red dashed line indicates the straight stem needed for FDF. The two red arrows indicate abscission zones A and C. (**B**) Example of a mechanical break detachment type. (**C**) Example of an abscised detachment type. (**D**) Nonlopsided fruit. (**E**) Lopsided fruit with visible deformation. (**F**) A severely symptomatic fruit. The red dashed line indicates the angle measured as the columella angle. The blue regions depict the surface area of the juice vesicles. The ratio of the surface area of the large half to the small half was calculated.

Scanning electron microscopy. The abscission zones of abscised and mechanically broken fruit were visualized using scanning electron microscopy (SEM) to confirm that the macroscopic differences observed were reflective of differences at the microscopic level. After FDF measurements were performed, the abscission zone and surrounding tissue of a subset of fruit were excised and prepared for SEM. Excess peel was removed from the fruit side of the abscission zone, and peduncles were trimmed to just above the calyx to image both sides of the abscission zone. Samples excised from the same fruit were paired in a vial containing 4% paraformaldehyde in 1× phosphate-buffered saline (PBS) and stored at 4 °C for up to 1 week. Samples were rinsed three times in 1× PBS.

Then, they were dehydrated by holding them for 15 min at each step of an increasing ethanol series (25%, 50%, 75%, 85%, 95%, 100%). Samples were stored overnight in fresh 100% ethanol at 4 °C. The 100% ethanol solution was changed again the next day, and the samples were allowed to warm to room temperature. Samples were transferred to baskets submerged in 100% ethanol and dried in a Ladd 28000 critical point dryer (Ladd Research Industries, Williston, Vt, USA). Once dried, samples were mounted on a 12-mm carbon sticker placed on a SEM stub (Electron Microscopy Sciences, Hatfield, PA, USA) so that the site of separation (abscission or mechanical break) was facing up. Then, the samples were sputter-coated with a gold/palladium mix using a Ladd 20802 sputter coater (Ladd Research Industries). Samples were imaged using a Hitachi S4000 scanning electron microscope (Hitachi High Technologies, Tokyo, Japan).

Statistical Analysis

All statistical analyses were performed using R (version 4.1.0; R Core Team; Vienna Austria) (R Core Team 2021). Two-way blocked analyses of variance using the tree as a blocking factor were performed to determine differences between the two detachment types. Pearson's correlation coefficients were calculated to identify significant relationships among the physiological measurements obtained. Chi-squared tests were performed to determine if there was a relationship between fruit shape and detachment type.

An α-value of 0.1 was used for all analyses because of the high degree of HLB symptom variability seen both within and across affected trees (Nehela and Killiny 2020).

Results

Overview and year comparison. Approximately one-third of the fruit had experienced partial to full separation at AZ-C (abscission) at both year 1 sampling dates, whereas one-half of the fruit at the year 2 timepoint displayed partial to full separation at AZ-C (Table 2). Many of the measured variables differed from year to year (Supplemental Fig. 1) as well as from tree to tree, as demonstrated by varying fruit diameters in Supplemental Table 1. The FDFs were, on average, higher at the year 1, 11 Mar timepoint compared with the other two timepoints (Supplemental Fig. 1A). The average fruit diameter and total soluble sugars content (°Brix) were also higher, on average, at the year 1, 11 Mar timepoint than at the other two timepoints (Supplemental Fig. 1B and D). The peduncle diameter was larger, on average, at the year 2, 22 Mar timepoint than at the other two timepoints (Supplemental Fig. 1C). These findings suggest that the year 2, 22 Mar timepoint is more similar to the year 1, 15 Apr timepoint than to the year 1, 11 Mar timepoint.

Detachment type. When imaged using SEM, differences were observed between the abscission zones of fruit likely to abscise and those that were mechanically broken. Fruit that were visually classified as mechanically broken were characterized by the contour and jagged tearing of tissue observed along the abscission zones on both the peel and peduncle sides of the samples (Fig. 2A and B). In contrast, fruit deemed likely to abscise had abscission zones characterized by less tearing of tissue (Fig. 2C and D).

Detachment type and FDF. At all three timepoints, the FDF was significantly lower in the fruit likely to abscise compared with that of the mechanically broken fruit (Fig. 3). In the abscised fruit, at all three timepoints, the average FDF was lower than 6 kgf, suggesting that the fruit were indeed likely to drop (Malladi and Burns 2008; Tang et al. 2019).

Fruit size. Fruit diameters were smaller on average in fruit likely to abscise compared with those of the mechanically broken fruit at the year 1, 11 Mar and year 2, 22 Mar timepoints (Fig. 4A and C). No difference in

Table 1. Lopsidedness scoring of internal fruit characteristics. Point assignment was based on the columella angle deviation from 180° and juice segment surface area ratio. Higher points correspond with a larger degree of lopsidedness. A normal fruit would have a straight columella (180°) and symmetrical sides (surface area ratio of 1).

Points	Columella angle deviationx = |measured angle − 180°|	Surface area ratio y = large:small
0	0° ≤ x < 5°	1 ≤ y < 1.1
1	5° ≤ x < 10°	1.1 ≤ y < 1.2
2	10° ≤ x < 20°	1.2 ≤ y < 1.3
3	20° ≤ x < 30°	1.3 ≤ y < 1.4
4	30° ≤ x < 40°	1.4 ≤ y < 1.5
5	40° ≤ x	1.5 ≤ y

Table 2. Overview of detachment type and lopsidedness. Fruit were identified as mechanically broken or abscised detachment types, and as having a lopsided or nonlopsided shape. Percentages refer to the total number of fruit in that year. In each year, fruit were treated as individual replicates and were collected from four trees (blocking factor).

	Yr 1 11 Mar 2022 (n = 640)		Yr 1 15 Apr 2022 (n = 248)		Yr 2 22 Mar 2023 (n = 511)	
	Broken	Abscised	Broken	Abscised	Broken	Abscised
Nonlopsided	9.5%	3.3%	16.9%	9.8%	9.4%	12.3%
Lopsided	55.3%	31.9%	46.4%	27.0%	39.7%	38.6%
Total	64.8%	35.2%	63.3%	36.7%	49.1%	50.9%

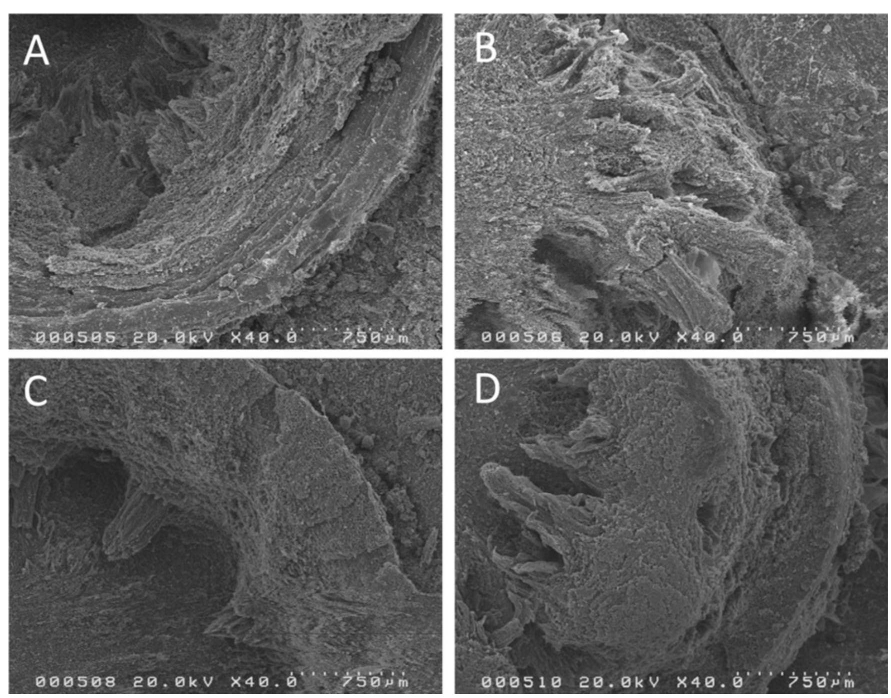

Fig. 2. Scanning electron microscopy (SEM) imaging of the site of separation of a fruit likely to abscise and a mechanically broken fruit. The site of separation from the peel (**A**) and calyx (**B**) sides of a fruit visually deemed mechanically broken during the fruit physiological measurements. The site of separation from the peel (**C**) and calyx (**D**) sides of a fruit visually deemed likely to abscise during the fruit physiological measurements.

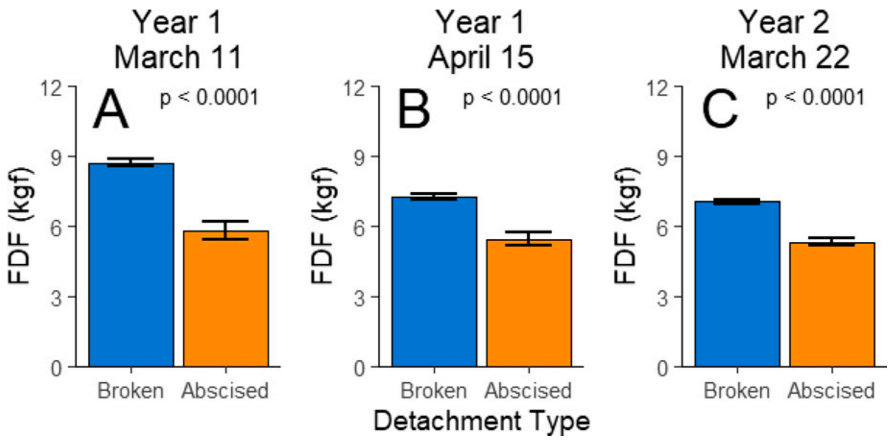

Fig. 3. Average fruit detachment force (FDF) of mechanically broken and abscised Huanglongbing (HLB)-affected 'Valencia' fruit at each timepoint. A two-way analysis of variance (ANOVA) using the tree as a blocking factor was used to calculate *P* values. This analysis was only conducted for the subset of fruit for which FDF measurements were possible. (**A**) 11 Mar 2022 (n = 227). (**B**) 15 Apr 2022 (n = 190). (**C**) 22 Mar 2023 (n = 484). Fruit were pulled from different trees at each timepoint.

average fruit diameter was seen at the later timepoint (year 1, 15 Apr) (Fig. 4B). The average fruit diameter also differed significantly between trees at each timepoint (Supplemental Table 1).

There was a consistent, significant, and positive relationship between fruit diameter and FDF (Fig. 5A,D and G). A moderate correlation existed between fruit diameter and FDF at the year 1, 11 Mar timepoint (R = 0.48) (Fig. 5A). A weak correlation existed between fruit diameter and FDF at the year 2, 22 Mar timepoint (R = 0.24) (Fig. 5G). A very weak correlation existed between fruit diameter and FDF at the later timepoint (year 1, 15 Apr) (R = 0.086) (Fig. 5D).

Upon isolating the mechanically broken fruit, a similar relationship between the fruit diameter and FDF was observed at all three timepoints. A strong correlation was observed at the year 1, 11 Mar timepoint (R = 0.62) (Fig. 5B), whereas a weak correlation was seen at the year 1, 15 Apr (R = 0.36) (Fig. 5E) and year 2, 22 Mar (R = 0.24) (Fig. 5H) timepoints.

Within the fruit likely to abscise, a significant, yet weak, correlation was observed between the fruit diameter and FDF at the year 1, 11 Mar timepoint (R = 0.27) (Fig. 5C) and the year 2, 22 Mar timepoint (R = 0.22) (Fig. 5I). No relationship was found between the fruit diameter and FDF at the later (year 1, 15 Apr) timepoint (Fig. 5F).

In year 2 (22 Mar 2023), the average fruit weight was significantly lower in abscising fruit than in fruit that mechanically broke (*P* = 0.022). Fruit weight and fruit diameter were also strongly correlated (R = 0.96), suggesting that fruit diameter was an appropriate means of measuring fruit size.

Peduncle diameter. The peduncle diameter did not differ significantly between fruit likely to abscise and fruit mechanically broken at any of the three timepoints (Supplemental Fig. 2). However, a significant positive correlation was found between the peduncle diameter and FDF. This correlation was weak at the year 1, 11 Mar timepoint (R = 0.25) (Fig. 6A) and very weak at the year 1, 15 Apr (R = 0.13) (Fig. 6D) and year 2, 22 Mar (R = 0.16) (Fig. 6G) timepoints.

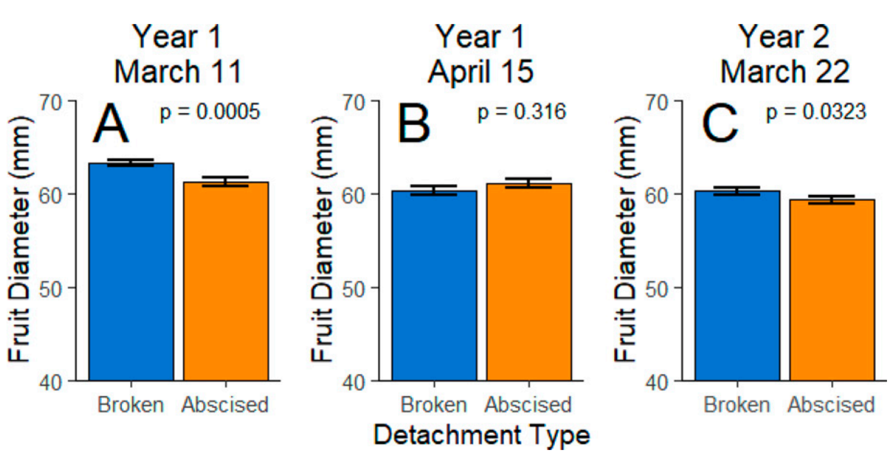

Fig. 4. Average fruit diameter of mechanically broken or abscised Huanglongbing (HLB)-affected 'Valencia' fruit at each timepoint. A two-way analysis of variance (ANOVA) using the tree as a blocking factor was used to calculate *P* values. This analysis was only conducted for the subset of fruit for which fruit detachment force (FDF) measurements were possible. (**A**) 11 Mar 2022 (n = 227). (**B**) 15 Apr 2022 (n = 190). (**C**) 22 Mar 2023 (n = 484). Fruit were pulled from different trees at each timepoint.

Within the mechanically broken fruit, a weak correlation was found between the peduncle diameter and FDF at the year 1, 11 Mar (R = 0.39) (Fig. 6B), year 1, 15 Apr (R = 0.40) (Fig. 6E), and year 2, 22 Mar (R = 0.20) (Fig. 6H) timepoints. Within the fruit likely to abscise, very weak, but significant, correlations were found between the peduncle diameter and FDF at the year 1, 11 Mar (R = 0.19) (Fig. 6C) and year 2, 22 Mar (R = 0.20) (Fig. 6I) timepoints. No relationship was found between the peduncle diameter and FDF in the fruit likely to abscise at the year 1, 15 Apr timepoint (R = −0.04) (Fig. 6F). The peduncle diameter was also positively correlated with fruit size at year 1, 11 Mar (R = 0.6), year 1, 15 Apr (R = 0.58), and year 2, 22 Mar (R = 0.68). However, the ratio of the fruit diameter to the peduncle diameter only differed significantly between mechanically broken and abscising fruit at the year 1, 11 Mar timepoint (*P* = 0.082), but not at the year 1, 15 Apr (*P* = 0.937) and year 2, 22 Mar (*P* = 0.136) timepoints.

Lopsidedness. At all three time points, most fruit surveyed were lopsided (>70%) (Table 2). At the year 1, 11 Mar timepoint, compared with lopsided fruit, normally shaped fruit required a significantly higher FDF to be removed from the stem (Fig. 7A). However, normally shaped and lopsided fruit did not have differing FDFs at the other two timepoints (Fig. 7B and C). At the year 1, 11 Mar timepoint and the year 1, 15 Apr timepoint, lopsided fruit were significantly smaller than normally shaped fruit (*P* = 0.0061 and *P* = 0.0156, respectively). On the contrary, at the year 2, 22 Mar timepoint, no difference in fruit size was found between lopsided and normally shaped fruit (*P* = 0.818). Moreover, the advanced scoring method used for the year 2, 22 Mar timepoint (a higher score indicated a more severe deformation of fruit shape) revealed a significant relationship between fruit shape and detachment type (Fig. 8); mechanically broken fruit tended to be the more severely lopsided fruit (score = 7–9), and the fruit likely to abscise were less lopsided (score 1–3) (Fig. 8), contrary to the trend seen at the year 1, 11 Mar timepoint. Altogether, there was no consistent trend between the fruit shape and abscission likelihood.

Juice quality. The total soluble solids content (°Brix) did not differ between fruit likely to abscise and mechanically broken fruit at any of the three timepoints [year 1, 11 Mar (*P* = 0.316); year 1, 15 Apr (*P* = 0.524); year 2, 22 Mar (*P* = 0.474)]. However, a weak positive correlation did exist between the total soluble solids content and fruit diameter at the year 1, 11 Mar timepoint (R = 0.27; *P* = 0.015) and the year 2, 22 Mar timepoint (R = 0.26; *P* = 0.019), but not at the year 1, 15 Apr timepoint (R = 0.09; *P* = 0.420). The total soluble solids content was similar for lopsided and normally shaped fruit at year 1, 11 Mar (*P* = 0.682), year 1, 15 Apr (*P* = 0.831), and year 2, 22 Mar (*P* = 0.911).

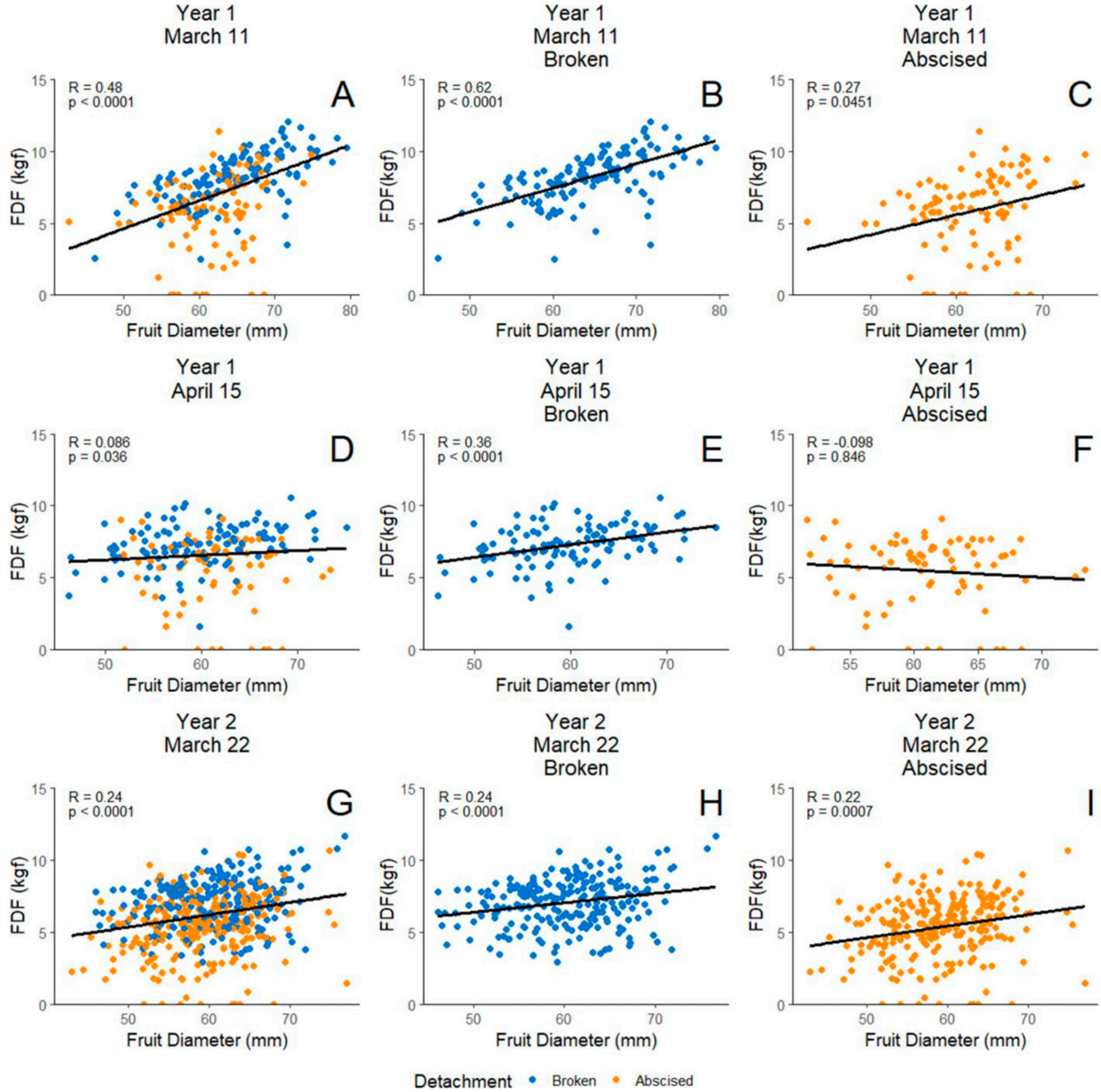

Fig. 5. Comparison of Huanglongbing (HLB)-affected 'Valencia' fruit diameter and fruit detachment force (FDF) at each timepoint and within each detachment type. Within each graph, the black line represents the linear model. Correlation coefficients and *P* values are reported for each comparison. (**A–C**) Comparisons made at the 11 Mar 2022 timepoint. (**D–F**) Comparisons made at the 15 Apr 2022 timepoint. (**G–I**) Comparisons made at the 22 Mar 2023 timepoint. (**A**, **D**, **G**) The overall trend of fruit ignoring the type of detachment. (**B**, **E**, **H**) Trend of the mechanically broken fruit. (**C**, **F**, **I**) Trend of the abscised fruit.

Discussion

A comparison of the three timepoints showed differences in FDF, fruit diameter, peduncle diameter, and total soluble solids content. Although the drop likelihood (FDF) varies naturally on a day-to-day basis because of the time of day, temperature, relative humidity, position in the tree, and stage of maturity (Hield et al. 1967; Kender and Hartmond 1999; Pozo et al. 2007 as reviewed by Dutta et al. 2023), other factors are also likely contributors. Different trees were used at each timepoint. Different locations (~35 km apart) were used in year 1 and year 2, which may have contributed to some of the differences observed between the two years. Hurricane Ian also made landfall as a category 4 (Florida Automated Weather Network 2023; National Hurricane Center 2023) in late Sep 2022. This hurricane resulted in more than $247 million in losses to the statewide citrus industry (Court et al. 2023). Hurricane Ian's path crossed through Central Florida, where the year 2 site is located. The growing fruit on the trees at this time would be the ones collected at the year 2, 22 Mar timepoint. Any mechanical damage sustained from hurricane-force winds likely induced ethylene production (Cooper 1970; Hyodo and Nishino 1981). Ethylene is a known effector of abscission, and ethephon applications have been shown to induce abscission of fruit and leaves (Alferez et al. 2006; Sawicki et al. 2015). Therefore, we can expect that some proportion of the crop load was lost prematurely as a direct result of the hurricane-force winds and subsequent injury-induced ethylene production. Temperatures also dropped below freezing in late Dec 2022 (Florida Automated Weather Network 2023). Freeze damage has also been reported to induce ethylene production and increase mature fruit drop (Lawless 1940; Young and Meredith 1971; Zekri et al. 2019). Therefore, it is possible that the fruit profile was initially altered by hurricane damage, and that fruit drop was further accelerated by the cold weather seen during the early winter. Together, this would contribute to the higher proportion of abscising fruit seen in year 2 compared with year 1, as well as the higher degree of similarity between the year 2, 22 Mar and year 1, 15 Apr timepoints than between the year 2, 22 Mar and year 1, 11 Mar timepoints, despite the year 1, 11 Mar and year 2, 22 Mar timepoints being closer in terms of the calendar date. These comparisons between the two

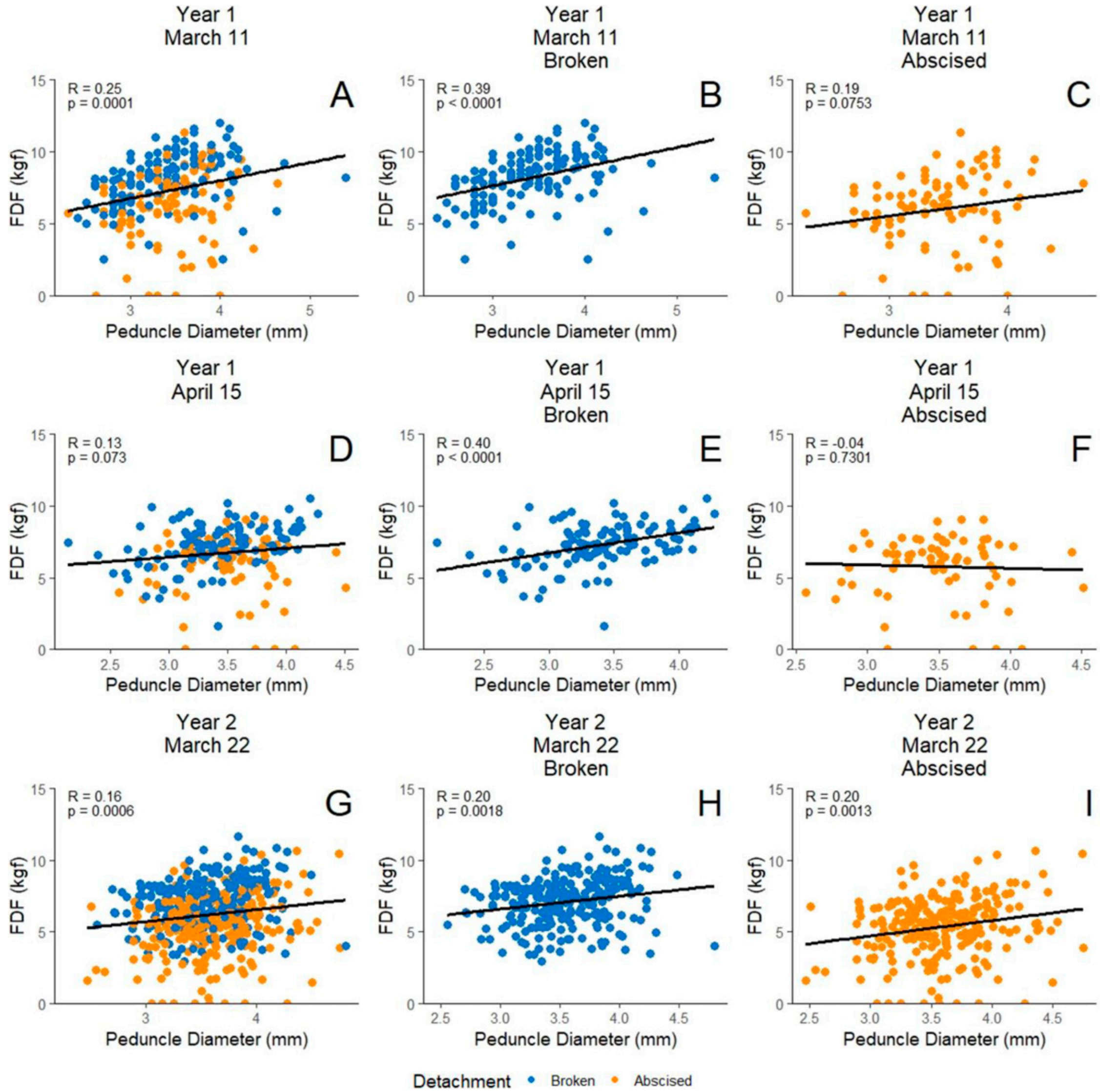

Fig. 6. Comparison of the Huanglongbing (HLB)-affected 'Valencia' peduncle diameter and fruit detachment force (FDF) at each timepoint and within the two detachment types. Within each graph, the black line represents the linear model. Correlation coefficients and *P* values are reported for each comparison. (**A–C**) Comparisons at the 11 Mar 2022 timepoint. (**D–F**) Comparisons made at the 15 Apr 2022 timepoint. (**G–I**) Comparisons at the 22 Mar 2023 timepoint. (**A**, **D**, **G**) The overall trend of fruit ignoring the type of detachment. (**B**, **E**, **H**) Trend of the mechanically broken fruit. (**C**, **F**, **I**) Trend of the abscised fruit.

years also demonstrated that a calendar date-based system is not a reliable method of predicting drop severity.

One of the critical stages of abscission comprises the dissolution of the middle lamella and cell walls within the abscission zone (Merelo et al. 2017; Patterson 2001). The changes associated with this cell breakdown have been shown to be highly correlated with FDF and berry drop in grapes (Deng et al. 2007) and in preharvest fruit drop in 'Temple' oranges (Zur and Goren 1977), suggesting that cell breakdown within the abscission zone corresponds with a weakening of the connection between the fruit and stem. Furthermore, cellulase activity has been linked to cell wall degradation during the abscission process (Merelo et al. 2017; Pandita and Jindal 1991), and FDF has been shown to be negatively correlated with cellulase activity in 'Temple' orange (Zur and Goren 1977). In the present study, fruit with the abscission detachment type consistently had lower average FDF than those with a mechanically broken detachment type. The average FDFs of the abscission and mechanically broken detachment types fell below and above the conventional cutoff of 6 kgf, respectively (Malladi and Burns 2008; Tang et al. 2019). The lower FDF of the abscission detachment type fruit was likely the result of the partial to full breakdown of cells within the abscission zone indicating the fruit would have soon dropped on their own. Likewise, the higher FDF of the mechanically broken fruit suggested that the cells within their abscission zones had not begun to breakdown. If an external force had not been applied, then these fruit would likely not have dropped on their own. Therefore, the mechanically broken fruit are those that are not succumbing to HLB-associated preharvest drop.

The likelihood of this is further supported by our SEM imaging, in which mechanically broken detachment types showed jagged tearing of the tissue in and around the abscission zone, whereas the abscission detachment types showed a smoother separation along the abscission zone. A similar visual distinction between mechanical breakage and abscission has been described for blueberry (Vashisth et al. 2015). The smoother separation in the abscission detachment type compared with the mechanically broken detachment type suggested the separation layer had, at least, begun to dissolve in the abscission detachment type fruit, whereas the separation layer was still fully intact in the mechanically broken fruit. The degree of cell breakdown may also affect FDF, as seen in the reduced strength of the correlation between the FDF and fruit diameter and between the FDF and peduncle diameter of abscising fruit. Although the type of detachment, mechanically broken or abscised, could be determined, the time when the separation layer began to dissolve could not be determined. Nonetheless, a lower FDF could be expected for fruit when the separation layer had fully dissolved compared to when the separation layer was still

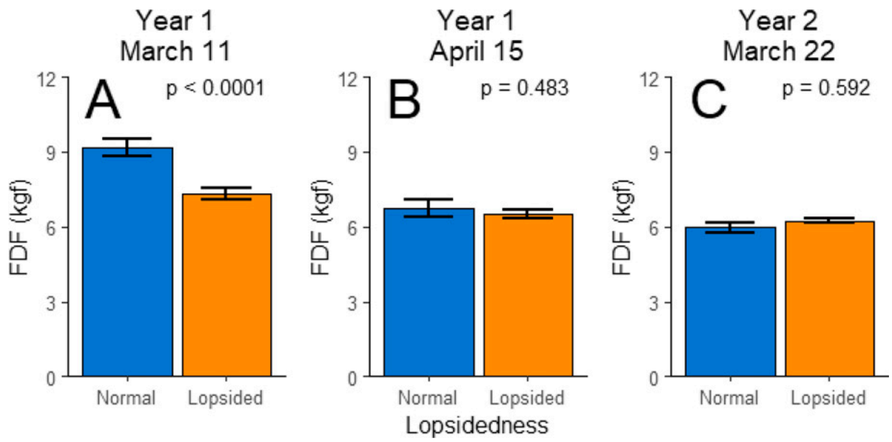

Fig. 7. Average fruit detachment force (FDF) of nonlopsided and lopsided Huanglongbing (HLB)-affected 'Valencia' fruit at each timepoint. A two-way analysis of variance (ANOVA) using the tree as a blocking factor was performed to calculate *P* values. This analysis was only conducted for the subset of fruit for which FDF measurements were possible. (**A**) 11 Mar 2022 (n = 227). (**B**) 15 Apr 2022 (n = 190). (**C**) 22 Mar 2023 (n = 484).

partially intact. Therefore, the generally weak correlations observed for abscised fruit were likely the result of varying degrees of cell wall dissolution and the time since full dissolution of the separation layer.

Regarding healthy citrus, a positive correlation has been historically observed between mature fruit size and FDF (Hield et al. 1967; Kender and Hartmond 1999). A similar relationship between the peduncle diameter and FDF has been reported (Hield et al. 1967). In the present study, similar positive relationships between the fruit diameter and FDF and between the peduncle diameter and FDF were seen at the year 1, 11 Mar timepoint. Although these findings are consistent with historical (Hield et al. 1967; Kender and Hartmond 1999) and current (Tang et al. 2019, 2020) data, the strength of these relationships breakdown as the season progresses, as reflected in the weaker correlations observed at the year 1, 15 Apr and year 2, 22 Mar timepoints. The altered fruit profile caused by the large amount of fruit drop that had already occurred by the late sampling date (15 Apr 2022) and the destructive climatic events in the second year (22 Mar 2023) likely contribute to the weakening of this relationship.

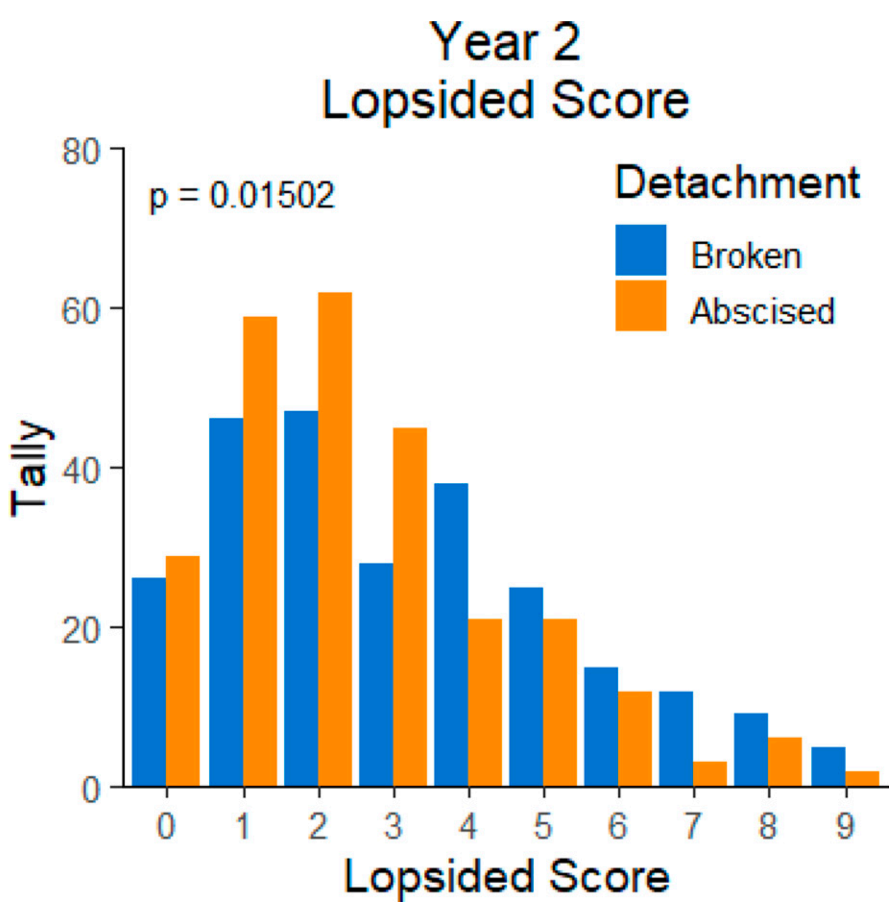

Fig. 8. Tally of the number of Huanglongbing (HLB)-affected 'Valencia' fruit that received each lopsided score. Higher scores correspond to a higher degree of lopsidedness. A chi-square test was performed to determine whether the detachment types had differing score breakdowns.

In healthy citrus trees, fruit maturity can vary within the same tree based on its location within the canopy (Kender and Hartmond 1999). A similar phenomenon has been reported for apple (Ward 2004). Arseneault and Cline (2016) suggested that fruit that with more advanced maturity on the tree may be able to respond to abscission signals sooner than those that are less advanced in maturity, resulting in preharvest drop. In the present study, this relationship between fruit maturity and drop likelihood may have been reflected in the significantly lower average FDF seen at the year 1, 15 Apr timepoint compared with that seen at the year 1, 11 Mar timepoint; as more fruit reach maturity and begin senescing, more fruit respond to abscission signals and drop. Therefore, the more advanced fruit on the tree are likely the ones that would drop during preharvest drop. It has been previously documented that HLB-affected leaves (Neupane et al. 2023) and roots (Johnson et al. 2014; Kumar et al. 2018) have shorter lifespans than those of healthy trees. A similar phenomenon may occur in the fruit of HLB-affected trees. Generally, a shorter fruit lifespan would account for the increased rates of preharvest fruit drop seen as the season progresses. Furthermore, more severely symptomatic fruit (i.e., extremely small and lopsided) (Fig. 1E) may have a shorter life span than their less symptomatic or asymptomatic counterparts, which may lead to more rapid transitions from one developmental stage to the next (e.g., I: cell division; II: rapid cell enlargement; III: maturation). Because HLB symptoms are unevenly distributed throughout the tree (Liao and Burns 2012; Tatineni et al. 2008), this may contribute to fruit of greatly varying maturity on a tree and a prolonged window of preharvest drop as seen in HLB-affected trees. Furthermore, HLB-affected trees experience a prolonged period of flowering and fruit set and often experience off-bloom flowering (Alferez and Vashisth 2020; Dewdney 2015). Thus, this altered flowering and fruit set pattern could be speculated to further contribute to the prolonged window of preharvest drop seen in HLB-affected trees. Nonetheless, in our study, the abscising fruit had maturity similar to that of the mechanically broken fruit, as evidenced by the lack of differences in the total soluble solids content. This suggests that the smaller abscising fruit were not small because they were developmentally delayed and immature; rather, the small fruit may represent the weaker sinks on the tree because the fruit size is indicative of sink strength and growth rate (Marcelis 1996). Therefore, it is possible that smaller fruit sizes resulted from lower fruit growth rates during phase II growth (rapid cell enlargement), which could cause them to cease growing earlier and enter phase III (maturation) first.

Fruit size continues to decrease with increasing HLB severity (Tang et al. 2020), and HLB symptoms can vary within a canopy (Nehela and Killiny 2020). Therefore, it is possible that the relatively smaller fruit on the tree that were more likely to drop early during the preharvest period are also the ones more severely affected by HLB. In this case, fruit size is indicative of both HLB severity and likelihood of drop during the preharvest drop period. Interestingly, a lopsided fruit shape was not consistently related to the detachment type or FDF, despite being another common HLB symptom. Further work is needed to determine whether the fruit shape is related to the preharvest fruit drop of HLB-affected 'Valencia'.

Finally, a high degree of variation in fruit size was observed between trees and between timepoints. Therefore, there is no size cutoff for fruit abscission; rather, the relatively smaller fruit on a tree are more prone to abscission early during the preharvest period than the larger fruit on the same tree.

Conclusion

Of the fruit characteristics studied, only fruit diameter was consistently correlated with FDF. The smaller fruit sizes may represent slow-growing fruit with an early transition from phase II to phase III growth. This early transition may allow them to respond to abscission signals earlier than the later-transitioning larger fruit. Therefore, smaller fruit are more likely to abscise during the preharvest period, especially during the start of preharvest drop. Closer to harvest, fruit of all sizes begin dropping as more fruit reach maturity and can respond to abscission signals. Future work should focus on management practices to reduce the early preharvest drop of small fruit.

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