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Juvenility Obscures Relative Citrus Tolerance to Huanglongbing: Juvenility-associated Tolerance Reveals New Opportunities

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Abstract. **The bacterial disease Huanglongbing (HLB) has had devastating effects on citrus industries worldwide, and tools used to manage the disease are costly and of limited effectiveness. Although some tolerant cultivars are available, they generally do not provide the critically necessary fruit traits for widespread commercial use. Development of new superior HLB-tolerant cultivars through hybridization, genetic transformation, or gene editing is a high priority in many citrus breeding programs. Developing and identifying highly tolerant cultivars with other essential traits and evaluating the new genotypes quickly often begin in the field before they transition from juvenility to maturity. Our study identifies clear evidence that juvenile clones appear much more HLB-tolerant compared to mature clones of the same cultivar, potentially confounding early field evaluations of tolerance. We also suggest that these observations may provide opportunities to manage juvenility to reduce HLB disease effects during tree establishment and identify strategies that may increase the juvenility-associated tolerance in fruiting cultivars.**

Citrus production in most parts of the world is declining or threatened by huanglongbing disease (HLB) caused by the bacteria *Candidatus* Liberibacter asiaticus (*C*Las). Florida has a long history of large-scale citrus production, but annual production of sweet orange has declined to less than 10% of the fruit volume observed before 2005, when *C*Las was first found in Florida (Singerman 2024). Although some resistance and tolerance to *C*Las have been identified in the citrus gene pool, most important citrus cultivars are susceptible and severely affected, and sweet orange cultivars exhibit significantly reduced tree growth, tree health, fruit production, and fruit quality following infection with *C*Las. Utilization of a suitable rootstock can provide improved sweet orange performance in the HLB environment (Bowman et al. 2016b, 2023; Kunwar et al. 2021; Singerman et al. 2021); however, even with the best rootstocks, profitable orange production remains difficult to achieve. Management strategies to reduce transmission of *C*Las by the vector Asian citrus psyllid (Chen et al. 2022; Pérez-Hedo et al. 2025) reduce bacterial titer in tree tissues through the injection of antibiotics (Albrecht et al. 2025) and improve tree health through applications of nutritional materials and growth regulators (Kwakye and Kadyampakeni 2022; Shahzad et al. 2024) have shown some benefits, but they are expensive and generally not considered suitable long-term solutions.

Citrus breeding by sexual hybridization has a long history of success in developing new commercial cultivars (Barry et al. 2020; Bowman and Joubert 2020), and efforts in some breeding programs over the past 20 years have focused heavily on creating new hybrids with resistance or tolerance to huanglongbing (Bowman 2023; Bowman et al. 2021; Huang et al. 2018; Killiny et al. 2018). Genetic transformation and gene editing have been widely described by researchers (Alquézar et al. 2021; Soares et al. 2020; Tiwari et al. 2024; Wang 2019) and citrus industry professionals (Dantzler 2024; Rogers 2023; Teiken et al. 2015) as the most likely long-term solutions to HBD. Conventional breeding, genetic transformation, and gene editing of citrus often include a multiyear juvenile period after creation, when the new hybrid or clone does not fruit and often exhibits different traits (such as increased thorniness) than those that will be observed in the mature fruiting cultivar. During the evaluation of both conventionally produced hybrids and products of genetic modification and editing for the HLB environment, rapid evaluations to determine resistance or tolerance to HLB are highly prioritized. Consequently, greenhouse and field evaluations of the new clones by challenges with *C*Las often begin while the new clone is still juvenile. One previously described characteristic of juvenility in tree species is high vigor (Wendling et al. 2014), and one clear effect of HLB on citrus trees is the reduction of vigor (Bowman and Albrecht 2020; Bowman et al. 2016b). Our observations have indicated significant differences in vigor and associated symptoms of HLB between citrus trees derived from juvenile and mature tissues. This research was initiated to quantify how juvenility affects tree vigor and the appearance of tolerance and/or resistance to HLB, as may be observed in field trials. The goal was to identify suitable controls (or reference corrections) in field trials with juvenile products from breeding or gene modification efforts. We also aimed to gain a better understanding of the effects of citrus juvenile vigor in *C*Las-infected trees and consider opportunities to use or stimulate the beneficial effects of juvenility in cropping trees.

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Materials and Methods

Plant material. Nine scion/rootstock combinations were included in this study (Table 1), including the following six different rootstocks: US-812 (*Citrus reticulata* × *Poncirus trifoliata*); US-802 (*Citrus maxima* × *Poncirus trifoliata*); standard sour orange (*C. aurantium*); Cleopatra (*C. reticulata*); Valencia sweet orange (*C. sinensis*); and Hamlin sweet orange (*C. sinensis*). Rootstocks for all trees included in the trial were grown from selected nucellar seedlings of the respective cultivars and using racks of 3.8 cm × 21 cm cells (Cone-tainers; Stuewe and Sons, Tangent, OR, USA) and soilless potting mix (Pro Mix BX; Premier Horticulture, Inc., Quakertown, PA, USA). Healthy seedling liners of each rootstock were transplanted into 2.54-L pots (Treepots; Stuewe and Sons) using soilless potting mix (Pro Mix BX). Rootstock liners were budded during Spring 2020 in the certified greenhouse nursery at the US Department of Agriculture Agricultural Research Service (Fort Pierce, FL, USA) using certified disease-free budwood of the Valencia sweet orange mature clone 1-14-19 or a true-to-type juvenile seedling of Valencia 1-14-19 during the first year of growth. One scion–root combination included in the study was a nucellar seedling of Valencia 1-14-19 that was not grafted with any scion (ungrafted) and, consequently, was composed of a juvenile Valencia trunk, branches, and leaves on Valencia seedling roots. Tree propagation occurred in certified greenhouses under *C*Las-free and psyllid-free conditions, and common greenhouse methods were used for nursery tree care during propagation and until trees were planted in the field.

Field planting. Ten *C*Las-free trees of each scion/rootstock combination were planted in a field trial at the US Department of Agriculture research farm (St. Lucie County, Fort Pierce, FL, USA) in Sep 2020 (center of the trial at lat. 27.435616°N, long. 80.424366°W). Trees were on double-row raised beds at a spacing of 2.1 m within each row and 6.1 m between rows. The experimental design was a randomized complete block with 10 single-tree replications and border trees on all sides. Trees

Table 1. Scion/rootstock combinations that were tested.

Scion (maturity)	Rootstock	Graft combination code
Valencia (juvenile)	US-812 seedling	JuvVal/US-812
Valencia (juvenile)	US-802 seedling	JuvVal/US-802
Valencia seedling (juvenile)	Ungrafted	JuvVal seedling
Valencia 1-14-19 (mature)	US-812 seedling	MatVal/US-812
Valencia 1-14-19 (mature)	US-802 seedling	MatVal/US-802
Valencia 1-14-19 (mature)	Valencia seedling	MatVal/Val
Valencia 1-14-19 (mature)	Hamlin seedling	MatVal/Ham
Valencia 1-14-19 (mature)	Cleopatra seedling	MatVal/Cleo
Valencia 1-14-19 (mature)	Standard sour orange seedling	MatVal/Sour

were irrigated by microjet and maintained using standard production practices, as previously described (Bowman et al. 2023). In Florida, HLB has been endemic since 2013 (Graham et al. 2020) and trees were exposed to natural *C*Las disease pressure that began immediately after planting.

Tree survival and size. Tree survival and tree canopy height, tree canopy diameter N–S, and tree canopy diameter E–W were measured in Jan 2021, Jan 2022, Jan 2023, and Apr 2025. Tree canopy volume was calculated as previously described (Bowman et al. 2023). In Apr 2025, scion trunk diameters were also measured on each tree at 5 cm above the graft union, with two measurements for each tree (one in the N–S direction and one in the E–W direction). In Apr 2025, rootstock trunk diameters were measured for each tree at 5 cm below the graft union, with two measurements per tree. For ungrafted Valencia seedling trees, trunk diameter was measured at the same height above the soil surface as the height for the corresponding measurement on grafted trees. Trunk cross-sectional areas (TCSAs) were calculated for both scion and rootstock and used to determine corresponding TCSA scion/rootstock ratios.

Tree canopy health. Canopy health was determined on 11 dates between 2021 and 2025 by visually assessing all trees. Visual ratings of tree canopy health were conducted using a scale of 1 to 5, as previously described (Bowman 2023; Bowman et al. 2023), with 1 indicating the most yellow tree color and thinnest canopy and 5 indicating the best healthy green color and thickest canopy. Canopy health values for each tree were averaged for the time periods 4 to 28 months and 32 to 55 months after planting.

CLas quantification. Six leaves per tree were randomly collected from the most recent mature flush in different areas of the canopy for each tree in Oct 2022 and stored at −20 °C until analysis by Southern Gardens Diagnostic Laboratory (Clewiston, FL, USA). Briefly, 100 mg of leaf petiole tissue was extracted using a bead beater to homogenize the tissue with the Qiagen BioSprint Robot and the BioSprint DNA plant kits (Qiagen, Germantown, MD, USA) according to the manufacturer's recommendations. After extraction, DNA concentrations were determined using PicoGreen (Life Technologies, Eugene, OR, USA) and a BioTek Synergy HT fluorescence microplate reader (Agilent Technologies, Santa Clara, CA, USA). The DNA concentrations were adjusted to 10 ng/uL using a Tecan liquid handling robot (Tecan Genomics, Morgan Hill, CA, USA), and a quantitative polymerase chain reaction was performed using the 16S primers for *C*Las as previously described (Li et al. 2006). On each plate, in addition to the unknown samples, a series of 10-fold dilutions of a known concentration of a plasmid containing the target sequence were also present. Copy number was determined by a regression analysis against the values obtained from the plasmid dilution series. Values were calculated as *C*Las genomes per mg of leaf tissue.

Fruit crop. On 28 Feb 2025, the number of fruit on each tree was recorded.

Statistical analysis. The experiment was analyzed as a completely randomized block design with 10 single-tree replications. A one-factor analysis of variance (ANOVA) of canopy volume of all nine scion/rootstock combinations was conducted at 4, 16, 28, and 55 months. A two-factor ANOVA of canopy volume at 55 months and all other traits of the six scion/rootstock combinations with paired juvenile and mature scions was conducted. Comparisons of means were performed by Tukey's honestly significant difference test when $P < 0.05$. Data were analyzed using Statistica version 14.0.0.15 (TIBCO Software, Palo Alto, CA, USA).

Results

Tree survival. One of the 90 trees planted from the combination of MatVal/US-812 died by 55 months of age.

Tree growth. Tree canopy volumes at 4, 16. 28, and 55 months were significantly influenced by the scion/rootstock combination (Table 2). At 4 months, a significant difference in canopy volume was observed between the largest trees [juvenile Valencia (JuvVal) seedlings at 33,464 cm^3] and the smallest trees composed of mature Valencia grafted on standard sour orange rootstock (MatVal/Sour at 17,727 cm^3). Beginning at 16 months and continuing through 55 months, the largest canopy volumes were observed for trees containing a juvenile Valencia scion on the three rootstocks, while the smallest trees at each timepoint were those of mature Valencia scion on Valencia rootstock. At 16 months, the tree canopy volume of JuvVal/US-812 was significantly larger than that of trees of MatVal/US-812 and three of the other rootstocks with mature Valencia scion. At 28 months, the tree canopy volume of JuvVal/US-812 was significantly larger than that of all other combinations except JuvVal/US-802. At 55 months, tree canopy volumes for both JuvVal/US-812 and JuvVal/US-802 were significantly larger than those of all other combinations except for the JuvVal seedling.

The additional results were evaluated by a two-factor ANOVA (scion maturity and rootstock) and using only the scion/rootstock combinations represented as both juvenile and mature scions on the same rootstock. Tree canopy volume at 55 months after planting exhibited a highly significant effect from scion maturity (Table 3); the canopy volume for a juvenile scion was approximately threefold the volume of a mature scion on the same rootstock. A significant rootstock cultivar effect on canopy volume was observed at this timepoint; trees on US-812 and US-802 were significantly larger than trees on Valencia rootstock. No interaction between scion maturity × rootstock cultivar for canopy volume was observed.

TCSA. Scion TCSA comparisons at 55 months showed a similar large contrast in growth between trees with juvenile Valencia scion and mature Valencia scion (Table 3). Scion maturity had a significant effect on scion TCSA, with juvenile scions averaging a TCSA of 9420 mm^2 and mature scions averaging a TCSA of 4490 mm^2. Rootstock cultivar did not have a significant effect on scion TCSA, and no interaction between scion

Table 2. Tree canopy volume (cm^3) of the nine different graft combinations at 4, 16, 28, and 55 months after field planting.

Graft combination	Canopy volume (4 mo[i])	Canopy volume (16 mo)	Canopy volume (28 mo)	Canopy volume (55 mo)
JuvVal/US-812	23,381 ab	307,760 a	619,423 a	2,185,530 a
JuvVal/US-802	27,624 ab	221,011 ab	472,580 ab	1,989,827 ab
JuvVal seedling	33,464 a	179,850 ab	310,556 bc	1,130,719 bc
MatVal/US-802	23,818 ab	136,069 b	275,267 bc	795,607 c
MatVal/Sour	17,727 b	175,666 ab	228,204 bc	636,030 c
MatVal/Cleo	19,806 ab	139,316 b	198,737 bc	628,393 c
MatVal/US-812	25,973 ab	135,181 b	187,859 bc	618,031 c
MatVal/Ham	20,934 ab	175,194 ab	203,550 bc	506,672 c
MatVal/Val	21,719 ab	121,988 b	133,331 c	376,558 c
P value	0.0464	0.0131	<0.0001	<0.0001

[i] mo = months after field planting.

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test when $P < 0.05$.

Table 3. Comparison of trees with juvenile and mature Valencia scion on three rootstocks.

	Canopy volume at 55 mo[i] ($cm^3 \times 10^6$)	Scion TCSA[ii] ($mm^2 \times 10^3$)	Rootstock TCSA[iii] ($mm^2 \times 10^3$)	Scion-to-rootstock TCSA ratio	Canopy health rating[iv]	*C*Las genomes in leaf tissue[v]
Scion maturity						
Juvenile	1.769 a	9.42 a	18.81 a	0.542 a	3.92 a	8.09
Mature	0.596 b	4.49 b	10.25 b	0.487 b	3.39 b	6.65
P value	<0.0001	<0.0001	<0.0001	0.0002	0.0001	0.5486
Rootstock cultivar						
US-812	1.443 a	8.07	15.39 a	0.518 b	3.83 a	8.27
US-802	1.393 a	7.10	20.02 a	0.351 c	3.79 a	8.22
Valencia	0.754 b	5.88	8.43 b	0.676 a	3.36 b	5.69
P value	0.0095	0.0500	<0.0001	<0.0001	0.0074	0.5882
Maturity × rootstock cultivar						
P value	0.2322	0.6976	0.4473	0.0277	0.8396	0.9941

[i] mo = months after field planting.
[ii] Scion TCSA = trunk cross-sectional area of scion 5 cm above the graft union at 55 months after field planting.
[iii] Rootstock TCSA = trunk cross-sectional area of rootstock 5 cm below the graft union at 55 months after field planting.
[iv] Canopy health = average of canopy health ratings between 32 and 55 months after field planting.
[v] Number of *C*Las genomes $\times 10^6$ per 100 mg leaf tissue at 25 months after field planting.
Different letters within columns indicate significant differences according to Tukey's honestly significant difference test, when $P < 0.05$.

maturity × rootstock cultivar for scion TCSA was observed.

Rootstock TCSA comparisons at 55 months also indicated a highly significant effect from scion maturity; the juvenile scion TCSA was significantly larger than the mature scion TCSA on the same rootstock. Rootstock cultivar had a highly significant effect on the TCSA of the rootstock, with TCSAs of 20,200 to 15,390 mm^2 for US-802 and US-812 rootstocks and 8430 mm^2 for Valencia rootstocks. An interaction between scion maturity × rootstock cultivar was not observed for rootstock TCSA.

Both scion maturity and rootstock cultivar had highly significant effects on the scion-to-Rootstock TCSA ratio. A significant interaction between scion maturity × rootstock cultivar was observed.

Tree health. During the first 28 months, no significant differences in the average canopy health attributable to either scion maturity or rootstock cultivar were observed. From 32 to 55 months, average canopy health scores indicated a highly significant effect of scion maturity on canopy health, with significantly healthier canopies on the trees containing a juvenile scion (Table 3). During this time period, rootstock cultivar also had a significant effect on canopy health; trees on US-812 and US-802 rootstocks had health scores that were significantly higher than those of trees on Valencia rootstock. No interaction between scion maturity × rootstock cultivar was observed for canopy health.

CLas. When tested at 25 months, *C*Las detection in leaves indicated that all 89 trees in the trial had already become naturally infected. There were no significant effects of scion maturity or rootstock cultivar on the number of *C*Las genomes in leaf tissue (Table 3), and there was no interaction between scion maturity × rootstock cultivar.

Fruit. All combinations with mature Valencia scion had some fruit on the trees at 54 months (28 Feb 2025), with the average number of fruit ranging from 1.8 to 4.8 per tree, but there were no significant rootstock effects. None of the trees with juvenile Valencia scion had fruit at 54 months.

Discussion

All trees in the trial were infected with *C*Las by 25 months after field planting (Oct 2022), with an average Ct_{CLas} of 32.5, suggesting no significant differences in Asian citrus psyllids transmission or susceptibility to *C*Las infection among the scion/rootstock combinations tested. The number of *C*Las bacteria genomes detected in leaves was not significantly different between juvenile and mature Valencia scion or between rootstock combinations, suggesting that "tolerance" to HLB is the basis for observed tree growth and canopy health differences observed in this trial, not active "resistance" to *C*Las bacterial survival or multiplication.

Trees of all scion/rootstock combinations survived field conditions in the trial; only one of 90 trees died by 4.5 years after field planting. Tree canopy size differed visibly among the combinations tested at 16 months after planting, with the size for juvenile canopies being approximately two-fold those of trees with mature canopies on the same rootstocks. Tree size differences became more obvious among the combinations as time passed after planting; trees with juvenile Valencia had canopies approximately three-fold the volume of those of trees with mature Valencia scion on the same rootstock at 55 months (Fig. 1).

Performance during this trial of mature Valencia on sour orange and Cleopatra, which are rootstocks of worldwide importance, were not significantly different from the performance of mature Valencia on Valencia rootstock. The performance of mature Valencia on Hamlin rootstock was also similar to its performance on Valencia rootstock. These observations suggest that the observed beneficial effect of juvenility on the grafted scion will likely be applicable across the range of rootstocks in commercial use.

Scion and rootstock TCSA measurements at 55 months confirmed the much greater growth of all combinations with juvenile Valencia scion as compared with that of combinations containing mature Valencia scion. The significantly larger rootstock TCSA for the two hybrid rootstocks, US-812 and US-802, as compared with Valencia rootstock, corresponded to the greater canopy growth

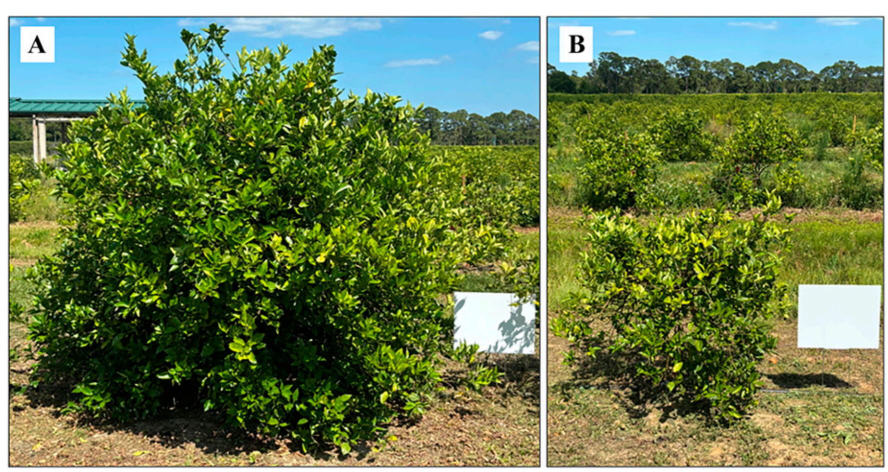

Fig. 1. Comparison of (**A**) juvenile Valencia/US-812 and (**B**) mature Valencia/US-812 at 55 months of age. White sign is 35.5 cm × 45.7 cm.

on those hybrid rootstocks and suggested an advantage to the use of the hybrid rootstocks for Valencia scion as compared with a Valencia root system, even at this early stage of field performance.

A relative comparison (ratio) of scion and rootstock trunk diameter (or TCSA) has been suggested to be informative of the likelihood of future graft incompatibility problems between particular scion/rootstock combinations (Smith et al. 2025), with some trifoliate hybrid rootstocks showing notable overgrowth as compared with some citrus scions, that will eventually result in graft union weakening. We have previously reported different levels of rootstock overgrowth for US-812 and US-802 hybrid rootstocks with citrus scions (Bowman and Rouse 2006; Bowman et al. 2016a), although in Florida neither of these has resulted in graft union problems with grafted citrus scions. The results of the scion-to-rootstock ratio in our study (Table 3) with overgrowth of US-802 > US-812 > Valencia rootstocks reconfirmed these earlier observations of relative rootstock overgrowth with citrus scions. Furthermore, the results of this study indicated that a juvenile scion resulted in significantly less rootstock overgrowth than a mature scion. We propose that this is the result of the greater vigor of the juvenile scion reducing the differential in vigor between the hybrid rootstock and the citrus scion.

Canopy health evaluations did not detect significant differences among the combinations during the first 2.5 years. However, from 32 to 55 months, the combinations with juvenile Valencia scion began to demonstrate significantly better canopy health than the combinations with mature Valencia scion. In the HLB-endemic environment, canopy health scoring is considered mainly for assessing tree tolerance to HLB (Bowman et al. 2021, 2023; Kunwar et al. 2021); therefore, at least part of this delay in visible canopy health differences between the juvenile and mature scion trees is likely because trees only became infected with *C*Las sometime during 2021 or 2022 (by 25 months). It can be noted that rootstock also had a significant effect on canopy health, which has been previously described as a result of both better maintenance of leaf area and improved leaf color in *C*Las-infected trees on particular rootstocks (Bowman and Albrecht 2020; Bowman et al. 2023).

Trees with mature Valencia scion grew weakly and had relatively unhealthy canopies with all of the rootstocks included in the study (Table 1). Although the mature trees flowered each season (2022–24), relatively few fruit set or developed to maturity. One symptom of HLB in sweet orange trees is premature fruit drop; therefore, most of the fruit initially set on mature trees during this study dropped before harvest time. As expected, the trees planted with juvenile scions in 2020 continued growing as juvenile and did not flower or set fruit through Feb 2025. During the last harvest season included in this study (Feb 2025), the average number of fruit (that did not drop prematurely) per mature tree was less than five for all rootstock combinations, which would not be sufficient for commercial harvest. Therefore, although the juvenile scion trees produced no fruit during the first 55 months, it can be noted that the mature scion trees also did not produce any marketable crop during this same time period.

One important observation from this study is that a tree with juvenile Valencia scion will clearly appear more tolerant to HLB (in canopy growth and canopy health) than a Valencia tree with a mature clone of the same scion cultivar. Because of the urgency caused by the HLB disease crisis to create new tolerant citrus scion cultivars by conventional breeding, genetic transformation, and gene editing as well as evaluate these cultivars for field performance as quickly as possible, significant value has sometimes been placed on the strong and healthy appearance of new clones in the field and infected with *C*Las, even before the new clones began to fruit. Based on our results, the appearance of HLB tolerance in a juvenile (unfruiting) clone may not be indicative of stable HLB tolerance for the mature and fruiting clone; therefore, great caution is warranted for this issue. A 300% greater canopy growth for juvenile sweet orange and a healthier appearance to the canopy, as compared with those of the mature clone, were observed in HLB-affected trees during the first 5 years in the field (Fig. 1).

Furthermore, our anecdotal observations in the breeding program suggest that some new hybrids continue to appear notably HLB-tolerant for at least several years after they begin to fruit, even though during eventual growth in replicated plantings the clone will not show continuing indications of good HLB tolerance. It is unclear, with the current information, whether this increase over time in visible severity of HLB disease in some clones is attributable to gradually declining HLB tolerance of the clone following the beginning of fruiting (as it continues to mature) or simply a significant delay in the appearance of the HLB-induced decline in large established trees.

Because three of the most common citrus cultivar types, sweet orange, grapefruit, and lemon, produce apomictic nucellar seedlings, there is a long history of using such apomictic seedlings to re-invigorate old budlines (Cameron et al. 1957). During the early 20th century, this re-invigoration was often thought to be associated with the elimination of graft-transmissible diseases that are not transmitted through seed. However, improvements in tree health and cropping among juvenile-line clones derived from nucellar seedlings of sweet oranges have continued since the widespread use of shoot tip grafting for disease elimination, indicating that rejuvenation of the budline by itself results in improvements in tree vigor, tree health, and fruit cropping (Rouse and Maxwell 1988). More recent studies have demonstrated recovery of higher-performing clones of sweet orange through juvenile nucellar seedlings of old budlines (Chater et al. 2024), and the performance improvement was often associated with higher vigor in the new seedling clones and better fruit quality. The rejuvenation of a citrus budline through juvenile nucellar seedlings appears a viable method to improve tolerance to HLB, at least during the early years.

One explanation of greater apparent HLB tolerance of juvenile citrus clones than that of mature clones is the metabolic cost of fruit development. The increased carbohydrate load on the citrus tree from developing fruit may overwhelm the capacity of a young fruiting tree that is already under stress from HLB. A similar observation was previously reported for watermelon plants infected by squash vein yellowing virus that showed no decline from the disease when fruits were removed (Adkins et al. 2013).

A second possible explanation for observed greater HLB tolerance of juvenile citrus clones may be that more rapid growth of juvenile citrus may impart a higher tolerance through more rapid regeneration of phloem damaged by the bacteria. The citrus tree may be able to outpace the bacteria's ability to multiply and spread in the phloem. Rapid phloem regeneration was previously proposed as the mechanism for greater HLB tolerance of specific lemon and mandarin cultivars (Deng et al. 2019). This mechanism of tolerance would result in declining tolerance as tree vigor declines during maturation or as the budline ages. It can also be noted that many of the management tools used to help improve citrus growth with HLB, such as nutritional, growth stimulant, and hormone treatments, will result in greater tree vigor.

A third possible explanation for observed higher tolerance in juvenile clones may be that juvenile citrus tree defense strategies (innate physiological conditions or responses to infection) may be different from the defense strategies of mature citrus trees. Along with greater thorniness and more rapid tree growth, juvenility may maintain or trigger physiological conditions that block the development of some important HLB disease responses, such as phloem plugging. As the clone transitions to maturity, the tree defense strategy may change to one that is less able to successfully block the HLB disease effects.

The greater HLB tolerance of juvenile citrus clones adds to the complexity and uncertainty in the evaluation of newly created hybrids and the products of genetic modification. However, it also may offer new opportunities to take advantage of juvenile vigor as a source of greater HLB tolerance through budline rejuvenation, and that may also be stimulated in existing mature cultivars. We suggest that HLB tolerance expressed during the juvenile phase or early maturation phase should be referred to as juvenility-associated tolerance (JAT), and that JAT is a potential tool in the HLB-solution toolbox. Juvenility has been associated with increased disease resistance or tolerance in diverse plant species, including tobacco, watermelon, and grapevine (Barna 2022), suggesting that JAT may be widespread among crop species. Future studies may aim to develop hormonal or other treatments to stimulate the high vigor and improved disease tolerance of juvenility in citrus and also allow fruiting. Alternatively, late-stage juvenile clones of Valencia and other cultivars (nearing the end of juvenility) may be used for field planting to facilitate rapid juvenile tree growth and development for the first 3 to 5 years and then be transitioned to the mature stage for fruiting after the tree is large and well-established. Several possible alternative explanations for observed JAT lead to several important areas of future research designed to identify the basis of JAT and effectively use this potent source of HLB tolerance.

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