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Oviposition of the Asian Citrus Psyllid on Six Citrus Cultivars: Associations with Flush Availability, Volatile Profiles, and Leaf Surface Chemistry

MARIA EUGENIA AMORÓS
Facultad de Química, Universidad de la República
ANNA P. BURGUEÑO
Facultad de Química, Universidad de la República
MARÍA L. UMPIÉRREZ
Facultad de Química, Universidad de la República
GUILLERMO BRAGUNDE
Facultad de Química, Universidad de la República
VERÓNICA GALVÁN
Instituto Nacional de Investigación Agropecuaria, INIA Salto Grande
ABEL RODRÍGUEZ
Instituto Nacional de Investigación Agropecuaria, INIA Salto Grande
FERNANDO RIVAS
Instituto Nacional de Investigación Agropecuaria, INIA Salto Grande
VIRGINIA PEREIRA DAS NEVES
Instituto Nacional de Investigación Agropecuaria, INIA Salto Grande
JOSÉ BUENAHORA
Instituto Nacional de Investigación Agropecuaria, INIA Salto Grande
ANDRÉS GONZÁLEZ
Facultad de Química, Universidad de la República
CARMEN ROSSINI
crossini@fq.edu.uy

Facultad de Química, Universidad de la República

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Abstract

Diaphorina citri Kuwayama (Hemiptera: Liviidae) is the vector of Huanglongbing, the most destructive citrus disease worldwide. Host selection and oviposition in this species integrate visual, olfactory, and contact cues from young flushing shoots. However, the plant traits underlying cultivar-dependent oviposition preference remain poorly understood. Here, we evaluated oviposition preference among six citrus cultivars and explored associations between chemical traits and oviposition patterns. In greenhouse choice assays, mandarin cultivars received significantly more eggs than the other cultivars, and oviposition positively correlated with the number of tender shoots. Although eggs per shoot did not differ among cultivars, mandarins produced more flushes, confirming the importance of shoot availability as a major determinant of oviposition. Exploring additional cues, potentially associated with host selection, volatile organic compounds and epicuticular waxes were characterized by gas chromatography-mass spectrometry. Both volatile and wax profiles differed among cultivars, citrus types, and oviposition preference categories. Several terpenoids (such as limonene, 1,8-cineole, γ-terpinene, *E*-β-farnesene, trans-α-bergamotene) and long-chain lipid compounds were consistently associated with preferred or less-preferred genotypes, suggesting that cultivar-dependent chemical traits may contribute to host discrimination by *D. citri*. Together, our results indicate that oviposition patterns in *D. citri* are associated with an interaction between plant phenology and cultivar-specific chemical characteristics. While the chemical associations reported here do not demonstrate a causal role in host selection, they identify candidate volatile and surface chemical markers that warrant further behavioral and electrophysiological validation. By integrating shoot development, in vivo volatile emissions, and epicuticular wax composition, this study provides new insights into the plant traits associated with *D. citri* host selection and may contribute to the development of cultivar-informed management strategies for Huanglongbing.

INTRODUCTION

Huanglongbing (HLB), also known as citrus greening disease, is currently the most significant citrus disease worldwide, and one of the top challenges facing plant pathology for the next decade (Hosseinzadeh and Heck 2023). It is caused by the phloem-associated bacteria complex *Candidatus* Liberibacter spp., and there is no known cure once trees are infected (Pérez-Hedo et al. 2025). Disease symptoms evolve rapidly, leading to decreased fruit production and quality, and often causing tree death (reviewed by Pérez-Haedo *et al*. (2025) (Pérez-Hedo et al. 2025)). The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is one of the primary vectors of HLB. Adult dispersal in search of feeding and oviposition sites facilitates the spread of HLB among plants (Halbert and Manjunath 2004). After its initial detection in South America (Brazil, 2004) and North America (Florida, USA) (Cervantes-Santos et al. 2025), HLB rapidly spread throughout the Americas (Grafton-Cardwell et al. 2013), causing significant management challenges (Halbert and Manjunath 2004) and economic losses (Belasque Jr et al. 2010).

Current HLB management relies on the use of healthy citrus grafts, the eradication of infected plants (Guo et al. 2024), and vector control (Gaire et al. 2024). Vector control relies on broad-spectrum pesticides applied repeatedly to suppress populations (Hall et al. 2012), a strategy that is costly, environmentally detrimental (Michaud 2004), and increasingly compromised by reduced psyllid susceptibility to several insecticides (Grafton-Cardwell et al. 2013). Hence, alternative products (Amorós et al. 2024; Yamamoto and Parra 2005), and cultural practices such as trap cropping through topping trees are being explored to attract *D. citri* adults and reduce vector pressure (de Carvalho et al. 2024). There is international consensus that *D. citri* control must be implemented within Integrated Pest Management (IPM) programs (Gaire et al. 2024; Santos Silva et al. 2023), combining resistant cultivars, improved monitoring, selective pesticides, semiochemicals, and conservation of natural enemies (Cortez-Madrigal 2024; Santos Silva et al. 2023; Tiwari et al. 2011; Yamamoto and Parra 2005).

Effective IPM implementation requires a detailed understanding of *D. citri* biology, disease transmission, and host selection. The psyllid is closely associated with new flushes, where oviposition and nymphal development occur on tender shoots (Bové 2006; Halbert and Manjunath 2004). Females are highly prolific, and development can be completed within 15–47 days depending on temperature (Asplanato et al. 2011; Ebert et al. 2023). Both nymphs and adults can acquire or transmit the pathogen through phloem feeding on infected or healthy plants, facilitating rapid disease spread (Asplanato et al. 2011; Bentancourt 2014; Guo et al. 2024). The host range of *D. citri* is largely restricted to Rutaceae, mainly *Citrus* and *Murraya*, with differential oviposition preference among species (Meng et al. 2022; Sétamou et al. 2016). Like many hemipterans, ACP relies on visual, contact, and olfactory cues during host selection (Setamou et al. 2016; Wenninger et al. 2009). Host plant volatiles play a key role in mediating ACP–host interactions and in discriminating suitable hosts for feeding or oviposition. Adults are attracted to volatile blends emitted by preferred citrus hosts such as *Citrus sinensis* compared with less preferred or non-host plants (Amorós et al. 2019; Fancelli et al. 2018). These blends are dominated by mono- and sesquiterpenes that elicit antennal and behavioral responses (Amorós et al. 2019; Fancelli et al. 2018; Martini et al. 2021). For instance, Oliveira et al. 2025 (2025) identified sabinene, β-myrcene, and (*E*)-β-farnesene as contributors to the attractiveness of some citrus cultivars, suggesting that host preference is influenced by chemical cues. Improved knowledge of citrus volatile chemistry and psyllid olfactory responses may therefore contribute to IPM development (Santos Silva et al. 2023). However, citrus epicuticular wax chemistry has been largely understudied as a source of cues to *D. citri* after landing on potential hosts, even though epicuticular leaf waxes play an important role in host recognition and oviposition site selection by many hemipteran herbivores, modulating landing, walking and oviposition after plant contact (Chapman 2003; Eigenbrode and Espelie 1995; Müller and Riederer 2005). While differences in wax composition among citrus genotypes have been documented (Abbas et al. 2022; Baker et al. 1975; Freeman et al. 1979; Haas and Schönherr 1979; Riederer and Schneider 1989), there is little functional evidence demonstrating whether these differences influence ACP host preference or oviposition behavior. Consequently, the identity of wax compounds potentially involved in host recognition, as well as their relative importance compared with other plant cues, remains largely unknown. Within this framework, we investigated the oviposition preference of the ACP among six citrus

cultivars. By integrating behavioral assays with host plant characterization -including chemical profiles and shoot development patterns- this study aims to improve understanding of ACP host-plant selection and identify putative citrus-derived kairomonal cues influencing oviposition.

METHODS AND MATERIALS

Chemicals. Chemicals were obtained from Sigma-Aldrich, and solvents from Mallinckrodt, Inc.

Plants and Insects. All plants and insects were reared in the Experimental Station of INIA Salto Grande (Salto, Uruguay, -31.2739°, -57.8908°). Insects were reared in *Citrus limon* cv. 'Cravo' seedlings under controlled conditions (26 ± 2°C, 62 ± 12% HR, 14:10 L:D). Experiments were performed using 6 citrus cultivars reared under the same conditions: *Citrus clementina* 'Clemenules' and *Citrus reticulata* 'Murcott' mandarins, *Citrus limon* 'Lisbon' and *Citrus sinensis* 'Valencia', *Citrus sinensis* 'Navelina' and *Citrus sinensis* 'Lanelate'. These cultivars represent important commercial segments of Uruguayan citriculture, covering early-, mid-, and late-season cultivars. All citrus plants were grown in individual 3-L pots, fertilized once a week and watered according to demand.

All assays were performed with 2-year-old seedlings. For performing shoot chemical extracts and volatile collections, plants were transported to the Chemical Ecology Laboratory of the Chemistry School-UdelaR (Montevideo, Uruguay, -34.88854°, -56.18576°), where they were kept under the above same conditions.

Preference Bioassays and Shoot Development Pattern. A bioassay was performed to assess *D. citri* oviposition preference in selected citrus cultivars. Potted seedlings (24-months old) were used (1 plant/pot). Murcott and Clemenules mandarins; Valencia, Navelina and Lanelate oranges; and Lisbon lemon seedlings were pruned making the same number of cuts on each plant, to boost homogeneous bud bursting. The experiments were set up after 7–10 days when bud breaks were present. Prior to the assay shoots per plant were counted and measured. One plant of each cultivar was then placed inside a white mesh cage (58 x 58 x 89 cm), and 50 adults were then released. Adults were aspirated from 2-week-old rearing cages, no sex ratios were controlled. Plant positions were randomized among cages (replicates), different plants were used in all cases. After 1 week the adults were removed with a manual aspirator and all shoots were excised, counted and measured. All eggs per shoot were counted under a stereoscopic lens (Olympus SZH10). Each cage represented one replicate; 17 replicates were performed over 8 months, and experimental dates were considered as blocks in statistical analyses.

Plant Volatile and Leaf Wax Extracts. Prior to chemistry studies, plants were pruned to stimulate shoot development. Approximately 15–20 days later, once the plants had developed young shoots not exceeding 5 cm, volatiles were collected and foliar waxes extracted. Volatiles were collected for 24 h by enclosing shoot-bearing plant sections in polyester bags (WYDA, 41 x 33 cm) at 22 ^{o}C. A background volatile control was done in parallel for each group of three sampled plants, using empty bags. Eight different plants for each cultivar were evaluated (n = 6).

Charcoal-filtered air was pushed through the system at 2 L min^{-1} using an electric air compressor (Toshiba TOSCON) and pulled from the chamber at 1 L min^{-1} using a CASELLA Apex 2 pump. Volatiles were adsorbed on 50 mg HayeSep Q (Hayes Separations Inc.), eluted with 1 mL double-distilled hexane and spiked with tridecane (24 µg) as internal standard. Extracts were concentrated to 100 µL under N_2 and stored at − 20°C. From the same plants, shoots were weighed and epicuticular waxes extracted by immersion in dichloromethane for 30 s (2 mL/shoot). Extracts were concentrated to 100 µL for gas chromatography-mass spectrometry (GC–MS) analysis.

Volatile and Wax Extract Analyses. Chemical characterization was performed using a Shimadzu QP2010 Plus GC–MS equipped with an Rtx-5MS column (30 m × 0.25 mm × 0.25 µm; Restek), He carrier gas (1 mL min^{-1}), ionization energy 70 eV, and scan ranges of 30–350 m/z (volatiles) and 35–500 m/z (waxes). For volatile extracts the oven temperature program was: 40°C (1 min), 150°C (5°C min^{-1}, hold 10 min), 250°C (10°C min^{-1}, hold 1 min) and 290°C (10°C min^{-1}, hold 10 min). For wax extracts: 40°C (4 min), 150°C (15°C min^{-1}) and 300°C (8°C min^{-1}). Injector and interface temperatures were 250°C and ion source 200°C. Injections were made in splitless mode (1 µL, sampling time 1 min).

Chromatographic data were processed using the PARADISe software (PARAFAC2-based Deconvolution and Identification System; University of Copenhagen) (Johnsen et al. 2017). For processing, raw GC–MS data were converted to NetCDF (.cdf) format. The following operations were made baseline correction by polynomial order = 2; chromatograms alignment within ± 0.1 min and smoothed with a Savitzky–Golay filter (9-point window). Data were segmented into retention windows of 0.2–0.3 min and modeled independently by PARAFAC2 with up to 2000 iterations.

When performed, volatiles were annotated by comparing (± 5 units) calculated Retention Indices (RI) with those reported by Adams (2007) and by comparison (match criterium > 90% similarity) of mass spectrum acquired with those in NIST 08 (Linstorm and Mallard 2005) and Adams (2007) libraries. (RI were calculated by injecting, under identical conditions, a homologous series of n-alkanes (C8–C32, including even-numbered alkanes and n-nonadecane). Compounds fulfilling both criteria were considered tentatively annotated, corresponding to Level 2 identification according to the Metabolomics Standards Initiative (Sumner et al. 2007). Whenever available, these tentative identifications were further compared with authentic standards analyzed under identical GC–MS conditions using the same chromatographic column.

Statistical Analyses. Analyses were performed in R (R Core Team 2025). Data sets were evaluated for normality and overdispersion using half-normal plots from the hnp package 1.2–6 (Moral et al. 2017). Numbers of shoots were analyzed with a generalized linear model with quasipoisson error (GLM-qp) followed by Tukey's HSD ($p < 0.05$). Shoot length data were log + 1 transformed and analyzed with ANOVA followed by Tukey's HSD ($p < 0.05$). Total eggs and eggs per shoot were analyzed with GLM-qp followed by Tukey's HSD.

Volatile and wax profiles were analyzed using the Metaboanalyst platform (Chong et al. 2019). The GC-MS data were not filtered before multivariate analyses. Normalization, scaling and centering procedures

are specified in Results (Chong et al. 2019). Outliers were not detected. Principal component analyses (PCA) were first used for exploratory analysis, followed by Partial Least Squares Discriminant Analysis (PLS-DA) to identify peaks contributing to sample differentiation. Three supervised models used classification variables of (i) citrus cultivar (six levels), (ii) citrus type (oranges, mandarins, lemon), and (iii) oviposition preference (preferred vs. less-preferred cultivars based on bioassay results). Models were validated by permutation tests. Variable influence on projection (VIP) scores were used to identify peaks contributing to group differentiation. Variables with VIP values > 1.5 (Xia et al. 2009) were considered the strongest contributors to class separation. Model performance and robustness were evaluated independently through cross-validation (R^2 and Q^2) and permutation tests, as reported in the Results section.

RESULTS

Shoot Development Pattern and Oviposition Bioassay. Seven days after pruning (start of the oviposition experiment), cultivars showed significant differences in shoot number (*GLM-qp, Tukey's HSD*, $P < 0.05$, Fig. 1A), ranging from 12 ± 9 in Clemenules to 4 ± 2 in Lisbon. Shoot length did not significantly differ among cultivars at the beginning of the trial (log + 1 transf, *ANOVA, Tukey's HSD*, $P = 0.07$). After seven days, both mandarin cultivars and Valencia orange developed the highest number of new shoots, Murcott mandarin reaching 29 ± 12, which also exhibited the shortest shoots, followed by Lisbon lemon and Valencia orange (Fig. 1B, log + 1 transf, *ANOVA, Tukey's HSD*, $P < 0.05$). Overall, Murcott mandarin produced more numerous and shorter shoots, Clemenules produced numerous but longer shoots, whereas lemon and oranges produced fewer but longer shoots.

Regarding *D. citri* oviposition, Murcott mandarin followed by Clemenules mandarin, Lisbon lemon and Valencia orange received more eggs (Fig. 2A, *GLM-qp, Tukey's HSD*, $P < 0.05$). However, eggs/shoot did not differ significantly among cultivars (Fig. 2B, *GLM-qp, Tukey's HSD*, $P = 0.137$). At the end of the experiment, a positive correlation was found between the number of eggs laid and the number of shoots (*Spearman correlation test*, $\rho = 0.55$, $P < 0.001$, Fig. 3), indicating that *D. citri* oviposition was greater on cultivars producing more tender shoots, particularly the Murcott mandarin. Considering the results of the oviposition bioassay (Fig. 2–3), we classified the cultivars into two groups: preferred (P), which includes both mandarin genotypes, and less preferred (LP), comprising the remaining cultivars studied. This classification was subsequently used as the grouping variable for subsequent chemical analyses. *This classification was subsequently used as the grouping variable* in PLS-DA models applied to the volatile and wax datasets.

Volatiles and Waxes Chemical Analyses. GC-MS analyses of volatile collections and wax extracts revealed numerous peaks (Fig. S1). After automatic peak detection, mass spectra deconvolution and alignment of the GC-MS raw data, matrices of 84 features (peak intensities) × 50 samples and of 138 features × 96 samples (Tables S1 and S2) were obtained for Volatiles and waxes respectively.

For Volatiles, data were cube-root transformed and Pareto-scaled prior to statistical analyses (Alaerts et al. 2010). Among the 84 detected features, 10 exhibited at least one significant difference between a pair of citrus cultivars (*ANOVA* for each feature, $P < 0.05$ in all cases, Table S3). Principal Component Analysis (PCA, singular value decomposition) indicated that 80% of variance was explained by 5 components (Component 1: 46%; Component 2: 11%; Component 3: 10%; Component 4: 7%; Component 5: 6%). Partial Least Squares Discriminant Analysis (PLS-DA; Fig. S2A-B) yielded a robust model discriminating the 6 citrus cultivars (validated model: 3 components; 2000-permutation test, $P = 0.002$; $R^2 = 0.72$; $Q^2 = 0.48$; accuracy = 0.44), identifying 8 discriminant features with Variable Importance in Projection (VIP) > 1.5 (Chong et al. 2019) (Table S4). When data were grouped by citrus type (Mandarins, Oranges, and Lemon), PLS-DA again produced a valid model to discriminate among types (2 components; $P = 0.009$; $R^2 = 0.53$; $Q^2 = 0.34$; accuracy = 0.45), with 10 features showing VIP > 1.5 (Table S4, Fig. S2C-D) (Chong et al. 2019). Finally, to explore associations with *D. citri* oviposition preference, a PLS-DA was performed using preference (preferred, P; less-preferred, LP) as the classification variable (Fig. 4). This model also proved reliable (5 components; $P = 0.0005$; $R^2 = 0.79$; $Q^2 = 0.35$; accuracy = 0.85), identifying 8 discriminant Volatiles (VIP > 1.5, Table 1 and Fig. 4). All these discriminating features corresponded to a total of 10 compounds annotated by their spectra and retention indexes (Tables 1 and S4). Remaining peaks that did not contribute to sample differentiation were not identified (Fig. S1 and Table S1). However, the volatile profiles obtained -as indicated by the mass spectra of the detected features (data not shown)- were composed of monoterpenes, sesquiterpenes, and specific oxygenated compounds.

For wax extracts, 138 features were detected across the 6 citrus species (Fig. S1B and Table S2). Prior to statistical analysis, data were square-root transformed and auto-scaled (Alaerts et al. 2010). Simple ANOVA for each compound revealed significant differences ($P < 0.05$, in all cases) for 89 of these features (Table S5), each showing at least one significant pairwise difference between species. PCA explained 76% of the total variance with five components (Component 1: 32%; Component 2: 16%; Component 3: 15%; Component 4: 7%; Component 5: 6%). PLS-DA (Fig. S3A-B) provided a robust discrimination among species (validated model: 4 components; 2000-permutation test, $P = 0.0005$; $R^2 = 0.82$; $Q^2 = 0.70$; accuracy = 0.95), identifying 15 features with VIP > 1.5 (Chong et al. 2019) (Table S6). When grouping wax data by citrus type (Mandarins, Oranges, and Lemon) PLS-DA also yielded a valid model (4 components; $P = 0.0005$; $R^2 = 0.76$; $Q^2 = 0.67$; accuracy = 0.83), highlighting 11 discriminant features (Fig. S3C-D; VIP > 1.5, Table S6). Finally, when analyzed according to *D. citri* oviposition preference (P vs. LP; Fig. 4C), the PLS-DA model was again significant (4 components; $P = 0.0005$; $R^2 = 0.83$; $Q^2 = 0.74$; accuracy = 0.95), identifying 12 discriminant wax compounds (VIP > 1.5 (Chong et al. 2019), Fig. 4D, Table 2). These discriminant features came from GC-MS peaks then annotated mainly to fatty alcohols, and organic acid derivatives (Table 2). The 17 compounds corresponding to discriminant features across cultivar, citrus type, and preference classifications are summarized in Tables 2 and S6.

DISCUSSION

The six cultivars differed markedly in shoot production between the beginning (7–10 days after pruning) and the end (14–17 days after pruning) of the assay (Fig. 1). Mandarin cultivars, particularly Murcott,

continued producing young flushes throughout the trial, resulting in a higher number of shorter shoots, which are preferred by *D. citri* (Setamou et al., 2016). Clemenules mandarins also produced a higher number of shoots; however, these were significantly longer than those of Murcott (*ANOVA, Tukey's HSD*, $P < 0.05$). Murcott mandarin exhibited the highest egg deposition, and Navelina orange showed the lowest (*GLM-qp, Tukey's HSD*, $P < 0.05$). These results agree with previous studies showing that *D. citri* oviposits exclusively on tender, younger shoots (Bové 2006; Halbert and Manjunath 2004) (Asplanato et al., 2011; Setamou et al., 2016; Li et al., 2019). Although this general pattern is well established, here we provide cultivar-specific information for genotypes not previously evaluated under standardized pruning. No cultivar difference was detected in eggs per shoot across genotypes. Moreover, total egg number increased with shoot number (Fig. 3), suggesting that total egg counts mainly reflected shoot availability. The assay evaluated oviposition only, leaving out egg hatching, nymphal development duration, adult emergence and the fitness of emerging adults. Consequently, the results do not establish cultivar resistance or susceptibility, nor do they demonstrate preference under mixed-cultivar field conditions. Rather, our findings reinforce that *D. citri* tends to oviposit more frequently where tender shoots are most abundant (Bové 2006; Halbert and Manjunath 2004).

Under comparable biotic and abiotic conditions, mandarins exhibited a higher sprouting rate and generated new shoots more rapidly than other cultivars. However, shoot availability is not the sole factor influencing the *D. citri* life cycle. Indeed, mandarin cultivars have been reported to be poorer hosts for *D. citri* regarding nymphal viability compared with sour orange, *Citrus limonia* (Rangpur lime), and *Murraya paniculata* (orange jasmine) (Nava et al. 2007; Tsagkarakis and Rogers 2010). In this context, Zhong et al. (2019) reported that the wild mandarin cv. Chongyi attracted more Asian citrus psyllids in olfactometer assays, attributing this response to a less saturated yellow coloration, reduced sensitivity of the jasmonic acid–mediated wound response, a higher abundance of attractive volatiles, and lower levels of repellent compounds. These studies support a multimodal view of host assessment and caution against attributing cultivar differences to any single plant trait.

The volatile blends characterized here were chemically complex (Table S1, Fig. S1), as is typical for citrus foliage (Azam et al. 2013; Deng et al. 2022; Hetka et al. 2018; Zhang et al. 2020b). Our annotations focused on the features retained by the supervised models (Table S1 and Table 1), where profiles were dominated (data not shown) by monoterpenes, sesquiterpenes, and oxygenated derivatives previously reported as constituents of citrus leaves (Azam et al. 2013; Deng et al. 2022; Hetka et al. 2018; Zhang et al. 2020b). *Citrus* volatile signatures vary among genotypes (Germanà et al. 2013; Hosni et al. 2013) and with developmental stage, environmental stress, and seasonal conditions (Asai et al. 2016; Azam et al. 2013; Deng et al. 2022). Across citrus cultivars and tissues, linalool, limonene, and citral are frequently prominent (Azam et al. 2013; Flamini et al. 2007). Leaf ontogeny is particularly important: linalool tends to predominate in young leaves, whereas limonene may increase as leaves mature (Azam et al. 2013). This orthogenic and developmental plasticity provides a dynamic chemical background for host assessment.

The PLS-DA score plots described cultivar- and citrus-type-associated variation in the volatile dataset (Table 1; Fig. S2). The post hoc P-versus-LP model also separated the groups defined from total egg counts (Fig. 4A). However, the P/LP contrast largely coincided with mandarin versus non-mandarin identity. The supervised results should therefore be interpreted as exploratory evidence of covariance among taxonomy, chemistry, and the observed egg-count pattern, not as independent causal evidence that the retained features caused oviposition differences.

Although ACP responses to citrus volatiles have been extensively studied (Santos Silva et al. 2023), most chemical characterizations concern *C. sinensis* (Santos Silva et al. 2023). Among the six cultivars examined here, descriptions of *in vivo* leaf volatile collections exist only for ‘Lanelate’ and ‘Valencia’ (Asai et al. 2016; Deng et al. 2022; Patt et al. 2018b). For ‘Lisbon’, ‘Clemenules’, ‘Murcott’, and ‘Navelina’, published data derive primarily from essential oils or excised leaves, which represent compositional pools rather than real-time emission dynamics.

Across the three volatile PLS-DA models, only a restricted subset of 10 features were retained with VIP > 1.5 (Table 1). Eight of the nine annotated discriminants—limonene, 1,8-cineole, γ-terpinene, neral, geranial, β-elemene, trans-α-bergamotene, and E-β-farnesene—are established constituents of citrus tissues (Baccati et al. 2021; Flamini et al. 2007; Lota et al. 2002; Santos Silva et al. 2023). In contrast, 3-methyl-2-butenyl butanoate, although reported in other plant species (Terry et al. 2021), has not been previously documented in citrus, suggesting it may represent a rare or underreported component of citrus volatilomes. All compounds discriminating cultivars or preference categories also contributed to separation among citrus types (mandarins, oranges, and lemon; Table 1), indicating that phylogenetic identity and oviposition preference may be chemically structured along overlapping axes. However, β-elemene and one unidentified oxygenated sesquiterpene differentiated cultivars but not citrus type or preference, whereas neral and geranial separated cultivars and citrus type but not preferred versus less-preferred genotypes (Table 1).

In the post hoc P-versus-LP model, limonene, 1,8-cineole, and trans-α-bergamotene were relatively higher in the LP group, whereas γ-terpinene, 3-methylbut-2-enyl butanoate, β-elemene, (E)-β-farnesene, and an unidentified oxygenated sesquiterpene were higher in the P group (Table 1, Fig. S4). Notably, limonene was higher in less-preferred genotypes and is known to increase in mature leaves (Azam et al. 2013), which are generally less suitable for oviposition. This convergence suggests that relative limonene enrichment may signal reduced host suitability. More broadly, our results support the view that quantitative shifts in key volatiles—rather than their mere presence—may function as chemical cues underlying ACP oviposition preferences and represent candidate biomarkers for host selection.

Consistent with the patterns described above, limonene and 1,8-cineole—both enriched in less-preferred genotypes—show context-dependent behavioral effects. Neither compound was attractive to *D. citri* in olfactometry assays (Mann et al. 2012). However, limonene was attractive in trap assays and elicited EAG responses (Liu et al. 2021), indicating dose- or blend-dependent activity. In contrast, 1,8-cineole has been suggested as a feeding and oviposition repellent (Killiny et al. 2018), consistent with its higher

abundance in less-preferred genotypes (Table 1, Fig. S4). Citral (neral + geranial) has been reported as attractive in cage assays (Liu et al. 2021), yet electrophysiological (in EAG) results are inconsistent (Liu et al. 2021) (George et al. 2016), and its degradation products can themselves be antennally active (George et al. 2016). In the present dataset, neral and geranial did not contribute to the P-versus-LP separation, providing no evidence that citral alone explains the egg-count pattern.

For trans-α-bergamotene, 3-methyl-2-butenyl butanoate, *E*-β-farnesene, β-elemene, and γ-terpinene, direct behavioral evidence in ACP is remains limited. γ-Terpinene shows moderate affinity for chemosensory proteins (Zhang et al. 2020a), whereas binding to odorant-binding proteins (OBP) has been demonstrated only for limonene and citral (Liu et al. 2021; Patt et al. 2018a; Zhang et al. 2020a). To date, 1,8-cineole, 3-methyl-2-butenyl butanoate, β-elemene, trans-α-bergamotene, and E-β-farnesene have not been evaluated in OBP or chemosensory protein assays, to our knowledge.

Together with previous studies (Amorós et al. 2019; Lapointe et al. 2016; Patt et al. 2011), these results support the idea that ACP host selection depends on quantitative shifts within complex blends rather than to single compounds in isolation. While some volatiles contribute to a general citrus background (e.g., caryophyllene, a ubiquitous sesquiterpene that binds ACP OBPs (Wang et al. 2020) but was not discriminant here), others may modulate oviposition through relative abundance differences. This limited but structured chemical subset may help explain the modest field performance of semiochemical-based tools, while highlighting cultivar-dependent emission patterns as a framework for identifying functional biomarkers.

In the case of leaf waxes, five of the 17 features retained across the three PLS-DA models were unidentified were not identified (Table 2). The putatively annotated compounds belong to typical leaf wax chemical classes, including long-chain aliphatic compounds, including alkanes, primary alcohols, aldehydes, free fatty acids, esters, and phenolic compounds as minor constituents) (Roma and Santos 2021). In the four species included in this study (*Citrus sinensis, C. clementina, C. reticulata, and C. limon*, without considering cultivar-level -often not reported- distinctions), epicuticular leaf waxes are dominated by long-chain aliphatic lipids of different oxidation state, particularly alkanes, free fatty acids, and primary alcohols (Abbas et al. 2022; Baker et al. 1975; Favaro et al. 2020; Freeman et al. 1979; Haas and Schönherr 1979; Riederer and Schneider 1989).

The putatively annotated compounds included 2-methoxy-4-vinylphenol, 4-hydroxy-benzaldehyde, n-pentadecanol, ficusin, hexadecanoic acid, n-docosanol, 1-tricosanol, 1-tetracosanol, tetracosanoic acid, methyl ester, 1-pentacosanol, 1-hexacosanol, 3-methylhentriacontane. Long-chain fatty acids, alcohols, and alkanes -such as the ones annotated- are consistent with reported citrus leaf waxes composition (Freeman et al. 1979; Haas and Schönherr 1979). The aromatic compounds 2-methoxy-4-vinylphenol and 4-hydroxybenzaldehyde, although not previously reported in citrus leaf waxes, occur as minor components in other citrus products (Dugo and Mondello 2010). Ficusin (psoralen), a furanocoumarin characteristic of several citrus species, has been reported in phenolic fractions and other citrus products (Dugrand-Judek et al. 2015).

Among the 17 compounds recognized as discriminant across the three biological classifications evaluated here, only 4-hydroxybenzaldehyde and hexadecanoic acid did not differentiate species or citrus type but contributed to the separation between preferred and less-preferred species (Table 2). In contrast, three compounds (phenolic compound 1, ficusin, and n-docosanol) discriminated among cultivars, yet did not differentiate either citrus type or preference category. UN2 and 1-pentacosanol discriminated citrus types or cultivars but did not separate preferred from less-preferred species (Table 2). Among the 12 putative preference biomarkers identified in waxes, all except two (UN1 and 3-methylhentriacontane) were present in higher amounts in preferred species. Five of these biomarkers were fatty alcohols (n-pentadecanol, 1-tricosanol, 1-tetracosanol, 1-pentacosanol, and 1-hexacosanol). In Clemenules, younger leaves—those typically preferred by *D. citri*—contain higher proportions of primary alcohols than older leaves (Favaro et al. 2020), a pattern consistent with a potential role of alcohol abundance in host preference, although this association remains to be experimentally evaluated.

While oviposition preference of ACP has been largely interpreted through volatile cues and shoot phenology, nonvolatile contact cues are an underexplored component of ACP host assessment. Plant surface lipids can influence attachment, walking, probing, and oviposition by hemipterans through both chemical and physical mechanisms (Chapman 2003; Eigenbrode and Espelie 1995; Müller and Riederer 2005). In citrus, genotype-dependent differences in host use are not always explained fully by volatile emissions or flush abundance, leaving open a role for post-landing surface traits (Patt et al. 2011; Poerwanto et al. 2012; Zhao et al. 2013). However, direct experimental evidence linking citrus leaf wax composition to ACP oviposition behavior is currently lacking. The present data show that leaf surface chemical profiles covary with cultivar identity, citrus type, and the post hoc egg-count grouping. Specific wax features consistently discriminated these classifications in supervised analyses, supporting a potential role of surface chemistry in *D. citri* host selection, and providing a rationale for future experiments in which surface extracts, reconstructed mixtures, or wax-removal and reconstitution treatments are tested directly in controlled oviposition assays.

Despite the integrative approach adopted here, some limitations should be acknowledged. The associations between volatile or wax compounds and oviposition preference are correlative, as no behavioral validation or manipulation of individual compounds was performed. To determine the effective role of these semiochemicals in host selection mechanisms, electrophysiological assays and behavior bioassays with specific volatiles should be addressed. Moreover, only oviposition was assessed, without evaluating egg viability or subsequent performance. Finally, experiments were conducted under greenhouse conditions, and field validation will be necessary to confirm the ecological relevance of the candidate biomarkers identified. However, by integrating plant phenology with volatile and surface chemical profiling, this study advances our mechanistic understanding of *D. citri* host selection and provides a framework for exploiting cultivar-specific chemical traits in sustainable HLB management.

Declarations

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Data Availability- The data sets generated and analyzed during the current study are available in the supplementary material.

Author contributions- **MEA.:** Conceptualization, Methodology (oviposition assays), Investigation, Formal analysis (behavioral data), Writing – original draft, Writing – review & editing. **APB:** Methodology (chemical extraction and GC–MS analyses), Investigation, Formal analysis (chemical data), Data curation. **MLU:** Funding acquisition, Investigation (chemical analyses). **G.B.:** Methodology (shoot-flushing assessment), Formal analysis (shoot development data). **VG, AR, FR, VPN:** Investigation (experimental assays and data collection). **JB:** Conceptualization, Methodology (experimental design), Project administration, Resources. **AG:** Methodology (GC–MS analyses), Investigation (instrumental analysis), Supervision. **CR:** Conceptualization, Supervision, Funding acquisition, Formal analysis (chemical data), Data curation, Writing – original draft, Writing – review & editing.

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Tables

Table 1 Volatile compounds tentatively identified in the PLS-DA model using the classification into preferred (mandarins) and less-preferred hosts (oranges and lemon) as grouping variables. Only compounds corresponding to features with VIP scores > 1.5 from each PLS-DA model are reported. See also Table S4 for compounds tentatively annotated using cultivar or citrus type as alternative grouping variables

Feature (rt)		Compound ID	RI (NIST 20)	RIexp	P vs LP ratio (t-tests)	Putative preference marker
1	10.625	limonene	1030 ± 2	1032	P < LP	LP
2	10.692	1,8-cineole	1032 ± 2	1034	P < LP	LP
3	11.533	g-terpinene	1060 ± 3	1063	P > LP	P
4	12.742	butanoic acid, 3-methylbut-2-enyl ester	NA	1104	P > LP	P
5	16.800	neral*	1240 ± 3	1245	NS	-
6	17.633	Geranial*	1276 ± NA (N = 1)	1276	NS	-
7	20.842	b-elemene	1391 ± 2	1394	P > LP	P
8	22.008	trans-á-bergamotene	1435 ± 13	1446	P < LP	LP
9	22.483	*E*-b-farnesene	1455 ± 10	1467	P > LP	P
10	24.133	Oxygenated sesquiterpene	-	1541	P > LP	P

RIexp: experimental retention index, RI (NIST 20): retention index reported by NIST 20, NA: not available data in NIST 20 (only estimated)

MM: Murcott Mandarin, CM: Clemenules Mandarin, VO: Valencia Orange, NO: Navelina Orange, LO: Lanelate Orange, LL: Lisbon Lemon. M: Mandarins, O: Oranges, L: Lemon

P: Preferred, LP: less-preferred species for oviposition by *D. citri*

NS: no significant differences

* Compounds found to be significantly different among cultivars and citrus type but that do not contribute to discrimination between preferred and less preferred genotypes (Table S4)

Table 2 Wax compounds tentatively annotated in the PLS-DA model using the classification into preferred (mandarins) and less-preferred hosts (oranges and lemon) as grouping variables. Only compounds corresponding to features with VIP scores > 1.5 from each PLS-DA model are reported. See

also Table S6 for compounds tentatively annotated using cultivar or citrus type as alternative grouping variables.

Feature (rt)		Compound ID	RI (NIST 20)	RIexp	P vs LP ratio (t-tests)	Putative Preference marker
1	8.383	phenolic compound 1	-	< 1200	P < LP*	-
2	12.067	2-methoxy-4-vinylphenol phenol	1317 ± 5	1326	P > LP	P
3	12.542	4-hydroxy-benzaldehyde	1364 ± -- (N = 1)	1372	P > LP	P
4	13.242	phenolic compound 2	-	1437	P > LP	P
5	14.867	C9H9NO	-	1581	P > LP	P
6	17.258	n-pentadecanol	1778 ± 7	1782	P > LP	P
7	17.650	UN1	-	1815	P < LP	LP
8	18.142	ficusin*	1836 ± 7	1857	NS	-
9	19.208	UN2*	-	1945	NS	-
10	19.392	hexadecanoic acid	1968 ± 7	1962	P > LP	P
11	24.908	n-docosanol*	2493 ± 19	2484	NS	-
12	25.058	1-tricosanol	2528 ± 50	2500	P > LP	P
13	26.017	1-tetracosanol	2698 ± 1	2694	P > LP	P
14	27.267	tetracosanoic acid, methyl ester	2728 ± 2	2728	P > LP	P
15	27.867	1-pentacosanol*	NA	2803	NS	-
16	28.417	1-hexacosanol	2852 ± 2	2849	P < LP	LP
17	31.008	3-methylhentriacontane	3175 ± 3	3178	P < LP	LP

RIexp: experimental retention index, RI (NIST 20): retention index reported by NIST 20, NA: not available data in NIST 20 (only estimated), * P < 0.05 in t-test (but VIP < 1.5)

MM: Murcott Mandarin, CM: Clemenules Mandarin, VO: Valencia Orange, NO: Navelina Orange, LO: Lanelate Orange, LL: Lisbon Lemon. M: Mandarin, O: Orange, L: Lemon

P: Preferred, LP: less-preferred species for oviposition by *D. citri*

UN: unidentified

* Compounds found to be significantly different among cultivars and citrus type but that do not contribute to discrimination between preferred and less preferred genotypes (Table S4)

Figures

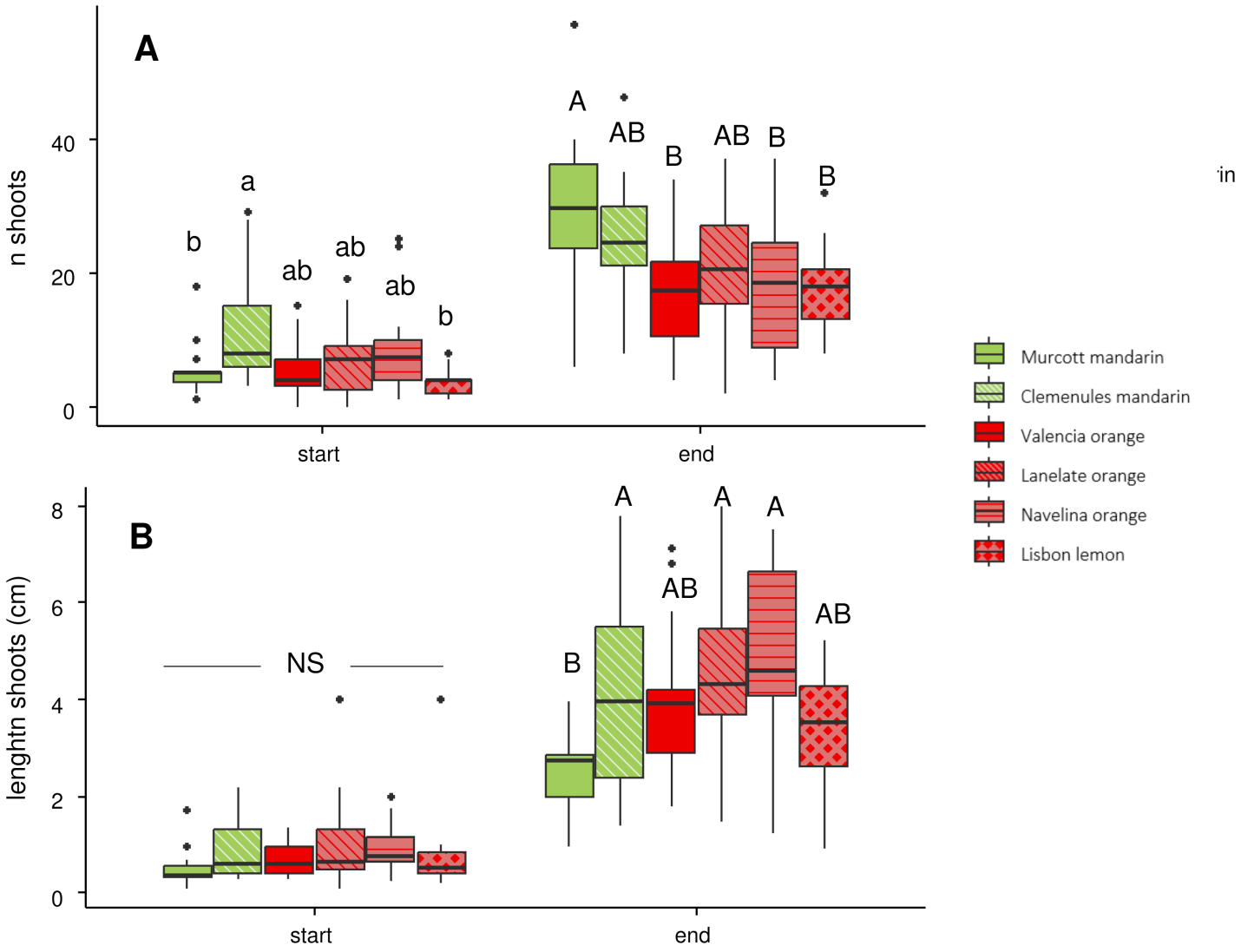


Figure 1

Number (**A**) and length (**B**) of shoots per cultivar at the start and end of the preference experiment. Different letters indicate significant differences (GLM-qp or ANOVA, Tukey's HSD, $p<0.05$); ns indicates non-significant differences. Start and end of the experiment were analyzed separately. Different colors indicate different cultivars. The boxes represent interquartile range, the horizontal line inside the box represents the median and the dots indicate outliers

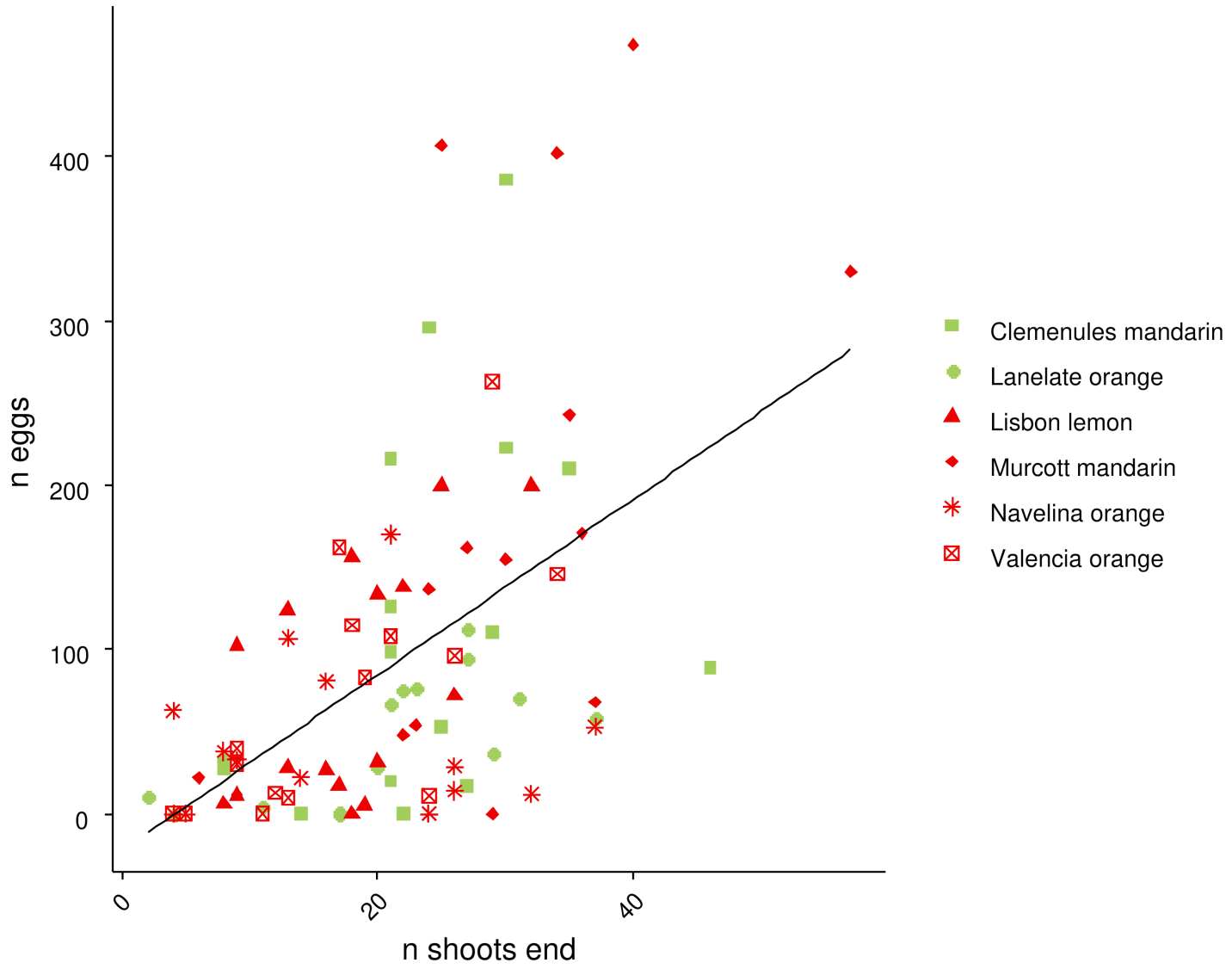


Figure 2

Oviposition bioassay results. Total eggs/cultivar (**A**) and eggs/shoot/cultivar (**B**) after 7 days are shown. Different letters indicate significant differences (GLM-qp, Tukey's HSD, $p<0.05$). Different colors indicate different cultivars. The boxes represent interquartile range, horizontal line inside the box represents the median and the dots indicate outliers

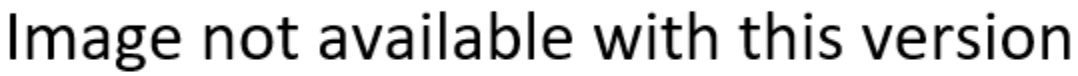


Figure 3

Correlation between egg number (n) and shoot number at the end of the assay (*Spearman correlation test*, ρ = 0.55, P < 0.001)

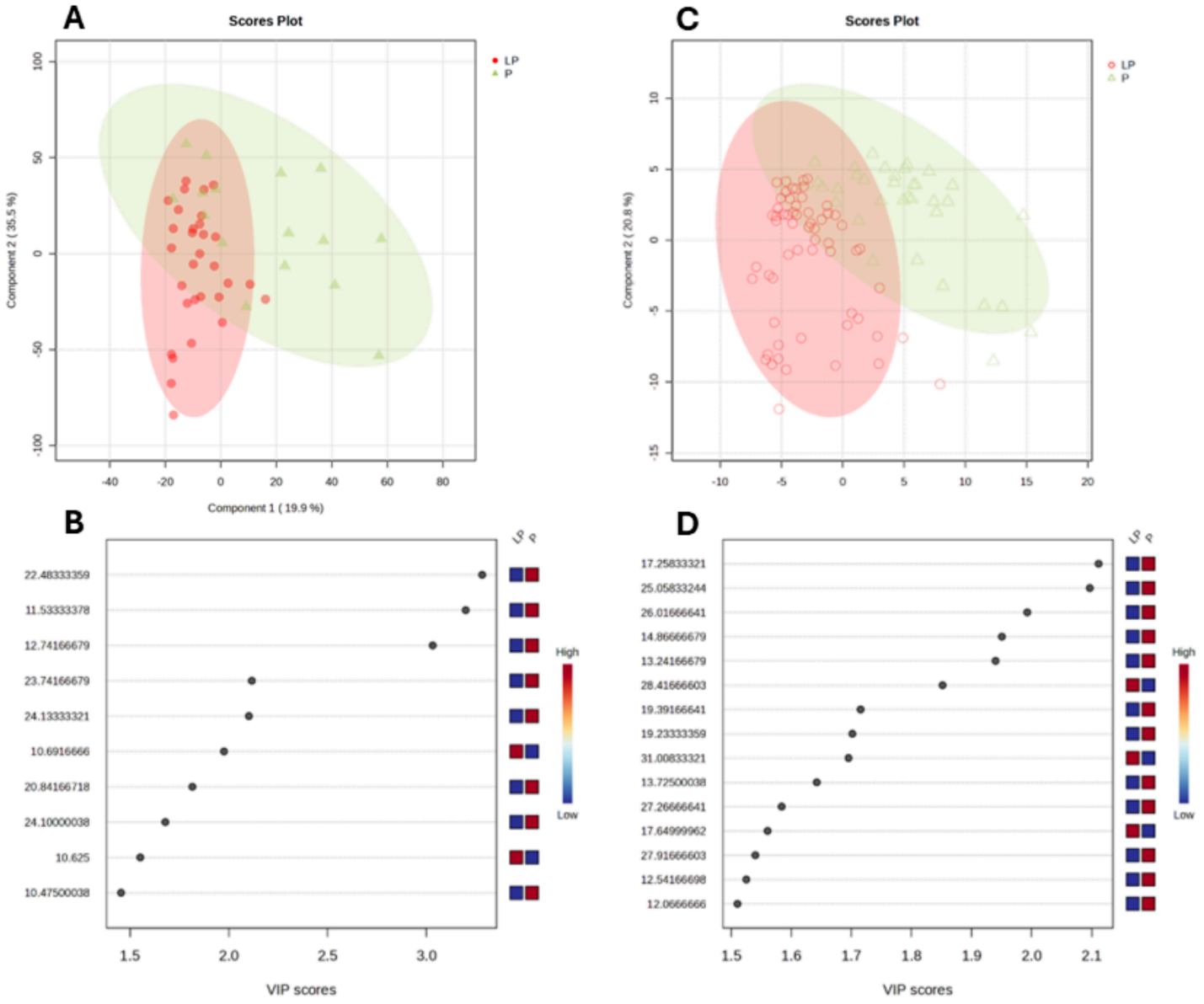


Figure 4

PLS-DA of volatiles (left panels, filled symbols) and waxes (right panels, open symbols) using oviposition preference as the classification variable (two levels). Preferred species (P, green) correspond to the two mandarins, whereas less-preferred species (LP, red) include oranges and lemon. (A) PLS-DA score plot for volatiles. (B) VIP scores of significant volatile features. (C) PLS-DA score plot for waxes. (D) VIP scores of significant wax features

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymateriallist.docx

- SupplementaryTablesvf.xlsx
- FigS1withlegend.tif
- FigS4withlegend.tif
- FigS5withlegend.tif
- FigS2withlegend.tif
- FigS3withlegend.tif