Title

Olfaction of leaf volatiles determines the most attractive host plant for *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae): potential pest management opportunities

Name(s) of the author(s)

C. Gunalan[1], S. Jeyarani[1,*], M. Murugan[1], S. Mohankumar[1], S. Haripriya[1], P. Saravan Kumar[2] and P. D. Kamala Jayanthi[2]

Affiliation(s) of the author(s)

[1]Tamil Nadu Agricultural University, Coimbatore 641 003, India

[2]ICAR-Indian Institute of Horticultural Research, Hesseraghatta Lake PO, Bengaluru 560 089, India

Corresponding author: Dr. S. Jeyarani, Professor and Head, Department of Crop Protection, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Kudumiyanmalai, 622 104, Tamil Nadu; E-mail: jayaranijawahar@gmail.com

The Rugose Spiraling Whitefly (RSW), an invasive polyphagous insect introduced into India during 2016, has threatened coconut and other crops. Natural infestation intensity data indicated, in order, the following most likely RSW hosts: *Cocos nucifera* L. (75.83%) > *Dypsis lutescens* H. Wendel (55.83%) > *Annona squamosa* L. (54.17%)> *Musa paradisiaca* L. (43.33%). A preference analysis of these four host plants found that coconut was the most favoured (8.17 spirals per 30 sq. cm and 33.04 eggs per spiral). Olfactometry of the headspace leaf volatiles revealed that *C. nucifera* (3.05 ± 0.27 min) and *D. lutescens* (1.67 ± 1.67 min) had longer residence durations and attracted more RSW females than other hosts. According to Principal Component Analysis (PCA), those potential hosts shared six volatile compounds, the most peculiar of which was 2-Ethyl-1-hexanol. GC-EAD analysis revealed that the substances 2-Ethyl-1,3-dioxolane, 1,3-Dioxolane, 2-propyl, Butanoic acid, 2-hydroxy-2-methyl-methyl ester, m-Ethyltoluene, p-Dichlorobenzene, and 2-Ethyl-1-hexanol evoked consistent olfactory responses in RSW. More studies into these chemicals might help develop parakairomones for managing RSW.

Keywords: Rugose Spiraling Whitefly, *Cocos nucifera*, Volatile organic compounds, GC-EAD, Principal component analysis

Rugose Spiraling Whitefly (RSW), *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) is a polyphagous pest described at the beginning of the twenty-first century in Belize, Central America[1]. The species soon expanded to sections of Central and North America[2-4], and Shanas *et al*.[5] documented its occurrence in the old world. This invasive pest was first reported on coconut, *Cocos nucifera* L. (Family: Arecaceae) in India during 2016 in the Southern peninsular region[6], and it has since spread to major coconut-growing regions across the country infesting other monocots and dicots as well[7,8]. It feeds on around 30 host plants in India and over 120 plant species worldwide, including numerous commercially significant horticultural and ornamental crops[9,10]. Its high reproduction rate, short life cycle, and broad host range enabled quick spread over several regions, causing severe losses up to 30.38 and 27.59 per cent on the coconut variety East Coast Tall[11]. Adult RSWs colonize the abaxial surface of the leaves, depositing eggs in spirals, while developing nymphs and adults feed on phloem sap. The honeydew expelled by the RSW attracts secondary growth of the fungi *Capnodium* spp., and *Leptoxyphium* sp. generating sooty mould on the adaxial surfaces of the lower crown canopy and interfering with photosynthesis[12,13]. Coconut palms with severe RSW infestations appear sick and turn blackish.

The RSW management in coconut plantations is exceptionally challenging due to the inaccessibility to plant canopy and impenetrability of pesticide applications. Although a diverse assemblage of RSW-associated natural enemies has been reported to effectively reduce the RSW populations, their performance often exhibits seasonal fluctuations, limiting sole reliance on such pest suppression agents[9,14-16]. Furthermore, RSW's rapid dissemination and persistent presence in infested environments necessitate developing alternate pest suppression measures. Semiochemical-based insect behaviour-modifying pest suppression approaches may pave the way for efficient and environmentally responsible RSW control[17].

Semiochemicals are critical for biologically managing insect pests as they are the principal source of interspecific interactions at different trophic levels[18]. Volatile Organic Compounds (VOCs) released by host plants play a major role in determining the herbivore's host plant location processes[19]. Determining the chemical cues responsible for the RSW attraction towards its host plant through detailed behavioural and electrophysiological studies involving Gas chromatography coupled electroantennogram detector (GC-EAD) can greatly aid in detecting EAD- responsive active chemical cues that may impact whitefly behaviour. The potent chemical cues thus discovered may be utilized to develop parakairomone based attractant formulations for managing whiteflies.

Materials and Methods

Host plant and insect multiplication

The RSW, *A. rugioperculatus* colony was established on coconut saplings (Cv. Chowghat Orange Dwarf). RSW adults were collected from the coconut palms in the research fields at coconut orchard, Tamil Nadu Agricultural University in Coimbatore, Tamil Nadu, India and were released into cages containing insect-free coconut saplings after taxonomic conformity and allowed to establish. The egg deposits were followed on alternate days for further life-stage advancements till the new adults emerged. These seedlings were watered twice a week, and polyfeed (19:19:19 - N:P:K) fertilizer @ 5g dissolved per litre of water was applied to the plants once in every two weeks. The colony was kept at 27 ± 1°C and 65 ± 10 % RH, and new saplings were introduced regularly to ensure stable colonies and a steady supply of test insects. RSW adult females from the cultured colony were used for all the experimental studies.

Assessment of whitefly infestation on different host plants

Degree of RSW infestation and adults per 20 sq. cm on various hosts were recorded during March 2022 at TNAU, Coimbatore, Tamil Nadu, India. Bleicher *et al*.[20] developed a rating

scale to quantify the degree of infestation by evaluating the branches at the median portion of plants and assigning scores to each plant. Host plants were scored from 0 to 4 based on insect colonisation and sooty mould development, with 0 indicating no infestation, 1 indicating the start of insect colonisation, 2 indicating a developing insect colony, 3 indicating a fully developed insect colony, and 4 indicating the presence of sooty mould in addition to a fully developed insect colony[20]. Observations were made on 30 plants per host, with each plant serving as a replication. Using the formula provided below, the degree of infestation was determined from the scores awarded to each host plant species.

$$\text{Degree of Infestation} = (\sum (S * F) / (N * Z)) * 100$$

where, S – Score value attributed per plant

F – Frequency of scores

Z – Maximum score value in rating scale

N – Total number of plants evaluated

RSW preference studies under caged conditions

The highly preferred host plants identified from field observations, *C. nucifera* (Arecaceae), *Musa paradisiaca* L. (Musaceae), *Annona squamosa* L. (Annonaceae), and *Dypsis lutescens* H. Wendel (Arecaceae), were obtained from the TNAU botanical garden nursery. One plant from each host was planted in a circular arrangement inside a net cage (2m x 2m x 2m: length x width x height) at the Department of Entomology, TNAU, Coimbatore. Before insect release, debris and dust on the leaf surface and insects etc inside the cage were eliminated. RSW-infested coconut leaf bits (6-8 inches long) with around 120 adult whiteflies from the colony were placed in the middle of the cage to ensure that all insects had equal access to every host. On the second day after the insects were released, each host plant was examined for egg spirals, and the cages were inspected for oviposition activity. With gentle air blowing, wax on the egg spiral was removed, and observations on egg spiral/30 sq. cm and eggs/spiral

were noted. The experiment was conducted with a completely randomized design and replicated twelve times.*Collection of host plant headspace volatiles*

Headspace volatiles from prospective host plants (*C. nucifera*, *M. paradisiaca*, *D. lutescens*, and *A. squamosa*) were collected using a custom designed field-based air entrainment device. A set-up consisting of polyvinyl acetate bags (150 cm x 75 cm in height x breadth) fitted with input and outflow silica tubes was designed to collect volatiles from host plants[21]. After passing through a humidifier and a charcoal filter, air from an air compressor reached the entrainment chamber through the input tube. Volatile trapping tubes constructed of Porapak Q (50 mg, 60/80 mesh; Supelco, Sigma Aldrich St Louis, United States) were installed inside the air outlet. These tubes were connected to the vacuum pump, and the airflow was set to 500 mL/min. The equipment was inverter-powered, and each host plant's volatile collection lasted 16 hours. The volatile substances trapped in Porapak Q were eluted in glass vials with 500µL of diethyl ether (purity > 99.5% pure, Merck) and stored in a freezer (−20◦C) until further use[22].

Olfactometer bioassays

RSW's behavioural responses to volatile extracts of four potential hosts (*C. nucifera*, *D. lutescens*, *A. squamosa*, and *M. paradisiaca*) were measured using four-arm clear acrylic olfactometers in single and multiple-choice experiments. In the single-choice olfactometer tests, one arm served as treatment arm (supplied with one of four different types of headspace volatiles), while remaining three arms served as control arms with solvent[23]. In multiple choice bioassays, all the four arms were provided with each host plant headspace sample. Each host's headspace extract (20µL) was swiftly deposited to the Whatman filter paper strips (4 2 cm, L B) and evaporated before being placed in the treatment arm. The control arm was set up with Whatman filter paper strips smeared with diethyl ether (20µL) following the evaporation step. After passing through a charcoal filter, clean air entered at 0.1 LPM through

each arm and was circulated to the insect release compartment. The proportion of responsive insects and the residence period of RSW females in each arm were determined. Continuous black and dotted lines were drawn on the olfactometer's clear lid to demarcate the zone of first choice and residence time[24]. The olfactometer was rotated 90° every 2 minutes to remove any directional bias. Thirty (n=30) RSW females were assessed in dual choice for their orientation to each plant volatile. In the multi-choice experiment with eighty (n=80) repetitions the most favoured host plant out of the four was confirmed.

Electrophysiological studies

Preparation of insect antenna

Young RSW female adults were collected in 2 m plastic vials (Eppendorf, United States) from laboratory grown RSW colonies and left to starve for 2 hours before conducting the electrophysiological investigation. An individual female RSW was inserted into a plastic micropipette (100 µL) tip for antennal preparation. With the head turned forward, modest air pressure was applied to wedge the RSW into the tip. The insect was pushed to thrust its head through the plastic tip. A pair of micro-scissors were used to remove the head, which was then placed on the EAD (Electroantennogram detector) probe holder (Syntech, Germany) with a small amount of electrode gel (Sigma Gel, United States) to make sure the antenna tip touched the recording electrode and the base of the antenna was on the neutral electrode. The generated EAD probe with the live antenna was then placed inside the pre-amplifier while being continuously sprayed with humidified air at a rate of 200 mL per minute[25].

Coupled Gas Chromatography-Electroantennographic Detection (GC-EAD)

The GC-EAD recordings (n =5) of RSW responses to coconut headspace volatiles were performed at Indian Council of Agricultural Research- Indian Institute of Horticultural Research (ICAR- IIHR)[26]. In a coupled GC-EAD system, the effluent from the GC column is routed simultaneously to the antennal preparation and the GC detector[27]. The host plant

volatiles were separated using an Agilent GC-7890 gas chromatography system with a flame ionization detector (FID) and an Agilent HP-5 (5%-phenyl)-methylpolysiloxane non-polar fused silica capillary tubing column (30 m length, 0.25 mm Diameter, and 0.25 µm film thickness). The thermal program was set at an initial step of 60°C for 1 minute with inlet temperature maintained at 250°C via splitless mode (40 mL/min ratio) and thereafter ramped at 15°C/min up to 240°C, held for 2 minutes using Nitrogen as the carrier gas at a flow rate of 1 mL/min. Humidified air and honey were utilized as negative and positive controls, respectively. AutoSpike software associated with the Syntech EAG Model IDAC-4 (Intelligent Data Acquisition Controller) was used to record odor stimulation data. Antennal responses for coconut headspace plant volatiles were recorded using SYNTECH Electroantennogram software based on the downward deflection signal (in mV), and signal means (in mV) of RSW antenna for coconut headspace plant volatiles were obtained[22]. The detected EAD compounds were authenticated by comparing the retention time and mass spectra to commercially accessible standards, and the remaining VOCs for which standards were unavailable were tentatively identified using the NIST spectral library 14.

Profiling of host plant volatiles through Gas Chromatography-Mass Spectrometry Analysis (GCMS)

Porapak Q elutes of headspace plant volatiles from four prospective hosts (*C. nucifera*, *D. lutescens*, *A. squamosa*, and *M. paradisiaca*) collected in the solvent (Diethyl ether, Merck, 99.97%) were analysed using GC-MS, Agilent 7890B GC system equipped with Mass Spectrometry, MS (Agilent 5977 MSD). The samples were examined using an Agilent (HP-5 MS UI) capillary column. The temperature setting was the same as indicated earlier. At a flow rate of 1 mL/min, helium was used as a carrier gas. The MS was set to full scan mode (70 eV) and the AMU range was set at 40-450. At a splitless mode ratio of 40 mL/min, one microliter of the sample was injected at a temperature of 250°C. Individual volatile chemicals

were identified by comparing the GC retention time and the MS spectra to the NIST 14 spectral database. Total volatile production was calculated as the sum of all GC-FID peak regions in the chromatogram, and specific compounds were quantified as a percentage of total volatile production[22].

Statistical analysis

The data were examined using IBM SPSS STATISTICS version 27.0.1 software. Whitefly responses in a single choice olfactometer tests to treated and control arms were compared using Chi square test ($\alpha = 0.05$). One way ANOVA with Tukey's HSD test ($\alpha = 0.05$) was used to compare the mean responses of whiteflies under caged conditions and multi choice olfactometer test. Principal Component Analysis (PCA) was used to assess the variability of volatile chemicals from headspace extracts of various hosts.

Results

Degree of infestation on different host plants

Field observations revealed that *C. nucifera* was the most preferred host, with a degree of infestation of 75.83±3.28 % and 12.59 adults per 20 sq. cm, followed by *D. lutescens* (55.83±3.92% with 7.61 adults/ 20 sq. cm) and *A. squamosa* (54.17±4.34% with 5.38 adults/20 sq. cm) (Table 1). Although there was a 49.17% infestation on *Psidium guajava* for which other invasive whitefly species, spiraling whitefly *A. dispersus*, and woolly whitefly *Aleurothrixus floccosus* were mainly responsible. *Musa paradisiaca*, with a degree of infestation of 43.33 % with 3.73 adults per 20 sq. cm, was ranked as the least favoured host plant.

Preference of RSW under caged conditions

Among the host plants observed, four host plants namely *C. nucifera, M. paradisiaca*, *D. lutescens*, and *A. squamosa* were identified as potential hosts. The preference was established through ovipositional experiments on young plants in cages. Studies on juvenile host plants in

cages showed that *C. nucifera* had the most egg spirals (8.17 egg spirals/30 sq. cm), with 33.04 eggs per spiral. The least amount of egg spirals (1.71 egg spirals/30 sq. cm) and eggs per spiral (14.58 eggs/spiral) were found in *D. lutescens*. The other hosts, *M. paradisiaca* and *A. squamosa* had 3.25 and 2.33 egg spirals/30 sq. cm and 20.29 and 18.58 eggs/spiral (Figure 1).

Single choice olfactometer bioassays

Three of the four the host plant volatiles when tested against the solvent control were found to be significantly attractive to the RSW (*A. squamosa*: Residence time: 5.74 ± 0.16 minutes; $\chi^2 = 13.07$; df = 1; $P < 0.001$; *C. nucifera*: 5.19 ± 0.13 minutes; $\chi^2 = 9.60$; df = 1; $P = 0.002$; *D. lutescens*: 4.92 ± 0.12 minutes; $\chi^2 = 1.07$; df = 1; $P = 0.30$ and *M. paradisiaca*: 4.54 ± 0.11 minutes; $\chi^2 = 6.67$; df = 1; $P = 0.010$) (Figure 2).

Multiple-choice olfactometer bioassay

RSW females exposed to the headspace volatiles of four prospective hosts demonstrated varied patterns of responses in the four-arm olfactometer. *C. nucifera* had the longest residence time (3.05 ± 0.27 minutes; $F = 29.44$, $P < 0.001$) and the highest proportion of female insects making their first choice (n =36) on arms, followed by *D. lutescens* and *A. squamosa*, while *M. paradisiaca* volatile extracts were the least attractive (Figure 3).

GC-EAD analysis of RSW

When headspace volatiles of the most preferred host plant, *C. nucifera* tested in GC-EAD analysis, the compounds namely 2-Ethyl-1,3-dioxolane, 1,3-Dioxolane,2-propyl, Butanoic acid-2-hydroxy-2-methyl-methyl ester, m-Ethyltoluene, p-Dichlorobenzene, and 2-Ethyl-1-hexanol elicited consistent responses in RSW antennae (Figure 4). Of these, p-Dichlorobenzene and 2-Ethyl-1-hexanol were the EAD active compounds that elicited the most robust olfactory response.

Headspace volatile profiling of potential hosts

Profiling of headspace volatiles revealed the presence of 56 VOCs in *C. nucifera*, 44 VOCs in *M. paradisiaca*, 30 VOCs in *D. lutescens*, and 43 VOCs (Figure 5) in *A. squamosa*, of which only six compounds α-Hydroxypropanoic acid, Hexadecane, n-Pentadecane, 2-Ethyl-1-hexanol, Tetradecane, and Butyl isobutyl phthalate were found to be common in the headspace extracts of all four potential hosts (Table 2), which would have been responsible for the insect attraction.

Discussion

Plant cues help insects to orient to suitable host plants[28]. Thus, the process of insect host selection is influenced by a combination of substances rather than a single primary compound[29]. Compared to the other three hosts investigated, blend of compounds with the 32 chemicals specific to *C. nucifera* would have increased the attractiveness of whiteflies. The concentration of various compounds in a complex mixture is also critical, as high and low concentrations might have a detrimental impact compared to the actual concentration of the same compound[30]. Less attractive hosts are distinguished by the absence of a specific volatile ingredient, or insufficient concentration of volatile compounds in a complex mixture, or by a higher proportion of neutral molecules that conceal the attractants[31,32].

When compared to other host plants such as coconut, *C. nucifera* L. (Arecaceae), avocado *Persea americana* Mill. (Lauraceae), black olive *Bucida buceras* L. (Combretaceae), and giant white bird of paradise *Strelitzia nicolai* Regel & Körn (Strelitziaceae), the Gumbo limbo, *Bursera simaruba* (L.) Sarg. (Burceraceae) was the most preferred host plant species for RSW oviposition[33]. Interestingly, coconut exhibited the highest RSW survival and adult emergence rates, trailing only gumbo limbo regarding ovipositional preference for RSW. The leaf size did not affect how many eggs were laid[33]. The most preferred host plant for RSW, whose presence is rare in the Nearctic region, is *C. nucifera*, which is widely produced in the

Oriental region. In many urban locations of the Nearctic region, RSW has caused annoyance and economic harm by overusing various plant species in the landscape and nurseries[33].

RSW behavioural responses were further corroborated by field studies, which revealed that RSW colonies were substantially higher in *C. nucifera* than in any other host plant. Previously, GC-EAD experiments on whiteflies demonstrated that the organic compounds α-terpinene, 7-epizingiberene, *R*-curcumene, methyl eugenol, heptadecane, n-undecane, 2, 6, 10, 15-tetramethyl, 6-methylhydrocoumarin, 4-ethoxy-, ethyl ester, 2-methoxy-1,3-dioxolane, sabinene, cyclofenchene, ethyl benzoate, benzoic acid, 2,6,11-trimethyldodecane, 5,6-dimethyldecane, n-decane, (E)-ocimenone, 4-ethyltetradecane, farnesane, linalool oxide, 2-methylheptadecane, 2-methylpentadecane, 3-carene, 2- methoxy-1,3-dioxolane, pcymene-2,5-diol, 8,9-dehydrothymol and 8-methylheptadecane produced antennal responses in *B. tabaci*[22,34-36], while *A. dispersus* consistently responded positively to (±)-2-hexanol[37]. Two compounds that generated stronger olfactory responses in RSW, p-Dichlorobenzene, and 2-ethyl-1-hexanol, have been shown to impact orientation in other insect species. Dichlorobenzene was utilized as an insect repellent, but 2-ethyl-1-hexanol was discovered to be a possible *B. tabaci* attractant[38]. Some of the 110 discovered volatile organic compounds identified in the current study from the four host plants had been documented to act as semiochemical (Table 3) in attracting or repelling other whitefly species. The variability of various volatile chemicals generated by the four hosts was examined using PCA. The three-dimensional scores (PC1 x PC2 x PC3) illustrate the association between different volatile compounds (Figure 6).

Among the six volatile molecules shared by four hosts, α-Hydroxypropanoic acid, Hexadecane, n-Pentadecane, Butyl isobutyl phthalate, and Tetradecane interacted positively, although 2-Ethyl-1-hexanol was unique among the compounds mentioned above.

Conclusion

Whiteflies' olfactory system is so advanced that they can distinguish between stereoisomers of volatile organic substances which enables successful exploitation of semiochemicals in eco-friendly pest management strategies. Behavioural responses of *Aleurodicus rugioperculatus* to headspace extracts of potential hosts revealed that *Cocos nucifera* was the most preferred host plant. The findings indicate that the volatile chemicals in *C. nucifera* are the key attracting factors for *A. rugioperculatus*. It seems probable that 2-ethyl-1-hexanol would be a possible attractant, which could be tested further using in-vivo and in-vitro experiments.

Conflict of interest: The authors declare no conflict of interest.

References

1. Martin, J. H., Whiteflies of Belize (Hemiptera: Aleyrodidae). Part 1- Introduction and account of the subfamily Aleurodicinae Quaintance & baker. *Zootaxa*, 2004, **681**(1), 1-19.
2. Evans, G. A., The whiteflies (Hemiptera: Aleyrodidae) of the world and their host plants and natural enemies. USDA, Animal Plant Health Inspection Service, 2007; http://keys.lucidcentral.org/keys/v3/whitefly/PDF_PwP%20ETC/world-whitefly-catalog-Evans.pdf. (accessed on 20 March 2023).
3. Stocks, I. C. and Hodges, G., The rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin, a new exotic whitefly in South Florida (Hemiptera: Aleyrodidae). Gainesville, Florida Department of Agriculture and Consumer Services, Division of Plant Industry, 2012; http://freshfromflorida.s3.amazonaws.com/*aleurodicusrugioperculatus*-pest-alert. Pdf. (accessed on 3 May 2022).
4. Kumar, V., McKenzie, C. L., Mannion, C., Stocks, I., Smith, T. and Osborne, L. S., Rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae). University of Florida, IFAS Extension, 2013; https://entnemdept. ufl. edu/creatures/orn/*Aleurodicus_rugioperculatus*. html. (accessed on 14 July 2022).
5. Shanas, S., Job, J., Joseph, T. and Anju Krishnan, G., First report of the invasive rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) from the old world. *Entomon*, 2016, **41**(4), 365-368.
6. Sundararaj, R. and Selvaraj, K., Invasion of rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae): a potential threat to coconut in India. *Phytoparasitica*, 2017, **45**, 71-74.

7. Rao, N. C., Roshan, D. R., Rao, G. K. and Ramanandam, G., A review on rugose spiraling whitefly, *Aleurodicus rugioperculatus* martin (Hemiptera: Aleyrodidae) in India. *J. pharmacogn. phytochem.*, 2018, **7**(5), 948-953.
8. Wankhede, S. M., Shinde, V. V. and Ghavale, S. L., Status of Rugose spiraling whitefly (*Aleurodicus rugioperculatus* Martin) in Konkan region of Maharashtra. *Pest manage. hortic. ecsyst.*, 2021, **27**(2), 190-195.
9. Francis, A. W., Stocks, I. C., Smith, T. R., Boughton, A. J., Mannion, C. M. and Osborne, L. S., Host plants and natural enemies of rugose spiraling whitefly (Hemiptera: Aleyrodidae) in Florida. *Fla. Entomol.*, 2016, **99**(1), 150-153.
10. Pradhan, S. K., Shylesha, A. N., Selvaraj, K. and Sumalatha, B. V., Distribution, Host Range and Status of Invasive Rugose Spiraling Whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) in Karnataka. *Agric. Res.* 2021, **11**, 499-505.
11. Puvvala, V. R., Nagulapati, B. V. C. R., Ede, P., Alwala, K., Kavuru, U. and Ganta, K. R., Yield loss in coconut variety East Coast Tall (ECT) owing to rugose spiralling whitefly (RSW), *Aleurodicus rugioperculatus* M. infestation. *Phytoparasitica*, 2023, **51**(5), 1017-1024.
12. Arun, K., Janeeshma. E., Job, J. and Puthur. J. T., Physiochemical responses in coconut leaves infected by spiraling whitefly and the associated sooty mould formation. *Acta. Physiol. Plant.*, 2021, **43**, 1-3.
13. Josephrajkumar, A., Mohan, C., Poorani, J., Babu, M., Daliyamol, Krishnakumar, V., Hegde, V. and Chowdappa, P., Discovery of a sooty mould scavenging beetle, *Leiochrinus nilgirianus* Kaszab (Coleoptera: Tenebrionidae) on coconut palms infested by the invasive rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae). *Phytoparasitica*, 2018, **46**, 57-61.

14. Selvaraj, K., Sundararaj, R., Venkatesan, T., Ballal, C. R., Jalali, S. K., Gupta, A. and Mrudula, H. K., Potential natural enemies of the invasive rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin in India. *Journal of Biological Control*, 2016, **30**(4), 236-239.

15. Kumar, V., Francis, A., Avery, P. B., McKenzie, C. L. and Osborne, L. S., Assessing compatibility of *Isaria fumosorosea* and buprofezin for mitigation of *Aleurodicus rugioperculatus* (Hemiptera: Aleyrodidae): An invasive pest in the Florida landscape. *J. Econ. Entomol.*, 2018, **111**(3), 1069-1079.

16. Visalakshi, M., Selvaraj, K., Sumalatha, B. P. and Poornesha, B., Biological control of invasive pest, rugose spiraling whitefly in coconut and impact on environment. *J. Entomol. Zool. Stud.*, 2021, **9**(1), 1215-1218.

17. Schlaeger, S., Pickett, J. A. and Birkett, M. A., Prospects for management of whitefly using plant semio-chemicals, compared with related pests. *Pest Manag. Sci.*, 2018, **74**(11), 2405-2411.

18. Smart, L. E., Aradottir, G. I. and Bruce, T. J. A., Role of semio-chemicals in integrated pest management. In: *Integrated pest management* (ed Abrol, D. P.), Academic Press, Cambridge, Massachusetts, USA, 2014, pp. 93-109.

19. Visser, J. H., Host odor perception in phytophagous insects. *Annu. Rev. Entomol.*, 1986, **31**(1), 121-144.

20. Bleicher, E., Melo, Q. M. and Furtado, I. P., Sugestões de técnicas de amostragem para as principais pragas do cajueiro. *EMBRAPA-CNPAT. Comunicado Técnico*., 1993, **6**: 1-5

21. Park, I., Schwarzländer, M., Hinz, H. L., Schaffner, U. and Eigenbrode, S. D., A simple approach to evaluate behavioural responses of insect herbivores to olfactory and visual cues simultaneously: the double stacked y-tube device and portable volatile collection system. *Arthropod Plant Interact.*, 2019, **13**, 139-149.

22. Yadav, R. K., Kambham, M. R., Parepally, S. K., Vyas, M., Manem, K. R. and Kamala Jayanthi, P. D., Encounter with a selfish virus sabotages its vector to orient toward requisite host plant: A case study with chili leaf curl virus-whitefly. *Front. Ecol. Evol.*, 2022, **10**, 819023; https://doi.org/10.3389/fevo.2022.819023.

23. Saraiva, W. V., Dias-Pini, N. S., Alves Filho, E. G., Melo, J. W., Fancelli, M., Coutinho, C. R., Zocolo, G. J., Rodrigues, T. H., Duarte, P. M., Macedo, V. H. and Goiana, E. S., Attraction of whitefly *Aleurodicus cocois* mediated by cashew volatiles. *Phytoparasitica*, 2022, **50**(2), 399-410.

24. Volpe, H. X., Zanardi, O. Z., Magnani, R. F., Luvizotto, R. A., Esperanca, V., Freitas, R. D., Delfino, J. Y., Mulinari, T. A., Carvalho, R. I., Wulff, N. A. and Miranda, M. P., Behavioral responses of *Diaphorina citri* to host plant volatiles in multiple-choice olfactometers are affected in interpretable ways by effects of background colors and airflows. *PLoS One*, 2020, **15**(7), e0235630; https://doi.org/10.1371/journal.pone.0235630.

25. Pagadala Damodaram, K. J., Kempraj, V., Aurade, R. M., Venkataramanappa, R. K., Nandagopal, B., Verghese, A. and Bruce, T., Oviposition site-selection by *Bactrocera dorsalis* is mediated through an innate recognition template tuned to γ-octalactone. *PLoS One*, 2014, **9**(1), e85764; https://doi.org/10.1371/journal.pone.0085764.

26. Kamala Jayanthi, P. D., Woodcock, C. M., Caulfield, J., Birkett, M. A. and Bruce, T. J., Isolation and identification of host cues from mango, *Mangifera indica*, that attract gravid female oriental fruit fly, *Bactrocera dorsalis*. *J. Chem. Ecol.*, 2012, **38**, 361-369.

27. Wadhams, L. J., The use of coupled gas chromatography: electrophysiological techniques in the identification of insect pheromones. In: *Chromatography and Isolation of Insect Hormones and Pheromones* (eds McCaffery, A. R. and Wilson, I. D.), Springer, New York, USA, 1990, pp. 289-298.

28. Sacchetti, P., Rossi, E., Bellini, L., Vernieri, P., Cioni, P. L. and Flamini, G., Volatile organic compounds emitted by bottlebrush species affect the behaviour of the sweet potato whitefly. *Arthropod Plant Interact.*, 2015, **9**, 393-403.
29. Bruce, T. J. and Pickett, J. A., Perception of plant volatile blends by herbivorous insects–finding the right mix. *Phytochemistry*, 2011, **72**(13), 1605-1611.
30. Sadeh, D., Nitzan, N., Shachter, A., Chaimovitsh, D., Dudai, N. and Ghanim, M., Whitefly attraction to rosemary (*Rosmarinus officinialis* L.) is associated with volatile composition and quantity. *PLoS One*, 2017, **12**(5), e0177483; https://doi.org/10.1371/journal.pone.0177483.
31. Togni, P. H, Laumann, R. A., Medeiros, M. A. and Sujii, E. R., Odour masking of tomato volatiles by coriander volatiles in host plant selection of *Bemisia tabaci* biotype B. *Entomol. Exp. Appl.*, 2010, **136**(2), 164-173.
32. Kergunteuil, A., Dugravot, S., Danner, H., Van Dam, N. M. and Cortesero, A. M., Characterizing volatiles and attractiveness of five brassicaceous plants with potential for a 'push-pull' strategy toward the cabbage root fly, *Delia radicum*. *J. Chem. Ecol.*, 2015, **41**, 330-339.
33. Taravati, S., Mannion, C., McKenzie, C. and Osborne, L., Oviposition preference of rugose spiraling whitefly (Hemiptera: Aleyrodidae) on five host plant species. *Fla. Entomol.*, 2018, **1**, 611-616.
34. Bleeker, P. M., Diergaarde, P. J., Ament, K., Guerra, J., Weidner, M., Schutz, S., De Both, M. T., Haring, M. A. and Schuurink, R. C., The role of specific tomato volatiles in tomato-whitefly interaction. *Plant Physiol.*, 2009, **151**(2), 925-935.
35. Bleeker, P. M., Diergaarde, P. J., Ament, K., Schütz, S., Johne, B., Dijkink, J., Hiemstra, H., De Gelder, R., De Both, M. T., Sabelis, M. W. and Haring, M. A., Tomato-produced

7-epizingiberene and R-curcumene act as repellents to whiteflies. *Phytochemistry*, 2011, **72**(1), 68-73.

36. Padala, V. K., Kumar, P., Ramya, N., Jayanthi, P. D., Aromatic plant odours of *Anethum graveolens* and *Coriandrum sativum* repel whitefly, *Bemisia tabaci* in tomato. *Curr. Sci.*, 2023, **124**(2), 231-238.
37. Zheng, L. X., Wu, W. J. and Fu Y. G., (±)-2-Hexanol from *Pterocarpus indicus* leaves as attractant for female *Aleurodicus dispersus* (Hemiptera: Aleyrodidae). *Afr. Entomol.*, 2014, **22**(2), 267-272.
38. Chen, G., Su, Q., Shi, X., Liu, X., Peng, Z., Zheng, H., Xie, W., Xu, B., Wang, S., Wu, Q. and Zhou, X., Odor, not performance, dictates *Bemisia tabaci*'s selection between healthy and virus infected plants. *Front. Physiol.*, 2017, **8**, 146; https://doi.org/10.3389/fphys.2017.00146.
39. Tu, H. and Qin, Y., Repellent effects of different celery varieties in *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotype Q. *J. Econ. Entomol.*, 2017, **110**(3), 1307-1316.
40. Matu, F. K., Murungi, L. K., Mohamed, S. and Deletre, E., Behavioral response of the greenhouse whitefly (*Trialeurodes vaporariorum*) to plant volatiles of *Ocimum basilicum* and *Tagetes minuta*. *Chemoecology*, **31**, 47-62.

Table 1. Host spectrum of RSW with all life stages and significant damage

Host Plant	Family	Degree of Infestation (%) ± SE*	Adults / 20 cm^2 **	Co-existing whitefly species at observational time
Annona reticulata	Annonaceae	31.67±3.16 (34.19) ^{bc}	3.44 (1.85) ^{d}	*Aleurodicus rugioperculatus*✦, *Paraleyrodes bondari*, *P. minei*
A. squamosa	Annonaceae	54.17±4.34 (47.38) ^{d}	5.38 (2.31) ^{e}	*A. rugioperculatus*✦, *A. dispersus*, *P. bondari*✦, *P. minei*, *Paelius sp.*,
Canna indica	Cannaceae	19.17±3.10 (25.92) ^{ab}	0.97 (0.98) ^{b}	*A. rugioperculatus*✦, *P. bondari*
Cocos nucifera	Arecaceae	75.83±3.28 (60.56) ^{e}	12.59 (3.54) ^{g}	*A. rugioperculatus*✦, *A. dispersus*, *P. bondari*, *P. minei*, *Aleurotrachelus atratus*
Dypsis lutescens	Arecaceae	55.83±3.92 (48.40) ^{d}	7.61 (2.75) ^{f}	*A. rugioperculatus*✦, *P. bondari*, *P. minei*, *A. atratus*
Manilkara zapota	Sapotaceae	11.67±2.32 (19.79) ^{a}	0.62 (0.78) ^{a}	*A. rugioperculatus*✦, *P. bondari*, *P. minei*
Musa paradisiaca	Musaceae	43.33±3.16 (41.13) ^{cd}	3.73 (1.92) ^{d}	*A. rugioperculatus*✦, *A. dispersus*, *P. bondari*
Psidium guajava	Myrtaceae	49.17±4.56 (44.50) ^{d}	0.79 (0.88) ^{ab}	*A. rugioperculatus*, *A. dispersus*✦, *P. bondari*, *P. minei*, *Aleurothrixus floccosus*✦
Terminalia catappa	Combretaceae	20.83±3.19 (27.02) ^{ab}	3.17 (1.77) ^{d}	*A. rugioperculatus*✦, *A. dispersus*, *P. bondari*
Theobroma cacao	Malvaceae	15.83±2.81 (23.43) ^{a}	1.60 (1.26) ^{c}	*A. rugioperculatus*✦, *P. bondari*
P value		< 0.0001	< 0.0001	
Coefficient of variation (%)		0.35	0.94	

* Values in the parentheses are arcsine transformed values; ** Values in the parentheses are square root transformed values; means followed by a common letter(s) are not significantly different by Tukey's HSD test (α = 0.05); ✦ Predominant whitefly species on the specific host plant

Table 2. Volatile compounds in headspace extracts of four potential host plants

Host Plant	Volatile organic compounds present
Annona squamosa *Cocos nucifera* *Dypsis lutescens* *Musa paradisiaca*	α-Hydroxypropanoic acid; n-Tetradecane; Hexadecane; 2-Ethyl-1-hexanol; n-Pentadecane; Butyl isobutyl phthalate
A. squamosa *C. nucifera* *D. lutescens*	Decanal; n-Nonanal
A. squamosa *C. nucifera* *M. paradisiaca*	Tridecane; Tetradecane, 2,6,10-trimethyl; Dodecane
A. squamosa *D. lutescens* *M. paradisiaca*	Dodecane, 2,6,11-trimethyl; Octadecane
C. nucifera *D. lutescens* *M. paradisiaca*	2,4-Di-tert-butylphenol; 2-Hexadecanol
A. squamosa *C. nucifera*	4-Methyldodecane; 3,7,11-Trimethyl-1-dodecanol
A. squamosa *D. lutescens*	3-Hexene, 1-(1-ethoxyethoxy)-, (Z); Diethyl disulfide; 5-Butyl-5-ethylheptadecane; Hexadecanoic acid, methyl ester; Isopropyl palmitate
A. squamosa *M. paradisiaca*	4,6-Dimethyldodecane; 2-Methylpentadecane; Decane, 2,3,5,8-tetramethyl; 2-Methylheptadecane; 5,5,7,7-Tetraethylundecane; Farnesane; β cis-Ocimene; Undecane
C. nucifera *D. lutescens*	Heneicosane
C. nucifera *M. paradisiaca*	1,3,5-Trioxepane; 3-Methylheptadecane; β-Caryophyllene; Humulene; 3-Methyltridecane; m-Xylene, 5-tert-butyl; 2-Methyldodecane; 2,3-Butanediol
D. lutescens *M. paradisiaca*	5,8-Diethyldodecane; Heptadecane; 2-Decen-1-ol, (E)
A. squamosa	β-Maaliene; Neoclovene; 10-Methylicosane; (E)-4,8-Dimethylnona-1,3,7-triene; 2-Methylicosane; α -Gurjunene; α -Terpinyl acetate; Ethanol, 2-isobutoxy; Eicosane; Nonane, 4,5-dimethyl; Isopropyl myristate; 2-Bromo dodecane; 1,3,7,11-Tridecatetraene, 4,8,12-trimethyl-, (3E,7E); Methyl salicylate; 2,6,10,14-Tetramethylhexadecane
C. nucifera	p-Diisopropylbenzene; 3-Hexanol, 3-methyl; 2-Heptadecanol; 2-Butyl-1-octanol; psi.-Cumene; o-Methylphenol; 2,5-di-tert-Butyl-1,4-benzoquinone; 9-Tetradecen-1-ol, acetate, (Z); Benzene, 1,3-bis(1-methylethyl); 2-Butanone, 3-hydroxy; 1,3,7-Octatriene, 3,7-dimethyl; Butanoic acid, 2-hydroxy-2-methyl, methyl ester; 1-Hexadecanol, acetate; α -Ethylhexanoic acid; Terpinolene; 6-Methyloctadecane; α -Copaene; Undecanal; Benzene, 1-(1,1-dimethylethyl)-4-ethyl; 3-Methylpentadecane; m-Ethyltoluene; 3,3-Diethyltridecane; 2-Ethyl-1,3-dioxolane; Cyclopentadecanol; 1,19-Eicosadiene; p-Dichlorobenzene; Geranyl isovalerate; α-Himachalene; β -Longipinene; m-Cresol; 3-Hexanol, 3,5-dimethyl; 1,3-Dioxolane, 2-propyl
D. lutescens	5-Hexene-1-ol, acetate; Benzothiazole, 2-(methylthio); Naphthalene; Dibutyl phthalate; Butane, 1-(1-ethoxyethoxy); Ethyl 3-methylbutan-2-yl carbonate; 1,3-Dioxolane, 2-butyl; Methyl Z-11-tetradecenoate; Decyl isopropyl ether
M. paradisiaca	Pentadecane, 2,6,10-trimethyl; 3,7-Dimethyldecane; 2,4,6-Octatriene, 2,6-

	dimethyl-, (E, Z); 2,6-Dimethyldecane; Benzene, 1,4-bis(1-methylethyl); Nonadecane; α -Pinene; 2-Hexyl-1-decanol; 7,7-Diethylheptadecane; β-Pinene; 2-Methyldecane; Phytane

Table 3. Identified volatile organic compounds with semiochemical role in different whitefly species

Volatile compound	Stated effect	Whitefly species	Reference
2-Ethyl-1-hexanol	Attractant	*Bemisia tabaci*	Chen *et al.*[38]
α-Pinene	Repellent	*B. tabaci*	Chen *et al.*[38]
β Caryophyllene	Attractant	*B. tabaci*	Sadeh *et al.*[30]
β cis-ocimene	Repellent	*B. tabaci*	Tu and Qin[39]
5-Hexene-1-ol, acetate	Attractant	*Trialeurodes vaporariorum*	Matu *et al.*[40]
β cis-ocimene	Repellent	*T. vaporariorum*	Matu *et al.*[40]

Figure 1. Host preference of RSW under caged condition. a) Number of egg spirals/ 30cm^2. b) Number of eggs/spiral. Means followed by a common letter(s) are not significantly different by Tukey's HSD test (α = 0.05).

Figure 2. Response of RSW in a dual choice olfactometer a) Percentual entries (Frequency) of RSW in each arm. b) Residence time of RSW females in minutes ± SE in treated and control arms. Means followed by a common letter(s) are not significantly different by Tukey's HSD test (α = 0.05).

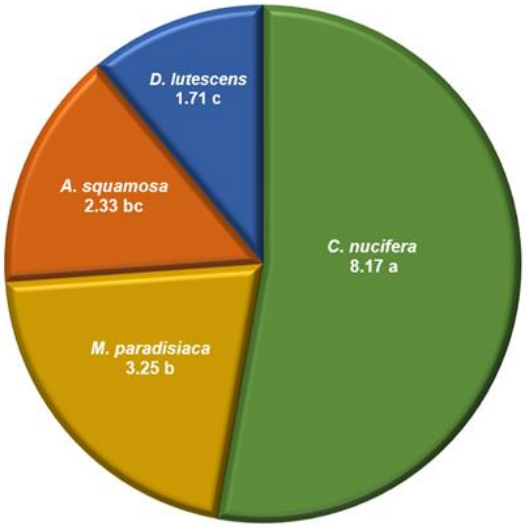
Figure 3. Residence time of RSW females in minutes ± SE in different arms of host plant volatile. Means followed by a common letter(s) are not significantly different by Tukey's HSD test (α = 0.05); n – total number of insects making their first choice.

Figure 4. GC-EAD responses of RSW to the headspace volatiles of *C. nucifera*

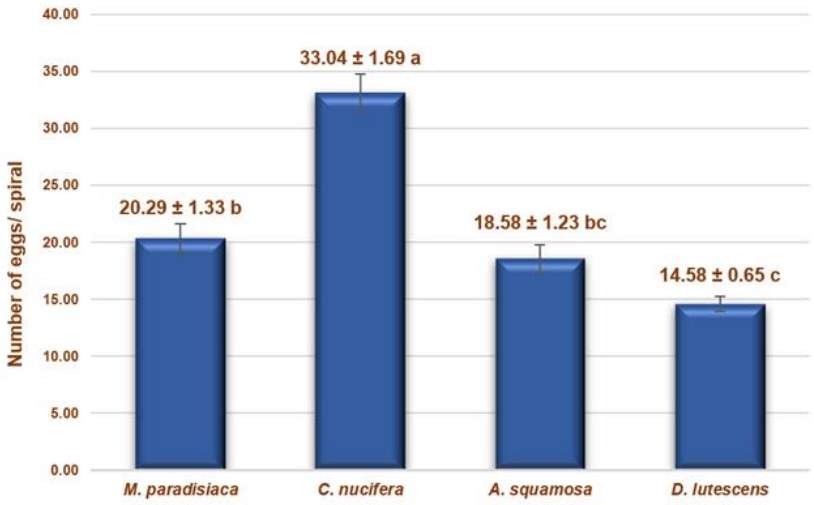
Figure 5. Venn diagram describing number of volatile compounds specific to potential hosts

Figure 6. Principal component analysis loading plots of volatile compounds in headspace extracts of potential hosts specifying the magnitude and correlation between the compounds. Compound codes: C1-1,3,5-Trioxepane; C2- 2,3-Butanediol; C3- α-Hydroxypropanoic acid; C4- Diethyl disulfide; C5- 2-Ethyl-1-hexanol; C6- β-cis-Ocimene; C7- 3-Hexene, 1-(1-ethoxyethoxy)-, (Z); C8- Undecane; C9- n-Nonanal; C10- m-Xylene, 5-tert-butyl; C11- Dodecane; C12- Decanal; C13- 4-Methyldodecane; C14- 2-Decen-1-ol, (E); C15- 2-Methyldodecane; C16- Dodecane, 2,6,11-trimethyl; C17- Tridecane; C18- Decane, 2,3,5,8-tetramethyl; C19- 4,6-Dimethyldodecane; C20- Farnesane; C21- 3-Methyltridecane; C22- n-Tetradecane; C23- β-Caryophyllene; C24- Humulene; C25- Pentadecane; C26- 2,4-Di-tert-butylphenol; C27- Tetradecane, 2,6,10-trimethyl; C28- 2-Methylpentadecane; C29- 5,8-Diethyldodecane; C30- 3,7,11-Trimethyl-1-dodecanol; C31- Hexadecane; C32- Heptadecane; C33- 2-Hexadecanol; C34- 2-Methylheptadecane; C35- 3-Methylheptadecane; C36- Octadecane; C37- Butyl isobutyl phthalate; C38- Hexadecanoic acid, 14-methyl, methyl ester; C39- Isopropyl palmitate; C40- Heneicosane; C41- 5-Butyl-5-ethylheptadecane

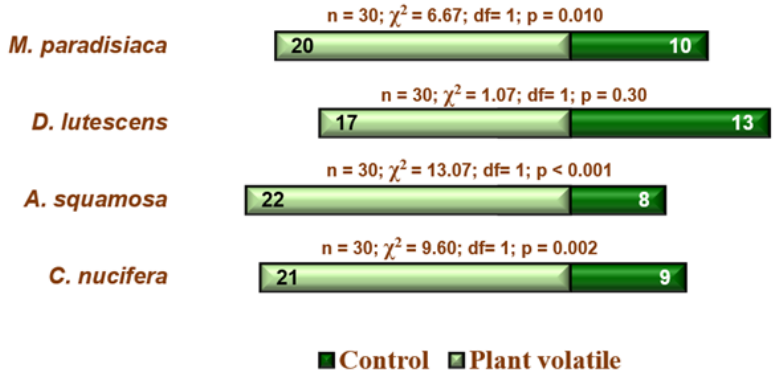
a


D. lutescens
1.71 c
A. squamosa
2.33 bc
C. nucifera
8.17 a
M. paradisiaca
3.25 b

b


Number of eggs/ spiral
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
20.29 ± 1.33 b
33.04 ± 1.69 a
18.58 ± 1.23 bc
14.58 ± 0.65 c
M. paradisiaca
C. nucifera
A. squamosa
D. lutescens

a



b