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Original Research Paper

Biochemical profiling of epidermal body wax and honeydew excretions in polyphagous mealybug species

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ABSTRACT

Mealybugs (Hemiptera: Pseudococcidae) are vulnerable soft bodied insects protected by an external hydrophobic wax coating, which shields them from extreme temperatures and pesticide applications. This study characterized the biochemical composition of mealybug body wax and sugar profile of honeydew, *viz*., *Maconellicoccus hirsutus* Green, *Planococcus citrii* Risso, *Ferrisia virgata* Cockerell, *Rastrococcus iceryoides* Green. Gas chromatography-mass spectrometry and liquid chromatography with tandem mass spectrometry profiling, revealed that mealybug body wax is primarily composed of long-chain hydrocarbons, alcohols, esters, aldehydes and ketones. The major hydrocarbons detected included phenyl tetramethyl cyclopentadiene, 1,19-eicosadiene tetratriacontane. Key wax esters identified included ä-methyl-2-(2-hydroxyethyl)-2-devinylchlorine 6 trimethyl ester, with notable interspecies variation in hydrocarbon composition. The honeydew primarily composed of sugars such as fructose, glucose and sucrose, with distinct dominance patterns among species. *Rastrococcus iceryoides* had glucose as the dominant sugar (32.12 g/100 g of honeydew), while fructose was most abundant in *M. hirsutus* (25.18 g/100 g) and *P. citrii* (16.1 g/100 g). Understanding the biochemical makeup of mealybug body wax and honeydew composition could aid in developing new management tactics for controlling these notoriously hard-to-kill pests.

Keywords: Honeydew, LC-MS/MS, mealybug wax, mealybugs, sugar profiling, wax profiling

INTRODCUTION

Mealybugs (Hemiptera: Pseudococcidae) are highly polyphagous pests that cause substantial economic losses in a wide range of horticultural crops. In India, the pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) is among the most destructive species and has been reported huge yield losses in grape under severe infestations (Mani et al., 2012; Mani & Shivaraju, 2016). Other major economically important mealybug species in India include the citrus mealybug *Planococcus citri* (Risso), mango mealybug *Rastrococcus iceryoides* (Green), cacao mealybug *Planococcus lilacinus* (Cockerell), and striped mealybug *Ferrisia virgata* (Cockerell). A major factor contributing to mealybug persistence is the secretion of a hydrophobic wax coating through dermal pores. This wax protects mealybugs from abiotic stresses, reducing pesticide penetration (Mwanauta et al., 2022; Sahito et al., 2022).

In addition to wax secretion, mealybugs excrete large quantities of honeydew as phloem sap feeders. This sugar rich exudate promotes the growth of sooty mould on plant surfaces, reducing photosynthetic efficiency and serves as a kairomonal resource for a wide range of predators and parasitoids (Heidari & Copland, 1993). Accordingly, mealybugs have long been managed through classical and augmentative biological control strategies employing natural enemies (Van Lenteren et al., 2018; Andreason et al., 2019; Mani et al., 2022). However, the effectiveness of these ecological interactions is strongly influenced by the chemical characteristics of honeydew as well as the protective wax layer that affects mealybug survival and susceptibility to control measures.

Despite the clear ecological and management relevance of mealybug body wax and honeydew, comparative biochemical information across major polyphagous species remains limited. The present study compared whether interspecific variation in wax and honeydew

composition with implications for insecticide penetration and interactions with natural enemies. Accordingly, we analysed the body wax and honeydew of *M. hirsutus*, *P. citri*, *R. iceryoides*, and *F. virgata.* The findings are expected to enhance our understanding of mealybug chemical ecology and contribute to the development of more effective and sustainable mealybug management strategies.

MATERIALS AND METHODS

Chemicals and reagents

Acetonitrile, methanol, hexane, chloroform, acetic acid water used for the study were LC/MS grade D(+)-sucrose (≤99.5%), D-(+)-maltose (≤99%), D-(+)-trehalose (≤99%), D-(–)-arabinose (≤98%), D-(+)-xylose (≤99%), D-(–)-ribose (≤99%), inositol (≤95%), D-(–)-sorbitol (≤98%), lactose (≤98%), D-(+)-glucose (≤99.5%), D-(+)-galactose (≤99%), D-(–)-ribose (≤99%), D-(+)-fructose (≤98%), L-(6-13c)-fucose, rhamnose (≤99%) were purchased from Sigma-Aldrich (India).

Laboratory culturing of mealybugs

Mealybug species (*M. hirsutus*, *P. citrii R. iceryoides*) were reared on fully matured, orange skinned pumpkin fruits (*Cucurbita moschata* D.) as laboratory hosts (Mineo 1967; Chacko et al.; 1978; Singh, 1978). Healthy pumpkins with ridges, grooves a small stalk were selected for mealybug culturing. The pumpkins were cleaned any wounds were sealed with paraffin wax to avoid secondary infections. For *F. virgata* rearing, mature, green-skinned *C. moschata* fruits were used. Ovisacs or crawlers of each mealybug species were separately distributed on the pumpkins, which were then placed on small tripod stands inside rearing cages maintained at a room temperature of 25±2°C for further development.

Wax extraction from mealybugs

Adult mealybugs covered with body wax were used in this study. A sample of 40 mg of mature adult mealybugs was gently collected using a size 2 camel hair brush placed into a glass vial. All glassware used for body wax extraction was thoroughly rinsed with 95% alcohol sterilized in a hot air oven at 180˚C for 12 h. The collected mealybugs were rinsed with double-distilled water for 5 min to remove honeydew other impurities, followed by a 6-7 second rinse in 1.5 mL of chloroform.

To optimize wax extraction, the amount of wax collected was initially quantified at different time intervals. Based on these observations, rinsing 40 mg of mealybugs with 1.5 mL of chloroform for 6-7 seconds yielded the highest wax content, approximately 6.37 mg/g. This procedure was subsequently adopted for wax extraction. After the chloroform rinse, the solution was evaporated to dryness on a water bath at 70°C. It was estimated that 40 mg of mealybugs could yield 0.5 g of wax. The dried mealybug wax was then diluted in 100 µL of hexane and 2 µL of this solution was injected into the GC-MS for analysis.

Collection of honeydew from mealybugs

The honeydew excreted by different mealybugs (*M. hirsutus*, *P. citrii*, *F. virgata*, *R. iceryoides*) was collected separately into glass vials using a dissection needle. The honeydew droplets were directly picked and placed into 1.5 mL glass vials, which were then stored at -20°C for further analysis.

Gas chromatography mass spectrometry (GC-MS) analysis of wax

GC-MS analysis of mealybug waxes was performed using a Varian 4000 GC-MS/MS (Varian 4000) ion-trap mass selective detector coupled with a Varian-3800 gas chromatograph equipped with a VF-5MS (Varian) column (30 m x 0.25 mm internal diameter with 0.25 mm film thickness) for flame ionization detector (FID) analysis. The mealybug wax compounds were determined using the following temperature program: the injection port was set to 300˚C, the FID was at 310˚C the column temperature was initially set at 100˚C for 2 min increased at 6˚C/min to 244˚C held for 2 min increased at 8˚C/min to 300˚C, where it was held isothermally for 30 min. Helium (1 mL min^{-1}) was used as the carrier gas, with all injections in split mode (20:1). The mass spectrometer was operated in external electron ionization mode with the trap temperature set to 200˚C, ion source heating at 200˚C transfer line temperature at 310˚C. The electron ionization (EI) mode was set to 70 eV, with a full mass scan range of 50-550 amu. Identification quantification of the mealybug wax composition were achieved by comparing the mass spectra with available libraries (NIST-2005 WILEY-2007). FID peak areas retention times/indices were compared with those of reference compounds, internal standard (pentadecane) a homologous series of hydrocarbons from C_{10} to C_{40} (Kovats,1965).

Table 1 : MRM of sugars standards

Sugar	Formula mass	Parent ion (m/z) $[M-H]^+$	Daughter ions	Cone voltage (V)	Collision energy (eV)	Ionisation mode
Fructose	180	178.97	89.09	18	16	ES-
Sucrose	342	341.03	89 .08	32	22	ES-
Galactose	180	178.97	89.09	18	16	ES-
Glucose	180	178.97	59.08	18	16	ES-
Maltose	342	341.03	161.05	12	8	ES-
Fucose	164	162.97	89.06	18	6	ES-
Rhamnose	164	162.97	103.06	18	6	ES-
Xylose	150	148.9	89.09	18	8	ES-
Arabinose	150	148.9	89.08	18	8	ES-
Mannose	180	178.97	119.03	20	8	ES-
Sorbitol	182	180.97	89.05	26	14	ES-
Inositol	180	178.97	161.11	28	8	ES-
Lactose	342	341.03	161.05	12	8	ES-
Ribose	150	148.9	89.09	18	8	ES-
Trehalose	342	341.03	89.08	32	22	ES-

Liquid chromatography mass spectrometry (LC/MS-MS) analysis of sugars in honeydew

A standard sugar solution was prepared in water. The organic solvents used as the mobile phase for liquid chromatography were of chromatographic/MS grade all other reagents were of analytical grade. Water purified through a Milli-Q (Millipore) system was used to prepare the mobile phases, which were filtered through 0.45 µm pore size membranes. For analysis, 4 mg of honeydew was placed in a sample vial homogenized with 3 mL of 80% ethanol. The mixture was sonicated for 15 minutes, then centrifuged at 4°C at 10000 rpm for 10 min. The supernatant was collected diluted to a total volume of 10 mL with 80% ethanol. An aliquot of 2 mL was evaporated to dryness re-dissolved in 1 mL mobile phase then filtered using a 0.2 µm nylon filter before injecting 3 µL into the ultra-performance liquid chromatography (UPLC) for LC-MS/MS analysis.

The evaluation parameters used to validate the methodology for determining water-soluble sugars included linearity, detection quantification limits repeatability. A series of sugar calibration standards were prepared in high-performance liquid chromatography (HPLC) grade water at various concentrations to create a calibration curve for each sugar of interest. Linearity was assessed by constructing calibration curves three injections performed at each concentration level. The mobile phase consisted of solvent A (80:20-Acetonitrile: water) solvent B (30:70-Acetonitrile: water) with 0.1% ammonium hydroxide. The initial gradient was set at 100% solvent A, held for one minute. The gradient then shifted to 88% A 12% B, held for one minute, followed by a change to 98% of A 2% of B at 15 min, held for 0.5 min. The system returned to initial conditions at 18 min, with a 2 min equilibration period before the next injection. The flow rate was set at 0.1 mL/min. The analytical column used was a 2.1 X 100 mm UPLC BEH (ethylene-bridged hybrid) Amide (Waters, USA) with 1.7 µm particle size, protected by a vanguard BEH-Amide with 1.7 µm particles (Waters, USA). The column temperature was maintained at 25°C. The detection system allowed for simultaneous detection using multiple reaction monitoring (MRM) methods developed for individual masses, as listed in Table 1. The entire system was controlled by Mass Lynx software, which also managed data collection processing. Elution was monitored using a (triple quadrupole detector) TQD-MS/MS (Waters, USA) system, optimized for sugar analysis.

RESULTS AND DISCUSSION

Mealybug wax profiling

This study examined the biochemical composition of epidermal waxes in four polyphagous mealybug species, *M. hirsutus, F. virgata, R. iceryoides,* and *P. citri* to understand how interspecific chemical variation may relate to ecological adaptation and pest management challenges. Across species, the wax layer was dominated by long-chain hydrocarbons, alcohols, esters, aldehydes, ketones, fatty acids, and nitrogen-containing compounds, although their relative proportions varied markedly (Fig. 1; Table 2).

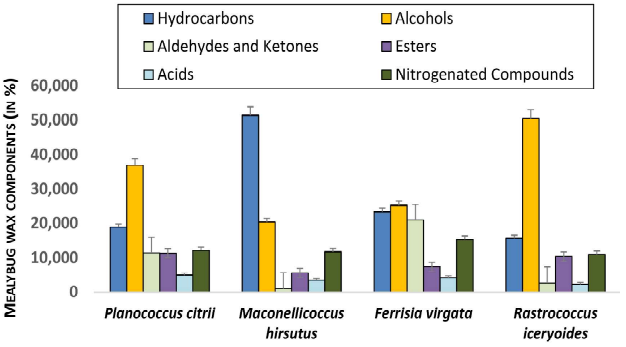

Fig. 1 : Different classes of mealybug wax component in different species of mealybug

The percentage composition varied across species, with *M. hirsutus* showing the highest hydrocarbon content at 51.31%, followed by *F. virgata* (23.33%), *P. citrii* (18.87%) *R. iceryoides* (15.73%). Among hydrocarbons, phenyl tetramethyl cyclopentadiene, 1,19-eicosadiene tetratriacontane were present at higher proportions. Specifically, 1,19-eicosadiene (23.41%), tetratriacontane (6.51%) were more abundant in *M. hirsutus* compared to other species, suggests a highly hydrophobic wax layer, which likely contributes to reduced insecticide penetration and enhanced protection against desiccation and environmental stress. Long-chain hydrocarbons (C15–C43), such as 1,19-eicosadiene and tetratriacontane, are known to confer waterproofing properties by reducing cuticular permeability and limiting water loss (Israelachvili, 2011). Similar relationships between hydrocarbon-rich cuticles and insecticide tolerance have been reported in scale insects and other hemipterans, where thick wax layers impede insecticide penetration and reduce contact toxicity (Venkatesan et al., 2016; Ulusoy et al., 2022). This biochemical trait may partly explain the notorious difficulty in managing *M. hirsutus* using conventional insecticides.

In contrast, the phenyl tetramethyl cyclopentadiene was detected at a very low concentration in *R. iceryoides* (3.39%) compared with other species where it ranged from 6.15-6.77% (Table 2). Overall, *F. virgata, P. citrii,* and *R. iceryoides* exhibited comparatively lower hydrocarbon content, indicating species-specific differences in wax barrier properties. Such variation may influence cuticular permeability and consequently, differential susceptibility to insecticides and environmental stressors, underscoring the role of wax chemistry in shaping species-specific ecological performance and pest management outcomes.

Interestingly, *R. iceryoides* exhibited a wax profile dominated by long-chain alcohols (50.55%), followed by *P. citrii* (36.91%), *F. virgata* (25.25%), *M. hirsutus* (20.41%) particularly diols and higher alcohols (tetracontane-1,40-diol,1-hentetracontanol, 1,30-triacontanediol). Such compounds may contribute to wax plasticity and adhesion, facilitating firm attachment to host surfaces and enhancing survival on exposed plant parts. Alcohol rich waxes have been reported in certain scale insects and are thought to balance waterproofing with flexibility, a trait advantageous for insects inhabiting variable microclimates (Blomquist & Bagnères, 2010).

Under the esters group, the highest proportion of δ-methyl-2-(2-hydroxyethyl)-2-devinylchlorine 6 trimethyl ester (8.63%) was detected in *P. citrii* followed by *R. iceryoides* (5.63%), *F. virgata* (5.34%), *M. hirsutus* (3.57%). Another compound, benzenepropanoic acid, 3, 5-bis(1, 1-dimethylethyl)-4-hydroxy-, octadecyl ester, was only detected in *R. iceryoides* (4.36%), but not in any of the other mealybug species examined. Decanoic acid, methyl ester hexadecanoic acid tetradecyl ester were detected, but in very low abundance across all the mealybug species tested. Two acids, (Z)-9-octadecanoic acid (Z)-13-docosenoic acid, were detected in trace amounts in all the mealybug species. Lower proportions of nitrogenated compounds were also detected.

Esters, though present in lower proportions than hydrocarbons and alcohols, also showed species-specific patterns. The exclusive detection of benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester in *R. iceryoides* suggests unique biochemical signatures that may influence interactions with microbes or natural enemies. Esters

Table 2 : Biochemical profiling of body wax from different mealybug species

Compounds Identified	Composition (%)				
	P. citrii	*M. hirsutus*	*F. virgata*	*R. iceryoides*	Molecular weight
Hydrocarbons					
Phenyl tetramethy lcyclo pentadiene	6.472	6.774	6.157	3.394	198
Nonadecane	0.219	0.067	0.062	0.029	268
1,19-Eicosadiene	4.618	23.414	4.317	2.522	278
1,2,3,4-tetrahydro-6, 11-bis (phenylethynyl)-aphthacene	0.000	0.709	0.000	0.000	322
9-Tricosene, (Z)-	3.071	0.000	0.400	0.397	392
Cyclo octacosane	0.000	0.529	0.000	0.000	422
H.C-30	0.000	0.939	0.394	0.526	432
H.C-32	0.231	10.439	0.760	8.714	450
Tetratria contane	0.977	6.514	0.349	0.153	478
3,5,24-Trimethyl tetracontane	1.764	0.283	2.326	0.000	490
17-Pentatria contene	0.704	0.878	8.564	0.000	492
Pentatria contane	0.818	0.769	0.000	0.000	604
Total	18.874	51.316	23.330	15.735	
2-Hexyl-1-decanol	0.188	0.145	0.197	0.114	242
1,16-Hexa decanediol	3.273	2.673	3.190	1.320	258
1-Dotria contanol	3.628	2.473	3.138	1.873	
1,22-Doco sanediol	3.966	1.898	2.546	1.523	342
1,30-Tria contanediol	0.000	0.951	0.934	1.007	454
Tetra contane-1,40-diol	1.328	0.721	1.225	0.847	466
1-Hentetra contanol	1.495	1.697	1.352	11.883	515
1,30-Tria contanediol	0.169	1.679	1.895	7.591	592
Tetracontane-1, 40-diol	0.337	0.756	1.277	13.852	594
Dipuupehetriol	22.526	7.419	9.495	10.546	
Total	36.911	20.413	25.250	50.556	
Aldehydes ketones					
(5β)-rostan-3-one	0.124	0.266	0.082	0.133	274
(Z)-6-Heneicosen-11-one	1.949	0.745	2.054	1.466	308
3-hydroxy-, (3α,5β,25R)-Spirostan-12-one	9.371	0.000	2.296	1.066	430
22-ethoxy-19-(2-ethoxy-3-formyl-5-methylphenyl)-5, 21-dihydro-9, 13, 17-trimethyl 15, 11-metheno-11H-tribenzo [c,g,n] [1,6] dioxa cyclopentadecin-7-carboxal dehyde	0.000	0.000	16.494	0.000	640
Total	11.445	1.011	20.926	2.665	
Esters					
Methyl 2 β, 6β-diphenylthio-3α, 4α-isopropylidene dioxy cyclohexan-1α-oate	0.000	0.959	0.000	0.000	430
Dodecanoic acid, methyl ester	0.696	0.062	0.437	0.163	214
Pthalicacid, 5-methoxy 3 methyl pentyl tridesyl ester	0.442	0.233	0.197	0.204	166
Hexadecanoic acid, tetradecyl ester	0.745	0.738	0.814	0.000	452
δ-Methyl-2-(2-hydroxyethyl)-2-devinylchlorine6 trimethyl ester	8.631	3.577	5.348	5.634	
Benzenepropanoic acid, 3, 5-bis (1, 1-dimethylethyl)-4-hydroxy-, octadecyl ester	0.000	0.000	0.000	4.363	530
20-Ethyl-1, 7, 8-trihydroxy-6, 16-dimethoxy-4-(methoxymethyl) aconitan-14-yl acetate	0.839	0.000	0.609	0.000	495
Total	11.352	5.568	7.405	10.364	
Acids					
(Z)-9-Octadecenoic acid	3.622	2.700	3.412	1.861	282
(Z)-13-Docosenoic acid	1.343	0.779	0.788	0.449	338
Total	4.964	3.479	4.199	2.311	
Nitrogenated Compounds					
Benzonitrile, 3, 5-di-tert-butyl-4-hydroxy-	0.235	0.000	0.173	0.081	231
(9E)-n-Propyl-9-octadecenamide	2.300	1.242	1.557	0.749	323
2-(2, 4-Dinitrobenzyl) pyridine	0.263	0.000	0.740	0.385	259
3-(ethoxycarbonyl)-1, 2-dihydro-2, 2-dimethyl-1, 4, 6-triphenylpyridine	8.873	10.107	6.627	2.812	409
4-cyano-7-phenyl-4, 5-dihydro-7H-thiopyrano [3,4-b] furan	0.105	0.082	0.072	0.081	241
2, 3-Distyryl-5, 8-dimethoxy-6-amino quinoxaline	0.358	0.325	0.410	0.173	409
n-trosyl-3 beta, 5 beta-(amino methano) Cholestan	0.000	0.000	5.808	0.000	250
bis-(5-cyano-2, 6-diphenyl-4-pyrimidinyl)-disulfide	0.000	0.000	0.000	6.706	576
Total	12.134	11.756	15.386	10.986	
Others					
1-Chloroheptacosane	0.000	3.135	0.000	2.588	

and fatty acid derivatives in insect waxes have been implicated in semiochemical communication and surface microbial regulation, highlighting their potential ecological relevance beyond structural roles (Howard & Blomquist, 2005; Blomquist & Bagnères, 2010; Pedrini et al., 2015).

In the current study, we have identified a number of long-chain hydrocarbons, includingphenyl tetramethyl cyclopentadiene ($C_{15}H_{18}$);1,19-eicosadiene ($C_{20}H_{38}$); tetratriacontane ($C_{34}H_{70}$); (Z)9-tricosene ($C_{23}H_{46}$); 17-pentatriacontene ($C_{35}H_{70}$); nonadecane ($C_{19}H_{40}$); 1,2,3,4-tetrahydro-6,11-bis (phenylethynyl)-naphthacene ($C_{34}H_{24}$); cyclooctacosane ($C_{28}H_{56}$); 3,5,24-trimethyltetracontane ($C_{43}H_{88}$) pentatriacontane ($C_{35}H_{72}$). Collectively, the dominance of long-chain hydrocarbons (C15–C43) across species underlines their role in conferring strong hydrophobicity to mealybug waxes.

The profiling of mealybug epidermal waxes can provide valuable insights into how the wax dissolves or resists dissolution. This understanding can aid in developing better management strategies. Since hydrocarbons are non-polar solutes, they will not dissolve in a polar solvent like water (Israelachvili, 2011). Therefore, solvents that can dissolve these hydrocarbons esters may be useful options for enhancing the effectiveness of insecticides in managing mealybug infestations. From a pest management perspective, this explains the limited efficacy of water-based insecticide formulations against mealybugs and supports the need for oil-based adjuvants or wax disrupting agents to improve control efficacy.

Sugar composition concentration in mealybug honeydew

In addition to wax chemistry, the LC-MS analysis of honeydew revealed distinct, species-specific sugar profiles, with fructose, glucose and sucrose being the dominant sugars across all mealybugs, albeit in differing proportions. The highest concentrations of sugars found in the honeydews were: glucose (32.12 g/100 g of honey dew hereafter) in *R. iceryoides*, sucrose (24.8 g/100 g) in *F. virgata* honeydew, fructose (25.18 g/100 g) in *M. hirsutus* honeydew and fructose (16.1 g/100 g) in *P. citrii* (Fig. 2). Such variation likely reflects differences in digestive physiology, phloem sap processing, and the metabolic contributions of endosymbionts.

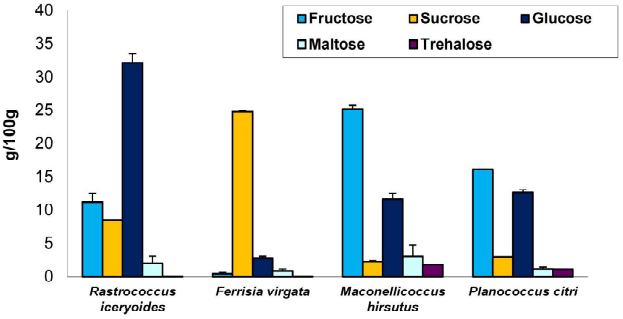

Fig. 2: Sugar profiling of honey dew in different mealybug species

The predominance of glucose in *R. iceryoides* honeydew contrasts with the sucrose rich honeydew of *F. virgata* and the fructose-dominated profiles of *M. hirsutus* and *P. citrii*. Comparable interspecific differences have been reported in other phloem feeders, including aphids and whiteflies, where honeydew sugar composition varies with insect species, host plant and symbiotic bacteria. Honeydew from the aphid *Metopeurum fuscoviride* was composed of glucose, fructose, trehalose, melizitose, where sucrose, maltose and erlose were present in lower concentrations (Fischer et al., 2002; Roopa et al., 2016). Compared to aphids and whiteflies, mealybug honeydew appears to be richer in readily utilizable sugars, potentially enhancing its attractiveness to ants and other insects.

Honeydew composition has important ecological and applied implications, particularly in shaping ant-mealybug mutualisms. Sugar rich honeydew promotes ant attendance, which in turn protects mealybugs from parasitoids and predators, thereby complicating biological control efforts (Daane et al., 2007; Mgocheki & Addison, 2009). At the same time, honeydew serves as a non-prey food resource and kairomonal cue for natural enemies (Wäckers et al., 2005). The longevity of the adult parasitoid *Anagyrus fusciventris* (Girault) (Hymenoptera: Encyrtidae) was increased in the presence of the honeydew from *Citrophilus* mealybugs (*Psuedococcus calceolariae* Maskell) (Chhagan et al., 2024). Previous studies have also reported that bacterial volatiles identified from the honeydew of *M. hirsutus, Nipaecoccus viridis* (Newstead) elicited positive responses in female parasitoids of *Anagyrus* species (Fand et al., 2020).

The sugar profiles identified in this study therefore provide a biochemical basis for understanding species-specific differences in ant attendance, parasitoid attraction, and overall pest severity. Moreover, these

data could facilitate the formulation of artificial honeydew mimics to enhance parasitoid survival and efficacy in biological control programs.

The analysis revealed distinct sugar profiles in mealybug honeydew, with fructose, glucose and sucrose being the predominant sugars, though their relative abundance varied between species. This variation may be attributed to differences in the digestive processes or the influence of endosymbionts. Studies suggested that honeydew can act as a kairomone, aiding in host selection by parasitoids and predators (Budenberg, 1990; Fand et al., 2020; Peñalver-Cruz, 2023). The mutualistic relationship between ants and mealybugs, where ants benefit from honeydew sugars and mealybugs receive protection from natural enemies, may impact the effectiveness of mealybug control by parasitoids and predators. The sugar profiling of mealybug honeydew in this study could also facilitate the synthesis of artificial honeydew for research and pest control applications. Furthermore, identifying the kairomones in honeydew may enhance mealybug parasitism, as shown by the increased longevity of parasitoids like *Anagyrus fusciventris* in the presence of honeydew.

CONCLUSION

Overall, combined analysis of epidermal wax and honeydew chemistry demonstrates that mealybug pest status is strongly influenced by biochemical traits that mediate protection, persistence, and multitrophic interactions. Hydrocarbon-rich waxes reduce insecticide efficacy, while sugar rich honeydew may alter interactions with ants and natural enemies. These findings emphasize the need for integrated pest management strategies that incorporate wax disrupting formulations, appropriate adjuvants, and biological control approaches that exploit honeydew mediated natural enemies attraction. Understanding species specific biochemical signatures provides a mechanistic basis for improving mealybug management and highlights the value of chemical ecology in addressing persistent pest challenges.

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REFERENCES

Andreason, S. A., Trjapitzin, S. V., & Perring, T. M. (2019). Untangling the *Anagyrus pseudococci* species complex (Hymenoptera: Encyrtidae), parasitoids of worldwide importance for biological control of mealybugs (Hemiptera: Pseudococcidae): Genetic data corroborates separation of two new, previously misidentified species. *Biological Control*, *129*, 65–82. doi: 10.1016/j.biocontrol.2018.09.010

Blomquist, G. J., & Bagnères, A. G. (Eds.). (2010). *Insect hydrocarbons*: *Biology*, *biochemistry*, *and chemical ecology*. Cambridge University Press. doi: 10.1017/CBO9780511717642

Budenberg, W. J. (1990). Honeydew as a contact kairomone for aphid parasitoids. *Entomologia Experimentalis et Applicata*, *55*(2), 139–148. doi: 10.1111/j.1570-7458.1990.tb01357.x

Chacko, M. J., Bhat, P. K., Rao, L. V. A., Singh, D., Ramanarayan, E. P., & Sreedharan, K. (1978). The use of ladybird beetle, *Cryptolaemus montrouzieri* for the control of coffee mealybugs. *Journal of Coffee Research*, *8*, 14–19.

Chhagan, A., Davis, V. A., Hunt, S., MacDonald, F., Santos, K., Richards, K., & Bell, V. A. (2024). Effect of floral resources mealybug honeydew on the longevity of the parasitoid, *Anagyrus fusciventris*. *Biocontrol Science and Technology*, *34*(3), 316–322. doi: 10.1080/09583157.2024.2340535

Daane, K. M., Sime, K. R., Fallon, J., & Cooper, M. L. (2007). Impacts of Argentine ants on mealybugs and their natural enemies in California's coastal vineyards. *Ecological Entomology*, *32*(6), 583–596. doi: 10.1111/j.1365-2311.2007.00910.x

Fand, B. B., Amala, U., Yadav, D. S., Rathi, G., Mhaske, S. H., Upadhyay, A., ... & Kumbhar, D. R. (2020). Bacterial volatiles from mealybug honeydew exhibit kairomonal activity toward solitary endoparasitoid *Anagyrus dactylopii*. *Journal of Pest Science*, *93*, 195–206. doi: 10.1007/s10340-019-01150-4

Fischer, M. K., Völkl, W., Schopf, R., & Hoffmann, K. H. (2002). Age-specific patterns in honeydew production honeydew composition in the aphid *Metopeurum fuscoviride*: Implications for ant-attendance. *Journal of Insect Physiology*, *48*(3), 319–326. doi: 10.1016/S0022-1910(01)00179-2

Heidari, M., & Copland, M. J. W. (1993). Honeydew: A food resource or arrestant for the mealybug predator *Cryptolaemus montrouzieri*? *Entomophaga*, *38*, 63–68. doi: 10.1007/BF02373140

Howard, R. W., & Blomquist, G. J. (2005). Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annual Review of Entomology*, *50*(1), 371–393. doi: 10.1146/annurev.ento.50.071803.130357

Israelachvili, J. N. (2011). *Intermolecular and surface forces* (3rd ed.). Academic Press. doi: 10.1016/C2009-0-21560-1

Kovats, E. S. (1965). Gas chromatographic characterization of organic substances in the retention index system. *Advances in Chromatography*, *1*, 229–247.

Mani, M., & Krishnamoorthy, A. (2012). Determination of release time of the Australian ladybird beetle, *Cryptolaemus montrouzieri* Mulsant for the suppression of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) on grapes. *Pest Management in Horticultural Ecosystems*, *18*(1), 98–99.

Mani, M., & Shivaraju, C. (Eds.). (2016). *Mealybugs and their management in agricultural and horticultural crops*. doi: 10.1007/978-81-322-2677-2

Mani, M., Krishnamoorthy, A., & Ramanujam, B. (2022). Trends in the biological control of horticultural crop pests in India. In M. Mani (Ed.), *Trends in Horticultural Entomology* (pp. 243–281). doi: 10.1007/978-981-19-0343-4_8

Mgocheki, N., & Addison, P. (2009). Interference of ants (Hymenoptera: Formicidae) with biological control of the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). *Biological Control*, *49*(2), 180–185. doi: 10.1016/j.biocontrol.2009.02.001

Mineo, G. (1967). On the entomophagous parasites of *P. citri* Risso and *I. purchasi* Mask. found in Sicily [Nuovi parassiti entomofagi di *P. citri* (Citrus moth) trovati in Sicilia]. *Bollettino dell'Istituto di Entomologia Agraria e dell'Osservatorio di Fitopatologia di Palermo*, *7*, 99–119.

Mwanauta, R. W., Ndakidemi, P. A., & Venkataramana, P. B. (2022). Characterization of farmer's knowledge management practices of papaya mealybug *Paracoccus marginatus* (Hemiptera: Pseudococcidae) in Tanzania. *Saudi Journal of Biological Sciences*, *29*(5), 3539–3545. doi: 10.1016/j.sjbs.2022.02.036

Pedrini, N., Ortiz-Urquiza, A., Huarte-Bonnet, C., Fan, Y., Juárez, M. P., & Keyhani, N. O. (2015). Tenebrionid secretions and a fungal benzoquinone oxidoreductase form competing components of an arms race between a host and pathogen. *Proceedings of the National Academy of Sciences*, *112*(28), E3651–E3660. doi: 10.1073/pnas.1504552112

Peñalver-Cruz, A., Satour, P., Jaloux, B., & Lavero, B. (2023). Honeydew is a food source and a contact kairomone for *Aphelinus mali*. *Insects*, *14*(5), Article 426. doi: 10.3390/insects14050426

Roltsch, W. J., Meyerdirk, D. E., Warkentin, R., Andress, E. R., & Carrera, K. (2006). Classical biological control of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), in southern California. *Biological Control*, *37*(2), 155–166. doi: 10.1016/j.biocontrol.2006.01.003

Roopa, H. K., Asokan, R., Krishna Kumar, N. K., Mahmood, R., Bakthavatsalam, N., Uprithi, K., & Raghavendra, A. (2016). Estimation of sugars and volatiles in the honeydew of *Bemisia tabaci* genetic groups Meam-I and Asia-I. *Journal of Bioscience and Biotechnology Discovery*, *1*, 66–73.

Sahito, H. A., Kousar, T., Mangrio, W. M., Mal, B., Mastoi, A. H., Jamali, Y. A., ... & Abro, A. H. (2022). Comparative crop production protection costs of cotton crop under different pest

management strategies of cotton mealybug, *Phenacoccus solenopsis* (Tinsley). *Annals of the Romanian Society for Cell Biology*, *26*(1), 763–775.

Singh, S. P. (1978). Propagation of a coccinellid beetle for the biological control of citrus and coffee mealybugs. *Proceedings of the Second Scientific Conference of CPA*, 2, 1–10.

Ulusoy, S., Kahya, D., Bilgin, M. G., & Apalak, A. (2022). The effectiveness of wax secretion on chemical control in some mealybug species. *Journal of Asia-Pacific Entomology*, *25*(3). doi: 10.1016/j.aspen.2022.101954

Van Lenteren, J. C., Bolckmans, K., Köhl, J., Ravensberg, W. J., & Urbaneja, A. (2018). Biological control using invertebrates and microorganisms: Plenty of new opportunities. *BioControl*, *63*, 39–59. doi: 10.1007/s10526-017-9801-4

Venkatesan, T., Jalali, S. K., Ramya, S. L., & Prathibha, M. (2016). Insecticide resistance and its management in mealybugs. In M. Mani & C. Shivaraju (Eds.), *Mealybugs and their management in agricultural and horticultural crops* (pp. 223–229). doi: 10.1007/978-81-322-2677-2_14

Wäckers, F. L., & Henry, F. (2005). The vegetarian side of carnivores: Use of non-prey food by parasitoids and predators. In F. L. Wäckers, P. C. J. van Rijn, & J. Bruin (Eds.), *Plant-provided food for carnivorous insects*: *A protective mutualism and its applications* (pp. 420–427). Cambridge University Press. doi: 10.1017/cbo9780511542206.014

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