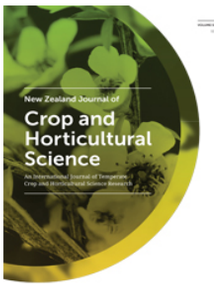

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Differential olfactory responses associated with host plant shift by the fruit-piercing moth, *Eudocima phalonia*, in the Pacific islands

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ABSTRACT

The fruit-piercing moth (FPM) *Eudocima phalonia* is widely present in the tropics, causing major damages to the citrus industry. In its ancestral distribution range, e.g. Australia, FPM develops on vines from the Menispermaceae family. But in more recently colonized islands, e.g. New Caledonia, FPM populations have shifted to new host plants of the Fabaceae family, *Erythrina* spp.. To understand this host shift, we studied the chemical ecology of FPM as a mechanism driving host plant acceptance. We collected volatile headspace samples of *Erythrina* species and Menispermaceae and compared their chemical spectra. We assessed the electrophysiological responses of FPM populations from the two countries to the plant chemical extracts and identified bioactive compounds. The volatile profiles from each species were quite different between and within each plant family. However, five compounds common across the two families triggered electrophysiological responses in both FPM populations. Those common bioactive compounds could have facilitated the host shift to completely different plant family. Furthermore, the diverging history between the two FPM populations may explain differences in electrophysiological sensitivity to other specific compounds. These results are discussed in the context of the production of a generic lure that could be attractive to all FPM populations.

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Eudocima phalonia; *Erythrina*; fruit pest; host shift; Menispermaceae

Introduction

Plant volatiles mediate host plant finding by phytophagous insects (Bruce et al. 2005). If insect survival and reproduction depend on finding a host plant, its olfactory receptors should be specifically tuned to the host plant volatile compounds. Therefore, studying

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the olfactory perception of an insect to host plant compounds should indicate how specialized an insect is and potentially its capability to shift to new host plants with similar compounds. Host plant shift by insects has been documented for several species and discussed in the context of host race formation potentially leading to speciation via prezygotic reproductive isolation and limited gene flow (Bush 1969; Mayhew 1997; Berlocher and Feder 2002; Drès and Mallet 2002).

The fruit-piercing moth (FPM), *Eudocima phalonia* (L.) (Lepidoptera: Erebidae), also known as *Othreis fullonia*, is a common pest of many fruits, including citrus, mango, carambola, guava, lychee and rambutan in most countries of the tropics (Waterhouse and Norris 1987; Leroy et al. 2021). Adults use their strong sclerotized proboscis to pierce the fruit skin to suck the juice, damaging the fruit and creating entry points for pathogen infections. During pest outbreaks, orchards can suffer up to 100% fruit loss, particularly Navel oranges, mandarins, grapefruit, mangos, guavas in New Caledonia (Cochereau 1977) and lychees and carambolas in Northern Queensland (Fay and Halfpapp 1993). FPM is believed to originate from the Indo-Malaysian region, including Australia (Waterhouse and Norris 1987). Larval development occurs on vines of the Menispermaceae family in Asia, Africa and Australia (Waterhouse and Norris 1987; Fay 1994); but in the Pacific Islands, such as New Caledonia, Hawaii, Papua New Guinea, Guam, States of Micronesia, Palau, Fiji, Vanuatu, Western Samoa, American Samoa, Tonga, the Cook Islands and French Polynesia, populations of FPM also breed on coral trees *Erythrina* spp., in the Fabaceae family (Muniappan et al. 1993). FPM populations from Australia or Asia do not develop on *Erythrina* spp. even though they are present in some of these regions (Fay 1994), whereas populations from the Pacific Islands can develop on both types of plants (Reddy et al. 2005). Sands and Chan (1996) have hypothesized that populations from the Pacific Islands have become adapted to *Erythrina* spp. through sympatric speciation with the transfer of larvae from vines growing on coral trees; those authors described them as a separate biotype from the Australian and African species (Sands and Chan 1996).

Menispermaceae is a family of about 75 genera with over 500 species of vine plants (Forman and Short 2011). In Australia, there are 13 genera and 24 species (Forman and Short 2011). In contrast, in the Pacific Islands, Menispermaceae vines are less diverse and less abundant. Only one species of Menispermaceae has been identified in Guam: *Tinospora homosepala* (Muniappan et al. 1994), while there are four genera with seven species reported in New Caledonia: *Stephania* spp., *Tinospora* spp., *Pachygone* spp. and *Hypserpa* spp., but with rare distribution (http://endemia.nc, accessed on March 2017). In contrast, *Erythrina* spp. occur all over the tropics with 120 known species, mainly studied for their alkaloid contents and pharmacopeia usage (Soto-Hernández et al. 2012). Two species, *Erythrina variegata* and *Erythrina fusca,* have been reported as the main hosts of FPM in New Caledonia. These species were introduced as ornamental plants in urban areas and utilised widely as shade and edge plants around cacao and coffee plantations by the end of the nineteenth century (Cochereau 1977). The abundance of this plant in comparison to the paucity of Menispermaceae in the islands has been suggested as a causative agent for the host shift from Menispermaceae to *Erythrina* spp. (Cochereau 1977). However, adaptation to a new host plant comes with compromise. Most *Erythrina* species lose their leaves in the dry season, potentially causing the

fluctuation of FPM populations on these islands (Cochereau 1977). Also, the invasion of the Erythrina gall wasp (*Quadrasticus erythrinae* Kim, Hymenoptera: Eulophidae: Tetrastichinae) in the mid-2000s has had a negative impact on the main host *E. variegata* in most Pacific Islands (Heu et al. 2008). These differences in distribution and abundance of host plants may explain why populations of FPM on the Pacific Islands have retained the ability to develop on Menispermaceae.

Understanding host shift and adaptation of FPM requires elucidating the mechanistic basis for host choice. Cochereau (1977) suggested in his thesis that host plant shift could have a chemio-ecological basis. In fact, some Menispermaceae species contain some tetracyclic alkaloids typical of *Erythrina* spp. (Thornber 1970). The ability of FPM populations from the Pacific Islands to exploit both Menispermaceae and *Erythrina* spp. suggests that adult moths can locate these plants in their environment. Plant volatiles are well known to be used by insects to locate their host, while non-volatile compounds like the tetracyclic alkaloids of both plants may facilitate palatability.

With the aim of developing an attractant based on the host plant volatiles (i.e. kairomones), we compared whether Menispermaceae and *Erythrina* spp. hosting FPM share similar volatile compounds that could have facilitated host shift. We also tested whether populations of FPM from Australia and New Caledonia respond differently to volatiles from each plant family, thus contributing to host race formation and the potential divergence toward speciation.

Materials and methods

Volatile headspace collection from host plants

The volatiles of FPM host plants were collected *in situ* in New Caledonia and Australia. In New Caledonia, volatile organic compounds (VOCs) from *E. fusca* and *E. variegata* were collected in the region of La Foa, in Farino County. In Australia, volatile collections were carried out at the Department of Agriculture and Fisheries (DAF) in Mareeba and Walkamin, Queensland. Volatiles from four species of Menispermaceae were collected *in situ*: *Tinospora smilacina* (inland and coastal species), *Stephania japonica*, *Legnephora moorei*, and *Sarcopetalum harveyanum* (Figure 1).

Oven bags were placed over coral tree branches and at the ends of Menispermaceae vines to seal them. Air was drawn through a charcoal filter, entering at the bottom and sucked out at the top by a pump with an airflow rate of 0.1 $ml.min^{-1}$. VOCs were absorbed over 24 hours onto 60 mg of Tenax-GR 35/60 (Alltech, Deerfield, IL, USA) contained in glass tubes and maintained by glass wool plugs and connected between the sealed oven bag and the pump. After 24 h, the Tenax was removed and eluted with 1 mL of *n*-hexane (BDH Laboratory Supplies, Poole, UK). An air control was collected through an empty oven bag at the same time. At least three replicates of each plant species and air control were collected at each location.

Chemical analysis

All headspace samples were analyzed with a Varian 3800 gas-chromatograph (GC) coupled to a Saturn 2200 mass-spectrometer (MS, Varian Inc., Walnut Creek, CA,

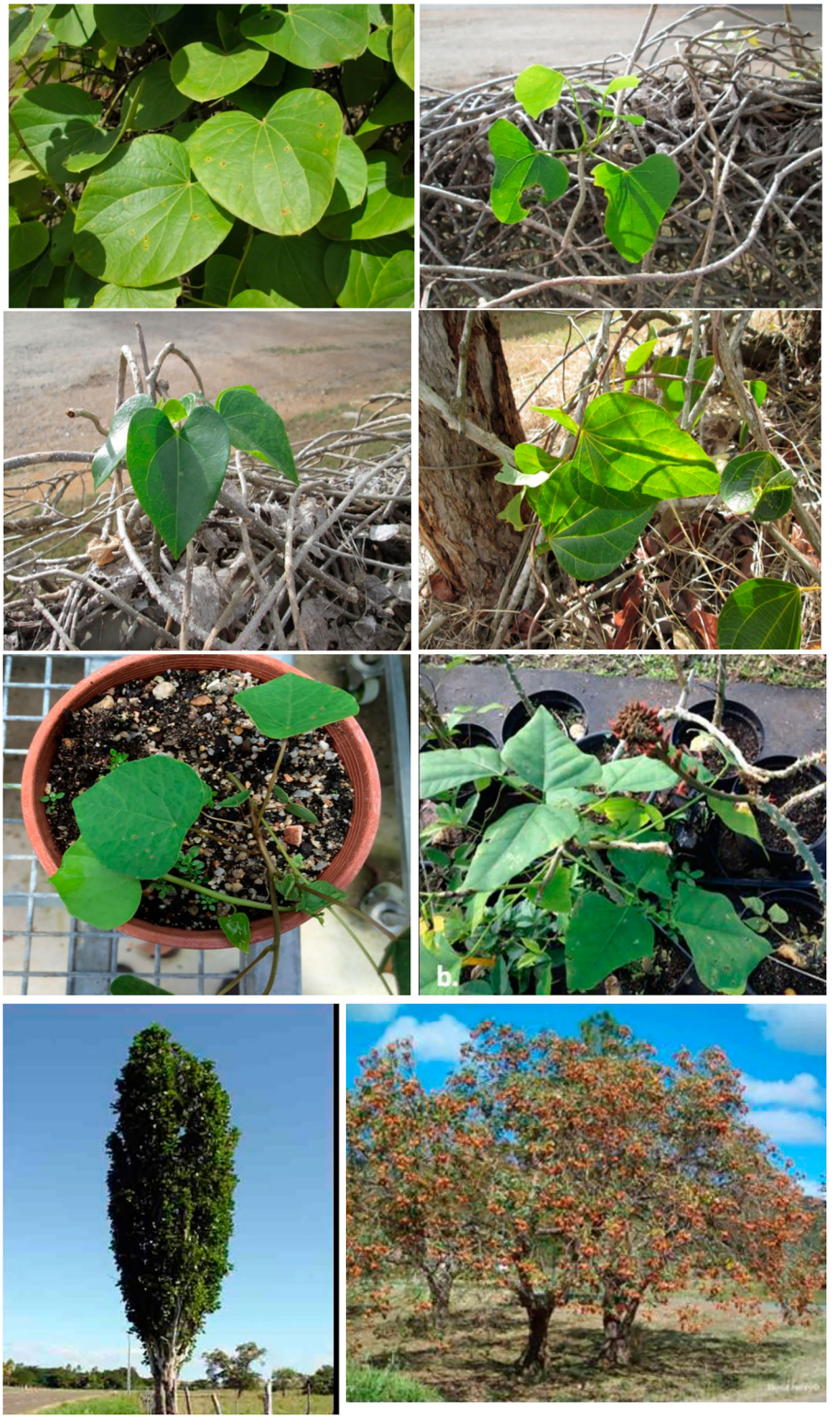

Figure 1 . Host plants (from top left to bottom right): Menispermaceae vines from Australia with *Tinospora smilacina (*inland*), Tinospora smilacina* (coastal), *Stephania japonica, Legnephora moorei, Sarcopetalum harveyanum,* and *Erythrina* from new Caledonia*, E. variegata* (leaves), *E. variegata* (tree) *and E. fusca* (tree). (credit: Flore Mas, Lise Leroy).

USA). Helium was used as the carrier gas (1 mL.min^{-1}) and injections were splitless for 0.6 min. Transfer line and ion trap temperatures were 250°C and 180°C respectively. The GC injector temperature was set at 220°C, and the oven temperature programme was set to 40°C for 2 min, and increased at 4°C min^{-1} to 240°C. A DB5-MS column (30 m × 0.25 mm i.d. × 0.25 µm film; Varian Inc., Walnut Creek, CA, USA) was used. A sub-sample of 200 µL from of each 1 ml plant extract was concentrated down to ca. 50 µL with a gentle stream of argon, A 1-µL aliquot from this concentrated sub-sample was injected into the GC-MS. Kováts retention indices (KI or RI) were calculated for each compound based on alkane series run with the same oven programme. Structural assignment of the compounds were made by comparing their mass spectra with the MS library NIST 2011 and Wiley 9th Ed. (McLafferty 2011), as well as comparing with KI published in the literature and an online library (PubMed (nih.gov)). Compound identities were confirmed by running synthetic standards when available from Sigma-Aldrich (compounds with an asterisk in Table 1). Relative proportions of each compound identified were calculated from the integrated area underneath each peak divided by the total sum of the area measured for each plant.

Fruit-piercing moth from New Caledonia and Australia

Females of FPM were collected at the tribe Hnaeu on Lifou Island, an island of the Loyalty Islands archipelago of New Caledonia. The sampling location was near a primitive forest with coordinates 21°0'00.18''S, 167°22' 28.4''E, Altitude 22 m. The females were then translocated in the greenhouse of the Institut Agronomique néo-Calédonien (IAC) in La Foa, where they were given *Erythrina* plants for egg laying. All larval stages were provided with fresh *E. variegata* or *E. fusca* leaves. FPM from Australia were cultured from larvae collected from the *T. smilacina* vine located at the DAF Mareeba facility (17°'25.21'' S 145°25'46.36'' E). Larvae were reared on leaves from the *T. smilacina* vines grown at the facility. First-generation laboratory-reared moths from New Caledonia and second-generation laboratory-reared moths from Australia were sent as pupae to the New Zealand Institute for Plant and Food Research Limited (PFR) under quarantine conditions, with an import permit (HSNO approval NOC100100). Pupae were kept in individual boxes in an incubator at 30°C and 40–70% relative humidity. After emergence, adults were provided with half a fresh mandarin as a food source. Unmated virgin females and males aged 2–6 days were used for electrophysiology.

Electrophysiology

To minimize the number of moth being killed and used for electrophysiology, we created pooled samples from each plant family (i.e. Menispermaceae or *Erythrina* spp.) by mixing together 50 µL of each sample within a family and adjusting the concentration by argon so that the two pooled family plant extracts were about the same total ion chromatogram when run on the GC-MS. Each pooled extract was tested with FPM from each population and sex. A gas chromatograph (GC) (Varian 3800) was coupled to a Syntech electro-antennogram detector (EAD) (Syntech Research and Equipment, Hilversum, The Netherlands). The capillary column was a DB5-MS, 30 m x 0.25 mm i.d. x 0.5 µm. The

Table 1. Volatile organic compounds from Menispermaceae host plants in Australia and *Erythrina* spp. in New Caledonia: a) relative peak area identified from headspace sample analysed with gas-chromatograph coupled with mass-spectrum (GC-MS) and b) electrophysiological responses (in mV) identified from gas-chromatography coupled with electroantennographic detection (GC-EAD). Numbers in bold are the largest in each column. Rows shadowed in grey highlight compounds triggering an electrophysiological response in both FPM populations.

a) Relative peak area from GC-MS

			Menispermaceae (Australia)						Erythrina (New Caledonia)		
Compounds	Retention time (min)	Kovats index	L.moorei	S. harveyanum	S. japonica	T. smilalina (coastal)	T.smilalina (inland)	*mean (pooled mix)*	E. fusca	E. variegata	*mean (pooled mix)*
(*E*)2-Hexenal*	8.36	858								0.55	*0.54*
Isoamyl acetate*	9.14	887		1.87			1.96	*1.55*		0.58	*0.53*
1-Octen-3-ol*	12.82	991								0.88	*0.88*
3-Octanone*	13.08	988			4.33		2.94	*1.00*		1.36	*1.29*
(*Z*)3-Hexenyl acetate*	13.86	1018		1.69	4.11		15.69	*5.85*		13.44	*13.32*
Hexyl acetate*	14.09	1025					5.17	*1.56*		2.86	*2.82*
(*Z*)2-Hexenyl acetate*	14.19	1027								2.13	*2.19*
Cymene	14.51	1036		3.99	2.52		1.18	*2.57*		0.40	*0.39*
Limonene*	14.68	1040		6.54	4.59		1.19	*3.99*		0.94	*0.93*
trans-β-ocimene*	15.02	1050					2.78	*0.84*		0.33	*0.32*
Phenylacetaldehyde	15.20	1055			9.45			*0.61*			
cis-β-ocimene	15.42	1060		4.85	9.56	**38.64**	**33.99**	***13.83***		**21.55**	***21.34***
γ-Terpinene	15.81	1071		6.25				*3.19*		1.13	*1.12*
4-Ethyl-o-xylene	16.78	1097		2.53		2.76	0.88	*1.64*		0.32	*0.32*
(+/-)Linalool*	17.31	1111					3.45	*1.39*		0.76	*0.75*
Nonanal*	17.46	1115	1.62	1.92			3.18	*2.12*		0.37	*0.36*
Benzeneethanol*	17.82	1125			**35.24**			*2.28*			
(*E*)−4,8-Dimethylnona-1,3,7-triene*	17.96	1129							14.76	16.54	*16.52*
unknown1	18.09	1133				7.49	0.58	*0.28*			
Ethyl benzoate*	19.90	1183			2.47			*0.16*		0.35	*0.34*
Methyl phenylacetate	20.18	1190		3.34			2.79	*2.56*			
Naphthalene	20.39	1196			3.40		0.93	*0.50*			
(*Z*)−3-Hexenyl butyrate*	20.49	1199		4.33			1.26	*2.64*	**72.40**	0.24	*0.94*
Butyl hexanoate*	20.65	1202		3.59			1.43	*2.12*			
Methyl salicylate*	20.81	1207	6.26	1.58			1.32	*1.89*		0.86	*0.85*
Dodecane*	20.99	1212			2.61			*0.17*			
Decanal*	21.18	1217	5.77	2.15			0.56	*1.82*	12.85	1.64	*1.74*
Isopropyl octanoate	22.13	1245								0.43	*0.42*
(*Z*)3-Hexenyl isovalerate*	22.26	1249		5.22			0.69	*2.90*		0.54	*0.54*

Phenethyl acetate*	22.57	1258		1.56			0.89	*1.70*			
Heptyl butanoate*	24.15	1302		**17.89**			9.17	*11.99*	1.64	*1.63*	
Tridecane*	24.48	1313	0.97	1.90			0.41	*1.15*	0.39	*0.36*	
Methyl o-anisate	25.80	1353			8.92			*0.58*			
Copaene	27.14	1394				4.25	0.93	*0.34*			
β-Elemene	27.65	1409					0.84	*0.25*	7.38	*7.31*	
Tetradecane*	27.79	1414	1.94	1.50	3.48		0.52	*1.35*	0.64	*0.64*	
β-Caryophyllene*	28.57	1439		14.12		9.23		*7.39*	3.28	*3.24*	
β-Copaene	28.88	1449		4.54				*2.85*	0.65	*0.65*	
α-Bergamotene	29.03	1454	**69.54**				0.46	*7.56*	0.98	*0.97*	
(*E*)-β-Farnesene*	29.64	1474	4.94	9.55		7.93		*5.55*	6.75	*6.69*	
(*E*,*E*)-α-Farnesene*	31.22	1525				3.27	5.28	*2.44*	8.82	*8.73*	
unknown2	33.89	1615	3.12		4.90		0.99	*0.94*			
unknown3	36.71	1717	2.66		2.87			*0.42*	0.74	*0.72*	
unknown4	36.88	1723	2.37		2.52			*0.42*	0.46	*0.46*	
unknown5	37.12	1732	0.82					*0.88*	0.34	*0.34*	

b) GC-EAD response (in mV)

	FPM-Australia		FPM-New Caledonia	
Compounds	**female (*n* = 7)**	**male (*n* = 7)**	**female (*n* = 9)**	**male (*n* = 6)**
(*E*)2-Hexenal*			0.23	0.33
Isoamyl acetate*				
1-Octen-3-ol*			0.28	0.33
3-Octanone*			0.25	0.25
(*Z*)3-Hexenyl acetate*	0.36	**0.31**	0.30	**0.46**
Hexyl acetate*			0.16	0.20
(*Z*)2-Hexenyl acetate*			0.13	0.20
Cymene				
Limonene*				
trans-β-ocimene*	0.27	0.25		
Phenylacetaldehyde				
cis-β-ocimene	**1.22**			
γ-Terpinene		0.18		
4-Ethyl-o-xylene			0.13	
(+/−)Linalool*	0.20	0.23	0.10	
Nonanal*	0.16	0.25	0.18	0.13
Benzeneethanol*		0.30		
(*E*)-4,8-Dimethylnona-1,3,7-triene*	0.24	0.16		
unknown1				0.23

(*Continued*)

Table 1. Continued.

	b) GC-EAD response (in mV)			
	FPM-Australia		FPM-New Caledonia	
Compounds	**female (*n* = 7)**	**male (*n* = 7)**	**female (*n* = 9)**	**male (*n* = 6)**
Ethyl benzoate*			0.19	0.06
Methyl phenylacetate				
Naphthalene				
(*Z*)-3-Hexenyl butyrate*			**0.40**	0.10
Butyl hexanoate*	0.22	0.14	0.10	0.12
Methyl salicylate*	0.32	0.20		
Dodecane*				
Decanal*	0.14	0.19	0.17	0.06
Isopropyl octanoate			0.28	
(*Z*)3-Hexenyl isovalerate*	0.20	0.12		
Phenethyl acetate*				
Heptyl butanoate*				
Tridecane*	0.16			
Methyl o-anisate				
Copaene			0.05	0.05
β-Elemene				
Tetradecane*				
β-Caryophyllene*				
β-Copaene				
α-Bergamotene	0.20	0.19		
(*E*)-β-Farnesene*				
(*E*,*E*)-α-Farnesene*	0.28	0.22		

*Compounds confirmed with synthetics.

oven temperature programme was held for 2 min at 40°C, then increased to 220°C at 10° C min-1 where it was held for 6 min. Helium was used as the carrier gas at a flow rate of 1 mL.min-1. The column effluent was split 1:1 between the flame ionization detector (FID) and EAD apparatus. For FPM from New Caledonia, antennal depolarization was detected by a high-resistance EAD probe, a signal interface box of type ID-02 and an Intelligent Data Acquisition Controller, type IDAC-02 (Syntech Research and Equipment, Hilversum, The Netherlands) and analysed with the GC-EAD software. For FPM from Australia received a year after, the antennal depolarization was detected and amplified using the IDAC 4 controller (Ockenfels Syntech, Germany) and analysed with the Autospike software (v3.9, Syntech, Germany). Antennae from 2 – to 6-day-old adults were excised at the base and attached to silver electrodes housed in saline-filled glass electrodes. When a volatile compound elutes from the gas column, it elicits depolarization in receptor neurons carrying the corresponding odorant receptor, which leads to a summed negative electric signal in the antenna. Following recordings of consistent antennal responses (i.e. at least half the individuals were responding) to some compounds, identification of these bioactive compounds was made by comparing with their respective mass spectra on the GC-MS. In total 14 FPM from Australia (7 females, 7 males) and 15 FPM from New Caledonia (9 females, 6 males) were tested with the two pooled host plant extracts with the GC-EAD.

Statistics

All statistical analyses were performed using R version 4.0.0 and packages 'ggplot2' for graphics and 'mixOmics' for multivariate analysis. Peak areas were extracted from each chromatogram and were log-transformed, adding a small constant to replace zeros such as $\log(X + 0.001)$, centred and scaled for running multivariate analysis and visualizing with principal component analysis (PCA).

Results

Chemical profile of host plants

After headspace collection of each plant *in situ* in each country, and analysis via GC-MS, we compared all chromatograms. The composition of volatile compounds was qualitatively and quantitatively quite different across all species, even within the same family (see Table 1 and Figure 2). The first two components of the PCA explained 57% of variation across all species (Figure 3). Even the most abundant compounds (see bold numbers in Table 1a) were different between species, apart for the two sub-species of *T. smilalina* and *E. variegata,* which all had cis-β-ocimene as their major compound.

FPM electrophysiological response to host plant volatiles

In order to identify compounds that are detected by the FPM, we run GC-EAD with both males and females originating from each country (Australia and New Caledonia) exposed to pooled samples from each family plant (Menispermaceae and *Erythrina*). Despite strong differences in plant volatile profiles, five compounds triggered an

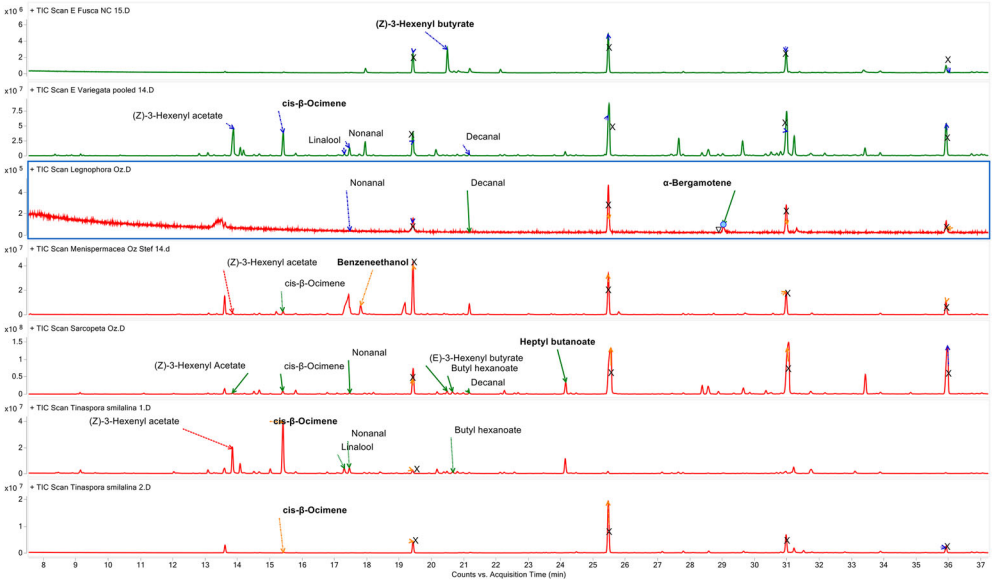

Figure 2 . Chromatograms from *Erythrina* plants from New Caledonia (in green from top to bottom: *E. fusca, E. variegata*) and Menispermaceae plants from Australia (in red from top to bottom: *Legnephora moorei, Stephania japonica, Sarcopetalum harveyanum, Tinospora smilacina (*inland*), Tinospora smilacina* (coastal)) with most abundant compound in bold and the five compounds that triggered an electroantennogram responses across the two fruit piercing moth populations indicated for each plant. Peaks crossed are contaminants.

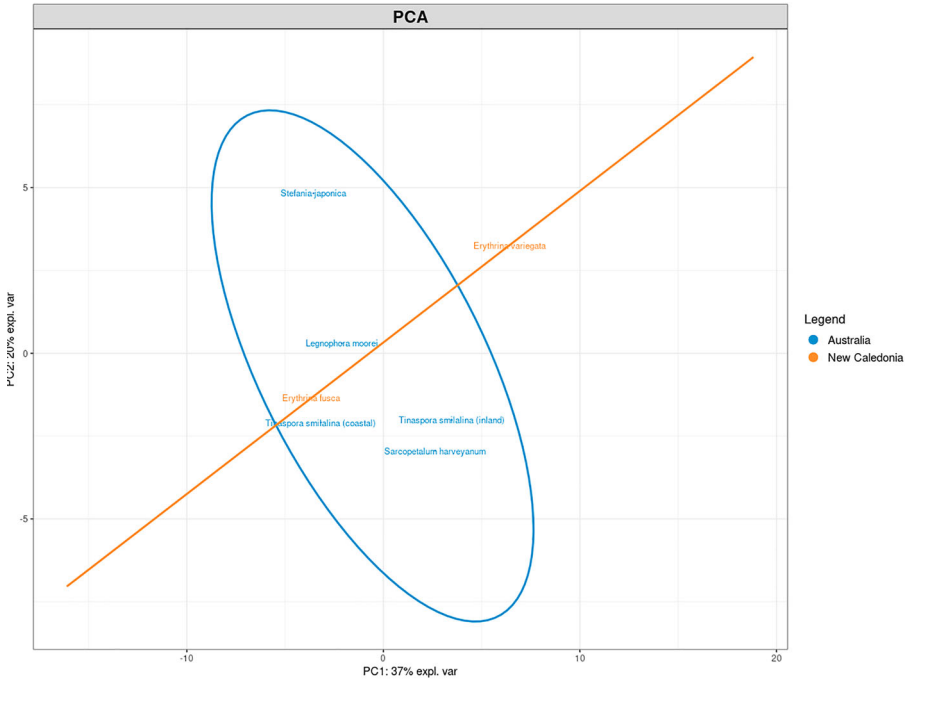

Figure 3 . Principal component analysis (PCA) of Menispermaceae species from Australia (in blue) and *Erythrina* spp. from New Caledonia (in orange).

electrophysiological response from either male or female FPM from the two populations (see shaded rows in Table 1 and compounds shown in figure 2 and S1–S6). Those volatiles in order of retention time were: *(Z)*−3-hexenyl acetate, (+/-) linalool, nonanal, butyl hexanoate and decanal. Those compounds were present in both plant families, except for butyl hexanoate, which was detected only in the Menispermaceae *S. harveyanum*.

(Z)−3-hexenyl acetate triggered on average the highest electrophysiological response from both FPM populations, although it was not the most abundant peak within each plant headspace. *(Z)*−3-hexenyl acetate was present in *E. variegata* from New Caledonia as well as the inland *T. smilalina*, *S. japonica* and *S. harveyanum* from the Australian Menispermaceae.

For each sex and population of FPM, the strongest electrophysiological responses were triggered by different compounds (see bold numbers in Table 1b). For example, FPM females from Australia responded strongly to cis-β-ocimene (the major compound of Menispermaceae overall) while FPM males from Australia responded uniquely to benzenethanol, which was found only in the Menispemeraceae *S. harveyanum and S. japonica*.

Also, there were population-specific responses, such as FPM from New Caledonia responding to *(Z)*−3-hexenyl butyrate, which was the main compound of *E. fusca* but also present in some Menispermaceae *(S.harveyanum* and the inland *T.smilalina)*. Similarly, only FPM from Australia responded to (*E*)−4,8-dimethylnona-1,3,7-triene, methyl salicylate, α-bergamotene and (*E,E)*-α-farnesene, which were all present in some of the plants from New Caledonia as well as in Australia, except for (*E*)−4,8-dimethylnona-1,3,7-triene only found in *Erythrina* species.. In contrast, butyl hexanoate was detected only in Menispermaceae but triggered a response by FPM from both populations.

Discussion

To our knowledge, this is the first time that volatile compounds from the FPM host plants have been screened to better understand host shift and local adaptation of FPM populations in the Pacific. In this study, we identified the VOCs from Menispermaceae tropical vines and from *Erythrina* coral trees that triggered electrophysiological responses by FPM from Australia and New Caledonia. Despite strong variation in VOC profiles between and within host plant families, we identified common VOCs detected by both FPM populations.

Common bioactive VOCs

Following the chemotype hypothesis, suggested by Cochereau (1977), similar compounds found in both groups of host plants would have facilitated the transition and adaptation from Menispermaceae to *Erythrina* spp. On average *cis-β*-ocimene was the major compound from all our samples, except *L. moorei,* and *E. fusca*. However, only FPM from Australia had an electrophysiological response to this compound. Nevertheless, we found five volatile compounds common across the two plant families which triggered an electrophysiological response from both FPM populations: *(Z)*−3-hexenyl acetate, (+/-) linalool, nonanal, butyl hexanoate and decanal. The presence of olfactory receptors in both populations responding to those common compounds could have facilitated the host shift between the two plant families and their localization in the environment. This is similar to the apple fruit moth *Argyresthia conjugella,* which lays

eggs on its non-favoured host apple fruit *Malus domestica* on the basis of common volatiles with its favoured host, rowan berries *Sorbus aucuparia* (Bengtsson et al. 2006).

Three other compounds found in both plant families (methyl salicylate, α-bergamotene and (*E,E)*-α-farnesene) were detected only by FPM from Australia. These compounds are usually induced by herbivory or pathogen infection as secondary defence compounds (Paré and Tumlinson 1999; Kessler and Baldwin 2001). The sensitivity of FPM from Australia to these compounds could suggest an acquired awareness to volatiles signalling the presence of other competitors. In contrast, populations from New Caledonia may have lost or not acquired the sensitivity to these induced compounds in the absence or rare presence of competitors on these plants in these Pacific Islands. The presence of these compounds resulting from the stress caused by our sampling can be excluded, since we collected the odours *in situ* without damaging the plant.

We did not find traces of the tetracyclic alkaloids that are known to be present in both families in our volatile sampling and were hypothesized to explain host shift by Cochereau (1977). These chemical compounds have a high boiling point (Chen et al. 2013) and therefore are not volatile enough to be detected by our method of sampling; hence they are not used either by FPM to locate their host plant from a distance.

Host plant-specific compounds

Only the population of FPM from Australia responded to (*E*)−4,8-dimethylnona-1,3,7-triene, uniquely found in *Erythrina* spp. Also, FPM females from Australia responded strongly to *cis-β-ocimene*, present in some Menispermaceae and identified as the main compound in *E. variegata.* Although this population is not known to use *Erythrina* spp. for egg laying and larval development in Australia (Sands and Schotz 1991), despite their presence (Stanley and Ros 1983), their olfactory sensitivity to these compounds could have facilitated host range expansion when colonizing the Pacific Islands where few Menispermaceae vines are present but where abundant *Erythrina* plants can be attractive.

In contrast, only the population of FPM from New Caledonia responded to compounds uniquely found in *Erythrina* spp (i.e (*E*)−2-hexenal, 1-octen-3-ol, (*Z*)−2-hexenl acetate, isopropyl octanoate). Thus, New Caledonian FPM seem to have acquired olfactory sensitivity to a new specific compound from its new host plant range. As hypothesized by Cochereau (1977), the larger availability of *Erythrina* tree species compared with Menispermaceae vines in New Caledonia might have prompted host shift and novel olfactory adaptation; this would have happened over a relatively short time, since the *Erythrina* spp. were introduced into these islands in the last century (Waterhouse and Norris 1987). This is a similar story to the well-known example of the apple maggot fly, *Rhagoletis pomonella* (Diptera: Tephritidae), which shifted from hawthorn (*Crateagus mollis*) to apple (*Malus domestica*) within a short evolutionary time (<150 years) owing to its host's natural scarcity in some locations (Bush 1969; Frey and Bush 1990; Linn et al. 2003).

Host race formation via chemosensory adaptation

According to the definition of Drès and Mallet (2002), the criteria for populations to be considered as host races which represent intermediate stages in the speciation continuum

are: that they are genetically differentiated, that populations use different hosts, and that between them there is appreciable gene flow.

The measured differential electrophysiological responses of the two FPM populations can be explained by chemosensory adaptation to a new host plant. Larvae of FPM from Australia would not directly feed on *Erythrina* spp. unless the opportunity is provided, whereas larvae from New Caledonia or Guam naturally feed on *Erythrina* spp. and can also develop on Menispermaceae (Waterhouse and Norris 1987; Sands and Chan 1996; Reddy et al. 2005). Therefore, host preference already exists at the larval stage. Furthermore, the capacity of FPM from New Caledonia to detect compounds specific to New Caledonian plants further supports the evolution of olfactory mechanisms specific to the new host, *Erythrina* spp. However, the electrophysiological responses of FPM from New Caledonia to common volatiles present in both plant families may reduce host plant fidelity. Since *Erythrina* spp. are dioecious trees which lose their leaves during the dry season, FPM populations move from low to high altitudes in order to find suitable host plants with leaves all year round. Therefore, populations growing on different host plants could interact at the feeding sites and potentially mate. Gene flow can then occur between sympatric populations and slow down speciation by habitat isolation. Unfortunately, little is known of their mating behaviour, and laboratory rearing has proven difficult to test for hybridization from different populations. The geographical distances between the populations of the Pacific Islands and the continental ones (Indo-Malaysian or Australian) should limit migration, thus supporting allopatric speciation via genetic drift associated with local adaptation. These pieces of evidence support that population of FPM from Australia and New Caledonia are slowly diverging and can be considered as two different host races or biotypes, as it was found between FPM populations of Guam and Australia with larval feeding experiments (Reddy et al. 2005). Adaptive divergence between the two biotypes via chemical mechanisms can lead with time to a pre-mating barrier and the formation of a new species (Smadja and Butlin 2009). This chemosensory divergence between the different populations of FPM across the Pacific Islands challenges the future development of a single generic attractant based on host plant volatiles that could be deployed for management across all the Pacific, as well as being used as a surveillance tool in New Zealand. However, a blend of common bioactive volatiles could be further tested in the field in each country.

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