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THE MONARCH BUTTERFLY CATERPILLAR (*DANAUS PLEXIPPUS*) WAVES AT PASSING HYMENOPTERA AND JET AIRCRAFT—ARE REPELLENT VOLATILES RELEASED SIMULTANEOUSLY?

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Key Word Index—*Asclepias syriaca*; Asclepiadaceae; *Danaus plexippus*; larvae; Hymenoptera; sound response; display jet aircraft; 3-hydroxy-2-butanone; *trans*-α-farnesene; 2-methoxy-3-alkyl-pyrazine; deterrents.

Abstract—*Danaus plexippus* larvae display to flying bumble-bees (*Bombus* spp.), wasps (*Vespula* sp., *Encarsia* sp.), human imitation of various 'buzzing' sounds, shouting, and the noise of jet aircraft passing overhead. We found that 33% of the total volatiles collected in the chæmosphere of fully grown larvae was 3-hydroxy-2-butanone, lacking in both food plants. *Trans*-α-farnesene (2%) also occurred and was present in *Asclepias syriaca*. Both substances may act as deterrents. Volatiles collected from the adult male and female butterflies also contained smaller amounts of the ketone. When roughly handled, the larvae emit a strong smell of 2-methoxy-3-alkylpyrazine—from the region behind the head collar—a widely distributed alerting signal found in both plants and animals. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

It has been known for over 200 years [1] that the larvae of Lepidoptera respond to sound, ranging from door-slamming to the strains of a violin [2, 3]. Setae distributed over the surface of the caterpillar's body are the organs of reception [3]. This has been verified by various authors; Tautz and Markl [4] showed that the larvae of *Barathra brassicae* detected flying wasps by hairs situated on the thorax, which were sensitive to airborne vibrations. If these filiform hairs were removed the caterpillars succumbed more often to the attacks of the parasitic wasp *Dolichovespula media*. White [5] reported that the larvae of *Cerura borealis* responded to a low frequency sound produced by the wing movements of the parasite *Cotesia* sp., by flicking the two caudal tentacles anteriorly, while extending eversable flagella from their tips. The authors surmised that these were defensive chemical dispersing organs.

The Large White caterpillar (*Pieris brassicae*) in their communal stages responded to a blackbird's whistle by synchronised violent head swinging [6]. After the 4th instar, when the caterpillars dispersed about the food plants, this reaction faded or disappeared altogether.

Various communal insects display in unison when threatened by the approach of predators, for example the aposematic Oleander aphid (*Aphis nerii*) respond by all 'jigging' together and the larval larch sawflies (*Pristiphora erichsonii*) and *P. westmoeli*, by 'snap-bending' and simultaneously releasing odorous volatiles [7]. These displays are often followed by a period of 'freezing' when the whole assembly remains motionless.

RESULTS

Response to sound by the Monarch caterpillar

This caterpillar responds to various sounds by sharp, sudden ducking or twitching movements of the head and a simultaneous agitation of the two anteriorly placed filiform tentacles (=tubercles) [8]. The head may be ducked up and down or twitched in spells of 35–37 times per min. The effect of this display, especially when a number of larvae are feeding in proximity on the food plant, is impressive. In the final instar, the response fades and may cease altogether.

The Monarch reacts to a greater variety and range of sound than the Large White, but it only occasion-

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ally appears stimulated by bird whistles or high pitched notes.

A large variety of imitation 'buzzing' sounds, human voices, shouting at close quarters, as well as the buzzing of flying bumble-bees, both males and workers, and wasps *Vespula germanica* and the minute *Encarsia formosa* (wing length 0.6 mm) introduced into their vicinity, will elicit the usual display. The wing beat frequency of the latter species reaches 370 Hz [9]. The most surprising exaggerated reaction was seen when jet aircraft (Hawker Harrier jump jets), passed overhead. Once or twice weekly, these machines sweep across the greenhouse, the pilots from the local base thus exhibiting their skill in low flying exercises. The loud aerial disturbance frightens the human bystanders if unaccustomed to the sudden rushing noise; and the vibration not infrequently cracks panes of glass in the roof of the greenhouse. The caterpillars, in unison, are then thrown into violent paroxysms of head jerking and tentacle waving, which subsides equally suddenly as the jets vanish. The effect is striking, occasionally on-lookers burst out laughing as the food plant appears momentarily to have come alive!

The larval volatiles

The caterpillar were reared at Ashton from eggs laid on *Asclepias syriaca* and *A. curassavica* by butterflies obtained in the pupal stage from San Jose, Costa Rica via the London Pupae Supplies, Oxford.

In order to investigate the larval volatiles, we adopted the methods described in *Chemoecology* [10]. Briefly, we enclosed 10 fully grown larvae in a transparent polyacetate (Pingvin) 'roasting bag', with adsorbent plugs with Porapak Q placed inside the bag. Air was drawn through with a mini-pump for 5–7 h. We enclosed 12 male and 12 female imagines in separate bags and subjected them to similar treatment. Chemical analyses was carried out at Göteborg (Sweden), see Tables 1 and 2. It should be noted that Schulz [11] extracted hair pencils and alar pockets of Danaid butterflies by isolation techniques and recorded compounds of lower volatility than revealed by our adsorption/desorption procedure. We aimed chiefly at capturing the highly volatile compounds.

The caterpillar were handled roughly before enclosing them in the container and were subjected to different types of 'buzzing' noises. Although occasionally responding with a feeble display, once enclosed in the bag they became quiescent (one or two attempted to escape) and did not duck their heads when subjected to the air currents nor the whirring sound of the pump.

The Table 1 lists the 16 principal volatiles we found in the chæmosphere around the larvae. 3-Hydroxy-2-butanone was present in large amounts: 33% of the total volatiles. This substance has a not unpleasant smell to the human nose but may, nevertheless, advertise its irritant qualities to a potential predator. It is also present in the adult butterfly's volatiles but in much smaller amounts, 1.2% in males and 0.7% in females. It is absent in the *Asclepias* host plants. The ketone was also found in odour collected in 1994. It was recorded by Sreng [12] as one of the three compounds in seducin, the male sex pheromone of a cockroach *Nauphoeta cinerea*, and is also produced

Table 1. Volatile compounds—16 principal volatiles from the chæmosphere around 10 5th instar *Danaus plexippus* larvae and their presence in food plants and imagines

	Larval volatile compounds	%	Food plant volatiles		Imago volatiles	
			A. curassavica	*A. syriaca*	♂ males	♀ females
1.	3-Hydroxy-2-butanone	33			+	+
2.	*Z*-3-hexenol	15	+	+	trace	trace
3.	Unidentified (chemically related to 1)	8				
4.	Methyl salicylate	5	+	+	+	
5.	Unidentified (chemically related to 1)	5				
6.	Unidentified	4				
7.	Unidentified	3				
8.	Germacrene D	3				
9.	Linalool	2.5		+	+	+
10.	Benzyl alcohol	2		+		
11.	Unidentified	2				
12.	Linalyl acetate	2				
13.	*Trans*-α-farnesene	2		+		
14.	Unidentified	1.5				
15.	Phenylacetaldehyde	1				
16.	*Trans*-β-ocimene	1	+	+		

Note: These 16 compounds make up 90% of the volatile material of *D. plexippus* larvae. The pyrazine present in *A. curassavica* was not recorded by the method used.

Table 2. Volatile compound—32 of the principal volatiles from the chæmosphere around larvae and imagines of *D. plexippus*

	Larvae %	Males %	Females %
mt	1.5		
Unidentified	4.8		
Trans-β-ocimene	1.3		
3-Methyl-3-butenol		15.4	2.4
3-Hydroxy-2-butanone	33.1	1.2	0.7
3-Methylbutyl 3-methyl butanoate			2.4
Tridecane			0.9
Z-3-Hexenyl acetate			0.7
f.a.der.	3.1		
f.a.der.	8.3		
f.a.der.		13.1	6.4
Z-3-Hexenol	15.1	trace	trace
Linalool oxide (furanoid isomer)		1.1	0.9
Linalool oxide (furanoid isomer)		1.0	1.0
Benzaldehyde			1.0
Linalool	2.6	1.6	0.5
Linalyl acetate	1.9		
Phenyl acetaldehyde	1.4		
f.a.der.	4.2		
Trans-verbenol		8.4	6.0
Hydrocarbon		5.3	3.6
Verbenone		6.7	5.0
Germacrene-D	2.0		
(*E*)-α-Farnesene	1.8		
Methyl salicylate	5.2	2.1	
Hydrocarbon	2.2		
Hexanoic acid?	trace	6.6	7.1
Unknown	0.5	6.1	7.9
Benzyl alcohol	2.3	4.0	4.3
f.a.der.		4.7	5.2
Octanoic acid?		2.6	3.6
Nonanoic acid?		6.8	2.8

Note: Percentages are from the total of 57 volatiles collected including smaller compounds not yet identified.

mt = monoterpene.

f.a.der. = fatty acid derivative

by various micro-organisms and is known to occur in beer, wine and butter [13].

Z-3-Hexenol, which is a 'grass odour', constitutes 15% of the volatiles, and has been recorded from the scent glands of various insects, but its function is uncertain [14]. It is present in only small amounts in the food plants. Methyl salicylate (5%) is present in the larvae, both food plants and the adult male (2%), but absent in the female. Boppré [15] found it in the Monarch's hair pencils. Its function is unknown. Germacrene D (3%) was present in the 5th larval instar and in *A. syriaca* (1.4% in seedlings), but lacking in *A. curassavica* and imagines. Curiously enough in *Papilio protenor* larvae [16] it is restricted to the 3rd and 4th instars [14]. Germacrene A has been reported as an alarm pheromone in the cornicle secretion of aphids [17], and it seems probable that it adds to the warning signals of *D. plexippus*. Benzyl alcohol (2%) is present in the caterpillar volatiles, in *Asclepias syriaca* in trace amounts (but not *A. curassavica*), and in male and female imagines. The terpene linalyl acetate (2%) was only found in the larvae, not in either food plants or adults.

The sesquiterpene *trans*-α-farnesene which, according to Schulz [11] is 'widespread in insects', is present (2%) in the caterpillar of *D. plexippus* and in the food plant *A. syriaca*. *Trans*-β-farnesene is present in *A. curassavica* (Table 3). It has also been recorded from the hair pencils of the Danaid butterflies *Amauris niavius* and *A. echeria* [11] and from the androconia of the Swift moth, *Hepialus humuli*. *Trans*-α-farnesene is considered an alarm signal in various aphids [18] and a volatile sexual attractant of the scarabid beetle *Maladena matreda* [19]. It has also been identified in Dufour's gland in various ants [20]. Boppré [15] extracted (*E*,*E*)-methyl-farnesenoate from the Monarch's hair pencils. Farnesol has been shown to possess juvenile hormone activity [21].

Table 3. Volatile compound—*Asclepias curassavica* (AC) and *A. syriaca* (AS) 22 major volatiles from leaves. y = young: o = old: s = seedlings. Numbers refer to percentages of total volatiles

	y.	o.	s.	o.
Compound	AC		AS	
Mycrene			0.3	0.3
Limonene			0.6	0.1
Cis-β-ocimene			0.5	0.1
Trans-β-ocimene	2.3	4.5	44.1	13.8
4,8-Dimethyl-1,(*E*)3,7-nonatriene	59.2	30.3	14.7	17.9
(*Z*)-3-Hexenyl acetate	2.9	1.8	3.2	12.6
Hexanol			0.4	0.8
(*E*)-3-Hexenol			trace	0.3
(*Z*)-3-Hexenol	trace	1.6	19.8	38.5
1,3,8-*Para*-menthatriene			1.4	0.9
α-Copaene			0.2	trace
Linalool			0.5	0.1
Caryophyllene			0.9	0.4
α-Humulene			0.3	0.7
Trans-β-farnesene	2.4			
Germacrene D			1.4	0.7
Trans-α-farnesene			5.9	0.5
δ-cadinene			0.3	0.3
Methyl salicylate	13.3	24.9	0.5	1.2
4,8,12-Trimethyl-1, (*Z*)3,(*E*)7,11-tridecatetraene		1.5		
4,8,12-Trimethyl-1, (*E*)3,(*E*)7,11-tridecatetraene	20.0	34.4	0.3	0.3
(*Z*)-3-Hexenyl benzoate			1.5	1.9

Note: This table is a condensed version of Tables 3/4 in Bergström *et al.* [10]. The pyrazine present in *A. curassavica* was not recorded (see text) by the method used.

Phenylacetaldehyde (1%) is present in the larva but was not found in the imago or food plants, while the terpene *trans*-β-ocimene (1%) is present in both plants and the larva but not in the adults. The unidentified numbers 3 and 5 (see Table 1 and 2) are closely related to 3-hydroxy-2-butanone.

We found if the anterior region of the larvae, near the head collar, was gently squeezed the unmistakable odour of 2-methoxy-3-alkylpyrazine became apparent, although no reflex bleeding or glandular exudate was noticeable. This substance is present in *Asclepias curassavica* [22]. We failed to detect pyrazine, however, in the chæmosphere of the host plant leaves, caterpillar or imago, but this was not unexpected as our system does not record some compounds, especially those occurring in very small amounts [10, Fig. 1].

2-Methoxy-3-alkylpyrazine is a widely distributed alerting signal in both plants and animals [23], and in the case of the Monarch is usually sequestered by the larvae from the food plant, but Trinidad Danaids are able to metabolize it when reared on plants lacking this pyrazine [22]. The leaves of *Asclepias curassavica* smell very strongly of pyrazine if scratched or injured. In the butterfly this substance is present in the haemolymph and any injury which brings blood to the surface releases its unmistakable odour.

It is known that the Monarch caterpillars also sequester and store cardiac glycosides from their food plant, and PAs if they are present. These pass via the pupal stage to the tissues of the adult butterflies [24, 25]. It is safe to assume that they likewise sequester and store *trans*-α-farnesene from *Asclepias syriaca* and may metabolise the *trans*-β-farnesene in *A. curassavica* into α-farnesene before metamorphosis.

The pyrazine probably circulates in the haemolymph of the larvae, as they do in the adult butterfly and appear to be concentrated in and released from the region of the head collar, at the site of the osmeteria of *Papilio* larvae.

The distribution within the tissues of the caterpillar of 3-hydroxy-2-butanone which is a short chain fatty acid derivative, and *trans*-α-farnesene is not known.

The pair of anterior filiform tentacles have a central canal, containing haemolymph but do not appear to excrete any defensive material; they are used continually by the larvae for investigating its immediate surroundings including the food plant. Their agitated thrashing movements during the display could disperse the volatile material emanating from the head collar region. This might well startle small parasitic wasps and induce them to move away. As weapons of defence the tentacles are singularly ineffectual against their larger wasp predators. One of us (MR) has watched *Vespula germanica* land alongside a three-quarter grown solitary larva of *D. plexippus*, sting it, cut it in two and carry off each half piecemeal.

DISCUSSION

We have found that the food plants and the larvae have relatively few specific volatiles in common (Tables 1 and 3), which is somewhat surprising. It should be borne in mind that we were working with limited material, and these must be regarded as preliminary results. The larger number of the major components in the imago (Table 2) clearly reflects the more active phase of the life-cycle, but only six of these were recorded in the larvae. Again the lack of specific volatiles collected from *A. curassavica*, eight compared with 20 from *A. syriaca*, is unexpected (Table 3). To the human nose the odour of *A. curassavica* is far more powerful, due chiefly to the strong smell of the pyrazine which, in addition, is well known to 'lift' and enhance the aroma of other odours present.

The relatively high concentrations of the short chain highly volatile ketone 3-hydroxy-2-butanone (33%) and the two unidentified related fatty acid derivatives **3** and **5** (Table 1: 8 and 5%, respectively) present in the larvae, is interesting. They are probably self-secretions of the caterpillar, rather than the production of micro-organisms, and are carried over in small quantities to the adults via the pupal stage. As we have noted (see Results) these substances were absent from both the food plants. It seems likely that 3-hydroxy-2-butanone acts as the major volatile deterrent for the Monarch caterpillar, but one is on more familiar ground in assuming *trans*-α-farnesene, although present in smaller amounts, functions as an alarm signal.

Butanones are rare defensive substances in insects generally, and when present as in *Cimex* [14] constitute trace components in all secretions. A related compound, 4-(4-hydroxyphenyl)-2-butanone, was found by Nishida [26] in the flowers of the Orchid *Dendrobium superbum*, collected and stored by the Melon fly *Dacus cucurbitae* in the rectal gland. It is worth noting that Eisner [27] identified the ketone 2-undecanone in defensive fluid from the cervical gland of a caterpillar of a Notodontid moth.

Flexibility is characteristic of the defensive compounds of insects; the same chemical frequently serves different purposes and is present in different organs and in different unrelated species. *Trans*-α-farnesene or isomers of farnesene, as we have noted, may function as an alarm signal, a sex attractant, a trail marker secreted in Dufour's gland, a marker along a flight path, secreted in a labial gland, and in male hair pencil scent organs.

In the majority of aposematic insects the major defence compounds are usually non-volatile, like the cardiac glycosides, or pyrrolizidine alkaloids in the Monarch imago, or the self-secreted toxins such as cajin in *Artica* and 'zygénin' in *Zygaena*.

It is evident that the majority of the volatile compounds released by the Monarch larvae, adults and the Asclepiad food plants (Tables 1–3), represent three chemical classes: fatty acid derivatives (for example in Table 1 1–3 and 5), benzenoids (4, 10 and 15) and isoprenoids (8, 9, 12, 13 and 16). These classes are usually well represented in both plants and insects [28] which once again illustrate their close interdependence.

Pyrazines, which are frequently associated with major toxins, are the most interesting alerting signal of the Monarch caterpillar. The ducking of its head during display exposes the area from which 2-methoxy-3-alkylpyrazine is released and the simultaneous agitation of the lateral tentacles would assist the distribution of the odour. An alerting signal must always be a forerunner, and function at a distance, even if only a small one, from the potential predator [22]. Thus the lateral tentacles are an important factor in the caterpillars' display.

There is a mild sense of disappointment that the defensive volatiles, especially the self-secreted volatiles of Lepidoptera, fail to give much assistance to the taxonomist. Many of these volatiles are widely distributed in unrelated insects, pertaining to different families and even Orders, of which pyrazines are a good example. They reflect the similar lifestyles of the species concerned rather than any phylogenetic relationship. Thus various Lepidoptera, Coleoptera, Hemiptera and Orthoptera which sequester or store plant toxins, employ pyrazines as warning or alerting signals. These substances may be either metabolised by the insect or sequestered from the food plant; this type of parallel evolution is linked to, or associated with, the plants' own defences. Once embarked on the aposematic lifestyle it is necessary for an insect to stockpile a variety of defence mechanisms in order to cope with predators attracted to exposed prey. In the lower taxonomic categories, such as species, genera and subfamilies, further investigation of the larval forms may well assist and support the systematist. Thus for example, Eisner has recorded nine species of Papilionid larvae secreting the odoriferous aliphatic acids 2-methylbutyric acid and isobutyric acid from the osmeterium [29, 30]. The species involved include *Baronia brevicornis* pertaining to a monobasic genus placed in a separate subfamily. On the other hand several Papilionid larvae produce fundamentally different substances in the osmeterium [14]. It will be interesting to see whether secretion of 3-hydroxy-2-butanone, or isomers thereof, by *D. plexippus* caterpillars, a substance which is not found in their food plants, will be common to various related Danaids.

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