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A STROPHANTHIDIN GLYCOSIDE IN SIBERIAN WALLFLOWER: A CONTACT DETERRENT FOR THE LARGE WHITE BUTTERFLY

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Key Word Index—*Cheiranthus* × *allionii*; Cruciferae; Siberian wallflower; *Pieris brassicae*; Large White butterfly; strophanthidin glycoside; cardenolide; contact deterrent; insect oviposition.

Abstract—In the greenhouse the Large White butterfly (*Pieris brassicae* L.) does not oviposit on the Siberian wallflower (*Cheiranthus* × *allionii*) or *Erysimum scoparium*. From the leaves of *Cheiranthus* × *allionii* we extracted a strophanthidin glycoside (also present in washings from the leaf surface) which when sprayed onto cabbage leaves inhibited egg laying. These extracts were tested in a dual choice chamber. Assayed on the butterfly's tarsal receptors the strophanthidin glycoside produced a high, to very high impulse frequency, which is phasi-tonic, with only one cell firing. It appears to be the first natural contact oviposition deterrent recorded for the Lepidoptera.

INTRODUCTION

The Large White butterfly (*Pieris brassicae* L.) does not oviposit on plants of either the genus *Cheiranthus* or *Erysimum* in the greenhouse, and only exceptionally in Nature, although both contain mustard oils (glucosinolates), their classical oviposition cues. It is of course well known [1] that both these plant genera contain cardiac glycosides within their tissues, whereas these are lacking in the majority of the Cruciferae, and have never been identified in the food plants of this butterfly. Cardenolides have been extracted from petals as well as the foliage and seeds of *Cheiranthus* [Reichstein, T., personal communication].

In a series of preliminary experiments by one of us (MR) cf. [2]* extracts of the foliage of a selection of various plants (Table 1) were sprayed onto the surface of fresh cabbage leaves which were offered to gravid or ovipositing Large Whites, for a period of two or three hr during the peak egg-laying period. The control was a cabbage leaf sprayed with extract from a similar cabbage leaf or with distilled water. Egg batches on both the experimental and control leaves were compared (Table 1).

Although a wide variety of plant species were used for comparison we chiefly selected those known to contain alkaloids or other substances believed to protect them from herbivores, whether by taste, smell or indigestible and toxic properties. Special attention was paid to species of the Asclepiadaceae which are also known to contain cardenolides [1].

It is recognised that quite apart from external influences like sunshine, humidity, light, and atmospheric pressure, a combination of plant characteristics influence oviposition. Vision (involving leaf shape, colour, size, habitus, etc.) as well as odour perception of the plant volatiles, and the surface quality of the morphology of the foliage (a tactile stimulus as opposed to substances affecting the chemical senses) are all involved. But it is not unusual to find that a group of chemicals such as the mustard oils, released from the plant tissues by scratching or drumming, exert a critical and dominating influence, whether as attractants or repellants. The evidence we had accumulated from our observations and experiments supported the hypothesis that the cardiac glycosides known to be present in *Cheiranthus* and *Erysimum* [1] were the principal oviposition deterrents and, perhaps, chiefly responsible for the rejection by the Large White butterfly of the plant and the extinction of the oviposition cue also present. We therefore sprayed cabbage leaves with a solution of ouabain in order to test the effect on oviposition of a cardenolide divorced from any of the plant volatiles and other chemical substances which could be present in our plant extracts. The ouabain spray was less potent as a deterrent than strophanthidin (unfortunately not available at that time for tests) but nonetheless proved effective.

A simple experiment was also performed to demonstrate that some element in the *Cheiranthus* foliage was lethal to young caterpillars of *P. brassicae*. An egg batch was placed on a leaf of the wallflower and the eggs hatched normally. Presumably the mustard oil feeding cue induced the larvae to feed, but none survived beyond the 2nd instar and most of them died during the first instar. This experiment was repeated several times with similar results. The same results were obtained with leaves sprayed with ouabain.

We therefore concentrated our attention on the strophanthidins present in the plants. Our findings set out below suggest that these substances, which are essentially non-volatile, are the first naturally occurring contact

*A synopsis of this paper only was published. The details presented here were embodied in the lecture itself [2].

Table 1. *Pieris brassicae*: Eggs laid on cabbage leaves sprayed with different plant extracts (selected samples)

Plant species	Plant extract (no. eggs batches or eggs)	Distilled water (no. egg batches or eggs)
Asclepias curassavica	0	30 (batches)
Calatropis procera	0	5 (batches)
Gomphocarpus fruticosus	0	5 (batches)
Hoya sp.	48	164
Stephanotis sp.	330	1051
Cheiranthus cheiri	0	50
Cheiranthus × *allionii*	0	395
Brassica oleracea	188	155
Brassica oleracea	482	462
Brassica oleracea	470	350
Peucedanum sp.	26	40
Digitalis purpurea	47	223
Euphorbia polychroma	347	77
Cassia sp.	44	63
Papaver sp.	45	68
Senecio jacobaea	182	139
Cannabis sativa (Mexican strain)	132	1493
Cannabis sativa (Turkish strain)	773	1421
Hippiastrum sp.	68	73
Brunsfelsia sp.	0	390
Nicotiana sp.	110	600
Acacia sp.	43	165
Lycopersicon esculentum	8 (batches)	9 (batches)

oviposition deterrents identified for the Lepidoptera. The lack of drumming and surface scratching by those female butterflies which merely brush against the plant, not settling to test them further but flying off immediately, can be explained by the fact that simple washing of the foliage reveals the presence of strophanthidin on the leaf surface.

RESULTS

Preliminary experiments in the greenhouse

Without exception, in all trials envolving leaves sprayed with plant extracts, eggs were laid on the control leaf. Only seven plant extracts (Table 1) completely inhibited egg laying on the experimental leaf, over-riding the glucosinolate cue of the cabbage leaf onto which the extract was sprayed. Of these seven plant species, six, i.e. *Asclepias curassavica, Calatropis procera, Gomphocarpus fruticosus, Cheiranthus cheiri* (some but not all cultivars), *Cheiranthus* × *allionii* and *Erysimum scoparium* are known to contain cardenolides in their tissues. The three first species belong to the Asclepiadaceae. Two other members of this family, *Hoya* and *Stephanotis*, which do not contain cardenolides, were extracted and sprayed onto cabbage leaves but only slightly reduced oviposition. On the other hand, extract of the foxglove (*Digitalis purpurea*) which we assumed contained cardenolides, also reduced but did not inhibit egglaying.

A few results were rather surprising. Thus the variation shown between the different cultivars and strains of *C. cheiri* was very striking (Table 2). (We did not test these plants for cardenolide content). It was also unexpected to find that extract of *Euphorbia polychroma* greatly increased oviposition compared with that of the control (347 eggs compared with 77), although in the greenhouse the Large White will not lay on the growing plant. However, the young larvae feed on the foliage if it is damaged. We therefore tested this species for the presence of glucosinolates, which have been recovered from one species of *Euphorbia* from India [3], but they were apparently absent. The oviposition and feeding stimulant in this plant therefore remains unidentified. It was also surprising to find that the extract of both ragwort and laurel did not depress oviposition. The former plant contains pyrrolizidine alkaloids and has a powerful odour, while laurel leaves contain a considerable concentration of HCN which smells strongly when the leaves are macerated. It was also interesting that the extract of cabbage increased the attraction of the sprayed leaf compared with the control which received only distilled water (Table 1).

We found that in the greenhouse free-flying Large White females laid on pot plants of cabbage, *Mattiola, Brassica napus, Tropaeolum* and *Reseda* but never on *Cheiranthus* × *allionii* or *Erysimum scoparium* (one exception) and only very rarely on *Cheiranthus cheiri*.

When searching for a food plant these free-flying females will drum on the foliage of a wide variety of unsuitable plants, such as *Pentas, Strelitzia, Eupatorium, Asclepias*, etc. Nor does the gravid female lay immediately

Table 2. *P. brassicae*: oviposition response to different strains of cultivated wallflower (*Cheiranthus cheiri*)

Plant species extracted	Plant extract (no. eggs or batches)	Cabbage leaf sprayed cabbage extract or distilled water (no. eggs or batches)
*Cheiranthus cheiri**	0	50
Strain A	2	135
Strain B	2	171
Strain C	1	48
Strain D	550	423
Reseda sp.*	8 (batches)	9 (batches)
Tropaeolum sp.*	4 (batches)	3 (batches)
Paper + *Brassica**	4 (batches)	green paper + distilled water O

*Contain glucosinolates.

after finding an acceptable host plant [4] but drums on average about 16 times before laying eggs. If well-matched contiguous plants of *Mattiola* sp. (which contain no cardenolides) and *Cheiranthus allionii* were offered, we found that no eggs were laid on the latter and fewer landings were made on *Cheiranthus*. If landings were followed by drumming, these were reduced in number before the plant was abandoned (Table 3 and 4).

We have reported previously [5] that the presence of eggs of the Large White on a host plant function as a deterrent. Experiments performed in the greenhouse with egg washings (500 eggs/ml in water) show that sprayed on the surface of cabbage leaves these will also reduce oviposition (Table 5). But leaves treated in this manner will always be chosen in preference to leaves sprayed with extract of *C. allionii*. The latter is a far more powerful deterrent than any of those occurring naturally which we have reported in the past, such as slug damage, the presence of feeding larvae [5] or egg batches.

Cardenolide identification

Tests of the various extracts in the dual choice chamber indicated that fraction 5 was the most effective oviposition deterrent and attention was therefore concentrated upon it. Isolation of this fraction was made from a base extract in 50% methanol subsequently subjected to clean-up. From fraction 5, ten HPLC fractions were further isolated, which contained 14 cardiac glycosides. Identification of the major component of fraction 40.15 was made by a comparison between the proton NMR spectrum (270 MHz) of this fraction (40.15) with that of strophanthidin, proving that the main cardenolide is a strophan-

Table 3. Ovipositing female *P. brassicae**: number of individual drumming sequences on matched contiguous plants

Matched plants		♀ drumming once after landing	♀ drumming twice	♀ drumming 3–5 times	♀ drumming 6 times and over	Egg batches
Stock (*Matthiola*)		454	131	174	143	161
V	33 trials					
Wallflower (*Cheiranthus*)		526	85	37	1	nil
Cabbage (*Brassica*)		6	12	6	9	21
V	1 trial					
Stock (*Matthiola*)		3	5	8	6	2
Cabbage (*Brassica*)		29	9	21	20	39
V	1 trial					
Erysimum sp.		4	4	7	1	1

*The butterflies were marked for purposes of identification but were free flying in the greenhouse.

Table 4. Ovipositing female *P. brassicae**: initial contact visits to single matched contiguous plants: April–August 38 trials

Plants†	Approach and initial contacts	Approach and sheering off without contact	Eggs laid (batches)
Stock (*Mattiola*) V Wallflower (*Cheiranthus cheiri*)	1198	129	185
	798	395	nil
Stock (*Mattiola*) V Cabbage‡ (*Brassica oleracea*)	45	not counted	2
	70	" "	21
Erysimum V Cabbage (*Brassica oleracea*)	16	" "	1
	67	" "	39
Rape (high glucosinolates) (*B. napus*) V	48	4	2169 eggs 80 batches
Rape (low glucosinolates) (*B. napus*) V	65	3	1358 eggs 56 batches
Siberian wallflower (*C. allionii*)	16	5	nil

*The females were marked for identification but were free flying in the greenhouse.

†Transposed every 15 m during trials.

‡Two trials only with cabbage.

thidin glycoside: elucidation of the structure of the two sugar residues is in progress. Also the mass spectra of fraction 40.15 and cymarin strophanthidin 3-cymaroside are virtually identical in the region m/z 300–405, reflecting the identity of the aglycone portion of the molecule. Ions were observed at m/z 405, 387, 368, 356, 340 and 322 and the intensities were identical in both spectra. Surface washings of the leaves (Fig. 1) also contained the main cardenolide, and such washings sprayed onto leaves in the dual choice chamber inhibited oviposition.

Dual choice chamber

The separated water and methanol phases of the basic extract from the foliage of young plants of *C. × allionii* was assayed for acitivty in the dual choice chamber. The water phase proved to contain the glucosinolates and when sprayed onto the surface of a dummy leaf elicited oviposition of the female Large White. The methanol phase was found to contain 12 deterrent HPLC fractions. Each of these inhibited egg laying to a greater or lesser extent when sprayed onto cabbage (*Brassica campestris* var *pekinensis*) leaves. Of these fractions, 5 (Fig. 2) proved to be the most effective and attention was henceforth concentrated upon it. Fraction 5 (40.15) was separated into a phenolic glycoside fraction and a cardiac glycoside. Both were tested in the dual choice chamber. The phenolic fraction was found to exert no effect on egg laying, both the cabbage leaf and its control carrying approximately the same number of eggs. The cardiac glycoside (strophanthidin type), on the other hand functioned as an oviposition deterrent. It was sprayed onto a cabbage leaf (both sides) and the similar control leaf was sprayed with the methanol carrier.

Three different concentrations were assayed:

Experimental leaf	Control
CA 300 ng cm^2 nil eggs	
CA 30 ng cm^2 nil eggs	795 eggs
CA 3 ng cm^2 112 eggs	

From the extract of fraction 5 (40.15) from which 10 HPLC fractions were isolated, five proved to be of the

Table 5. Oviposition of female *P. brassicae* on cabbage leaves sprayed with egg washings* and deterrent fractions† of *Cheiranthus* (control leaves sprayed with methanol carrier)

	Control leaves	Leaves sprayed with *P. brassicae* egg washings
Batches of eggs laid	745	343
Number of eggs laid	23963	8648
Experiments when no eggs were laid	0	0
		Leaves sprayed with different deterrent fractions of *Cheiranthus*
Batches of eggs laid	1087	195
Number of eggs laid	27027	5536
Experiments when no eggs were laid	0	10

*Supplied by Drs Brüggemann and Klijnstra.

†Supplied by Dr Lennart Lundgren.

These experiments substantiate the field observations that the oviposition deterrents in the wallflower are more effective than the egg pheromone deterrent.

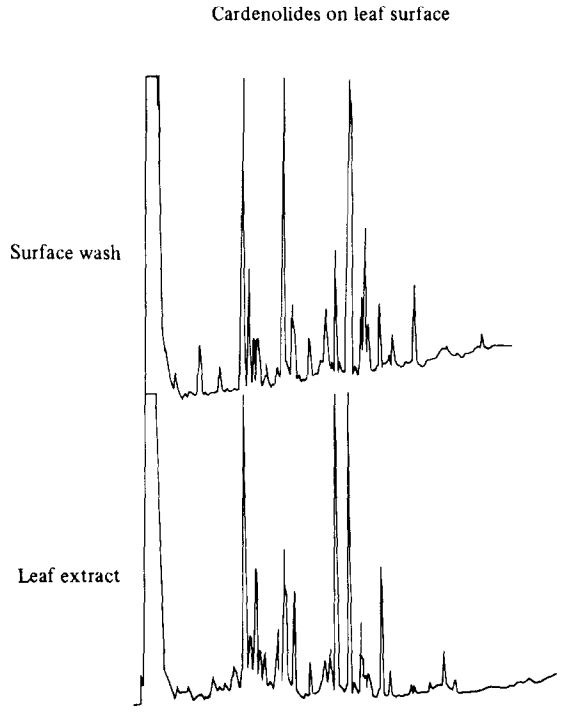

Fig. 1. Comparison of cardenolide contents in leaf extract and on leaf surface of *C. allionii*. Liquid chromatograms of A; 50% methanol–water extract and B; steam wash. Both samples with phenolics removed. Chromatographic column: analytical 15 cm 4.8 mm i.d., packed with 4 μm Novapac C18 (Waters). Flow: 1.0 ml/min. Oven temperature: 60°. Detection: UV adsorption at 220 nm. Mobile phase: gradient from 10 to 40% of methanol in water in 35 min.

strophanthidin type (24.50, 25.00, 36.75, 40.15, 46.35) and one (49.06) of the uzarigenin type. It will be seen from Table 6 that all ten fractions which were tested in the dual choice chamber at concentrations of 30 ng/cm^2 were found to deter egg laying. In the trial with the major cardiac glycoside (fraction 40.15) 48 eggs were laid on the experimental leaf and 3453 on the control leaf. The effect appears to be additive not synergistic. Thus a mixture of the four principal components (24.50 + 25.00 + 36.75 + 40.15, see Fig. 2) has the same effect as the same concentration of one of these components (24.50) while a similar concentration of the four small components (44.50 + 46.35 + 48.05 + 49.06, see Fig. 2) is equally effective.

Assay on foretarsal receptors

The foretarsal recordings of the B-hairs of *P. brassicae* (Fig. 3) show that they respond to 0.01 M NaCl in one or two cells, at very low frequencies. We found that the cardenolide produces a high to very high impulse frequency which is phasi-tonic, with predominantly only one cell firing. A crude extract of the oviposition deterrent pheromone (ODP) [5, 6] (egg washings 500 eggs/ml in water) evokes a medium to high frequency. Spike size and shape characteristics suggest it is the same cell responding to both the wallflower strophanthidin glycoside and ODP, a suggestion enhanced by the fact that we found that a mixture of both also stimulates only a single cell (Fig. 3).

After stimulation with the cardenolide solution, the cell's response to 0.01 NaCl becomes much more intense than before the application. The same applies to ODP reactions, although this proved more difficult to determine as the initial response was medium to high intensity. In some preparations the potentiating effect of one or

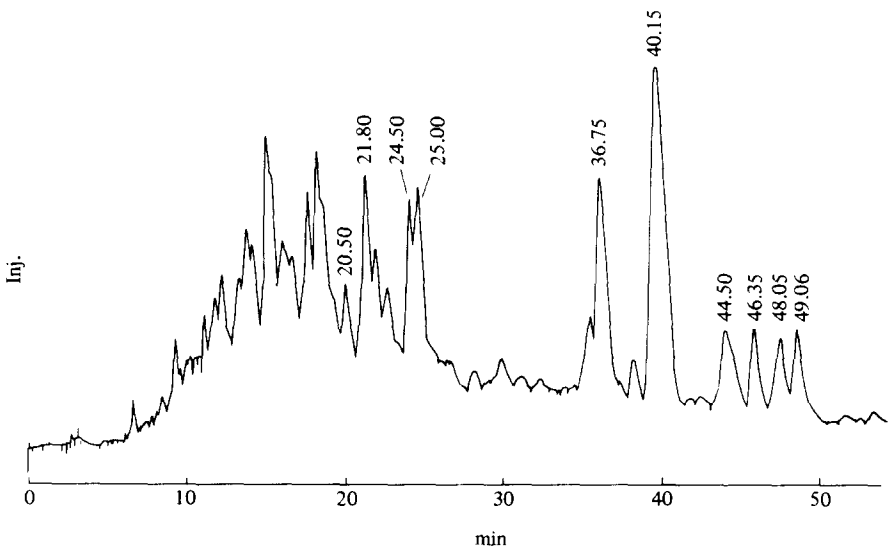

Fig. 2. HPLC of a purified extract obtained from *C.* × *allionii*. Column: 30 cm × 8 mm i.d. 10 μm Polygosil C18. Column temperature: 60C. Mobile phase: methanol–water gradient from 10 to 25% in 5 min followed by 25–45% in 45 min. Detection: UV adsorption at 220 nm. Fourteen cardenolides were isolated from the methanol/water extract of *C.* × *allionii*. Five are of the strophanthidin type and one of the uzarigenin type. The main cardenolide fraction (R_t 40.15 min) does not represent all the activity in the plant extract but it has the strongest deterrent effect on oviposition and was present in large amounts in the leaves of *C. cheiri*, *C.* × *allionii* and *Erysimum scoparium*

Table 6. Ovipositing of Large White butterflies *P. brassicae* in a dual choice chamber

Test fraction R_f (min)	Test leaves sprayed with fraction (no. of eggs)	Control leaves sprayed with carrier (no. of eggs)	Eggs on test leaves (% of total)
20.50	540	2931	15.6
21.80	208	3710	5.3
24.50	113	2442	4.4
25.00	58	2219	2.5
36.75	371	2431	13.2
40.15	48	3453	1.3
44.50	329	2672	11.0
46.35	167	2660	5.9
48.05	263	3237	7.5
49.06	359	2388	13.1
Sum:	2456	28143	8.0

Sum of data from six (three hr) experiments involving ten females.

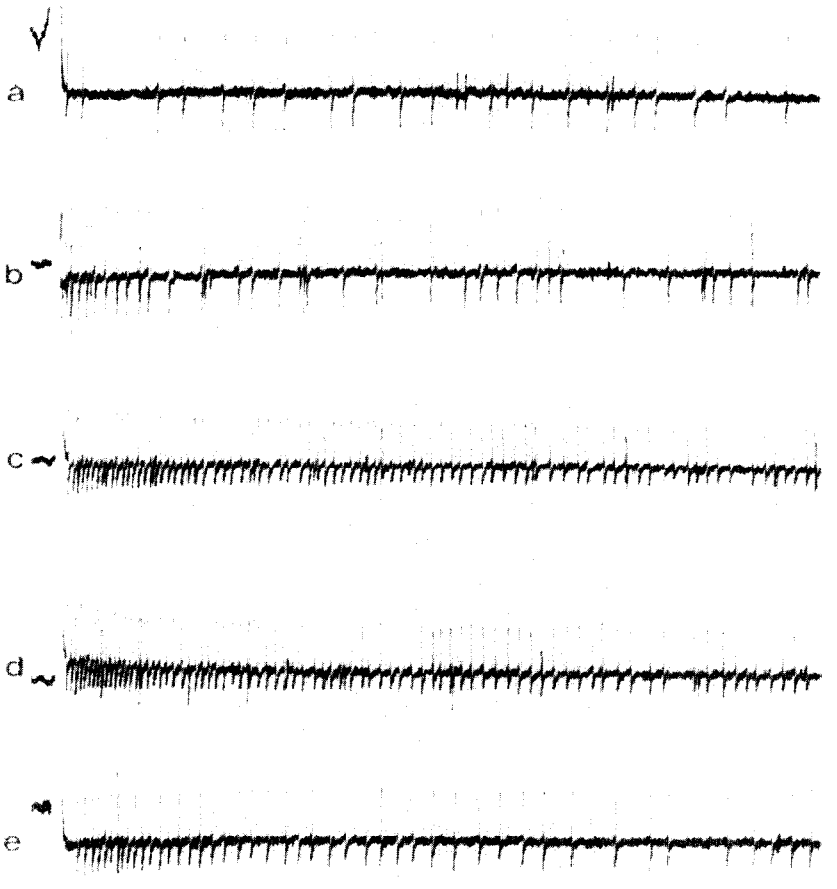

Fig. 3. Neural responses of a tarsal contact chemoreceptory hair of a female of *Pieris brassicae* to different stimuli: (a) 0.01 M NaCl, (b) ODP, eggwash from 500 eggs/ml in water, (c) 0.05% strophanthidin glycoside in 0.01 M NaCl, (d) 0.05% strophanthidin + eggwash from 500 eggs/ml, (e) 0.01% M NaCl after three prior stimulations with strophanthidin. In the latter case the hair was rinsed with aqua dest before stimulation with NaCl. All recordings last one sec.

more stimulations with cardenolide solution was spectacular.

DISCUSSION

Factors determining oviposition in butterflies, both with regard to attractants and deterrents, are varied and complicated. Writing about the insect's ability to overcome or avoid the toxic defence mechanisms of their host plants, Blum [7] states each one must be regarded as a unique entity for, "it appears that the methods for dealing with toxins are as numerous as the insects themselves" and again, "the mechanisms of host plant recognition may be equally unique and diverse".

The Monarch (*Danaus plexippus*) for instance, feeds mainly on plants of the Asclepiadaceae and if cardenolides are present in the tissues they are sequestered by the larvae and stored [8]. But these substances do not constitute obligatory oviposition cues (the oviposition cue of the Monarch is unknown) and it lays and feeds up successfully on species lacking cardenolides. The Large White, which is also a toxic, aposematic species [9–11], stores glucosinolates sequestered from its foodplant, but these substances also serve as compulsory oviposition cues, and the gravid female will only lay on plants containing them. Although in the laboratory egglaying can be induced by merely brushing the gravid females feet with sinigrin [12] in Nature other less imperative factors within this framework, are known to play a not unimportant role in determining the choice of oviposition sites [4, 5]. Thus the presence of butterfly's eggs on a leaf will render it unfavourable, over-riding otherwise various attractive features (Table 5).

Rodman and Chew [13] found that in *Pieris napi* oviposition correlates with glucosinolate profiles of plant species. This phenomenon—if indeed it exists for *P. brassicae*—is far less well defined for this species [4]. Furthermore cardenolides painted on their foodplant, has neither a repellent or a toxic effect on the larvae of *P. rapae* [Usher: thesis] whereas in our experiments they invariably proved lethal to the young larvae of the Large White. The picture is further complicated by the variation in the repellent quality (cardenolide concentrations ?) in many of the cultivated strains of *Cheiranthus cheiri* and also by the different effects of the different types of cardenolides on the butterflies. This angle of the problem is well illustrated by the Danaids which are selective storers of the host plant cardenolides; in some cases they metabolise, reduce and in others enhance the toxic qualities of the sequestered cardenolides [14, 15]. Thus it is interesting, but also something of a relief to find that the deterrent effect of the strophanthidin glycoside from *C. allionii* is so clear cut and unequivocal; the fact they are also present on the leaf surface (Fig. 1) ensures they function as 'instant' contact deterrents. They can alert the butterfly to the unsuitable nature of the potential host-plant without the female drumming or scratching the surface, and thus releasing the glucosinolates from the plant tissues. Should this occur, however, the cardenolide deterrent over-rides the glucosinolate cue. How this is brought about is at present a matter for speculation.

In addition to a mechano-receptive neuron, the B-hairs on the fifth tarsomere [16] contain three chemoreceptive cells as deduced from impulse amplitudes and frequency characteristics [6, 17]. They respond to salt, ODP, and glucosinolates. Our present observations suggest that the 'ODP receptor' appears to act as a general deterrent receptor. Certainly the cardenolide is very effective, and we suggest that the deterrent-sensitive receptors are potentiated or de-stabilised by the action of the strophanthidin glycoside. As far as we are aware this has not previously been reported for tarsal receptors, although a comparable phenomenon is known for the mouth-part sensilla of various insects, upon stimulation with copper ions, correlated with antefeedant activity.

Many questions remain unanswered. Are the other cells also de-stabilized? Is the sinigrin receptor affected? Does the sensitised deterrent cell fire spontaneously, thus depressing oviposition behaviour? Do other related compounds have similar effects?

Although strophanthidin appears to be such an unusually effective deterrent—in fact the only natural contact deterrent identified hitherto for the Lepidoptera—it may not be the sole factor involved in the repellent qualities of *Cheiranthus* or *Erysimum*. Observations of the ovipositing female, free-flying in the greenhouse, suggests that the butterfly makes fewer close approaches (Table 4) to these plants than to other species which also contain glucosinolates. This suggests that a volatile may have discouraged the ovipositing Large White before contact with the *Cheiranthus* plant surface; on the other hand it may have merely lacked an additional unidentified attractant present in *Mattiola*.

It is well known (see [18] p. 488) that the volatiles from tomato and Indian hemp, when these plants are grown in the proximity of the normal food plant, reduces laying by the Large White (*Pieris brassicae*). We have noticed that in the greenhouse the presence of plants of *Passiflora foetida* dramatically reduces oviposition of this butterfly. Further investigation will, in all probability, reveal other deterrent factors in addition to the presence of the major strophanthidin contact deterrent in *Cheiranthus* and *Erysimum*, but none which so effectively over-rides the glucosinolate cue.

EXPERIMENTAL

The butterflies. All the oviposition experiments and tests, both at Ashton and in Göteborg were made with stock derived from Brian Gardiner's Cambridge strain of the Large White butterfly (*Pieris brassicae*). Those reared at Ashton were fed on cabbage, (*Brassica oleracea*, Greyhound cultivar). Those reared in Göteborg were raised on artificial diet [19]. Three mercury halogen vapour lamps with wide 80 cm diameter reflectors were used to produce a light intensity in the breeding cages of 5000 lux.

The plants. The wallflower (*Cheiranthus cheiri*), the Siberian wallflower (*Cheiranthus* × *allionii*) and *Erysimum scoparium* were grown under glass at Ashton (England). *C.* × *allionii* had been in cultivation in this situation 12 consecutive years. Our original stock of *Erysimum scoparium* was collected in the Canary Isles by Tadeus Reichstein. Plants derived from Ashton seeds were subsequently grown at Göteborg (Sweden). We tested the deterrent oviposition effect of both living plants, living plant extracts and dried leaf extracts. Foliage was originally air-dried and sent by post to Sweden. Subsequently the plants were grown at Göteborg, and 5–10-week-old foliage dried for 12 hr at 40°. The cabbage used in oviposition experiments at Ashton was *Brassica oleracea* (Greyhound cultivar), and at Göteborg *Brassica campestris* var *pekinensis* (China King cultivar).

Oviposition tests. The preliminary tests were carried out in a large greenhouse (at Ashton) in which 100 specimens of the Large

White butterfly, which included a number of females paired three days previously, were released. For certain experiments the individual females were numbered.

They were offered two large similar cabbage leaves cut from the same plant, each presented horizontally, on a flower pot. These were transposed every 15 min. The experimental leaf was sprayed with the fluid derived from macerated leaves of *C.* × *allionii* and various other plants, filtered through No. 1 Whatman paper to which 50 ml of H_2O was added. The control was a leaf sprayed with the fluid from macerated cabbage leaves filtered through a No. 1 Whatman paper with H_2O added, or with H_2O only.

Extracts of plants. A preliminary examination was made of extracts obtained from the foliage and stems and flower petals of *C.* × *allionii*. Isolation methods included liquid extraction with different solvents, ultrafiltration, centrifugation and fractionation by different chromatographic systems. All the isolates obtained were bioassayed in the dual choice chamber. Drying the leaves did not destroy their deterrent quality. Leaves of young plants (5–10 weeks) were dried at 40° for 12 hr, powdered and extracted in H_2O (1:20) and vacuum filtered. This base extract was separated in a H_2O and MeOH phase with a preparative C18 column.

The dual-choice chamber. A plexiglass dual-choice chamber with an internal light source was used for the behaviour tests [20]. To reduce the possibility of volatiles spreading from one side to the other the test chamber was constructed with a small contact area between the airstreams.

Ten fertlized female *P. brassicae* 7–14-days-old were used in egglaying experiments. Each test ran from 10.00 a.m. to 2.00 p.m. The airsteam through the test chamber was 0.1 m/sec, the temp. 25–30° and the light intensity 5000 lux.

Matched cabbage leaves from the same plant, supported by a small metal wire were used in each trial. Dummy leaves were also used. These consisted of (i) A green plexiglass disc (10 cm) (acrylic plastic) with a track (2mm wide × 2mm deep) cut 1mm from the edge and filled with test solution. The surface was roughened round the edge. (ii) Green PVC plastic cut to leaf-shape, the surface of which was roughened. For the trials four such 'leaves' were mounted together on each side of the chamber.

Application of the solution and preparation. A micro spray was used to ensure even distribution of a known amount of the solution. The base extract consisted of powdered dried leaves (1 g) mixed with 20 ml of H_2O. It was vacuum filtered to a clear solution and pre-concentrated (1:10) on a reversed phase sample preparation column (Waters sep/pak). The H_2O fraction was also collected for tests.

Chemical isolation. The principal cardenolide fraction present in the air-dried leaves of *C.* × *allionii* was separated by a single extraction with 50% MeOH–H_2O. The filtered extract was treated with Pb and $Ba(OAc)_2$ acetate to ppt. the phenolic compounds. The extract was then evapd to half vol. and rough fractionated with a small preparative C18 column. The final chromatographic separation was made with a semi-preparative C18 column with slow gradient elution (from 30 to 49% MeOH H_2O in 35 min). The fraction (40.15) was collected together with 11 other fractions. To obtain sufficient material for the NMR spectra over 100 repeated runs were required. The proton NMR spectrum of this fraction is an adequate identification of the cardenolide and also shows the purity of the fraction.

A comparison between extracts of leaves from *C. cheiri*, *C.* × *allionii* and *Erysimum scoparium* show that fraction 40.15 is present in all three but it is dominant in *C. allionii*.

Foretarsal recordings of the B-hairs. The principal strophanthidin glycoside (Fig. 2, fraction 40.15, 5 mg purified) was dissolved in 0.1 ml MeOH and diluted × 100 with 0.01 M NaCl. The final cardenolide concentration was approximately 0.05‰. Neural responses from tarsal receptors were recorded using a modification of the hair-tip recovery technique of ref. [21]; the stimulus solution in a glass capillary served as the indifferent electrode. A silver wire inserted into an amputated foreleg functioned as recording electrode and was connected to a pre-amplifier adapted from ref. [22]. Nerve impulses were visualized using standard oscilloscopes and recording instruments.

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REFERENCES

1. Hoch, H. J. (1961) *A Survey of Cardiac Glycosides and Genins*, University of South Carolina Press.
2. Rothschild, M., Gardiner, B. O. C., Valadon, G. and Mummery, R. (1975) *Proc. R. Ent. Soc. C* **40**, 13.
3. Puntambekar, S. V. (1950) *Proc. Indian Acad. Sci.* **23A**, 114.
4. Rothschild, M. (1987) in *Proceedings in Life Sciences: Perspectives in Chemoreception and Behaviour*, p. 175. Springer, New York.
5. Rothschild, M. and Schoonhoven, L. M. (1977) *Nature, Lond.* **266**, 352.
6. Behan, M. and Schoonhoven, L. M. (1978) *Ent. Exp. Appl.* **24**, 163.
7. Blum, M. S. (1983) in *Plant Resistance to Insects* (Hedin, P. A. ed.) pp. 265–278. American Chemical Society, Washington.
8. Reichstein, T., Von Euw, J., Parsons, J. A. and Rothschild, M. (1968) *Science* **161**, 861.
9. Aplin, R. T., d'Arcy Ward, R. and Rothschild, M. (1975) *J. Ent. (A)* **50**, 73.
10. Marsh, N. and Rothschild, M. (1974) *J. Zool., Lond.* **174**, 89.
11. Marsh, N., Rothschild, M. & Evans, E. (1984) in *The Biology of Butterflies* (Vane-Wright, R. I. and Ackery, P. R. eds) p. 135. Academic Press, London.
12. David, W. A. L. and Gardiner, B. O. C. (1962) *Bull. Ent. Res.* **53**, 91.
13. Rodman, J. E. and Chew, F. S. (1980) *Biochem. Syst. Ecol.* **8**, 43.
14. Dixon, C. A., Erickson, J. M., Kellett, D. N. and Rothschild, M. (1978) *J. Zool., Lond.* **185**, 437.
15. Rothschild, M., Von Euw, J., Reichstein, T., Smith, D. A. S. and Pierre, J. (1975) *Proc. R. Soc. Lond. B.* **190**, 1.
16. Ma, W. C. and Schoonhoven, L. M. (1973) *Ent. Exp. Appl.* **16**, 343.
17. Kluijnstra, H. W. and Roessingh, P. (1986) *Ent. Exp. Appl.* **40**, 71.
18. Feltwell, J. (1982) *Large White Butterfly. The Biology, Biochemistry and Physiology of Pieris brassicae (Linnaeus)* p. 1. Dr. W. Junk, The Hague.
19. Lundgren, L. (1975) *Zool. Scripta* **4**, 253.
20. Alborn, H., Karlsson, H., Lundgren, L., Ruuth, P. and Stenhagen, G. (1985) *OIKOS* **44**, 61.
21. Hodgson, E. S., Lettvin, J. Y. and Roeder, K. D. (1955) *Science* **122**, 417.
22. Van Drongelen, W. (1978) *Ent. Exp. Appl.* **24**, 343.