

ECONOMICS OF CHEMICAL DEFENSE IN CHRYSOMELINAE

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Abstract—Chemical defense in chrysomelid larvae (subtribe Chrysomelina and Phyllodectina) is reviewed. Most species secrete autogenous monoterpenes. The diversity of their secretion is interpreted as a mechanism to reduce adaptation by predacious arthropods. The consequences of a host plant shift to the Salicaceae are explored. Salicin from these host plants is used as a precursor for the salicylaldehyde secreted by the larvae of many species. This offers several advantages. It provides the larvae with an inexpensive and efficient defense. The recovery of the glucose moiety of the salicin contributes significantly to the larval energy budget. Adults sequester salicin in the eggs at concentrations which are toxic to ants. Owing to this maternal provisioning, neonate larvae produce salicylaldehyde from hatching onwards, whereas other species secreting monoterpenes are not protected at hatching. The secretion of salicylaldehyde by different species is considered to be chemical mimicry reinforcing visual aposematic signals.

Key Words—Defensive secretion, egg defense, allomones, *Salix*, salicin, Coleoptera, Chrysomelidae, monoterpenes, salicylaldehyde.

INTRODUCTION

Chrysomelidae, or leaf beetles, are a family of phytophagous insects, often forming large aggregations on their food plants. This makes them particularly apparent to predators and parasitoids, and spectacular chemical, mechanical, and behavioral defensive mechanisms have evolved in this family (Deroe and Pasteels, 1982). Defensive glandular secretions of both larvae and adults are particularly prominent in the brightly colored Chrysomelini, a tribe of the subfamily Chrysomelinae.

In temperate regions, Chrysomelinae are often found in damp, open, relatively undisturbed habitats such as riversides, marshes, and meadows. There, the favored food plants include both herbs [docks (Polygonaceae), buttercups (Ranunculaceae) or mints (Labiatae)] and also some trees and shrubs [willows and poplars (Salicaceae) or alders (Betulaceae)]. The Chrysomelinae are monophagous or oligophagous on these plants.

Within the Chrysomelinae there is a fairly good parallel between systematic position and the distribution and chemical nature of chemical defense. This is true both in the larval and adult stages. At least in the larvae, the apparent exceptions can be explained by the influence of particular host plants. In the present paper, we will give an overview of larval chemical defense in this group and discuss in more detail the influence of host plant secondary chemistry.

DISTRIBUTION OF CHEMICAL DEFENSE IN CHRYSOMELINAE LARVAE

The larvae of the subtribe Chrysomelina and of the genus *Phratora* in the subtribe Phyllodectina all possess nine pairs of glands distributed over the meso- and metathorax and the first seven abdominal segments. The glands are eversible, and when the larvae are disturbed, a drop of secretion appears at the tip of the everted reservoirs. Most of this secretion can be withdrawn back into the reservoirs during the retraction of the latter into the body (Hollande, 1909; Garb, 1915). According to the descriptions of Cuénot (1896), Hollande (1909), Garb (1915), Berti (1968), and Renner (1970), there seems to be little morphological difference between the glands of the different genera.

The principal classes of compounds found so far in the larval secretions of Chrysomelini are listed in Table 1, together with the host plant of each species. Six different methylcyclopentanoid monoterpenes have been identified (Figure 1) (review in Pasteels et al., 1984). Many species secrete a mixture of these compounds which are most likely autogenously synthesized. The larvae producing them feed on a range of plants, and no direct host plant influence can be detected. The secretions of different species within a genus are, in some cases, more different than the secretions from species belonging to different genera. In addition quantitative and qualitative differences exist between geographically isolated populations of the same species.

In Table 2, the data for different populations of *Plagioderma versicolora* are summarized. The European samples are rather homogenous, characterized by the presence of plagiodial and plagiolactone. They differ strongly from both the Japanese and the previous North American samples. The Japanese *Plagioderma* is a different subspecies but *Pl. versicolora* was introduced into North America from Europe. The reader must be cautioned about possible artifacts; these monoterpenes are unstable, and, for example, plagiodial can be easily transformed

TABLE 1. OCCURRENCE OF PRINCIPAL CLASSES OF COMPOUNDS FOUND IN LARVAL SECRETIONS OF CHRYSOMELINI SPECIES^a

	Compound ^b					Host Plant
	M	S	B	P	J	
Subtribe Chrysomelina:						
8 <i>Chrysomela</i> sp.		7	(3)	1		Salicaceae, and <i>Alnus</i>
1 <i>Gastrolina</i> sp.					1	<i>Juglans</i>
3 <i>Gastrophysa</i> sp.	3					<i>Rumex</i>
1 <i>Hydrothassa</i> sp.	1					<i>Ranunculus</i>
1 <i>Linnaidea</i> sp.	1					<i>Alnus</i>
2 <i>Phaedon</i> sp.	2					<i>Brassica</i> and <i>Nasturtium</i>
1 <i>Prasocuris</i> sp.	1					<i>Ranunculus</i>
1 <i>Plagioderia</i> sp.	1	(1)				<i>Salix</i>
Subtribe Phyllodectina:						
4 <i>Phratora</i> sp.	3	1				Salicaceae

^aAbbreviated from Pasteels et al., 1984.

^bAbbreviations: M, methylcyclopentanoid monoterpenes; S, salicylaldehyde; B, benzaldehyde; P, phenylesters; J, juglone; For each genus, the number of species secreting one or the other classes of compounds are given. (), minor component or only present in some populations.

to chrysomelidial (reported from the American population) when stored at room temperature in the light. Even when stored for long periods in sealed capillaries at -20°C , degradation occurs. We recently analyzed freshly collected secretions from the United States (Wisconsin). This sample contained plagiodial and

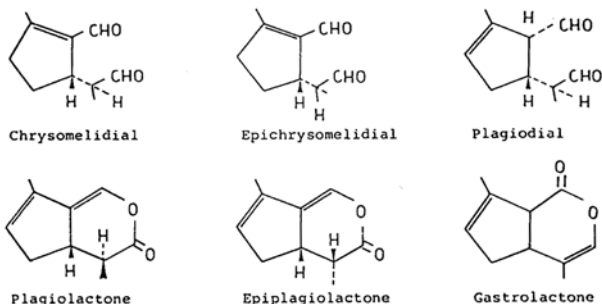


FIG. 1. Methylcyclopentanoid monoterpenes identified in the larval secretions of Chrysomelini.

TABLE 2. PROPORTIONS OF DIFFERENT METHYLCYCLOPENTANOID MONOTERPENES IN SEVERAL POPULATIONS OF *Plagioder a versicolor a*

Country	Methylcyclopentanoid monoterpenes ^a					Reference
	1	2	3	4	5	
Japan		3	90	3	3	Sugawara et al. (1979)
U.S.A.	66*			33		Meinwald et al. (1977)
U.S.A.			81	19		This paper
England			73	27		This paper
Belgium			70	30		Pasteels et al. (1984)
Switzerland			33	67		This paper
South of France			47	53		This paper

^a1: chrysolmelidial, 2: epichrysolmelidial, 3: plagiodial, 4: plagiolactone, 5: epiplagiolactone.
*66% of compound 1 and 2.

plagiolactone but no chrysolmelidial. It has been reported that some populations of European *Plagioder a* larvae also produce salicylaldehyde (Hollande, 1909; Pasteels et al., 1984). In view of the relative homogeneity of the secretions from larvae originating from England to the south of France, this observation needs to be confirmed.

The significance of the diversity of chemical defenses between sympatric species or between allopatric populations of the same species is not clear. In most cases, no pheromonal function is known which could explain the specificity of the blends. The larvae are often gregarious, but defensive secretions do not have an aggregating function. Groups of mixed species are easy to obtain in the laboratory. Larvae experimentally made devoid of secretion remain grouped. It has been suggested that the secretion of the larvae of *Gastrophysa viridula* may lower intraspecific competition. Indeed, the larvae evert their glands at the approach of adults of the same species which are repelled (Renner, 1970). This epideictic function does not necessarily imply highly specific pheromones. Indeed, Raupp et al. (1984) suggested that the secretion might also lower interspecific competition by repelling other herbivores.

The diversity of defensive secretions could be due to genetic drift in the absence of selective pressure for uniformity. Tschinkel (1975), in his study of the defensive secretions of tenebrionids, suggested that their precise composition was not critical as long as the secretion met certain physical requirements. Variation on the theme would then be possible without any particular ecological significance. On the other hand, it is also possible that intraspecific variation between geographically isolated populations reflects adaptation to the different communities in which the beetles are living. If this is the case, secretions from

different species inhabiting the same food plant should be more similar to each other than those of species living in different habitats. This does not seem to be true. In nature, larvae of *Hydrothassa marginella* and *Prasocuris phellandrii*, for example, are often found together on *Ranunculus* spp.. They are probably exposed to the same set of potential predators and parasitoids, but they produce different mixtures of compounds.

Selection for diversity would be another possible explanation of the specificity of the defensive secretions in sympatric species. So far, the activity of the defensive compounds has only been demonstrated with "standard laboratory predators" such as ants, for which at least chrysomelidial and salicylaldehyde are deterrent (Blum et al., 1978; Sugawara et al., 1979; Pasteels et al., 1983). Using adult sawflies (*Tenthredo olivacea*), predator observed to feed on chrysomeline larvae in nature, Pasteels and Grégorie (1984) demonstrated that the sawflies can be conditioned and show a preference for the larvae producing the secretion they have already experienced. such behavior of predators favors the maintenance or evolution of diversity of defensive secretions in sympatric species. Especially for species feeding next to each other on a plant, a high diversity of defensive blends may be a good strategy to prevent adaptation by the predator. Similarly, interspecific and intraspecific diversity of plant secondary metabolites is thought to limit the adaptation of herbivores to these plants (Dolinger et al., 1973; Atsatt and O'Dowd, 1976).

The wide distribution of methylcyclopentanoid monoterpenes in the secretions of the larvae of *Chrysomelina* and *Phratora* suggests that these compounds represent their primitive defensive secretion. As already stated, these products are most likely synthesized de novo, since they have no obvious precursors in the food plants; for example, chrysomelidial is secreted by the larvae of different species feeding on *Alnus*, *Rumex*, *Ranunculus*, *Brassica*, *Nasturtium* and *Salix*, respectively.

The larvae of some species of *Chrysomelina* and *Phratora*, however, secrete not monoterpenes but aromatic compounds such as salicylaldehyde or benzaldehyde. These species feed on trees belonging to the Salicaceae or Betulaceae. In contrast to the situation described for the species producing monoterpenes, in at least some species producing aromatic compounds, the defensive secretion is derived from a host plant precursor. In these species there is no longer a chemical diversity in the defensive blends.

Pasteels et al. (1982) suggested that the secretion of aromatic compounds by chrysomeline larvae can be best explained as a consequence of a shift of food plant, associated with speciation, from herbs to Salicaceae or Betulaceae. The secretion of salicylaldehyde would then be an adaptation to the secondary chemistry of the Salicaceae.

In the remainder of this paper we will examine in more detail the mechanism of such adaptation and its consequence for the willow-feeding leaf beetles.

FOLIAR SECONDARY CHEMISTRY OF THE SALICACEAE

Among north temperate woody plants, the European Salicaceae are remarkable for the presence of phenolglycosides in their bark and in the leaves of some species. The leaves of the species containing phenolglycosides do not contain proanthocyanidins, a form of condensed tannins found in the leaves of the remaining salicaceous species (Hegnauer, 1973).

Salicaceous bushes and trees might have been particularly likely objects of host plant switches of primitively herb feeding Chrysomelinae for various reasons. First, they are one of the most abundant woody plants in the biotopes favored by the beetles. Secondly, the leaves of these *Salix* species without proanthocyanidins might be a less formidable evolutionary barrier for an herbivore adapted to digesting herbs (Rowell-Rahier and Pasteels, 1982) than those of most woody plants, which typically contain tannin.

Using published food plant lists for the weevils, the sawflies, and the caterpillars of the British moths, we searched for a correlation between the presence or absence of phenolglycosides in the leaves and the degree of dietary specialization of the insects feeding on these leaves.

The results showed that the *Salix* spp. with phenolglycosides (*S. babylonica*, *S. fragilis*, *S. incana*, *S. nigricans*, *S. pentandra*, *S. purpurea*, *S. repens* and *S. triandra*) tend to be the hosts of specialized herbivores and are avoided by generalists. Conversely, *Salix* spp. without phenolglycosides in their leaves (*S. alba*, *S. aurita*, *S. caprea*, *S. cinerea*, and *S. viminalis*) tend to be eaten by more generalist insects and are avoided by the more specialized ones. Moreover, the faunas of the different *Salix* species with phenolglycosides in their leaves are more similar to each other than to the faunas of the *Salix* species having no phenolglycosides in their leaves (Rowell-Rahier, 1984).

Salicin is one of the most frequently occurring of these glycosides. It is known to be toxic (Marks et al., 1961) and, together with the other phenolglycosides present in the leaves, may act as a wide-range feeding deterrent against nonadapted herbivores (Edwards, 1978; Tahvanainen, 1985). Salicin also proved to be deterrent and highly toxic for ants. In a toxicity test, 50% mortality was reached after two days of experimental regime, during which the LD₅₀ may be estimated as 5 µg of salicin per ant in two days (Pasteels et al., in preparation).

INFLUENCE OF HOST PLANT ON DEFENSIVE SECRETION OF CHRYSOMELINE LARVAE

The best studied example within this group of beetles, whose defensive secretions are dependent on the host plant, is *Ph. vitellinae*, one of the species whose larvae secrete salicylaldehyde. It was suggested long ago that salicylal-

dehyde is derived from host plant phenolglycosides (Wain, 1943) such as salicin and populin. We tried to verify this hypothesis experimentally for *Ph. vitellinae* and *C. tremulae*, using two different approaches (Pasteels et al., 1983)

First, to confirm the role of salicin as a precursor of the salicylaldehyde secreted by the larvae of *Ph. vitellinae*, the consequences of a salicin-free diet on the defensive secretion of the larvae were examined. In nature, adult *Ph. vitellinae* are never seen on *S. caprea*, and the larvae do not normally accept these hairy leaves as food. After being denuded of trichomes, however, the leaves of *S. caprea* were readily accepted by the larvae of *Ph. vitellinae*. Larvae developed normally on "shaved" *S. caprea* leaves but did not secrete salicylaldehyde. Addition of salicin to *S. caprea* restored the secretion of salicylaldehyde. Salicin is thus a precursor for the larval defensive secretion, but it is not obligatory for food acceptance by the larvae. The hairiness of the leaves of *Salix* species containing no salicin is responsible for the mortality due to starvation of the young larvae reared on them.

Secondly, by feeding larvae of *C. tremulae* with labeled salicin, we demonstrated that also in this species salicylaldehyde is derived from salicin. This is most likely true for all species of the genus feeding on salicaceous plants.

After confirming the influence of the host plant secondary metabolite, we tried to establish the relationship between the quantity of precursor ingested and the quantity of defensive secretion produced and to determine where in the body this transformation occurred. The fate of the glucose moiety split from the original glycoside molecule was also examined.

The results show that the concentration of salicylaldehyde in the secretion is positively correlated with the amount of salicin in the food of the larvae. The transformation of salicin into salicylaldehyde probably occurs mostly in the defense glands, since the β -glucosidase activity is four times higher in the glands than in the gut (Pasteels et al., 1983).

The concentrations of glucose and of salicylaldehyde in the secretion are far from being equimolar, indicating that the glucose formed by salicin hydrolysis was in great part recovered by the larvae and transferred back into the blood (Pasteels et al., 1983).

WHEN A STRONG DEFENSE IS GOOD FOR THE ECONOMY

The energetic costs of defense are frequently referred to in discussions of the ecology and evolution of chemically defended organisms; it is generally assumed that any loss in fitness caused by these costs must be outweighed by the gain in fitness secured by an effective defense. However, there has been virtually no attempt to demonstrate these energetic costs or to measure them quantitatively, especially in animals (Blum, 1981).

Although no direct experimental data are available, it is very likely that

the methylcyclopentanoid monoterpenes secreted by the larvae of chrysomeline species (see above) arise from geranylpyrophosphate via mevalonic acid, as do other terpenes (Blum, 1981). This must involve finite, although perhaps small, costs. The salicylaldehyde secreted by other species is formed by the enzymatic hydrolysis of the glucosidic bond of salicin. A preliminary calculation shows that when the glands are emptied and refilled daily, this glucose could supply up to one third of the caloric requirements of the larva (see Table 3).

Both types of defense occur in closely comparable species of the same genera feeding on Salicaceae. This assemblage is thus very suitable for an investigation of the cost of defense, which would be expected to be maximal in those species synthesizing monoterpenes de novo and minimal in those secreting salicylaldehyde, which profit from the use of a plant precursor and also by gaining glucose.

Larvae of *Phratora tibialis*, *Ph. vitellinae*, *Plagioderma versicolora*, and *Chrysomela 20-punctata* were reared in the laboratory from egg to adult on appropriate *Salix* spp.; these, together with the chemical nature of the secretions of the beetles, are listed in the first three columns of Table 4. Each species sample was divided into control and experimental groups, which did not differ in population density, quantity, and quality of food provided, sex ratio, mortality during the experiment, or in time elapsed before eclosion as adults. Both groups were disturbed daily and induced to evert their glands: the exposed secretion was collected only from the experimental group, which then refilled their glands by the following day. The assumption is made that the control

TABLE 3. DERIVATION OF BENEFIT OF GLUCOSE RECOVERY IN *Ph. vitellinae* LARVAE^a

A. Energy requirements

Oxygen consumption: 0.5 ml O₂/g FW/hr

1 ml O₂ consumed = 5.05 cal

3.68 cal = 1 mg glucose, incorporating correction for energy loss during digestion.

Mean weight of field-collected third-instar larvae: 8.54 mg; thus, one larva requires 140.65 μg glucose/day

B. Energy gain from salicin metabolism

Daily salicin ingestion: 0.46 μM

Daily glucose ingestion in form of salicin: $0.46 \times 180 = 82.8 \mu\text{g}$ of which $(0.46 - 0.20^*) \times 180$ reach the glands = 46.8 μg

[*: the glands only start to produce secretion after ingestion of 0.20 μM of salicin].

99.9% of this glucose is recovered by the larvae; thus the larva recovers 46.7 μg glucose/day, covering 33.2% of the daily energy requirement.

Rather than the correction marked with an asterisk above, one could also take into account the fact that 20% of the total glucosidase activity is found in the gut. This would suggest that 80% (= 65.5 μg/day) of glucose present in the original salicin is recovered from the glands, covering 46.6% of the larval daily requirement.

^aData from Pasteels et al. (1983) and Keister and Buck (1974).

TABLE 4. MEAN WEIGHTS OF ADULT CHRYSOMELINAE WHEN DEFENSIVE SECRETION OF LARVAE HAS (EXPERIMENTAL) OR HAS NOT (CONTROL) BEEN REMOVED DAILY^a

Chrysomelinae species	Host-plant species	Defence nature	Mean weights (mg ± sd (N))		P
			Control	Experimental	
<i>Ph. tibialis</i>	<i>Salix purpurea</i>	M	5.89 ± 0.54 (52)	5.63 ± 0.64 (33)	*
<i>Pl. versicolora</i>	<i>S. purpurea</i>	M	7.09 ± 1.39 (82)	6.68 ± 1.11 (78)	*
<i>Ph. vitellinae</i>	<i>S. nigricans</i>	S	5.41 ± 0.63 (7)	6.25 ± 0.52 (14)	**
<i>Ph. vitellinae</i>	<i>S. caprea</i>	none	5.41 ± 0.63 (7)	5.39 ± 0.74 (7)	NS
<i>Ph. vitellinae</i> + reduced salicin		S	5.41 ± 0.63 (7)	5.49 ± 0.08 (13)	NS
<i>Ch. 20punctata</i>	<i>S. nigricans</i>	S + X	25.10 ± 5.19 (79)	25.60 ± 3.19 (26)	NS
<i>Ch. 20punctata</i>	<i>S. caprea</i>	X	25.20 ± 2.80 (8)	22.60 ± 3.70 (120)	*

^aMeans and standard deviations were calculated for each group (columns 4 and 5).

^bAbbreviations: M, methylcyclopentanoid monoterpenes; S, salicylaldehyde; X, unidentified compounds; N, sample size; P, probability levels of difference between experimental and control groups (Student's *t*-test); **, $P < 0.01$; *, $P < 0.05$; NS, $P > 0.05$.

group, which do not lose their secretion, will synthesize at a much lower rate than the experimental group.

Within 12 hr of eclosion, and before feeding, the resulting adults were weighed, and this value was used as a measure of larval growth and, by inference, of adult fitness. In those species secreting autogenous monoterpenes (*Ph. tibialis* and *Pl. versicolora*), the mean weight of the experimental group is significantly lower than that of the control: a daily renewal of the secretion is thus a drain on their metabolism. It is not excluded that this is due to the fluid lost occurring during collection of the secretion in the experimental group. In the species which secrete exclusively salicylaldehyde (*Ph. vitellinae*), the experimental group is significantly heavier than the control. This weight gain is probably associated with the extra glucose obtained from the hydrolysis of salicin. When the larvae of this species are completely deprived of salicin [by raising them on *S. caprea*, which does not contain this compound (Hegnauer, 1973)] or allowed only one fifth of their normal intake, the experimental imaginal fresh weights do not differ from those of the control group feeding on *S. nigricans*. Additionally, preliminary results suggest that when *Ph. vitellinae* is reared undisturbed on *S. nigricans* leaves with added glucose, the adult weights are comparable to those of the experimental group on the same plant. The last species (*Ch. 20-punctata*) reared on *S. nigricans*, secretes a mixture of both salicylaldehyde and various compounds which are as yet unidentified but most likely not directly derived from the host plant. Here there is no difference between the control and the experimental groups, suggesting that the glucose recovered from salicin degradation is balanced by the biosynthetic cost of the other compounds. This hypothesis is supported by the finding that in the same species raised on *S. caprea* (i.e., without salicin) the experimental animals weigh significantly less than the controls.

These results show, to our knowledge for the first time, that an autochthonously produced secretion entails an appreciable metabolic cost. This cost is expressed as a loss of weight of the adult, which in many other insects has been demonstrated to be associated with reduced fecundity and fitness (references in Raupp and Denno, 1984). These costs can be avoided by the use of an appropriate plant precursor: where this is a glucoside, as in the present example, the resultant glucose production can convert the loss into a profit. Leaves, because of their high fiber and cellulose content, usually have a low available calorific density (McNab, 1978), and this spin-off metabolic advantage could be of particular relevance to a folivorous insect. Plant glucosides are not, in general, available to herbivores as energy sources because of the potential toxicity of the aglycone; in chrysomeline larvae, it is the localization of the greater part of the degradation process in the external glands which makes this feasible. Lastly, larvae in the field are likely to be frequently disturbed by parasites and predators, or even by the movement of leaves in the wind, and will tend to lose

secretion by contact with these agents. If this is true, the laboratory situation, where the secretion is collected daily, may reflect the natural situation rather closely.

The transformation of salicin to salicylaldehyde does not only provide an inexpensive or even profitable defense for the larvae but also an effective one. Laboratory tests with a generalist predator such as the ant *Myrmica rubra*, often seen exploring the foliage of the host plants of various leaf beetles, show that salicylaldehyde is a potent deterrent, more active than salicin or its aglycone saligenin (Pasteels et al., 1983).

DEFENSE OF EGGS AND NEONATE LARVAE

At hatching, the larvae are clustered and immobile on the leaves. They are thus highly exposed to predation and also to cannibalism. An innate defense might therefore be critical. The observation that, on the one hand, the larvae of some species already produce secretion at hatching, before feeding, and that, on the other hand, some other species do not have "full" glands at the beginning of their larval life, is therefore very interesting. It is only the neonate larvae of those species secreting salicylaldehyde which have functional glands on hatching. No larvae of any species seem to be able to produce monoterpenes at eclosion. Indeed, chemical analysis demonstrated that, paradoxically, only those larvae which depend on salicin normally found in their food seem to be able to produce a defensive secretion before feeding. A possible explanation could be that salicin is sequestered in the eggs and used as a precursor by the neonate larvae. To test this hypothesis, we studied the occurrence of defensive allomones in chrysomelid eggs.

The eggs, often brightly colored, are laid in clusters on the foliage and are thus highly exposed to predation. In the field, survival of the eggs can be as low as 25% (Wade and Breden, personal communication). There might therefore be a strong selective pressure for the eggs to be protected. Indeed, chemical analysis showed that the two isoxazolinone glycosides characteristic of the adult defensive secretion (Pasteels et al., 1982) are present in the eggs of all species studied (Table 5). The occurrence of identical compounds in the eggs and in the adult defensive glands was already reported in the *Chrysomelina* producing cardenolides (Pasteels and Dalozé, 1977) and could represent a general feature of the *Chrysomelinae*. However, these isoxazolinones were never detected in neonate larvae, and, at least in one species, it was found in the egg shells left after hatching.

Additionally, the eggs of some of the *Salicaceae* feeders contain salicin, and those species are also those whose larvae secrete salicylaldehyde. Since the salicin was no longer detected in the neonate larvae, it must have been trans-

TABLE 5. PRESENCE (+) OR ABSENCE (-) OF SALICIN AND ISOXAZOLINONES IN EGGS OF SPECIES BELONGING TO SUBTRIBES CHRYSOMELINA AND PHYLLODECTINA^a

	Host plant	Salicin	Isoxazolinones
Subtribe Chrysomelina			
<i>Ch. populi</i> ^b	Salicaceae	+	+
<i>Ch. tremulae</i> ^b	Salicaceae	+	+
<i>Ch. saliceti</i> ^b	Salicaceae	+	+
<i>Ch. 20-punctata</i> ^b	Salicaceae	+	+
<i>G. viridula</i>	<i>Rumex</i>	-	+
<i>Pl. versicolora</i>	Salicaceae	-	+
Subtribe Phyllodectina			
<i>Ph. vitellinae</i> ^b	Salicaceae	+	+
<i>Ph. laticollis</i>	Salicaceae	-	+
<i>Ph. tibialis</i>	Salicaceae	-	+

^aAbbreviated from Pasteels et al. (submitted).

^bSpecies whose larvae produce salicylaldehyde from their defensive glands.

formed into salicylaldehyde. The sequestration of salicin in the eggs explains the fate of this plant toxin in the adult females. It protects the eggs themselves and also provides precursor for protection of the neonate larvae at a very vulnerable life-stage when other species are not protected. The amount of salicin found, on average, in one *Chrysomela* egg is about the LD₅₀ dose for an ant (Pasteels et al., 1985).

CONCLUSIONS

One of the challenges of chemical ecology is to interpret chemical results in an ecological context. Ecomones (semiochemicals) often appear as "wisdom" remaining from past history. The selective pressures from which they result are difficult to identify and might even be absent from the biotopes in which the organisms live today.

We would like to suggest a possible scenario for the evolution of chemical defense in the larvae of leaf beetles. The secretion of methylcyclopentanoid monoterpenes in species feeding on herbs could be a response to the pressure of numerous predacious arthropods (or parasitoids) exploring the foliage. These compounds, classified as volatile irritants, are well known ant repellents. The larvae of these beetle species are rather small and inconspicuous, feeding on the lower surface of the leaves. Larval defense is not associated with aposematism, and preliminary experiments suggest that birds are only slightly re-

pelled by their secretions. The chemical diversity of the secretion might prevent adaptation by predacious arthropods.

Shifts of host plants occurred during the evolution of chrysomeline beetles, and one of these shifts had a drastic influence on chemical defense: the shift to salicaceous species, with leaves rich in phenolglycosides. It has occurred at least three times (Pasteels et al., 1984), and it frequently results in the use of salicin as a precursor of the defensive secretion.

The utilization of salicin offers multiple advantages for chrysomelid beetles. First, it allows them to exploit a set of host plants otherwise well protected from generalist herbivores by phenolglycosides. Second, it provides the larvae with an inexpensive defense, an effective deterrent against generalist predators. Third, the recovered glucose contributes significantly to the larval energy budget and is positively correlated with an increased relative growth rate of the larvae. Fourth, the adults sequester salicin in the eggs at concentrations which are toxic for ants. Fifth, neonate larvae of the species producing salicylaldehyde are protected from hatching onwards, whereas other species secreting monoterpenes are not so protected.

The larvae of species feeding on Salicaceae produce a much larger volume of secretion than those synthesizing monoterpenes (three or four times larger for larvae of equal sizes). This is probably made possible by the relative availability of the salicylaldehyde derived from the host plant. Such abundant production of a volatile irritant deters predaceous birds as well as arthropods. These larvae (e.g., *Chrysomela* spp.) are large, gregarious, and aposematic, and several species feed on the upper surface of the leaf. The utilization of salicin has led to chemically homogenous secretions. This is probably advantageous against predacious birds. It reinforces the aposematic signal and can be considered as a case of chemical mimicry.

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