

Chemical Defences in Leaf Beetles and their Larvae: The Ecological, Evolutionary and Taxonomic Significance

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Key Word Index – Chrysomelinae; defensive secretions; host plant influence; predator spectrum; methylcyclopentanoid monoterpenes; salicylaldehyde; cardenolides; isoxazolin-5-one glucosides.

Abstract – The chemical defences of the Chrysomelinae are reviewed. Defensive glandular secretions have evolved independently in larvae and adults, and faster than the morphology of the glands. Both characters are used in a phylogenetic study of the Chrysomelini, disclosing suprageneric affinities. First, a close relationship between the Chrysomelina and *Phratora* is proposed. Secondly, *Leptinotarsa* and *Gonioctena* are probably more closely related to the Chrysolina than to the Chrysomelina and *Phratora*. The qualitative and quantitative composition of larval and adult secretions sometimes differs between geographically isolated populations of the same species, which are therefore probably genetically distinct. The host plant shows an influence which explains discrepancies between the current classification and the chemical nature of the secretions. Some beetles sequester secondary plant metabolites or use them as precursors for their own defences. The original biosynthetic pathway has been lost in these species. Other factors which could affect the chemical diversity of the secretions are discussed: random events such as neutral mutations and genetic drift, conditioning or adaptation of predators to specific defensive mixtures, and the diversity of potential predators. The latter could help to explain the divergence in the chemical nature of the larval and adult secretions. The volatile irritants produced by the larvae are well suited to repel small arthropods, such as ants, whereas poisons associated with aposematic coloration in the adults could be directed against small vertebrates, such as birds.

Introduction

Chrysomelidae, or leaf beetles, are 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 [1]. Defensive glandular secretions of both larvae and adults are particularly prominent in the brightly colored Chrysomelini (Chrysomelinae).

The classification of the Chrysomelinae into tribes and groups of genera is controversial. Data on the chemical ecology of the chrysomeline larvae and adults might bring new taxonomical criteria. In this paper, new and previously published (references in [2]) chemical data on the defensive secretions of the Chrysomelini are summarized, and their ecological and possible evolutionary implications are discussed (see also [3] for a first account of these aspects). We follow the new pan-global classification of Daccordi [4]; other classifications

based on more restricted faunas [5-9] are however also mentioned (summary in Table 1).

In addition to phylogenetic aspects, we attempt to relate the nature of the defensive secretions to ecological factors such as the host plants and the probable predator spectrum. 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 either monophagous or oligophagous on these plants. There is evidence that evolution within this habitat proceeds, at least, in part through sympatric speciation *via* shifts of host plants [10].

Glandular secretions of larvae

The larvae of the subtribe Chrysomelina and of the genus *Phratora* all possess 9 pairs of glands on the meso- and metathorax and on the first 7

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TABLE 1. CLASSIFICATIONS OF THE CHRYSOMELINE GENERA CITED IN THIS PAPER, MODIFIED FROM DACCORDI [4], COX [5], HENNIG [6], KIMOTO [7] AND WEISE [9] GIVEN IN CHRONOLOGICAL ORDER OF APPEARANCE

Weise on world species*	Hennig on German species †	Kimoto on Japanese species †	Cox on British species †	Daccordi on world species* †
Chrysomelini <i>Chrysolina</i> <i>Oreina</i> <i>Leptinotarsa</i>	Group 1 <i>Chrysolina</i> <i>Oreina</i> <i>Leptinotarsa</i>	Generic group Chrysolina <i>Chrysolina</i> <i>Oreina</i> <i>Leptinotarsa</i>	Chrysomelini <i>Chrysolina</i> <i>Leptinotarsa</i> <i>Goniocтена</i>	Chrysomelini Doryphorina <i>Leptinotarsa</i> Stål Chrysolinina <i>Chrysolina</i> Motschulsky <i>Oreina</i> Chevrolat
Phaedonini <i>Phaedon</i> <i>Gastrophysa</i> <i>Chrysomela</i> (incl. <i>Linæideae</i>) <i>Plagioderia</i> <i>Gastrolina</i> <i>Prasocuris</i> <i>Hydrothassa</i> <i>Phaedonia</i>	Group 3 <i>Phaedon</i> <i>Gastrophysa</i> <i>Chrysomela</i> (incl. <i>Linæideae</i>) <i>Hydrothassa</i> <i>Plagioderia</i> <i>Prasocuris</i> <i>Phratora</i>	Generic group Phaedon <i>Phaedon</i> <i>Gastrophysa</i> <i>Phratora</i> Generic group Chrysomela <i>Chrysomela</i> <i>Linæideae</i> <i>Plagioderia</i> <i>Gastrolina</i> Generic group Prasocuris <i>Prasocuris</i> <i>Hydrothassa</i>	Phaedonini <i>Phaedon</i> <i>Gastrophysa</i> <i>Phratora</i> <i>Chrysomela</i> <i>Plagioderia</i> <i>Prasocuris</i> <i>Hydrothassa</i>	Chrysomelina <i>Phaedon</i> Latreille <i>Gastrophysa</i> Chevrolat <i>Chrysomela</i> L. <i>Linæideae</i> Motschulsky <i>Plagioderia</i> Chevrolat <i>Gastrolina</i> Baly <i>Prasocuris</i> Latreille <i>Hydrothassa</i> Thomson <i>Phaedonia</i> Weise
Phyllodectini <i>Phratora</i> <i>Goniocтена</i>	Group 2 <i>Goniocтена</i>	Generic group Goniocтена <i>Goniocтена</i>		Goniocтенina <i>Goniocтена</i> Chevrolat Paropsina <i>Paropsis</i> Olivier <i>Chrysotharta</i> Weise Phyllodectina <i>Phratora</i> Chevrolat

*Chiefly on adult criteria.

† Chiefly on larval criteria.

To avoid confusion due to synonymy we have adopted the generic nomenclature of Daccordi (Column 5) in all classifications.

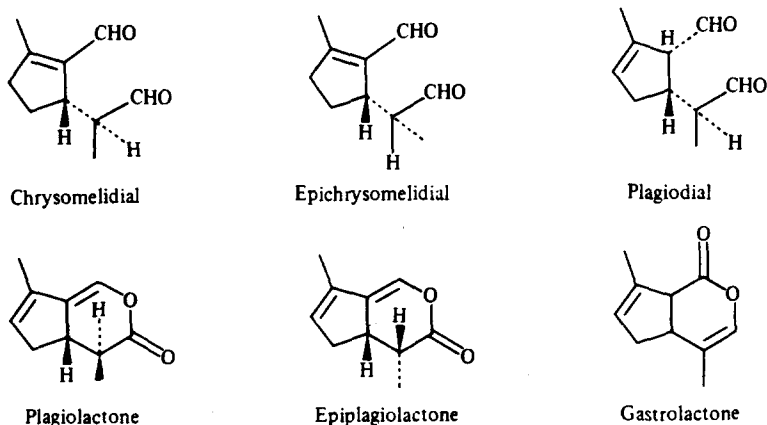


FIG. 1. METHYLCYCLOPENTANOID MONOTERPENES IDENTIFIED IN THE LARVAL SECRETIONS OF CHRYSOMELINI.

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 reservoir during the retraction of the latter into the body [11, 12]. According to the descriptions of Cuénot [13], Hollande [12], Garb [11], Berti [14] and Renner [15] there seems to be little morphological difference between the glands of the different genera. For Takizawa [8] the genera with such glands belong to the "glanduliferous group".

The Paropsina have only one single pair of well developed glands between the 8th and 9th abdominal tergites [16]. One pair of glands, often

very reduced, is also present in other groups (e.g. *Chrysolina* and *Gonioctena* species) but have not yet been studied in detail. Takizawa [8] put these species with only one pair of glands or without glands in a "non-glanduliferous group".

The principal classes of compounds found so far in the larval secretions of Chrysomelini are listed in Table 2 together with the host plant of each species. The structures of these compounds are given in Figs 1 and 2. Table 3 gives the composition of the mixtures of methycyclopentanoid monoterpenes secreted by species of the subtribe Chrysomelina and the genus *Phratora*, including data for geographically isolated populations of some species.

TABLE 2. PRINCIPAL CLASSES OF COMPOUNDS FOUND IN THE GLANDULAR SECRETIONS OF LARVAL CHRYSOMELINI*

	Methycyclopentanoid monoterpenes	Salicylaldehyde	Phenylethyl esters	Benzaldehyde	HCN	Juglone	Alkyl and alkenyl acetates	Host plants	References
<i>Chrysomelina</i>									
<i>Chrysomela populi</i>		+						<i>Populus</i>	45
<i>C. tremulae</i>		+						<i>Populus</i>	2
<i>C. saliceti</i>		+		+				<i>Salix</i>	†
<i>C. 20-punctata costella</i>		+		+				<i>Salix</i>	32
<i>C. 20-punctata</i>		+		+ †				<i>Salix</i>	2
<i>C. scripta</i>		+						<i>Salix</i>	46
<i>C. aenicollis</i>		+						<i>Salix, Populus</i>	†
<i>C. interrupta</i>			+					<i>Alnus</i>	31
<i>Gastrolina depressa</i>						+		<i>Juglans</i>	32
<i>Gastrophysa atrocyanea</i>	+						+	<i>Rumex</i>	36, 47
<i>G. cyanea</i>	+							<i>Rumex</i>	35
<i>G. viridula</i>	+							<i>Rumex</i>	2
<i>Hydrothassa marginella</i>	+							<i>Ranunculus</i>	2
<i>Linaeidea aenea</i>	+						+	<i>Alnus</i>	37
<i>Phaedon brassicae</i>	+							<i>Brassica</i>	36
<i>P. cochleariae</i>	+							<i>Nasturtium</i>	2
<i>Plagioderia versicolora</i>	+	+ † ‡						<i>Salix</i>	48
<i>Prasocuris phellandrii</i>	+							<i>Ranunculus</i>	2
<i>Phyllodectina</i>									
<i>Phratora vitellinae</i>		+						<i>Salix, Populus</i>	49
<i>P. laticollis</i>	+							<i>Populus</i>	†
<i>P. tibialis</i>	+							<i>Salix</i>	†
<i>P. vulgatissima</i>	+							<i>Salix</i>	†
<i>Paropsina</i>									
<i>Paropsis atomaria</i>				+	+			<i>Eucalyptus</i>	16
<i>Chrysophtharta varicollis</i>				+	+			<i>Eucalyptus</i>	16
<i>C. amoena</i>				+	+			<i>Eucalyptus</i>	16

*See figures.

† This paper.

‡ Present in only one of the four populations studied.

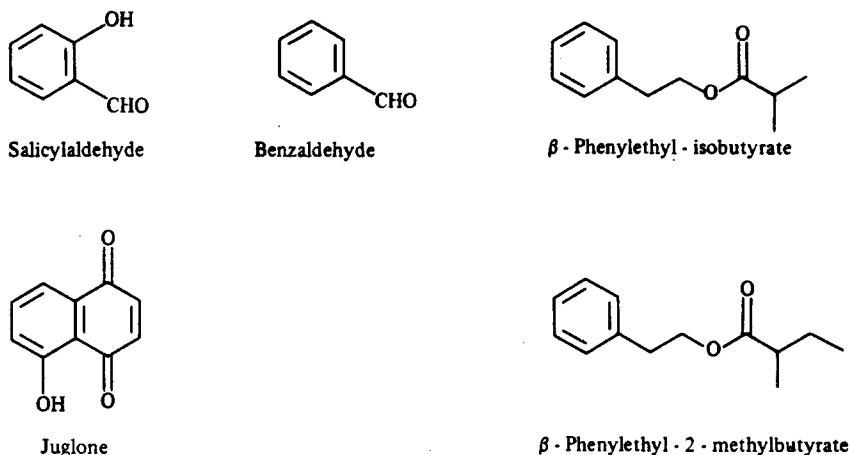


FIG. 2. AROMATIC COMPOUNDS IDENTIFIED IN THE LARVAL SECRETIONS OF CHRYSOMELINI.

Chemical defence in adults

In adults, the defensive apparatus consists of clusters of glands opening into grooves on the surface of the pronotum and the elytra [1]. These glands are found not only in the Chrysomelinae but also in the Criocerinae, Alticinae and Galerucinae, forming one phyletic line [17]. They have been briefly described by Cuénot [13], Tower [18], Hollande [12] and Patay [19]. Deroe and Pasteels [20] made a detailed histological study of the glands of *Leptinotarsa decemlineata*.

When the beetles are disturbed the secretion oozes from the openings of the glands, covers the integument and accumulates in the marginal grooves. The different classes of compounds recognized so far in the secretion of adult Chrysomelini are listed in Table 4. The structures of the cardenolides and the isoxazolin-5-one glucosides identified in the secretions are given in Figs 3 and 4.

Taxonomic implications

As can be seen from Tables 1, 2 and 4 the taxonomic grouping of Daccordi [4] accords well with the distribution of the different classes of compounds found in the defensive secretions of both larvae and adults.

Paropsina

The Paropsina (*Paropsis* and *Chrysophtharta*) form a distinct group. This is consistent with their single pair of defensive glands in the larvae, their

unique food plant (*Eucalyptus* species) and the defensive secretion of the larvae. The presence of benzaldehyde together with hydrocyanic acid in this secretion suggests a biosynthetic pathway starting from phenylalanine, and with mandelonitrile as an intermediate (review [21]). In the secretion of *Chrysomela 20-punctata* and *C. saliceti*, benzaldehyde is associated with salicylaldehyde rather than hydrocyanic acid. This implies a different metabolic pathway and does not indicate a close taxonomic relationship between the Paropsina and those *Chrysomela*. No defensive secretion has been reported as yet in adult Paropsina.

Chrysolinina

The adults possess secretions characterized by the presence of cardenolides, which so far have not been detected in other chrysomelid taxa, either within or without the Chrysomelini [22, 23]. Since cardenolides were not detected in the food plants (e.g. *Mentha* and *Galeopsis*) they are probably synthesized by the insects, presumably from a plant sterol precursor [22].

Five species of *Chrysolina* (*C. brunsvicensis*, *C. varians*, *C. hyperici*, *C. geminata* and *C. carnifex*) do not produce cardenolides. This could represent an adaptation to a particular host plant (see next section).

Another characteristic of the Chrysolinina is the presence in the secretions of the adults of amino acid derivatives and of small quantities of lipids.

No larval secretion is known in this group, but cardenolides were detected in extracts from whole individuals of all immature stages, in increasing quantity with age [23].

Doryphorina

In the *Doryphorina*, the adult secretion of only one species has been studied. It does not contain any cardenolides but amino acid derivatives and relatively small quantities of saturated hydrocarbons are present. This suggests a closer relationship between the *Doryphorina* and the *Chrysolinina* than between the *Doryphorina* and the *Chrysolina*. Indeed, *Leptinotarsa* and *Chrysolina* have been put in the same taxon by many authors [5-7, 9; Table 1], on morphological criteria.

No larval secretion has yet been described or analyzed.

Chrysomelina

The *Chrysomelina* seem also to be a natural category on the basis of their defensive secretions. Methylcyclopentanoid monoterpenes have been found in the larval secretions of 6 of

the 8 genera studied (Table 2). The exceptions can be explained by a secondary adaptation to particular host plants (see next section).

The secretion of the adults is characterized by the presence of isoxazolin-5-one glucosides and a large quantity of lipids, unlike the *Chrysolinina*, the *Doryphorina* and *Goniocтена*.

Phyllodectina

In the *Phyllodectina*, defensive compounds have been fully identified only in *Phratora*. The larvae, like those of the *Chrysomelina*, secrete methylcyclopentanoid monoterpenes (*P. vitellinae* being an exception) and the adults produce isoxazolin-5-one glucosides.

The taxonomic affinities of *Phratora* are controversial (Table 1). Weise [9], using morphological characteristics of adults, grouped *Phratora* with *Goniocтена* (= *Phytodecta*) in a distinct tribe, the *Phyllodectini*. This subdivision has been followed by Chen [24], Derenne [25] and Jolivet and Petitpierre [26]. However, on the basis of larval characteristics Cox [5], Hennig [6], Kimoto [7] and Takizawa [8] recognized that the *Phyllodectini* of Weise do not seem to be a natural tribe,

TABLE 3. PROPORTIONS (%) OF THE DIFFERENT IDENTIFIED METHYLCYCLOPENTANOID MONOTERPENES IN THE SECRETIONS OF LARVAL CHRYSOMELINI*

	Chrysomelidial	Epichrysomelidial	Plagioidial	Plagiolactone	Epipiagiolactone	Gastrolectone	Origin (reference)
<i>Gastrophysa cyanea</i>	50	-	-	-	-	50	U.S.A. [35]
<i>G. atrocyanea</i>	100	-	-	-	-	-	Japan [36]
<i>G. viridula</i>	90	10	-	-	-	-	Belgium [2]
<i>Hydrothassa marginella</i>	90	90	-	10	-	-	Belgium [2]
<i>Linnaeidea aenea</i>	35	-	-	15	50	-	Japan [37]
<i>L. aenea</i>	-	-	85	15	-	-	Belgium [2]
<i>Phaedon brassicae</i>	100	-	-	-	-	-	Japan [36]
<i>P. cochleariae</i>	100	100	-	-	-	-	Belgium [2]
<i>Plagiiodera versicolora</i>	66	66	-	33	-	-	U.S.A. [48]
<i>P. versicolora</i>	-	-	70	30	-	-	Belgium [2]
<i>P. versicolora distincta</i>	-	3	90	3	3	-	Japan [37]
<i>P. versicolora</i> † §	2	2	4	17	-	-	Germany †
<i>Prasocuris phellandri</i>	-	-	100	-	-	-	Belgium [2]
<i>Phratora tibialis</i> §	7	7	91	tr	-	-	Belgium †
<i>P. laticollis</i> §	3	3	79	1	-	-	Belgium †
<i>P. vulgatissima</i> §	88	88	tr	-	-	-	Switzerland †

*For formulae see Fig. 1.

† This paper.

‡ 68% of salicylaldehyde also present.

§ Minor compounds remain unidentified.

- The compound has not been detected in the secretion.

Phratora being more closely related to the Chrysomelina than to *Goniocтена*. This is consistent with the presence of 9 pairs of defensive glands in the larvae of *Phratora* but not in those of *Goniocтена*. The composition of the defensive secretions of both larvae and adults confirms this opinion. The precise nature of the secretion of adult *Goniocтена* is unknown. The isoxazolin-5-one glucosides characteristic of *Phratora* and of the Chrysomelina are not present, but amino acid derivatives were detected as well as small amount of lipids. On the basis of chemical data from both larvae and adults, we favor the idea that *Phratora* should be included within the Chrysomelina. Kimoto [7] and Takizawa [8] put *Phratora* in the same generic group as *Phaedon* and *Gastrophysa*. Defensive

chemistry neither supports nor contradicts this association. It is, however, interesting to note that in captivity *P. polaris* accepts as food plant *Polygonum viviparum*, a member of the same family (Polygonaceae) as the food plants of *Gastrophysa* [27]. As already suggested by Cox [5] (Table 1), *Goniocтена* might have some affinities with both Doryphorina and Chrysolinina.

Secondarily derived secretions and the influence of the host plant

The wide distribution of methylcyclopentanoid monoterpenes in the secretions of the Chrysomelina and *Phratora* larvae suggests that these compounds represent their primitive defensive secretion. These monoterpenes are most likely synthesized *de novo*, since they have no obvious

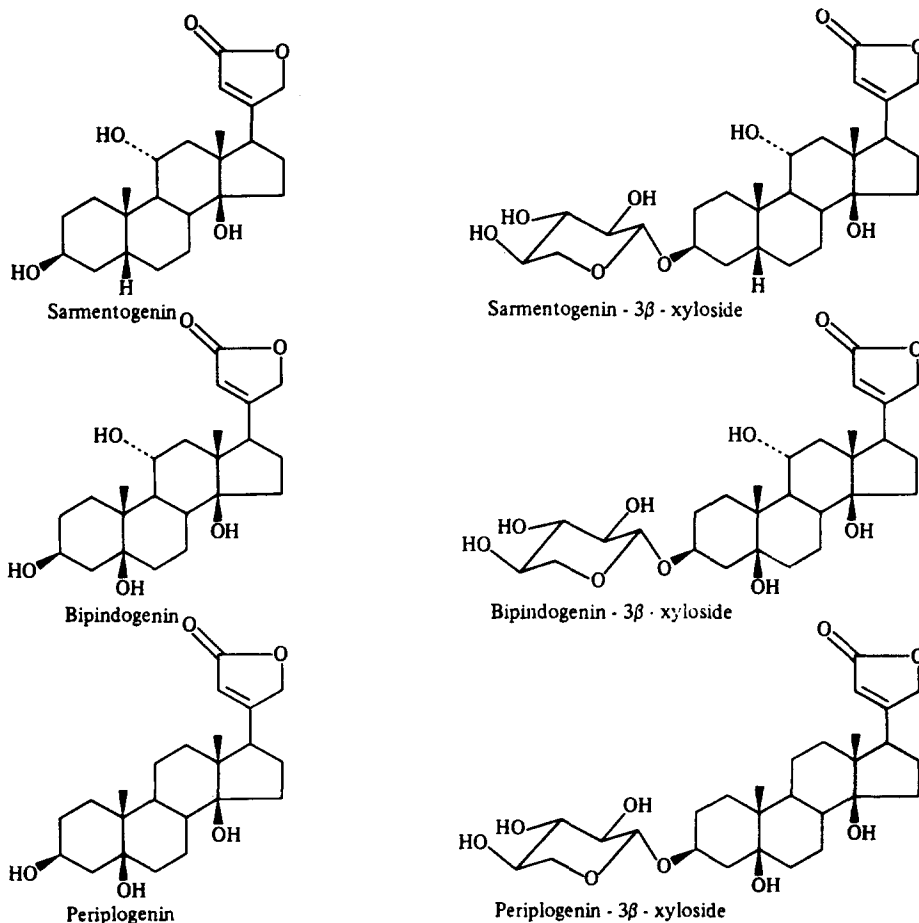


FIG. 3. CARDENOLIDES IDENTIFIED IN THE SECRETIONS OF ADULT *CHRYSOLINA COERULANS* AND *C. HERBACEA*.

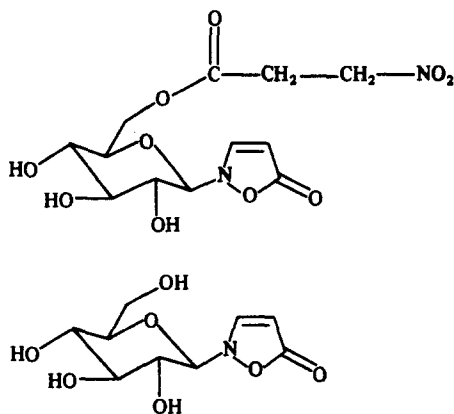


FIG. 4. ISOXAZOLIN-5-ONE GLUCOSIDES IDENTIFIED IN THE SECRETIONS OF ADULT CHRYSOMELINA AND *PHRATORA*.

precursors in the food plants of the larvae and their presence is independent of the food plant; e.g. 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 instead aromatic compounds; most of them feed on trees belonging to the Salicaceae or Betulaceae. For both *C. tremulae* and *P. vitellinae* the salicylaldehyde secreted by the larvae has been shown to be derived from salicin, a phenylglucoside present in the leaves of their salicaceous food plants [28, 29]. Thus in contrast to the situation described for the species producing monoterpenes, in at least some of the species producing aromatic compounds the defensive secretion is dependent upon the host plant.

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 be an adaptation to the secondary chemistry of the Salicaceae, enabling the larvae not only to produce cheap defensive compounds largely presynthesized by the food plant, but also to mobilize an otherwise unexploited energy source, the glucose moiety of salicin [29]. 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 some *Salix* species, in contrast to those of most woody plants, do not contain proanthocyanidins [30] and their secondary chemistry might therefore be more favorable for a herbivore adapted to digesting herbs [28]. That the secretion of salicylaldehyde is advantageous to the larvae feeding on Salicaceae is supported by the fact that it has occurred in at least three genera: *Chrysomela*, *Phratora* and in one German Upper-Rhein population of *Plagioderma versicolora*. The latter is particularly interesting because it secretes a mixture of salicylaldehyde (as major constituent) and monoterpenes, thus differing from the previously studied American, Japanese and Belgian populations (Table 1) which secrete only monoterpenes. This mixture of salicylaldehyde and monoterpenes in the secretion of the larvae of only one of the populations studied could represent an intermediary step in the evolution of the secretion of aromatic compounds. Hollande [12], working in Grenoble (France), also reported the presence of salicylaldehyde in the secretions of *P. versicolora* larvae; unfortunately he does not give precise information on the chemical methods used nor on the exact origin of the insects studied. The change from the secretion of monoterpenes to that of salicylaldehyde has probably occurred early in the evolution of the genus *Chrysomela*, since none of its present studied species are still producing monoterpenes. The change must have occurred more recently in the genus *Phratora*, because only one of its four studied species secretes salicylaldehyde; and even more recently and not completely in *Plagioderma*.

The *Alnus*-feeding *C. interrupta* also produces aromatic compounds, but these are phenylethyl-esters, not salicylaldehyde. It has already been shown that *C. interrupta* belongs to a group of sibling species which have apparently evolved through ecological isolation, some feeding on *Alnus* and others on *Populus* or *Salix* [10]. Brown successfully fed the larvae of the *Populus*-feeder on *Alnus* and obtained hybrids between the *Alnus*- and the *Populus*-feeders. We suggest that *C. interrupta* originated through a secondary ecological speciation from a Salicaceae-feeder which had lost the ability to synthesize monoterpenes for defence but had acquired the capacity

to incorporate aromatic compounds in its defensive secretion. *Alnus* being devoid of salicin, it is not salicylaldehyde but phenylethylesters which are produced. These phenylethylesters are not themselves present in *Alnus* leaves [31] and their origin remains unknown.

Another example of the influence of host plant chemistry on the composition of the defensive secretion of the larvae is that afforded by *Gastrolina depressa* [32] which secretes juglone, a naphthoquinone that could be easily derived from a 1,4,5-trihydroxynaphtalene glucoside present in the walnut tree (*Juglans*) on which it feeds.

The sporadic occurrence in the secretions of compounds derived from plant precursors supports the idea that their production is a secondary development within the lineage.

In adults, the host plant may also influence the nature of the chemical defence. The five species of *Chrysolina* not producing cardenolides (Table 4) feed on chemically protected plants such as *Hypericum* and *Artemisia*. They could rely on plant toxins for their defence [23] rather than synthesize costly cardenolides. Indeed, *C. brunsvicensis* sequesters hypericin from *Hypericum* [33] but does not incorporate it in an exocrine secretion. *C. didymata*, another species feeding on

TABLE 4. CLASSES OF DEFENSIVE COMPOUNDS IN THE GLANDULAR SECRETIONS OF ADULT CHRYSOMELINI*

	Cardenolides	Amino-acid derivatives	Lipids	Isoxazolin-5-one glucosides	References
<i>Doryphorina</i>					
<i>Leptinotarsa decemlineata</i>	-	+	+	-	2, 22
<i>Chrysolinina</i>					
<i>Chrysolina americana</i>	++			-	2,22
<i>banksi</i>	++			-	2, 23
<i>brunsvicensis</i>	-			-	2,22
<i>carnifex</i>	-			-	2, 23
<i>cerealis</i>	++			-	2, 22
<i>coerulans</i>	++	+	+	-	2, 22, 23, 51
<i>diversipes</i>	++			-	2, 23
<i>didymata</i>	+			-	2, 23
<i>fastuosa</i>	++			-	2, 22
<i>germinata</i>	-	+		-	†
<i>grossa</i>	++			-	2, 22
<i>haemoptera</i>	++			-	2, 23
<i>herbacea</i>	++	+		-	2, 22, 51
<i>hyperici</i>	-	+		-	2, 22
<i>polita</i>	++	†		-	2, 22, 23
<i>sanguinolenta</i>	++			-	2, 22
<i>staphylaea</i>	++			-	2, 23
<i>varians</i>	-	†		-	2, 22
<i>Oreina cacaliae</i>	++	†		-	2, 22
<i>tristis</i>	++			-	2, 22
<i>Chrysomelina</i>					
<i>Chrysomela populi</i>	-	-	++	++	2
<i>tremulae</i>	-	-	++	++	2, 23
<i>20-punctata</i>	-	-	++	++	2, 23
<i>saliceti</i>	-	-	++	++	†
<i>Gastrophysa viridula</i>	-	-	++	++	2, 22
<i>cyanea</i>	-	-	++		50
<i>Hydrothassa marginella</i>	-	-		++	2, 22
<i>Phaedon brassicae</i>	-	-		++	2, 22
<i>Phaedonia circumcincta</i>	- [23]	-		++	†
<i>Plagiodera versicolora</i>	- [22]	-		++	†
<i>Phyllodectina</i>					
<i>Phratora vitellinae</i>	-	-		++	2
<i>laticollis</i>	-	-		++	2, 22
<i>tibialis</i>	-	-		++	2
<i>Gonioctenina</i>					
<i>Gonioctena olivacea</i>	- [21]	+		-	†

*See figures.

† This paper.

- The compound has not been detected in the secretion.

Hypericum secretes only one cardenolide, sarmantogenin, whereas a mixture of 4–12 different cardenolides is usually present in the secretion of other *Chrysolina* species. *C. didymata* could represent an intermediate stage in the secondary loss of cardenolide production by insects feeding on toxic plants [23].

Inter- and intraspecific diversity

As seen above, the chemical defence of both larvae and adults provides good taxonomic characters at the supragenetic level for the Chrysomelini. When the precise composition of the defensive mixtures is considered, however, great variation is observed between genera and species.

It is clear from Table 3 that the larvae of most species produce a different blend of methylcyclopentanoid monoterpenes. The secretions of different species within a genus are in some cases more different than the secretions from species belonging to different genera. Quantitative and qualitative differences exist between geographically isolated populations, e.g. *Linaeida aenea* in Japan and Belgium, and *P. versicolora* in North America, Japan, Belgium and Germany. The Japanese *P. versicolora* is usually considered a distinct subspecies, but both the American and the European forms belong to the same subspecies, the American having probably originated from insects introduced from Europe. These differences are not due to sampling error: most of the proportions were calculated from the pooled secretions of hundreds of larvae. At least in *P. versicolora*, individual or year to year variations are smaller than the differences observed between species or distant populations of the same species [2].

Similarly, in the secretions of adult *Chrysolina*, the spectrum of cardenolides varies quantitatively and qualitatively from species to species. The mixture of cardenolides can also differ between isolated populations of the same species, e.g. between Belgian and Greek populations of both *C. polita* and *C. herbacea*. As judged by thin layer chromatography, however, there is little variation in the proportion of the different cardenolides between individuals of one population, from one generation to the next, or between individuals fed on different food plant species [23].

The significance of the diversity of chemical defence between different sympatric species or between allopatric races 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 groups of mixed species are easy to obtain in the laboratory (e.g. *P. versicolora* with *P. vitellinae*). Larvae of *P. vitellinae* experimentally made devoid of defensive 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 strongly repelled [15]. Defensive secretions of the larvae may also reduce interspecific competition. In the spring, the same *Salix* bushes are colonized first by adults of *C. 20-punctata* coming out of hibernation and a couple of weeks later by *P. vitellinae*. The larvae of the new generation of *C. 20-punctata* develop rapidly and smell strongly of salicylaldehyde when they reach the third larval instar or the pupal stage. A decrease in the population of *P. vitellinae* adults on the initially favored trees is positively correlated with the number of larvae and pupae of *C. 20-punctata* (Rowell-Rahier, unpublished observations).

The diversity of defensive secretions could be due to genetic drift in the absence of selective pressure for uniformity. Tschinkel [34], in his study of the defensive secretions of tenebrionids, suggested that their precise composition was not critical within a given range of compounds as long as the secretion meets certain physical requirements (e.g. lipophilicity, volatility, melting point, irritant value). 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. No data supporting this hypothesis are presently available.

Selection for diversity would be another possible explanation of the specificity of the defensive secretions in sympatric species. In nature, larvae of *Hydrothassa marginella* and *Prasocuris phellandrii*, for example, are often found together on *Ranunculus*. They are probably exposed to the same set of potential predators and

parasitoids but they produce different mixtures of compounds. 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 [29, 35–37]. Using adult sawflies (*Tenthredo olivacea*), a predator observed to feed on chrysomeline larvae in nature, Pasteels and Grégoire (in preparation) 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 of the predator. Similarly, interspecific and intraspecific diversity of plant secondary metabolites is thought to limit the adaptation of herbivores to these plants [38, 39].

Differences between the chemical nature of the defensive secretion of the larvae and of the adults

The glands of the *Chrysomelina* larvae are not morphologically homologous to those of the adults. Moreover, the chemical nature of their secretion is very different. Although they live together on the same plant, the chemical defence of the larvae and of the adults might be a response to different selective pressures or, alternatively, there might be no selection for them to be identical.

The small soft larvae of the *Chrysomelinae* are easy prey for predators such as ants. A small wound can be fatal to a larva and it is therefore important for them to repel the potential predator at a distance. The secretion of a small volatile molecule is appropriate in this case [40]. Volatile monoterpenes appear to be very effective against arthropods, acting as irritants at a distance. Salicylaldehyde, more volatile than its precursors saligenin and salicin, has been shown to be an effective deterrent against ants, even at low concentration [29]. On the other hand, volatile compounds may not provide an efficient defence against larger predators such as birds. The small size of most larvae, their cryptic coloration and their feeding habit on the lower side of the leaves are probably more effective in protecting the larvae against predation by birds. It is worth

noticing that the relatively aposematic adults of *P. vitellinae* feed on the upper surface of the poplar and willow leaves whereas the larvae which are more cryptic feed on the lower surface.

The adults, on the other hand, are mechanically protected by their hard cuticle against small predators like ants. Further, many species fall to the ground on the slightest mechanical stimulus; this behavior pattern is probably effective against birds. The adults which are mostly brightly colored tend to clump together. The association of highly toxic compounds and aposematic coloration has been shown to be a good defence against birds [41–43]. The toxicity of cardenolides for vertebrates is well known. The physiological effect of the isoxazolin-5-one glucosides secreted by the adults of the *Chrysomelina* and *Phratora* remains to be investigated. β -Nitropropionic acid (Fig. 4), present as an ester in one of the major constituents of the secretions, is known to be very toxic for both vertebrates and insects [44]. In some legumes it is also present as glucosides and responsible for their toxicity to mammals.

We do not claim that larval defence is invariably inefficient against vertebrates, or adult defence against ants, but rather that one group of defensive chemicals could be more adequate against one group of predators. This might be sufficient to explain the divergence in the chemical nature of the secretions of larvae and adults.

Conclusions

Enough data are presently available to allow some taxonomic and evolutionary deductions from comparative studies of chemical defence of the *Chrysomelini*, especially when they are analyzed in their ecological context.

Chemical defence has evolved separately in larvae and adults. In both, the evolution of the chemical nature of the secretions is not strictly linked to the evolution of the glandular structures. Independent morphological and chemical information can thus be used to assess taxonomical affinities.

So far, the existing information on chemical defence is in agreement with Daccordi's classification of the *Chrysomelini* from temperate regions at the suprageneric level. The defence mechanism of *Phratora* shows more similarities with that of the *Chrysomelina* than with

Gonioctena; and we consider the grouping of *Phratora* and *Gonioctena* in a distinct subtribe to be unnatural. Unfortunately, there is not enough information on the chemical ecology of tropical species to suggest a rearrangement of the Chrysomelini on a world-wide basis.

Some discrepancy between classification and the chemical nature of defensive secretions can be interpreted as secondary adaptation to new host plants; such close adaptation to ecological conditions may thus mask taxonomical affinity.

The precise composition of the secretions offers no reliable taxonomical characters at the generic or specific levels. Differences are as great between species of different genera as between congeneric species, or even between distinct populations of the same species. This high diversity can be interpreted as the result of several factors: first, the absence of selective pressure for uniformity, leading to a certain degree of genetic drift; secondly, a selective pressure for diversity in sympatric species, to take advantage of the establishment of preferences in the predators.

The dissimilarity of the chemical defences of the larvae and adults living in the same habitat could be explained by differences in the type of predators to which they are most vulnerable, e.g. ants or other small arthropods for the larvae and birds or other vertebrates for the adults. This last hypothesis remains to be supported by further ecological and experimental data.

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