Twenty-five years of chemical ecology

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1 Introduction

Chemical ecology has been defined as 'the promotion of an ecological understanding of the origin, function and significance of natural chemicals that mediate interactions within and between organisms'.[1] One major strand in the development of chemical ecology was the discovery of insect pheromones, and the recognition that trace amounts (*e.g.* 1 μg or less) of simple organic molecules could exert profound effects on the mating behaviour of Lepidoptera and other insects.[2] Equally important was the discovery of kairomones, volatile signals released by plants to attract or repel insects to feed or oviposit on those plants.[3]

Another major strand in the origin of chemical ecology was the increasing need to explain the enormously rich variation in secondary metabolism encountered specifically in plants rather than in animals. Fraenkel in 1959[4] argued that the *raison d'être* of these so-called secondary metabolites could not be accommodated by the idea that they are simply "waste products" of primary metabolism, accumulating in the plant cell because of the absence of an efficient excretory system. Instead, Fraenkel described these metabolites as "trigger" substances, which induce or prevent the uptake of nutrients by animal herbivores.

In 1964, Ehrlich and Raven[5] were among the first to propose a defined ecological role for plant products as defence agents against insect herbivory. They proposed that, through the process of co-evolution, insects are able to detoxify certain defensive agents that deter feeding so that, eventually, the same deterrent substances become feeding attractants. Such a hypothesis helps to explain the relatively restricted feeding preferences of many insects, *e.g.* the restriction of the cabbage white butterfly to plants which contain glucosinolate. Since then, this basic hypothesis has undergone modifications to take into account the ecological difference between so-called apparent (trees) and non-apparent (herbs) plants[6] and the fact that secondary compounds are costly for the plant to synthesise.[7,8]

A third important event in the history of chemical ecology was the recognition that Lepidoptera with warning coloration have the ability to utilise plant toxins for their own protection. Thus, Reichstein *et al.* in 1968[9] established that Monarch butterflies sequester and store the heart poisons or cardiac glycosides present in their food plants, namely species of *Asclepias*. Brower[10] at the same time showed that the cardiac glycosides in the butterfly were emetic to blue jays so that the Monarchs were able to avoid bird predation.

Chemical ecology has been the subject of a number of key publications that have been influential in its development. The founding of the *Journal of Chemical Ecology* in 1976 by Sondheimer and Simeone was an important event. The first multi-author treatise on the subject appeared in 1970.[11] A significant publication on the interaction between herbivores and different classes of secondary metabolite appeared in 1979[12] and was revised in 1993.[13] An introductory textbook was published in 1977 and is now in its fourth edition.[14] Regular reviews covering recent advances have appeared in *Natural Product Reports* (*e.g.* refs. 15–19).

In this historical account of chemical ecology, attention will be concentrated on (a) the ability of aposematic insects to store

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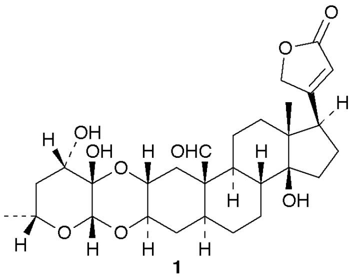
Table 1 Plant toxins sequestered from plants by Monarch butterflies

Toxins	Source	Metabolites (if any)
Cardiac glycosides		
Calactin	*Asclepias*	Calotropagenin
Calotropin (**1**)	*curassavica*	
Calotoxin, *etc.*	leaf	
Pyrrolizidine alkaloids[a]		
Retronecine (**5**)	*Senecio*	Danaidone (**6**)
Integerrimine	*jacobaea*	Hydroxydanaidal (**7**)
Seneciphylline	nectar	(male pheromones)
Intermedine		
Lycopsamine		
Echinatine		
Senecionine		
Pyrazine alkaloids[b]		
3-Isopropylpyrazine	*Asclepias*	
3-sec-butylpyrazine	*curassavica*	
3-Isobutyl-2-methoxypyrazine	leaf	

[a] Present both in the free state and as the *N*-oxides. [b] Amounts detected in the Monarch vary from a trace to 5 mg per insect.



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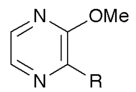

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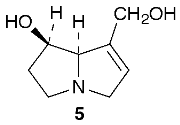

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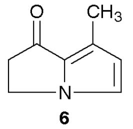

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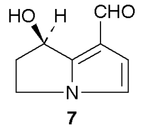

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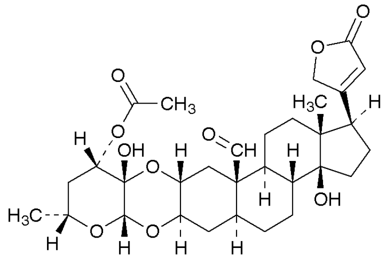

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secondary plant constituents; and (b) the role of chemistry in plant defence. In addition, it is planned to discuss pollination biochemistry, oviposition cues and the ecological role of plant chemistry in fruit development and seed dispersal.

2 Sequestration of plant substances by insects

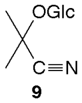
One of the most remarkable features of plant–animal interactions in the natural world is the ability of certain insects to sequester plant toxins from their food plants in the larval stage. They then move these toxins into the adult imago and both larva and adult generally gain protection from bird predation. The best known and most widely studied example is the Monarch butterfly, *Danaus plexippus*, the larvae of which absorb cardiac glycosides (*e.g.* calotropin **1**) from the food plant, typically *Asclepias curassavica*, so that the adult butterfly is protected from bird and mice predation.[9,10]

What is particularly striking about the Monarch butterfly is the fact that it borrows *three different classes* of plant toxin for its protection (Table 1). Thus, besides borrowing a range of cardiac glycosides at the larval stage from the food plant, it also absorbs a mixture of three pyrazine alkaloids. These are transferred to the adult and provide a mixture of warning odour secretions. The pyrazines (**2**)–(**4**) are volatile and are released immediately as a bird starts feeding on the butterfly and are sufficient to arrest that feeding.[20] A second class of alkaloid, the pyrrolizidines (*e.g.* retronecine, **5**), are taken up by the adult butterfly either from drinking the alkaloid-laced nectar of *Senecio* plants or by "sucking up" the leaf alkaloids present in borage plants. These alkaloids are dual purpose. They provide useful extra protection from bird predation, as a back up to the cardiac glycosides, but secondly they are precursors for male pheromone production. They are converted into danaidone (**6**) and hydroxydanaidal (**7**), two pheromonal structures, which are stored in the wing pencils. They are released as a "love dust" onto the wings of a female butterfly just before mating.[21]

Important features of the uptake and sequestration of plant products by insects are (1) the selective absorption of only certain metabolites, (2) the control in the concentrations of metabolites taken up, (3) the further metabolism of these metabolites, and (4) their conjugation and storage in a modified form. In the case of the cardiac glycosides of the Monarch it is known that each species of the several *Asclepias* taxa commonly used as food plants has a specific pattern of toxins, which are modified *in vivo* to a different mixture of cardiac glycosides. For example, asclepin (**8**) in the plant is hydrolysed by the insect into calotropin (**1**). By means of such patterns, readily revealed by one-dimensional TLC, it is possible to recognise, from the spot pattern of the adult, which one of several food plant species the larva fed upon earlier in its life history.[22]

The borrowing of plant toxins by the Monarch for defence has important implications for the conservation of this important North American butterfly and this is discussed at length in a symposium volume published in 1984.[23] The success in solving the origin of the toxic protection present in this aposematic insect stimulated the chemical analysis of other insects with warning coloration and their corresponding food plants. In fact, a range of toxic molecules have now been variously characterised in many different insects (Table 2).

In spite of the range of chemicals that can thus be sequestered from plant sources by insects, it should not be forgotten that many aposematic insects synthesise their own animal-derived toxins. These may be simple in structure (*e.g.* histamine, acetylcholine) or proteinaceous.[24] Occasionally, too, it has been discovered that an insect may both borrow a plant toxin and synthesise the same molecule *de novo*. This is true in the case of the burnet moth, *Zygaena trifolii*.[25] It synthesises the cyanogenic glycosides linamarin (**9**) and lotaustralin (**10**) from valine and isoleucine, respectively. It also acquires the same two cyanogens from its food plant *Lotus corniculatus*. As a result of this duplication, the adult moth, with its bright red and black coloured wings, contains exceptionally high concentrations of cyanogenic glycoside to protect it from bird predation. It will rest undisturbed on a favoured nectar source (*e.g. Centaurea nigrans*) for minutes at a time.



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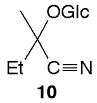

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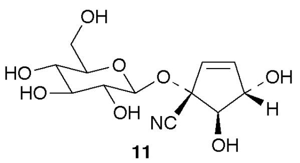
The source of cyanogenesis in insects is particularly variable. In *Zygaena*, discussed above, the cyanogens are of both plant and insect origin.[25] And yet, in Heliconiine butterflies, which feed on plants of the Passifloraceae, which are rich in cyanogens (*e.g.* gynocardin **11**), the insect toxins are chemically different from those of the food plants and are entirely produced within

Table 2 Classes of plant toxin sequestered by insects and stored for defence

Class of chemical	Typical structure	Plant source	Insect storing it
Acetogenins	Bullatasin	*Asamina triloba*	Butterfly, *Eurytides marcellus*
Aliphatic acids	Siphonidin	*Euonymus europaeus*	Moth, *Yponomenta cagnagellus*
Aristolochic acids	Aristolochic acid	*Aristolochia* spp.	Butterfly, *Battusa archidamus*
Bianthraquinones	Hypericin	*Hypericum hirsutum*	Beetle, *Chrysolina brunsvicensis*
Cardiac glycosides	Calotropin (**1**)	*Asclepias* spp.	Butterfly, *Danaus plexippus*
Cucurbitacins	Cucurbitacin D	*Cucurbita* spp.	Beetle, *Diabrotica balteata*
Cyanogenic glycosides	Linamarin (**9**)	*Lotus corniculatus*	Moth, *Zygaena trifolii*
Diterpenoids	Clerodendron D	*Clerodendrum trichotomum*	Sawfly
Fluoracetate	Fluoroacetate	*Dichapetalum cymosum*	Moth, *Sindrisa albimaculata*
Glucosinolates	Sinigrin	*Brassica oleracea*	Butterfly, *Pieris brassicae*
Iridoids	Aucubin	*Plantago lanceolata*	Butterfly, *Euphydryas cynthia*
Methylazoxymethanols	Cycasin	*Zamia floridana*	Butterfly, *Eumaeus atala*
Phenols	Salicin	*Salix* spp.	Beetle, *Chrysomela aenicollis*
Polyhydroxy alkaloids	2,5-DM-3,4-DP	*Omphalea* spp.	Moth, *Urania fulgens*
Pyrazines	3-Isopropyl-2-methoxypyrazine	*Asclepias curassavica*	Butterfly, *Danaus plexippus*
Pyrrolizidine alkaloids	Retronecine	*Senecio* spp.	Moth, *Arctia caja*
Quinolizidine alkaloids	Cytisine (**13**)	*Cytisus scoparius*	Aphid, *Aphis cytisorum*
Steroidal alkaloids	3-Acetylzygadenine	*Veratrum album*	Sawfly, *Rhadinocarpa nodicornis*
Tropane alkaloids	Calystegine A_3	*Datura wrightii*	Hawkmoth, *Acherantia atropus*

the butterfly.[26] In a third example, the Apollo butterfly *Parnassus phoebus*, the cyanogenic glycoside present, sarmentosin (**12**), also occurs in the food plant *Sedum stenopetalum*, so that it would seem to be solely plant-derived.[27]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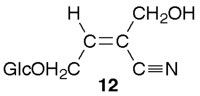
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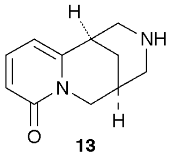
Insects may selectively borrow one plant toxin from several that may be available to it from the dietary viewpoint. Thus, the broom aphid *Aphis cytisorum*, feeding on the legume *Pelteria ramentaica* in the larval stage, only sequesters the alkaloid cytisine (**13**), rejecting at the same time anagyrine and *N*-methylcytisine, both of which are also present in the food plant.[28] Such plant-derived toxins may be passed on in the food chain. This happens with the ragwort aphid, *Aphis jacobaeae*, which borrows pyrrolizidine alkaloids from the ragwort *Senecio jacobaea*. When the ladybird *Coccinella septempunctata* feeds on these aphids it is found to have stored the aphid's alkaloids in its knee-joint protective secretions.[29]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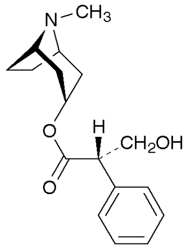
A defence based on toxins borrowed at the larval stage and transferred to the imago is by no means universal in aposematic insects. A comparison between the two large butterfly groups, the Danaids and the Ithomiines, shows that while the Danaids are adapted to borrow cardiac glycosides from their food plants, the Ithomiines do not. This is somewhat surprising, since the latter butterflies feed extensively on plants of the Solanaceae, known to be rich in both tropane and steroidal alkaloids. An extensive screening of Ithomiine species at first failed to yield any insect containing solanaceous alkaloids.[30] More recent experiments have exceptionally provided one example of an Ithomiine butterfly with tropane alkaloids, but this was only one of hundreds screened. The exceptional butterfly is *Placidula euryanassan*, which feeds in the larval stage on *Brugmansia suaveolens* (Solanaceae). In doing so, it borrows hyoscyamine (**14**), norscopolamine and scopolamine, passing them on through the pupae to the freshly emerged adults. This storage of tropane alkaloids protects the butterflies from being eaten by chickens or monkeys.[31] However, they are still susceptible to predation by the orb-weaving spider. To avoid spider attack, it additionally borrows a second alkaloid defence, *via* pyrrolizidines, which are imbibed from the nectar of *Eupatorium* species in the adult state.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The borrowing of plant alkaloids by an insect may serve several purposes. In the case of the Monarch butterfly, pyrrolizidine alkaloids may be stored for defence or converted, in the male, into pheromonal material. In some insects, protection from bird or spider predation may be required during a lengthy breeding session. This is true of the wasp moth *Cosmosoma myrodora*, which spends nine hours at a time in the process of copulation. Here, the male secretes pyrrolizidine alkaloids borrowed from *Eupatorium capillifolium* and, when courting, discharges over the female a mass of intricate fibres impregnated with alkaloid. This is sufficient to deter spider attack during the whole of the mating period.[32]

A characteristic feature of most plant toxins borrowed by insects is their bitter taste. This is true, for example, of the iridoids borrowed by *Euphydryas* butterflies (Table 3).[33] There is selectivity in uptake and only some of the iridoids present in the plant are sequestered. Catalpol (**15**), a commonly sequestered iridoid (Table 3), is particularly bitter-tasting. This may be formed in the insect from ingested iridoid esters. Thus both the plants *Besseya plantaginea* and *Penstemon virgatus* contain esters of catalpol with benzoic and cinnamic acids, but larvae of the *Euphydryas* species feeding on these plants contain only catalpol.[33]

The dietary sequestration of secondary compounds by insects is not confined to toxins, and a number of other classes of plant substance can be found, especially in butterflies and moths. Carotenoids are widely present, the concentrations

Table 3 Iridoid-sequestering butterflies and their host plants

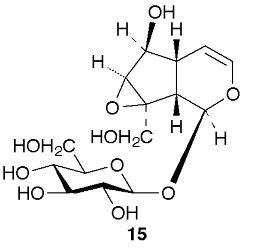
Lepidoptera[a]	Host plant	Sequestered iridoid[b]
Euphydryas anicia	*Besseya plantaginea*	Catalpol (**15**)
	B. alpina	Catalpol (**15**)
	Castilleja integra	Macfadienoside, catalpol
E. phaeton	*Chelone glabra*	Catalpol
	Plantago lanceolata	Catalpol, aucubin
	Aureolaria flava	Aucubin (**16**)
E. chalcedona	*Scrophularia californica*	Aucubin
E. cynthia	*Plantago alpina*	Catalpol, aucubin, 6-glucosylaucubin
Junonia coenia	*P. lanceolata*	Aucubin, catalpol
Poladryas erachne	*Penstemon virgatus*	Catalpol

[a] Three moths, a leaf beetle and two flies also sequester aucubin or catalpol from appropriate host plants (see ref. 33). [b] Iridoids usually detected in larvae, pupae and adults.

Table 4 Flavonoids incorporated by butterflies from their food plants

Butterfly and food plant(s)	Flavonoids of food plant	Flavonoids of butterfly
Marbled white (*Melanargia galathea*) on *Festuca* spp. (Gramineae)	Tricin 7-glycosides and glycosylflavones[a]	Tricin, tricin 7-glucoside, tricin 7-diglucoside, tricin 4′-glucoside, tricin 4′-conjugate and glycosylflavones
Zebra swallowtail (*Eurytides marcellus*) on *Asimina triloba* (Annonaceae)	Quercetin 3-glucoside, quercetin 3-rutinoside, quercetin 3-rutinoside-7-glucoside	Quercetin 3-glucoside (**18**)
Chalkhill blue (*Lysandra coridon*) on *Hippocrepis comosa*, *Lotus corniculatus* and *Anthyllis vulneraria* (Leguminosae)	Kaempferol, quercetin and isorhamnetin 3,7- and 3,4′-diglycosides, apigenin 7-glucoside	Kaempferol 3-glucoside, 3-rhamnoside, 7-rhamnoside, 3-glucoside-7-rhamnoside, and quercetin and isorhamnetin analogues
Small heath (*Coenonympha pamphilus*) on Gramineae	Tricin 7-glucoside, glycosylflavones	Tricin, tricin 7-glycosides

[a] Small amounts of apigenin and luteolin 7-glycosides are also present in the food plant and appear in the butterfly as such and in the free state. Glycosylflavones identified in both food plant and butterfly include orientin, isoorientin and isovitexin and their corresponding 7-glucosides, together with vitexin 7-glucoside.



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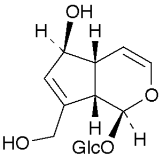

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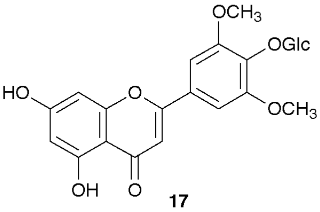
varying considerably from species to species. Certain aposematic Lepidopterans can accumulate large quantities, and Rothschild *et al.*[34] have suggested that, in such cases, the carotenoids might have a protective function, *e.g.* by preventing free-radical oxidation of phenolic materials. Such protection appears to occur in the *Aristolochia*-feeding butterflies which store nitrophenanthrenes; for example, *Battus philenor* has 726 µg of carotenoid per gram of dry weight. Evidence in support of this hypothesis is that related butterflies, which mimic them in colouring but do not store toxins, have significantly lower concentrations of carotenoid in their tissues. On the other hand, the Monarch butterfly has low concentrations of carotenoid, although it stores cardenolides; perhaps, in this case, the butterfly has no need of photo-protection.

Dietary flavonoids are also sequestered and stored by about 10% of butterflies, although the purpose of this is not yet clear.[35] Flavonoids may have a function in wing coloration but they are unlikely to be distasteful to avian predators, although this has not yet been tested in detail. Studies on flavonoid-ingesting butterflies and their food plants indicate that metabolism or a change in conjugation occurs in most cases (Table 4). This is true of the marbled white butterfly (*Melanargia galathea*), where the dietary flavone tricin 7-*O*-glucoside is recovered in the insect as the 4′-*O*-glucoside (**17**) and possibly as the 4′-*O*-sulfate.[36] It is also true of the swallowtail *Eurytides marcellus*, the body and wings of which contain the flavonol quercetin 3-*O*-glucoside (**18**), whereas the larval food plant, *Asimina triloba*, contains the 3-*O*-rutinoside 7-*O*-glucoside, the 3-*O*-rutinoside, and the 3-*O*-glucoside of quercetin.[37] Likewise, the chalkhill blue butterfly (*Lysandra coridon*) contains a simpler mixture of kaempferol, quercetin, and isorhamnetin glycosides than its leguminous food plants, so some hydrolysis of glycosidic links appears to occur *in vivo*.[38]



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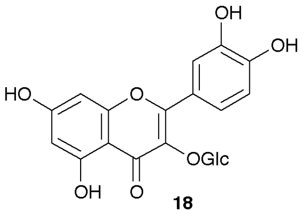

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Plant flavonoids, well known to be sequestered and stored by butterflies, have been identified in grasshoppers. Hopkins and Ahmad[39] showed that six North American species accumulate quercetin and its 3-glucoside chiefly in the wing cuticle. The concentration in the Carolina grasshopper *Dissostera carolina* makes up to 2% of the live weight. A fat body UDP-glucosyltransferase preferentially catalyses glucosylation at the 3-hydroxyl of quercetin and it is assumed that this flavonol is absorbed by the larvae from the food plant, although this has not yet been established experimentally. Yet to be explained is the dietary source of quercetin since this flavonol has a restricted distribution in grasses, the main food plants of grasshoppers. Grasses, on the other hand, are known to be rich sources of glycosylflavones and tricin derivatives (see Table 4). Why are these flavones not taken up by the grasshoppers?

Table 5 Plant species showing increased synthesis of toxins induced by leaf damage

Plant species	Toxins involved	Increases observed
Nicotiana sylvestris	Nicotine and nornicotine	220% on larval feeding, 170% on mechanical damage
Atropa acuminata	Hyoscyamine and related alkaloids	153–160% on mechanical, damage and slug attack
Pastinaca sativa	Xanthotoxin and related furanocoumarins	215% on insect feeding, 162% on mechanical damage
Brassica napus	Glucosinolates	Increases in indole glucosinolates; decrease in aliphatic glucosinolates

Plants are also rich sources of simple phenolic constituents, such as chlorogenic acid. Are simple phenolics ever sequestered and stored? Simple hydroxycoumarins have been detected in larvae of *Yponomenta mahalebellus* feeding on *Prunus mahaleb*.[40] One suspects that other examples will eventually be brought to light.

3 Role of chemistry in plant defence

More attention has been given to studying the role of secondary metabolites in defending plants from insect herbivory or mammalian grazing than to any other aspect of chemical ecology. This is because establishing the *raison d'être* of secondary metabolism is central to our understanding of plant–animal interactions. Also, defence is rarely absolute and for every example where there is resistance to animal feeding, there will be some animal that can overcome that barrier to feeding.

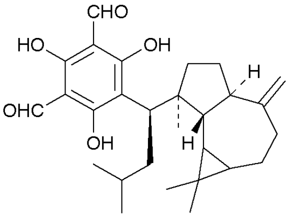
One of the most important advances in our appreciation of plant–animal interactions has been the discovery of induced defence. Thus some plants respond to either mechanical damage or insect feeding by increasing the synthesis of a particular plant toxin or plant toxins. Some examples of plants where induced defence has been established are given in Table 5. It should, however, be borne in mind that induced defence is not a universal feature of plant metabolism and, up to the present, only a relatively few plants have been shown unambiguously to respond to herbivory in this way.[41,42]

Two other types of induced defence have also been encountered in plants. The first involves the *de novo* synthesis of proteinase inhibitors, which typically occurs in the potato during Colorado beetle feeding.[43] A complex set of signalling systems are set off in the process. The ecological consequences of this novel type of plant defence have so far not been extensively explored.[44] The second type of induced defence involves the release of predator-attracting volatiles by the plant. This occurs specifically in tritrophic interactions where a herbivore feeding on a plant causes the release of newly synthesised volatiles, which specifically attract parasitoids of that herbivore to attack it and destroy it. This systemic release of volatile chemicals which mediate in plant–herbivore–predator interactions has been extensively explored from the biochemical viewpoint, but its ecological effectiveness in the field to limit insect herbivory has yet to be determined.[45]

In this present review of chemical defence in plants, attention will be given in turn to (a) phenolic constituents, (b) terpenoid toxins, (c) nitrogen-containing toxins and (d) miscellaneous barriers.

3.1 The phenolic barrier

Traditionally in ecological studies, methods for demonstrating that constitutive phenolics are involved in plant defence have depended on measuring either total phenolics or total tannins or both.[46] While such measurements might occasionally show some correlation, experience shows that it is an individual subclass of phenolic or an individual structure that is active against a particular herbivore. Good examples are the ringtail possum and the koala bear feeding on leaves of *Eucalyptus ovata* and *E. viminalis*. Ecological investigations revealed considerable variations in the amounts of leaf consumed of individual trees of the same species, caused by some feeding deterrent. *Eucalyptus* trees are rich in phenolic constituents; yet there were no observable correlations between feeding and nutritional quality or total phenolics or total tannin. It was only possible by bioassay to show what was happening and to incriminate two diformylphloroglucinol molecules as the missing links. Two structures, macrocarpal G (**19**) and jensenone (**20**), emerged as being strongly antifeedant to possum and koala.[47] These belong to a group of phloroglucinol-based phenolics only relatively recently characterised in *Eucalyptus* species.[48]



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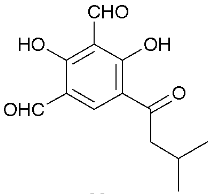

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Further studies on the ringtail possum feeding on *Eucalyptus ovata* foliage confirmed that macrocarpal G is the active agent in browse-resistant trees. In feeding experiments with an artificial diet, it was found that a concentration of 2.1% macrocarpal G causes 90% reduction in voluntary food intake by this possum.[49] A later survey of other *Eucalyptus* species showed that 27 of 41 species contained appreciable quantities of this new class of feeding deterrent. Thus, the evolution of formylated phloroglucinols in this genus has become a key determinant in the restriction in feeding of marsupial folivores.[50]

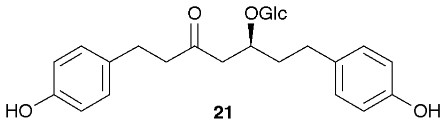
Some examples of simple phenolics being feeding barriers to insect and mammalian herbivores are shown in Table 6. There has been some concentration of effort on the salicylic acid-based phenols of beech and willow trees and the herbivores that feed on them.[51,52] There is evidence in the case of *Betula platyphylla* that a metabolite of the naturally occurring phenol platyphylloside (**21**) is the harmful agent, interfering with the digestion in the animal of the normal dietary nutrients. Thus, platyphylloside undergoes stepwise reduction *in vitro* to centrolobol (**22**) and it is the latter compound which builds up in the gut of the hare to cause feeding inhibition.[53] Similarly, metabolism also takes place with salicortin (**23**) which occurs in the leaf of *Populus*. It is hydrolysed *in vivo* to 6-hydroxycyclohex-2-enone (**24**), which is the active agent.[54]

The concentration of the phenolic in the plant is a key factor in deterrence and it is the accumulation of phenols in particular parts of the plant which represents a feeding barrier (Table 6). Such concentrations of toxin can sometimes be circumvented. Thus, the meadow vole, *Microtus pennsylvanicus*, is able to reduce the phenolic toxicity of the gymnosperm tree *Picea glauca* by cutting branches off and leaving them to stand on the winter snow for 2–3 days before eating. During this time, phenolic levels drop from 2.8% to 1.5% dry weight. Additionally, the terpenoid content (see the next section) may also affect feeding and, in the case of this meadow vole, it completely avoids feeding on leaves of another gymnosperm, *Pinus strobus*, irrespective of the phenolic content because of the high content of myrcene and bornyl acetate.[55]

Insects can overcome phenolic barriers to their feeding by tolerating or otherwise metabolising a particular toxin. This

Table 6 Simple phenolics that have been implicated as defensive agents against herbivores

Phenolic	Occurrence	Effect on herbivore
o-Pentadecenylsalicylic acid	Leaf trichomes of *Pelargonium* × *hortorum*	Toxic to two-spotted spider mite *Tetranychus urticae*
Magnolol	Leaf of *Magnolia virginiana*	Toxic to larvae of the moth *Callosomia promethea*
Miconidin and primin	Leaf trichomes of *Primula obconica*	Antifeedant to larvae of *Heliothis armigera*
Salicortin and tremulacin	Leaves of *Populus* spp.	Toxic to large willow beetle *Phratora vulgatissima*
Salicylaldehyde	Leaf of *Populus balsamifera*	Antifeedant to snowshoe hare *Lepus americanus*
Pinosylvin and its methyl ether	Buds of *Alnus crispa*	
Platyphylloside	Buds of internodes of *Betula platyphylla*	Feeding inhibitor to mountain hare, moose and goat
Coniferyl benzoate	Flower buds and catkins of *Populus tremuloides*	Feeding deterrent to ruffed grouse *Bonasa umbellus*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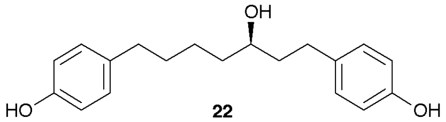
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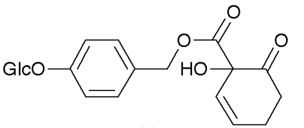
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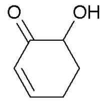
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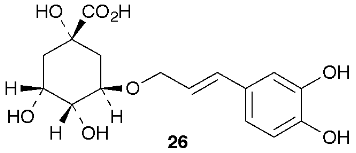
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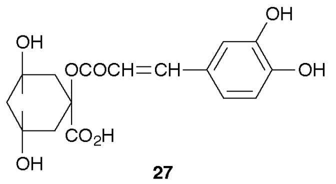
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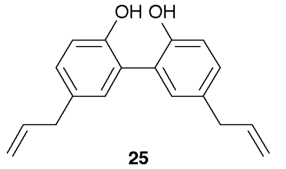
happens, for example, with the silkworm *Callosamia securifera*, which is monophagous on the magnolia tree, *M. virginiana*. Two related generalist silkworms, *C. angustifera* and *C. promethea*, do not survive, because of the toxic lignans present, magnolol (**25**) and a related biphenyl ether. In laboratory feeding experiments in which host leaf acceptable to all three species is painted with magnolol at the same concentration as in the magnolia leaf, the specialist *C. securifera* survives but the two generalists lose out.[56]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Some phenylpropanoids, caffeic acid esters, and related structures are of widespread occurrence in the plant kingdom. They are less likely to be useful defensive agents in that many insects may be adapted to them and hence would be expected to tolerate their dietary presence. Nevertheless, feeding inhibition has been observed for caffeic acid, the 3′-methyl ether ferulic acid, and various derivatives. For example, ferulic acid is released from a bound form in maize seed and is antifeedant at a concentration of 0.05 mg g^{-1} to the maize weevil, *Sitophilus zeasmais*. Similarly, chlorogenic acid (**26**) is a feeding deterrent to the leaf beetle, *Lochmaea capreae cribrata*, feeding on the Salicaceae.[57] Chlorogenic acid also occurs in leaf trichomes of tomato and reduces growth of early instars of the cotton bollworm, *Helicoverpa zea*. Again, the chlorogenic acid analogue 1-caffeoyl-4-deoxyquinic acid (**27**), present in leaves of the wild groundnut *Arachis paraguaensis*, inhibits growth of the tobacco armyworm *Spodoptera litura*.[58]

The effectiveness of phenolics as a resistance factor to animal feeding is enhanced, as above, by oxidation to polymers, which reduce digestibility, palatability and nutritional value. This happens in the case of the Colorado beetle feeding on the potato. The potato is rich in chlorogenic acid, which can produce harmful polymers in the presence of oxidising enzymes. Thus high levels of polyphenol oxidase, the major phenolic oxidising enzyme of plants, can be correlated with high beetle resistance among various potato genotypes.[59]

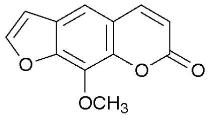
Turning to the ruminant–plant phenolic interface, it appears that the efficiency of phenolics in inhibiting digestion depends on whether they are present free or in combined form. This conclusion followed from a study of the effect of the simple phenols orcinol, quinol and arbutin present in heather on rumen microbial activity. In particular, arbutin is hydrolysed to quinol and glucose in the gastrointestinal tract through the activity of glucosidase. The negative digestive effects of quinol are counterbalanced by the positive effects of the liberated glucose.[60]

One group of masked phenolics, the furanocoumarins, have been implicated as defensive agents in the Umbelliferae, a family where they occur regularly. They are notable in being phototoxic, namely that their toxicity to animal life is enhanced in the presence of sunlight. Furanocoumarins usually occur in plants as mixtures of related structures. Berenbaum *et al.* have demonstrated that such mixtures act synergistically in the interaction between *Helicoverpa zea* and the fruits of the wild parsnip *Pastinaca sativa*. They are more protective against this herbivore than when a single structure xanthotoxin is applied at the same concentration as the furanocoumarin mixture.[61] Such experiments nicely explain why plants tend to synthesise and accumulate a suite of closely related toxins, rather than always rely on the production of a single secondary metabolite in quantity.

The toxicity of the furanocoumarin xanthotoxin (**28**) to insects is related to the relative rate of detoxification. The black swallowtail *Papilio polyxenes* tolerates it, because it can metabolise 95% of a dietary dose within 1.5 h to an open-chain compound. By contrast, the armyworm *Spodoptera frugiperda* is sensitive to its toxic effects because it detoxifies it much more slowly.[62] Xanthotoxin is a linear coumarin, whereas isopsoralen is an angular coumarin and even umbellifer specialist insects find it difficult to metabolise these structural analogues. Furanocoumarins are also toxic to mammals. The rock hyrax *Procaria capensis syriaca* dies within 20 h if fed with shoots of *Pituranthos triradiata*, which contain between 0.6 and 1.7% dry weight of furanocoumarin.

Table 7 Flavonoids as insect feeding deterrents

Flavonoid	Plant source	Insect feeding deterrence
Cyanidin 3-glucoside	*Gossypium* spp.	*Helicoverpa zea*
Rutin	*Glycine hispidum*	*Trichoplusia ni*
Vitexin	*Triticum sativum*	*Myzus persicae*
Phloridzin	*Malus domestica*	*Acyrthosiphon pisum*
(+)-Catechin	*Rosa* spp.	*Macrosiphum pisum*
Procyanidin	*Sorghum bicolor*	*Schizaphis graminum*
Schaftoside	*Oryza sativa*	*Nilaparvata lugens*



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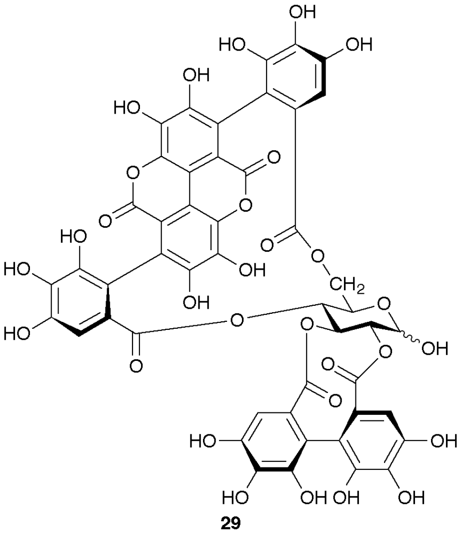
Most flavonoids are water-soluble, occurring in the vacuoles of leaves and flowers. Their defensive role seems to be limited, since for most phytophagous insects they are regular dietary components. Indeed, larvae of ~10% butterfly species sequester and store them in their tissues. Nevertheless, there are some Lepidopteran species sensitive to dietary flavonoids, notably *Helicoverpa zea* and *H. virescens*. Typically, cyanidin 3-glucoside, a common anthocyanin, added to the diet at a 0.07% concentration causes 50% inhibition of larval growth during 5 days of feeding. The reason for this is not clear, but it may be related to the inability of the insect to absorb its nutritional requirements from such a diet. Other examples of flavonoids acting as feeding deterrents are shown in Table 7. Nevertheless, there are an equal number of situations where water-soluble flavonoids act as feeding stimulants. One well-known example isoquercitrin, a quercetin glycoside which occurs in *Morus alba* leaves and stimulates the silkworm, *Bombyx mori*, to feed on that tissue.[63]



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There are, as well, lipophilic flavonoids in plants with a more restricted distribution and here there is only evidence of toxicity and feeding deterrence. The rotenoids which occur in the roots of legumes such as *Derris elliptica*, are well known to be insecticidal and toxic to fish. Again, the prenylated flavanones present in *Lonchocarpus* seed are toxic to the predating mouse, *Liomys salvini*. In captivity, this mouse prefers to starve rather than feed on the seeds of this plant.[64]

Much attention has been devoted to the plant tannins as feeding barriers, because of the very widespread occurrence of one class, the condensed tannins (or proanthocyanidins), in most woody plants. That they are biologically active is evident from their well-known ability to bind to proteins. There have been considerable difficulties in measuring their quantities in leaf and other plant tissue, but these have been overcome and several methods of accurate determination are now available.[65] The majority of experiments carried out since the late 1980s confirm the view that the condensed tannins at least are significant and powerful feeding barriers to both phytophagous insects and grazing animals. Some of the more important supporting evidence follows.

(i) The toxic effects of condensed tannins in unadapted animals are well established. Typically, weaning hamsters that are treated with a diet containing 4% dry weight of sorghum tannin suffer weight loss and then perish within 3–21 days.

(ii) Co-evolution adaptation in mammals to high tannin diets is well established. This involves the increased synthesis of a series of unique proline-rich proteins in the parotid glands. These salivary proteins have a high affinity for condensed tannins and remove them by binding them at an early stage in the digestive process. These PR-proteins are typically found in the saliva of mammalian herbivores (*e.g.* rats, snowshoe hares, deer, moose) but do not occur in the saliva of carnivores (*e.g.* dogs).[66]

(iii) Adapted animals may still avoid feeding on plants, when certain types of tannin are present. Thus, snowshoe hares in Alaska show a threefold preference for leaves of *Purshia tridentata* over those of *Coleogne ramossissima*. This difference in feeding behaviour is due to chemical variations in the procyanidins present. In *Coleogne*, the polymers are based on epicatechin units, whereas in *Purshia* they are based on both catechin and epicatechin units in a 1 : 1 ratio. The deterrent effect on feeding of the epicatechin-based tannins in *Coleogne* is probably not a direct one, but is related to the adverse effects of the flavans liberated in the gut following ingestion and depolymerisation of the procyanidin.[67]

(iv) In moths and butterflies, dietary tannins lower the growth rate, although it is only plants with more than average tannin concentrations which deter feeding. Experiments with *Aphis craccivora*, a pest of the groundnut *Arachis hypogaea*, show that it is deterred from feeding when the procyanidin content in the phloem of the petiole reaches more than 0.3% fresh weight. Aphids forced to feed on cultivars with a high tannin content show a twofold decline in reproductive rates. Interestingly, tannin production in the groundnut is channelled towards the phloem to provide aphid resistance, since other parts of the plant are essentially tannin free.[68]

(v) The response of primates to dietary tannin depends on whether they are adapted (Table 8). All the monkey species examined are sensitive to dietary tannins above 2–4% dry weight.[69] By contrast, both chimpanzee and gorilla are adapted and are able to survive on tannin-rich leaves and fruits.[70,71] However, the chimpanzee cannot cope with the high concentration of tannin in wild fig seed (25.65 mg g^{-1} compared to 9.94 mg g^{-1} in fruit pulp). On feeding, it discards the seed tissue as a "wadge" that is spat out during feeding.[70] Interestingly, chimpanzees do appear to tolerate higher levels of dietary tannin than human beings. Human diets are almost without exception low in condensed tannin.

Although the chemical ecology of condensed tannins has received considerable attention, that of the hydrolysable tannins has barely been considered. And yet an increasing number, over 600, of hydrolysable tannins have now been characterised in plants. One such example is β-punicalagin (**29**), which has been characterised along with terminalin in the leaves of the bushy tree *Terminalia oblongata*.[72] Eating the leaves of this plant by cattle and sheep produces yellow-wood poisoning, caused by liver damage. The two tannins are responsible for these toxic effects and the hydrolysable tannins in leaves of the oak and of

Table 8 Feeding preferences of primates

Primate	Feeding response
Chimpanzee	Tolerates fig fruits with medium tannin levels; fig seeds rich in tannin are discarded. Eats *Khaya* tree bark with 4% dry wt. tannin
Gorilla	Eats a wide range of leaves and fruits containing tannin. Not known to "wadge" fig seeds
Blue monkey Red-tail monkey Black and white Colobus monkey	Prefers fruits low in tannin. Leaf tissue mainly consumed at juvenile stage, when low in tannin and in alkaloid. Alkaloid levels up to 8 μg g(dry wt.)$^{-1}$ tolerated but tannin levels of 0.2% dry wt. rejected

Table 9 Terpenoids identified as barriers to herbivore feeding

Terpenoid	Occurrence	Effect on herbivore
Camphor	White spruce leaf	Antifeedant to snowshoe hare
Limonene	Bark of *Pinus ponderosa*	Feeding deterrent to pine bark beetle, *Dendroctonus brevicomis*
Pulegone and carvone	*Satureja douglasii* leaf	Feeding deterrent to slug, *Ariolimus dolichophallus*
Lactucin (**33**) and 8-deoxylactucin	*Cichorium intybus* leaf	Antifeedant to locust, *Schistocerca gregaria*
Caryophyllene epoxide	*Melampodium divaricatum* leaf	Arrests leaf-cutting ant, *Atta cephalotes*
Zingiberene	Leaf trichome of *Lycopersicon hirsutum*	Toxic to the Colorado beetle
Parthenin	*Parthenium hysterophorus* leaf	Toxic to flour beetle, *Tribolium confusum*
Germacrone	*Ledum groenlandicum* leaf	Grazing deterrent to snowshoe hare
Petasin (**31**) and furanopetasin (**32**)	*Petasites hybridus* leaf	Feeding deterrents to snails
Kaurenoic and trachylobanoic acids	Floret of sunflower, *Helianthus annuus*	Toxic to larvae of moth, *Homeosoma electellum*
Papyriferic acid	Paper birch, *Betula resinifolia*	Antifeedant to snowshoe hare

Thiloa glaucocarpa have similarly been identified as being toxic to farm animals.[73]

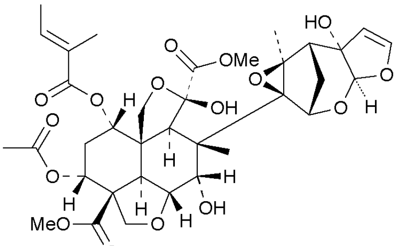
It has sometimes been assumed that all tannins act as feeding deterrent allelochemicals in a uniform manner. Mole *et al.*[74] have now punctured this hypothesis. Feeding trials with rats showed that differences were apparent in the way that tannins affected digestion and the post-digestive assimilation of nutrients into the body. In particular, while condensed tannins pass through the rat bound as PR-protein complexes, hydrolysable tannins appear to undergo hydrolysis during digestion.

Unlike farm animals, insects can adapt themselves to eating oak leaves and most oak trees have a well-developed larval fauna. Nevertheless, as Feeny[75] has shown, Lepidoptera feeding on oak leaves, *e.g.* the winter oak moth *Operopthera brumata*, stop feeding when the level of leaf tannin (a mixture of hydrolysable and condensed tannins) reaches a critical level of over 4% dry wt. content. It is interesting that one particular moth, *Nemoria arizonaria*, uses the presence of tannin in the leaves to determine which of two larval forms it will adopt. Thus, larvae born in the spring feed on the oak catkins, which are low in tannin, and are yellow in colour, mimicking the catkins in colour and form. Those born in the summer eat the leaves, which are rich in tannin and are green–grey, resembling in shape the twigs of the tree. The triggering role of the tannin was demonstrated in laboratory feeding experiments when 94% of larvae raised on catkins plus tannins developed into the twig form, while 94% of larvae raised on catkins alone took up the catkin form.[76]

3.2 Toxic terpenoids

Terpenoids, from volatile monoterpenoids to involatile triterpenoids, are broadly defensive against herbivory on plants. Not only have individual compounds been implicated as being toxic or antifeedant (Table 9), but also mixtures of related structures often synergise to produce a deterrent or toxic effect. Most groups of herbivores can be inhibited from feeding including insects, molluscs, birds, and especially geese, and many browsing and grazing animals. The defensive role of terpenoids has been reviewed in detail elsewhere.[77] Only the salient points will be mentioned here.

Monoterpenoids are generally toxic to unadapted insects. This has been demonstrated with locusts, which reject a range of monoterpenes which have been tested by applying them to artificial diets at 0.01% dry weight. Adapted insects may use monoterpene mixtures as feeding cues, but they can become susceptible to high concentrations or to non-host plant compounds. The pine bark beetle, *Dendroctonus brevicomis*, for example, is adapted to pine trees high in α- and β-pinene, myrcene, and 3-carene, but avoids feeding on trees which are high in limonene. What is true of monoterpenoids is true of the higher terpenoids (see Table 9). Sesquiterpene lactones, in particular, appear to discourage insects feeding on the plants where they are present. Several diterpenoids are antifeedants and the triterpenoid azadirachtin (**30**) is well known to be a potent insecticidal agent.



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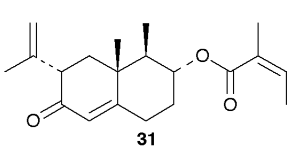
Slugs and snails have the reputation of being able to feed on a wide variety of plant species, irrespective of the chemical barriers that may be present. But some chemicals, in this case sesquiterpenes, stop them feeding. It has been shown that both petasin (**31**) and furanopetasin (**32**) which occur in *Petasites hybridus* repel snails feeding on this plant. Leaf extracts containing petasin proved to be deterrent, while leaf discs with low petasin content were preferred to leaf discs with higher petasin content. Furthermore, the range of petasin concentrations in the leaves of the plant (0.07–0.72% dry wt.) coincided with the level of deterrence (0.05%) of the pure compound. Furanopetasin (**32**) also played an important role in snail deterrence. Interestingly, the snails became more sensitive to dietary sesquiterpene with time. This could be the result of a rapid long-term associative learning process.[76]

The concentration of terpenoid in the plant is also critical for restricting the feeding of insects on plant leaves. Thus, in chicory, in feeding tests, 0.2% lactucin (**33**) was a deterrent to



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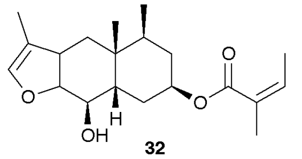

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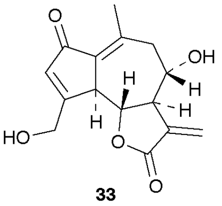
feeding, while upper leaves of the plant never had less than 0.45% dry weight of sesquiterpene lactone. Interestingly, the effectiveness of lactucin as a feeding deterrent was increased by the presence of a phenolic coumarin, cichoriin, in the same plant.[78]



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Seasonal variation or distribution within the plant can determine the effectiveness of terpenoid toxins. For example, juvenile leaves of the holly *Ilex opaca* contain 135 mg g^{-1} dry weight of saponins, which arrests feeding by the Southern red mite *Oligonychus ilicis.* Mature leaves have much lower levels (30 mg g^{-1} dry weight) but these are then protected by physical structures. In the leek, *Allium porrum*, saponins are concentrated in the flowers, driving the leek moth larva *Acrolepiopsis assectella* to feed exclusively on the more expendable leaves. The relative amounts of saponin are 0.03% dry weight in leaves and 0.2–0.4% dry weight in the flowers.

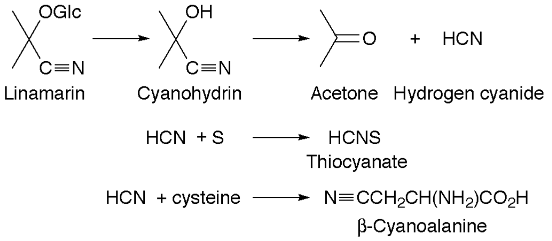
The leaves of most gymnosperms and of many angiosperm trees and shrubs are rich in monoterpenoid and sesquiterpenoid mixtures and there is increasing evidence that they are defensive against many mammalian feeders, including deer, hares, and voles. The concentration is a major factor in defence and this may vary seasonally, as happens in the shrub *Chrysothamnus nauseasus.* Leaves are not eaten in the summer, when the sesquiterpenes such as (*E*)-β-farnesene, β-humulene, and (γ)-muurolene reach a total concentration of 80 μg g^{-1} dry weight. This level drops to 18 μg g^{-1} dry weight in the winter, when the leaves are browsed by the mule deer, *Odocoileus nemionus.*[79]

Adaptation to a terpene-rich diet has been observed in possums and gliders feeding on *Eucalyptus* leaves. This occurs by efficient detoxification (as in ringtail possum *Pseudocheirus peregrinus*) or by avoiding the inhibitory effect of terpenes on the microorganisms of the rumen. Thus the greater glider *Petauroides volans* avoids the deleterious effect on the microbial population in its hindguts by absorbing the terpenes through the stomach and small intestine and detoxifying them *via* the liver.[80]

3.3 Nitrogen-based toxins

Many plant alkaloids are both bitter tasting and acutely toxic and therefore appear to be obvious barriers to animal feeding. Yet ironically, the defensive role of alkaloids has not been explored to the same extent as that of other nitrogen-containing plant toxins. Thus, more attention has been devoted by ecologists to cyanogenic glycosides, glucosinolates, and non-protein amino acids. These compounds will be considered here first before passing on to the alkaloids.

Cyanogenic glycosides have been likened to a two-edged sword in that on enzymic breakdown they yield two different classes of toxin, namely cyanide, which is a respiratory inhibitor, and an aldehyde or ketone, which is directly toxic. Typically, the bound glucoside linamarin is broken down in a two-stage process to yield cyanide and acetone (Scheme 1).

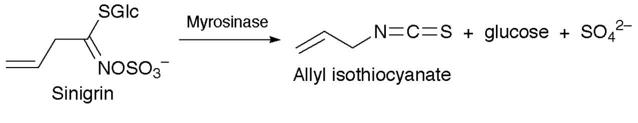


Scheme 1 Release of cyanide and ketone from the cyanogenic glucoside linamarin and detoxification of cyanide to thiocyanate or β-cyanoalanine.

The defensive role of linamarin, lotaustralin, and other commonly occurring cyanogens has been reviewed[81] and they have been shown to be effective variously in deterring feeding by molluscs, Lepidoptera, deer, sheep, rabbits, and voles. Detoxification of cyanide is possible, either by the enzyme rhodanese, which converts it into thiocyanate, or by the enzyme β-cyanoalanine synthase to produce β-cyanoalanine (Scheme 1). Unfortunately, both of these detoxification products can have harmful effects; thiocyanate, a metabolite of cassava cyanogen in humans, has goitrogenic effects; β-cyanoalanine, a metabolite of cyanogen in insects, is a neurotoxin.

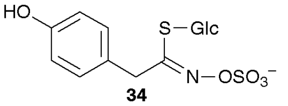
A special feature of cyanogenesis in many plants where it occurs is its variable nature. This is especially pronounced in both clover and birdsfoot trefoil, where populations can vary from having about 5% cyanogenic to those with 100% cyanogenic. Much argument has been spent over this feature, but most ecologists recognise the advantages to a plant of maintaining of a variable toxic defense in keeping the herbivore "guessing" about the palatibility of a particular plant and having to adapt *via* induced detoxification enzymes to sampling cyanogenic forms. One final point may be made about the protective value of cyanogenesis in young seedlings of clover. Measurements of cyanogenic content within the plant indicate larger amounts in the growing stem and cotyledons than in the leaves. Thus, garden slugs will restrict their feeding to the leaves, leaving the more vital organs to regenerate following grazing.[82]

Like cyanogenic glycosides, the glucosinolates or mustard oil glycosides (Scheme 2) are bound toxins which yield the free toxin, an isothiocyanate, following enzymic hydrolysis. Over 120 different glucosinolates have now been identified.[83] Their distribution in nature is limited to 16 plant families, but in many cases, as in the Cruciferae, they occur universally and in considerable abundance. Overall, the evidence is strong that glucosinolates and their products function in plant defence against generalised consumers, including mammals, birds, aphids, grasshoppers, beetles, flies, and mites. Even more telling is the fact that crucifer specialists are restricted in their ability to feed on their chosen food plants by the high concentration that are often present in young tissues. The toxicity of sinigrin, a common glucosinolate of crucifers (Scheme 2), to unadapted insects has been demonstrated by feeding it to larvae of the black swallowtail butterfly *Papilio polyxenes* by infiltrating a 0.1% solution into a normal food plant, namely celery. This was sufficient to cause 100% mortality to the larvae.[84]



Scheme 2 Release of acrid toxin, allyl isothiocyanate, by enzymic breakdown of the glucosinolate sinigrin.

The ecological strategy in defence of the mustard plant *Sinapis alba* is to protect the vulnerable young tissues with *p*-hydroxybenzylglucosinolate (sinalbin **34**).[34] The high concentrations in young cotyledons (20 mmol dm^{-3}) and young leaves (up to 10 mmol dm^{-3}) effectively deter feeding by both specialist insect, *e.g.* the flea beetle *Phyllotetra cruciferae*, and a generalist insect such as an armyworm. As the plant grows, the concentration drops so that older leaves have between 2 and 3 mmol dm^{-3} sinalbin. At these levels, there will be some stimulation for the flea beetle to feed, but the more generalist insect will still be deterred from feeding.[85] A similar strategy operates in the wild crucifer *Schouwia purpurea*, which is fed upon by the locust *Schistocerca gregaria* so that the locust is forced to feed on the dried senescing leaves rather than the fresh green leaves.[86]



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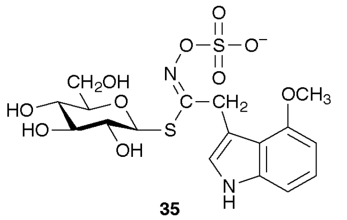
In some crucifers, glucosinolate levels may be increased by herbivory or mechanical damage. In such cases, the signal chemical methyl jasmonate is the trigger for increased glucosinolate synthesis. McConn *et al.*[87] have shown that the blocking of jasmonate synthesis in *Arabidopsis thaliana* is sufficient to convert a resistant plant into a susceptible one. Thus, mutants of *Arabidopsis* deficient in the synthesis of linolenic acid, the immediate precursor of jasmonate, are low in glucosinolate. They become infested by the gnat *Bradysia impatiens* and perish as a result.

The application of methyl jasmonate to crucifers can cause a switch in glucosinolate synthesis, to produce the more toxic indole-based glucosinolates, such as 4-methoxyglucobrassicin (**35**). Thus, treatment of oil seed rape, *Brassica napus*, with methyl jasmonate increases the concentration of glucosinolate 20-fold, with 90% of the toxins being indole-based.[88]



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The next group of nitrogen-containing toxins to be considered are the non-protein amino acids. They accumulate especially in the seeds of the Leguminosae but are also widely distributed in other plant families. These compounds are largely structural analogues of one or other of the protein amino acids and are antimetabolites in their mode of action. They are liable to be incorporated into protein synthesis, when imbibed dietetically, and this usually has disastrous consequences. A number of non-protein amino acids of legume seeds are notably neurotoxic and cause neurolathyrism in humans and domestic animals. Canavanine (**36**) and L-Dopa (**37**), both of which are regularly found in some quantity in seeds of tropical legume trees, have an ecological role in protecting these seeds from bruchid beetle attack. Two legume tree species may be growing adjacent to each other; the seeds of one, protected by L-Dopa at a concentration of 6–9% dry weight, will be free of bruchid infestation, whereas the seeds of the second species lacking a protective chemical are riddled with bruchid borings.[89]

$NH_2C{=}NH\cdot NHO(CH_2)_2CHNH_2CO_2H$

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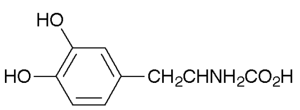

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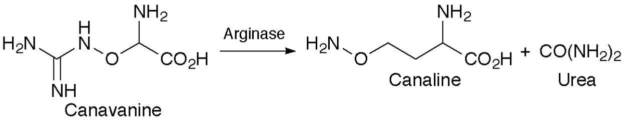
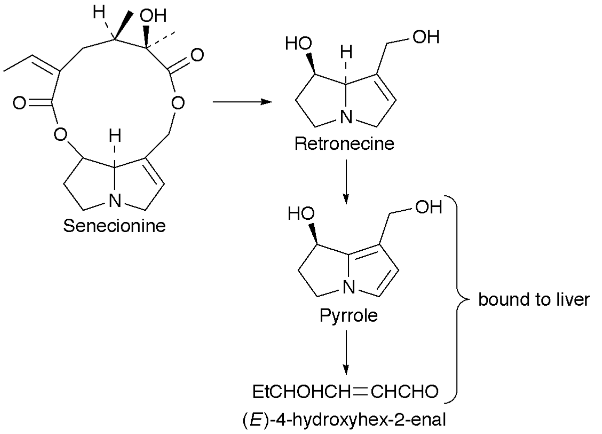
While the presence of non-protein amino acids may provide a general defence against insect predation on seeds, some individual species may co-evolve to overcome the barrier and survive the harmful effects of the toxin. This happens with larvae of the bruchid beetle *Caryedes brasiliensis*, which in Costa Rica feeds exclusively on seeds of *Dioclea megacarpa* that contain large amounts of canavanine. The beetle is able to detoxify the canavanine with the enzyme arginase, which produces canaline and urea (Scheme 3). The canaline is further broken down, providing more useful nitrogen, together with the urea, for the further growth of the larva. In spite of this success, it is still true that canavanine and other related structures are highly damaging dietary constituents to the majority of insects. Rosenthal[90] has shown, for example, that feeding larvae of the tobacco hornworm, *Manduca sexta*, with an agar-based diet containing 0.05% canavanine creates dramatic growth aberrations in the pupae and adults and renders them infertile.



Scheme 3 Detoxification of canavanine to canaline by a brucid beetle.

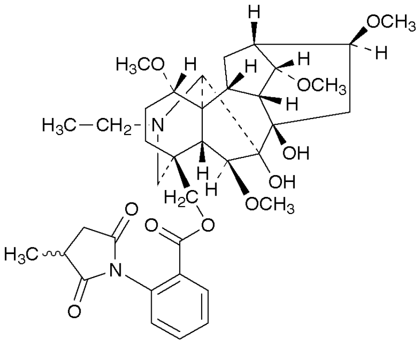
Considering that over 10 000 plant alkaloids are known and that these may be present in over 20% of angiosperm families, it is remarkable how little is known of their ecological significance. Most attention has been devoted to the accidental poisoning of domestic animals, especially cows, which graze on alkaloid-containing plants in the Leguminosae (*e.g. Lupinus* spp.) and in the Compositae (*e.g. Senecio* spp.). Wild animals such as deer and rabbits avoid feeding on them in general. The toxicity of the pyrrolizidine alkaloids (PAs) of the ragwort *Senecio jacobeae* and other *Senecio* plants is notorious and ~50% of all domestic cattle deaths worldwide are due to poisoning by these alkaloids. They are dangerous to life because they are metabolised *in vivo* to produce a more toxic agent, which has the ability to bind to macromolecules such as DNA in the liver. Typically, a *Senecio* alkaloid such as senecionine undergoes hydrolysis to retronecine and this undergoes dehydrogenation to the related pyrrole. This, and a further breakdown product, (*E*)-4-hydroxyhex-2-enal, are responsible for liver damage (Scheme 4). Hence PAs are known to be hepatotoxic.[91]



Scheme 4 Metabolism *in vivo* in mammals of pyrrolizidine alkaloids.

Many attempts have been made to establish a role in defence for the pyrrolizidine alkaloids synthesised in such profusion in the ragwort *Senecio jacobaea* and other *Senecio* species. Hartmann and Dierich[92] have provided convincing biochemical evidence for a protective role, by feeding ^{14}C-labelled senecionine *N*-oxide and ^{14}C putrescine to *Senecio* plants and following the fate of the labelled alkaloids with time. They established beyond dispute that the alkaloids were *not* turned over at all for periods of up to 29 days. Altogether, the lack of degradation suggested that the alkaloids provide 'a powerful strategy to successfully defend the plant from herbivory'. The alkaloids are being constantly synthesised in the roots, there is long distance transportation into the shoots, a continuing allocation of alkaloid to plant organs and a highly efficient use of alkaloid concentration to defend vulnerable tissues throughout the life cycle.

Another group of toxic alkaloids are the diterpenoid-based alkaloids, methyllycaconitine (**38**) and 19-deacetylnudicauline (**39**), which occur in tall larkspur (*Delphinium*) species growing in the Western ranges of the USA. The toxic alkaloids co-occur with related structures, which are not toxic. While the total alkaloid content ranges from 9.3 to 38.8 mg g^{-1} dry weight, the amounts of **38** and **39** vary between 4.0 to 7.1 mg g^{-1} dry weight.



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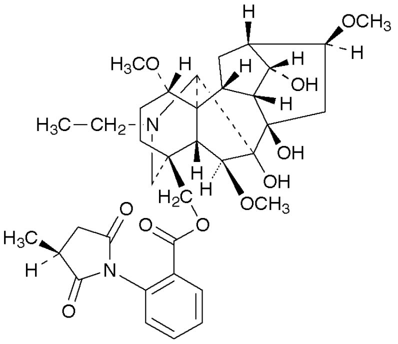

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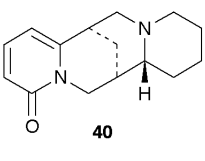
Attempts to directly relate cattle and sheep feeding on larkspur to alkaloid content failed.[93] And then it was realised that cattle in particular regulate their feeding on these plants by never taking in more than safe levels of tissue. Grazing studies showed that larkspur consumption above 25–30% of dietary requirements for 1–2 days leads inevitably to reduced larkspur consumption on subsequent days. This allows the cattle to detoxify the alkaloid before taking in further plant material. At the same time, the plants gain a respite from grazing to allow continued growth.[94]

A third group of toxic alkaloids are the quinolizidine alkaloids (QAs), *e.g.* anagyrine (**40**) and cytisine (**13**), of lupin plants. They are directly toxic to sheep and have also been implicated as teratogenic agents. Their protective role in plants has been demonstrated by offering rabbits and hares the choice of feeding on low-QA "sweet" lupins or high-QA "bitter" lupins, with the latter being largely avoided in favour of the sweet-tasting plants.[95]



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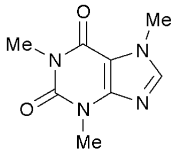
There is some evidence that, in certain plants, alkaloids are formed in high concentration to protect juvenile tissues and that these concentrations drop dramatically as the tissues mature and physical structure takes over to defend them from herbivore feeding. The pattern of accumulation of caffeine (**41**) and theobromine (**42**) in the coffee plant *Coffea arabica* is closely correlated with such a defence strategy. During leaf development, the alkaloid content reaches 4% dry weight, whereas in the maturing leaf the rate of biosynthesis decreases exponentially from 17 mg d^{-1} to 0.016 mg d^{-1} per gram of leaf.[96] Soft young fruit (bean) tissue is similarly protected, and there is a drop in alkaloid content as the coffee bean ripens.[96]



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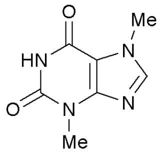

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A similar study of ergot alkaloid synthesis in *Ipomoea parasitica* showed a high concentration in young seedlings and also during flowering, but low amounts of alkaloid in between. Feeding experiments with the larvae of the moth *Heliothis virescens* showed that these ergot alkaloids, such as lysergol, were a deterrent to feeding and also reduced fertility. Other classes of alkaloid, *e.g.* harman, lupin, and indole, have been shown to be antifeedant or harmful to phytophagous insects, so that there is circumstantial evidence for a useful defence role for these organic bases.[97] Nevertheless, there is still much to be done to be sure that the many alkaloids reported up to now in plants generally have a useful defensive role.

3.4 Other aspects of plant defence

Many other plant compounds, which do not fall into the three main categories listed above, are also potentially defensive against herbivory. Fluoroacetate and oxalate ions must at least be mentioned because of their considerable toxicity. Fluoroacetate, CH_2FCO_2, occurs in *Dichapetalum cymosum* (Dichapetalaceae), in species of *Gastrolobium* and *Oxylobium* (Leguminosae), and in a variety of other sources. It is highly toxic to mammals, since it is incorporated into the Krebs cycle and then blocks it at the fluorocitrate stage. Cattle-poisoning is well known in South Africa and Australia after animals have grazed on these plants. Interestingly, some native fauna in Australia have co-evolved with these poisonous plants and can feed on them without harm. This is true of the grey kangaroo, *Macropus fuliginosus*, although it is still not entirely clear how it is able to cope with the fluoracetate poison.[98] The potential threat of the organic acid anion oxalate depends on which cation it is associated with in the plant. Thus plants with calcium oxalate, which is insoluble, are relatively safe to eat, whereas plants with the soluble potassium oxalate (*e.g. Setaria sphacelata*) may be toxic.[99]

If secondary compounds do have a protective function against herbivory, they are most likely to be located where they are most readily perceived by animals, namely at the leaf surface. Hence, a first line of defence, especially against insect feeders, are secondary metabolites localised in glandular hairs or trichomes principally on the upper surface. Some examples of phenolics and terpenoids that are so located have been given in earlier sections. Further examples are quoted in a book edited by Juniper and Southwood.[100] A second line of defence in some plants is provided by the leaf wax, which itself may be a barrier to feeding. Additionally ~50% of angiosperm species contain "extra" lipophilic secondary constituents mixed in the wax. It is likely that many of these constituents are repellent to insects. There is certainly evidence in the case of some varieties

Table 10 Plant-derived oviposition stimulants of insects

Oviposition stimulants	Plant source	Female adult insect
Methyleugenol, asarone, xanthotoxin, falcarindiol	*Daucus carota*	Carrot fly, *Psila rosa*
Luteolin 7-malonylglucoside (**43**) and chlorogenic acid	*D. carota*	Black swallowtail, *Papilio polyxenes*
Tyramine, chlorogenic acid, *etc.*	*Pastinaca sativa*	
Vicenin-2, narirutin, rutin (**45**) adenosine and bufotenine	*Citrus unshui*	Swallowtail, *Papilio xuthus*
Sterols, steryl ferulate	*Oryza sativa*	Rice grain weevil, *Sitophilus zeamais*
Sinigrin, glucobrassicin	*Brassica oleracea*	Cabbage white, *Pieris brassicae*
Allyl isothiocyanate	*B. oleracea*	Diamond back moth, *Plutella maculipennis*
Aristolochic acids and sequoyitol	*Aristolochia debilis*	Piperine swallowtail, *Atrophaneura alcinane*
Aucubin and catalpol	*Plantago lanceolata*	Buckeye butterfly, *Junonia coenia*
Rutin and other flavonol glycosides	*Asclepias curassavica*	Monarch, *Danaus plexippus*
Cardenolides	*A. humistrata*	Monarch, *Danaus plexippus*
Moracin C	*Morus alba*	Mulberry pyralid
(+)-Isocotylocrebine, 7-demethyltylophaorine	*Tylophora tanakae*	Danaid butterfly, *Ideopsis similar*
$2'$-Acetylsalicin	*Salix pentandra*	Shoot gall sawfly, *Enura amarinae*
Isorhamnetin 3-(glucosyl-1→6 galactoside)-7-glucoside	*Heterotropa aspera*	Swallowtail, *Luchdorfia japonica*

of *Sorghum* that the leaf wax alkanes themselves are distasteful to *Locusta migratoria*.

A third line of defence in plants from insect grazing is latex production. Latex has been reported in over 12 000 plant species and one of its main functions appears to be to protect those plants which contain it from herbivory. The effectiveness of latex, a viscous liquid consisting of a suspension of rubber particles, as a feeding deterrent is often reinforced by the presence of terpenoid toxins (*e.g.* sesquiterpenes or diterpenes) or of alkaloids. Experimental evidence for a defensive role has been mainly confined to studies with ants. However, Dussourd and Eisner[101] have established that many mandibular insects, in order to feed on latex-bearing plants, have to overcome the latex defence by vein-cutting behaviour. Thus, larvae of the Monarch butterfly, *Danaus plexippus*, feeding on milkweed plants cut the leaf veins before feeding distal to the cuts. Vein cutting blocks the flow of latex to the feeding sites and represents a counter-adaptation by the insect to the plant's defence. Unadapted insects such as the armyworm, *Spodoptera eridania*, have not learned this behaviour and are repelled from feeding by latex droplets.

Other barriers to both insect feeding and mammalian grazing involve the physical make-up of plant tissues and especially the extent of lignified cell walls in the leaves. Monkeys, for example, tend to concentrate their feeding on young flush leaves of trees to avoid the toughness and rigidity of the mature tissues.

Finally, it is important to point out that the effectiveness of secondary metabolites as defence agents may be strengthened by the presence of inorganic compounds. Many grasses contain crystalline occlusions, known as raphides, composed of calcium carbonate, and these probably restrict mammalian grazing on such plants. Calcium chloride may also contribute to plant resistance. Harada *et al.*[102] reported that calcium chloride interacts with the leaf diacylglycerols in providing the resistance in *Nicotiana benthamiana* to aphid feeding. Resistant forms have 10–100 times more calcium chloride in the leaf than susceptible cultivars.

Some plants, growing on particular soils, have the ability to accumulate toxic metal ions in their tissues, usually by chelating them with either organic acids (citrate, oxalate) or with small peptides, called phytochelatins. Such plants are therefore toxic to grazing animals and hence will be protected from herbivory. This has been demonstrated in the case of the nickel accumulator *Thlaspi montanum*, which may contain up to 3000 ppm nickel in its tissues. Several Lepidopteran and grasshopper larvae, when fed on these leaves or on an artificial diet containing nickel, showed acute toxicity to nickel at 1000 ppm.[103]

Protection from herbivory by metal accumulators, however, does not extend to all insect feeders. Thus, aphids, which are phloem-feeders, are not affected by feeding on *Streptanthus polygaloides* plants when they accumulate nickel ion. Plants with elevated levels (up to 88-fold over low nickel plants) have no detectable negative effect on the pea aphid *Acyrthrosiphon pisum* when it feeds.[104] Protection from herbivory is not restricted to nickel-accumulators and both copper and zinc accumulators show toxic effects. The copper-accumulating *Silene vulgaris* is resistant to feeding by the moth *Hedena cucubalis*, while the zinc-accumulating *Thlaspi caerulescens* repels feeding by larvae of the cabbage white butterfly, *Pieris brassicae.*[105]

4 Plant compounds involved in insect oviposition

4.1 Oviposition stimulants

Plant chemicals play an important role, together with visual cues, in attracting phytophagous insects to their chosen host plants for both feeding and oviposition. In fact, in most cases, the association depends on the female adult butterfly or moth locating the right plant species, laying her eggs on the leaves, and for the eggs to hatch out and the larvae to feed. We know now in a reasonable number of examples that particular secondary constituents characteristic of these host plants are the major attractants to such oviposition (Table 10). What is unexpected is that the chemicals are largely involatile components on the surface or within the leaf and are not volatile in nature. Recognition therefore depends on direct contact with the chemistry of the leaf.

Much is known about the choice of plant species for oviposition by the female Monarch butterfly. An important feature is the concentration of cardiac glycoside, since this determines the survival of the subsequent generation. Plants containing 200–500 μg g^{-1} wet weight are preferred. Those with lower levels are avoided, since the larvae subsequently would not be able to absorb enough cardenolide to be properly defended. Equally, higher levels of cardenolide are disadvantageous since larvae are liable to suffer physiological strain trying to absorb excessive amounts of toxin. Flavonol glycosides also seem to be important oviposition attractants in the case of *Asclepias curassavica* and a mixture of four such glycosides have been characterised as oviposition stimulants to the Monarch. A survey of other host plants of the Monarch has revealed that three main classes of quercetin glycoside act as oviposition stimulants: (1) glycosides based on galactose, glucose and rhamnose; (2) glycosides based on galactose, glucose and xylose; and (3) glycosides based on all four of the above sugars.[106]

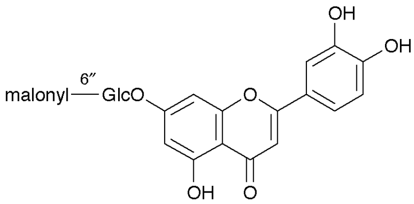
Ovipositing females of the Monarch clearly distinguish by odour between young and old leaves of *A. curassavica*, always laying on young leaves. Headspace analysis has failed to reveal any one volatile as a cue for the young leaves. However, there are quantitative differences, which may be operative. Thus,

Table 11 Plant-derived oviposition deterrents in butterflies

Oviposition deterrent	Plant source	Butterfly
Two strophanthidin glycosides	Wallflower, *Cheiranthus* × *allianii*	*Pieris brassicae*, *P. rapae*
Four cardiac glycosides	*Erysinum cheiranthoides*	*Pieris rapae*
Quercetin 3-(2^G-xylosylrutinoside) (**44**)	*Orixa japonica*	*Papilio xuthus*
8-Prenyldihydrokaempferol 7-glucoside	*Phellodendron amurense*	*Papilio xuthus*, *P. protenor*

trans-α-farnesene and linalool accumulate in young leaf odour, whereas α-thujene and methyl salicylate are dominant in the odour of older leaves and may be the reason for the rejection of old leaves.[107]

Chemical specificity of the leaf attractants has been established in the use of black swallowtail butterfly ovipositing on carrot leaves. A mixture of luteolin 7-(6″-malonylglucoside) (**43**) and chlorogenic acid is most effective. The related luteolin 7-glucoside is also a component of the carrot leaf, but it is quite inactive. Each species visited by the black swallowtail would seem to have its characteristic chemical signature. Thus, the stimulants in leaves of the wild parsnip, *Pastinaca sativa*, which induce oviposition are chlorogenic acid, tyramine and several as yet unidentified components.[108] Whereas this swallowtail depends on two phenolic constituents for recognising the carrot leaf, the carrot fly relies on a mixture of phenylpropenes, furanocoumarins, and a polyalkyne in the leaf wax (Table 10) to find its food plant.[109]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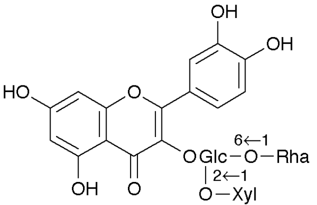
It is apparent in some cases that mixtures of unrelated structures may synergise to provide oviposition stimulants (Table 10). This is particularly apparent in *Papilio xuthus* and *P. protenor*, two related swallowtails living on rutaceous plants, where mixtures of flavanone glycosides, glycosylflavones, and organic bases provide the attractive cocktail for egg laying.

4.2 Oviposition deterrents

Less can be said about the chemical factors which guide ovipositing female Lepidoptera away from unsuitable host plants, since only a few deterrents have so far been identified (Table 11). The role of cardiac glycosides in *Cheiranthus* and *Erysimum* in discouraging egg laying on these two crucifer plants is fairly clear. These compounds are toxic to the larvae and would be fatal as soon as the eggs hatched out.[110] These plants are avoided by the females in spite of the fact that they both contain glucosinolates, which under other circumstances would be attractive (see Table 10).

Oviposition deterrence in the swallowtail, *Papilio xuthus*, can be produced by a small change in the structure of the flavonol glycoside present in the leaves of potential host plants. Thus the insect is stimulated to oviposit on *Citrus* plants by the presence of quercetin 3-rutinoside (rutin) among others. It is, however, deterred from oviposition on the non-host plant *Orixa japonica* because the leaves contain quercetin 3-(2^G-xylosylrutinoside) (**44**). Thus, the simple addition of the extra sugar xylose apparently turns rutin (**45**) from an attractant into a repellent.[111]

Oviposition deterrence in beetles is induced by the acylsugars occurring in leaf trichomes of the wild tomato *Lycopersicon pennellii.* These chemicals have been shown to reduce oviposition on this plant by the homopteran *Beneisia argentifolii.*[112]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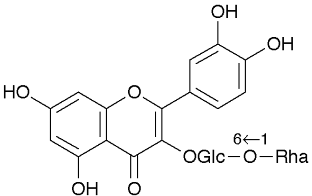
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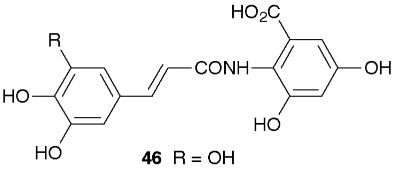
Oviposition deterrence on plant leaves can be caused by a female insect depositing on its eggs a special oviposition-deterring pheromone. This turns away a second female from laying her eggs on the same leaf or the same plant.[113] Such a pheromone has been characterised in the cabbage white butterfly, *Pieris brassicae*. Three active substances are present: miriamide (**46**), miriamide 5-glucoside (**47**) and 5-deoxymiriamide (**48**). These three caffeic acid derivatives are unique to this particular butterfly.


46 R = OH
47 R = OGlc
48 R = H

5 Floral volatiles and pollination

The floral volatiles play an important role in attracting pollinators to the plant. They may attract a pollinating bee or wasp from a distance of several metres. Fruity or aminoid odours are attractive to beetles, sweet smells to bees, moths, and butterflies, musty or fruity odours to bats, and fecal odours to dung-flies. Research using headspace analysis has indicated the major floral volatiles in a representative sample of flowering plants (Table 12).

The chemistry of aroid odours has been somewhat controversial in that simple amines such as hexylamine were earlier reported from *Arum maculatum* and several related species. A reinvestigation of *A. maculatum* failed to indicate any amines in the headspace. Instead, indole, *p*-cresol, germacrene B (**49**), and heptan-2-one were detected as major constituents. The plant is pollinated by females of the owl midge, *Psychoda phalaenoides*, which otherwise feeds on cow dung. Both indole and *p*-cresol were detected in the headspace of the dung, so these two compounds appear to be the most important attractants.[114] Incidentally, indole and skatole are the major "distasteful" odours of another aroid plant, the voodoo lily *Sauromatum guttatum.* Odours unpleasant to the human nose are also dominant in bat-pollinated flowers, and a series of methyl sulfides (Table 12) have been identified in *Crescentia cujeta* and several other bat-pollinated plants.[115]

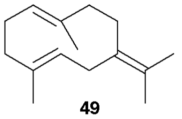

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Table 12 Floral volatiles of bat-, bee-, beetle-, butterfly-, moth-, and fly-pollinated plants

Floral volatiles[a]	Plant species	Pollinator
Dimethyl trisulfide (24.3%), dimethyl disulfide, dimethyl tetrasulfide, *etc.*	*Crescentia cujeta*	Bat
Squalene (26.5%) nerol, geraniol, hydrocarbons	*Dactylanthus taylorii*	Bat
Geraniol, citral, farnesol, *etc.*	*Ophrys* spp.	*Andrena* male bee
Carvone oxide	*Catasetum maculatum*	*Eulaema* male bee
Linalool (95%) + its oxides	*Daphne mezereum*	*Colletes* bee
Indole, 1,2,4-trimethoxybenzene, cinnamaldehyde	*Cucurbita* spp.	Diabroticite beetle
Methyl anthranilate and isoeugenol	*Cimifuga simplex*	Butterfly
Methyl benzoate (25%), linalool (50%), geraniol (12%)	*Platanthera chlorantha*	Moth
Ethyl acetate, monoterpenes, and aliphatics	*Zygogymum* spp.	Moth
trans-Ocimene (**50**) (46%), 1,8-cineole (12%)[b]	*Brugmansia* × *candida*	Hawkmoth
Heptan-2-one (16%), indole (16%), germacrene B (**49**) (18%), *p*-cresol (3%)	*Arum maculatum*	Dung-fly

[a] Only major components are listed: values in parentheses are average percentages of total floral odour. [b] Tropane alkaloids, thought to be present, could not be detected.

Many species with carrion smells produce mixtures of dimethyl oligosulfides. Kite and Hetterschieid[116] analysed by headspace techniques the inflorescence odours of 18 *Amorphophallus* and two *Pseudodracontium* species. Fourteen of these species had nauseating odours based on dimethyl disulfide and dimethyl trisulfide. *A. brachyphyllus* with an anise-like odour contained trimethylamine, while *A. elatus* with a cheese smell produced isocaproic acid.

A parallel investigation of 11 bat-pollinated species by Bestmann *et al.*[117] showed that six of the 11 were sulfide producers. Nine sulfur-based volatiles were variously detected. Not all bat-pollinated species, however, necessarily produce such vile-smelling compounds and the remaining five species surveyed contained more expectable aliphatic, monoterpenoid and sesquiterpenoid volatiles.

One other fetid-smelling plant species to be investigated is *Senecio articulatus* (Compositae), which is fly-pollinated. The flower of this plant produces 3-methylbutanoic acid, with minor amounts of linalool and its oxides.[118]

Turning to plants with fragrant odours, it may be mentioned that pleasant-smelling species can be found in families such as the Araceae, where nauseous odours dominate. Investigation of five *Anthurium* species showed none with sulfides present. In fact, all of them yielded terpenoids such as α- and β-pinenes, limonene, 1,8-cineole and linalool and the odours were characterised as floral, minty, pine and sweet.[119]

In sweet-smelling plants, individual constituents may dominate (*e.g.* linalool in *Daphne mezereum*), but more usually there are several components, which act synergistically to attract the pollinator (*e.g.* as in the moth-pollinated *Platanthera*) (Table 12). The floral scent is usually released at the right time of day for the particular pollinator, *e.g.* during the day for bee-pollinated flowers. For moth-pollinated species, it may be at dusk or even later in the night. Thus, ocimene (**50**) is released from flowers of *Mirabilis jalara* at night between 6.00 p.m. and 8.00 p.m. Different parts of the flower may have slightly different odours. This is true in *Rosa rugosa* and *R. canina*, where bees can select out pollen for collection from the rest of the flower. The compound geranylacetone (**51**), for example, is specific to the pollen and is not found in the floral odour.[120]


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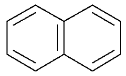
In the distinctive pollination of orchid flowers of the genus *Ophrys* by male bees of the genus *Andrena*, a large number of scent constituents are involved. Pseudocopulation of the flower by the male bee depends on the flower having the same shape, same scent, and same colour as the female bee. In *Ophrys lutea*, for example, octan-1-ol, decyl acetate, and linalool are common to the floral volatiles and to the pheromonal odour of the female bee. Studies on the *Ophrys–Andrena* volatiles were described by Borg-Karlson *et al.*[121]

The pleasant floral volatiles of *Ligustrum japonicum* were investigated to see which of the odour molecules were particularly attractive to the foraging adult small white butterfly, *Pieris rapae*. Five of the 30 volatiles were implicated: phenylacetaldehyde, 2-phenylethanol, 6-methylhept-5-en-2-one, benzaldehyde and methyl phenylacetate. These substances acted synergistically to attract the insect to feed on the nectar.[122]

A survey of floral fragrances in nine species of *Narcissus* native to southern Spain divided them into two groups. One group of species pollinated by butterflies and moths have fragrances typical of moth-pollination, *i.e.* indole and aromatic esters. The second group pollinated by bees and flies have monoterpenoids but lack the components of moth-pollination. One species, *Narcissus assoanus*, is unusual in having both fragrance chemotypes and is pollinated by both moths and solitary bees.[123]

A strange discovery is the presence of the moth-repellent naphthalene (**52**) in *Magnolia* flowers.[124] It occurs in petals, gynoecia and leaves of five out of nine species surveyed. Its role in beetle pollination is not clear. Is it an attractant or does it stop beetles from chewing the petals? Its function deserves further investigation. Otherwise, *Magnolia* flowers contain more expectable pleasant-smelling volatiles; a range of terpenoids, benzenoids and fatty acid esters have been characterised variously in flowers of Magnoliaceae.[125]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6 Nectar and pollen constituents

Nectar is an important source of food for most animal pollinators. Nectar chemistry does vary within certain limits and it is possible to suggest that many plant species modify the nectar components, through natural selection, to suit the needs of particular pollinators. The major components of nectars are simple sugars in solution, the sugar content varying from 15 to 75% by weight. The three common sugars are glucose, fructose, and sucrose, but traces of various oligosaccharides (*e.g.* raffinose) are sometimes present. There are distinct quantitative differences in the proportions of the three common sugars and angiosperm species can be divided into three groups, according to whether sucrose is dominant, glucose and fructose are dominant, or all three sugars occur in equal amounts. There is thus an evolutionary trend from nectar that is mainly sucrose, to nectar that is mainly glucose and fructose. Such a trend would correspond to some extent to the sugar preferences of the particular pollinators which vary from butterflies and bees to flies and bats.

Table 13 Toxins of plant nectars

Class	Compound	Plant nectar
Alkaloid	Hyoscyamine	*Atropa belladonna*
Phenolic	Arbutin	*Arbutus unedo*
Alkaloid	Hyoscyamine	*Brugmansia aurea*
Alkaloid	Pyrrolizidines	*Eupatorium* spp.
Alkaloid	Quinolizidines	*Lupinus polyphyllus*
Alkaloid	Nicotine	*Nicotiana tabacum*
Iridoids	Catalpol	*Catalpa speciosa*
Diterpenoid	Acetylandromedol[a]	*Rhododendron ponticum*
Alkaloid	Pyrrolizidines[a]	*Senecio jacobaea* and other spp.
Alkaloid	Quinolizidines	*Sophora microphylla*
Sugar	Mannose[b]	*Tilia cordata*

[a] These toxins are carried through from nectar to the honey stored by bees in their hives. [b] Toxic to bees, since they are unable to metabolise it.

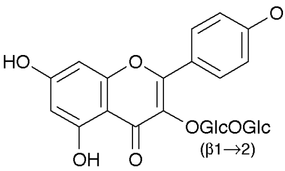
Lipid is an alternative source of energy to sugar, and lipid bodies replace nectar sugar in some 49 genera of the Scrophulariaceae, Iridaceae, Krameriaceae, Malpighiaceae, and Orchidaceae. These are all bee-pollinated and the oil is mainly used by the bees for feeding their young. These lipids appear to be chemically distinct from the triglyceride seed oils. Indeed, in species of *Krameria*, free fatty acids have been characterised. These are all saturated acids with chain lengths C_{16} and C_{22} and all have an acetate substituent in the β-position.[126]

Small amounts of protein amino acids are also present in nearly all nectars. The ten amino acids essential for insect nutrition are often present and there is no doubt that nectars are a useful source of nitrogen, especially to insects such as butterflies, which have few other ways of acquiring amino acids at the adult stage. It is much less important for bird pollinators and there are indications that amino acid concentrations are related to the needs of the different pollinating vectors.[127]

Plant nectars may contain toxins, which are presumably derived from their synthesis in other plant parts. Alkaloids have been most frequently detected, but several other classes have also been noted (Table 13). The alkaloid content may vary from the traces (0.106 μg g^{-1} fresh weight) in the tobacco plant nectar to as much as 273 μg g^{-1} fresh weight of tropane alkaloids in the deadly nightshade, *Atropa belladonna*.[128] The purpose of toxin accumulation in nectars is still uncertain, although a defensive role aganst herbivores or an undesirable animal visitor is certainly possible. The formation of iridoids in the nectar of the plant *Catalpa speciosa* is apparently to protect the plant from ants, which are nectar thieves.[129]

Occasionally, the toxins in the nectar may be collected during the process of pollination by certain butterflies. This is true of adult Ithomiines and Danaids, which have a requirement for pyrrolizidine alkaloids both for defence and for pheromone production. These alkaloids are obtained from nectar of *Eupatorium* and *Senecio* species, which are grown in their respective habitats (see Section 2).

Pollen, like nectar, is largely nutritional and is collected and eaten by bees and beetles. Carotenoids are present in many pollens, providing yellow colour, and function in improving pollen detection by the pollinator. All pollens also contain small amounts of flavonol glycosides, particularly such compounds as kaempferol 3-sophoroside (**53**) and isorhamnetin 3-sophoroside (**54**). Until recently, the occurrence of these flavonol glycosides was obscure. However, there is now evidence in the *Petunia* flower that the pollen flavonol **53** has an essential role in assisting the germination of the pollen when it lands on the stigma. During the process, a specific β-glycosidase removes the protecting sugars to release the free aglycone. The kaempferol formed is probably a growth promoter and at the same time prevents the introduction of pathogens into the pistil.[130]



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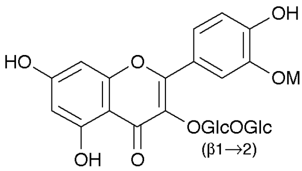

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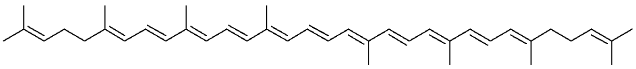
7 Fruit chemistry and seed dispersal by animals

The ripe fruit is the one part of the plant which is likely to be undefended chemically, since it is provided for animals in return for the widespread dispersal of the seed that lies within the fruit. By contrast, the seed and the seedcoat usually possess some chemical toxins, although they are often also well protected by physical structures. This is to ensure that the seed is not consumed along with the fruit.

The unripe fruit will, however, differ from the ripe fruit in being protected to some degree from herbivory, since the seed within is not yet ready for distribution. The green chlorophylls of the leaf may, for example, camouflage the unripe fruit so that it is not seen by a herbivore. There may be alkaloids present, as in green tomato fruits, which discourage animal feeding. Other chemical traits, such as acidity, bitterness, or astringency, may deter the majority of herbivores. Chemical changes during ripening will reduce or eliminate these barriers. Attractive colours, odours, and flavours will develop during ripening and advertise the readiness of the fruit for eating.

Although our knowledge of the chemistry of cultivated fruits is considerable, we know much less about that of wild species. The chemical ecology of fruits and their seeds has not been as intensively investigated as that of plant leaves, so that it is sometimes necessary to extrapolate from what we know of the cultivated species. Here, it is intended to review briefly the chemical attractants of fruits—the colours, the odour principles, and the flavours—and then to consider the chemical defence of plant seeds.

The ripe fruit is usually exposed to herbivores by its attractive and distinctive colour, which may be provided largely by carotenoids and anthocyanins. Green fruits will contain chlorophyll, but most orange and red fruits are coloured by a range of carotenoids. β-Carotene, which is yellow, is often abundant in yellow-coloured fruits and the red lycopene (**55**) is a common pigment of red fruits. Red to purple black fruits generally have anthocyanins present. The red range of colours, which overlaps with that of carotenoids, is usually due to the presence of cyanidin-derived structures, while most blue to purple–black fruits are based on delphinidin. Much information is available on fruit pigments of cultivated plants[131] but less is known about fruit colour in wild plants.



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Some examples of fruits eaten by birds and their pigments are given in Table 14. Red and black colours are much preferred by birds, with blue coming a poor third (5–7% of wild fruits). Most blue fruits are coloured by anthocyanins, although some additional factor (co-pigmentation or metal for chelation) must be present to shift the usual purple anthocyanin towards the blue region. The presence of anthocyanins in blue fruited species has been confirmed by a survey of 26 species in 18 genera in Costa Rica, India, Florida, and Malaysia.[132] The absence of anthocyanin from the blue fruits of *Elaeocarpus angustifolius* is striking. Microscopic investigation showed that colour is produced by the presence of an iridosome structure beneath the outer cell wall of the adaxial epidermis. This unique iridescent blue appears to be of selective advantage in

Table 14 Pigments of some fruits eaten by frugivorous birds

Colour class	Plant species	Pigments
Red	*Empetrum rubrum*	Anthocyanins; cyanidin 3-glycosides
	Rosa canina	Carotenoids, *e.g.* lycopene (**55**)
	Solanum dulcamara	Carotenoids, *e.g.* lycopene (**55**)
	Taxus baccata	Carotenoids, *e.g.* lycopene (**55**)
Blue	*Elaeocarpus angustifolius*	None (structural colour)
	Vaccinium spp.	Anthocyanins; delphinidin glycosides
Black	*Atropa belladonna*	Anthocyanin; petunidin triglycoside
	Empetrum nigrum	Anthocyanins; delphinidin 3-glyocosides
	Vitis vinifera	Anthocyanins; malvidin 3-glucoside

Table 15 Chemical principles of fruit odours

Fruit	Components identified as aroma principles[a]
Almond	Benzaldehyde
Apple	Ethyl 2-methylbutanoate
Banana	Amyl acetate, amyl propionate, and eugenol
Coconut	γ-Nonalactone
Cucumber	$CH_3CH_2CH{=}CHCH_2CH_2CH{=}CHCHO$
Ginkgo	Butanoic and hexanoic acid
Grapefruit	(+)-Nootkatone, 1-*p*-menthene-8-thiol
Lemon	Citral
Mandarin orange	Methyl *N*-methylanthranilate and thymol
Mango	Car-3-ene, dimethylstyrene
Quince	Ethyl 2-methylbutanoate
Passion fruit	Methyl salicylate, eugenol, and isoeugenol
Peach	γ-Undecalactone
Pear	Ethyl *trans*-2,*cis*-4-decadienoate
Pepper	2-Isobutyl-3-methoxypyrazine
Pineapple	Furaneol and mesifurane
Raspberry	1-(*p*-Hydroxyphenyl)butan-3-one
Vanilla	Vanillin

[a] Many fruits have several other minor aroma principles in addition to those listed. For further details, see ref. 134.

ensuring that the fruit is readily apparent to the cassowary bird, *Casuarius casuarius*, and to fruit-eating pigeons. The brilliant blue colour persists even when the mesocarp is almost completely senescent or has been consumed by beetles. This plant species produces a structural colour in the fruit which is presumably superior to and more stable than the usual anthocyanin pigmentation.[133]

Many fruits have attractive odours, which are pleasant to humans (Table 15).[134] This is not surprising considering that humans and other primates have an ecological role in dispersing the seed of such plants. Chimpanzees living in African rainforest areas depend on fruits of trees such as figs for much of their diet. Through their faeces, they distribute the seeds along the forest floor and their food choice will determine those tree species which are able to regenerate through seed and those which cannot.

The odour principles of most fruits consumed by humans have been identified and much is known of the chemistry involved. Some fruits have single aroma principles (*e.g.* citral in lemons), others have mixtures of several components (*e.g.* banana, Table 15), and yet others have complex mixtures (*e.g.* 10 or more terpenes in apricots). Many of these odour components occur in glycosidic form in the unripe fruit and are released by enzymic hydrolysis during ripening.

Some fruits have odours repellent to humans, reflecting the fact that other animals besides primates may be responsible for eating the fruit and dispersing the seed. This is true of the durian fruit, *Duria zibethium*, which is rejected by some humans because of its offensive sewage-like smell. Many animals consume the fruit in the Malaysian rainforest, but elephants are particularly attracted to the foul odour and are also good seed dispersers. The reason why the fruit of that most ancient of gymnosperms, *Ginkgo biloba*, smells of rancid butter, due to a mixture of organic acids, is more obscure but it was presumably attractive to the seed disperser (a reptile?) of a past age.

In addition to the aromas, fruits contain many non-volatile constituents which contribute to taste and flavour. The most important and universal is sugar, providing sweetness of taste. Sugar is in the form of sucrose, glucose, and fructose, with either sucrose or glucose and fructose being dominant. Sucrose-rich fruits can provide problems of digestion to some birds which lack the enzyme sucrase, which hydrolyses sucrose to glucose and fructose. This is true of starlings, which are liable to develop osmotic diarrhoea if they eat such fruits, and this can be fatal. Sucrose-rich fruits include the peach and the apricot. Sweetness can occasionally be provided in fruit more efficiently by the presence of sweet proteins. Such proteins may be 3000 times sweeter than sucrose. Examples of fruits with sweet protein are the plants *Dioscoreophyllum cumminsii* and *Thaumatococcus daniellii*.

Acidity or sourness is another characteristic of fruit flavour. This is due to the accumulation of simple organic acids, such as citrate, malate, tartrate, and oxaloacetate. The concentrations of such acids is largely reduced during the ripening process and any remaining acidity is often counterbalanced by the sugar that is present in the ripe fruit.

Astringency in fruits is largely due to the presence of condensed tannins or flavolans (see Section 3.1). In small amounts, tannins may provide an attractive feature of ripe fruits, providing a counterbalance to the blandness of sweetness. In larger amounts (*e.g.* 1.6% wet weight), the fruit with high levels of tannin is the persimmon, *Diospyros kaki*, and here efforts have been made to reduce the astringency by chemical treatment. There is some evidence that tannin levels change during fruit ripening, so that tannins become inactivated either by polymerisation or by complexing with pectin. As a result, they become less astringent and hence more attractive for eating.

It has been proposed by Janzen[135] that ripe fruits may still contain some chemical constituents which may have a harmful effect on the herbivore because of the need to deter the "wrong" seed dispersers. This would apply to animals which either damage the seed in eating the fruit or which fail to distribute the seed away from the parent plant. The example quoted by Janzen is of ripe *Andira inermis* fruits, which have a potent antibiotic in the juicy pulp. This has no effect on the Costa Rican fruit bats, which avidly eat the fruit and properly disperse the seed. However, such fruits are rejected by cattle and pigs, because the antibiotic inhibits the gut flora required for proper digestion. Such animals would feed under the parent tree and not move away to disperse the seed.

How far protective chemistry is involved generally in fruits in selecting out the "right" seed dispersal agents is still undetermined. However, Barnea *et al.*[136] have evidence that fruits eaten by frugivorous birds are mildly toxic, even when ripe. The purpose here would seem to be to prevent the consumption of too many fruits in any one foraging bout and

Table 16 Some toxins of seeds

Compound	Class[a]	Source
Alkaloid	Caffeine	Guarana, *Paullinia cupana*
	Ajaconine	Delphinium, *Delphinium ajacis*
	Atropine	Deadly nightshade, *Atropa belladonna*
	Cytisine	*Laburnum anagyroides*
Cyanogen	Amygdalin	Bitter almond, *Prunus amygdalus*
Furanocoumarin	Xanthotoxin	Wild parsnip, *Pastinaca sativa*
Monoterpene	α-Thujone	White cedar, *Thuja occidentalis*
Diterpene	Columbin	Serendipity berry, *Dioscoreophyllum cumminsii*
Sesquiterpene lactone	Anisatin	Japanese star anise, *Illicium anisatum*
Protein	Abrin	Jequirity, *Abrus precatorius*

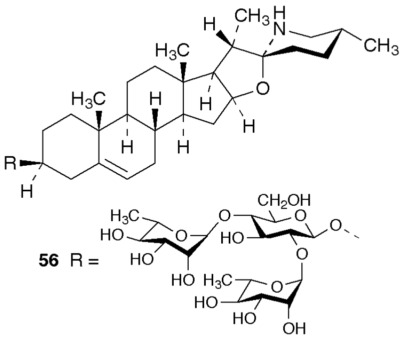
[a] For references, see ref. 93.

hence to regulate seed retention time. This in turn ensures better seed dispersal by the bird, since only a few seeds will be deposited at any one site at one time. Chemical analysis of ripe fruits of ivy, holly and hawthorn confirmed that saponins, flavonoids, and cyanogens were present in the pulp and were mildly distasteful to foraging blackbirds, starlings and redwings. Likewise, ornithological observations showed that feeding bouts were limited to 1.3–5.3 min and numbers of seeds eaten per bout varied between four and six.[136]

Turning finally in this section to the defensive chemistry of seeds, we have the situation where much is known about the occurrence of toxins in seeds but little is known about the defensive role. Many toxic constituents from alkaloids to cyanogens and monoterpenoids to diterpenoids have been encountered in seeds.[137] A few examples are given in Table 16. Unfortunately, in many cases, there is only circumstantial evidence that these toxins protect the seed from herbivory. Those examples where something is known of defensive chemistry will be described.

The protective role of the purine alkaloid caffeine (**41**) in the seed coat (at a concentration of 1.64% dry weight) of the guarana fruit has been established by Baumann *et al.*[137] Their experiments show that caffeine is not released from the seed while the fruit is being digested by toucans and guans, because there is a powerful diffusion barrier preventing the birds' intoxication. The level of caffeine in the seed is 3–5 times that of the coffee bean and this would be toxic to the bird if the seed coat were damaged during eating the fruit.

An interesting contrast is provided by some experiments of Levy and Cipollini[138] on the effects of alkaloids on cedar waxwings. Here, a typical fruit steroidal alkaloid, α-solamargine (**56**), was a deterrent to the birds feeding on an artificial fruit agar. The alkaloid was clearly deterrent, irrespective of its concentration (between 0.1 and 0.3% wet weight) and irrespective of the nutritional benefit offered in the agar. While some British birds appear to be impervious to the toxicity of tropane alkaloids in the berries of *Atropa belladonna*, it is clear that some tropical bird species are very sensitive to the presence of alkaloids in their food plants.



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8 Conclusion

Some of the advances in our understanding of the chemistry of plant–animal interactions over the last twenty-five years have been outlined in this review. Progress in no small measure has been due to the successful application of modern chemical techniques to the traces of chemicals released from biological systems. For example, the collection of floral volatiles through "headspace analysis" of living plants has revolutionised our appreciation of the chemical signals that attract the various pollinating vectors to plants.

Only a limited selection of many excellent literature reports in the field of chemical ecology are mentioned here. Some aspects, such as plant–microbial interactions, have been omitted since this area has been well reviewed elsewhere. Even with the chemical defence of plants as a major theme, some ecological aspects, especially induced defence systems, have not been covered in depth. This is a subject of intensive current interest. Undoubtedly, a clearer picture of the ecological effects of increased synthesis of secondary metabolites by plants will emerge in the next five years or so.

Remarkable progress has been made in ascribing functional value to a range of complex plant metabolites. Volatiles in flowers and fruits serve important ecological roles in specifically attracting animals to plants for pollination and seed dispersal. Many unexpected structures have turned up in this context—such as dimethyl trisulfide, naphthalene, *p*-cresol, squalene and methyl benzoate. Non-volatile chemicals in leaves may provide feeding barriers to many herbivores, although there are always individual species who, by detoxification, can overcome such defences. Included here are pyrrolizidine, quinolizidine and diterpenoid alkaloids. Perhaps the most surprising barriers are simple phenolics, which sometimes appear to be more effective feeding deterrents than the more complex phenolic tannins of higher molecular weight. Examples are coniferyl benzoate, jensenone and platyphylloside.

A female insect intent on laying its eggs on a suitable food plant is able to monitor the chemistry of potential candidate plants very precisely. Visual and olfactory signals may be involved, but a critical feature is the non-volatile leaf chemistry. There will be some chemicals that attract, but others that deter egg-laying on particular species. A range of chemicals from flavonoids and organic bases to alkaloids and cardenolides have now been implicated as chemical triggers of insect oviposition.

One area that deserves more experimental development is the chemical ecology of plant fruits and seeds and of the animals involved. The limited progress that has been made up to now is summarised in Section 7. However, much more needs to be done, especially with plants and animals in their natural environments.

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