Chapter 11

Chemical Ecology of Alkaloids

Michael Wink

1. INTRODUCTION

In previous chapters the authors considered the distribution of alkaloids within the plant kingdom and how the plants synthesize, transport, and store them. In this chapter I shall concentrate on the question of the role and function of alkaloids, to what purpose they are produced.

Let us consider the food chain in our ecosystem first (Fig. 1). Plants constitute the major group of photoautotrophic organisms which utilize solar energy to fix CO_2 into sugar and produce ATP as fuel and $NADPH_2$ as reduction equivalents, which serve to build up all of the other essential components of a cell. Animals and most microorganisms (except the chemo- or photoautotrophic bacteria) are heterotrophic organisms, which rely on complex, plant-made organic molecules for their energy requirement or other metabolic functions. Thus, plants serve as a major and the ultimate source of food for animals and microorganisms (Fig. 1). Plants struggle for life as do other organisms and have evolved strategies against herbivorous animals, microorganisms, and viruses. Plants also compete with other plants (of the same or of different species) for space, light, water, and nutrients.

How plants defend themselves against microorganisms (bacteria and fungi), viruses, herbivores and other plants is an old but still controversial topic in botany, ecology, and evolutionary studies. As plants do rather well in nature, the need for defense is not obvious. We are well aware of the defense strategies of higher animals (including man) against microbes and predators (Table I). The complex immune system with its cellular and humoral components is a well-studied and well-documented area in the context of animal–microbe interactions. Against predators animals evolved weapons, armor, crypsis, thanatosis, deimatic behavior, flight, or defense chemicals (usually called "poisons") (see Edmunds, 1974, for an overview).

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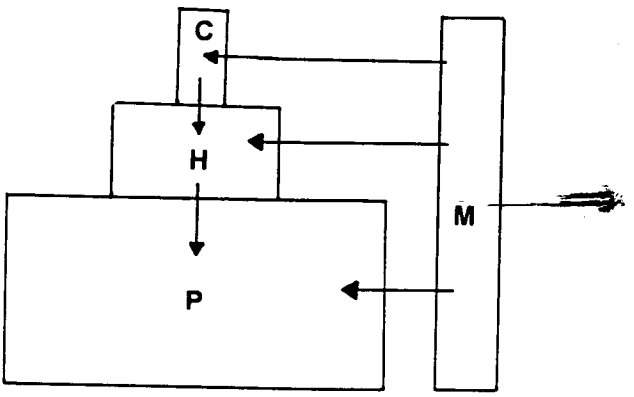

Figure 1. Schematic illustration of the terrestrial food pyramid (biomass). Main trophic levels include plants (P), herbivores (H), carnivores (C), and microorganisms (M). Arrows indicate the direction of attack or predation.

Table I
Defense Strategies of Animals versus Those of Plants and Fungi

	Defense strategies					
Measure	Mammals	Birds	Amphibia	Insects	Plants	Fungi
Carnivores/herbivores						
Flight	++	++	++	++	–	–
Weapons	++	+	*	+	*	–
Armor	*	–	–	*	++	–
Anachoresis	*	*	*	*	–	–
Crypsis	*	*	*	*	–	–
Aposematism	*	*	*	*	?	?
Thanatosis	*	*	*	*	–	–
Deimatic behavior	*	*	*	*	–	–
Group defense	*	*	–	*	–	–
Open growth	–	–	(*)	–	++	++
Toxins	(*)	(*)	+	++	++	++
Microorganisms						
Antibodies	++	++	++	–	–	–
Macrophages	++	++	++	++	–	–
Lytic enzymes	++	++	++	++	++	++
Toxins	–	–	*	?	++	++

++ = major strategy
+ = used by many but not all organisms
– = not used
* = restricted for few groups of organisms
(*) = very restricted to special cases

Table II
Selected Defense Strategies of Plants

Open growth	Replacement of leaves or branches on wounding
Physical protection	
"Armour"	Indigestible cell walls containing cellulose, lignin, suberin, callose
	Presence of hydrophobic cuticular layers (as a penetration barrier directed against microbes)
	Formation of a thick bark of roots and stems
"Weapons"	Mechanical weapons
	Spines, thorns, hooks, trichomes
	Glandular and stinging hairs (sometimes filled with defense chemicals)
	Laticifers and resin ducts (often filled with defense chemicals)
Chemical protection	Inhibitory or toxic proteins
	Lectins
	Protease inhibitors
	Toxalbumins
	Chitinase (against fungal cell walls)
	β-1,3-Glucanase (against bacteria)
	Peroxidase and phenolase (to degrade microbial toxins)
	PR proteins
	Secondary metabolites
	Constitutive defense chemicals
	Preformed chemicals activated by attack or wounding (cyanogenic glycosides, glucosinolates, coumaroylglycosides, alliin, ranunculin)
	"Phytoalexins" induced by elicitors or wounding

It is obvious that sessile plants cannot rely on many strategies that animals apply for defense. Table II shows some of the defense strategies of plants (Harborne, 1993; Wink, 1988, 1992, 1993a–d).

Natural products (or allelochemicals) are the major means involved in the overall defense strategy of plants. For many years secondary metabolites, of which more than 50,000 (including 12,000 alkaloids) have been described, were considered to be waste products or otherwise functionless molecules, illustrating the biochemical virtuosity of nature (Mothes *et al.*, 1985).

Toward the end of the 19th century, Stahl (1888) had advocated that natural products are used by plants for chemical defense against herbivores. The plant physiologists of that period had not accepted the Darwinian view of evolution and therefore were not inclined to accept the defense concept. As a consequence, the chemical defense hypothesis was ignored and remained forgotten for 60–70 years until the debate was reopened by Fraenkel in 1959. Although the defense hypothesis has presently become more and more accepted, critics from chemistry and botany can be heard regularly. While we don't know the function of each secondary metabolite, we can summarize the present argument as follows.

Most secondary metabolites are important for the fitness of the plant producing them, in that they serve as defense compounds against microbes and viruses, competing plants, and/or herbivores. In some instances, other functions, e.g., as signal compounds, include the attraction of pollinating or seed-dispersing animals, e.g., by colored compounds [betalains (within the Centrospermae), anthocyanins, carotenoids, flavonoids], fragrances (terpenes, amines, and aldehydes), or sweet substances (sugars). Physiological roles have

often been discussed, such as UV protection, N transport or N storage (Wink and Witte, 1984, 1985), or pigments (carotenoids). As secondary metabolites have rather a group-specific distribution, these physiological functions cannot be universal but special and additional. Furthermore, many natural products have multiple functions, a fact that is easily overlooked as most scientists usually specialize within a very narrow range, i.e., a microbiologist is not usually curious as to whether an antibiotic alkaloid also deters the feeding of caterpillars. To understand all of the interactions we need to adopt a holistic and interdisciplinary approach. Although the defense and signal hypotheses are probably valid for most compounds, it is likely that other compounds exist for which we have no identified function, especially if they occur in minor quantities.

Overviews on the field of chemical ecology are found in Swain (1977), Levin (1976), Harborne (1993), Schlee (1992), Rosenthal and Janzen (1979), Rosenthal and Berenbaum (1991), and Fritz and Simms (1992).

2. FUNCTION OF ALKALOIDS

About 20–30% of higher plants accumulate alkaloids (see Chapter 4). Within systematic alkaloid-accumulating groups, most members produce alkaloids, e.g., 60–70% of the Solanaceae and Apocynaceae species produce alkaloids. Some alkaloids have a wide distribution in nature: caffeine occurs in the largest number of families, lycorine in the largest number of genera, and berberine in the largest number of species. Alkaloids are not restricted to higher plants (although they are here most numerous), but are also present in club mosses (*Lycopodium*), horsetail (*Equisetum*), fungi, and animals, such as marine sponges, worms (e.g., Nereidae), bryozoa, snails, insects (e.g., Coccinellidae, Solenopsidae), amphibia (toads, frogs, salamanders), fishes, and even birds and mammals (see Chapters 15 and 16).

Alkaloids represent one of the largest group of natural products and were considered to be waste products for a long time (even by eminent alkaloid researchers such as W. O. James and Kurt Mothes). The waste product argument probably derived from animal physiology: Animals take up relatively large amounts of proteins and nucleic acids, containing more nitrogen than is needed for metabolism which is eliminated via uric acid or urea (Urich, 1990). As nitrogen is a limiting nutrient for most plants, a nitrogenous waste product would be *a priori* unlikely. If alkaloids were waste products, we would expect an accumulation in old organs being shed; in contrast, many plants (especially perennial species) remobilize their nitrogenous natural products (including alkaloids) from senescing organs such as old leaves or aerial parts. It is noteworthy that the alkaloids stored by animals were never considered to be waste products by zoologists but were always regarded as toxins, i.e., defense chemicals (see Chapter 15).

Although more than 12,000 alkaloids have been described so far, only about 600 have been partly analyzed for their biochemical properties and even fewer for their ecophysiological roles (Wink, 1993a; Brown and Trigo, 1995). This does not imply that the other alkaloids are inactive or without a function; they just have not been studied in detail. Research projects have addressed the question of whether a given alkaloid was a potential candidate for treating bacterial, fungal, or viral diseases, or killing parasites and cancer cells. Although not intended as ecological studies, the results can be interpreted and extrapolated in an ecological sense. We can safely assume that many more functions

and activities will become evident if alkaloids are analyzed with a more ecological or biological perspective, i.e., using ecologically relevant test organisms and employing adequate alkaloid concentrations. The last point needs to be stressed: because of the toxic properties of most alkaloids, many pharmacological experiments apply much lower doses than are found in the plant. As a consequence, ecologically relevant properties may be easily overlooked.

In conclusion, we can definitely accept the entirely plausible hypothesis that most alkaloids of plants, microorganisms, and animals serve, like other allelochemicals, as defense and signal compounds. This idea is intuitively straightforward, because many alkaloids are known to be human and animal poisons.

In this chapter, the functions of alkaloids in plant–herbivore interactions will be discussed in more detail. Plant–plant and plant–microbe interactions and the distribution and function of alkaloids in animals will only be treated briefly, as these aspects are extended in Chapters 13–17.

3. PLANT–HERBIVORE INTERACTIONS

3.1. Invertebrates

Insects are extremely successful from the evolutionary point of view and represent the largest class of organisms; they comprise at least 1 million and perhaps as many as 20–30 million species.

Most insects are herbivores and their adaptation to host plants and their chemistry is often very close and complex (Bernays, 1982; Bernays and Chapman, 1994). As many plants need insects for pollination and seed dispersal, but try to avoid insect herbivory, the interplay between attraction and deterrence can be very complicated. In the latter context it can be observed that plants attract insects by chemical means (colors, fragrances, sugar, amino acids) and at the same time other secondary metabolites are employed to prevent herbivory on flowers and seeds. The close association between plants, especially the Angiospermae, and insects evolved during the last 200 million years (Swain, 1977). Some scientists have called this phenomenon a "coevolutionary" process (Ehrlich and Raven, 1964), but it has to be recalled that the associations seen today are not necessarily those in which the chemical interactions originally evolved, i.e., the current associations may be quite recent.

Insect herbivores can be divided in two large groups, whose strategies with respect to the plant's defense chemistry differ substantially (Bernays and Chapman, 1994): The polyphagous species exploit a wide range of host plants, whereas the mono-/oligophagous insects often specialize on one or a small number of host plants which are often systematically related and accumulate the same class of secondary compounds. For these "specialists" the originally noxious defense compounds are no longer toxic but often attractive feeding and oviposition stimulants (Duffey, 1980; Schoonhoven, 1972; Bernays and Chapman, 1994).

Insects cope with dietary allelochemicals using one of several strategies:

1. Insects are commonly endowed with fantastic and powerful olfactory receptors and can select between plants with high or low amounts of "toxins" and also can ascertain

the food quality present, such as lipid, protein, or carbohydrate contents (Bernays and Chapman, 1994). The polyphagous "generalists" are usually deterred from feeding on plants sequestering high amounts of toxic allelochemicals and either select those with less active ones (Schoonhoven, 1972) or change host plants rapidly, consuming small portions of a particular poison at a given time and thus avoid severe intoxication.

2. A species "learns"; or more accurately, during evolution variants have been favored by natural selection which can tolerate a noxious defense compound: (a) by developing a mechanism to avoid toxin resorption in the gut. (b) If resorption cannot be prevented, to eliminate the toxin quickly via the Malpighian tubules or degrade it by detoxifying microsomal and other enzymes. Most polyphagous species have evolved active detoxification mechanisms, such as microsomal oxidases, glutathione transferase, and peroxidase, which promote rapid detoxification and elimination of dietary secondary products (Ahmad, 1983; Brattsten, 1988; Brattsten and Ahmad, 1986). (c) By developing a target site that is resistant to the toxin, i.e., a receptor that does not bind the exogenous ligand any longer. For example, in the monarch butterfly (*Danaus plexippus*) which stores dietary cardiac glycosides, the ouabain binding site of Na^+/K^+-ATPase has been made ouabain insensitive by a single point mutation (Holzinger *et al.*, 1992; Holzinger and Wink, 1996).

3. Alternatively, a species not only tolerates a plant's defense compound, but it also exploits it for its own chemical protection or for other purposes, such as pheromones (see Section 3.2.)

3.1.1. INSECT FEEDING DETERRENCE AND ALKALOID TOXICITY

In general, we would expect that alkaloids are active feeding deterrents against most insects. The examples compiled in Table III indicate that this assumption is valid, i.e., many alkaloids can act as feeding deterrents at higher concentrations (0.5%), although only a limited number of alkaloids have been assessed in this context. Given the choice, polyphagous insects tend to select a diet with no or only a small dose of alkaloids. Also the specialists avoid most "toxins" except those of their host plants. These findings indicate that under natural conditions, plants with a high load of alkaloids should be safe from most herbivorous insects (which is indeed the case) with the exception of particular monophagous species or a few very resistant polyphagous ones.

If animals have no choice or if they are very hungry, the deterrency threshold value is much reduced and they often feed on a diet containing alkaloids that they would normally avoid. In this case the toxicity of an ingested alkaloid can be assessed. Alternatively, alkaloid toxicity can be determined to some degree by topical application, although such data are of limited ecological relevance.

A substantial number of alkaloids display significant insect toxicity: examples include nicotine, piperine, lupin alkaloids, caffeine, and rayanodine. The toxic effects of alkaloids on insects (Table III) can be caused by their interference with a diversity of cellular and intracellular targets. As most mechanisms have not yet been elucidated for insects, we can only extrapolate from the mechanisms outlined in Chapter 12.

3.1.2. SEQUESTRATION OF ALKALOIDS BY INSECTS

Plants that defend themselves effectively constitute an ecological niche, almost devoid of herbivores and pathogens. It is not surprising that during evolution a number of

Table III
Activity of Alkaloids Toward Insects

Alkaloid	Effect[a]	ED_{50}
Ajmalicine	FD for polyphagous *Syntomis* (Lep.)	1%
Ajmaline	FD for *Syntomis*	0.1%
Anabasine	FD for *Syntomis*	0.1%
Arecoline	FD for *Syntomis*	<0.1%
	FD for *Phormia*	10 mM
Aristolochic acid	FD for *Spodoptera, Lymantria*	0.25–0.5%
	I for *Papilio* (Lep.)	0.5%
Atropine	FD for *Phormia*	0.6 mM
	I for *Bruchidius*	0.1%
Berberine	I for *Euxoa* (Lep.)	0.3%
	FD for *Phormia*	0.6 mM
	FD for bees	0.01%
	I for bees	0.003%
Boldine	FD for *Syntomis*	0.01%
Brucine	FD for *Syntomis*	1%
	FD for bees	0.05%
	I for bees	0.02%
Caffeine	FD for *Phormia*	2.5 mM
	FD for *Syntomis*	<0.1%
	FD for bees	0.03%
	I for *Bruchidius*	1%
	I for bees	0.2%
Castanospermine	FD for aphids	0.1 mM
Chaconine	FD for *Choristoneura*	0.1 mM
Chelidonine	FD for *Syntomis*	0.01%
Cinchonidine	FD for *Syntomis*	0.1%
	FD for bees	0.04%
Cinchonine	FD for bees	0.007%
Codeine	FD for *Phormia*	10 mM
Colchicine	FD for *Locusta* (Orth.)	0.001%
	I for *Bruchidius*	0.1%
	FD for *Syntomis*	<0.01%
	FD for bees	0.2%
	I for bees	0.03%
Coniine	FD for *Phormia*	5 mM
Cytisine	FD for *Syntomis*	0.01%
	FD for *Acyrthosiphon* (Hom.)	0.02%
	FD for *Formica* (Hym.)	0.01%
Deoxynojirimycine	FD for aphids	2.5 mM
Emetine	FD for *Syntomis*	<0.1%
L-Ephedrine	I for *Bruchidius*	0.1%
	FD for *Syntomis*	0.01%
	FD for bees	0.09%
Ergometrine	FD for *Syntomis*	1%
Ergotamine	FD for *Syntomis*	<0.1%
Glaucine	FD for *Spodoptera, Lymantria*	0.25-0.5%
Gramine	FD for aphids	<1 mM
	I for *Schizaphis* (Hom.)	0.01%
Harmaline	FD for *Syntomis*	<1%

(continued)

Table III
(Continued)

Alkaloid	Effect[a]	ED_{50}
Harmine	FD for *Syntomis*	<1%
	FD for bees	0.08%
	phototoxic for *Trichoplusia*	
Heliotrine	FD for *Choristoneura*	1.6 mM
	FD for bees	0.09%
	I for bees	0.1%
Hyoscyamine	FD for *Syntomis*	0.01%
	FD for bees	0.005%
	I for bees	0.1%
Jacobine	FD for *Locusta*	0.001%
Lasiocarpine	FD for *Choristoneura*	1.2 mM
Lobeline	FD for *Syntomis*	<1%
	FD for bees	0.008%
Lupanine	FD for *Syntomis*	0.1%
	I for *Plutella* (Lep.)	6 mM
	I for *Dysdercus* (Hom.)	12 mM
	I for *Ceratitis* (Dipt.)	3 mM
	I for *Phaedon* (Col.)	12 mM
Lupinine	FD for *Acyrthosiphon*	0.08%
Nicotine	FD for *Syntomis*	<0.1%
	FD for bees	0.03%
	I for *Bruchidius*	0.1%
	I for bees	0.2%
Papaverine	FD for *Spodoptera, Lymantria*	0.25–0.5%
	FD for *Phormia*	10 mM
	FD for *Syntomis*	<0.1%
Perloline	FD for *Locusta*	0.1%
Physostigmine	FD for *Syntomis*	0.01%
Pilocarpine	FD for *Phormia*	2.5 mM
	FD for *Syntomis*	0.1%
Protoveratrine B	FD for *Syntomis*	0.01%
Quinine	FD for bees	0.01%
	I for bees	0.02%
	FD for *Phormia* (Dipt.)	0.6 mM
	FD for *Locusta* (Orth.)	0.01%
Reserpine	I for *Bruchidius* (Col.)	0.1%
	FD for *Syntomis*	1%
Sanguinarine	FD for *Spodoptera, Lymantria*	0.25–0.5%
	FD for *Syntomis*	<1%
Scopolamine	FD for *Syntomis*	0.01%
	FD for bees	0.03%
Senecionine	FD for *Choristoneura*	1.6 mM
Solanidine	FD for *Choristoneura*	0.1 mM
Solanine	FD for *Choristoneura*	1 mM
Sparteine	FD for *Syntomis*	0.1%
	FD for bees	0.03%
	FD for *Acyrthosiphon*	0.01%
	I for bees	0.05%
	I for *Plutella*	50 mM
	I for *Dysdercus*	50 mM
	I for *Ceratitis*	9 mM

Table III
(Continued)

Alkaloid	Effect[a]	ED_{50}
Strychnine	I for Bruchidius	0.1%
	FD for *Phormia*	10 mM
	FD for *Syntomis*	<1%
	FD for bees	0.02%
	I for bees	0.2%
13-Tigloyloxylupanine	FD for *Choristoneura* (Lep.)	1.4 mM
	I for *Plutella*	12 mM
	I for *Dysdercus*	6 mM
	I for *Phaedon*	6 mM
	I for *Ceratitis*	6 mM
Tomatidine	FD for *Choristoneura*	1 mM
	FD for *Syntomis*	1%
Tomatine	FD for *Locusta*	0.1%
	FD for *Choristoneura*	0.1 mM
	FD for *Phormia*	10 mM
Vincamine	FD for *Syntomis*	0.01%
	FD for bees	0.08%
Yohimbine	FD for *Phormia*	2.5 mM
	FD for bees	0.008%

[a]FD, feeding deterrent; I, insecticidal (after Detzel and Wink, 1993; Wink and Schneider, 1990; Wink, 1993b).

organisms were selected that have specialized on a particular host plant species and found ways to tolerate or even to exploit the defense chemistry of their hosts. As compared to the huge number of potential enemies, the number of adapted specialists is usually small and in general a "status quo" or equilibrium can be observed between specialists (or parasites) and their hosts. A specialist is well advised not to kill its host, for to do so would destroy its own resources; a mutualism is more productive.

Superficially, these observations seem to contradict the working hypothesis, that secondary metabolites are primarily defense compounds. But these specialists are only the exceptions to the general rule. In this context we should recall that our immune system is fantastic in warding off bacteria, fungi, viruses, and parasites. We usually take notice of its existence only when it fails, i.e., when a specialized pathogen has found a way to undergo the immune response. Nobody would call the immune system ineffective because of this! Considering the specialized herbivores that have overcome the chemical defense barrier of plants a similar logic applies.

On a basic evolutionary level we find insects that can tolerate the defense chemistry of their host plants. One such example is *Manduca sexta*, whose larvae live on *Nicotiana* and other solanaceous plants. The tobacco hornworm can even grow on a diet with more than 1% nicotine without any adverse effects. The alkaloids present, such as nicotine or hyoscyamine, are not stored by the insects but degraded or directly eliminated with the feces. In order to avoid toxicity it has been postulated either that nicotine may not diffuse into nerve cells or that the ACH receptor no longer binds nicotine, as in "normal" animals. Recent experiments from my laboratory have shown that *Manduca* has ACh receptors that can bind nicotine. Furthermore, we have sequenced the alpha subunit of the receptor which does not show a substantial target site modification as compared to other moths

Table IV
Examples of the Storage of Dietary Quinolizidine and Pyrrolizidine Alkaloids by Insects[a,b]

Insects	Host plant	Acquisition		Utilized as pheromone
		By larvae	By adults	
PA (natural sources)				
Lepidoptera				
Euploea treitschkei	*Parsonia*	+		+
Gnophaela latipennis	*Hackelia*	+		
Hyalurga syma	*Heliotropium*	+		
Idea leuconoe	*Parsonia*	+		+
Mechanitis polymnia	Eupatorieae, Boraginaceae	+	+	?
Thitorea spp.	*Prestonia*	+		+
Tyria jacobaeae	*Senecio*	+		
	Adenostyles	+		
	Petasites	−		
Utetheisa ornatrix	*Crotalaria*	+		+
U. pulchelloides	*Heliotropium*	+		+
Coleoptera				
Oreina cacaliae	*Adenostyles*	+	+	
O. speciosissima	*Adenostyles*	+	+	
Coccinella spp.	*Senecio/Aphis*	+	+	
Homoptera				
Aphis jacobaeae	*Senecio*	+	+	
Aphis cacaliaster	*Senecio*	+	+	
PA (experimental feeding)				
Lepidoptera				
Amauris sp.	P		+	+
Arctia caja	P, S	+		
Callimorpha dominula	S	+		
Creatonotos transiens	P, S	+		+
Danaus spp.	P		+	+
Diacrisia sannio	S	+		
Euploea spp.	P		+	+
Nyctemera coleta	P, S	+	+	
Phragmatobia fuliginosa	S	+		+
Spilosoma lubricipeda	S	+		
Tyria jacobaeae	S	+		
Orthoptera				
Zonocercus variegatus	P, S	+	+	
QA (natural sources)				
Lepidoptera				
Uresiphita reversalis	*Teline*	+		
Homoptera				
Macrosiphumn albifrons	*Lupinus*	+	+	
Aphis cytisorum	*Laburnum*	+	+	
	Petteria	+	+	
	Cytisus	+	+	
Aphis genistae	*Spartium*	+	+	
	Sophora	+	+	
	Genista	+	+	

[a]After Hartmann and Witte (1995), Nickisch-Rosenegk and Wink (1993a), Wink (1992, 1993b), Brown and Trigo (1995).
[b]P, PA-rich plant material; S, isolated PA (mostly heliotrine, senecionine, or monocrotaline).

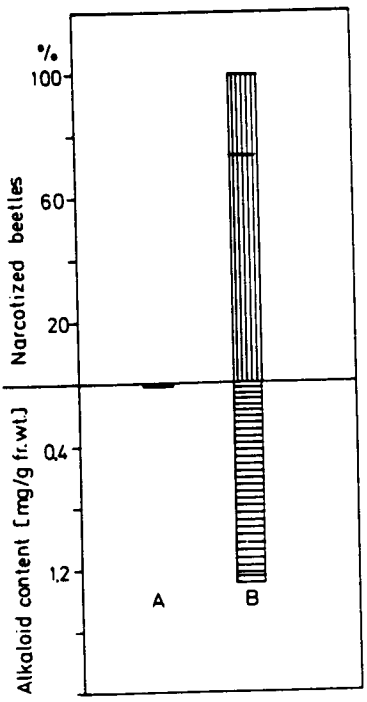

Figure 2. Effects of quinolizidine alkaloids stored by the lupin aphid, *Macrosiphum albifrons*, on a carnivorous beetle (*Carabus problemataicus*) (after Wink and Römer, 1986). About 12 individually kept beetles were given aphids without QA (control: A) or aphids with a QA content of 1.3 mg g^{-1} fresh weight (B). Experiments were evaluated after 16 hr; in B all beetles lay on their backs and remained narcotized for more than 48 hr, whereas the control beetles showed no symptoms.

(V. Theile, T. Schmeller, and M. Wink, unpublished). The potato beetle (*Leptinotarsa decemlineata*) lives on *Solanum* species containing steroidal alkaloids, which are tolerated but not stored by this species. The bruchid beetle, *Bruchidius villosus*, predates seeds of quinolizidine alkaloid (QA)-rich plants, such as *Laburnum anagyroides*. This beetle eliminates most of the dietary cytisine with the feces (Szentesi and Wink, 1991).

Insect herbivores, which not only feed on alkaloidal plants but also sequester the dietary alkaloids and exploit them for their own defense (Blum, 1981; Duffey, 1980; Rosenthal and Janzen, 1979; Rosenthal and Berenbaum, 1991; Brown and Trigo, 1995), have further evolved.

In a number of plants alkaloids are translocated via the phloem (e.g., quinolizidine, pyrrolizidine, and polyhydroxy alkaloids; Wink, 1987a, 1990; Dreyer *et al.*, 1985; Hartmann and Witte, 1995; Vrieling, 1991). If aphids live on these plants, they come in direct contact with the alkaloids present. A few adapted aphids can store the dietary alkaloids (Table IV). Examples are QA in *Aphis cytisorum*, *A. genistae*, and *Macrosiphum albifrons* (Wink and Witte, 1991) and pyrrolizidine alkaloids (PA) in *Aphis jacobaeae* and *A. cacaliaster* (Hartmann and Witte, 1995; Wink, unpublished). For alkaloid-storing *M. albifrons* it was shown experimentally that the QA stored provide protection against carnivorous beetles, such as *Carabus problematicus* (Fig. 2), or *Coccinella septempunctata* or syrphids (*Episyrphus balteatus*). *Acyrthosiphon spartii* prefers sparteine-rich *Cytisus scoparius* plants; it is likely that this species also stores QA.

A further (tritrophic) interaction has also become evident: *Aphis cytisorum* and *A. genistae* colonies are regularly visited by ants, which collect honey dew. Regarding *Lasius niger* collected from an *A. cytisorum* colony, the ants contained about 45 μg cytisine g^{-1}

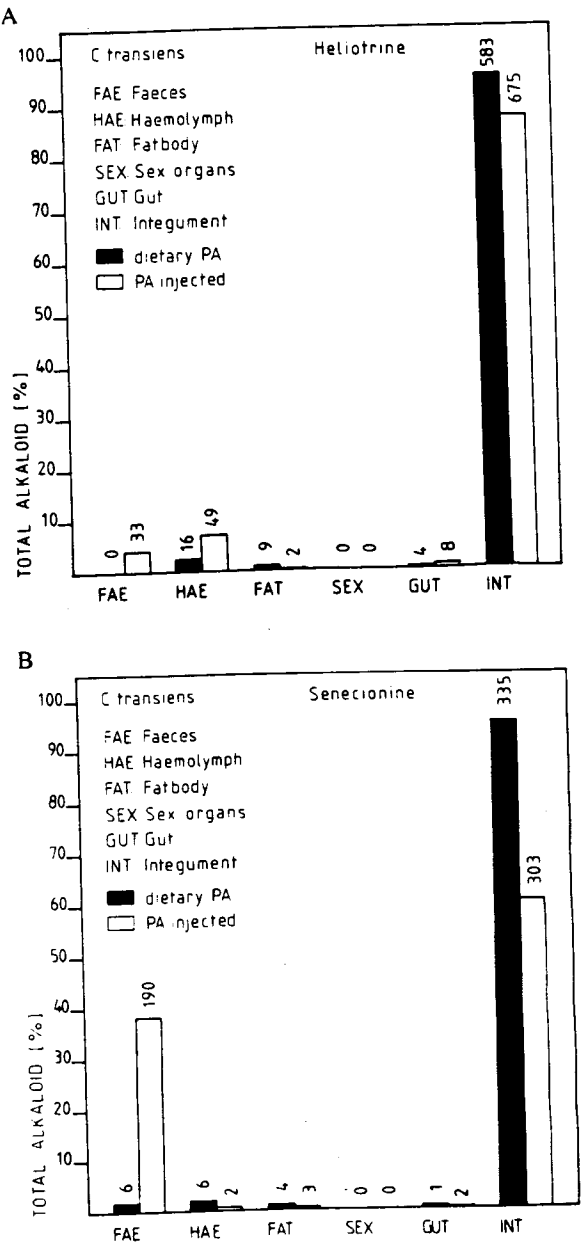

Figure 3. Distribution of pyrrolizidine alkaloid in larvae of the arctiid moth, *Creatonotos transiens* (after Nickisch-Rosenegk *et al.*, 1990). (A, B) PAs [heliotrine (A) or senecionine (B)] were administered orally or via injection into the hemolymph. After 48 hr PA distributions in the different parts of the insects were determined. (C, D) Kinetics of heliotrine within the first 48 hr after feeding, differentiated for males (C) and females (D). (By permission of *Zeitschrift der Naturforschung*.)

fresh weight (Szentesi and Wink, 1991). As cytisine is a very toxic alkaloid, it would be interesting to find out whether the ants gain protection from the alkaloids obtained from aphids. Regarding PA a tritrophic interaction has also been reported (Hartmann and Witte, 1995): Ladybirds (*Coccinella*) sequestered PA from *Aphis jacobaeae* feeding on PA-rich *Senecio jacobaea*. It is likely that, in ladybirds, besides the endogenously produced coccinellines the PA also serve as chemical protectants

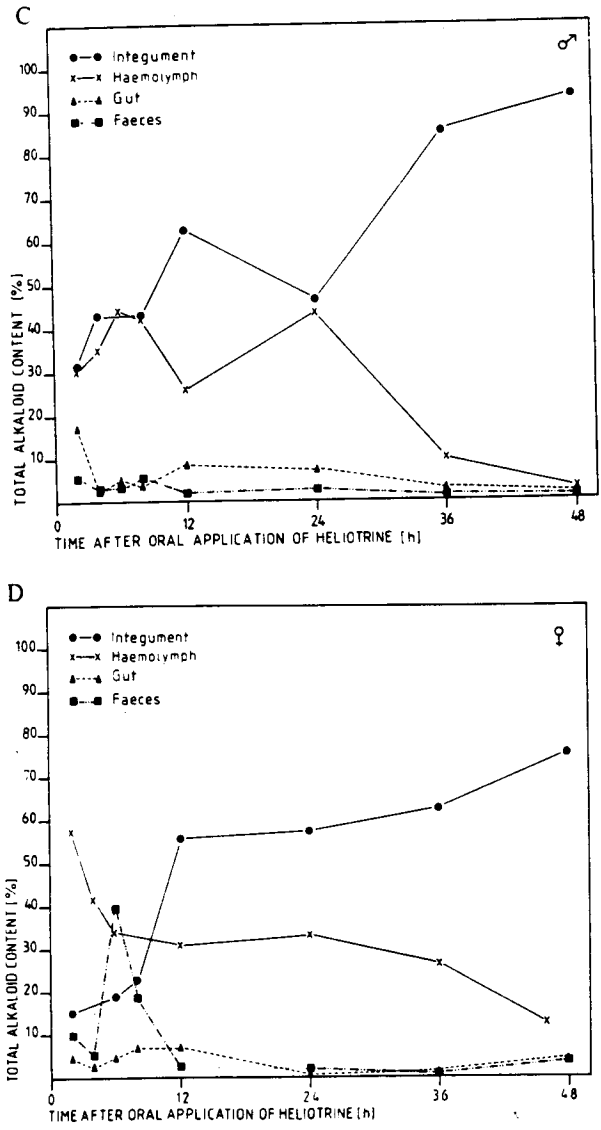

Figure 3 *(Continued)*

Larvae of the pyralid moth, *Uresiphita reversalis*, live on QA-producing plants, such as *Teline monspessulana* (Table IV). The larvae store some of the dietary alkaloids, especially in the integument and silk glands. The uptake is both specific and selective and achieved by a carrier mechanism: Whereas alkaloids of the 10-oxosparteine type dominate in the plant, it is the more toxic cytisine that is accumulated by the larvae and the 10-oxosparteines are eliminated with the feces (Wink *et al.*, 1991). These larvae gain some protection from storing QA as was shown in experiments with predatory ants (*Iridomyrmex humilis*) and the paper wasp (*Mischocyttarus flavitarsus*). When the larvae pupate, most of their stored alkaloids are used to impregnate the silk of the cocoon, thus providing defense for this critical developmental stage. The emerging moth lives cryp-

tically and has no aposematic coloring and does not contain alkaloids. In contrast, the alkaloid-rich larvae were aposematically colored and live openly on the plants (Bernays and Montllor, 1989; Montllor *et al.*, 1990, 1991).

Especially among lepidopteran larvae, many examples have been reported of insects sequestering PA of their host plants (Table IV) and probably making use of them for their own defense (Schneider, 1987; Hartmann and Witte, 1995; Brown and Trigo, 1995). PA are almost always stored as their *N*-oxides and not as a free base. Because PA *N*-oxides cannot diffuse freely across biomembranes, they can be easily stored and retained in specific organs or tissues, in general, the integuments (Nickisch-Rosenegk *et al.*, 1990; Nickisch-Rosenegk and Wink, 1993). In addition, because they also affect muscarinic ACh and serotonergic receptors, the *N*-oxides probably contribute to the deterrence and toxicity of PA (Table III) (Schmeller *et al.*, 1997). Following resorption from the midgut (probably with the aid of a carrier mechanism; Wink and Schneider, 1988), PA remain transiently in the hemolymph before they are transferred to the integuments (Fig. 3). In larvae of the arctiid moth, *Creatonotos transiens*, we could show that during metamorphosis the PA are partly transferred to the ovary in females and to the spermatophore in males. During copulation, females obtain the PA-rich spermatophore (as a "nuptial gift") and transfer the alkaloids to the eggs. Thus, both sexes contribute their PA to the clutch which may benefit from the PA as a chemical protectant (Nickisch-Rosenegk *et al.*, 1990). A similar phenomenon has been reported for *Utetheisa ornatrix* (Dussourd *et al.*, 1988; Conner *et al.*, 1981).

Besides their use as chemical defense compounds, some insects exploit PA as precursors for pheromones (Fig. 5). In *C. transiens* PA are converted into hydroxydanaidal (the hydroxyl group at C-7 is *R*-configured), which is dissipated via the inflatable scent organs of the male (Fig. 4) (Schneider *et al.*, 1982). Because the pheromone content depends on the storage of dietary PA during the larval stages, PA-rich males should be especially attractive to females. If males with a high content of hydroxydanaidal are selected, this behavior would ensure that females can obtain a PA-rich nuptial gift during copulation. Normally, PA are 7*R*-configured in nature. In the event that PA are present in the 7*S* configuration, larvae of *C. transiens* and a few other species can invert the configuration to the correct 7*R* form (Wink *et al.*, 1988, 1990; Schulz *et al.*, 1993; Nickisch-Rosenegk *et al.*, 1993). In androconial organs of Danainae and Ithomiinae, PA-derived male courtship pheromones have been found, such as hydroxydanaidal, danaidal, danaidone, and ithominae lactone. In these butterflies PA are often acquired as adults while feeding on nectar or wilting PA-containing plants (Boppré, 1990; Hartmann and Witte, 1995).

In *C. transiens* the PA adaptation even went one step further: Only if the larvae feed on a PA-rich diet are the males able to develop their big inflatable scent organs (corema) (Fig. 4). In this case, PA serve as a morphogen which triggers the morphological development of the coremata (Schneider *et al.*, 1982; Boppré, 1990; Schneider, 1993).

A number of other alkaloids (e.g., aconitine, cinchonine, aristolochic acid, cocaine, polyhydroxy alkaloids, β-carbolines, cycasin) have also been reported to be sequestered by insects (extensive review in Brown and Trago, 1995).

It is worth recalling that a number of animals (sponges, nudibranchs, worms, insects, toads, frogs, and salamanders) are able to synthesize their own defense compounds, among which are several alkaloids (see Chapters 15 and 16). The endogenously produced and the acquired alkaloids appear to serve as chemical defense compounds, in analogy to the situation found in plants. In animals we observe the trend that sessile species, such as

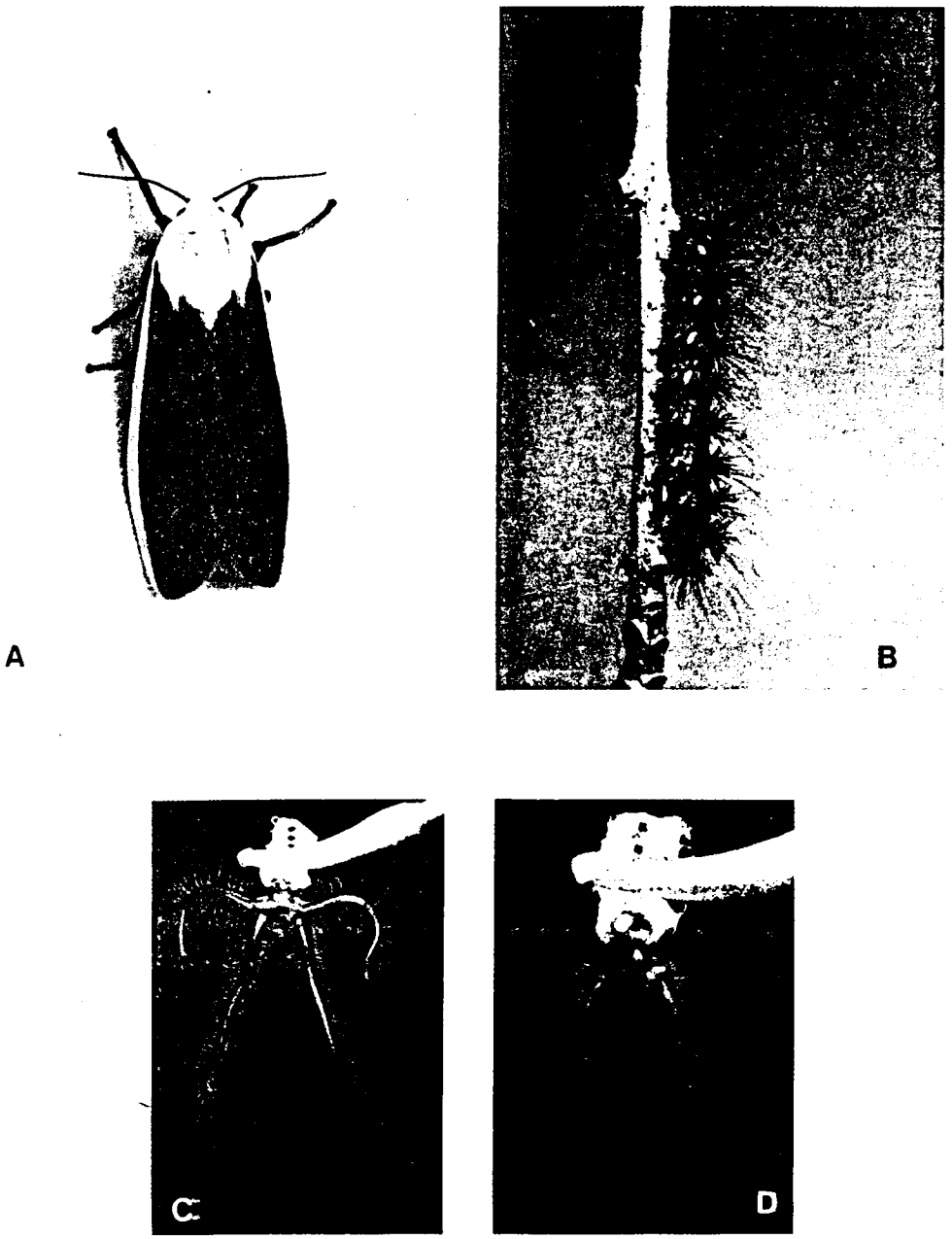

Figure 4. Corema development in *Creatonotos transiens* and alkaloid feeding. (A) Adult moth; (B) larva; (C) male corema (larva had PA as a diet); (D) corema of an insect raised without PA.

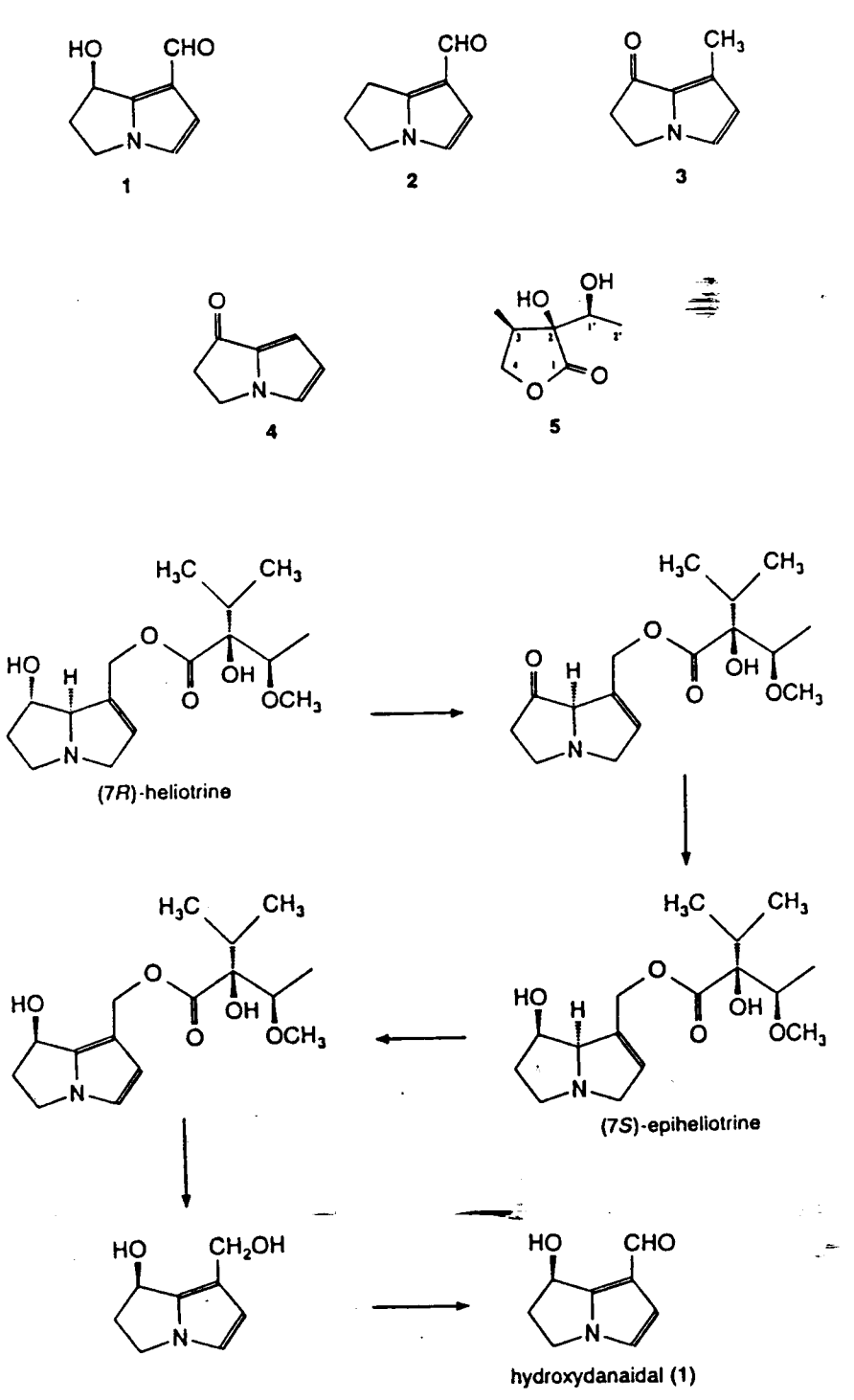

Figure 5. Structures of PA-derived pheromones [**1** = (7*R*)-hydroxydanaidal, **2** = danaidal, **3** = danaidone, **4** = nordanaidone, **5** = ithominae lactone] and pathway of heliotrine transformation in *Creatonotos transiens* and other arctiid moths (after Wink *et al.*, 1988; Schulz *et al.*, 1993; Hartmann and Witte, 1995).

bryozoa, or slow moving ones without armor, such as worms, nudibranchs, frogs, toads, and salamanders, produce active allelochemicals but not so animals with weapons, armor, or the possibility of escape by flight. Plants just share their strategy with sessile animal species (cf. Table I). In this context it seems amazing that hardly anyone has doubted the defense role of animal alkaloids, whereas people did and still do doubt the defense role regarding alkaloids in plants.

3.2. Vertebrate Herbivores

As man and his livestock are herbivores rather than carnivores, a large body of information on adverse effects of alkaloids and other dietary secondary metabolites has been accumulated over the centuries (see also Chapters 1, 12, and 17).

Many alkaloids exhibit a bitter or pungent taste for vertebrates and a bitter or pungent diet is normally instinctively avoided. Examples of bitter-tasting alkaloids (at least in man) are quinine, strychnine, brucine, emetine, and sparteine and for pungent alkaloids, capsaicine and piperine. It should be recalled that these taste properties are not identical for all animals. For example, geese, which are obligate herbivores, hardly avoid food with alkaloids or smelly compounds (amines, mercaptoethanol) which are strong repellents for humans. On the other hand, fragrances that are attractive to us, are highly repellent to geese (Wink *et al.*, 1993). Even within a population taste can differ significantly: a substantial proportion of humans cannot detect the smell of HCN, whereas others are highly sensitive. Furthermore, olfactory sensitivity can differ with age, sex, and hormonal cycles.

The bitterness varies with the chemical structure: in the case of QA the following scale was determined for man: Mean detection levels are 0.00085% for sparteine, 0.0021% for lupanine, and 0.017% for hydroxylupanine (Wink, 1992). Whereas we know most parameters of olfactory qualities in *Homo sapiens*, much less or hardly anything is known for most other vertebrates.

Alkaloids are infamous for their toxic properties in vertebrates (see Chapters 2 and 3) and plants that produce alkaloids are often classified as poisonous or toxic. For a number of alkaloids the respective LD_{50} values have been determined with laboratory animals, especially mice, but also with rats, guinea pigs, cats, rabbits, dogs, or pigeons (Table X in Chapter 12). As rodents are herbivores (and thus adapted to allelochemicals), they are not especially sensitive to alkaloids as toxins and some of the data may be misleading. The toxic effects observed with complete animals have their counterpart in the cytotoxic effect determined for some alkaloids (Table V). Most of these data have been obtained by screening many natural products for anticancer activity. But an alkaloid that can kill a cancer cell is usually also toxic for "normal" cells and the complete animal. Therefore, the data shown in Table V are another indication of the general toxicity of alkaloids toward animals. The mechanisms underlying the toxic effects have been elucidated in some detail. Often molecular targets and processes are involved that are important for all cells, such as DNA, RNA, proteins, replication, transcription, protein biosynthesis, membrane assembly and stability, electron chains, or metabolically important enzymes or proteins, such as receptors, hormones, signal compounds (see Chapter 12 for a more detailed discussion).

Whereas many insect herbivores are "specialists," vertebrate herbivores are rather polyphagous, although some specialization may occur. For example, grouse (*Lagopus*

Table V
Cytotoxic Properties of Selected Alkaloids[a]

Alkaloid	Target cells
Acronycine	L1210 cells, *Plasmodium*
Arecoline	*Trypanosoma*
Berberine	*Trypanosoma, Plasmodium*
Boldine	Human epidermoid carcinoma cells
Camptothecine	L1210 Walker sarcoma cells, KB and P388 leukemia cells
Chelerythrine	Tumor cells
Cinchonine	*Plasmodium*
Colchicine	General cytotoxicity
Coptisine	Cytotoxic
Echinatine *N*-oxide	P388 leukemia cells
Ellipticine	L1210 sarcoma cells
Emetine	Trypanosoma
Fagaronine	KB, L1210, P388 leukemia cells
Harmaline	*Trypanosoma*
Harmine	*Trypanosoma*
Harringtonine	Tumor cells
Heliotrine	Tumor cells
Indicine *N*-oxide	P388 cells
Jatrorrhizine	*Plasmodium*
Liriodenine	A-549, HCT-8, KB, P388 leukemia cells
Lycorine	NIH/3T3 cells
Matrine	Ascites tumor, mouse sarcoma 180
Olivacine	*Trypanosoma*, L1210, KB cells
Palmatine	*Plasmodium*
Quinine	*Plasmodium, Trypanosoma*
Sanguinarine	Tumor cells
Senecionine	Tumor cells
Solamargine	PLC, PRF cells
Tabernamine	P388 leukemia cells
Vinblastine	*Trypanosoma*, Wilms' tumor, lymphoma cells

[a]More data are found in Wink (1993a).

lagopus) or capercaillies (*Tetrao urogallus*) prefer plants of the Ericaceae or Coniferae; crossbills, the seeds of *Picea* and *Abies*, which are rich in terpenes. The Australian koala is oligophagous and consumes certain terpene-rich species of the genus *Eucalyptus*. While a single plant can be a host for hundreds of insect larvae, hundreds of plants comprise a daily menu for a larger grazing mammal.

Vertebrates share a few strategies with insects in coping with allelochemicals. But a sequestration and storage of dietary alkaloids has hardly been reported (as opposed to its regular occurrence in insects; see above); the storage of quinolizidine-type alkaloids in castoreum (derived from food plants) is an exception rather than a rule. Strategies of vertebrates include:

- Avoidance of alkaloid-rich plants (usually labeled toxic or poisonous by man) which is facilitated by the bitter or pungent taste of most alkaloids.
- Sampling of food from a wide variety of sources and thus minimizing the ingestion of high amounts of a single toxin.

- Detoxification of dietary alkaloids, which can be achieved by symbiotic bacteria or protozoa (Dowd, 1992), living in the rumen or intestines, or by liver enzymes, which are specialized for the chemical modification of xenobiotics. Carnivorous animals, such as cats, are known to be much more sensitive than herbivores toward plant poisons. It has been suggested that animals that do not face the problem of toxic food are not adapted to the handling of allelochemicals.
- Some animals such as ungulates, monkeys, parrots, or geese ingest soil (so-called "geophagy"). For geese and chimpanzees it was shown that the ingested soil binds dietary allelochemicals, especially alkaloids (Table VI) (Wink *et al.*, 1993; Zippin *et al.*, 1998).
- Animals are intelligent organisms, able to learn. The role of learning in food and toxin avoidance is rather important but is not understood in most species.

How most vertebrate herbivores manage to avoid, tolerate, or detoxify their dietary allelochemicals has not been explored. Sometimes, only domesticated animals were used in experiments, but they tend to make more mistakes in food choice than the wild ones. More evidence is available for humans; as with most other herbivores, alkaloid-rich diets are avoided, but if toxins have been ingested, they are normally detoxified in the liver. This evolutionary trait is very helpful for *Homo sapiens*, as it endowed us with a way of coping with man-made chemicals that pollute the environment. Besides these biological adaptations, man has evolved a number of "tricks," some of them obviously not anticipated by evolution:

Many fruits or vegetables are peeled before consumption. Because many alkaloids and other natural products are stored in the epidermis, such as steroidal alkaloids in potato tubers, or cucurbitacins in cucumbers, peeling eliminates some of the compounds.

Most food is boiled in water. This leads to the thermal destruction of a number of toxic allelochemicals, such as lectins, protease inhibitors, and some esters and glycosides.

Table VI
Absorption of Alkaloids to Soil and Charcoal[a]

	Adsorption (%)				
	Soil			Charcoal	
Alkaloid	1	2	3	1	2
Cytisine	75	74	n.d.[b]	n.d.	100
Harmine	99	99	n.d.	n.d.	100
Heliotrine	92	87	n.d.	n.d.	100
Nicotine	93	92	n.d.	n.d.	100
Quinine	99	99	n.d.	n.d.	100
Scopolamine	73	71	n.d.	n.d.	100
Sparteine	93	92	74	85	100
Thebaine	94	93	n.d.	n.d.	100

[a]Soils 1–3 and charcoal 1 were collected from places where the geese had ingested similar material. Charcoal 2 was activated charcoal, known for optimal binding capacities. Alkaloids were dissolved in aqueous solutions. After adding 100 mg of soil or charcoal, solutions were centrifuged and the alkaloid content of the supernatant was determined (after Wink *et al.*, 1993).
[b]n.d. = not determined.

Furthermore, many water-soluble compounds are leached out into the cooking water and are discarded after cooking (e.g., steroidal and polyhydroxy alkaloids in potatoes, quinolizidines in lupins, or cyanogenic glycosides in yams).

South American native Indians ingest clay when alkaloid-rich potato tubers are on the menu. As clay binds steroidal alkaloids, geophagy is an ingenious way to detoxify toxins (Johns, 1990).

Man has modified the allelochemical composition of his crop plants, in that he has reduced unpleasant taste components by plant breeding. From the point of view of avoidance this strategy is plausible, but from the point of view of chemical ecology, as will be discussed later (Section 6), not so. These plants often lose their resistance vis-à-vis herbivores and pathogens in this way, resistance that must be replaced by man-made pesticides.

In general, only a few plants are exploited by man as food, out of the more than 300,000 existing species. Thus, despite all of our intelligence, we have only achieved a rather moderate success in the utilization of plants as a staple diet, indicating the importance and power of chemical plant defenses.

4. PLANT–MICROBE INTERACTIONS

It is a common observation that dead plants rot easily under the action of bacteria and fungi, whereas metabolically active, intact plants usually resist a microbial attack. Thus, plants (despite lacking an immune system) must have a means of defending themselves against microorganisms. Plants have an epidermis that is covered by a more or less thick cuticle consisting of waxes, alkanes, and other lipophilic natural products. This cuticle layer is water repellent and chemically rather inert and thus constitutes an important penetration barrier against most bacteria and fungi. In perennial plants and in roots we find another variation of this principle in that they often form resistant bark tissues (Table II).

The only way for microbes to enter a healthy plant is via the stomata or at sites of injury inflicted by herbivory, wind, or other accidents. Immediately after wounding, most plants start to accumulate suberin, lignin, callose, gums, or other resinous substances which seal the injured areas. In addition, antimicrobial agents are produced, such as 1,3-glucanase and chitinase, i.e., lytic enzymes stored in the vacuole which can degrade bacterial and fungal cell walls, protease inhibitors which can inhibit microbial proteases or just secondary metabolites with antimicrobial activity (Table II) (compare Chapter 9 and 17). These antimicrobial natural products, which can be either constitutive or inducible, often also interfere with herbivores; i.e., they often exhibit multiple functions (an observation often overlooked in the phytopathology literature).

Secondary compounds with antimicrobial activity include many phenolics (such as flavonoids, isoflavones, tannins, and simple phenolics), glucosinolates, nonproteinogenic amino acids, cyanogenic glycosides, acids, aldehydes, saponins, triterpenes, sesquiterpenes and last but not least alkaloids (see Chapter 17). It is likely (although not determined in many instances) that a substantial number of the 12,000 alkaloids have antimicrobial properties, directed against ubiquitous and generalist microbes, which have not specialized on a particular host plant.

Most plants are known to be parasitized or infected by at least a few specialized bacteria or fungi which often form close associations. In these circumstances the antimicrobial effect expected from the secondary metabolites present in the plant is rarely

observed. We suggest that these specialists have adapted to the chemistry of their host plants and found a way to handle it. Mechanisms (which are yet to be determined in most instances) can be the inhibition of biosynthesis or the degradation of the respective secondary compounds. Many phytopathogenic bacteria and fungi produce their own secondary metabolites, which are often toxic to plants. It is assumed that these phytotoxins serve to weaken the host plants' defense, but this may not be the whole story.

Many grasses are infected with fungi that produce ergot or pyrrolizidine (loline-type) alkaloids. It has been assumed that these fungi are proper parasites. But recent experimental evidence suggests that the interaction between grasses and fungi may be of a symbiotic nature (Clay, 1990): For example, ergot alkaloids are strong vertebrate toxins, mimicking the activity of neurotransmitters such as dopamine, serotonin, and noradrenaline (Table I of Chapter 12). In fact, herbivorous impact on populations that were highly infected by fungi was much less than those without. These fungi exploit the nutrients of their host plants but supply them with strong poisons, which are not produced by the plants themselves. As these fungi do not kill their hosts, this commensalism seems to be of mutual benefit.

Some other associations between plants and fungi are symbiotic in nature, such as *rhizobia* in root nodules of legumes or microrhizal fungi in many species. In lupins, N-fixing *rhizobia* are present in both alkaloid-rich and alkaloid-free plants; they must thus be able to tolerate the alkaloids that are also present in the root.

Like animals, plants are hosts for a substantial number of viruses which are often transmitted by sucking insects, such as aphids and bugs (Heteroptera). Resistance to viral infection can be achieved either by biochemical mechanisms that inhibit viral development and multiplication or by warding off vectors, such as aphids, in the first place.

In two instances it was directly shown that alkaloids, such as quinine and sparteine, can inhibit the multiplication of a plant virus, here the potato X virus (Wink, 1987b, 1993a–d). All other evidence for antiviral activities (Table VII) of alkaloids comes from

Table VII
Examples of Antiviral Activities[a]

Alkaloid	Virus
Acronycine	Herpes simplex virus
Camptothecine	Herpes and others
Castanospermine	Cytomegalovirus and retroviruses
Cinchonidine	Potato X virus
Citracridone I	Herpes simplex virus
Crytopleurine	Herpes simplex virus
Didemnin	Herpes simplex virus
Grandisine	Herpes simplex virus
Harmine	Sindbis and murine cytomegalovirus
Hippeastrine	Herpes simplex virus
Lycorine	Herpes simplex and Rauscher virus
Maytansine	Murine sarcoma virus
Narciclasine	Rauscher virus
Norharman	Herpes simplex virus
Pretazettine	Herpes simplex and Rauscher virus
Sparteine	Potato X virus

[a]After Wink (1993a).

experiments with animal viruses. As viral life strategies are related in plants and animals, we suggest that a wider number of plant viruses may be controlled by alkaloids in nature than our limited data imply.

Fungal and bacterial multiplication can be controlled at the level of replication, transcription, protein biosynthesis, posttranslational protein modification, and membrane/cell wall integrity, whereas viruses are more difficult to inhibit (see Chapters 12 and 18).

5. PLANT–PLANT INTERACTIONS

Plants often compete with other plants, either of the same or of a different species, for space, light, water, and nutrients. This phenomenon becomes especially evident in deserts or semideserts where resources are limited and thus competition intensive. A number of biological mechanisms to avoid competition have been described, such as temporal spacing of the vegetation period, i.e., some species flower when others are still dormant or ungerminated.

Plants can inhibit each other also by their secondary metabolites (so-called "allelopathy") (Rice, 1984; Waller, 1987; Inderjit *et al.*, 1995). Secondary products are often excreted by the root or rhizophore or they are leached from the surface of intact leaves or from decaying dead leaves by rain. Both processes will increase the concentration of allelochemicals in the soil surrounding a plant, where the germination of a potential competitor may occur. The area of allelopathy is well documented at the level of controlled *in vitro* experiments but how it works in the ecosystem is still often a matter of controversy (Waller, 1987; Inderjit *et al.*, 1995). Allelopathic natural products have been recorded in all classes of secondary metabolites; however, the alkaloid group was somewhat neglected probably because their remarkable animal toxicities were more obvious.

As discussed in more detail in Chapter 14, nearly all structural types of alkaloids can exhibit allelopathic activities. At higher concentrations, a marked reduction in the germination rate of seeds can be recorded. More sensitive, however, are radicles and hypocotyls. They respond to alkaloids at a much lower level, usually showing growth inhibition or overstimulation. Both effects reduce the fitness of a seedling. The inhibitory effect can be absent for the endogenously produced alkaloid, as was reported for quinolizidine alkaloids in lupins (Wink, 1983a) and colchicine in *Colchicum autumnale*. Some of the cellular targets, discussed under plant–animal interactions, may also be affected by alkaloids in allelopathy.

A special case of plant–plant interactions can be seen in parasitic or hemiparasitic plants. The role of alkaloids in these interactions is discussed in Chapter 13.

6. ECOLOGICAL RELEVANCE OF ALKALOIDS

Because many of the allelochemical properties discussed above were determined in *in vitro* systems, it might be argued that they are not relevant under field conditions. In the following, evidence that supports the ecological relevance of alkaloids is discussed in more detail. For an alkaloid to serve as a chemical defense compound, the following criteria should be met:

1. The alkaloid or alkaloid mixture should have significant effects against microbes and/or animals
2. The compounds should be present in the plant in concentrations that are of the same order or even higher than those determined in the bioassays or animal experiments.
3. The compound should be present in the plant at the right time and right place.
4. Experimental evidence should show that an alkaloid or alkaloidal mixture promotes the fitness of a plant.

Many examples have been given concerning the first criterion; the second, third, and fourth will be explained below.

6.1. Are Alkaloid Concentrations in Plants Sufficiently High?

The accumulation of secondary metabolites only makes sense in view of their ecological function as defense compounds; however, they can fulfill these functions only if the amount stored is appropriate. The synthesis and maintenance of high levels of a defense compound is very demanding from the point of view of physiology and biochemistry. In view of the activities of these compounds, it can be assumed that many natural products would probably interfere with the metabolism of the producing plant if these metabolites were accumulated in the same compartments where they were made. Whereas biosynthesis takes place in the cytoplasm, in vesicles (berberine) or organelles such as chloroplasts [QA (Wink and Hartmann, 1982), coniceine (Roberts, 1981)], the site of accumulation of water-soluble alkaloids is the central vacuole; that of lipophilic compounds, the latex, resin ducts, or glandular hairs (see Chapter 10).

In general, all parts of an alkaloidal plant accumulate alkaloids (Table VIII). Although the site of synthesis is often restricted to a particular organ, such as roots or leaves, a translocation via the phloem, xylem, or apoplastically must occur (Wink, 1987a, 1993c) (see Chapter 10). Alkaloid levels can vary with respect to organ and development and even diurnal fluctuations have been observed for quinolizidine (see Fig. 9) and tropane alkaloids (Waller and Nowacki, 1978; Wink and Witte, 1984; Sporer *et al.*, 1993). Alkaloid levels are usually highest during the time of flowering and fruit/seed formation. In annual species the leaves, flowers, and seeds are often alkaloid-rich, whereas in perennial ones, like shrubs and trees, we find alkaloid-rich stem and root barks as well. All of these plant parts and organs have in common that they are important for fitness or reproduction and thus for long-term survival of the species. Spiny species, which invest in mechanical defense as well, accumulate less alkaloid than soft-bodied ones; examples are isoquinoline alkaloids in cacti or QA in legumes (Fig. 6). If a plant produces few and large seeds, their alkaloid levels tends to be higher than in species with many and small seeds (Fig. 7).

Summarizing the relevant phytochemical literature we find that alkaloid levels are between 0.1 and 15% (dw), which is equivalent to 0.01–1.5% fresh weight or 0.1–15 mg g^{-1} fw. As a representative example, Table VIII lists QA concentrations in different legume species and in some of their tissues. As compared with the inhibitory concentrations determined in plant–plant, plant–microbe, and plant–herbivore interactions, plant alkaloid levels are of the same order or one order of magnitude greater (Wink, 1992, 1993a–d).

Table VIII
Organ-Specific QA Concentrations in Selected Legumes

Species	Organ/Tissue	Total alkaloid (per g fresh wt)
Cytisus scoparius	Stem epidermis	46 mg/g; 200 mM
	Shoots	2 mg
	Leaves	0.2–1 mg
	Seeds	2 mg[a]
	Roots	0.03 mg
Lupinus polyphyllus	Petiole epidermis	1.7-10 mg
	Stem epidermis	6.3 mg
	Leaves	1–4 mg
	Stems	1–2 mg
	Flower	
	Pollen	1.8 mg
	Carpels	1.3 mg
	Petals	0.4 mg
	Fruits	1.6 mg
	Seeds	30–40 mg[a]
	Roots	0.2 mg
L. mutabilis	Stem epidermis	5.3 mg
L. albus	Stem epidermis	6.3 mg
	Phloem sap	0.5–1.2 mg/ml
	Leaves	2.8 mg
	Stem	0.7 mg
	Flower	4.1 mg
	Fruit	3.1 mg
	Seed	43.0 mg[a]
	Roots	0.5 mg
L. angustifolius	Phloem sap	0.8 mg/ml
	Xylem sap	0.05 mg/ml
Laburnum anagyroides	Leaves	0.3 mg
	Twigs	
	Bark	11.1 mg
	Wood	0.5 mg
	Flower	0.4 mg
	Fruit	0.5 mg
	Seed	10–30 mg[a]
	Endosperm	21 mg
	Testa	2 mg

[a]Dry weight.

The specific characteristics of individual molecular species, as well as the absolute amounts accumulated are important, and need to be addressed. Usually one to five main alkaloids dominate in a plant, but are accompanied by several (up to 80) minor alkaloids. As the alkaloids present in a particular plant usually share a biosynthetic pathway, their basic skeletons are often similar. To facilitate phytochemical investigations these compounds are usually classified as belonging to a particular alkaloid group, such as pyrrolizidine or quinolizidine alkaloids. This does not mean, however, that the biological activities of individual alkaloids are identical, i.e., that they are addressed to the same

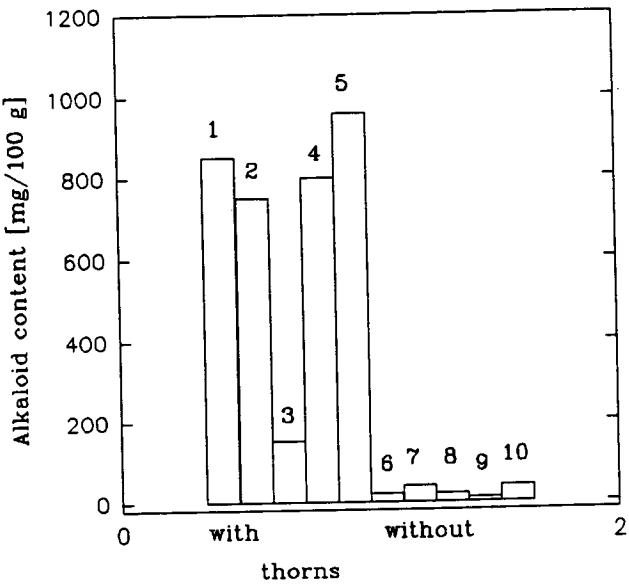

Figure 6. Relationship between mechanical and chemical defense: alkaloid content versus thorns in brooms. 1. *Genista radiata;* 2, *G. lydia;* 3, *G. sagittalis;* 4, *Teline monspessulana;* 5, *T. linifolia;* 6, *G. silvestris;* 7, *G. hispida;* 8, *Callicotome spinosa;* 9, *Ulex europaeus;* 10, *Echinospartum horridum.* After R. Greinwald, 1988. Untersuchungen zur chemotaxonomischen Bedeutung von Leguminosenalkaloiden und zum Alkaloidstoffwechsel in transformierten Geweben und Zellkulturen, Ph.D. dissertation, Universität Würzburg.

molecular target (Chapter 12, Fig. 9). On the contrary, the addition of small substituents to a molecule, such as a lipophilic side chain, while it seems to be a small and insignificant variation from a phytochemical point of view, may render the compound more lipophilic and thus more resorbable. In consequence, its toxicity may be higher.

The qualitative patterns are not constant but usually differ between organs, developmental stages, individuals, populations and, species. For a herbivore or pathogen the variable alkaloid profiles are very demanding, as these organisms not only have to adapt to one group of chemicals but also to most of the individual compounds (at least the major alkaloids) present. Because the composition of these chemicals also change quantitatively, it is even more difficult for them to cope. Thus, we suggest that structural diversity and its continuous variation is a means of counteracting the selection of adapted specialists.

In medicine we apply a related strategy to control microbial infections: In order to prevent bacteria developing resistance toward a particular antibiotic, mixtures of structurally different antibiotics are often applied which are oriented to various molecular targets. If only one antibiotic were given to all patients, the development of resistance would be much favored.

6.2. Occurrence of Alkaloids at the Right Site and Right Time

Intuitively, a valuable plant organ must be more protected than others. The preferential storage of alkaloids in very actively growing young tissue, seeds, petals, carpels, or

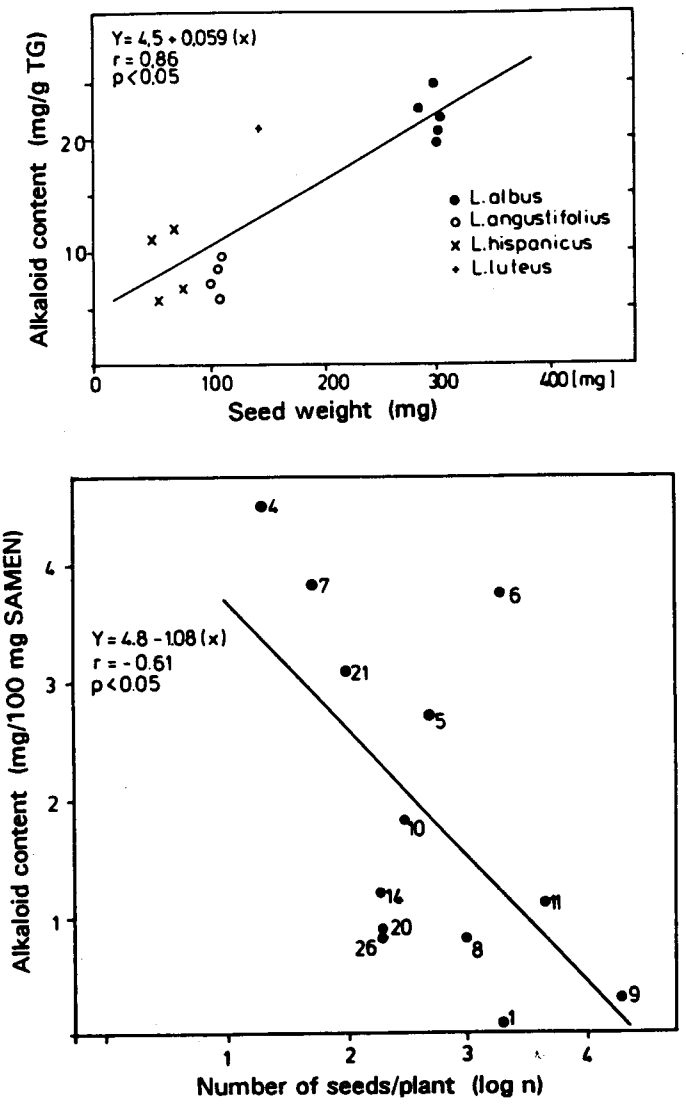

Figure 7. Correlation between alkaloid content and seed size and seed numbers in legumes (after Wink, 1985). (Top) Relation between seed weight and alkaloid content: bigger seeds contain proportionally more QA than smaller seeds. (Bottom) Relation between alkaloid content and seed numbers per plant: seeds from plants with few seeds are alkaloid-rich, whereas plants having many small seeds contain rather low levels of QA. Numbers refer to different legume species (Wink, 1985). By permission of Springer-Verlag.

pollen (Table VIII) provides evidence that alkaloids are stored at the right site, as these organs are important for reproduction and thus species survival.

At the tissue level we can also see these constraints: As discussed in Chapter 10, alkaloids are often accumulated in a cell- and tissue-specific fashion. For example, isoquinoline alkaloids in the Papaveraceae are abundant in the latex, where they are sequestered in many small latex vesicles. In latex vesicles of *Chelidonium majus* the concentration of protoberberine and benzophenanthridine alkaloids can be in the range of 0.6–1.2 M,

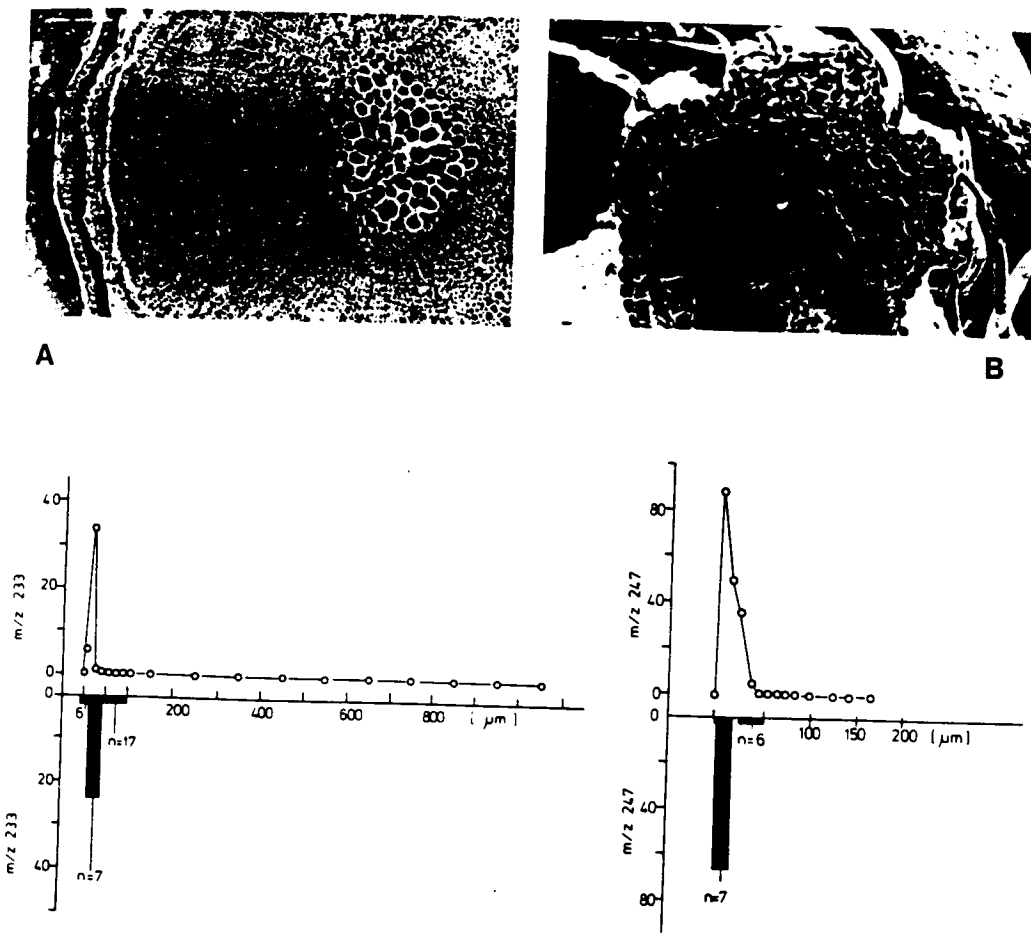

Figure 8. Epidermal storage of quinolizidine alkaloids in *Lupinus polyphyllus* and *Cytisus scoparius* (after Wink *et al.*, 1984). Distribution of sparteine (A) and lupanine (B) in cross sections of *C. scoparius* stems and *L. polyphyllus* petioles analyzed by laser desorption mass spectrometry. SEM photos show the cross sections analyzed by LAMMA 1000. The graphs demonstrate the occurrence of the molecular ions of sparteine (*m/z* 233) and lupanine (*m/z* 247) in scans carried out from epidermal to central cell layers. The lower panel presents a statistical analysis of several LAMMA measurements (means ± SD).

M, which is achieved by their complexation with equal amounts of chelidonic acid. If a herbivore wounds such a plant, the latex spills out immediately. Besides gluing the mandibles of an insect, the high concentration of deterrent and toxic alkaloids will usually do the rest. Indeed, *Chelidonium* plants are hardly attacked by herbivores. In addition, as these alkaloids are also highly antimicrobial, the site of wounding is quickly sealed and impregnated with natural antibiotics.

Lupin alkaloids are preferentially stored in the epidermal, subepidermal, and hypodermal tissues of stems and leaves, reaching local concentrations between 20 and 200 mM (Fig. 8) (Wink *et al.*, 1984; Wink, 1983a,b). A small herbivore or pathogen will enter the plant by injuring the epidermis first, where it is stopped by a chemical barrier. Epidermal storage of alkaloids does not seem to be restricted to legume species, but is probably a

common phenomenon, although hardly studied with modern techniques. The accumulation of many alkaloids in root or stem bark, such as berberine, cinchonine, and quinine, can be interpreted in a similar way, i.e., that alkaloids are deposited at strategically important sites, where they can ward off an intruder at the first opportunity.

The biosynthesis and accumulation of alkaloids can be regarded as constitutive processes, ensuring that alkaloids are present when needed for defense. The diurnal cycles (Fig. 9) and alkaloid turnover rates (see Chapter 10), however, seem to contradict this observation. To explain this phenomenon, in the case of alkaloids that have been selected as analogues of neurotransmitters (see examples in Chapter 12), it is essential that the structure and configuration of the molecule does not change. For L-hyoscyamine, which binds to mAChR, a racemization to atropine can occur under physiological conditions, which would halve its activity. Other alkaloids, which are stored in vacuoles, could be oxidized by peroxidase and thus would lose their intrinsic activity. A steady turnover and/or a diurnal fluctuation would ensure that the level of the correctly configured alkaloid is always present. It should be recalled that other molecules that are important for the correct function of a cell (e.g., enzymes and receptors) exhibit a pronounced turnover.

Plants appear to be reactive with respect to challenges by pathogens and herbivores. When infected by microorganisms, many plants begin to synthesize a series of antimicrobial compounds, ranging from proteins to secondary products including alkaloids (Table II). Chapter 9 considered the role of alkaloids in this context.

But what is the situation after wounding by a herbivore? When eaten by large herbivores, a *de novo* synthesis of defense compounds would be almost useless for a plant (except perhaps for trees), for the reaction would not be quick enough. But the situation is different for small herbivores such as insects or worms, which may feed on a particular plant for days or weeks. Here the *de novo* production of an allelochemical would be worthwhile. There are, indeed, experimental data supporting this view: In *Liriodendron tulipa* several aporphine alkaloids accumulate after wounding which are otherwise not present. In tobacco the production of nicotine, in lupins that of QA and in *Atropa belladonna* that of hyoscyamine is induced by wounding, thus increasing the already high levels of alkaloids by factors between 1.2 and 5. While the response was seen after 2–4 hr in lupins (Wink, 1983b), it took days in *Nicotiana* (Baldwin, 1989) and in *Atropa* (Harborne, 1993). We suggest that the wound-induced stimulation of alkaloid formation is not an isolated phenomenon, but part of the chemical defense system (see examples in Tallamy and Raupp, 1991).

Summarizing, it can be assumed that alkaloids are present at the right place, at the right time, and in the right concentrations to fulfill their ecological defense functions.

6.3. Evidence for Alkaloid-Mediated Fitness

The arguments and circumstantial evidence discussed in the last paragraphs appear convincing and support the hypothesis that alkaloids serve as defense compounds. It is, however, difficult to prove that alkaloids are important for the fitness and survival of the plants producing them.

For one group of alkaloids, though, critical data are available. As mentioned before, QA constitute the main secondary products of many Leguminosae, especially in the genera *Lupinus, Genista, Cytisus, Baptisia, Thermopsis, Sophora, Ormosia,* and others

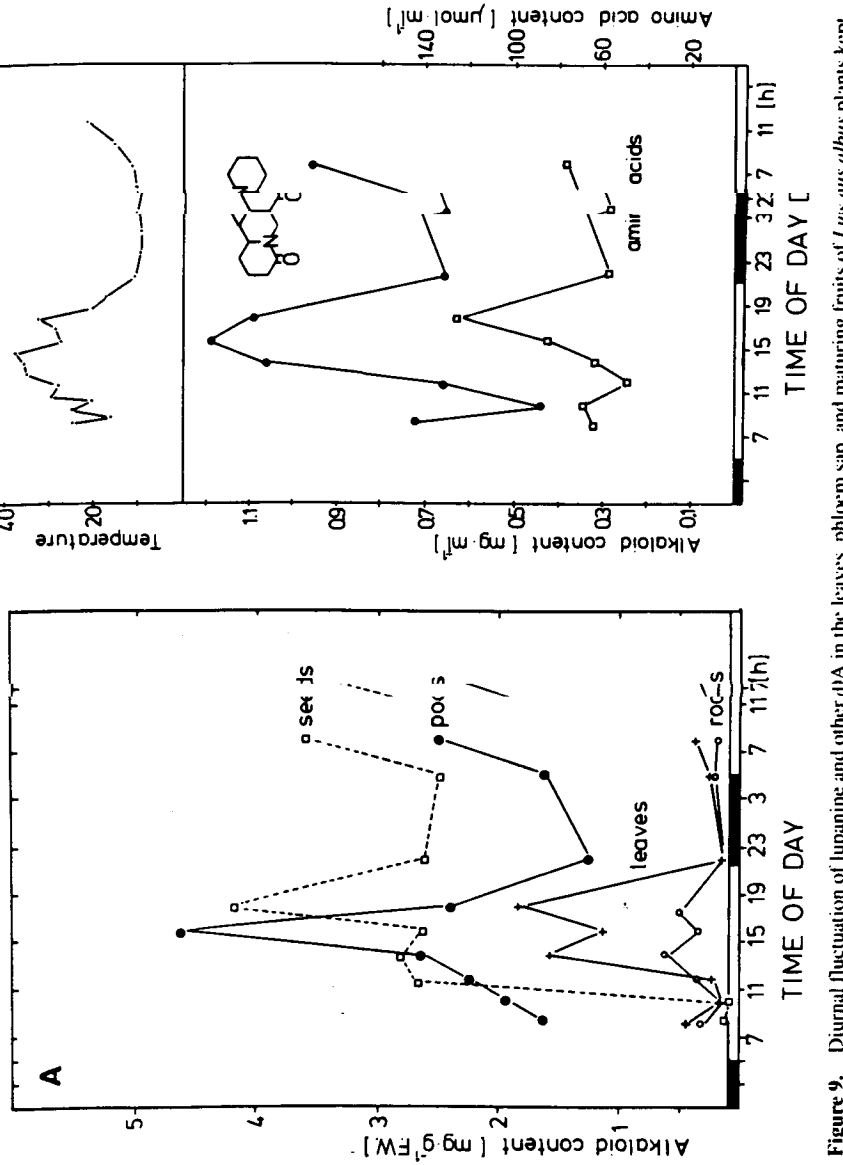

Figure 9. Diurnal fluctuation of lupanine and other dA in the leaves, phloem sap, and maturing fruits of *Lupinus albus* plants kept under natural day–night conditions (after Wink and Witte, 1984). (A) QA fluctuations in leaves, pods, peds, and roots. (B) Lupanine and amino acids in the phloem sap.

(Kinghorn and Balandrin, 1984; Wink, 1993a). Lupins have relatively large seeds that contain up to 40–50% protein, up to 20% lipids, and 2–8% alkaloids. In order to use lupin seed for animal or human nutrition, our ancestors cooked the seeds and leached out the alkaloids in running water. This practice has been reported from Egypt and Greece in the Old World and for the native Indians and Incas of the New World and is still in use today. The treated seeds taste sweet, in contrast to the alkaloid-rich ones, which are very bitter.

At the turn of this century, German plant breeders set out to grow alkaloid-free lupins, the so-called "sweet lupins." Although extremely rare in nature (frequency $<10^{-4}$), the efforts were successful and at present, "sweet" varieties with an alkaloid content lower than 0.01% exist for *Lupinus albus, L. mutabilis, L. luteus, L. angustifolius,* and *L. polyphyllus.* As far as it has been determined, the sweet varieties differ chemically from their original bitter wild forms, only in their degree of alkaloid accumulation. This offers the chance to test whether bitter lupins have a higher ecological fitness than sweet ones.

The results of these experiments were clear cut: When lupins were grown without being fenced in and without protection from man-made chemicals, a dramatic effect was regularly observed, especially with regard to herbivores: Rabbits (*Cuniculus europaeus*) (Fig. 10A) and hares (*Lepus europaeus*) clearly prefer the sweet plants and leave the bitter plants almost untouched, at least as long as there was an alternative food source. A similar result was seen for a number of insect species, such as aphids, beetles, thrips, and leaf-mining flies (Fig. 10B, Table IX), i.e., the sweet forms are attacked, whereas the alkaloid-rich ones were largely protected. For the specialized and adapted aphid (*Macrosiphum albifrous*) the opposite behavior was observed as expected.

A sweet variety of *L. luteus* was infested by *Acyrthosiphon pisii* in Poland. The invasion of the aphids became a serious problem not only because the aphid enfeebled the plant by sucking its phloem sap, but also because it transferred a viral disease (lupin narrow leafness). In a mixed population of sweet and bitter lupins, sweet plants are at a disadvantage and after a few generations will disappear. The infestation by the aphid and the following viral infection accelerated the elimination of alkaloid-poor plants, which, even without infection, are already inferior in seed production. This observation again stresses the importance of alkaloid for the fitness of lupins.

Plant breeders have observed that bacterial, fungal, and viral diseases are more abundant in the sweet forms, but this effect has not been documented in necessary detail. These experiments and observations clearly prove the importance of alkaloids for lupins, but it should be recalled that other secondary metabolites, such as phenolics, isoflavones, terpenes, saponins, stachyose, erucic acid, and phytic acid, are present in lupins which may have additional or even synergistic effects.

The lupin example also illustrates an intrinsic problem of traditional plant breeding. Knowing the ecological importance of QA for the fitness of lupins, it seems doubtful whether the selection of sweet lupins was a wise strategy. In order to grow sweet lupins, fences are needed and worse, man-made chemical pesticides (which have a number of well-documented disadvantages) are needed to substitute for the alkaloids no longer present. It can be assumed that similar strategies, i.e., breeding away unwanted chemical traits, have been chosen with our other agricultural crops, with the consequent reduction in overall fitness. We can easily observe their reduced fitness by trying to leave crop species to themselves in the wild: they will quickly disappear and not colonize new habitats. There are, however, alternatives: Taking lupins as an example, we could devise large-scale

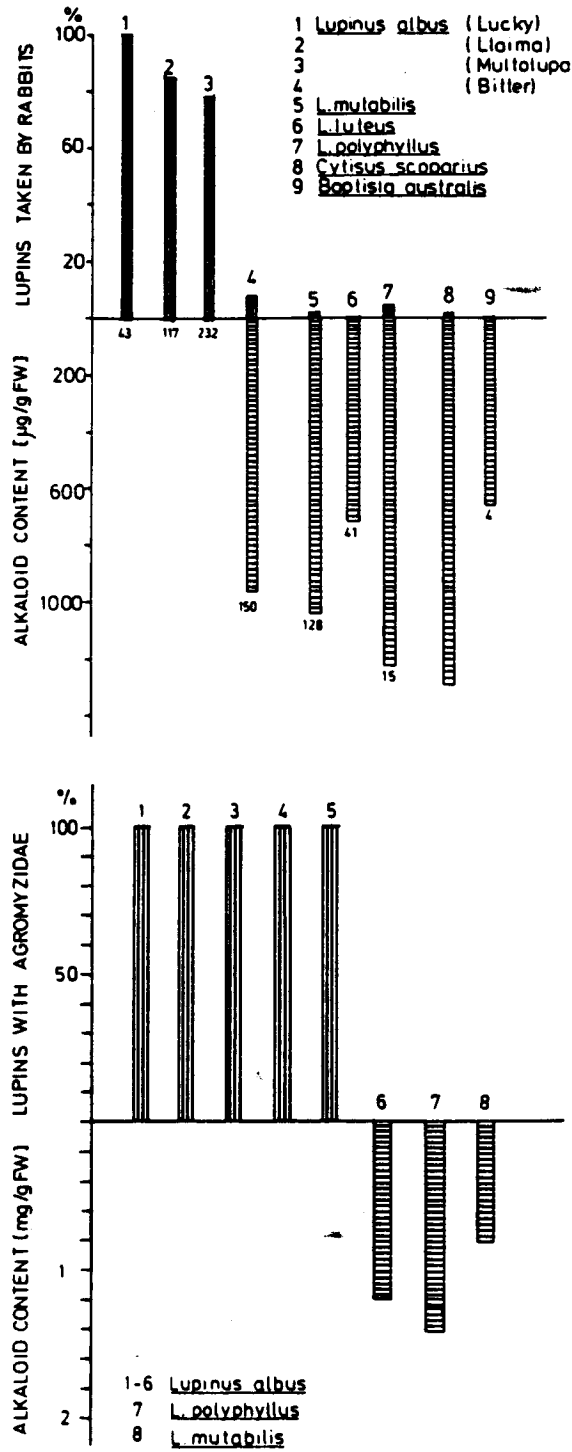

Figure 10. Examples of alkaloid-mediated fitness in alkaloid-rich and alkaloid-poor (bitter versus sweet) lupins (after Wink, 1985, 1987b, 1988). (A) Differential grazing by rabbits; (B) differential infestation by leaf-mining flies (Agromyzidae). Upper panels indicate the degree of herbivory, lower panels the alkaloid content of the respective plants.

Table IX
Correlation between Alkaloid Content and Herbivory in Polyphagous and Specialized Herbivores[a]

Species	Lupin	Alkaloid content	Effect
Nonadapted herbivores			
Vertebrates			
Sheep		Sweet	Sweet lupins are preferred, bitter
		Bitter	discriminated
Lepus europaeus		Sweet	Sweet lupins are preferred, bitter
		Bitter	discriminated
Oryctolagus europaeus	*L. albus*	0.01 mg/g	Herbivory almost 100%
		2.0 mg/g	Herbivory <10%
Insects			
Agromyzidae	*L. albus*	0.01 mg/g	Heavy infestation, 100% incidence
		2.0 mg/g	Infestation < 1%
Sitona lineatus	*L. albus*	<0.02 mg/g	100% herbivory
		1500 mg/g	Low or no herbivory
	L. mutabilis	2500 mg/g	Low or no herbivory
Myzus sp.	*L. luteus*	0.01 mg/g	Infestation 100%
		>0.7 mg/g	Infestation < 1%
Aphis fabae	*L. polyphyllus*	Sweet	Infestation
		Bitter	No infestation
Frankliniella tritici	*Lupinus*	Sweet	Heavy infestation
		Bitter	No infestation
F. bispinosa	*Lupinus*	Sweet	Heavy infestation
		Bitter	No infestation
Adapted herbivores			
Macrosiphum albifrons	*L. albus*	0.01 mg/g	Infestation < 10%
		2.0 mg/g	Infestation 100%
	L. polyphyllus	>1 mg/g	Infestation 80%
	L. angustifolius	1.5 mg/g	Infestation 100%
	L. mutabilis	2.5 mg/g	Infestation 30%

[a]Note that generalists are deterred by alkaloids, whereas specialists are attracted.
Sweet = low-alkaloid variety; bitter = high-alkaloid variety (>0.5%) (after Wink and Römer, 1986; Wink, 1988, 1992).

procedures to remove alkaloids from the seeds after harvest (similar to refining sugar from sugar beets) and to economically produce more valuable products, such as pure protein, lipid, and dietary fibers from bitter seeds. A spin-off product would be alkaloids, which could be used either in medicine (sparteine is exploited as a drug to treat heart antiarrhythmias) or in agriculture as a natural biorational plant protective (Wink, 1993d).

7. CONCLUSION

Although the biological activities of many alkaloids have not yet been studied and their ecological functions remain to be elucidated or proven in most instances, we can, nevertheless, safely conclude that alkaloids are neither waste nor functionless molecules, but that they are important fitness components, probably primarily antiherbivore com-

pounds. As nature obviously favors multitasking, additional activities such as allelopathic or antimicrobial activities are plausible.

As pointed out above, many features of alkaloid physiology and biochemistry only become plausible if this concept is applied. The role of alkaloids in human history (Chapters 2, 3) and their present application in agriculture and medicine (Chapters 17, 18) can be regarded as a benefit that evolved in an ecological context.

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