

## 9B. Ecological Biochemistry: Allelopathy and Defense Against Herbivores

### 1. Introduction

Plants contain a vast array of compounds referred to as **secondary metabolites** that play no role in primary catabolic or biosynthetic pathways. Many of these metabolites influence important ecological interactions (e.g., deterring herbivores, protection against pathogens, allelopathy, symbiotic associations, seed germination of parasites, or interactions with pollinators). Others provide protection against ultraviolet radiation or high temperatures. Some of these roles have already been discussed. This chapter discusses the role of secondary compounds in allelopathic and plant–herbivore interactions. Plant–pathogen interactions are discussed in Chapter 9C.

### 2. Allelopathy (Interference Competition)

Some plants harm the growth or development of surrounding plants by the release of chemical compounds: **allelopathic compounds** or **allelochemicals**. These **allelopathic** effects are invariably negative, and the compounds may come from living roots or leaves or from decomposing plant remains (Fig. 1). Other released compounds may have positive effects, such as the carboxylates that solubilize

phosphate in the rhizosphere or chelate Al metals and avoid Al toxicity (Sects. 2.2.5 and 3.1.2 of Chapter 6 on mineral nutrition). These positive effects are *not* referred to as **interference competition** or **allelopathy** [the word allelopathy is derived from two Greek words: *allelon* (of each other) and *pathos* (to suffer)]. The chemicals involved in positive interactions, however, may still be referred to as allelochemicals.

Many allegedly allelopathic interactions can be explained in other ways. For example, the absence of seedlings near aromatic shrubs that produce volatile growth inhibitors suggested that allelopathy might be involved (Muller et al. 1964), but closer investigation showed that seed-eating animals prefer to graze in the shelter of the shrub, where they are in less danger from predatory birds (Bartholomew 1970). There is general agreement in the literature, however, that allelopathic interactions do exist and can be ecologically important. Both water-soluble compounds (mainly of a phenolic nature) and volatiles (mainly terpenoids) can have an allelopathic effect (Birkett et al. 2001, Bais et al. 2006).

Activated carbon, which adsorbs allelochemicals, has been used to assess the significance of allelopathic interactions in natural ecosystems, for example to study the allelopathic potential of an **invasive weed**, *Centaurea maculosa* (spotted knapweed) in western North America. Root elongation and biomass production of *Festuca idahoensis* (Idaho fescue) plants that were grown together with this

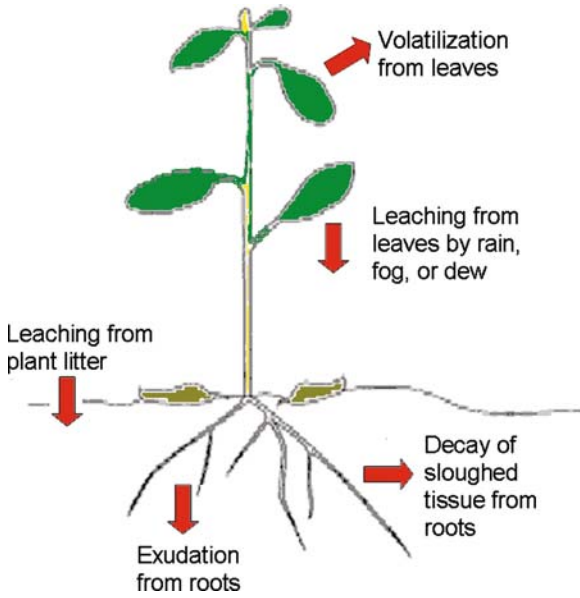


FIGURE 1. Routes of entry of allelochemicals from plants into the rhizosphere.

invasive weed was enhanced in the presence of activated carbon in the root environment (Fig. 2). Using activated carbon, it can be shown that allelopathy accounts for a substantial proportion of the total interference of *Centaurea maculosa* on *Festuca idahoensis*, shifting the balance of competition in favor of the invasive weed. However, *Centaurea maculosa* outperforms *Festuca idahoensis* even in the absence of activated carbon, which shows the combined roles of **resource competition** and allelopathy. Some species, e.g., *Lupinus sericeus* (silky lupine)

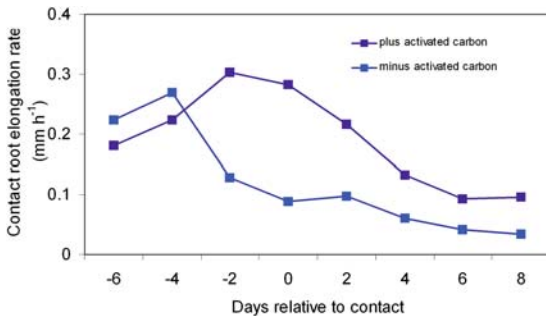


FIGURE 2. Elongation rates of *Festuca idahoensis* (Idaho fescue) roots that made physical contact with *Centaurea maculosa* (spotted knapweed) roots in root observation chambers, with or without activated carbon, from 6 days before until 8 days after contact. Elongation rates of all roots were converted to mm h<sup>-1</sup> and standardized in time by aligning their days of contact as “day 0” (Ridennour & Callaway 2001).

and *Gaillardia grandiflora* (blanketflower) are resistant to the allelochemical [(+)-catechin] released by *Centaurea maculosa*, because they release increased amounts of **oxalate** upon exposure to catechin. Oxalate blocks generation of **reactive oxygen species** and reduces oxidative damage generated in response to catechin (Weir et al. 2006).

Genotypes of one species, e.g., *Triticum aestivum* (wheat) differ substantially in the rate at which they release allelochemical phenolics (Wu et al. 2000a). This characteristic has potential in integrated weed management, because the wheat genotypes that release most phenolics tend to have the greatest capacity to suppress the weedy grass *Lolium rigidum* (annual ryegrass) (Wu et al. 2000b). Benzoxazinoids (cyclic hydroxamic acids) are common allelochemicals in root exudates from *Triticum aestivum* (wheat), *Zea mays* (corn), and *Secale cereale* (rye) (Understrup et al. 2005). In soil, the exudates may be converted into other benzoxazinoids, many with a similar phytotoxic effect (Macías et al. 2005).

Allelopathic compounds may have originally evolved as compounds that deter pathogens or herbivores and subsequently become involved in interactions between higher plants. Secretory glands were well developed in the early gymnosperms and angiosperms of the Paleozoic before there were terrestrial herbivores, but after the evolution of terrestrial fungi which suggests that early defense systems may have been directed at pathogens (Chapter 9C on microbial pathogens; Bais et al. 2004).

The mode of action of most allelopathic compounds is unknown. Many phenolic compounds inhibit seed germination of grasses and herbs, and they may inhibit ion uptake or respiration. Volatile terpenoids can inhibit cell division. Potentially allelopathic compounds can be **detoxified** by some species through mechanisms discussed in Sect. 5.

The allelopathic effects of *Juglans nigra* (black walnut) illustrate the multiplicity of ecological effects. In a zone up to 27 m from the tree trunk, many plants [e.g., *Solanum lycopersicum* (tomato), *Medicago sativa* (alfalfa)] die. The toxic effects are due to the leaching from the leaves, stems, branches, and roots of a bound phenolic compound, which undergoes hydrolysis and oxidation in the soil. The bound compound, which is nontoxic itself, is the 4-glucoside of 1,4,5-trihydroxy-naphthalene. It is converted to the toxic compound juglone (5-hydroxynaphthoquinone). Some species are resistant to juglone [e.g., *Poa pratensis* (Kentucky bluegrass)], probably because they detoxify this allelochemical (Sect. 5). Juglone severely inhibits the relative growth rate, photosynthesis, stomatal conductance, and respiration of *Zea mays* (corn) and *Glycine max* (soybean), when applied at a concentration of 10  $\mu\text{M}$  or more. This concentration can be found in soil under black walnut when it is used in alley cropping (Jose & Gillespie 1998a,b).

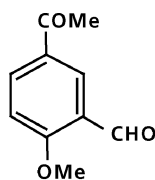
*Sorghum* species have a reputation for suppressing weed growth, due to the exudation of allelochemicals. One of these is a dihydroxyquinone (sorgoleone), which inhibits mitochondrial respiration (Rasmussen et al. 1992) and electron transport in photosystem II (Nimbal et al. 1996), presumably due to the structural similarity between sorgoleone and both ubiquinone and plastoquinone (Sect. 2.1 of Chapter 9D on parasitic associations). Similarly, very few weeds occur under trees of *Leucaena leucocephala* (white leadtree) plantations in Taiwan. This has been ascribed to the presence of high concentrations of mimosine (a toxic nonprotein amino acid) as well as a range of phenolic compounds, which originate from the tree leaves and inhibit germination and growth of many forest species (Table 1).

Allelopathic interactions also appear to play a major role in desert plants [e.g., between *Encelia farinosa* (brittlebush) and its surrounding plants in the Mojave desert in California, USA]. In many of these plants, a simple benzene derivative is produced, primarily in the leaves (Fig. 3). It is released when the leaves fall to the ground and decompose.

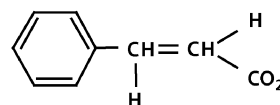
An example of growth inhibition by a toxin produced in roots, rather than leaves, is that of the rubber plant guayule (*Parthenium argentatum*). The aromatic compound (Fig. 3), remarkably, causes inhibition of plants of the same species (**autotoxicity**). Similar examples of autotoxicity have been found for cultivars of *Triticum aestivum* (wheat) in bioassays under laboratory conditions; this suggests that cultivars may have to be selected carefully if wheat is to be used in a continuous cropping system (Wu et al. 2007). In several cucurbit crops [e.g., *Citrullus lanatus* (watermelon), *Cucumis melo* (melon), and *Cucumis sativus* (cucumber)], autotoxicity contributes to "soil sickness"; that is, a reduction in yield when crops are grown on the same plot without rotation (Yu et al. 2000). Cinnamic acid is one of the autotoxic compounds in cucumber; it induces formation of **reactive oxygen species** (ROS) (Ding et al. 2007).

Allelopathic and autotoxic effects probably play a role in many environments; however, it is hard to estimate their ecological significance. Some of the released compounds are probably decomposed rather rapidly by microorganisms, thus diminishing their potential effects. Other allelopathic compounds decompose rather slowly, including a group of phenolic compounds mostly referred to as tannins (Sect. 3.1). The consequences of this slow decomposition for nutrient cycling are discussed in Chapter 10A on decomposition.

Allelochemicals may also affect soil microorganisms and thus indirectly affect surrounding plants. For example, monoterpenes from *Picea abies* (Norway spruce) inhibit **nitrification**, either directly or indirectly due to immobilization of mineral nitrogen (Sect. 2.1.1 in Chapter 6 on mineral nutrition) (Paavolainen et al. 1998). Allelochemicals released



**3-acetyl-6-methoxybenzaldehyde**  
*Encelia farinosa*



**trans-cinnamic acid**  
*Parthenium argentatum*

**FIGURE 3.** Two examples of toxins produced in desert shrubs (Harborne 1988).

**TABLE 1.** The effects of *Leucaena leucocephala* (white leadtree) leaves mixed with 150 g of soil or mulched and spread on the soil surface on survival of seedlings of a number of plant species.\*

Species	Survival (% of the control)		
	Leaves mixed with soil	2 g	Leaf mulch added
	1 g	2 g	5 g
<i>Leucaena leucocephala</i>	100	100	87
<i>Alnus formosana</i>	72	44	37
<i>Acacia confuse</i>	30	19	14
<i>Liquidamber formosana</i>	5	9	31
<i>Casuarina glauca</i>	0	0	0
<i>Mimosa pudica</i>	0	0	0

Source: Chou & Kuo (1986).

\* The data are expressed as percent survival relative to that in the soil alone.

from grass roots may also inhibit **nitrification** (Lata et al. 2004, Subbarao et al. 2006, 2007a). Since  $\text{NO}_3^-$  is far more prone to losses by denitrification and leaching, the biological nitrification inhibitors may enhance the efficiency of N use at the ecosystem level, both in natural and in managed systems. This is why their potential is currently being explored in wild relatives of *Triticum aestivum* (wheat) (Subbarao et al. 2007b).

Exudates released by some plants, e.g., *Eragrostis curvula* (weeping lovegrass) are antagonistic against plant-parasitic nematodes (Chitwood 2002). These **nematicidal** compounds include polythienyls, isothyanatyes, glucosinolates, and a range of other compounds (Sect. 3). Grass species such as *Eragrostis curvula* can be used in rotations to manage nematode problems in cropping systems (Katsvairo et al. 2006).

### 3. Chemical Defense Mechanisms

Many secondary plant compounds play a role in deterring herbivores; however, some herbivores have found ways “to get around the problem” or even prefer the plants that contain specific secondary compounds: **food selection**. Both topics will be discussed in this section.

#### 3.1 Defense Against Herbivores

Chemical defense is quite obvious in poison ivy (*Toxicodendron radicans*) as well as in the stinging

nettle (*Urtica dioica*) and closely related members of the Urticaceae. Touching the nettle breaks off the tip of the hairs on leaves or stem. The walls of these hairs are thin, and contain silica, which gives the cut hair a sharp end to penetrate the skin. The contents of the hair are then released, giving local pain and swelling of the skin. This is a clear example of a **direct defense**. The exact nature of the content of the stinging hairs of *Urtica dioica* is unknown; the older literature suggests biogenic amines, including serotonin, whereas a tropical member of the Urticaceae, *Laporta moroides*, accumulates peptides, including a tricyclic octapeptide (moroidin) (Leung et al. 1986). The number of stinging hairs varies widely in *Urtica dioica*; some plants have none at all. Grazing by large herbivores is negatively correlated with the number of hairs (Pollard & Briggs 1984).

Some secondary compounds inhibit specific steps in mitochondrial respiration. For example, HCN, which blocks cytochrome oxidase, is released from **cyanogenic** compounds that are present in a wide range of species. **Fluoroacetate** (1080), after conversion to fluorocitrate, blocks aconitase, which is an enzyme in the TCA cycle. **Rotenone**, which is an isoflavonoid in roots of *Derris*, *Lonchocarpus*, and *Tephrosia* species (Fabaceae) (Yenesew et al. 2005), blocks the mitochondrial internal NADH dehydrogenase, and **platanetin**, which is a flavonoid from the bud scales of *Platanus acerifolia* (plane tree) (Ravanel et al. 1986), inhibits the mitochondrial external NADH dehydrogenase (Sect. 2.3.1 of Chapter 2B on plant respiration; Roberts et al. 1996). Seeds of a wide range of *Phaseolus* (bean) and *Cicer arietinum* (chickpea) species contain specific inhibitors of  $\alpha$ -amylase, which is a digestive enzyme that hydrolyzes starch (Pueyo & Delgado-Salinas 1997), or of proteinases (Giri et al. 1998). Other secondary plant compounds are much less specific; for example, **tannins** precipitate proteins and thus interfere with food digestion. Toxic phenolic glycosides in *Salix* (willow) species deter herbivores. Others [e.g., glucosinolates in Brassicaceae (cabbage family)] probably evolved as secondary metabolites in plants because they are toxic to most herbivores. The stored glucosinolate **sinigrin** is converted enzymatically to highly toxic allyl isothiocyanate, which gives mustard its distinct sharpness (Fig. 4). In some herbivores mechanisms have evolved, however, that defy this chemical defense and use glucosinolates as **attractants**. In *Brassica oleracea* (cabbage) and other Brassicaceae, sinigrin attracts butterflies of *Pieris brassicae* (cabbage moth) as well as certain aphids (e.g., *Brevicoryne brassicae*) and cabbage-root flies (*Delia radicum*). Cabbage moths normally deposit their eggs only on plants

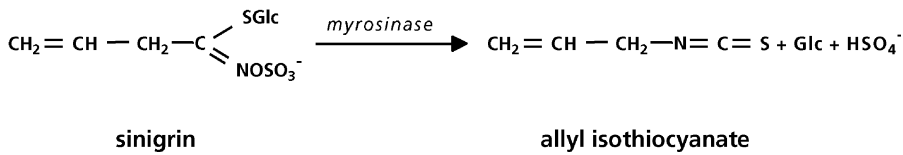


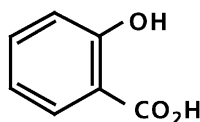
FIGURE 4. The chemical structure of sinigrin, which is a glucosinolate in *Brassica* (cabbage) species, and allyl isothiocyanate, into which it can be converted. The reaction is catalyzed by endogenous  $\beta$ -thioglucosidases

(myrosinases) that are localized in “myrosin” cells, scattered throughout most plant tissues. Within these cells the enzyme is stored inside myrosin grains (Rask et al. 2000).

that contain sinigrin, but accept filter paper that contains this compound as a substitute. Their larvae exclusively eat food that contains sinigrin, either naturally or experimentally added (Van Loon et al. 1992).

**Cyanogenic glucosides** are widespread in the plant kingdom, whereas **glucosinolates** are evolutionarily younger and found in Brassicaceae and one outgroup, the genus *Drypetes* of the Euphorbiaceae (Halkier & Gershenzon 2006). Because both groups of natural products are derived from **amino acids** and have aldoximes as intermediates, it has been hypothesized that glucosinolates developed based on a predisposition for making cyanogenic glucosides. Consistent with an evolutionary relationship between the cyanogenic glucoside and glucosinolate pathways, the aldoxime-metabolizing enzymes in both pathways belong to the same gene family. A mutation in the aldoxime-metabolizing enzyme in the cyanogenic pathway may have resulted in the production of toxic compounds, which the plant subsequently had to get rid of, instead of the original hydroxynitrile in the pathway toward cyanogenic glucosides (Halkier & Gershenzon 2006).

Many plants contain defensive phenolics [e.g., tannins in leaves of *Quercus* (oak)]. In the bark of *Picea abies* (Norway spruce) clones that are resistant to *Ceratocystis polonica* (a fungal pathogen that is transmitted through bark beetles) specialized phloem-parenchyma cells contain deposits of



salicylic acid

FIGURE 5. The chemical structure of salicylic acid, which is produced after ingestion from some of the phenolic glycosides that regularly occur in *Populus* and *Salix* species. Salicylic acid is closely related to acetylsalicylic acid, which is the active ingredient of aspirin.

polyphenols. These parenchyma cells are enriched in phenylalanine ammonia lyase, which is a key enzyme in the synthesis of phenolics. Susceptible clones have much less of these **polyphenol-containing parenchyma cells**. The phenolics in the resistant clone are mobilized upon fungal attack which indicates that the specialized parenchyma cells are an important site of both **constitutive** and **inducible** defense (Franceschi et al. 1998).

Both *Populus* (poplar) and *Salix* (willow) plants contain a wide range of toxic phenolic glycosides, including salicin (Clausen et al. 1989). After ingestion, salicin is hydrolyzed and oxidized, producing **salicylic acid** (Fig. 5), which **uncouples oxidative phosphorylation** in mitochondrial preparations. In addition, salicylic acid is associated with stress signaling and systemic acquired resistance (Heil & Baldwin 2002). The structure of phenolic glycosides resembles that of many allelopathic compounds which suggests that the driving force in evolution for the formation of allelopathic compounds may well have been their role in deterring herbivores or pathogens (Bais et al. 2004, 2006). Both the total phenolic glycoside concentration in the leaves and the composition of these compounds vary among *Salix* species (Table 2).

The role of phenolic glycosides in the **food-selection** pattern of beetles feeding on willow leaves has been investigated extensively. Leaves of the eight willow species shown in Table 2 were used for laboratory feeding experiments with four beetle species. In all cases, the leaves of the *Salix* species that is chemically most related to the preferred species are fed on to the highest degree (Fig. 6). Both the total amount and the quality of the phenolic glycosides determine the food-selection pattern of the investigated beetles.

Mammals have been important selective influences for the patterns of defense in woody plants, which are vulnerable to mammalian herbivory throughout the winter. Mammalian herbivory is a major cause of mortality in woody plants, in part because mammals remain active and often have

TABLE 2. Phenolic glycoside concentration [mg g<sup>-1</sup> (dry mass)] in the leaves of eight *Salix* (willow) species that are native to Finland or have been introduced to this area.\*

	Salicortin	Salicilin	Fragilin	Triandrin	Salidroside	Picein	Total
<b>Native willows</b>							
<i>S. nigricans</i>	48	3	0.2				51
<i>S. phylicifolia</i>	0.5	0.1	0.1	0.3	0.1		1.8
<i>S. caprea</i>	0.3	0.2	0.1	0.1			1.2
<i>S. pendandra</i>		0.7	0.7				7.6
<b>Introduced willows</b>							
<i>S. cv. aquatica</i>	6.4	1.3	0.1				7.8
<i>S. dasylados</i>	9.9	2.0	0.2				12.1
<i>S. viminalis</i>	0.1		0.1	0.1		0.2	1.5
<i>S. triandra</i>		0.3			7.4		7.8

Source: Tahvanainen (1985).

\* Apart from these identified compounds, some others were present, so that the total amount differs from the sum of the identified ones.

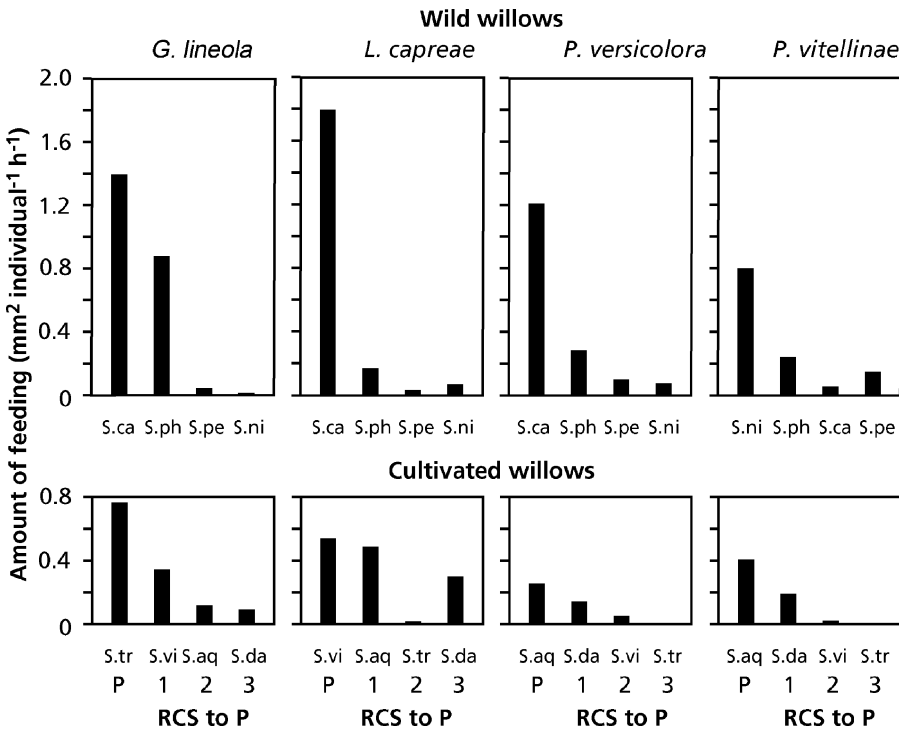


FIGURE 6. Food-selection pattern by four beetle species (*Galerucella lineola*, *Lochmaea capreae*, *Plagiodera versicolora*, and *Pratora vitellinae*) when leaves of four native and four introduced *Salix* (willow) species are offered in two separate food choice experiments. The

preferred species is placed at the left; the others are ranked according to their chemical similarity to the preferred species. The species are the same as those presented in Table 2 (Tahvanainen et al. 1985).

highest energy demand in winter, when plants cannot grow to compensate for tissues lost to herbivores. Woody plant defenses are better developed in regions with a long history of vertebrate browsing than in regions that were glaciated during the Pleistocene (Bryant et al. 1989). There is strong developmental control over defenses in woody plants, with these being most strongly expressed in juvenile woody plants that grow in a height range where they are vulnerable to mammalian herbivores. After browsing, juvenile shoots are produced that have higher levels of secondary metabolites that deter further browsing. These defenses include ether-soluble terpenes [e.g., papyriferic acid in *Betula resinifera* (paper birch) and pinosylvin in *Alnus viridis* subsp. *fruticosa* (green alder)] that deter feeding below levels required for weight maintenance, and, if consumed, result in a negative N and Na<sup>+</sup> balance (Bryant et al. 1992).

There is some evidence that plants that hyperaccumulate **heavy metals** [e.g., Ni-accumulating Brassicaceae (cabbage family); Sect. 3.3 of Chapter 6 on mineral nutrition] are better protected against herbivores (Jhee et al. 2005).

### 3.2 Qualitative and Quantitative Defense Compounds

Secondary metabolites involved in deterring herbivores can be divided into two categories:

1. **Qualitatively** important secondary plant compounds. These are **toxins**, which are usually present in low concentrations, but may constitute up to 10% of the fresh weight of some leaves or seeds. Numerous compounds belong to this category, including **alkaloids** (Fig. 7), **cyanogenic glycosides**, **nonprotein amino acids**, **cardiac glycosides**, **glucosinolates** (Fig. 4), and proteins. Their mode of action varies widely.
2. **Quantitatively** important secondary plant compounds. These reduce the **digestibility** and/or **palatability** of the food source and invariably make up a major fraction of the biomass. They

are mostly phenolic compounds (phenolic acids, tannins, lignin; Fig. 8) or terpenoid resins (Dell & McComb 1974). Tannins and some other phenolics reduce the digestibility of plant tissues by blocking the action of digestive enzymes, binding to proteins being digested, or interfering with protein activity in the gut wall. Tannins, as well as lignin, also increase the leaf's toughness.

If one considers that relatively few resources are required to acquire protection against herbivores by toxic compounds, one may wonder why the alternative strategy of the digestibility-reducing compounds, which requires far greater investment of carbon resources, has evolved at all. The answer to this question is that there are numerous examples of herbivores in which mechanisms have evolved to cope with the toxic compounds that are effective against most herbivores. These herbivores may metabolize the toxin to an extent that it is used as a food source, they may store the toxin, sometimes after slight modification, and thus gain protection themselves, or they rapidly excrete the toxic compound. Such combinations of toxic plants and animals that cope with the toxin provide examples of **co-evolution** of plants and animals in an ever-continuing "arms race" (Ehrlich & Raven 1964).

Although the distinction between qualitative and quantitative defenses is a useful starting point, it is not a clear-cut dichotomy. Many phenolic compounds also have toxic effects on herbivores and may be more toxic against some herbivores than others (Ayres et al. 1997), and some cyanogenic glycosides or alkaloids accumulate to rather high levels in some species [e.g., prunasin in *Eucalyptus cladocalyx* (sugar gum) (Gleadow et al. 1998) and nicotine in *Nicotiana attenuata* (wild tobacco) (Baldwin 1999)].

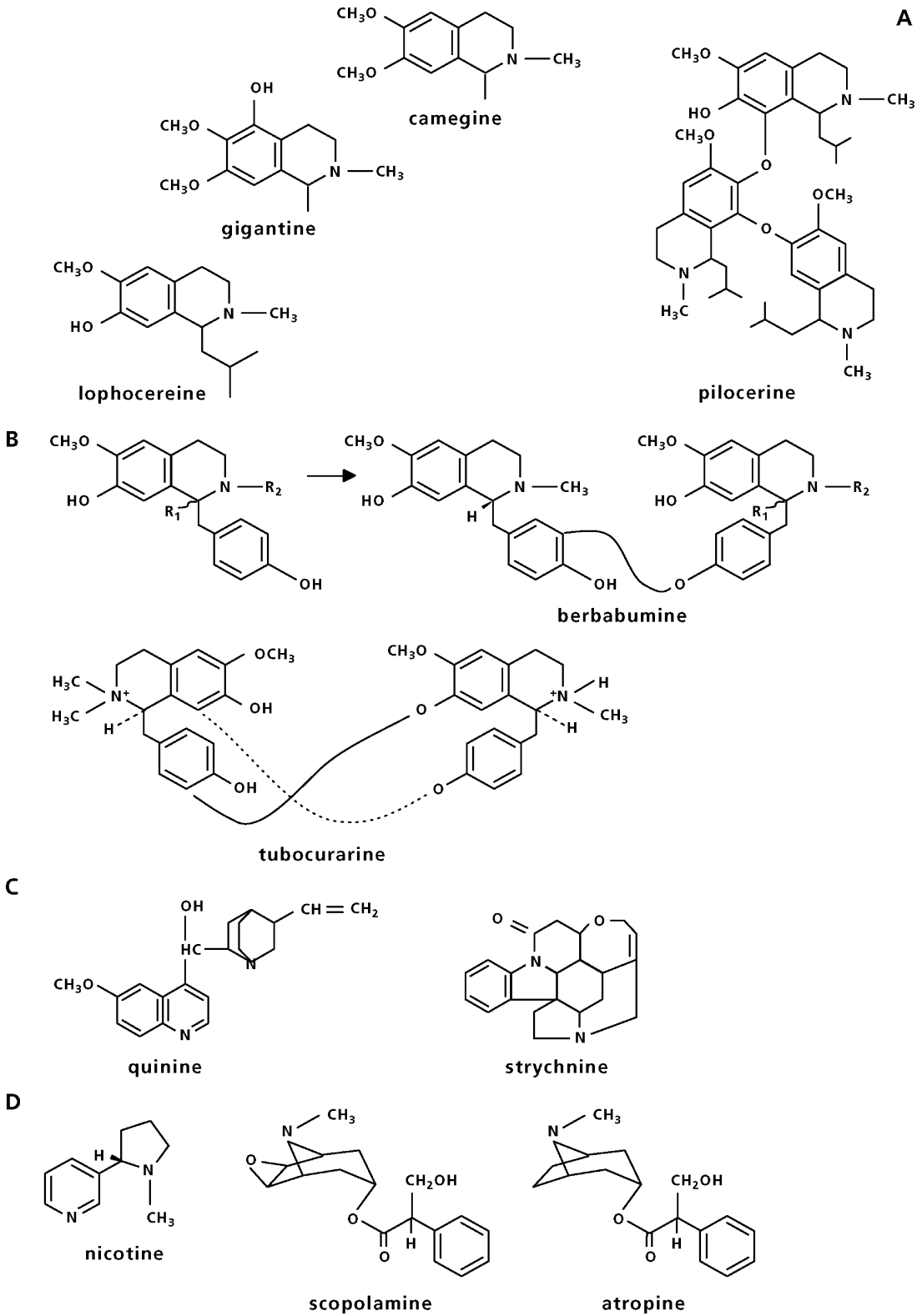
### 3.3 The Arms Race of Plants and Herbivores

The expression "arms race" graphically describes the continuous evolution of ever more toxic

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**FIGURE 7.** Alkaloid subclasses. (A) Isoquinoline alkaloids. These are synthesized (e.g., carnegine and gigantine in a species-specific manner in saguaro (*Carnegia gigantea*) and cardon (*Pachycereus pringlei*) cacti). Sentia cactus (*Lophocereus schottii*) contains as much as 30–150 mg g<sup>-1</sup> (DM) lophocerine and its trimers, pilocereine and piloceredine. (B) Bisbenzylisoquinoline alkaloids. Examples include berbaminine from barberry (*Berberis stolonifera*) and tubocurarine, an arrow poison, from

*Chondrodendron tomentosum*. (C) Monoterpene indole alkaloids, including quinine (from *Cinchona officinalis*) and strychnine (from *Strychnos nux-vomica*). (D) Nicotine and tropane alkaloids. These are naturally occurring insecticides and feeding deterrents in Solanaceae [e.g., nicotine in *Nicotiana tabacum* (tobacco), scopolamine in *Hyoscyamus niger* (henbane), and atropine in *Atropa belladonna* (deadly nightshade)] (Harborne 1988, Schuler 1996).





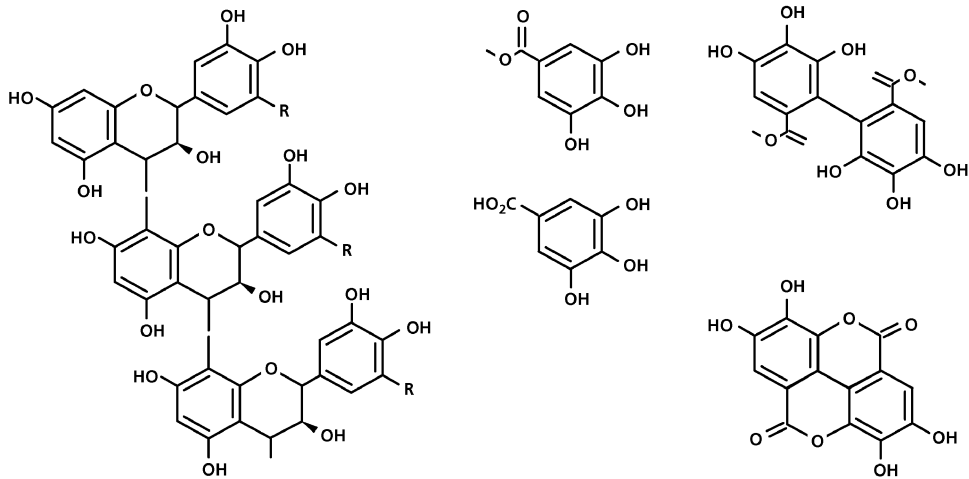


FIGURE 8. The chemical structure of proanthocyanidin (condensed tannin) (left). Gallotannin (top, middle) and ellagitannin (top, right) are hydrolyzable tannins,

releasing gallic acid (bottom, middle) and ellagic acid (bottom, right), respectively, and the esterified sugar(s), mostly glucose, upon hydrolysis.

defense compounds in plants and of more mechanisms to cope with these compounds in herbivores. Numerous examples of such a **co-evolution** exist, but they appear to be restricted to mechanisms in herbivores that store, detoxify, or excrete qualitative defense compounds, with very little evidence for evolutionary escape from quantitative defenses. We will first present a number of striking examples of co-evolution of predators coping with qualitative defenses.

Whereas the stinging hairs on members of the Urticaceae protect the plants against large herbivores, some caterpillars (e.g., those of *Inachis io*, *Vanessa atalanta*, and *Aglais urticae*) are not affected by them. Some of these caterpillars simply bite the hairs off. Snails (e.g., *Arion ater* and *Agriolimax columbianus*) are also little affected by the leaf hairs on *Urtica dioica* (stinging nettle) (Cates & Orians 1975). Plants and herbivores, particularly insects, are in a continuous battle. From a plant's perspective, success in this interaction is determined by its ability to defend itself from devastation by insect feeding. From an insect's perspective, success is measured by its ability to protect itself from a variety of toxic plants defense compounds, thereby allowing it to use specific plants as its sole food source.

One example of co-evolution that involves defensive secondary plant compounds is that of marsupials and several other native animal species in Western Australia that are resistant to the very poisonous **fluoroacetate**, which is a potent inhibitor of an enzyme of the TCA cycle (aconitase). Fluoroacetate occurs in some leguminous shrubs (mainly

*Gastrolobium* species) of the Western Australian flora, and is poisonous to introduced cows, sheep, and feral animals (Twigg & King 1991, Twigg et al. 1999). Consequently, fluoroacetate (1080) can be used to control feral animals, e.g., rabbits, foxes, and pigs, without harming native animals; however, there is a looming risk of resistance building up in rabbits (Twigg et al. 2002). Another well-studied example of co-evolution is the combination of *Senecio jacobaea* (tansy ragwort) and *Tyria jacobaea* (cinnabar moth) (Hartmann 1999). The *Senecio jacobaea* plants contain at least six pyrrolizidine **alkaloids** (Fig. 9). Alkaloids are characterized by a N-containing heterocyclic ring and their alkaline reaction. They represent the largest (>12000 structures) and one of the most structurally diverse groups of substances that serve as plant defense agents (Schuler 1996, De Luca & St Pierre 2000). The highly toxic alkaloids from *Senecio* may cause damage to the liver. The larvae of *Tyria jacobaea* are not harmed by these alkaloids and use *Senecio jacobaea* as a **preferred food source**; they sometimes consume the leaves of *Senecio vulgaris* (groundsel) or *Petasites hybridus* (coltsfoot) as alternative food sources. They accumulate the toxins, which even end up in the mature butterfly. Both the larvae and the butterfly are poisonous to birds. The toxic nature of these animals coincides with black and bright yellow warning coloration (visual advertisement). In addition to the larvae of *Tyria jacobaea*, there are some other animals that cope with the toxic alkaloids in *Senecio* [e.g., the tiger moth (*Arctia caja*) and the flea beetle (*Longitarsus jacobaea*)].

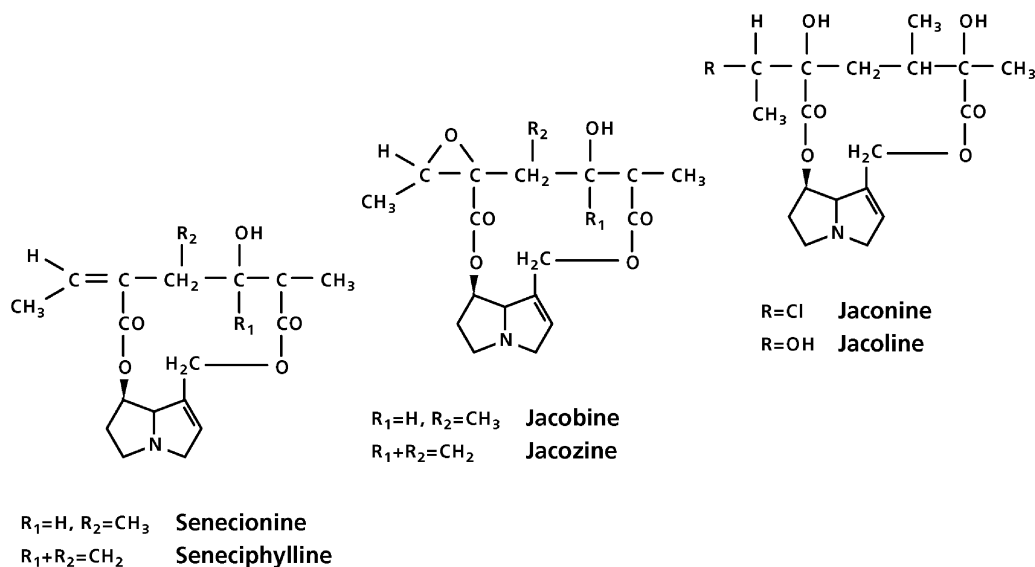


FIGURE 9. The chemical structure of some pyrrolizidine alkaloids from *Senecio jacobaea*.

The interaction of *Asclepias curassavica* (milkweed) and *Danaus plexippus* (the monarch butterfly) is similar to that of *Senecio jacobaea* and *Tyria jacobaea*; the *Asclepias curassavica*–*Danaus plexippus* interaction has an interesting additional dimension in that it is exploited by *Limenitis archippus* (the viceroy butterfly). The milk sap of *Asclepias curassavica* plants contains **cardiac glycosides** (calotropine and calactine). Cardiac glycosides (cardenolides) are bitter compounds that stimulate the heart when applied in small doses, but are lethal in slightly higher doses; the structure of some cardiac glycosides is given in Fig. 10. The presence of these toxic compounds in the larvae of *Danaus plexippus* is again advertised; moreover, caterpillars of the viceroy butterfly have similar colors, but without containing any cardiac glycosides (**mimicry**).

Being able to cope with toxic plants does not invariably lead to accumulation of the toxin. Larvae of the beetle *Caryedes brasiliensis* from Costa Rica largely feed on the seeds of *Dioclea megacarpa*. These seeds contain canavanine, a toxic **nonprotein amino acid** that resembles arginine (Fig. 11) and may constitute as much as 7–10% of the seed fresh mass. Nonprotein amino acids are toxic because they act as “antimetabolites”. That is, their structure is recognized as the same as that of the amino acid they resemble which leads to proteins without the same tertiary structure and function of the protein containing the normal amino acid. Resistance of the larvae of *Caryedes brasiliensis* is based on two

principles. First, the larvae have a slightly different tRNA synthetase, which recognizes arginine as being different from canavanine. Second, they have high levels of the enzyme urease, which breaks down canavanine. Thus, the toxin is a major N source for the larvae.

These few examples selected from a wide range show that one or more animal species have invariably co-evolved with a plant species producing a toxin. Thus, while **qualitative defense** against herbivores requires relatively little investment of resources, it is also a vulnerable strategy. Although there are some examples of animals coping with large quantities of digestibility-reducing and unpalatable compounds (**quantitative defense**), these examples are rare. Hence, the strategy that requires a major investment of carbon is most certainly the safest. A large investment of carbon in protective compounds and structures inevitably goes at the expense of the possibility of investment of carbon in growth. It is therefore most predominant in slow-growing species, especially those with evergreen leaves with a long life span (Bryant et al. 1983, Wright & Cannon 2001, Lambers & Poorter 2004). On the other hand, toxins are found in both fast-growing and slow-growing species. In the evergreen *Ilex opaca* (American holly) the toxic saponins are only found in young leaves and in the mesophyll cells of older leaves. Nonmesophyll cells of older leaves contain digestibility-reducing compounds like lignin, crystals, and tannin (Kimmerer & Potter 1987).

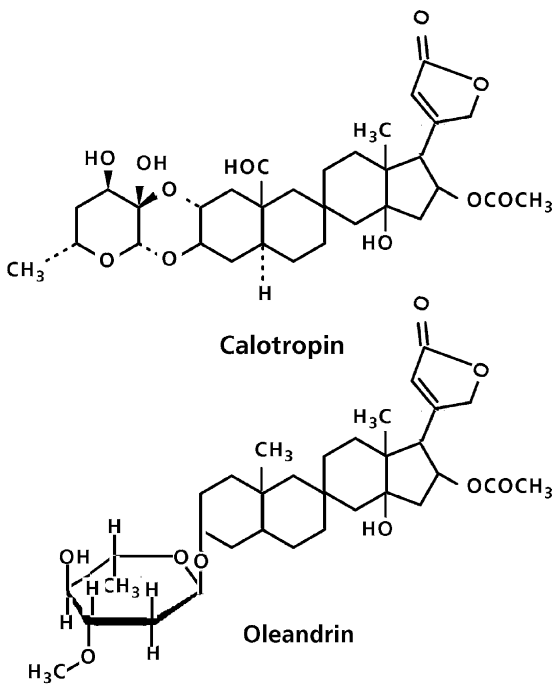


FIGURE 10. The chemical structure of some cardiac glycosides, including calotropin from *Asclepias curassavica* (milkweed).

### 3.4 How Do Plants Avoid Being Killed by Their Own Poisons?

Most secondary plant compounds that deter herbivores are also toxic to the plants themselves. Prussic acid (HCN) is produced upon ingestion of plant material of approximately 2000 species from some 110 families, including genotypes of *Trifolium* spp. (clover), *Linum usitatissimum* (flax), *Sorghum bicolor* (millet), *Pteridium aquilinum* (bracken fern), and *Manihot esculenta* (cassava). If HCN inhibits several enzymes in both animals and plants (e.g., cytochrome oxidase and catalase), and this also holds for plants that contain the cyanogenic compounds, how do cyanogenic plants protect themselves from this toxic HCN?

Cyanogenic plants do not actually store HCN, but contain **cyanogenic glycosides** (i.e., cyanide attached to a sugar moiety) or **cyanogenic lipids** (in Sapindaceae), and these only produce HCN upon hydrolysis. The reaction is catalyzed by specific enzymes (e.g., linamarase, which catalyzes hydrolysis of linamarin in some legumes) (Fig. 12). Synthesis of many cyanogenic compounds requires amino acids as precursors, as Fig. 12 illustrates for

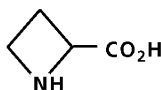
the synthesis of linamarin from valine. The enzymes responsible for the breakdown of the cyanogenic compound and the cyanogenic compounds themselves occur in different cell compartments. Upon damage of the cells, such as after ingestion, the enzyme and its substrate come into contact. For example, dhurrin, which is a cyanogenic glycoside in *Sorghum* species, occurs exclusively in the vacuole of leaf epidermal cells, whereas the enzyme responsible for its hydrolysis is located in mesophyll cells. Linamarase, hydrolyzing linamarin, occurs in the walls of mesophyll cells, whereas its substrate is stored inside the cell. As long as this strict **compartmentation** between cyanogenic compounds and hydrolyzing enzymes is maintained, no problem arises for the plant itself. The linamarin (monoglucoside of acetone cyanohydrin) that is found in the roots of *Hevea brasiliensis* (rubber tree) and *Manihot esculenta* (cassava), however, is synthesized in the shoot and imported via the phloem. In the rubber tree the transport compound is linustatin, which is a nonhydrolyzable diglucoside of acetone cyanohydrin, rather than the hydrolyzable linamarin itself. Transport as the diglucoside avoids the risk of HCN production during transport from leaf cells, via the phloem, to the roots (Selmar 1993).

Although avoidance of damage by compartmentation is the best strategy, some **detoxification mechanisms** may be needed. Detoxification of HCN in plants is possible; it is catalyzed by  $\beta$ -cyano-alanine synthase, transforming L-cysteine + HCN into  $\beta$ -cyano-alanine. The N in cyanogenic compounds that are stored in seeds, can therefore be remobilized and incorporated into primary nitrogenous metabolites (Selmar et al. 1988, 1990). In addition, in vegetative plant organs, cyanogenic compounds may be subject to some turnover.

**Resistance** against cyanogenic glycosides in animals is based on the presence of the enzyme rhodanese (e.g., in sheep and cattle). It catalyzes the transformation of cyanide to thiocyanate. The sulfur required for this reaction comes from mercaptopyruvate. Treatment of patients suffering from HCN poisoning is based on the same principle when thiosulfate is administered to the victim.

In *Trifolium* (clover) species, as well as in others, polymorphism for cyanogenesis has been found. Genotypes are cyanogenic only when they are homozygous for both the recessive gene responsible for the production of linamarase and for the recessive gene responsible for linamarin hydrolysis. In southern Europe cyanogenic genotypes are predominant, except at higher locations. In northern and western Europe, most genotypes are acyanogenic

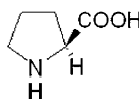
## Nonprotein amino acid

 $\beta$ -cyanoalanineazetidine  
2-carboxylic acid

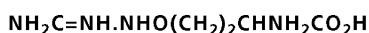
## Protein amino acid



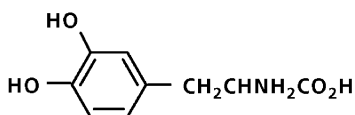
alanine



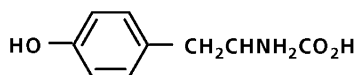
proline



canavanine

3,4-dihydroxyphenylalanine  
(L-DOPA)

arginine



tyrosine

FIGURE 11. Some examples of nonprotein amino acids from higher plants, including canavanine from *Dioclea megacarpa*. The structure of the corresponding ordinary

amino acids is also given for comparison (Harborne 1988).

(Kakes 1990). This correlation (with temperature), however, has not yet been explained in a satisfactory manner. There may be other factors involved, such as in the case of *Hevea brasiliensis* (rubber tree), which releases HCN when it is infected by a pathogenic fungus (*Microcyclus ulei*). HCN then interferes with both the plant host and the fungal pathogen. Because of its inhibition of cytochrome oxidase, this inhibits energy-requiring defense responses, hampering the plant's ability to ward off the fungus (Lieberi et al. 1989). Being cyanogenic would then have a disadvantage. It is therefore possible that the correlation of genotype with temperature reflects the temperature dependence of a pathogenic organism.

Like cyanogenic compounds, many alkaloids are also stored in specific compartments (i.e., either

the vacuole or smaller vesicles in which they are produced). In *Papaver somnifera* (opium poppy), laticifers contain abundant vesicles that both contain morphine and the enzymes to synthesize and metabolize it. In *Berberis wilsoniae* (barberry), *Thalictrum glaucum* (rue), and many other species cells have similar "alkaloid vesicles", which contain berberin or other alkaloids and some of the enzymes of the pathway that produce them. The "alkaloid vesicles" may fuse with the central vacuole and thus deposit the alkaloids there (Hashimoto & Yamada 1994).

**Ricin** is a highly toxic and abundant protein in seeds of *Ricinus communis* (**castor bean**). Ricin is a ribosome-inactivating protein; similar proteins occur in taxonomically and ecologically diverse species, including crop plants (Hartley et al. 1996). Ricin

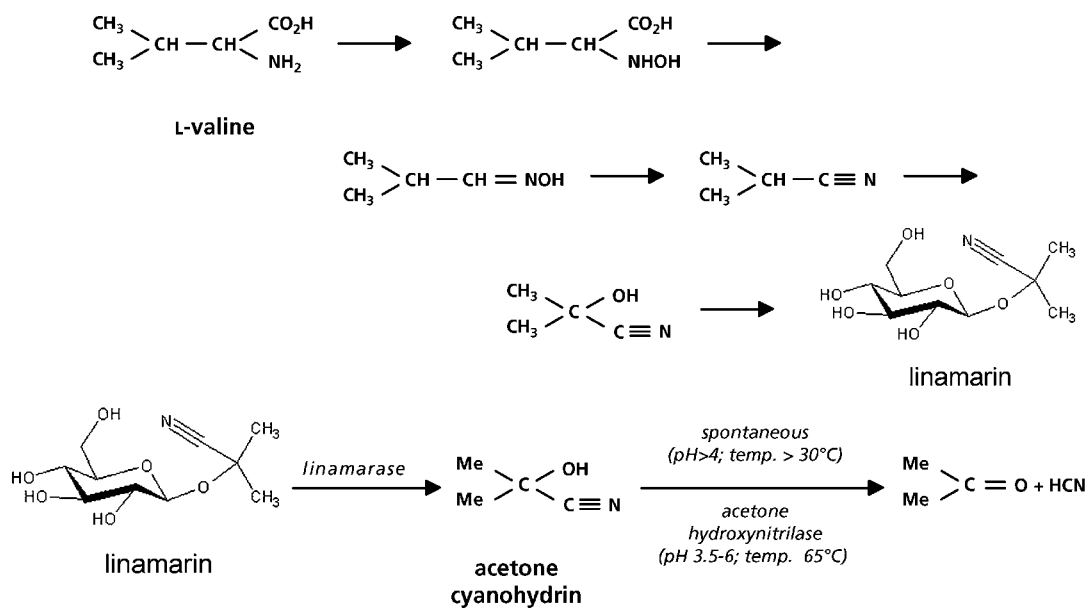


FIGURE 12. In the synthesis of linamarin (a cyanogenic glucoside) the amino acid valine is used as a precursor. The release of HCN from linamarin is catalyzed by a specific enzyme, linamarase (McMahon et al. 1995).

is a heterodimeric protein that consists of an enzymatic polypeptide that destroys ribosomal RNA; it is covalently bound to a galactose-binding lectin [lectins are proteins with noncatalytic sugar-binding domains; the first ones were discovered in *Ricinus communis* (castor bean) more than a century ago; numerous other plants were found to contain lectins since then (Etzler 1985); see also Sect. 3.3]. This bipartite structure and functional properties allow ricin to bind to galactosides on the cell surface. Upon binding, ricin enters the cell via endocytotic uptake and traverses an intracellular membrane to deliver the enzymatic component to the cytosol. Once it is there, it irreversibly inhibits protein synthesis, followed by death of the cell. Ricin is one of the most potently toxic compounds known, and entry of a single toxin molecule into the cytosol may be sufficient to kill the cell. *Ricinus* ribosomes that synthesize ricin are also susceptible to the catalytic action of this protein. How, then, does *Ricinus* avoid suicide? The subunits of which the heterodimer is composed are originally synthesized together in the form of a single precursor protein: proricin. Proricin is an active lectin, but it does not bind to ribosomal RNA. It is transported to the vacuole, where acidic endoproteases remove amino acid residues to generate the heterodimer: ricin. None of the ricin appears to escape from the vacuole (Lord & Roberts 1996).

### 3.5 Secondary Metabolites for Medicines and Crop Protection

Secondary metabolites that deter herbivores or inhibit pathogens have been used by humans for a very long time. The bark of willow (*Salix*) contains **salicylic acid** (Fig. 5), which is closely related to acetylsalicylic acid (**aspirin**) and has been used as medicine. Quinine, which is an alkaloid from the bark of *Cinchona officinalis* (quinine), has been used for centuries to combat **malaria**. Artemisinins are extracted from *Artemisia annua* (sweet wormwood); they are potent antimalarials, rapidly killing all asexual stages of *Plasmodium falciparum* (Eckstein-Ludwig et al. 2003). Other examples of secondary compounds used as medicine are included in Table 3; some of these are still used [e.g., atropine from *Atropa belladonna* (deadly nightshade)]. Others are used because of their antitumor activity [e.g., the diterpene taxol from *Taxus brevifolia* (western yew), and other *Taxus* species (Heinstein & Chang 1994)]. Many more compounds, as-yet-undiscovered, may well be found to have similar effects, as long as the species that contain them do not become extinct, thus offering a strong argument for plant conservation. About 25% of currently prescribed medicines originate from plant compounds that evolved as defenses against herbivores (Dirzo and Raven 2003).

TABLE 3. Examples of secondary metabolites for which man has found some use.

Chemical compound	Species	Applications
Salicylic acid	<i>Salix</i> sp., <i>Populus</i> sp.	Pain killer
Aconitine	<i>Aconitum napellus</i>	Pain killer
Atropine	<i>Atropa belladonna</i>	Ophthalmology
Cytisine	<i>Cytisus laburnum</i>	Migraine
Germerine, protoveratrine	<i>Veratrum album</i>	Muscle diseases, pain killer
Cardiac glycosides	<i>Digitalis</i> sp., <i>Asclepias</i> sp.	Heart diseases
Linarine, linine	<i>Linaria vulgaris</i>	Hemorrhoids
Quinine	<i>Cinchona officinalis</i>	Malaria
Atropine	<i>Atropa belladonna</i>	Poisoning
Taxine	<i>Taxus baccata</i>	Poisoning (arrowheads of Celts)
Cicutoxin	<i>Cicuta virosa</i>	Poisoning (of Socrates)
Hyoscyamine, scopolamine	<i>Hyoscyamus niger</i>	Poisoning (in Shakespeare's "Hamlet")
Pyrethrins	<i>Chrysanthemum cinearifolium</i>	Insecticide
Rotenone	<i>Derris</i> sp., <i>Lonchocarpus</i> sp.	Rat and fish poisoning, pesticide
Camphor	<i>Cinnamomum camphora</i>	Moth balls

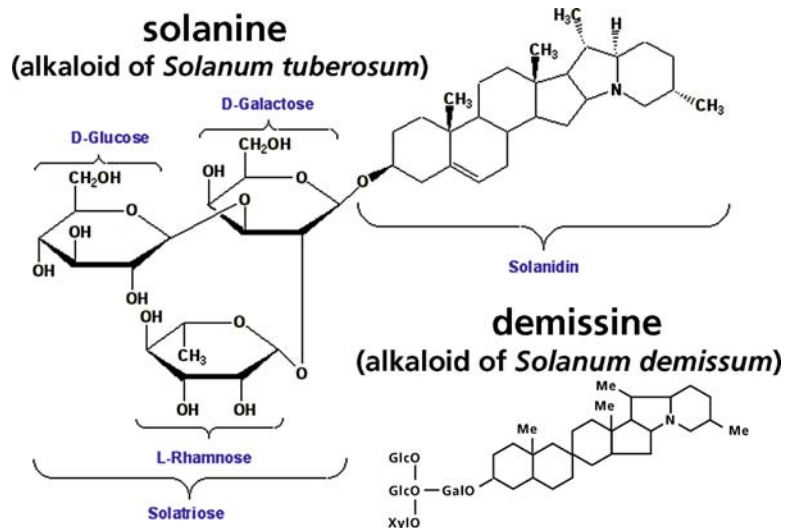
Humans have also found other uses for secondary metabolites, some of these in ancient history, such as taxine [from *Taxus baccata* (yew)] to make arrowheads poisonous, and alkaloids [from *Conium maculatum* (poison hemlock)] to poison Socrates. One of the more recent applications includes the now widespread use of pyrethrins from *Chrysanthemum cinerariifolium* (Dalmatian chrysanthemum) as an "environmentally friendly" insecticide. Over 800 compounds have been reported in the Asteraceae, including nematicides [e.g., thiarubrine and terthieryl in the roots of *Calendula officinalis* (marigold)], fungicides, and bactericides (Flores et al. 1999).

The ancestors of our food plants also contain many toxic compounds, including alkaloids in *Solanum lycopersicum* (tomato) and *Solanum tuberosum* (potato) (Fig. 13). Breeding has greatly reduced the alkaloid levels in tomato and potato, so that food poisoning by potatoes, which was known until the beginning of the 20th century, no longer occurs. Whenever wild species are used to make new crosses, however, new cultivars emerge that may produce poisonous solanine. It is well known that the majority of pyrrolizidine alkaloids cause serious diseases in domestic animals and humans through liver bioactivation. Grazing animals, however, usually avoid plants with high levels of pyrrolizidine alkaloids, unless there is shortage of other herbaceous food, apparently because of their deterrent taste (Hartmann 1999).

Cyanogenic glycosides (Sect. 3.4) in *Manihot esculenta* (cassava), *Sorghum bicolor* (millet), and *Vicia faba* (broad bean) are made harmless during food preparation. This also holds for many inhibitors of digestive enzymes (proteases, amylases), if the food is properly prepared. Eating raw or insufficiently cooked beans is an unhealthy affair because they will still contain large amounts of secondary compounds. Some compounds in herbs that are commonly used to flavor our food are on the black list. These include safrole (in nutmeg, cacao, black pepper) and capsaicin (in red pepper, hot pepper), but taken in small doses they do not cause problems.

There are certainly compounds, however, that should be avoided at all costs (e.g., **aflatoxin**). This is a fungal compound produced by *Aspergillus flavus* growing on peanuts (*Arachis hypogaea*), corn (*Zea mays*), and some other crop plants. This compound may cause severe liver damage or cancer. Other secondary compounds have a distinctly positive effect on our health in that they reduce the risks for certain forms of cancer. These include the flavonoids in a so-called fiber-rich diet. These phenolics likely inhibit the production of sex hormones; hence, they appear to reduce the incidence of cancers in which these hormones play a role, including breast cancer and prostate cancer. The alkaloid camptothecin, from the roots of the Chinese medicinal herb *Camptotheca acuminata*, is a recent anticancer drug (Flores et al. 1999). Isothiocyanates, which are produced

FIGURE 13. The chemical structures of two alkaloids: solanine from *Solanum tuberosum* (cultivated potato) and demissine from *Solanum demissum* (wild potato) (Bennett & Wallsgrove 1994).



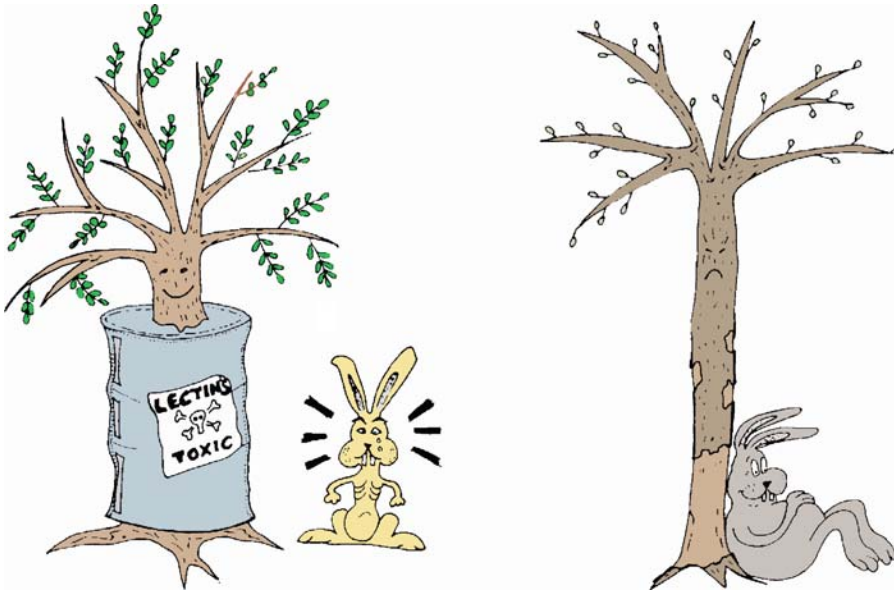
upon degradation of glucosinolates, induce anticarcinogenic enzymes which suggests that high consumption of *Brassica* (cabbage) species could reduce the risk of developing cancer. The roles of fruit, vegetables, and red wine in disease prevention have been attributed, in part, to the **antioxidant** (radical-scavenging) properties of their constituent phenol compounds (polyphenols; Sect. 3.1), some of which are more effective antioxidants in vitro than are vitamin C (ascorbic acid) and vitamin E ( $\alpha$ -tocopherol) (Rice-Evans et al. 1997).

Breeding or genetically modifying genotypes of crop species that contain antiherbivore compounds is of increasing economic importance and may lead to more environmentally friendly methods in agriculture. The tendency to breed for oilseed varieties with low glucosinolate levels to improve the feeding quality of rape meal is an excellent example how *not* to go about it. Such a breeding approach makes the crop more vulnerable to herbivores and makes agriculture more dependent on pesticides. It would be better instead, to aim for oilseed varieties that have their leaves well protected against herbivores, while having a reduced level of glucosinolates only in their seeds (Halkier & Gershenzon 2006). This promising strategy has been taken on board in more recent breeding efforts.

There are increasingly positive developments in breeding resistant cultivars. For example, *Leptinotarsa decemlineata* (Colorado beetle) is a well-known predator of *Solanum tuberosum* (potato) and may cause severe damage to potato crops in North America and Western Europe. A closely related species of

our cultivated potato, *Solanum demissum*, is not affected by the beetle. It contains an alkaloid (demissine) that is slightly different from solanine in *Solanum tuberosum* (Fig. 13; Bennett & Wallsgrove 1994). Close relatives of crop species can be used for breeding of resistant crop cultivars. One striking example of the application of ecophysiological information on plant-herbivore interactions is the incorporation of a gene from *Phaseolus vulgaris* (common bean), encoding an amylase inhibitor, into *Pisum sativum* (garden pea). The transgenic plants suffer considerably less from attack by pea weevils (*Bruchus pisorum*) than do the wild type (Schroeder et al. 1995). Similarly, genes encoding a proteinase inhibitor or lectins have been inserted.

Herbivores may acclimate and possibly even adapt to an increased level of a specific proteinase or amylase inhibitor. They do so by producing other proteinases or amylases, whose activity is not inhibited by the plant-produced inhibitor. For example, one type of  $\alpha$ -amylase inhibitor protects seeds of the common bean (*Phaseolus vulgaris*) against predation by the cowpea weevil (*Callosobruchus maculatus*) and the azuki bean weevil (*Callosobruchus chinensis*), but not against predation by the bean weevil (*Acanthoscelides obtectus*) or the Mexican bean weevil (*Zabrotus subfasciatus*). A serine protease in midgut extracts of the larvae of the Mexican bean weevil rapidly digests and inactivates  $\alpha$ -amylase from *Phaseolus vulgaris* as well as from *Phaseolus coccineus* (scarlet runner bean), but not the  $\alpha$ -amylase from wild common bean accessions or from *Phaseolus acutifolius* (teparty bean) (Ishimoto & Chrispeels 1996).



**FIGURE 14.** Lectins are carbohydrate-precipitating proteins. Some of these give plants protection against insects as well as vertebrates. When present in bark [e.g., in

*Sambucus nigra* (elderberry)] they offer good protection against rodents and deer (after Peumans & Van Damme 1995). Copyright American Society of Plant Biologists.

Lectins bind carbohydrates (by definition). As such they play a role as defense compounds (Fig. 14; Peumans & Van Damme 1995). Lectins occur in many plants, including *Sambucus nigra* (elderberry), *Hevea brasiliensis* (rubber tree), *Galanthus nivalis* (snowdrop), and *Datura stramonium* (thorn apple) (Raikhel et al. 1993). In *Sambucus nigra* lectin is located in protein bodies in the phloem parenchyma of the bark (Greenwood et al. 1986). Some lectins are highly toxic to many animals and also offer good protection against viruses and some fungi (Sect. 2 of Chapter 9C on effects of microbial pathogens). Although some insects appear to tolerate lectins, sucking insects like aphids are highly sensitive.

The gene encoding the lectin from *Galanthus nivalis* (snowdrop) has been linked to a promoter that ensures expression of the gene in the phloem (Hilder et al. 1995). It has been inserted in *Oryza sativa* (rice) in an attempt to develop a plant that contains its own insecticide to enhance its resistance to aphids and brown plant-hoppers (Sudhakar et al. 1998, Wu et al. 2002). Ever-increasing numbers of transgenic plants with a range of different resistance genes inserted are now being produced (Petersen et al. 2001, Tattersall et al. 2001, Carlini & Crossi-de-Sá 2002).

## 4. Environmental Effects on the Production of Secondary Plant Metabolites

Although specific secondary metabolites tend to be specific for certain species, the concentration of these compounds may vary greatly, depending on environmental conditions.

### 4.1 Abiotic Factors

The concentration of secondary plant compounds depends on plant age as well as on abiotic environmental factors (e.g., light intensity, water stress, waterlogging, frost, pollution, and nutrient supply). In *Leucaena retusa* (goldenball leadtree) the production of organic sulfur compounds (COS and CS<sub>2</sub>) from crushed roots increases with increasing supply of sulfate, especially in young seedlings (Feng & Hartel 1996). The concentration of caffeine (an alkaloid) in the shoot of *Camellia sinensis* (tea) is higher when the plants are grown at high irradiance, rather than in the shade. *Pinus sylvestris* (Scots pine) trees exposed to water stress produce less resin and are affected more by herbivorous beetles. Exposure of



*Toxicodendron radicans* (poison ivy) to elevated atmospheric CO<sub>2</sub> concentrations enhances its growth as well as the production of urushiol, suggesting the rate of spread of poison ivy and its ability to recover from herbivory may be enhanced in a future environment with higher CO<sub>2</sub> concentrations (Ziska et al. 2007). Defoliation of *Picea abies* (Norway spruce) trees reduces the production of terpenoids which is associated with an increased attack on their bark. In other plants, stress enhances the production of secondary metabolites, e.g., in *Salix aquatica* (willow) the concentration of tannin and lignin is enhanced when plants are grown under N limitation as compared with an optimum supply (Waring et al. 1985, Northup et al. 1995). In a cross between *Festuca* and *Lolium*, the alkaloid concentration declines when plants are exposed to water stress, whereas that in *Nicotiana tabacum* (tobacco) increases. These effects may be mediated via carbohydrate-modulated gene expression (Sect. 12.1 of Chapter 2A on photosynthesis). Whereas genes that encode photosynthetic enzymes are down-regulated by carbohydrates, evidence is accumulating that a number of defense genes are positively modulated by carbohydrates (Koch 1996).

Two hypotheses have been advanced to explain patterns of environmental effects on plant secondary metabolites. The **carbon/nutrient balance** (CNB) hypothesis explains the level of investment in **carbon-based secondary metabolites** (i.e., those that contain only C, H, and O) as a balance between photosynthesis and growth, which, in turn, is sensitive to the carbon/nutrient balance of the plant (Bryant et al. 1983, Gershenzon 1984, Tuomi et al. 1984). According to the CNB hypothesis, plants allocate carbon preferentially to growth when nutrients are available. Low nutrient availability constrains growth more than it reduces photosynthesis (Sect. 5 of Chapter 7 on growth and allocation), however, leading to a build-up of carbohydrates that are funneled into production of carbon-based secondary metabolites (broadly synonymous with quantitative defenses). This hypothesis explains the high levels of plant defenses typically found in plants that grow on infertile soils, and the reductions in defense that occur in response to both nutrient addition or shading. For example, tropical trees that grow on infertile soils have higher concentrations of phenolic compounds and less herbivory than do trees that grow on more fertile sites (McKey et al. 1978). The hypothesis predicts that plants that grow more rapidly should invest less carbon in defense, as observed among seedling of the tropical tree *Cecropia peltata*

(Coley 1986). However, although the hypothesis successfully predicts outcomes in some cases, there are enough exceptions that it cannot be considered a predictive tool (Hamilton et al. 2001).

The **growth-differentiation balance** (GDB) hypothesis was advanced to explain seasonal and interannual variations in rates of production of carbon-based secondary metabolites (Loomis 1932, Lorio 1986). According to this hypothesis, growth is the primary path of carbon investment as long as conditions permit cell division and expansion; however, once water stress, photoperiod, or any other environmental factor constrains growth, cells differentiate, resin ducts form, and plants switch allocation of carbon to production of resins and other secondary metabolites. This hypothesis accounts for the greater vulnerability of *Picea mariana* (black spruce) and *Pinus banksiana* (jack pine) to attack by beetles early in the growing season, and it explains why emission of monoterpenes and resin production increase late in the year, particularly in years when water stress constrains growth (Lorio 1986, Lerdaun et al. 1997).

Herms & Mattson (1992) integrated the two hypotheses discussed above into an expanded version of the GDB hypothesis, which suggests that scarcity of any resource that restricts growth more than photosynthesis should enhance secondary metabolite production (Fig. 15). At extremely low resource availability, assimilation rate may be so low that maintenance respiration consumes most carbon, so that both growth and secondary metabolite production are limited (Waring & Pitman 1985). In the expanded GDB model, which is supported by recent evidence (Lambers & Poorter 2004), fast-growing species invest less carbon in secondary plant compounds than do slow-growing ones, when compared at a high resource availability. Herms and Mattson emphasized that further testing of their model is necessary. It may well be valid for one class of secondary compounds only (e.g., the quantitatively important defense compounds of a phenolic nature).

The CNB and GDB hypotheses provide a plausible mechanism for a pattern that should be strongly selected for: long-lived leaves of slow-growing plants should be well protected against pathogens and herbivores to minimize tissue loss (Sect. 4.1 of Chapter 9E on interactions among plants). The actual biochemical allocation to specific pathways of synthesis of individual secondary metabolites is undoubtedly regulated much more specifically than is implied by the CNB and GDB hypotheses.

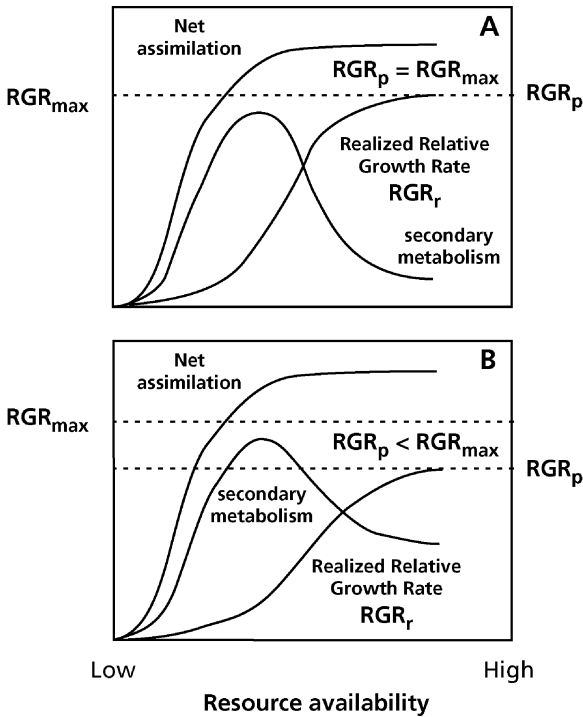


FIGURE 15. A hypothetical model that shows the realized relative growth rate ( $RGR_r$ ), the net assimilation rate, and the investment of carbon in secondary plant compounds as a function of the availability of resources. Two populations (A and B) are depicted that differ with respect to the RGR that they can achieve at optimal resource availability ( $RGR_p$ ).  $RGR_{max}$  in these figures denotes the maximum possible RGR of population A at the most favorable resource supply in the environment given its investment in secondary plant metabolites. Population B does not reach this  $RGR_{max}$ , due to a greater allocation to secondary metabolites (after Herms & Mattson 1992).

## 4.2 Induced Defense and Communication Between Neighboring Plants

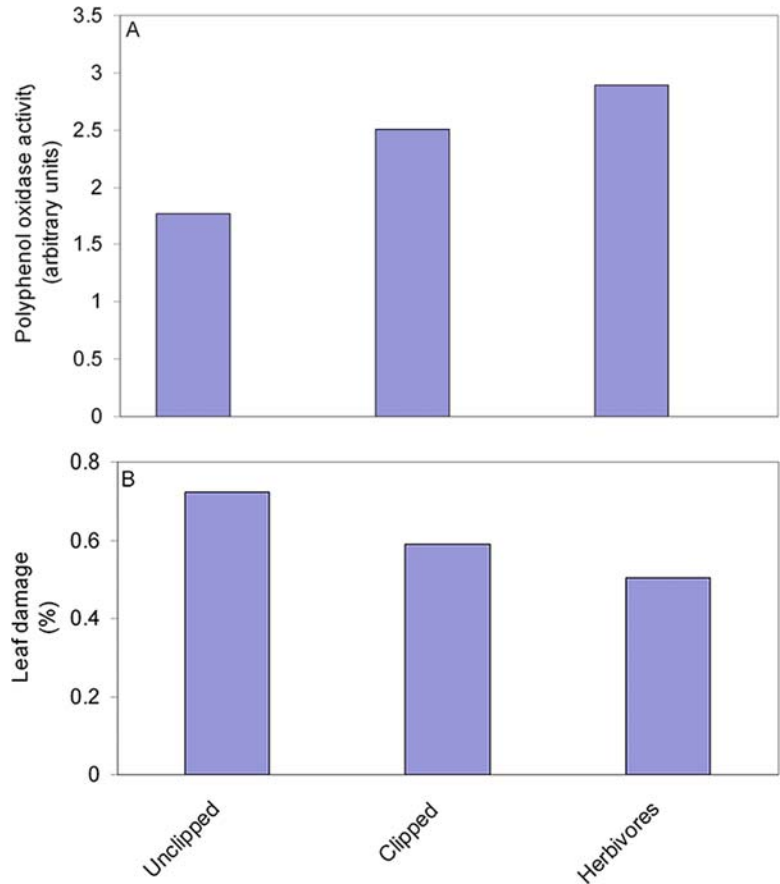
The production of secondary metabolites depends on abiotic environmental factors as well as on the presence of herbivores: **induced defense**. Physical damage of leaves often enhances the transcription of genes encoding polyphenol oxidase [e.g., in *Populus* (poplar) species] (Constabel et al. 2000). It also induces the formation of tannins, and the production of proteinase inhibitors [e.g., in *Solanum tuberosum* (potato) and *Solanum lycopersicum* (tomato)], especially when it is due to insect attack (Koiwa et al. 1997, Korth & Dixon 1997). These plant responses reduce the quality of both the attacked and other leaves on the same plant as a food source. Jasmonate, ABA, salicylic acid, and ethylene play a role as signaling molecules in the systemic induction of defense (Wasternack & Partheir 1997, Gatehouse 2002). This response sometimes occurs within minutes to hours (**short-term induction**), as a result of reactions among precursors already present in the leaf. For example, chewing of *Populus tremuloides* (quaking aspen) leaves causes enzymatic hydrolysis of two phenolic glycosides (salicortin to salicin, and tremulacin to tremuloidin) with the release of 6-HCH (6-hydroxycyclohex-2-ene-1-one), which then becomes converted to phenol or catechol (potent

toxins) in the gut of the insect (Clausen et al. 1989). As a result, insects cannot feed continuously on a few leaves; rather, they must constantly move among leaves which makes them more vulnerable to predators. Short-term induced defenses are effective against those herbivores that cause the initial damage.

There are also **long-term induced defenses** produced by the next cohort of leaves after severe insect outbreaks. These serve to protect plants against catastrophic herbivory by insects with large population outbreaks. Long-term induction is typically associated with increases in phenolics or fiber, less leaf N, and often smaller leaves. Long-term induced defenses are best developed in tree populations with an evolutionary history of outbreaking insects. In some cases they are induced more strongly by insect feeding than they are by comparable amounts of physical damage which suggests a tight evolutionary linkage with insect herbivores (Haukioja 1980, Haukioja & Neuvonen 1985). Both long- and short-term induced defenses are best developed in rapidly growing woody plants, whereas slow-growing species have higher levels of background (**constitutive**) defenses that are always present to deter herbivores (Coley et al. 1985, Bryant et al. 1991).

There is increasing evidence that neighboring, unattacked plants respond by increasing the

FIGURE 16. Eavesdropping among plants in nature. (A) *Artemisia tridentata* (sagebrush) plants induce increased activity of polyphenol oxidase in neighboring *Nicotiana attenuata* (wild tobacco) plants when the sagebrush neighbors are either clipped manually or damaged by real herbivores. (B) Maximum proportion of tobacco leaves that are damaged by herbivores on tobacco plants with sagebrush neighbors that were unclipped, clipped artificially, or clipped by real herbivores (Karban et al. 2003). Copyright Blackwell Science Ltd.



concentration of defensive compounds (Fig. 16) and become less attractive to herbivores (Dicke et al. 2003, Baldwin et al. 2006, Paschold et al. 2006). Dolch & Tschardt (2000) investigated the effects of manual defoliation, to simulate herbivory, of *Alnus glutinosa* (black alder) on subsequent herbivory by the alder leaf beetle (*Agelastica alni*) in northern Germany (Fig. 17). Subsequent damage by the leaf beetle is less when the trees are close to the manually defoliated tree. In addition, the extent of leaf consumption in laboratory feeding-preference tests and the number of eggs oviposited per leaf in another laboratory test are positively correlated with distance from the defoliated tree. **Resistance** is therefore **induced**, both in defoliated alders and in their undamaged neighbors, demonstrating that defoliation triggers interplant resistance transfer, and therefore reduces herbivory in whole alder stands. This indicates that plants **communicate** with each other after herbivore attack.

Effects of leaf damage on neighboring trees of *Acer saccharum* (sugar maple) involve volatile signal transfer between leaves, because these effects are

also found when plants are grown in separate pots. Volatile compounds play a role in this type of **communication** between plants, including octadecanoid-derived "green leaf volatiles", volatile terpenoids and phenols (Tschardt et al. 2001, Turlings & Ton 2006). **Jasmonate** is also involved; it primes defense-related genes for induction upon subsequent defense elicitation (Ton et al. 2007). Plants of different species can also respond to signals released from damaged plants. For example, *Nicotiana attenuata* (wild tobacco) plants next to damaged *Artemisia tridentata* (sagebrush) plants have higher levels of the defensive enzyme polyphenol oxidase and reduced levels of insect damage, compared with control plants next to undamaged sagebrush plants (Karban et al. 2000, 2003). In addition to signaling via volatiles released from damaged leaves, plants also communicate via signals released from roots (Dicke & Dijkman 2001, Guerrieri et al. 2002). The relative importance of airborne and soil-borne signals as well as unknown effects of intensified nutrient absorption of defoliated trees, possibly reducing foliage quality of

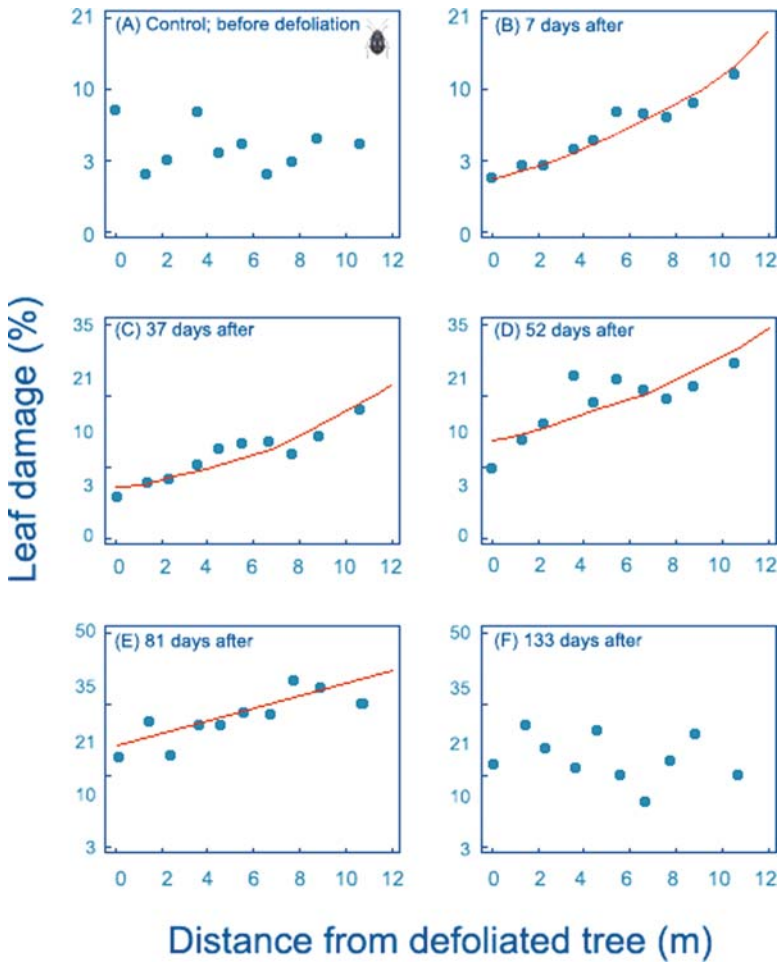


FIGURE 17. Relationship between leaf damage by *Agelastica alni* (alder leaf beetle) to *Alnus glutinosa* (black alder) and distance from the manually defoliated tree in the field. (A) Control: before defoliation, the amount of leaf damage within each plot is randomly distributed. (B–E) 7–81 days after defoliation: herbivory by *Agelastica alni* is greater at increasing distance from the manually defoliated tree. (F) 133 days after defoliation: the distribution pattern of leaf damage no longer depends on distance from the manually defoliated tree (Dolch & Tschartke 2000).

undamaged neighbors, remains to be further investigated (Fig. 18).

There is a wide variation in the extent to which plants respond to browsing with an increased concentration of phenolics. Of three South African Karoo shrubs, the deciduous species [*Osteospermum sinuatum* (African daisy)] is the most palatable. It contains very few polyphenols, does not enhance this level upon browsing, but has a high **regrowth capacity**. On the other hand, the evergreen succulent species (*Ruschia spinosa*) shows almost no regrowth after browsing, but contains the highest level of constitutive and browser-induced levels of polyphenols, condensed tannins, and protein-precipitating tannins. The evergreen sclerophyllous species [*Pteronia pallens* (scholtz bush)] shows an intermediate response in terms of regrowth capacity and browser-induced phenols. It also contains intermediate levels of phenols before browsing (Stock et al. 1993). This suggests a trade-off between

allocation to (induced) defense (**avoidance**) and regrowth capacity (**tolerance**) upon attack by herbivores.

In *Leucaena* (leadtree) species, damaging the roots or shoots greatly enhances the production of organic sulfur compounds (COS and CS<sub>2</sub>), which are foul-smelling compounds that are toxic to bacteria, fungi, and animals like nematodes and insects (Feng & Hartel 1996). The suggestion to use some of these species as potential animal fodder should therefore be viewed with some skepticism.

### 4.3 Communication Between Plants and Their Bodyguards

Volatile compounds play a role in communication between neighboring plants, when attacked by herbivores (Sect. 4.2), as well as between plants and predatory mites or parasitic wasps. These

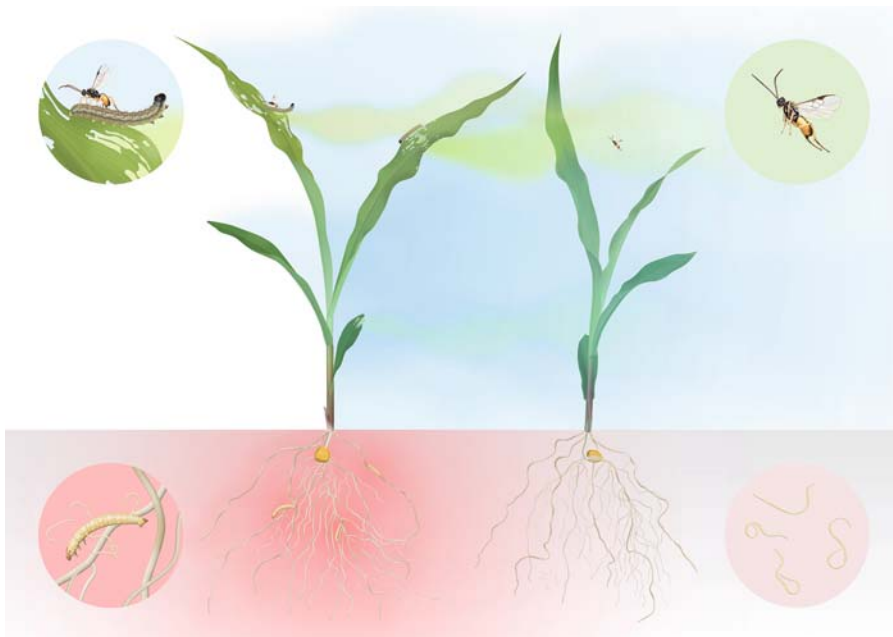


FIGURE 18. When damaged by caterpillars, young plants of *Zea mays* (corn) immediately release several typical octadecanoid-derived “green leaf volatiles” from the damaged sites (indicated in green). In addition, elicitors in the caterpillar’s oral secretions cause the induction of a systemic release of volatiles that mainly comprise terpenoids but also include some phenolics, such as indole and methyl salicylate (indicated in blue). This blend of herbivore-induced volatiles is highly attractive to various parasitic wasps that lay their eggs in the caterpillars. Below-ground beetle larvae might cause the emission of similar signals by damaged roots

(indicated in red). Corn roots release one dominating compound, (E)- $\beta$ -caryophyllene, in response to root feeding. This sesquiterpene is attractive to entomopathogenic nematodes and increases the effectiveness of these nematodes in finding and killing herbivore larvae. In addition, the herbivore-induced volatiles might repel other herbivores and can induce or prime defense responses in neighboring plants. All of these effects might be exploitable for the control of agricultural pests (after Turlings & Ton 2006; copyright Elsevier Science, Ltd.).

**tritrophic systems** offer another fascinating example of co-evolution in the arms race between plants and herbivores, except now there is an ally involved: **indirect defense**, as opposed to the **direct defense** responses that were discussed above in this chapter. The volatiles that are released by leaves upon attack by herbivorous mites or caterpillars attract predatory mites or parasitic wasps, respectively. These predatory mites and parasitic wasps then act as **bodyguards**. The attractants produced by plants upon attack are specific in that they are not produced upon artificially damaging the leaves or are produced in much smaller quantities. Upon attack of *Brassica oleracea* (cabbage) plants by caterpillars of *Pieris brassicae* (cabbage moth) the plant responds to a specific caterpillar enzyme ( $\beta$ -galactosidase) with the synthesis of a mixture of volatiles, which are highly specific for a parasitic wasp, *Cotesia*

*glomerata*. Leaves treated with  $\beta$ -galactosidase from almonds respond in a similar manner, which shows that this compound acts as an “elicitor” (Mattiacci et al. 1995). *Zea mays* plants attacked by larvae of *Spodoptera frugiperda* and *Spodoptera exigua* (armyworms) respond to a specific compound [*N*-(17-hydroxylinolenoyl)-L-glutamine, or volicitin] (Alborn et al. 1997). Upon attack, they emit terpenoids and indole that attract a parasitic wasp, *Cotesia marginiventris*. Mechanical damage, without application of volicitin, does not trigger the same blend of compounds. When infested by the larvae of *Pseudaletia separata*, the corn plants emit terpenoids, indole, oximes, and nitriles that attract *Cotesia kariyai*. The production of the attractants is **systemic**. In other words, it is not restricted to the damaged parts of the plant, but also occurs in undamaged leaves; a similar systemic response occurs in *Gossypium hirsutum* (cotton) that are

attacked by larvae of the beet armyworm (*Spodoptera exigua*) (Röse et al. 1996).

Several crop species infested by the herbivorous two-spotted spider mite, *Tetranychus urticae*, or larvae of *Spodoptera exigua* (beet armyworm) become attractive to a predatory mite, *Phytoseiulus persimilis* and *Cotesia marginiventris*, respectively (Fig. 18). Many plant species respond to arthropod attack with the release of a blend of volatiles that attract predators or parasitic wasps. Each species, however, produces its own blend of chemicals that attract their **bodyguards**. Feeding of the two-spotted spider mite on the leaves of *Phaseolus lunatus* (lima bean) or *Cucumis sativus* (cucumber) strongly induces a sesquiterpene synthase, which catalyzes the formation of a volatile attractant from a precursor (Bouwmeester et al. 1999). The bodyguards can learn to distinguish between herbivore-induced volatiles emitted by different species. The attractants produced by *Phaseolus lunatus* are presented in Fig. 19. There is substantial genetic variation in the amount of attractants produced upon attack on which natural selection can act (Baldwin et al. 2006). This provides substantial scope for breeding efforts to exploit this aspect of ecological biochemistry. Tritrophic interactions are not restricted to above-ground plant organs and interacting animals. For example, *Thuja occidentalis* releases chemicals upon attack by larvae of *Otiiorhynchus sulcatus* (a weevil) and thus attracts *Heterorhabditis megidis* (a parasitic nematode), which then preys on the weevil larvae (Van Tol et al. 2001). Similar **below-ground tritrophic interactions** occur in *Zea mays* (corn). Upon attack by beetle larvae, their roots release a sesquiterpene, (*E*)- $\beta$ -caryophyllene, which attracts entomopathogenic nematodes and increases the effectiveness of these nematodes in finding and killing herbivore larvae (Rasmann et al. 2005, Turlings & Ton 2006). Improved knowledge in this area should provide opportunities for applications in plant management systems, similar to those existing for above-ground tritrophic interactions (Turlings & Wäckers 2004).

A fascinating example of a tritrophic interaction is found in *Nicotiana attenuata* (wild tobacco), which contains high levels of the alkaloid nicotine (up to 12% of the dry mass of leaves). Upon attack by most herbivores, **jasmonic acid** is produced, which is transported via the phloem to the roots. Here, it induces the production of more nicotine, which is transported to the leaves, via the xylem, where it accumulates to even higher levels than in control plants. When a specialist caterpillar, *Manduca sexta* (tobacco hawkmoth) attacks *Nicotiana attenuata*, however, there is no increased synthesis and accumulation of nicotine. Rather,

**bodyguards** are attracted, involving specific signals, like in the examples given above. The bodyguards can kill the specialist caterpillar, without being affected by increased nicotine levels in the caterpillar. That is, in this case, suppression of the transduction pathway that leads to increased nicotine levels in the leaves is advantageous for the host plant (Kahl et al. 2000).

## 5. The Costs of Chemical Defense

The production of secondary plant compounds requires **investment** of carbon, as well as some other elements. Does this mean that a gram of biomass is more costly to produce if it contains large quantities of secondary plant compounds? This is not so when costs are expressed in terms of grams of glucose required for carbon skeletons and for production of energy to produce the biomass. Approximately equal amounts of glucose are needed to produce 1 g of dry mass in slow-growing herbaceous species (which contain relatively small amounts of phenolic compounds) and fast-growing ones (Fig. 20; Sect. 5 of Chapter 2B on plant respiration). Per gram of fresh mass or per unit leaf area, the situation is different, but this is due to the lower water content or thicker leaves of the slow-growing species.

### 5.1 Diversion of Resources from Primary Growth

There are **costs** associated with the strategy of accumulating vast quantities of secondary plant compounds. This can best be illustrated by imagining a leaf with a certain amount of protein. If half of this protein were to be replaced by lignin or tannin, then its physiological performance would probably be less. It is quite likely that its photosynthetic capacity would decline by approximately half. The higher costs of well-protected leaves, therefore, do not reflect high costs of the production of new leaves. Rather, defense is costly because it diverts resources from primary growth (an opportunity cost, i.e., the cost of resources that would otherwise be gained by an alternative allocation) (Herms & Mattson 1992) which reduces the potential growth rate of the plant.

**Investment** of large quantities of carbon in secondary plant compounds that reduce herbivory will lead to greater plant fitness only when the costs of repairing the damage incurred by herbivory exceed those needed for protection. This explains why quantitatively important secondary plant

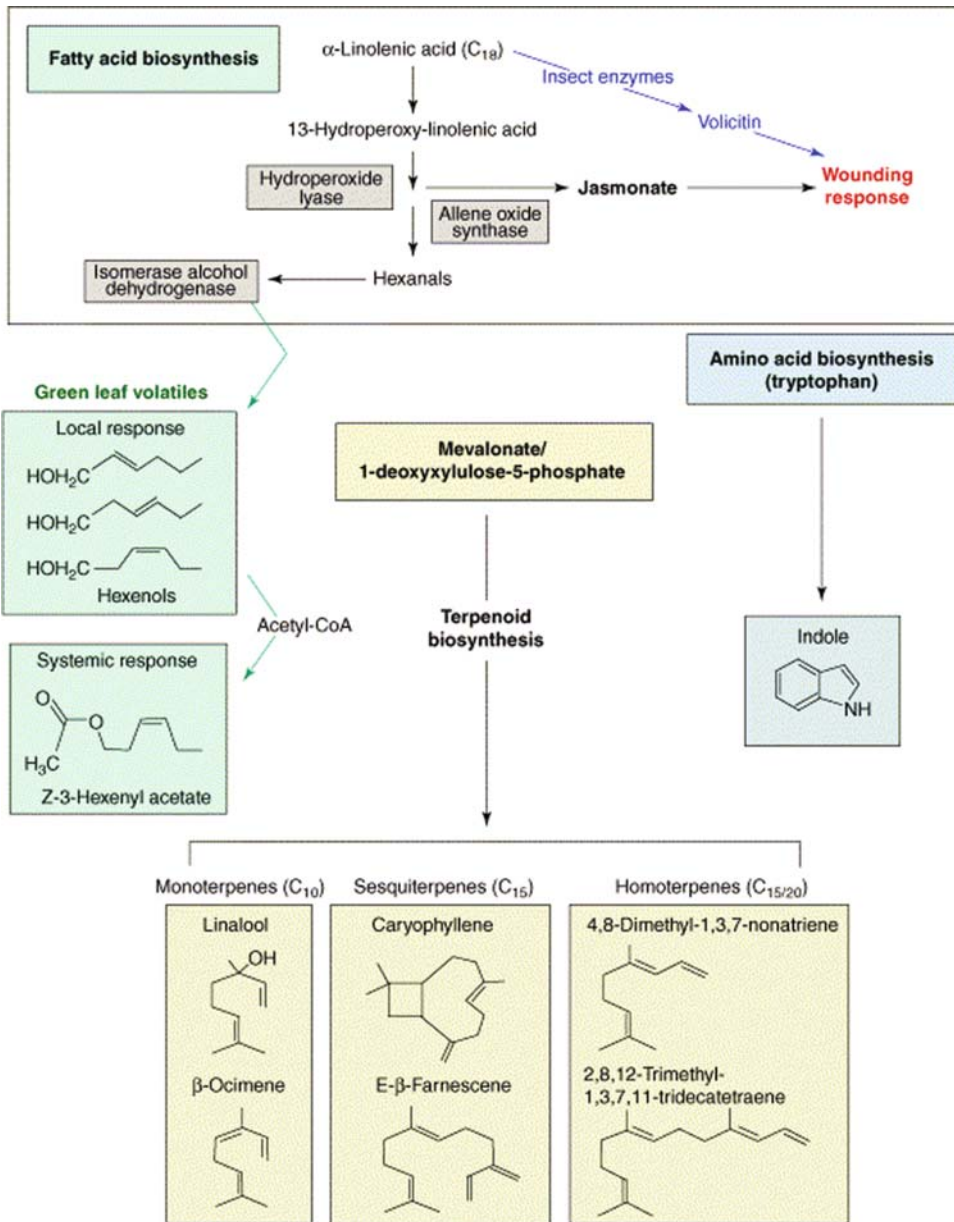


FIGURE 19. General overview of plant volatiles synthesized in response to insect attack, either both locally or systemically (after Ferry et al. 2004; copyright Elsevier Science, Ltd.).

compounds are more pronounced in inherently slow-growing species from low-productivity environments than they are in fast-growing ones from more productive habitats. On one hand, costs select against defensive adaptations, whereas on the other hand herbivore pressure leads to investment in defense. Defensive adaptations may then lead to offensive adaptations in

animals (e.g., the co-evolution of fluoroacetate-bearing legumes and Western Australian native animals) (Fig. 21). When costs of defense have been evaluated by comparing fitness of resistant and susceptible genotypes in the absence of herbivores or pathogens, the costs of resistance appear small (Vrieling & Wijk 1994, Bergelson & Purrington 1996); however, most of these tests

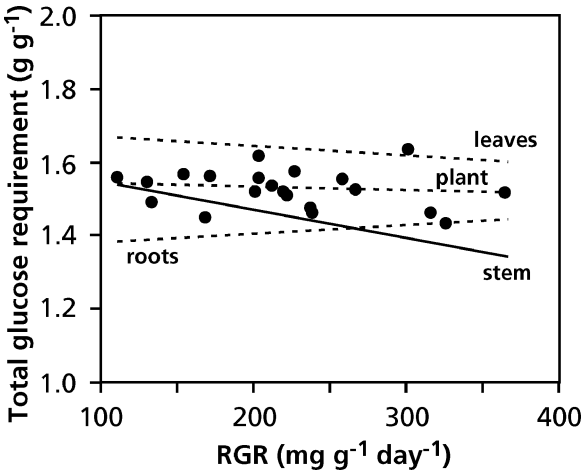


FIGURE 20. The amount of glucose required to produce biomass in slow-growing and fast-growing herbaceous species, all grown with free access to nutrients. Glucose costs include the costs for the carbon in the biomass as well as those associated with the formation of biomass, for which glucose has to be catabolized to generate ATP and NAD(P)H (Poorter & Bergkotte 1992). Copyright Blackwell Science Ltd.

have been done on rapidly growing species where we would not expect a large cost of defense.

### 5.2 Strategies of Predators

Two strategies may be discerned among the offensive adaptations of animals (Fig. 22). The evolutionary response to communication between plants which leads to the accumulation of protective compounds in neighboring plants may be to **suppress the communication** or to emit **countersignals**. The response to the accumulation of protective compounds in plants upon recognition of a predator

may be either to **suppress recognition** of the predator or to **consume** the plant quickly and so prevent protection (surprise). Inducible defenses may be counteracted by **suppression** of the induced defense or by decreasing the defense. Constitutive defense may be counteracted by detoxification or avoidance of the most toxic plant parts (Karban & Agrawal 2002). In addition, prior attack of *Nicotiana attenuata* (wild tobacco) by some insects, e.g., the sap-feeding *Tupiocoris notatus*, results in “**vaccination**” of the tobacco plant against subsequent attacks by chewing hornworms (*Manduca sexta*). This vaccination is mediated by a combination of direct and indirect defenses (Voelckel & Baldwin 2004, Kessler 2006).

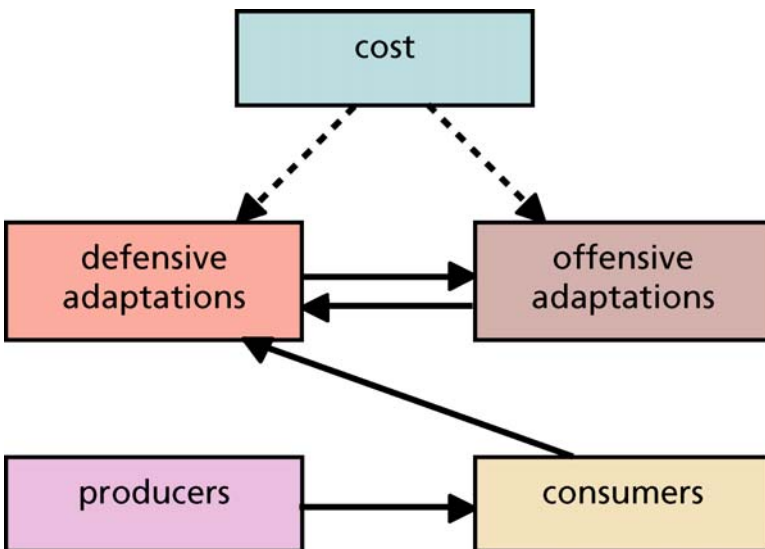


FIGURE 21. Interactions between higher plants and animals involving secondary plant compounds. Attack by herbivores leads to the evolution of protection with defense compounds (defensive adaptations in producers). At the same time, there is a selection against production of defense compounds because it incurs a cost. Defensive adaptations in plants lead to the evolution of offensive adaptations in consumers. These offensive adaptations are selected against because they incur some costs (after Rhoades 1985).



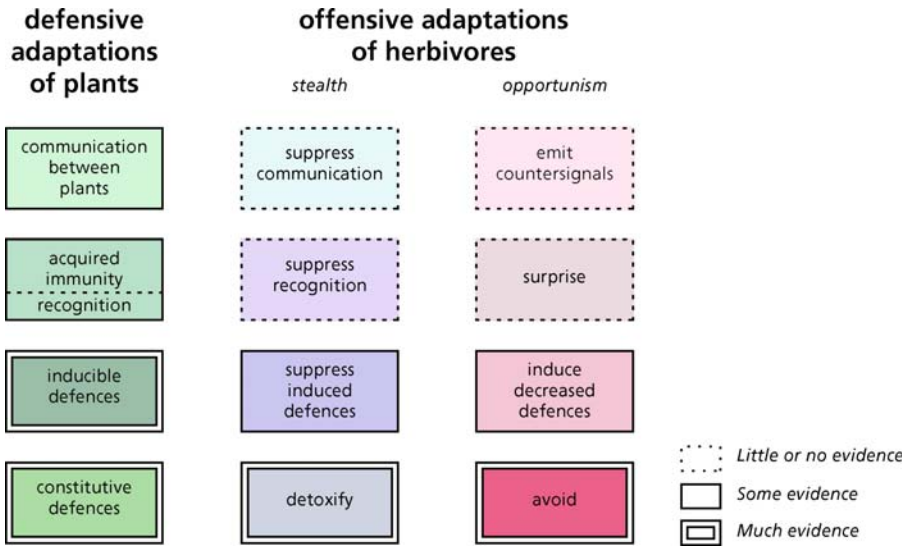


FIGURE 22. The evolutionary strategy of “stealthy” and “opportunistic” animals to cope with the defensive adaptations of plants (after Rhoades 1985).

### 5.3 Mutualistic Associations with Ants and Mites

Instead of investing in defense compounds, plants can also form a **mutualistic association** with animals that protect them. Several thousand seed plants have extrafloral nectaries that indicate some level of **ant defense**. Most of these mutualistic associations involve a limited co-evolved specialization between the partners. A small number of plant species that attract ants for their defense [e.g., species belonging to the genera *Acacia* (wattle), *Cecropia*, and *Macaranga*] have obligate or facultative relationships with a single ant species. *Acacia* species that form an obligate relationship provide their allies with nectar, lipids, and proteins in special structures, and shelter in special plant parts (**domatia**). The resident ants are very aggressive and defend the tree against both invertebrate and vertebrate herbivores. Some of these species have lost their major line of chemical defense against herbivores, and the tree is quickly destroyed if the ants are removed. The costs of ant defense (production of extrafloral nectaries), therefore, are partly compensated for by lower costs of chemical defense (Heil et al. 2001). However, there are additional benefits in that the ants bring in substantial resources, and most of the N that is accumulated in *Cecropia peltata* (trumpet tree) trees is derived from debris deposited by its mutualistic *Azteca* ants (Fig. 23; Sagers et al. 2000).

The defending ants form a potential risk, however, because the plants still need a suite of insect **pollinators** for cross-pollination (Pellmyr 1997). Observations on the African *Acacia zanzibarica* reveal that ants quickly abandon first-day flowers when they encounter them, and return after pollinator activity ceases. It is likely that a volatile that triggers alarm behavior in ants is produced by flowers before pollination has occurred, but this has yet to be confirmed (Willmer & Stone 1997).

In addition to ants, predatory mites may also inhabit domatia, e.g., on leaves of *Cupania vernalis* in south-east Brazil. Blocking leaf domatia shows that leaf domatia can benefit plants against herbivory in a natural system (Romero & Benson 2004).

## 6. Detoxification of Xenobiotics by Plants: Phytoremediation

Plants, like any other organisms in the environment, are continually exposed to potentially toxic chemicals: **xenobiotics**. These xenobiotics may be natural secondary plant chemicals, which we discussed in this chapter, industrial pollutants, or agrochemicals. Many xenobiotics are lipophilic; they are therefore readily absorbed and accumulate to toxic levels within the plant, unless effective means of detoxification are present. If plants have pathways to



FIGURE 23. An example of a mutualistic association between an ant plant and an ant. (*Top left*) *Cecropia peltata* growing in the cerrado in Brazil. (*Bottom left*) Trunk of *Cecropia peltata* showing one of the many entry points for *Azteca xanthochroa* (Aztec ant, an ant species defending the tree). The base of each petiole bears a trichilium, a pad of densely packed trichomes,

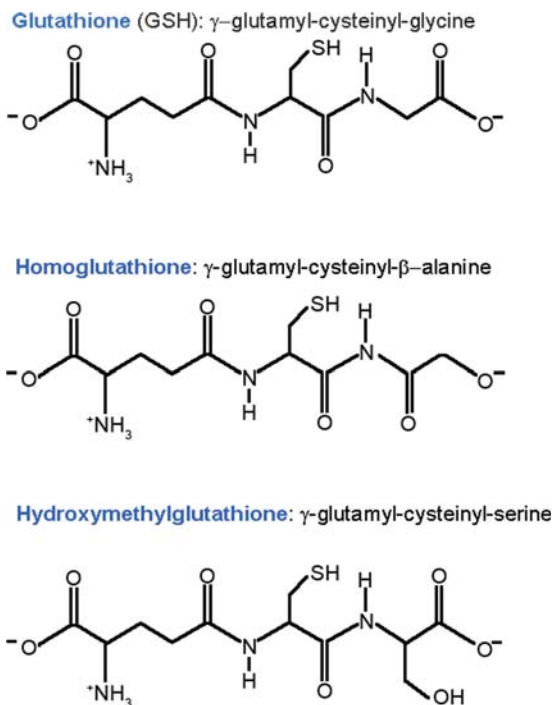
from which emerge 1–2 mm long glycogen-containing beads called Muellerian bodies. (*Top right*) An individual of an Aztec ant exiting the special hole in the stem, and another one descending from the stem. (*Bottom right*) Cross-section of the stem of *Cecropia peltata*, showing the hollow stem and perforated internodes, large enough for Aztec ants to move up and down the stem.

produce and cope with a vast array of natural secondary chemicals, can they also be put to use to clean up environmental pollutants? In Sect. 3.3.2 of Chapter 6 on mineral nutrition, we discuss the capacity of **metallophytes** to clean up inorganic pollutants. In this section we discuss the capacity of some plants to detoxify **organic pollutants** (Cunningham & Berti 1993).

The cellular detoxification systems of plants dispose of the xenobiotics by two sequential processes (Coleman et al. 1997):

1. Chemical modification
2. Compartmentmentation

The reactions responsible for chemical modification of lipophilic xenobiotics involve hydrolysis or oxidation that makes the chemicals more hydrophilic and creates reactive sites by the addition or exposure of functional groups (e.g., hydroxyl or carboxyl groups) (step I); the modified chemicals may still be toxic. If the xenobiotic already has a functional group that is suitable for conjugation, then there is no need for step I. The next step is the conjugation of



**FIGURE 24. Structures of  $\gamma$ -glutamyl-cysteinyl tripeptides that act as protective chemicals in plants. Glutathione is the major protective tripeptide in most plants. Some leguminous species [e.g., *Vigna unguiculata* (mung bean) and *Glycine max* (soybean)] produce homoglutathione. In some grasses hydroxymethyl glutathione is a major constituent (Coleman et al. 1997).**

the modified xenobiotic (phase II), followed by export from the cytosol (step III).

Hydrolysis of the xenobiotics in phase I is catalyzed by various esterases and amidases, but the major reactions are oxidations catalyzed by the **cytochrome P-450** system, which involves mono-oxygenases that insert one atom of oxygen into inert hydrophobic molecules to make them more reactive and water-soluble (Werck-Reichhardt et al. 2000). The rates of chemical transformation and the types of metabolites that are formed depend on plant genotype and accounts for variation in **herbicide resistance** and **tolerance to pollutants**. In phase II, the (modified) xenobiotic is deactivated by covalent linkage to endogenous hydrophilic molecules (e.g., glucose, malonate, or glutathione) which produces a water-soluble nontoxic conjugate. Export of the conjugates from the cytosol to the vacuole or apoplast (phase III) occurs by membrane-located transport proteins. This detoxification pathway shares many features with the pathway used by plants for the vacuolar deposition of secondary metabolites (e.g., anthocyanins).

One important detoxification mechanism is chemical modification of the xenobiotic by covalent linkage to tripeptides like **glutathione** (Fig. 24). Conjugation with xenobiotics may take place spontaneously or may require catalysis by glutathione-S-transferase. Glutathione is an important plant metabolite that acts both as a reducing agent that protects the cell against oxidative stress (Sects. 2.2.2 and 3.1 of Chapter 4B on effects of radiation and temperature) and guards against chemical toxicity via the modification reactions of phase II. Glutathione conjugates that are deposited in the vacuole can undergo further metabolism. For example, the glycine residue of the glutathione moiety may be removed enzymatically which is sometimes followed by enzymatic removal of the glutamic acid residue (Fig. 25).

The glutathione-mediated and related detoxification systems probably evolved for the metabolism and compartmentation of natural substrates. For example, a glutathione-S-transferase is required for the synthesis of **anthocyanins**; it produces a glutathione conjugate that can be transported to the vacuole. Cytochrome P-450 is, similarly, involved in anthocyanin biosynthesis. Therefore, the selective mechanisms that led to the catalytic proteins of the pathway that has an apparent specificity for industrial chemicals are probably associated with the metabolism of natural secondary plant products, including allelochemicals and pigments (Alfenito et al. 1998).

Higher plants, unlike microorganisms and animals, are unable to catabolize xenobiotics; instead, detoxification mechanisms have evolved that lead to the formation of water-soluble conjugates that are compartmented in the vacuole or deposited in the apoplast. The residues may persist in plant tissues for a considerable time, and may affect consumers of the plant tissues. A thorough understanding of the metabolic fate of xenobiotics is therefore important. Genetic engineering of crops with plant or bacterial genes has already produced transgenics that are resistant to herbicides and air pollutants. In time, similar approaches may lead to workable strategies to develop the **phytoremediation** of land polluted by industrial chemicals (Cunningham & Berti 1993, Coleman et al. 1997).

Plants can also detoxify air pollutants, for example, ozone, which is increasing in the lower atmosphere as a result of human activity. Ozone damage of sensitive plants is a common phenomenon in North America and Europe. Exposure of the needles of *Picea abies* (Norway spruce) enhances the levels of three enzymes involved in ozone detoxification:

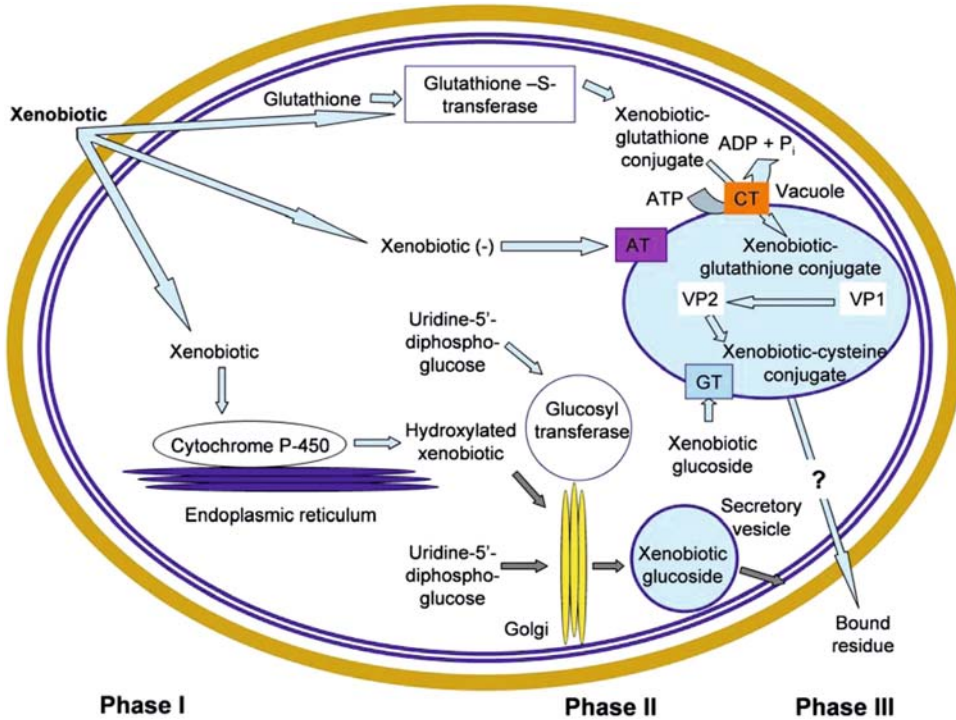


FIGURE 25. Enzyme-catalyzed reactions that are responsible for the detoxification of xenobiotics in plants are localized in or associated with several organelles and cellular compartments. The gray arrows represent a proposed pathway for the glucosylation of xenobiotics in the Golgi, followed by release of the metabolites via exocytosis. CT, glutathione-conjugate; AT, ATP-

dependent xenobiotic anion transporter; GT, ATP-dependent glucoside-conjugate transporter; VP, vacuolar peptidases that catalyze the removal of glycine (VP1) and glutamic acid (VP2) from the glutathione moiety of the conjugate. For further explanation, see text (after Coleman 1997).

superoxide dismutase, ascorbate peroxidase, and glutathione reductase (Sehmer et al. 1998).

## 7. Secondary Chemicals and Messages That Emerge from This Chapter

Plants produce a wealth of secondary plant compounds that play a pivotal role in defense and communication. We are only just beginning to understand how plants communicate with their neighbors, symbionts, pathogens, herbivores, and with their personal "bodyguards", both above and below ground, via chemical signals, which are often very specific. This new area is fascinating from an ecological point of view, and it has tremendous potential for major applications in agriculture, forestry, and environmental science. For example, **intercrops** can be selected that protect a crop in an

environmentally friendly manner (Sect. 6.2 in Chapter 9E on interactions among plants). For the intercrop to be of maximum benefit, however, intercrops should not compete to any great extent with the crop plant. It is up to ecophysiologists to help define desirable traits of an intercrop, with respect to its secondary chemistry, and also in terms of root traits that minimize competitive ability of the intercrop or, even better, that are beneficial to the crop. Numerous pertinent traits can be found in this and preceding chapters to help identify a desirable intercrop. Plants can also be used for phytoremediation, to remove organic pollutants from the environment or to reduce the concentration of air pollutants, such as ozone.

Knowledge of the chemical compounds that protect plants, preferably with full identification of the genes encoding the traits, will allow us to design crop plants that are better protected against herbivores. Such plants will reduce the need for pesticides, and many examples are now available of

transgenic plants with enhanced protection. We should be aware, however, that the arms race between plants and herbivores will continue, and that for every newly designed crop genotype resistant herbivores will coevolve. A thorough understanding of the intricate chemical interactions between plants and their herbivores is required to optimize the production of new crops.

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