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Review

Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores

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1 Summary. Chemical protection plays a decisive role in the resistance of plants against pathogens and herbivores. The so-called secondary metabolites, which are a characteristic feature of plants, are especially important and can protect plants against a wide variety of microorganisms (viruses, bacteria, fungi) and herbivores (arthropods, vertebrates). As is the situation with all defense systems of plants and animals, a few specialized pathogens have evolved in plants and have overcome the chemical defense barrier. Furthermore, they are often attracted by a given plant toxin. During domestication of our crop and food plants secondary metabolites have sometimes been eliminated. Taking lupins as an example, it is illustrated that quinolizidine alkaloids are important as chemical defense compounds and that the alkaloid-free varieties ("sweet lupins"), which have been selected by plant breeders, are highly susceptible to a wide range of herbivores to which the alkaloid-rich wild types were resistant. The potential of secondary metabolites for plant breeding and agriculture is discussed.

Key words: Secondary metabolites – Resistance – Herbivore – Pathogen – Lupinus

2 Introduction

A characteristic feature of higher plants is their capacity to synthesize an enormous variety of organic molecules, the so-called secondary metabolites (Table 1). Only 5% to 15% of plant species have been chemically analyzed so far. As sophisticated new analytical tools such as NMR (^1H, ^{13}C), MS (EI, CI, FD, FAB), HPLC, and capillary GLC become more common and available to natural product chemists and biologists, we can anticipate that the number of secondary metabolites will increase substantially in the near future. Secondary metabolites are widely used (Table 2) and have an important economic impact (Balandrin et al. 1985; Curtin 1983). Therefore, chemical and biotechnological strategies have been advanced for the production of these compounds. The improvement of product yields

Table 1. Number of known secondary metabolites of higher plants

Compounds	No. of structures
Monoterpenes	1,000
Sesquiterpenes	1,500
Diterpenes	1,000
Triterpenes/steroids	800
Tetraterpenes	350
Polyketides	700
Polyacetylenes	750
Flavonoids	1,200
Phenylpropanoids	500
Amines	100
Alkaloids	7,000
Nonprotein amino acids	400
Cyanogenic glycosides	50
Glucosinolates	100

Table 2. Exploitation of secondary metabolites by man. Examples are given for respective compounds and the plant species in which they are produced

Use	Compound	Species
Pharmaceuticals	Atropine	Atropa[a]
	Scopolamine	Datura[a]
	Quinine	Cinchona
	Cardenolides	Digitalis[a]
	Codeine	Papaver[a]
Fragrances	Rose oil	Rosa[a]
	Lavender oil	Lavendula[a]
Flavours	Vanillin	Vanilla
	Capsaicin	Capsicum[a]
Colours	Indigo	Indigofera
	Shikonine	Erythrorhyzon
Poisons	Coniine	Conium
	Strychnine	Strychnos
Stimulants	Caffeine	Coffea[a]
	Theophylline	Thea[a]
	Nicotine	Nicotiana[a]
Hallucinogen	Cocaine	Erythroxylon
	Cannabinol	Cannabis[a]
Insecticide	Nicotine	Nicotiana[a]
	Pyrethrin	Pyrethrum[a]
	Piperine	Piper

[a] Species which have been selected for higher product yields by plant breeders

through selection by plant breeders was specially successful (Table 2). However, secondary metabolites could also be important in another field of economical importance, i.e. agriculture and plant breeding. It is not my intention in this presentation to cover the literature exhaustively, but rather to present an overview of the most significant and interesting aspects of secondary metabolism in relation to plant breeding and agriculture.

3 Defense strategies of plants

To be able to understand and interpret the functions of secondary metabolism we have to discuss the defense measures of plants against herbivores and microbial pathogens. Most of the main defense mechanisms of animals (Edmunds 1974, Table 3) are not valid for plants. Although plants serve as the main source of energy for animals and microorganisms, plant life has survived and is abundant on earth. Therefore, efficient defense strategies must exist, which can also be seen from the fact that dead plant parts usually decay (i.e. they are degraded by animals and microorganisms) in a relatively short time. We can distinguish the following main strategies in plants, which are not independent and which may react cooperatively and synergistically:

Table 3. Defense strategies in vertebrates and insects as compared to plants and fungi

Defense against	Organisms					
	Mammals	Birds	Amphibia	Insects	Plants	Fungi
1. Carnivores/Herbivores						
Flight	0	0	0	0	–	–
Weapons	0	+	*	+	*	–
Secondary metabolites	*	–	+	+	0	0
Armour	*	–	–	+	*	–
Anachoresis	*	*	*	*	–	–
Crypsis	*	*	*	*	–	–
Aposematism	*	–	*	*	–	–
Batesian mimikry	–	–	–	*	–	–
Thanatosis	*	*	*	*	–	–
Deimatic behaviour	*	*	*	*	–	–
Group defence	*	*	–	*	–	–
Open growth	–	–	(*)	–	0	+
2. Microorganisms						
Antibodies	0	0	0	–	–	–
Macrophages	0	0	0	0	–	–
Secondary metabolites	–	–	*	?	0	0
Lysozyme and other proteins	0	0	0	0	0	0
Cuticle	0	0	0	0	0	0

0 = Common and regular feature; + = widely used mechanism; * = used by a few species; – = not used

Table 4. Chemical protection of plants (general strategies)

Defense level	Compounds	Biological activity
1. Surface	Cuticular waxes	repellent, antibiotic, hydrophobic barrier
2. Carbohydrates/polymers	Cellwall (cellulose, lignin)	reduced digestibility
	Callose, lignin (wounds)	penetration barrier, antibiotic
4. Proteins	Lectins	cytotoxic,
	Protease inhibitors	reduced digestibility
	Hydrolytic enzymes (Lysozyme, chitinase, Esterase, DNAse, RNAse, Phosphatase, glycosidases)	degradation of microbial constituents
	Oxidases (Peroxidase, Phenoloxidase)	Degradation of microbial phytotoxins
4. Secondary metabolites	Flavonoids/anthocyanins	antimicrobial, insectistatic,
	Phenylpropanoids	antimicrobial
	Alkaloids	toxic for vertebrates, arthropods, antimicrobial
	Non-protein amino acids	toxic for animals, antimicrobial
	Cyanogenic glycosides	toxic for animals, antimicrobial
	Glucosinolates	repellent for animals, antimicrobial
	Terpenes	repellent for animals, antimicrobial

3.1 Mechanical protection

The evolution of thorns, spikes, trichomes, glandular hairs [in combination with 3.3 (chemical protection)], bark (especially in woody perennials) etc. has been interpreted as an antipredatory device (in analogy to weapons and shells in animals).

3.2 Growth strategies

Plants (especially perennials) are able to regenerate parts which have been diseased or wounded.

3.3 Chemical protection (Table 4)

I. Plant surfaces are usually covered by a hydrophobic layer consisting of antibiotic and repellent cuticular waxes (Levin 1976; Harborne 1982) which can contain other secondary metabolites such as flavonoids (see *3.3.IV*). *II.* Presence of carbohydrates and lignin: a) constitutively in the cell wall and b) after induction at the site of infection or wounding (Kauss 1985; Deverall 1977). *III.* Synthesis of inhibitory proteins or enzymes which could degrade microbial cell walls and other microbial constituents, or peroxidase and phenoloxidase which could help to inactivate phytotoxins of microbial origin. These enzymes are either stored in the vacuole (Boller and Wiemken 1986) or are present as exoenzymes outside the cells (Boller and Wiemken 1986; Wink 1984). In addition, proteins (e.g. storage proteins of legumes and cereals) are often deficient in amino acids, such as lysine or methionine, which are essential in the metabolism of animals. This trait can also be regarded as an antiherbivore strategy. *IV.* Synthesis of low-molecular-weight compounds ("secondary metabolites", Table 1) with repellent or toxic properties against microorganisms and/or herbivores (Swain 1977; Levin 1976; Harborne 1982, 1986). These compounds can be constitutively expressed, they may be activated by wounding (e.g. cyanogenic glycosides, glucosinolates, coumarins), or their de-novo synthesis may be induced by infection or herbivory (e.g. "phytoalexins") (Grisebach and Ebel 1978; Deverall 1977; Harborne 1982, 1986; Brooks and Watson 1985). All these products are often synthesized or stored at strategically important sites (epidermal tissues or in cells adjacent to an infection), or plant parts that are valuable for reproduction and survival (flowers, fruits, seeds, bark, roots).

In animals we can observe the analogous situation in that many insects, but also vertebrates, produce "secondary metabolites" for their defense (Table 3), which are often similar in structure with plant metabolites (Habermehl 1983; Schildknecht 1977). In many instances the animals have obtained these toxins from their host plants (Schneider 1986; Boppre 1986; Wink and Römer 1986; Nahrstedt 1985; Harborne 1986).

4 Function of secondary metabolites

As is obvious from the last chapter, secondary metabolites play an important role in the defense of plants. This has been a controversial concept over the last 100 years, and even today has not been generally accepted.

It was often argued that secondary metabolites are waste products or have no function at all (Mothes 1955; Paech 1950). However, the "waste product" or "no-function" hypothesis fails to explain a number of observations:

1) Waste products are characteristic and necessary for heterotrophic organisms, e.g. animals, which have to take up organic material of plant, microbial and animal origin. Since this material cannot be degraded completely for energy production or reused for biosynthesis, heterotrophs excrete waste products that are often rich in nitrogen. Plants, however, are essential autotrophs and therefore do not need comparable elaborated degradative and excretory activities as do animals. Furthermore, plants are usually limited in their nitrogen supply. Consequently, the production of *N*-containing metabolites, such as alkaloids, non-protein amino acids, or cyanogenic glycosides (Table 1), which have been considered as waste products (Paech 1950), would be difficult to explain. In addition, alkaloids are often found in young and metabolically active tissue, but not in dying or senescing cells (Waller and Nowacki 1978). According to the waste product hypothesis just the opposite would have been expected.
2) Secondary metabolites are often not inert end products of metabolism (which would be a consequence of the waste product hypothesis), but many of them can be metabolized by plant cells (Barz 1977; Barz and Köster 1981). For example, *N*-containing secondary metabolites, such as alkaloids or non-protein amino acids, are often stored in legume seeds. During germination a degradation of these compounds has been recorded, indicating that the nitrogen was reused for the seedling's metabolism (Rosenthal 1982; Wink and Witte 1985).
3) Secondary metabolism of plants is often very complex and regulated in a tissue and developmentally specific manner (Wiermann 1981), which would be surprising for waste products or products without any function.

Because the "waste product" and "no function" hypothesis did not satisfy either, the original "defense" hypothesis of Stahl (1888) was rediscovered and further developed by Fraenkel (1959), Ehrlich and Raven (1964), Whittaker and Feeny (1971), Swain (1977), Levin (1976), Levinson (1976), Schildknecht (1977), Rosenthal and Janzen (1979), and Harborne (1982, 1986). Today this hypothesis is the basis of a biological discipline, i.e. "Chemical Ecology", which is actively studied in North America. Several research conferences (American Chemical society; Gordon conference) were devoted to this topic in recent years (Green and Hedin 1986; Waller 1987).

According to this concept, secondary compounds have evolved during evolution as a defense against microorganisms (viruses, bacteria, fungi), against herbivores (molluscs, arthropods, vertebrates), and against competing plants (allelopathy) (Table 5). Another function can be the attraction of animals for pollination (fragrances, colours) or seed dispersal (Table 5). Secondary metabolites are often not directed against a single organism but generally against a variety of potential enemies, or they may combine the roles of both repellents and attractants [e.g. anthocyanins or volatile terpenes (essential oils) can be attractants in flowers, but are also insecticidal and antimicrobial]. In addition, some secondary metabolites concomitantly display metabolic functions, e.g. nitrogen transport (Wink and Witte 1984), nitrogen storage (Rosenthal 1982; Wink and Witte 1985), or UV-protection (Harborne 1982).

Table 5. Biological activities of some secondary metabolites of plants (Schlee 1986; Levin 1976; Swain 1977; Wink 1987)

Biological activity	Compounds
Antiviral	Lycorine (A); sparteine (A);
Antibacterial	Citronellal (T); cineole (T); many essential oils (T); canavanine (NP); azetidine-2-carboxylic acid (NP); glucosinolates; alliin (NP); berberine (A);
Antifungal	Protocatechuic acid (P); Nobeletin (F); Chlorogenic acid (P); tannin (P); solanine (A); phellandrine (T); gossypol (T); limonene (T); geraniol (T); citrol (T); saponines (S); canavanine (NP); arbutine (Q); cyanogenic glycosides (CG); glucosinolates; juglone (Q); pisatin (F); lupanine (A); furanocoumarine; rishitin (T); polyacetylenes; stilbenes;
Allelopathy	simple phenols; juglone (Q); phloridzin (P); non-protein amino acids; coumarins; quercetin (F); polyacetylenes; monoterpenes (T); sesquiterpene lactones;
Toxicity/repellence:	
Insects	Quercetin (F); morin (F); rutin (F); ferulic acid (P); caffeic acid (P); juglone (Q); tannine (P); limonene (T); gossypol (T); cucurbitacin (T); saponins (T); phytoecdysone (T); glucosinolates; monoterpenes; steroid-, quinolizidine alkaloids, nicotine (A); aconitine (A); colchicine (A); strychnine (A); atropine (A); morphine (A); berberine (A); piperine (A); most non-protein amino acids; cyanogenic glycosides; sesquiterpene lactones (T); azadirachtin (T); protease inhibitors, lectins; pyrethrins; rotenone; coumarins;
Vertebrates	Isoflavones (F); coumarins; juglone (Q); tannin; hypericin (F); gossypol (T); essential oils (T); saponins (T); sesquiterpene lactones (T); cardenolides (T); glucosinolates; most alkaloids; non-protein amino acids; cyanogenic glycosides;
Attractants	Flavonoids; anthocyanins; monoterpenes; sesquiterpenes; Carotenoids (T); betalaines (A); quinones; phenols; amines; sugars; amino acids; lipids;

A = alkaloid; F = flavonoid; P = simple phenol; S = saponine; T = terpene; NP = non-protein amino acids; Q = quinone

A number of arguments are often given which seem to contradict the ecological significance of secondary metabolites. It might be argued that the defense hypothesis cannot be valid since most of our plants, even those with extremely poisonous metabolites (from the human point of view), are nevertheless attacked by pathogens and herbivores. We have to understand

and accept that chemical defense can only constitute a general barrier which will be effective in the majority of cases. Plants with strong toxins at the same time constitute an ecological niche for potential pathogens and herbivores. During evolution[1] a few organisms have generally been successful in specializing in a given toxic plant in that they found a way to sequester the toxins or become immune to them. This is especially evident in the largest class of animals, the insects (more than a million species), which are usually very host specific. The number of these specialists is exceedingly small for a given plant species as compared to the number of potential enemies. We have to compare this situation with our own immune system: it works against the majority of microorganisms, but fails towards few viruses, bacteria and fungi, which have overcome this defense barrier by clever strategies. Nobody would call the immune system and the antibodies useless because of these few adapted specialists! We should adopt the same argumentation when we consider plants' defenses by secondary metabolites.

Those specialized pathogens do not only tolerate a given toxin, they may use it for their own defense or communication and they often select host plants according to this particular metabolite.

Within a group of secondary metabolites, a high diversity of chemical modifications (hydroxylation, methylations, epoxides, esters, glycosides) can be generally observed, which has been interpreted as a molecular game of otherwise functionless compounds (Mothes 1976). Is there any explanation for this diversity or the existence of so many metabolites (Table 1) in view of the defense hypothesis?

Chemical diversity could be a strategy against adaptation: metabolite patterns often differ between species but even vary for different plant organs, developmental stages, and within populations. Since these metabolites can differ in their toxicity and biological activity (although they are chemically related and often similar), it is more difficult for a herbivore to adapt and cope with a diverse and everchanging mixture as compared to the situation in which all individuals or species contain only one type of a toxin molecule. An analogy can be observed in medicine: resistance of microbial pathogens increases if only a single antibiotic is given over a longer time period and resistance is often overcome by applying a mixture of different antibiotics or by variation of antibiotics used.

5 Importance of secondary metabolites for plant breeding

Plant breeders try to select varieties which provide maximal yields in combination with optimal quality and resistance against pathogens, herbivores, and other environmental stress. One of the major problems of modern agriculture (both economically and ecologically) is its need for herbicides, insecticides and other pesticides.

Are agricultural crops less resistant against pathogens and herbivores than wild plants? It can be observed regularly that wild plants which have been introduced into gardens often escape to the wild again and can establish new populations. Although crop species are grown on a large scale, these species hardly ever escape to the wild and form new populations, indicating their reduced fitness.

We might argue that crop species have lost their original protection during domestication. In the next section evidence will be presented that the lost resistance factor can be the accumulation of secondary metabolites.

5.1 Lupinus

Seeds of lupins, such as *Lupinus albus* and *L. mutabilis*, contain up to 40% protein and up to 20% lipids are nutritionally comparable to soy beans. But lupin seeds also contain up to 5% quinolizidine alkaloids, which taste bitter and are toxic to vertebrates (including man) and insects. Therefore, plant breeders (notably M. Baur and R. von Sengbusch) were ambitious to select varieties devoid of alkaloids, the socalled sweet lupins (Hackbarth and Husfeld 1939). Sweet lupins are extraordinarily rare in nature, but sweet varieties have been obtained which are currently grown in France, Poland, USSR, South America, South Africa and especially in Australia (Gross and Bunting 1982; Bellido 1982, 1984). These new varieties can only be cultivated if pesticides are applied, since sweet lupins are susceptible to a wide variety of pathogens and herbivores to which their bitter wild-types are resistant (Table 6). Therefore, this species offers the opportunity to study the biological functions of alkaloids and the integration of alkaloid metabolism in the defense concept of a plant.

QA are synthesized in the aerial green parts of lupins and other legumes (Schütte 1969; Wink and Hartmann 1985) and the chloroplast could be determined as the intracellular site of alkaloid formation (Wink and Hartmann 1985). Like other chloroplast processes, QA-formation is regulated by light and shows a diurnal rhythm (Wink and Witte 1984). QA are translocated after synthesis from the mesophyll cells to the neighbouring epidermal cells or by the phloem all over the plant, so that all plant parts contain alkaloids (Wink and Witte 1984; Wink 1987). Especially rich in alkaloids are the epidermal tissues and the organs important for reproduction, e.g. flowers and seeds. QA are stored in concentrations of 1 mM–10 mM in meso-

1 Even today we can observe this evolutionary process: If plant species are introduced to a new continent or island, it usually takes a long time before new pathogens or herbivores become adapted and specialized on this new species. For example, *Lupinus polyphyllus* from North America has a number of specialized herbivores, but it is hardly attacked by herbivores in Europe (Wink and Römer 1986). This lupin obviously left its enemies behind when it was transferred to Europe three centuries ago. Or *Cytisus monspessulanus*, which has been introduced in North America: whereas this species has a number of enemies in its mediterranean homeland, it has only one regular pest species in California about 100 years after having been introduced there (E. Bernays, pers. commun. 1986)

Table 6. Predation of lupins in relation to their alkaloid content (Wink 1985, 1987; Wink and Römer 1986). Lupins were grown in an experimental garden or in a greenhouse (more than 100 specimens each). Alkaloid-rich lupins and "alkaloid-free" plants had an equal chance of predation ("herbivory") which is indicated as the percentage of plants that were attacked or infested. Herbivory is expressed as % of the individuals which were attacked or eaten by the herbivores. Predators were: rabbits (*Oryctolagus cuniculus*), leaf miners (*Agromyzidae*), and aphids. For aphids a polyphageous species, *Myzus spec.*, and a monophageous species, *Macrosiphon albifrons* (which is specialized on lupanine-richt plants), were compared

Species[a]	Alkaloid content mg/g fresh weight	Herbivory (%)			
		Rabbits	Leaf miners	Myzus spec.	Macrosiphon
Lupinus albus					
var. lucky	0.01	100	100	20	< 5
var. lutop	0.01	100	100	15	< 5
var. lublanc	0.01	100	100	15	< 5
var. multoplupa	0.03	80	100	15	< 10
var. llaima	0.01	85	100	n.d.	n.d.
var. Syria	2.0	< 10	< 1	0	100
var. Crete	2.2	n.d.	< 1	0	100
Lupinus luteus	0.01	n.d.	n.d.	100	n.d.
	0.25	n.d.	n.d.	50	n.d.
	> 0.7	< 5	n.d.	< 1	0
Lupinus polyphyllus	> 1.0	< 5	< 1	0	80
Lupinus angustifolius	1.5	< 10	< 1	0	100
Lupinus mutabilis	2.5	< 5	< 1	0	30

[a] Seeds were kindly provided by Dr. E. v. Baer, Dr. R. v. Sengbusch, INRA Lusignan, FAL Braunschweig
n.d. = not determined

phyll tissue, of 25 mM–200 mM in epidermal cells, and of 100 mM–200 mM in seeds (Wink 1985; Wink 1987). QA have a number of defined biological properties: They can inhibit the multiplication of plant viruses, of bacteria, of fungi, (such as mildew). They can repel herbivores or are toxic to them, e.g. molluscs, insects and mammals (Waller and Nowacki 1978; Dreyer et al. 1985; Wink 1985, 1987). QA also inhibit the germination of other plants (Wink 1985, 1987). The inhibitory concentrations determined experimentally (ED_{50}) were usually in the range of 0.1 mM–3 mM, which would indicate that the alkaloid levels in the plant, especially those in epidermal tissue (which has to ward off an attack in the first instance) and in seeds, exceed the inhibitory concentrations by far. Thus, it is likely that QA play a decisive role in the defense system of lupins. As shown in Table 6 it is indeed the alkaloids which seem to be the relevant factor for resistance, since specimens, which were low in QA, were selectively attacked by herbivores.

In this case plant breeders have eliminated the alkaloids deliberately without knowing their functions. Today we would probably devise a different strategy: lupins should be selected which store QA in their tissues but not in their seeds. This sort of selection is not utopic but has been achieved with another important crop species, the potato.

5.2 Other crop species

Potatoes (*Solanum tuberosum*) contain steroid alkaloids as characteristic secondary metabolites which have been shown to be active against insects, mammals and microorganisms (Levinson 1976; Bentley et al. 1984; Wolters 1966; Tingey 1984; Sinden et al. 1986). Since these steroid alkaloids are toxic and even teratogenic for man, alkaloid-free tubers have been one goal of the domestication of potatoes. The alkaloids have been successfully reduced in tubers (Johns 1985, 1986), but were maintained in the green parts. In this case natural resistance against enemies has been only partly eliminated during domestication, which seems to be wise from the point of view of chemical ecology. A specialized herbivore of potato is the Colorado potato beetle (*Leptinotarsa decemlineata*), which is not repelled by low and medium concentrations of steroid alkaloids, such as solanine. When plant breeders selected for plants which were resistant to *Leptinotarsa,* it turned out that these plants had elevated alkaloid contents and were no longer useful for human consumption (Max-Planck-Institut für Züchtungsforschung, Köln). It was recently found that plants of *Solanum chacoense* which contained acetylated glycoalkaloids (leptine) were highly resistant against the potato beetle. In the next step, plant breeders intend to cross these varieties with

S. tuberosum to confer the resistance to a crop species (Sinden et al. 1986).

There are over 200,000 species of higher plants, but only a very small percentage has been selected as food plants by man. From the angle of chemical ecology, we can distinguish two groups of plants:

1) A number of species have evolved fruits which contain sweet compounds (sucrose, glucose, fructose) and which are advertized by attractive colours (mainly red and blue anthocyanins, yellow flavonoids or carotenoids) when they are mature (e.g. apple, peach, plum, or raspberry). The mature fruits often do not contain vertebrate-toxic ingredients, but only antimicrobial compounds (e.g. quinones in bilberry) (Harborne 1982). Vertebrates, and sometimes insects, are attracted to eat the fruits and since most seeds are resistant to digestion, these animal species help in the dispersion of seeds and thus improve the survival of the species. While the fruit pulp is usually free from toxins, the respective seeds are often poisonous (e.g. cyanogenic glycosides in *Prunus* which liberate the toxic HCN upon destruction). Most of our food plants which are not exploited as staple diets can be grouped in this class. During cultivation the aim was to increase the size of the fruits and often their sugar content. In general, there was no need to eliminate protective secondary substances which was advantageous from the point of view of chemical ecology.

2) For staple diets (tubers, seeds) rich in carbohydrates (starch), lipids, or protein (i.e. the seeds of Gramineae and Fabaceae), other strategies were followed, since these food items usually contain a number of active secondary metabolites (see *3.3.IV*). Man has overcome the toxicity problem by: *a* boiling food which will destroy heat labile compounds (e.g. lectins, protease protease inhibitors of legumes, glycosides etc.), and by discarding the boiling water which contains the majority of the hydrophilic toxins (i.e. alkaloids, phenolics, cyanogenic glycosides). Special leaching processes have been invented for cyanogenic glycosides (in yams and cassava) and for alkaloids (in lupins) (Wink 1985). These diets are kept under running water for several days to eliminate water soluble natural products. *b* As mentioned earlier, many secondary compounds are stored in the epidermis or other peripheral tissues which have to ward off an attack in the first instance. Thus, the toxic or repellent principle can be removed by peeling or cutting off the peripheral tissues in many instances [e.g. potatoes (especially the green tissue, which contains steroid alkaloids), cucumbers (cucurbitacins), raddishes (Glucosinolates); oranges (essential oils); cereals (phenolics); pomegranate (pelletierine, tannine)]. *c* Man has eaten clay with his food (e.g. potatoes), which could absorb toxic alkaloids (Johns 1986). *d* Man has bred for varieties which contain less or no bitter or toxic secondary metabolites (e.g. cucumbers/cucurbitacins; lettuce/bitter terpenes; potatoes/steroid alkaloids; *Brassica*/glucosinolates; *Cassava*/cyanogenic glycosides; grape fruit/sesquiterpenes; lupins/quinolizidine alkaloids). As shown for lupins, the natural resistance of a plant can thus be eliminated. This is probably true for a number of other crop species, but needs to be studied in detail. *e* Like many other phytophageous organisms man has evolved powerful enzymatic detoxification systems, in which a cytochrome p 450 dependent mixed function oxygenase plays a major role (Harborne 1982; Schlee 1986). (This adaption also enables man to cope with his synthetic chemistry).

6 Conclusion

Considering the available data on the chemical ecology of secondary metabolites, we can assume that these compounds can indeed contribute to the protection of a plant against microorganisms and herbivores in concert with other chemical and morphological features. If we want to select resistant plant varieties (Wenzel 1985) secondary metabolites should be taken into consideration. In North America this concept has been followed by the Western Region Research Centre, USDA, Berkeley, CA.

Often the wild-type or a nearly related species is still resistant to microorganisms or herbivores. As illustrated for potatoes, (see *5.2*) the plant breeder can try to cross these types with a cultivated variety. Modern methods of cell biology, e.g. protoplast fusion and chromosome transfer technique could be very helpful in this context (Wenzel 1985). If the resistance is due to a secondary metabolite which is unpalatable or toxic to man, these compounds can often be eliminated by food processing (cooking, leaching, peeling).

If this elimination is impossible, the plant breeder has to select for a toxin-free variety but could concomitantly try to substitute the unwanted chemical trait by another metabolite-mediated repellence which does not lead to unpalatable or toxic food. Many phenolics (simple cinnamic acids, flavonoids, anthocyanins), and terpenes (essential oils sesquiterpenes, ecdysones) (Table 5) confer resistance to microorganisms and insects, but are usually not toxic for man.

If we want to strengthen the resistance of a plant against a pathogen which is a specialized species, it is often of no use to increase the concentration of the secondary metabolites to which the species is adapted (see *5.2*). The selection of plants with an additional chemically different inhibitor could be the solution.

How can we breed for plants with a new chemical trait? The genome of plants is very large and probably contains a large number of "silent genes", some of which will code for enzymes of secondary metabolism. We have recently speculated that the plant genome harbors the genes for the basic pathways of most secondary metabolites (Wink and Witte 1983): quinolizidine alkaloids are found in many species of the *Fabaceae*, but a few isolated occurrences are known from other unrelated families (Wink 1985). Lupin alkaloid formation could be stimulated in lupin cell cultures by the addition of low-molecular weight "elicitors", including DNA-active alkaloids (which are unrelated to lupin alkaloids) (Wink and Witte 1983; Wink 1985). When these compounds were used in cell cultures of *Daucus, Spinacia, Symphytum, Conium* and *Chenopodium* (which are not known to contain quinolizidine alkaloids), nanogramm amounts of lupanine could be recorded. This means that the genes for lupin alkaloid biosynthesis should be present in these species. This evidence, together with the occurrence of QA in other unrelated species, induced us to assume that the respective genes are probably widely distributed but usually "silent" in the plant kingdom. A similar situation could exist for the basic pathways of other alkaloids. Most of the other groups of secondary metabolites (e.g. flavonoids, terpenes) are distributed widely in the plant kingdom anyway. This would mean that a way to find new chemical traits is to "wake up" silent genes, which could be done by stress chemical (DNA-active alkaloids, azacytidine), or mutagenetic treatment. It is important, however, that we monitor for the occurrence of plants (haploids should be especially useful) with new secondary metabolites (Hedin et al. 1985). This would involve the screening of many thousands of individuals by immunological methods such as radio immuno assay or ELISA (these tests exist for a number of secondary products already, or could be developed rather quickly) and visually if the compounds are coloured (e.g. anthocyanins) or by GLC, HPLC or TLC. If such a plant has been detected, it's chemical content can be further increased by breeding (selection, haploid techniques), as has been done with many other species (compare Table 2). Traditionally, the plant breeder looks for a resistant phenotype without knowing the underlying chemistry, and will discard any plant in which a new chemical trait is not fully developed or enhanced (because he cannot detect it).

A second strategy would be the transfer of the respective genetic information. Probably the easiest way would be by modern cell techniques, e.g. protoplast fusion or chromosome transfer. More difficult, but also feasable in the long run, would be the direct genetic transformation of a plant with respective "resistance genes". This would mean, however, that we have to know and clone the genes of the pathways of secondary metabolism beforehand (in the case of protease inhibitors respective gene transfer experiments seem to work). At the present time only a few genes of secondary metabolism have been cloned, e.g. phenylalanine ammonium lyase (PAL), 4-coumarate: CoA ligase (4-CL) and chalcone synthase (CHS) (Chappell and Hahlbrock 1984, Bell et al. 1984), so that genetic engineering of secondary pathways is still in its infancy. An additional difficulty would be the fact that resistance due to secondary metabolites needs the concomitant expression of a number of genes (i.e. those for biosynthesis and for accumulation, such as carrier proteins) which have to be regulated in the correct spatial and temporal sequence. Basic research in the principles of plant secondary metabolism and its molecular biology will be an ultimate requirement for this approach.

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