BIOLOGICAL ACTIVITIES AND POTENTIAL APPLICATION OF LUPIN ALKALOIDS

Wink, M.
Institut für Pharmazeutische Biologie der Universität Heidelberg,
Im Neuenheimer Feld 364, D-69120 Heidelberg, FRG.

ABSTRACT

A characteristic feature of lupins is the production of quinolizidine alkaloids, such as lupanine, sparteine, lupinine, 13-hydroxy lupanine, angustifoline and ester alkaloids. Especially rich in alkaloids are the seeds, which are of nutritional importance because of their protein and oil content. Since the alkaloids are toxic for vertebrates, they have to be eliminated for human and animal consumption. Thus, large amounts of alkaloids will be a by-product of the refinement of bitter lupin seeds. Is there a utilization for these alkaloids?

The biochemical activities of lupin alkaloids will be reviewed. The existing data imply that lupin alkaloids might be interesting in medicine (the quinolizidine alkaloid sparteine is used as an anti arrhythmie drug already) and in agriculture (exploitation as a natural plant protective against insect and maybe against fungi and weeds).

1. INTRODUCTION

In order to survive plants have developed defences against herbivorous animals or phytopathogenic microorganisms during evolution. Furthermore, plants compete with other plants (of the same or of different species) for light, water and nutrients. The main defence strategies (Harborne 1988, Levin 1976, Swain 1977, Deverall 1977, Wink 1988, 1992a,b, 1993a-c) in which alkaloids and other secondary metabolites are of ultimate importance are summarized in Table 1.

More than 30,000 "natural products" or "secondary metabolites" have been reported from plants so far. Although the biological function of many plant-derived secondary metabolites has not been studied experimentally, it is now generally accepted that these compounds are important for the survival and fitness of a plant and that they are not useless waste products, as was suggested earlier in this century. Allelochemicals are often not directed against a single organism, but against a variety of potential enemies, or they may combine the roles of both deterrents and attractants. Thus, many natural products have multiple functions.

It might be argued that the defence hypothesis cannot be valid since most plants, even those with extremely poisonous metabolites, are nevertheless attacked by pathogens and herbivores. However, chemical defence is not an absolute process. Rather, it constitutes a general barrier which will be effective in most circumstances, i.e. most potential enemies are repelled or deterred. Plants with allelochemicals at the same time represent an ecological niche for potential pathogens and herbivores. During evolution a few organisms have generally been successful in specializing towards that niche (so-called specialists). This is especially apparent in the largest class of animals, the insects (probably with several million species), which are often highly host plant specific and ingenious to utilize the chemical defence of their host plants. The number of these "specialists" is exceedingly small for a given plant species as compared to the number of potential enemies that are present in the ecosystem. We can compare this situation with our immune system: it works against the majority of microorganisms, but fails towards a few viruses, bacteria, fungi and protozoa, which have overcome this defence 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 the plants' defences by secondary metabolites.

Table 1. Defence strategies of plants against herbivores, microorganisms and competing plants (after Wink 1988, 1992a,b, 1993a-c)

- Replacement of leaves and branches after herbivory ("open growth").
- Mechanical protection by hooks, thorns, spikes, trichomes, glandular hairs and stinging hairs
- Formation of penetration barriers (bark, hydrophobic cuticle)
- Laticifers and resin ducts (filled with latex or resin)
- Cell walls with reduced digestibility (cellulose, pectin, suberin, callose and lignin)
- Production and storage of defence chemicals

a.) Plant surfaces with cuticular waxes which often incorporate other antibiotic and deterrent/repellent allelochemicals such as flavonoids.

b.) Synthesis of inhibitory or toxic proteins (e.g., lectins, protease inhibitors, toxalbumins) or enzymes (e.g., chitinase, β-1,3-glucanase, hydrolases, nucleases, peroxidase and phenolase) which are either stored in the vacuole or are secreted as exoenzymes into the cell wall or the extracellular space (review: Chrispeels 1991, Wink 1993c).

c.) Storage proteins (of cereals and legumes) are often deficient in particular essential amino acids, such as lysine or methionine.

d.) Secondary metabolites with deterrent/repellent or toxic properties against microorganisms, viruses and/or herbivores. These compounds can be constitutively expressed, they may be activated by wounding (e.g. cyanogenic glycosides, glucosinolates, coumaryl glycosides, alliin, ranunculin, etc.), or their de-novo synthesis may be induced by elicitors (so-called "phytoalexins"), infection or herbivory. Storage in strategically important positions (epidermal tissues or in cells adjacent to an infection), or in plant parts that are especially important for reproduction and survival (flowers, fruits, seeds, bark, roots).

2. QUINOLIZIDINE ALKALOIDS (QA) IN LUPINS

2.1. Structures, occurrence and biochemistry of lupin alkaloids

Quinolizidine alkaloids are predominantly distributed within the Leguminosae, the third largest family of flowering plants after the Compositae and Orchidaceae, which consists of about 650 genera and 18,000 species. Tribes with QA are: Sophoreae, Dalbergieae, Euchrestaeae, Thermopsidae, Genistaeae, Bossiaeae, Brongniartieae, Podalyrieae, Liparieae and Crotalarieae.

More than 170 structures of quinolizidine alkaloids have been reported from plants (overviews in Kinghorn and Balandrin 1984, Wink 1992, 1993a,b). The main structural types of QA belong to lupanine/sparteine, multiflorine, aphylline, anagyrine/ctisiine, lupinine, matrine and respective derivatives (Kinghorn and Balandrin 1984, Wink 1993b) (Fig.1).


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Figure 1: Structures of quinolizidine alkaloids from lupins.
In Table 2 the alkaloid composition of lupins used in agriculture is summarized. A number of phytochemical methods have been developed for the qualitative and quantitative determination of lupin alkaloids (reviews: Kinghorn & Balandrin; Wink 1992, 1993a,b). Method of choice for the evaluation of complex alkaloid mixture is capillary gas-liquid chromatography combined with mass spectrometry (Wink 1992, 1993). Depending on the task HPLC, TLC, colorimetry, NMR, RIA and ELISA are additional helpful analytical techniques (Wink 1992, 1993a).

Table 2. Composition of alkaloids in seeds of bitter lupins used in agriculture (after Wink 1993b; Wink et al., in preparation)

<table>
<thead>
<tr>
<th>Species</th>
<th>Alkaloid composition (total =100%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>L. albus</td>
<td>+</td>
</tr>
<tr>
<td>L. angustifolius</td>
<td>+</td>
</tr>
<tr>
<td>L. atlanticus</td>
<td>15</td>
</tr>
<tr>
<td>L. consenstini</td>
<td>15</td>
</tr>
<tr>
<td>L. hispanicus</td>
<td>50</td>
</tr>
<tr>
<td>L. luteus</td>
<td>60</td>
</tr>
<tr>
<td>L. micranthus</td>
<td></td>
</tr>
<tr>
<td>L. palaestinus</td>
<td>2</td>
</tr>
<tr>
<td>L. varius</td>
<td>25</td>
</tr>
<tr>
<td>L. mutabilis</td>
<td>+</td>
</tr>
<tr>
<td>L. polyphyllus</td>
<td>+</td>
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</table>

\[1=\text{lupinine}, \quad 2=\text{spartine}, \quad 3=\text{albine}, \quad 4=\text{angustifoline}, \quad 5=\text{lupanine}, \quad 6=11,12-\text{seco-12,13-didehydromultiflorine}, \quad 7=\text{multiflorine}, \quad 8=13\alpha-\text{hydroxylupanine}, \quad 9=13-\text{tigloyloxy} lupanine, \quad 10=13-\text{cinnamoyloxy} lupanine, \quad 11=\text{tetrahydrotrohombifoline}, \quad 12=\text{gramine}; \quad + = <1\%\]


2.2. Role of QA as chemical defence compounds

We have studied the functions of QA in lupins and found that their main role is that of chemical defence besides minor functions in nitrogen transport in the phloem and nitrogen storage in the seed (Wink and Witte 1984). Their main function, however, is that of chemical defence against herbivores. In the following experimental evidence for the defensive role of QA is summarized.

2.2.1. Insecticidal and deterrent properties of QA

QA were found to be feeding deterrents for a number of oligo- and polyphagous insects, including aphids, moth- and butterfly larvae, beetles, grasshoppers, flies, bees and ants, i.e. QA are deterrents over a wide range of insect orders (Table 4) (Wink 1985, 1987a, 1988,1992a, 1993a,b). The few electrophysiological experiments performed with QA so far indicate that spartine and other QA elicit responses at chemical sensilla of Entomoscelis, Phormia and Pieris which lead to food rejection (Schoonhoven 1972, Mitchell and Sutcliffe
1984, Blades and Mitchell, 1986). In addition to deterrence, QA seem to be toxic to many insects (Table 4). The mechanisms underlying the toxic effects observed in insects have not been elucidated. However, it was shown that QA can interfere with the acetylcholine receptor (Fig.2) and with protein biosynthesis (Korcz et al., 1987, Wink and Twardowski 1992, Wink 1992a, 1993b). Since synaptic signal transduction and protein biosynthesis are important and vulnerable processes in most animals, it is not surprising that QA have toxic properties over a wide range of organisms (Table 4). Other targets could be K⁺-channels which are influenced by sparteine in vertebrates or glutamate receptors which can be modulated by matrine in crayfish neurons (Tab.4; Kinghorn and Balandrin 1984; Wink 1992a,b, 1993a,b). The list is certainly far from complete.

Table 3: Overview of the biochemistry of quinolizidine alkaloids (after Wink 1984a,b, 1985, 1987c, 1991, 1992a-c)

- **OCCURRENCE:** Fabaceae: Lupinus, Cytisus, Genista, Laburnum, Thermopsis, Baptisia, Sophora etc.
- **BIOSYNTHESIS:** Sequence: Lysine = cadaverine = lupanine
  - Enzymes involved: Lysine decarboxylase, oxosparteine synthase
  - Alkaloid synthesis only in green tissue
  - Localisation of alkaloid biosynthesis in leaf chloroplast
  - Regulation by light (pH, thioredoxin, precursor availability)
  - Diurnal fluctuation of enzymes and alkaloid contents
- **ACCUMULATION:** All parts of a plant accumulate alkaloids
  - Vacuole functions as intracellular storage compartment
  - Epidermal/subepidermal tissues main site of alkaloid storage in leaves and stems
  - Storage in seeds
- **TRANSPORT:** Long distance transport in phloem
  - Passage across tonoplast with aid of an alkaloid transporter; Mg-ATP and K⁺ necessary for uptake
  - Also carrier-mediated transport across plasmalemma
- **DEGRADATION:** Diurnal degradation in all organs
  - During germination and growth of the seedling (Mobilization of alkaloidal nitrogen)

2.2.2. Toxicity and pharmacology

QA deter or repel the feeding of a number of non-insect herbivores (and other animals), e.g. nematodes, snails, rabbits, cows or are directly toxic or mutagenic to them (Table 4). For example, anagyrine causes malformations, the so-called "crooked calf disease" in young sheep and calves, when their mothers feed on lupins or broom containing anagyrine (ingested at 7-11 mg/kg per day during day 40 and 75 of gestation) (Keeler 1976). Alkaloid extracts of Lupinus angustifolius which contain lupanine, angustifoline and 13-hydroxylupanine did not show mutagenic activity in a Salmonella and Chinese hamster cell system (Culvenor and Petterson 1986). This suggests that the mutagenic effect is limited to anagyrine and perhaps other α-pyridone alkaloids, but not caused by the lupanine-type QA. Vertebrate toxicity was assessed for those QA that are abundant and frequently found in plants (Tab.4): alkaloids of the sparteine-lupanine-type are relatively toxic when injected (i.v or i.p.) but less so when given orally, an important fact, if we consider using these alkaloids as natural pesticides. Lupanine and other lupin alkaloids show a moderate toxicity in vertebrates, whereas α-pyridone alkaloids, such as clytine and anagyrine are strong poisons. It is likely that the molecular target of QA poisoning is the ACH-receptor (Fig.2), since QA display similar agonistic receptor activities as the alkaloid nicotine.
<table>
<thead>
<tr>
<th>ACTIVITY</th>
<th>ALKALOID</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt;</th>
<th>ACTIVECONC.</th>
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</thead>
<tbody>
<tr>
<td><strong>Activities against animals</strong></td>
<td></td>
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<tr>
<td>Reduction of nematode and</td>
<td>N-methylcytisine</td>
<td>&lt;0.1 mM</td>
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<tr>
<td>helminth motility</td>
<td>Matrine</td>
<td>&lt;1.2 mM</td>
<td></td>
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<tr>
<td>Feeding deterrent in snails</td>
<td>Sparteine</td>
<td>&lt;0.7 mM</td>
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<tr>
<td></td>
<td>Lupanine</td>
<td>&lt;7 mM</td>
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<tr>
<td></td>
<td>Cytisine</td>
<td>2.5 mM</td>
<td></td>
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<tr>
<td>Insect feeding deterrence</td>
<td>Sparteine</td>
<td>&lt;0.05%</td>
<td></td>
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<tr>
<td>(several species)</td>
<td>Lupanine</td>
<td>&gt;0.1%</td>
<td></td>
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<tr>
<td></td>
<td>13-Tigloyloxylanine</td>
<td>&lt;0.1 mM</td>
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<td></td>
<td>Cytisine</td>
<td>&lt;0.1%</td>
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<tr>
<td></td>
<td>Lupinine</td>
<td>0.08%</td>
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<tr>
<td>Insect toxicity (LD&lt;sub&gt;100&lt;/sub&gt;)</td>
<td>Sparteine</td>
<td>9-50 mM</td>
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<tr>
<td>(several species)</td>
<td>Lupanine</td>
<td>3-12 mM</td>
<td></td>
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<tr>
<td></td>
<td>13-Tigloyloxylanine</td>
<td>6 mM</td>
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<tr>
<td><strong>Vertebrate toxicity</strong></td>
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<tr>
<td>General toxicity</td>
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<tr>
<td>(Mouse, i.p. LD&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>Sparteine</td>
<td>55-67 mg/kg</td>
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<td></td>
<td>Lupanine</td>
<td>80-175 mg/kg</td>
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<tr>
<td></td>
<td>13-Hydroxylanine</td>
<td>175 mg/kg</td>
<td></td>
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<tr>
<td></td>
<td>17-Oxolupanine</td>
<td>690 mg/kg</td>
<td></td>
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<tr>
<td></td>
<td>Matrine</td>
<td>150 mg/kg</td>
<td></td>
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<tr>
<td>(Mouse, p.o. LD&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>Sparteine</td>
<td>350-510 mg/kg</td>
<td></td>
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<tr>
<td></td>
<td>Lupanine</td>
<td>410 mg/kg</td>
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<tr>
<td><strong>Antimicrobial activities</strong></td>
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<tr>
<td>Inhibition of viral multiplication</td>
<td>Sparteine</td>
<td>n.d.</td>
<td>10 mM</td>
</tr>
<tr>
<td>Inhibition of bacterial growth</td>
<td>Sparteine</td>
<td>0.5-10 mM</td>
<td></td>
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<tr>
<td></td>
<td>Lupanine</td>
<td>&lt;5 mM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13-Tigloyloxylanine</td>
<td>&lt;5 mM</td>
<td></td>
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<tr>
<td></td>
<td>Angustifoline</td>
<td></td>
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<tr>
<td></td>
<td>13-Hydroxylanine</td>
<td>50 mM</td>
<td></td>
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<tr>
<td>Inhibition of fungal growth</td>
<td>13-Hydroxylanine</td>
<td>50 mM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sparteine</td>
<td>&gt;15 mM</td>
<td></td>
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<tr>
<td><strong>Allelopathic activities</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of seed germination</td>
<td>QA mixture</td>
<td>1.5 mM</td>
<td>&gt;4 mM</td>
</tr>
<tr>
<td></td>
<td>Sparteine</td>
<td>&lt;10 mM</td>
<td></td>
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<tr>
<td></td>
<td>Lupanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13-Tigloyloxylanine</td>
<td>&lt;10 mM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytisine</td>
<td>6 mM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sparteine</td>
<td>0.1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytisine</td>
<td>0.1%</td>
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</table>

QA are obviously bitter to man and other mammals: Mean detection levels in man are 0.00085% for sparteine, 0.0021% for lupanine and 0.017% for 13-hydroxylanine. In animals, the sensitivity to bitter lupins increases from sheep, over rabbit, Guinea pig, to mouse and pig (Wink 1992a, 1993a). This indicates that mammalian herbivores are able to detect and to avoid harmful levels of dietary QA. Interestingly, young and adult geese (*Anser anser*, *A.*
indicus and Branta canadensis) do not detect the bitter sparteine and feed deliberately on an artificial diet with up to 1% sparteine (Wink et al., 1993).

Figure 2: Binding of lupanine to the ACH receptor. Binding is measured as the amount of radiolabelled nicotine which is replaced by lupanine (T. Schmeller and Wink, in preparation). Binding of lupanine, tetrahydrorhombifoline and 13-hydroxy-lupanine are compared to a standard reference compound hexamethonium-bromide.

In addition to toxic and repellent properties, lupin alkaloids have a number of pharmacological activities (Kinghorn and Balandrin 1984, Wink 1992a,b, 1993a): Sparteine, lupanine and other QA have antiarrhythmic properties (Fig.2). Since only sparteine can be easily isolated from broom (Cytisus scoparius) it is the only lupin alkaloid that is commercially available and exploited in medicine as an antiarrhythmic drug. However, about 10% of all patients are unable to metabolize sparteine and suffer from sparteine intoxication. Because of these side effects and the availability of more reliable synthetic heart drugs the use of sparteine in modern medicine is declining and restricted. The utilisation of sparteine as a uterus contractive has been abandoned for the same reasons. Sparteine, lupanine and 13-hydroxy-lupanine have hypotensive and CNS-depressant properties and furthermore, are hypoglycemic, i.e. they reduce the level of blood glucose. In addition to QA, also the alkaloid-free fraction of L. albus seeds seems to have antidiabetic activities. Matrine and cytisine are amoebicidal. Matrine and 17-oxolupanine are effective inflammatory compounds. Some of
these pharmacological properties can be explained through modulation of the ACH receptor (Fig.2) and K⁺/Na⁺-channels.

2.2.3. Further biological activities of QA

Lupin alkaloids show a wide range of defensive activities (Tab.4) against other competing plants (Wink 1983a, 1985) and against microorganisms such as viruses (Wink 1987), bacteria (Wink 1984b, Tyski et al., 1988) and fungi (Wink 1984b, Wippich and Wink 1985). As compared to antiherbivoral activities, the antimicrobial and herbicidal defences seem to be of minor importance. Whereas lupin alkaloids are effective growth regulators in other plants they do not influence germination and root growth in lupin seeds or lupin seedlings. Thus, lupins must have evolved mechanisms to overcome the effects of their own defence chemicals.

2.3. Ecological relevance of alkaloid accumulation

QA concentrations are sufficiently high in lupins to guarantee their inhibitory effects observed in vitro. (Wink 1985, 1988, 1993a,b). In addition, QA contents can be increased by wounding; this short-term effect was highest under greenhouse conditions, but also measurable in the field (Wink 1983b, Johnson et al., 1989). In this context QA localisation in epidermal tissues (Wink et al., 1984) can be interpreted as a strategically important adaptation, since this tissue has to ward off small herbivores and pathogens in the first instance.

Plants which produce QA such as Cytisus, Genista, Chamaecytisus, Laburnum, Lupinus, Baptisia and Thermopsis, are relatively abundant in temperate climate zones of the Old and New World and are often dominant members of plant associations, present in pastures, meadows, heath and ruderal habitats. QA plants seldom suffer severely from the attack of herbivore generalists and comparably few adapted insects have been described.

Therefore, it seems likely that QA are definitely important for the fitness of lupins, an assumption which could be tested experimentally: Lupins have relatively large seeds which contain up to 40-50% protein, up to 20% lipids and 2-8% alkaloids. In order to use lupin seed for animal or human nutrition, Homo sapiens, for several thousand years, used to cook the seeds and leach out the alkaloids in running water. (Gross 1987, Bleitgen et al., 1979). The resulting seeds taste sweet, in contrast to the alkaloid-rich ones which are very bitter.

At the turn of this century, German plant breeders initiated a program to grow alkaloid-free lupins, the so-called "sweet lupins". Although extremely rare in Nature, the efforts were largely successful, and at present, "sweet" varieties with an alkaloid content lower than 0.01% exist for Lupinus albus, L. mutabilis, L. luteus, L. angustifolius and L. polyphyllus. As far as we know, the sweet varieties only differ from their original bitter wild forms, by their degree of alkaloid accumulation. This offers the chance to test experimentally, whether bitter lupins have a higher fitness than sweet ones with regard to microorganisms and herbivores (Table 5).

The results of these experiments were clearcut (Table 5): When planted in the field, without being fenced in and without man-made chemical protection sweet lupins suffered substantially from herbivores: Rabbits (Cuniculus europaeus) and hares (Lepus europaeus) clearly prefer the sweet plants and leave the bitter plants almost untouched, at least as long as there was an alternative food source (Waller and Nowacki 1978, Wink 1985, 1987a, 1988). A
similar picture was seen for a number of insect species, such as aphids, beetles, thrips and leaf-mining flies (Table 5), i.e. the sweet forms were attacked, whereas the alkaloid-rich ones were largely protected. The list of insects attracted by sweet lupins is even longer and includes Agrotis spec. (Lep.), Phorbia platura (Dipt.), Tipula spec. (Dipt.), Agriotes spec. (Col.), Smithurus viridis (Colembola), Ciampa arietaria (Lep.), Heliothis punctigera, H. armigera, (Lep.), Phytomyza horticola (Dip.), Sitona griseus (Coll.), Acyrthosiphon pisi (Hom.), A. kondoi (Hom.), A. craccivora (Hom.), Myzus persicae (Hom.), Calocoris norvegicus (Het.), Adelphocoris lineolatus (Het.), and Lygus rugulipennis (Het.). Plant breeders have observed that also bacterial, fungal and viral diseases are more abundant in the sweet forms, but this effect has not been documented in sufficient detail.

These data clearly support the importance of alkaloid production for chemical defence against herbivores. Since other allelochemicals (such as flavonoids, isoflavones, anthocyanins, phenolics, saponins, protease inhibitors, stachyose and verbascose, erucic acid, phytic acid etc.) are present in both forms, some residual resistance is still maintained even in the absence of QA. This is especially true for roots, which are a rich source for antifeedant and antifungal isoflavones.

No defence is absolute. A number of specialized pathogens and herbivore always evolved which have adapted to the special chemical situation of their host plant. These specialists are usually not deterred or intoxicated by the natural products of the host, instead these compounds often serve as an attractant. But as compared to the large number of potential enemies the number of specialized pests is usually rather small. For lupins such a specialized enemy is the lupin aphid, Macrosiphum albifrons.

The North American Lupin Aphid (Macrosiphum albifrons) appeared in Europe for the first time in England in 1981. Since then it has spread over large parts of Great Britain and since 1984 also over Germany. This aphid infests alkaloid-rich lupins which produce lupanine as the major alkaloid (Wink and Römer 1986, Emrich and Wink 1992). M. albifrons exploits the phloem which translocates the alkaloids from the site of synthesis (the leaves) to the sites of accumulation (stems, fruits, seeds). M. albifrons excretes QA with the honey dew but is also able to store substantial amounts of them. In a previous study we found alkaloid contents between 0.6 and 1.8 mg/g FW (equivalent to 2.4 - 7.2 mM) in aphids living on L. polyphyllus, L. albus, L. mutabilis L. arboresus and L. angustifolius (Wink and Witte 1991). It could be shown experimentally that the aphids obviously gain chemical protection against predators by storing lupin alkaloids (Wink and Römer 1986, Wink 1992a,b).

2.4. Sweet or bitter lupins?

The lupin example tells us also about the standard philosophy and problems of plant breeding. With our present knowledge of the ecological importance of QA's for the fitness of lupins, it seems doubtful whether the selection of sweet lupins was a wise decision. In order to grow them we have to built fences and worse, to employ man-made chemical pesticides, which have a number of well-documented disadvantages. It can be assumed that similar strategies, i.e. to breed away unwanted chemical traits, have been chosen with our other agricultural crops (such as cabbage, turnip, rape seed, tomato, potato, cassava or barley), with the consequence, that the overall fitness was much reduced (Wink 1988). We can easily observe their reduced fitness by trying to leave crop species to themselves in the wild: they will quickly disappear and not colonize new habitats. If we do the same with wild species,
usually the opposite is observed and these "newcomers" are often very successful in establishing new grounds.

Table 5. Importance of lupin alkaloids for the resistance of lupins against herbivores (after Wink 1988, 1992b)

<table>
<thead>
<tr>
<th>Herbivore species</th>
<th>Effect Alkaloid content of lupins</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>high (&quot;bitter&quot;)</td>
</tr>
<tr>
<td>Non-adapted herbivores:</td>
<td></td>
</tr>
<tr>
<td>1. Vertebrates</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>-</td>
</tr>
<tr>
<td>Hares (Lepus europaeus)</td>
<td>-</td>
</tr>
<tr>
<td>Rabbits (Oryctolagus europaeus)</td>
<td>-</td>
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<tr>
<td>2. Insects</td>
<td></td>
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<tr>
<td>Leaf miner (Agromyzidae)</td>
<td>-</td>
</tr>
<tr>
<td>Sitona lineatus (Coleoptera)</td>
<td>-</td>
</tr>
<tr>
<td>Acyrthosiphon pisum (Aphidae)</td>
<td>-</td>
</tr>
<tr>
<td>Aphis fabae (Aphidae)</td>
<td>-</td>
</tr>
<tr>
<td>Myzus spec. (Aphidae)</td>
<td>-</td>
</tr>
<tr>
<td>Thrips (Frankliniella spec.)</td>
<td>-</td>
</tr>
<tr>
<td>Adapted herbivores:</td>
<td></td>
</tr>
<tr>
<td>3. Insects</td>
<td></td>
</tr>
<tr>
<td>Macrosiphum albifrons (Aphidae)</td>
<td>+</td>
</tr>
</tbody>
</table>

- = no or very low infestation/herbivory; + = high or complete infestation/herbivory

Bitter lupins have alkaloid levels in their leaves of 1-4 mg/ g FW, sweet lupins of 0.01-0.05 mg/g FW

For lupins, we have proposed two alternatives (Wink 1991, 1992b): 1. To select for lupin mutants which do not translocate the alkaloids to the seeds, since seeds do not produce QA but store them. In this case, the plant would retain its chemical resistance but would provide the valuable alkaloid-free seeds at the same time. 2. To grow alkaloid-rich plants but to process the seeds, which are important because of their high levels of proteins and oil (in case of L. mutabilis). Large-scale technological procedures to remove alkaloids from the seeds after harvest (similarly to sugar raffination from sugar beets) would be the solution. At present few companies actively explore these possibilities, such as the Mittex Anlagenbau in Ravensburg, FRG (Jaeggle 1992). One idea is to produce simultaneously pure protein, lipids, amino acids, and dietary fibres from bitter seeds. A spin-off product would be alkaloids, which could be either used in medicine or in agriculture as a natural plant protective, i.e. insecticide. A pilot lupin refinery will be built soon by Mittex company with help of the EC.

3. POTENTIAL APPLICATION OF LUPIN ALKALOIDS

3.1. Utilization of natural products in agriculture

Before considering the question whether lupin alkaloids might be useful as natural plant protectives, we should ask whether natural products in general have a future in this field. Since many secondary metabolites have evolved in Nature as biologically active compounds with defensive properties, some of them are useful to mankind as pharmaceuticals or plant protectants.

Our modern agriculture can only exist if chemical means are employed to protect crops against microorganisms, especially fungi (fungicides), competing plants (herbicides) and
insects (⇒insecticides) and sometimes nematodes (⇒nematicides) and snails and slugs (⇒molluscsicides). The reasons why our crops are so much more susceptible against their enemies than their wild counterparts, are manyfold:

a) Mass cultivation of any plant provides an attractive ecological niche for potential enemies and thus the selection of them is favoured by modern large-scale agriculture.

b) Many crop plants have lost their natural resistance that was mediated through secondary metabolites (see above). These traits were neglected during plant breeding for high yields or were abolished by purpose, as outlined already for lupins.

c) Synthetic pesticides usually contain one active ingredient, whereas natural resistance is often mediated by a cocktail of poisons which make adaptations by herbivores much more difficult.

d) The equilibrium between pests and their natural enemies is often disturbed in modern agriculture.

Because of the increasing resistance of pests against synthetic pesticides, the persistancy of synthetic pesticides in soil and water (especially halogenated hydrocarbons) and their accumulation in the food chain, safe alternatives are always in demand.

Farming in third-world countries is confronted with even more severe problems. Most of these countries have a fast growing population which needs to be fed. On the other hand, the economy of many of these countries does often not allow to buy safe and efficient pesticides, which are in use in Europe or the USA. Instead, cheap alternatives (such as DDT) are used which, however, have been already banned in our countries because of the inherent environmental or health problems. Here, a cheap and safe strategy is of urgent importance to promote food self-sufficiency, which can be achieved better by "low-input agriculture" than by the "high-tech agriculture" of our Western world.

The question is therefore, whether plant-derived pesticides could be an alternative. These compounds are also active pesticides and usually do not persist in the environment for long, since they are rapidly degraded by microorganisms or sunlight. Furthermore, if these compounds can be obtained from plants grown locally, this would help third world countries to become food-self-sufficient and more independent from the second- and first world countries and petrochemical industries. However, since these allelochemicals evolved in nature as defence compounds, they cannot be considered as harmless and a thorough investigation of their safety and toxicology will be necessary before exploiting them in the field.

In order to appreciate the problems and chances of applying natural insecticides, we should recall the experiences with a small number of phytochemicals which have been used in agriculture already before synthetic compounds became available (Frear 1948, Heal et al., 1950, Jacobson & Crosby 1971, Jacobson 1975, 1988, Schmutterer et al., 1981, Schmutterer and Ascher 1984, 1987, Stoll 1986). The compounds mentioned do not necessarily represent the natural products which are best suited for their purpose, but at least some experience exists regarding their exploitation as natural insecticides (Wink 1993b).

### 3.1.1. Nicotine

Nicotine is the main alkaloid of many *Nicotiana* species (concentrations in leaves 2-8% DW). Nicotine is a lipophilic alkaloid, that can easily penetrate biomembranes by simple
diffusion. Thus, it can be quickly sorbed by target organisms. Nicotine is a strong agonist of the acetylcholine (ACH) receptor in insects and vertebrates, and acts as a deadly poison already at low concentrations (lethal dose (LD50) in mice after intravenous application (i.v.) is 0.3 mg/kg). Extracts from *Nicotiana* species can be effectively used to kill ectoparasites and agricultural pests (insects of various classes). Whereas nicotine was commonly used until the second world war, it was widely abandoned, because of its toxicity, in the US and Europe, when synthetic insecticides became more available.

### 3.1.2. Rotenone

The isoflavonone rotenone is produced by plants of the genus *Derris* (Fabaceae), such as *D. elliptica*, a small scrub originating from tropical rain forests of the Malaysian archipelago. Roots of *Derris* species are especially rich in rotenone (up to 6-8%). Rotenone is a powerful inhibitor of the electron chain in mitochondria and thus a potent toxin for all animals, including pests. After 1848 until the advent of DDT, rotenone became one of the most widely used insecticides worldwide (Atwal, 1976). Within Europe (e.g. in Austria and Switzerland), *Derris* extracts were usually combined with pyrethrins. *Derris* preparations are effective in the control of various insects.

### 3.1.3. Quassin

*Quassia amara* and *Picrosma excelsa* (Simaroubaceae) contain bitter-tasting seco-triterpenes, such as quassin, neоquassin and 18-hydroxyquassin which were used as a bitter tonic, a hop imitate, an anthelmintic and as an insecticide. *Quassia* extracts, prepared from the wood of both trees, were widely used against ectoparasites (lice) and flies. Quassin acts as a contact, stomach and systemic poison in insects (ectoparasites, lepidopteran larvae, beetles, and flies) and nematodes. *Quassia* extracts are also used for fly poison on flypaper. Quassin seems to be a selective pesticide with no recorded toxic effects on vertebrates.

### 3.1.4. Ryanodine

*Ryania speciosa* (Flacourtiaeaceae) is a tropical American (Amazonian basin, Caribean) small tree and produces the insecticidal ryanodine which is a stomach and contact poison and constitutes a selective pesticide against cockroaches, *Tribolium*, other beetles and lepidopteran larvae. Ryanodine was patented in 1946 by Merck & Co ("Ryanex, Ryanicide"), but its use is limited due to the shortage of raw material.

### 3.1.5. Pyrethrins

Flowers of the perennial *Chrysanthemum cinerariifolium* (syn. *Pyrethrum cinerariifolium*) (Asteraceae) which originate from Dalmatia and Montenegro, produce a series of terpene esters (pyrethrin, cinerin, pyrethrosin, pyretol, chrysanthemine, etc) with insecticidal properties. At present, *Chrysanthemum* species are cultivated throughout the world, e.g. in Japan, Kenya, Tanzania, Rwanda, Ecuador, Yugoslavia, California, Mexico, Chile, Brasil, and Russia). The insecticidal compounds include pyrethrin I and II and cinerin I and II and are especially abundant in carpels of young flowers. Pyrethrins are quickly inactivated by light and oxygen but rather few resistant pests have developed as compared to synthetic pesticides. Pyrethrins are active as a nervous and contact poison in insects, but also
act as a deterrent. Pyrethrins have been found to be effective against many insects (beetles, lepidopteran larvae, aphids, flies, cockroaches, ants, mosquitos, locusts, thrips, etc.). The insecticidal activity can be enhanced by the use of synergists, like sesamine from sesame oil or piperonylbutoxid (which inhibits mixed function cytochrome oxidases p450), by antioxidants such as hydroquinone and tannin, or by activators such as the ethyleneglycolether of pinene.

### 3.1.6. Azadirachtins

The neem tree (*Azadirachta indica* (Syn. *Melia azadirachta*) and the chinaberry tree (*Melia azederach*) (Meliaceae) produce seeds which are rich in insect deterrent and insecticidal tetranortriterpenoids, such as "azadirachtin" (Schmutterer *et al.*, 1981, Schmutterer & Ascher 1984, 1987, Schmutterer 1990, Dreyer & Hellpap 1991). This plant is already widely used Asian countries and will have a wide impact in other countries of the third world (Schmutterer 1990) as a cheap and efficient pesticide, especially in the small-scale cultivation of vegetables. Extracts of the neem tree are effective in the control of a wide range (*n >200 species*) of insects as contact and stomach poisons, because azadirachtin interferes with the metamorphosis (especially with the action of the moulting hormone ecdysone) of insects. In addition, azadirachtin can act as a feeding deterrent. Resistance of pests against azadirachtin and other neem products have not been recorded so far.

### 3.2. Utilization of lupin alkaloids

At present only sparteine is used as an antiarrhythmic drug and is thus of some medical importance. The other QA are not in use pharmaceutically since they are not available commercially.

Evaluating the biological activities of QA (Tab.4) (insecticidal in solutions with 0.1-0.5% alkaloid), it seems most promising to exploit the alkaloids as natural plant protectants, similar to the use of pyrethroids or the constituents of the Neem tree mentioned above. QA have the following advantages: QA do not cumulate in the soil, but are degraded within 10 d (Gross and Wink 1986). Experiments with cell cultures indicate that QA can be metabolized rapidly by plants (Wink 1985b, Wink and Witte 1985), thus an accumulation in crop plants is not very likely. Since QA will be produced as a byproduct of a normal crop, which is grown for proteins, lipids and fibres (see above), lupin alkaloids can be a comparably cheap insecticide. Since both, proteins and insecticides, are in shortage in many countries around the world (especially in the third world), lupin cultivation and processing might be a valuable alternative. However, it might be argued that QA are toxic alkaloids in higher animals. As compared to nicotine or rotenone and to many other synthetic insecticides, QA are of moderate toxicity (Table 4) which can be tolerated if necessary precautions are taken. Thus, lupin alkaloids might be a promising newcomer in the field of natural pesticides.

On a longterm perspective it might be interesting to transfer the genes which are responsible for production of lupin alkaloids from one species to another in order to improve the fitness of the receiving species (Hanke & Wink 1992). But much more basic knowledge is necessary to control all the relevant factors e.g. biosynthesis, transport and degradation) involved.
Figure 3. Antiarrhythmic activity of lupanine as compared to sparteine (V. Pfahlert and M. Wink, unpublished)

3. CONCLUSIONS

There is an urgent demand for effective and ecologically tolerable plant protectants in developing countries, but also in the Western world. "Natural pesticides" should be an interesting alternative. Since natural products evolved as defence compounds, some adverse effects towards animals and man should be expected. Therefore, natural phytochemicals must not be applied without proper evaluation of short- and long term toxicity and environmental compatibility; [which include acute toxicity tests (LD50), ninety days oral tests (performed with rats w/ different amounts of a pesticide), mutagenicity tests, residue tests (if residues appear in the plant or water, they have to be tested for carcinogenicity and mutagenicity), environmental side-effects fate of pesticides in water, soil, and air]. At present, natural mixtures are not dealt with separately and each single compound which is present needs to be evaluated. A: far as lupin alkaloids are concerned it will be certainly interesting to exploit this by-product of raffination as a cheap and environmentally friendly plant protectant.

Besides developing new pesticides, we should not forget to improve our present agricultural praxis, to avoid large-scale monocultures, to develop biological strategies against herbivores and pathogens, as done in the integrated pest control, to breed for more resistant crops. Or in the long run, to use genetic engineering to establish transgenic plants which are more resistant towards insects, because they have obtained the genes for the biosynthesis of insecticidal allelochemicals, such as lectins, protease inhibitors or even alkaloids. We can
anticipate, that full pathways will be cloned in the future which hopefully leads to plants with improved defence qualities.

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