Interrelationship between Quinolizidine Alkaloid Producing Legumes and Infesting Insects: Exploitation of the Alkaloid-Containing Phloem Sap of *Cytisus scoparius* by the Broom Aphid *Aphis cytisorum*

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Aphids *(Aphis cytisorum)* which infest broom plants *(Cytisus scoparius)* accumulate up to 500 µg alkaloid/g fr. wt. The alkaloids, which are similar to those of the plants, consist of 17-oxo-sparteine, sparteine, 12,13-dehydrosparteine, and lupanine. Infested plants contain >50% less alkaloids than aphid-free plants. In *Lupinus* aphid resistance which is due to their high alkaloid content is more expressed: Whereas bitter varieties are free from aphids, only the sweet alkaloid-free plants are susceptible to aphid infestation. The accumulation of alkaloids in aphids indicates that the quinolizidine alkaloids are translocated via the phloem in legume plants. This assumption is supported by direct evidence: analysis of phloem sap from *Lupinus* contains up to 5 mg alkaloid whereas xylem sap is virtually free of alkaloids. The interrelationship between quinolizidine alkaloids and hervivores is discussed.

Introduction

The biosynthesis of quinolizidine alkaloids takes place in the leaf chloroplast of lupin plants [1, 2]. Since alkaloids accumulate also in other plant organs such as roots and fruits, an alkaloid translocation within the plant has to be assumed [3]. Photosynthates are usually exported from the leaves via the phloem. Therefore, it appears likely that quinolizidine alkaloids are also translocated via the phloem. A simple method to study the composition of the phloem sap employs aphids, which exploit only the phloem sap of a plant [4].

Unfortunately most of our lupin species studied *(Lupinus polyphyllus, L. albus, L. angustifolius,* and *L. luteus)* were almost totally devoid of aphids. However, we found a population of broom *(Cytisus scoparius)* which accumulates quinolizidine alkaloids to a high degree [5]. About 10% of the broom plants were heavily infested by the broom aphid, *Aphis cytisorum,* a monophagous aphid specialized on legumes such as *Laburnum* and *Cytisus.*

In this communication we report on the alkaloid accumulation of broom plants and their infesting aphids in order to get prove for phloem transport of quinolizidine alkaloids in legume plants. Furthermore the interrelationship between quinolizidine alkaloid producing plants and infesting insects and its ecological significance is discussed.

Material and Methods

Plants: Lupinus polyphyllus Lindl., *L. albus* L., *L. angustifolius L., L. luteus* L. were grown in the green house and outside in a field. A *Cytisus scoparius* (L.) Link *(Sarothamnus scoparius* (L.) Wimmer ex Koch) population growing near Braunschweig at a natural habitat was studied in May and June during the time of flowering.

Insects: About 100 to 800 mg aphids *(Aphis cytisorum* Hartig) each were collected from the upper stems of about 12 flowering *C. scoparius* plants. They were frozen and kept at – 20 °C. Additionally other phytophagous insects (coccidae, beetles, cicadas and leaf bugs) feeding on *C. scoparius* plants were killed with ethylacetate and then stored at – 20 °C.

Alkaloid extraction: Plant material (5 – 20 g) was homogenized in 0.5 M HCl, alkalinized with ammonium hydroxide and extracted with methylene chloride as outlined in [3]. Insects were homogenized in a mortar in 0.5 M HCl. Alkalinized

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extracts were applied onto an Extrelut column (Merck) and eluted with methylene chloride. The solvent was evaporated in vacuo and the alkaloid mixture obtained studied by capillary GLC. Sparteine was used as an external standard.

N-Oxides

Sparteine N-oxide was synthesized from sparteine by oxidation with H_2O_2. Quinolizidine alkaloids and their respective N-oxides are easily separated by TLC (Cl_2CH_2 : MeOH : NH_4^+/6 : 4 : 1). Respective Rf values are 0.9 for sparteine and 0.25 for sparteine-N-oxide. About 700 mg of insects were homogenized in MeOH and aliquots of the filtered extracts were chromatographed by TLC. Alkaloid detection was carried out with Dragendorff's reagent.

Capillary gas-liquid chromatography and GLC-mass spectrometry

Alkaloid extracts were separated by capillary GLC on fused silica SE 30 wall-coated columns (15 m × 0.25 mm) (J & W Scientific). A Perkin Elmer gas chromatograph was employed equipped with FID and nitrogen specific detectors (SIGMA 1) combined with the data system SIGMA 10. Injector: 250 °C, Detector: 320 °C, Carrier gas: Helium 1.1 bar, split ratio: 1 : 30, Temperature program: 150 – 300°, 6 °C/min, at 300 °C isothermal for 20 min.

Capillary GLC-mass spectrometry was performed using the same column and a Kratos MS 30 mass spectrometer combined with the Data system AEI DS 50. Mass spectra were recorded every 4 sec at 24 eV.

Results and Discussion

Identification of quinolizidine alkaloids from plants and insects

The alkaloid composition of *Cytisus scoparius* plants has been studied in details previously [5]. Sparteine (1) 12,13-dehydrosparteine (2) 17-oxosparteine (3) and lupanine (4) figure as the main alkaloids of stems and leaflets (Fig. 1 A).

Extracts of *Aphis cytisorum* feeding on *C. scoparius*, separated by high resolution GLC (Fig. 1 B), revealed at least 4 nitrogen containing compounds, presumably alkaloids. From comparison with the specific retention indexes of known quinolizidine alkaloids (5) we concluded that GLC-peak 1 was sparteine, 2 = 12,13-dehydrosparteine, 3 = 17-oxosparteine and 4 = lupanine. This assumption was unequivocally confirmed by GLC-MS (Table I).

In alkaloid extracts from other insects feeding on the same *C. scoparius* plants only traces of alkaloids

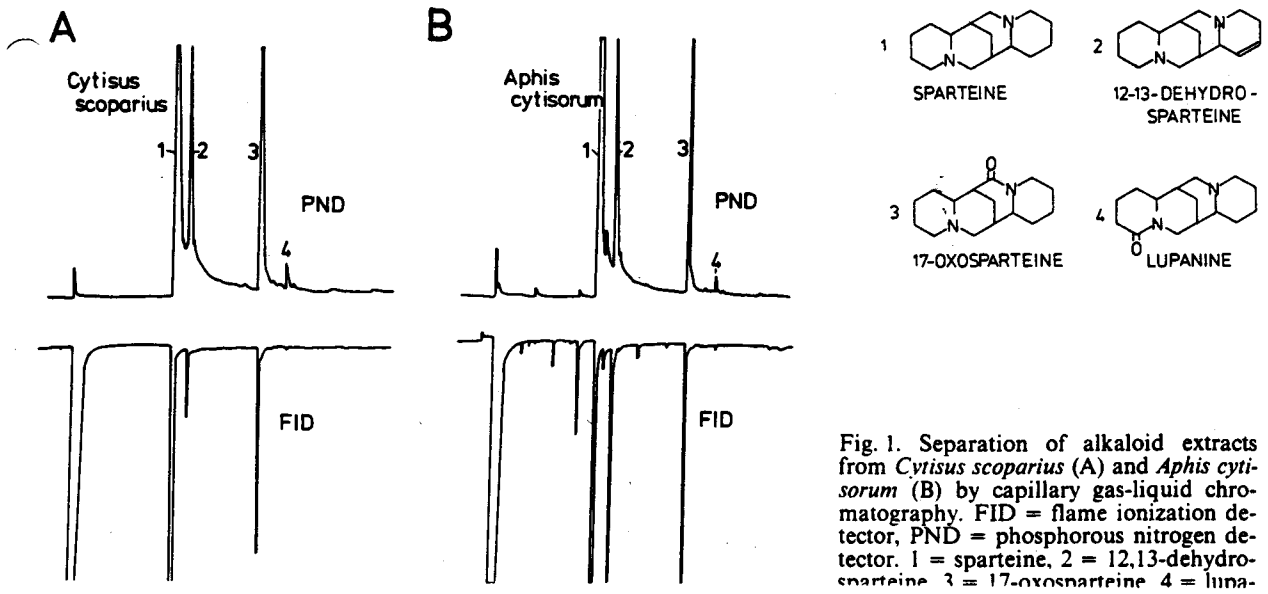

Fig. 1. Separation of alkaloid extracts from *Cytisus scoparius* (A) and *Aphis cytisorum* (B) by capillary gas-liquid chromatography. FID = flame ionization detector, PND = phosphorous nitrogen detector. 1 = sparteine, 2 = 12,13-dehydrosparteine, 3 = 17-oxosparteine, 4 = lupa-

Table I. Identification of the quinolizidine alkaloids from *Cytisus scoparius* and *Aphis cytisorum* by capillary GLC/mass spectrometry.

GLC Peak	Alkaloid	M^+	Characteristic ions [% abundance]					
1	sparteine	234	137 (100)	98 (85)	193 (20)	234 (30)		
2	12,13-dehydrosparteine	232	97 (100)	134 (85)	232 (38)	148 (18)		
3	17-oxosparteine	248	97 (100)	98 (85)	110 (60)	136 (50)	220 (45)	248 (55)
4	lupanine	248	136 (100)	149 (60)	248 (40)	219 (5)	97 (40)	

Table II. Distribution of phytophagous insects on broom plants (*Cytisus scoparius*) in relation to the alkaloid content of the infested plant part.

Plant part	Alkaloid content µg/g fr. wt.	Infestation by insects
Lower stems	930–2260[a]	Coccidae
Middle stems	4400–5200[a]	Coccidae
Upper stems	24400	–
Small green top stems (flowering)	41000	*Aphis cytisorum*
Young small branches	8600	–
Leaflets	5500	–
Flowers	300	–
Pods	1300	Curculionidae

[a] In the older stems (diameter 10 mm) about 97% of the alkaloids were present in the outer bark.

were detected when separated by GLC (Table III). The alkaloids found were sparteine (**1**) and 17-oxosparteine (**3**). Only in the case of the curculionid beetle *Sitona tibialis* there was sufficient material to confirm sparteine by GLC-MS.

Analyzing MeOH extracts of *Aphis cytisorum* and *Pulvinaria vitis* by TLC no evidence was obtained for the presence of alkaloid N-oxides. Sparteine N-oxide can be isolated from mammalian organisms after application of sparteine [6].

Accumulation of alkaloids in Aphis cytisorum

An infested broom plant harbours broom aphids usually in the upper third part of the plants, relatively rich in alkaloids (Table II). The aphids use to suck on young stems and not on leaflets. Often the branching point of a younger stem with the axis is most heavily infested.

Broom aphids were collected from 12 different plants and analyzed for their alkaloid content, which accounted for 30 – 500 µg/g fresh weight. The alkaloid pattern of the aphid is different as compared to that of its host plants: whereas sparteine is the most abundant alkaloid of the broom plant, it is 17-oxosparteine in the aphid (Fig. 2, Table III). It is not clear if the 17-oxosparteine found in the aphid was directly taken up or if it derived from sparteine taken up before. It should be recalled that a similar biotransformation can be observed in plant cell cultures which had been fed with sparteine [7].

Table III. Accumulation of quinolizidine alkaloids in phytophagous insects feeding on *Cytisus scoparius*. Alkaloids were usually extracted from 100–700 mg insects and analyzed by capillary GLC and GLC/MS.

Insect species	Family	Alkaloid content µg/g fr. wt.	Alkaloid composition [%]			
			sparteine	dehydro-sparteine	17-oxo-sparteine	lupanine
Aphis cytisorum	Aphidae	30–500	36[a]	16[a]	48[a]	2[a]
Pulvinaria vitis	Coccidae	10	–	–	tr.	–
Sitona tibialis	Curculionidae	1	tr.[a]	–	–	–
Apion striatum	Curculionidae	1	–	–	tr.	–
Phytodecta olivacea	Chrysomelidae	0	–	–	–	–
Formica pratensis	Formicidae[b]	0	–	–	–	–

tr. = trace amounts; – = not detected.
[a] confirmed by GLC/MS.
[b] feeding on the honey dew of *Aphis cytisorum*.

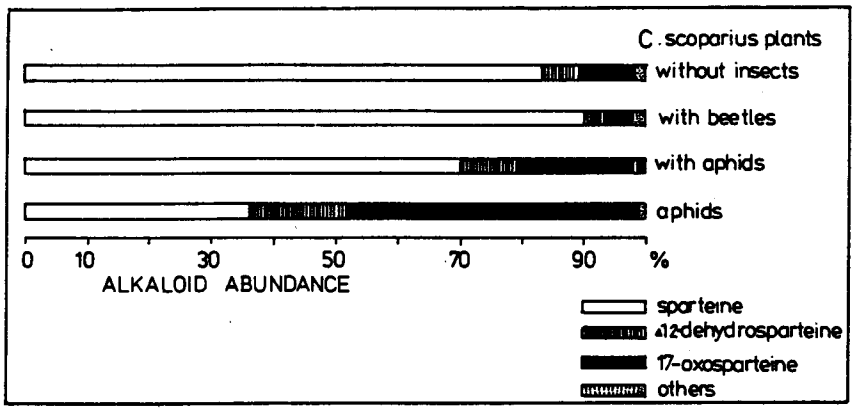

Fig. 2. Composition of the alkaloid pattern found in *Cytisus scoparius* plants and infesting *Aphis cytisorum*.

About 5% of the broom plants were infested with *Pulvinaria vitis* (Coccidae), which were found at the lower stems predominantly (Table II, III). They accumulated only very low amounts of alkaloids. The predominant alkaloid is 17-oxosparteine.

The alkaloid accumulation of other phytophagous insects was also very low as compared to *Aphis cytisorum;* these species do not exploit the phloem but other plant parts, probably containing less alkaloids.

Infestation by aphids in relation to alkaloid content of the plants

Only 10% of the broom population was infested by *Aphis cytisorum.* The alkaloid content of 12 infested plants was significantly lower (<50%) than that of aphid-free plants (t-test, $p<0.01$). About 50% of the plants harboured other phytophagous insects, too. Also these plants had a lower alkaloid contents than uninfested plants (Fig. 3).

That high alkaloid content may be the reason for insect resistance is indicated from the observation that "bitter" lupins were not infested by polyphagous aphids, whereas "sweet", alkaloid-free varieties were heavily infested (Table IV).

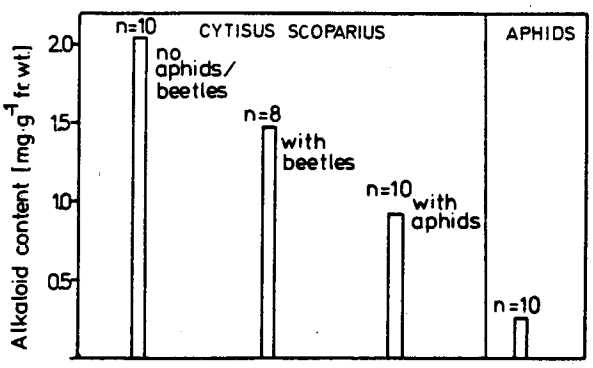

Fig. 3. Quinolizidine alkaloid content of *Cytisus scoparius* plants and of its infesting *Aphis cytisorum.*

Evidence for phloem transport of quinolizidine alkaloids

From our finding that the broom aphids contain significant amounts of alkaloids (0.1 to 2 mM alkaloid) we assume that quinolizidine alkaloids are translocated via the phloem.

Another experimental approach, which will be dealt with in detail in another communication, offers further evidence for this assumption: Phloem sap was collected from *Lupinus consentinii* Guss. and *L. angustifolius* L. plants and it contained up to 5 mg alkaloid per ml (about 20 mM). In contrast, xylem sap had only 10 – 50 µg alkaloid/ml sap (Fig. 4) (Wink, Hartmann, Pate, unpublished). Both examples strongly indicate that quinolizidine alkaloids are translocated via the phloem similar to the situation of other photosynthates.

Table IV. Aphid resistance in relation to alkaloid content of Lupin plants (leaflets).

Lupin species	Alkaloid content [µg · g^{-1} fr. wt.]	Number of aphids
Lupinus luteus	15	++++
	20	+++
	250	++
	695	0
	1000	+
Lupinus polyphyllus	500–3000	0
Lupinus albus	300–3000	0
Lupinus angustifolius	120	+
	500–1500	0

+ = few aphids; ++ = some; +++ = many; ++++ = very abundant.

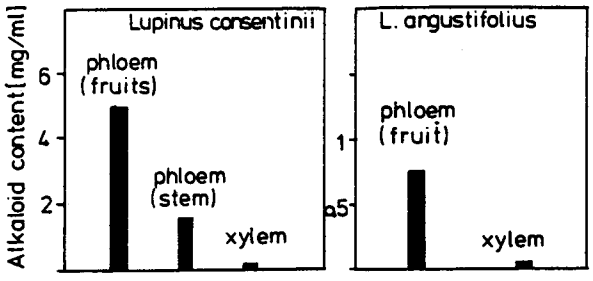

Fig. 4. Quinolizidine alkaloid content of phloem and xylem sap of *Lupinus consentinii* and *L. angustifolius*. Samples of 50 µl of phloem and xylem excudate were collected (by J. S. Pate) and freeze dried, followed by standard alkaloid extraction and GLC-analysis.

Ecological significance of alkaloid accumulation in Aphis cytisorum

Similar to the situation in other groups of plant secondary compounds, quinolizidine alkaloids play a role in plant herbivore interactions [8, 9]. Some experiments show that herbivorous vertebrates are deterred from feeding on bitter lupins due to their high content of quinolizidine alkaloids whereas sheep and hares readily graze on "sweet" alkaloid-poor varieties [10, 11]. Obviously quinolizidine alkaloids are also effective against arthropods. Thrips infestations were only recorded in sweet lupin varieties, but not in bitter ones [12]. The polyphagous grasshopper *(Melanoplus bivittatus)* is deterred from feeding on plants containing the quinolizidine alkaloid lupinine [13]. Some lupins are infested by butterfly larvae of *Glaucopsyche lygdanus*. The infestation rate was markedly decreased in plants with a high variability in the pattern of quinolizidine alkaloids. It was assumed that high variability of the alkaloid pattern makes a counter adaption by the herbivores less likely [14].

Legumes which accumulate quinolizidine alkaloids are usually devoid of aphids (Table I). Only plants which contain low levels of alkaloids, the so called "sweet" lupins, were infested by polyphagous aphids. In this example a high alkaloid content obviously offers the advantage of aphid resistance. Wengorek and Kschimanska [15] came to a similar conclusion studying different varieties of *L. albus*. Plants were infiltrated with alkaloid solutions of different concentration and composition. The authors could clearly show that the nonspecific *Acyrthosiphon pisum* was deterred from lupin plants which contained elevated levels of various quinolizidine alkaloids.

On the other hand the *Aphis cytisorum* example shows that most antifeeding devices developed by a plant species can be overcome by a specialized insect pest. In this case the insect species is usually monophagous and is specially adapted to its host species. Therefore it is not surprising that Smith [16] could show that sparteine may even act as a feeding stimulant for *Acyrthosiphon spartii*, another monophagous aphid which is specialized on *C. scoparius*.

This finding implies that quinolizidine alkaloids may leed the broom aphid to its proper host, to which it is accustomed. From our findings it is evident, however, that even the monophagous aphids avoid broom plants with high alkaloid content and selects plant which contain about 50% less alkaloids than the average uninfested plants.

Sparteine and other quinolizidine alkaloids taste bitter and are known to exhibit toxic properties in vertebrates which are of pharmacological interest. Sparteine is frequently used as an antiarrhythmic heart drug. The precise effects of quinolizidine alkaloids on insects are unknown as well as the question how the monophagous *A. cytisorum* and *A. spartii* are able to detoxificate or to stand the relative high alkaloid levels found in the phloem sap.

The accumulation of high alkaloid levels (about 2 mM) may be advantageous even for the aphid itself. Since this level could provide some sort of protection against predating animals similar to the example of Danaid butterflies feeding on Asclepiadaceae plants containing cardiac glycosides [17]. Maybe birds are deterred by the bitter taste of the alkaloid containing aphids.

These aspects show how interrelated the ecological interactions may be between a secondary compound and a coevolved animal species.

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