Uptake and Transport of Quinolizidine Alkaloids in *Cuscuta reflexa* Parasitizing on *Lupinus angustifolius*

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During parasitism on *Lupinus angustifolius* the phanerogamic parasite *Cuscuta reflexa* takes up quinolizidine alkaloids from *L. angustifolius via* haustoria that tap xylem and phloem vessels of the host. The alkaloid pattern of the haustorial region of *C. reflexa* as revealed by GLC as well as by GLC-MS closely resembles that of infested lupin stems. With increasing distance from the haustoria towards the apex of *C. reflexa* the complex alkaloid pattern of the host is significantly reduced. Whereas alkaloid esters such as 13-benzoyloxylupanine and 13-cinnamoyloxy-lupanine are prominent peaks in the alkaloid profiles of the host as well as of the haustorial region of the parasite they are not detected in the apex of *C. reflexa* but are replaced by their respective alcohol component 13-hydroxylupanine. This change of alkaloid profiles is accompanied by a strong decline of alkaloid concentration which drops from approximately 11 mg/g dry wt. in the haustorial region of *C. reflexa* to 0.2 mg/g dry wt. in the apex. Alkaloid analyses of xylem sap and phloem exudate from *L. angustifolius* suggest that uptake of host plant alkaloids by *C. reflexa* is mainly *via* the phloem even though a contribution of xylem sap to the supply of alkaloids to the parasite can not be excluded.

Introduction

The phanerogamic stem parasite *Cuscuta* is well known due to its detrimental effects on various crop plants [1]. While twining on its host *Cuscuta* produces haustoria which tap xylem as well as phloem elements [2, 3] thereby securing nutrients and water from the host. *Cuscuta* spp. such as *Cuscuta platyloba* Progel or *Cuscuta reflexa* Roxb. which are usually devoid of alkaloids are nevertheless able to grow on a variety of host plants rich in alkaloids including for example *Lupinus albus* L., *Nicotiana xanthi* L. or *Duboisia myoporoides* R. Br. [4, 5]. Even though many alkaloids are known for their strong physiological effects in vertebrates (including man) and invertebrates (*e.g.* insects) as well as for allelopathic properties [6−8] these alkaloids are apparently not able to prevent parasitism by *Cuscuta* spp. even though they are passed over to the parasite through the haustoria [5]. Detailed studies on this interspecific flow of host plant alkaloids to *Cuscuta* spp., however, are so far lacking. It is therefore the aim of the present report to elucidate the fate of quinolizidine alkaloids accumulated by *C. reflexa* while parasitizing on *L. angustifolius*.

Results

Microscopical analysis

The alkaloid-free parasitic plant *C. reflexa* is able to grow on the alkaloid-rich host plant *L. angustifolius*. There was no difference in growth (measured as increase of the branches in terms of length) whether *C. reflexa* grew on the alkaloid-free host *Coleus blumei* or on lupin. Under optimal conditions a daily increase of the shoot length of *C. reflexa* of up to 10 cm could be observed. Parasitism on lupins could be accelerated by keeping the connection of *C. reflexa* to *C. blumei* for 6−7 days until *Cuscuta* had developed a functioning "haustorium" on lupin host plants. From the tip of the haustorial endophyte several "searching hyphae" [2] extend in various directions in the host
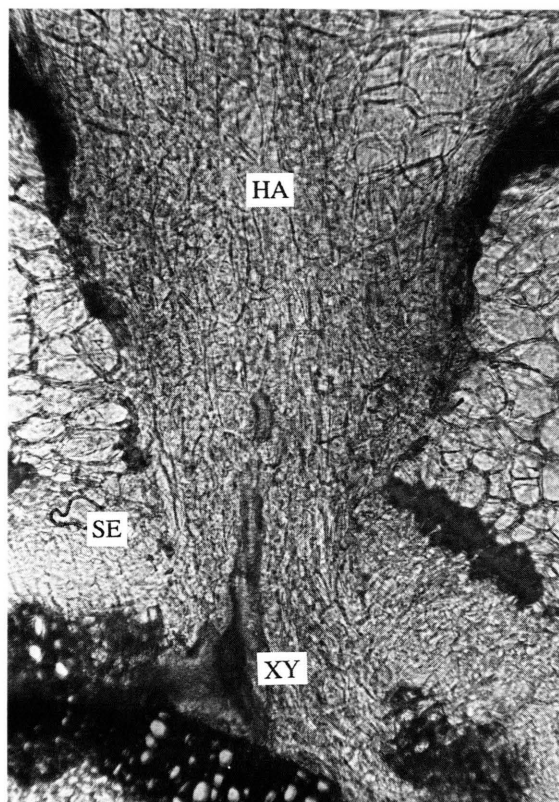
Fig. 1. Light microscopical survey of a haustorium of *C. reflexa* (HA) situated in the stem of *L. angustifolius*. The parasitic shoot was cut longitudinally, the host crosswise and stained with FSA [9]. Searching hyphae (SE) penetrate the host tissue occasionally getting contact to the vascular bundles of the host (see contact xylem (XY) in the innermost part of the haustorium). Enlargement: × 31.25.

Fig. 2. Haustorial tracheary elements of *C. reflexa* contact host xylem. Cross section treated as in Fig. 1. Light microscopy. Enlargement: × 160.

tissue sometimes intruding cells of the host tissue and finally tapping the vascular bundles of lupin (Fig. 1 and 2). At a later stage these differentiated tracheary elements are able to form a xylem bridge between the xylem of the host and the xylem axis of *Cuscuta* in the base of the haustorium. On both sides the central xylem strand is accompanied by sieve elements (Fig. 1). A direct connection of phloem elements of *C. reflexa* to the phloem of *L. angustifolius*, however, could not be detected by microscopical analysis during the present study.

Identification of quinolizidine alkaloids from C. reflexa and L. angustifolius

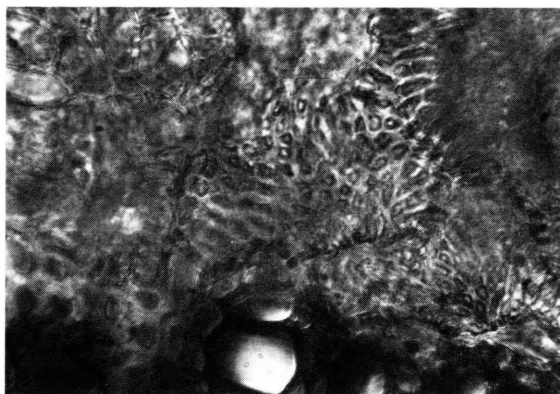The alkaloid composition of *L. angustifolius* has already been subject of previous studies [10, 11].

Nevertheless possible influence of the attack of *Cuscuta* on alkaloid biosynthesis and transport within lupin required detailed analysis of alkaloid composition of the host plant. The highest concentrations of alkaloids within the host plants could be detected in the shoot apex (consisting of the first cm of stem and uppermost leaves) and in the roots. The concentrations of alkaloids within the stems of uninfested 25 days old lupins continuously increased towards the apex. The same was observed for the alkaloid concentrations in leaves at the respective nodes. Similar results were obtained for infested 25 days old lupins (data not shown). However, an infested plant usually contained less alkaloids in total than an uninfested lupin. When the total amounts of alkaloids present in the host plant and in the parasite were added they were found to resemble the alkaloid content of an uninfested lupin (Table I).

Table I. Comparison of total amounts of alkaloids in uninfested and infested lupins (age: 25 days) as well as in *C. reflexa* parasitizing on lupins for 8 days.

	Total amount of alkaloids [µg/plant]
Uninfested lupin	1960 ± 67
Infested lupin	1655 ± 108
C. reflexa	499 ± 190

Total amount of alkaloids was determined for the whole host plant (stem, leaves and root) and the parasite, respectively, in 3 independent experiments.

Fig. 3 illustrates the alkaloid pattern of *L. angustifolius* and *C. reflexa* parasitizing on lupins for a total of 8 days. The alkaloid pattern detected in the haustorial region of the parasite closely corresponded to that found in lupin stems. According to mass spectra and Kovats retention indices [10, 11] the following main alkaloids could be identified both in the host plant and in the parasite: ammodendrine (**1**), isoangustifoline (**2**), tetrahydrorhombifoline (**3**), angustifoline (**4**), α-isolupanine (**5**), lupanine (**6**), dehydrolupanine (**7**), 13-hydroxylupanine (**8**), 13-tigloyloxylupanine (**9**), 13-benzoyloxylupanine (**10**), 13-*cis*-cinnamoyloxylupanine (**11**), 13-*trans*-cinnamoyloxylupanine (**12**)

(Fig. 4). Comparison of the haustorial region of *C. reflexa* with the corresponding infested stem part of *L. angustifolius,* however, revealed several differences with regard to the alkaloid profiles. The haustorial region of *Cuscuta* is characterized by a significant increase of angustifoline (**4**) and by a less prominent increase for 13-tigloyloxylupanine (**9**) compared to the alkaloid pattern of the host plant, whereas 13-hydroxylupanine (**8**) as well as 13-*trans*-cinnamoyloxylupanine (**12**) decrease in the parasite compared to *L. angustifolius* (Table II).

The presence of alkaloids within *C. reflexa,* however, is not restricted to the haustorial region. Lupin alkaloids are also found in the apex of the
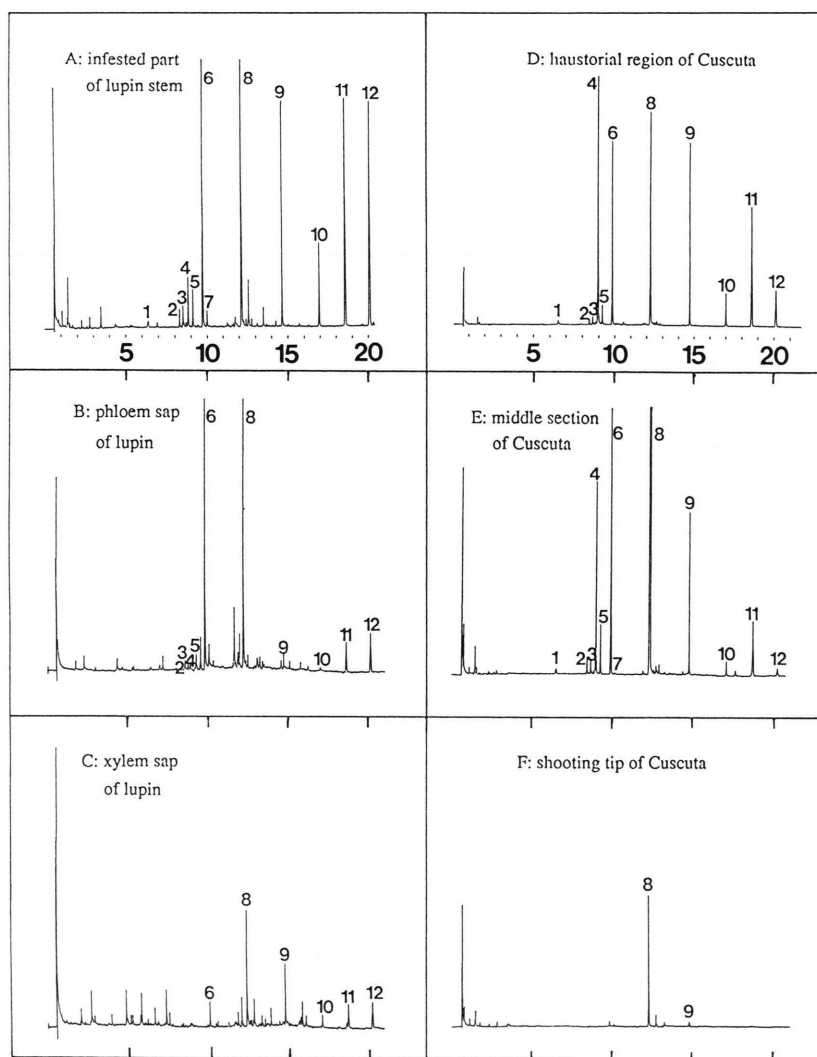

Fig. 3. Separation of alkaloids from the host plant *L. angustifolius* and the parasite *C. reflexa* by capillary GLC and detection by a nitrogen specific detector. GLC conditions see Methods and Materials. Numbering of the GLC-peaks is according to Table II and III.

angustifoline

13 — hydroxylupanine

lupanine

13−O−tigloyllupanine

13−O−cis−cinnamoyllupanine

13−O−trans−cinnamoyllupanine
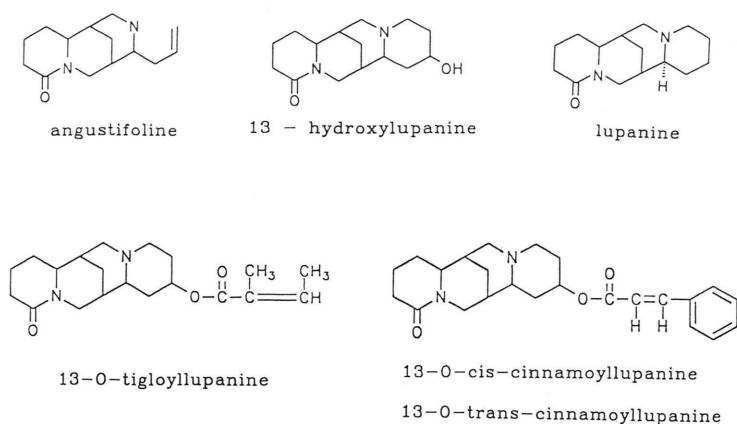
Fig. 4. Main alkaloid structures of *L. angustifolius* and *C. reflexa*.

parasite. In branches of *C. reflexa* the total concentration of alkaloids decreased from 11 mg/g dry weight in the haustorial region to less than 1 mg/g dry weight in the shoot apex. In addition to the declining alkaloid concentrations the alkaloid patterns in various segments of *C. reflexa* simplified from the haustorial region towards the apex. Alkaloid esters such as 13-benzoyloxylupanine (**10**), 13-*cis*- and 13-*trans*-cinnamoyloxylupanine (**11, 12**) gradually decreased below detection limit whereas their corresponding alcohol component 13-hydroxylupanine (**8**) as well as traces of

Table II. Distribution and concentrations of alkaloids in infested stem parts of *L. angustifolius* and in various segments of *C. reflexa*.

Peak number	Lupin Infested stem 3.5 cm	Abundance of alkaloids [%] in Cuscuta			
		Haustorial region 4.9 cm	Middle section 28 cm	Apical tissue 13 cm	Below haustorial region* 8.9 cm
1	+	0.5	0.4	−	+
2	1.0	0.6	0.7	−	+
3	1.3	0.7	0.6	−	2.1
4	2.6	23.2	8.1	−	2.5
5	2.0	1.6	2.1	−	1.8
6	16.6	14.9	13.5	−	15.6
7	0.9	0.2	+	−	+
8	24.8	19,9	64.9	100	62.4
9	9.0	12.0	5.2	+	11.2
10	4.7	3.5	0.7	−	+
11	16.7	16.9	3.1	−	4.5
12	20.4	6.1	0.5	−	+
Yield of alkaloids [mg/g dw]	2.1	11.2	2.2	0.2	0.6

Alkaloids were analyzed by capillary GLC and GLC-MS.
1, ammodendrine; 2, isoangustifoline; 3, tetrahydrorhombifoline; 4, angustifoline; 5, α-isolupanine; 6, lupanine; 7, dehydrolupanine; 8, 13-hydroxylupanine; 9, 13-tigloyloxylupanine; 10, 13-benzoyloxylupanine; 11, 13-*cis*-cinnamoyloxylupanine; 12, 13-*trans*-cinnamoyloxylupanine.
+: Traces (<0.01 mg/g dry wt.).
−: Not detected.
*: Chromatogram not shown in Fig. 3.
Conc.: concentration; dw: dry weight.

13-tigloyloxylupanine (**9**) remained as the only alkaloids detected in the apex of *C. reflexa*. Transport of alkaloids within *C. reflexa* is apparently not restricted towards the apex only since lupin alkaloids are also found in sections of *Cuscuta* below the haustorial region (Table II).

In addition to the alkaloid analysis of different host plant organs xylem sap and phloem EDTA exudate of *L. angustifolius* were separately collected and likewise analyzed for quinolizidine alkaloids (Fig. 3). In xylem sap a concentration of alkaloids of about 2 µg/ml was found mainly consisting of lupanine (**6**), 13-hydroxylupanine (**8**) and the esters 13-benzoyl- (**10**), 13-tigloyl- (**9**), 13-*cis*- (**11**) and 13-*trans*-cinnamoyloxylupanine (**12**). The relative proportions of the respective alkaloids showed considerable variation in two independent experiments (Table III). Phloem EDTA exudate of *L. angustifolius* could not be quantified accurately with regard to alkaloid concentration (excepting relative statements) by the method of Tully and Hanson [12] which was employed in this study. However, it was striking that in contrast to xylem sap the complete alkaloid pattern found in stems of *L. angustifolius* (Table III) − as well as several additional minor alkaloid components − was identified in the phloem EDTA exudate.

Discussion

The luxuriant growth of *C. reflexa* on *L. angustifolius* confirms the compatibility of the parasite and the host plant which is also revealed by microscopic analysis demonstrating functioning haustoria (Fig. 1). Obviously *C. reflexa* manages to overcome the chemical barrier of the quinolizidine alkaloids which are present in appreciable quantities especially in the epidermal and subepidermal layers of lupin stems [13]. This tolerance of *C. reflexa* is remarkable since various lupin alkaloids including 13-tigloyloxylupanine or 13-hydroxylupanine are known for their allelopathic properties in other species including for example *Lactuca sativa* L. [8] and comparatively large concentrations of lupin alkaloids are accumulated by *C. reflexa* during parasitism on *L. angustifolius* (Table II). Similar distinct compatibilities to lupin alkaloids have previously only been demonstrated for other parasitic plants (*e.g. Orobranche rapumgenistae* and *Castilleja* spp.) and for specialized herbivorous insects including the aphids *Macrosiphon albifrons* and *Aphis cytisorum* [14−17].

Within *C. reflexa* the highest concentrations of quinolizidine alkaloids reaching approximately 11 mg/g dry wt. are detected in the haustorial re-

Table III. Patterns of quinolizidine alkaloids in xylem sap and phloem exudate of *L. angustifolius* as revealed by GLC.

Compound	Abundance of alkaloids [%] in			
	Xylem sap		Phloem exudate	
	Sample 1	Sample 2	Sample 1	Sample 2
Isoangustifoline (**2**)	−	−	1.1	1.0
Tetrahydrorhombifoline (**3**)	−	−	2.7	2.3
Angustifoline (**4**)	−	−	12.2	8.2
α-Isolupanine (**5**)	−	−	4.5	0.9
Lupanine (**6**)	8.1	26.0	7.8	39.9
13-Hydroxylupanine (**8**)	35.9	50.0	55.8	23.4
13-Tigloyloxylupanine (**9**)	21.1	5.2	9.8	2.1
13-Benzoyloxylupanine (**10**)	5.2	10.0	2.1	0.7
13-*cis*-cinnamoyloxylupanine (**11**)	19.8	0.5	1.7	1.1
13-*trans*-cinnamoyloxylupanine (**12**)	9.8	5.7	2.3	20.5

Sample 1 and sample 2 represent samples of independent experiments. The abundance of alkaloids is based on total of alkaloids revealed by GLC patterns (concentration of alkaloids in xylem sap: about 2 µg/ml, in phloem sap: not determined).
Numbers in brackets refer to peaks in Fig. 3.
−: Not detected.

gion (Table II). With regard to qualitative composition the alkaloid pattern of the haustorial region of *C. reflexa* as revealed by GLC closely resembles that of infested lupin stems. This indicates no discrimination of alkaloid uptake by the parasite. From the haustorial region of *C. reflexa* the alkaloids are transported towards the apex of the parasite. This transport is accompanied by a decline of alkaloid concentration (Fig. 3). In the apex of *C. reflexa* only a marginal concentration of 0.2 mg alkaloids/g dry wt. is detected (Table II). This significant difference in alkaloid concentration within *C. reflexa* may be due to either an increased rate of alkaloid degradation in growing parts of *C. reflexa* or to alkaloid trapping in the haustorial region of the parasite. In addition to this decline of alkaloid concentration the alkaloid patterns of the various segments of *C. reflexa* simplified towards the apex (Fig. 3). This was most obvious for several esters originally present as prominent peaks in the haustorial region but replaced in the apex by the corresponding alcohol 13-hydroxylupanine (**8**) (Table II). Presently it is not known if the alkaloid esters are hydrolyzed by *C. reflexa* yielding 13-hydroxylupanine or if the latter compound is transported preferentially towards the apex thereby giving rise to the different alkaloid profiles as observed in the GLC analysis (Fig. 3). Furthermore the increase of angustifoline (**4**) in the haustorial region of *Cuscuta* was striking indicating either a preferential uptake of this component or metabolism of host plant alkaloids by the parasite. The compartments responsible for alkaloid storage in *Cuscuta* are likewise not known at present even though the vacuoles are likely candidates based on previous studies on subcellular alkaloid localization in other plants [18, 19].

Accumulation of quinolizidine alkaloids in *C. reflexa* demonstrates alkaloid transport through the parasitic haustorium which is likely to occur mainly *via* phloem based on previous studies on alkaloid transport in lupins [20]. GLC analysis of the phloem exudate of *L. angustifolius* revealed a similar pattern of alkaloids as present in stem extracts or in the haustorial region of *C. reflexa* (Fig. 3). This obvious similarity with regard to alkaloid patterns points to a phloem connection of host and parasite which has previously only been demonstrated for *Cuscuta odorata* R. et P. parasitizing on *Pelargonium zonale* [3]. The minute concentrations of alkaloids present in xylem sap of *L. angustifolius* as well as the reduced pattern (in comparison to the alkaloid pattern in the phloem exudate, Fig. 3) suggest that xylem transport of alkaloids is only of minor importance in *L. angustifolius*. However, the amount of alkaloids originating from the phloem and the xylem, respectively, would depend on the relative flow of saps from either source to the parasite. The high abundance of No. 9 in xylem sap as well as in *Cuscuta* tissues argues for a contribution of xylem sap to the supply of alkaloids to the parasite in addition to that from the phloem sap. Further experiments by analyzing phloem sap rather than exudate are planned to resolve this point. Nevertheless, an uptake of alkaloids from epidermal cells or from the parenchyma of lupins by the parasite can so far not be excluded [21].

Materials and Methods

Plant material

C. reflexa (originally established by Prof. Dr. O. H. Volk, Würzburg [4]) was cultivated on the alkaloid-free host plant *C. blumei* in the greenhouse of the Botanical Garden Würzburg at an ambient temperature of 20–25 °C and a relative humidity of about 70%. The short day plant requires additional light (mercury vapour lamp, 16 h daylight, 8 h darkness) to prevent it from flowering. Seeds of the host plant *L. angustifolius* were obtained from a local market. Plants were grown from seedlings in the greenhouse. The correct taxonomic identification of the plant was secured by Prof. Dr. W. D. Jeschke. In order to transfer *Cuscuta* to *L. angustifolius* shoots of the parasite were either separated from the stock culture and put directly on lupin plants or the connecting branches to *C. blumei* were cut after prehaustoria on *L. angustifolius* had been produced. *C. reflexa* and *L. angustifolius* were harvested separately after different time intervals, directly frozen (−20 °C) and lyophilized previous to alkaloid extraction.

Extraction of xylem sap and phloem exudate from *L. angustifolius*

Phloem exudate

Because of unfavourable seasonal conditions bleeding of phloem didn't occur [22]. Thus phloem exudate was collected following the method de-

scribed in [12] using a solution of Li_2EDTA and sorbitol (10 mM, pH 7) as exudation medium. Incubation of excised plant organs was performed in small polyethylene tubes filled with the exudation medium. In order to create a saturated atmosphere a beaker was lined with $KHCO_3$-solution soaked filter paper and put over the polyethylene tube. During exudation experiments, which lasted 8–10 h, plants were illuminated additionally. Control plants were dipped in distilled water and thereafter treated as described above.

Xylem sap

For these experiments lupin seedlings were grown first in perlite, after a week transferred into pots fitting for pressure chambers and daily watered with fresh Hoagland nutrient solution [23]. After placing the pot in the pressure chamber xylem sap was extracted from different plant organs as reported by [24]. Each experiment was terminated by collecting root pressure exudate following decapitation of the aerial plant parts.

Alkaloid extraction

Lyophilized plant material was finely ground in a mortar. Usually a total of 15 ml of 1 N sulfuric acid was added to 200 mg plant material, the mixture was left standing at room temperature for 45 min followed by filtration. Subsequently the filtrate was alkalinized with 25% ammonia to pH 10–11 and put onto a "ChemElut" column (ICT, Frankfurt, F.R.G.). After 15 min alkaloids were eluted with dichloromethane and the solvent concentrated *in vacuo*. For GLC analysis the residue was dissolved in a known volume of methanol. Xylem and phloem sap samples were made alkaline without treatment with sulfuric acid and extracted as described above [25].

Capillary GLC and GLC-MS

Alkaloid extracts were separated on a DB 1 fused silica capillary column (15 m × 0.25 mm i.d.; film thickness 0.25 μm) with He as carrier gas (flow: 2.5 ml/min; split ratio 1:20; inj. temp. 290 °C) and detection with a nitrogen specific detector (PND at 310 °C). Quinolizidine alkaloids were chromatographed using a temperature program starting with 150 °C (2 min isothermal,

10 °C/min to 250 °C, 20 °C/min to 300 °C, 10 min isothermal) [25].

For capillary GLC-MS the following conditions were used: GC: carrier gas: He; DB 1 capillary column (0.32 mm × 30 m); inj. temp. 250 °C; split ratio 1:20; temperature program: 150–300 °C, 6 °C/min; GC-MS transline: 250–220 °C to a quadrupol instrument Finnigan MAT 4515; temperature of electron impact ion source: 120 °C; electron energy: 45 eV.

Identification of quinolizidine alkaloids

Alkaloids were identified by comparing their Kovats retention indices (RI) and their mass spectra (MS) with literature data [10, 11].

Ammodendrine: RI = 1862; MS: 208 (M^+) (50), 191 (25), 179 (60), 165 (100), 136 (82), 123 (65); isoangustifoline: RI = 2032; MS: 234 (M^+) (5), 193 (100), 150 (16), 112 (58), 94 (14), 55 (23); tetrahydrorhombifoline: RI = 2048; MS: 248 (M^+) (1), 207 (100), 136 (6), 112 (26), 108 (10), 58 (92); angustifoline: RI = 2083; MS: (M^+) (n.d.), 193 (100), 150 (18), 112 (87), 94 (11), 55 (25); α-isolupanine: RI = 2102; MS: 248 (M^+) (42), 247 (33), 149 (55), 136 (100), 98 (31), 55 (27); lupanine: RI = 2168; MS: 248 (M^+) (42), 219 (8), 149 (55), 136 (100), 98 (26), 55 (25); dehydrolupanine: RI = 2190; MS: 246 (M^+) (100), 231 (18), 148 (46), 134 (87), 112 (17), 55 (22); 13-hydroxylupanine: RI = 2400; MS: 264 (M^+) (17), 247 (15), 246 (30), 165 (37), 152 (100), 134 (37); 13-tigloyloxylupanine: RI = 2748; MS: 346 (M^+) (1), 246 (100), 148 (17), 134 (30), 112 (15), 55 (16); 13-benzoyloxylupanine: RI = 3090; MS: 368 (M^+) (1), 246 (100), 148 (10), 134 (42), 112 (20), 55 (18); 13-*cis*-cinnamoyloxylupanine: RI = 3264; MS: 394 (M^+) (0,1), 246 (100), 148 (14), 134 (30), 112 (20), 55 (12); 13-*trans*-cinnamoyloxylupanine: RI = 3397; MS: 394 (M^+) (0,1), 246 (100), 148 (12), 134 (28), 112 (20), 55 (8).

For quantification of the alkaloids by GLC sparteine was used as external standard.

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