Quinolizidine Alkaloids as Nitrogen Source for Lupin Seedlings and Cell Cultures

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Alkaloid Turnover, Quinolizidine Alkaloids, *Lupinus,* Germination, Nitrogen Source

The alkaloid patterns during germination and seedling development of *Lupinus polyphyllus, L. angustifolius, L. albus, L. pubescens, Cytisus scoparius, Baptisia australis, Spartium junceum* and *Laburnum anagyroides* were studied by capillary glc and EI-MS and CI-MS. The alkaloid contents were relatively high in the seeds and decreased by 20–100% during germination and the early developmental stages. The plants with fully developed leaves were able to synthesize new alkaloids. The decrease of alkaloid concentrations during germination was interpreted in terms of alkaloid turnover and use of the alkaloidal nitrogen for seedling development. The ability of plants to rely on the alkaloidal nitrogen as a nitrogen source could also be shown in lupin cell cultures which could survive and even grow on media which contained sparteine as the sole nitrogen source.

Introduction

Quinolizidine alkaloids (QA) are common natural products of the Fabaceae, but are also present in a few unrelated families [1–4]. QA are produced in the aerial green parts of lupins [1, 5], especially in the leaf chloroplast [6, 7]. The alkaloids, which are synthesized in a light-dependent diurnal rhythm [8, 9], are translocated *via* the phloem [9, 10] to the other plant organs. In consequence all plant parts contain alkaloids [1, 3, 5] which are preferentially stored in epidermal tissue [11, 12]. Alkaloids are especially abundant in the fruits, and the ripe seeds contain up to 5% alkaloid (dry weight) [3, 4].

QA like other nitrogen-containing natural products [13, 14] are not inert end products of metabolism but dynamic compounds with a high degree of turnover [8, 9]. The turnover is especially evident in cell suspension cultures of lupins which are capable to degrade endogenously produced or added alkaloids [8, 15, 16]. In this communication we provide evidence that plants can use the nitrogen stored in the alkaloid molecule: We report on the alkaloid metabolism of developing seedlings from 9 species of the genus *Lupinus, Baptisia, Laburnum, Spartium* and *Cytisus* and on the survival of lupin callus cultures on media with the lupin alkaloid sparteine as sole nitrogen source.

Abbreviations: CI, Chemical ionization; CI-MS, chemical ionization – mass spectrometry; EI-MS, electron impact – mass spectrometry; glc gasliquid chromatography; QA, quinolizidine alkaloids; M^+, molecular ion; RI, Kovats retention index.

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Material and Methods

Plants

Seeds of *Laburnum anagyroides* (Medicus), *Cytisus scoparius* (L.) Link, *Lupinus polyphyllus* Lindl. were collected from plants growing in gardens near Braunschweig. *Spartium junceum* L. seeds were from Crete, *Lupinus albus* L. seeds from Syria. Seeds of *Lupinus hartwegii* Lindl., *L. pubescens* Benth., and *Baptisia australis* (L.) R. Br. were obtained from the Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben. *L. angustifolius* L. seeds were from the local market.

Seeds were imbibed in water for 6–12 h and afterwards sown in garden soil. The seedlings were grown in a greenhouse with 14 h light and approx. 20 °C and 60% relative humidity. Seedlings were harvested as indicated in the figures and were stored at −20 °C.

Cell cultures

Cell suspension and callus cultures of *L. polyphyllus* were kept according to Wink *et al.* [17, 18]. For the experiments with media containing sparteine as sole nitrogen source, the standard agar medium [17] was made without ammonium salts and nitrate. Filter-sterilized sparteine (1 or 5 mmol/l) was added instead.

Alkaloid extraction

Plant material was homogenized in 50 ml 0.5 mol/l HCl in a Waring blendor and left standing at room temperature for 30 min. Then the homogenate was filtered through nylon gauze (100 μm mesh). The filtrate was made alkaline with 25% ammonium hydroxide and applied onto a standard extrelut column (Merck, Darmstadt). The alkaloids were eluted with 50 ml methylene chloride and the extracts were evaporated to dryness.

Alkaloid analysis

High-resolution gas-liquid chromatography: Alkaloids were separated on fused silica capillary columns (15 or 30 m × 0.25 mm) coated with SE 30 or DB1 (J & W Scientific) employing a Perkin Elmer gas chromatograph (Sigma 1b) with flame ionization and nitrogen specific detectors [17, 18]. The chromatograms were evaluated with the data system Sigma 10 and sparteine was used as an external standard. GLC-MS: Using the same columns as above, GLC-MS measurements were performed with a Perkin Elmer F 22 gas chromatograph, a Kratos MS 30 mass spectrometer and the data system DS 50 according to our previous studies [9, 10, 17–20]. For CI-MS measurements isobutane was used as a reactand gas.

Identification of quinolizidine alkaloids

Capillary-GLC is a convenient and powerful method for the separation and identification of complex QA mixtures. Owing to the high reproducibility of the columns most compounds could be identified by their Kovats retention indexes determined in previous studies [17–20]. For each plant these tentative identifications were confirmed by GLC-MS measurements. Since QA have clear and informative mass spectra a quick and unambiguous identification is usually possible [2, 9, 10, 17–20].

Lists of the characteristic fragment ions have been published recently [2, 17–20], so that we present here only the mass spectral data which have not been previously reported from our laboratory. In addition we have determined the chemical ionization (CI) mass spectra of some alkaloids, which have not been recorded previously. CI-MS is especially suitable for the identification of compounds which only give rise to molecular ions of low intensity in ordinary electron impact-(EI)-mass spectrometry, *i.e.* the ester alkaloids and tricyclic alkaloids such as angustifoline, tetrahydrorhombifoline, rhombifoline, tinctorine, albine, and N-methylalbine. In CI-MS we obtain a $[M + H]^+$ ion with an abundance of 100%.

1. Lupanine: EI-MS, RI as in [17, 18]; CI-MS: $[M + H]^+ = m/z$ 249 (relative abundance = 100%), 98 (5), 136 (5). – 2. α-Isolupanine, EI-MS, RI as in [18]; CI-MS as lupanine. – 3. 13-hydroxylupanine: EI-MS, RI as in [17, 18]; CI-MS: 265 (100), 245 (30), 151 (20). – 4. 4-Hydroxylupanine: EI-MS, RI as in [18]; CI-MS: 265 (100), 136 (5). – 5. 13-Angeloyloxy-lupanine: EI-MS, RI as in [18, 21]; CI-MS: 347 (100), 247 (15). – 6. 13-Tigloyloxy-lupanine: EI-MS, RI as in [18, 21]; CI-MS as no 5. – 7. 13-Benzoyloxy-lupanine: EI-MS, RI as in [18, 21]; CI-MS: 369 (100), 246 (80). – 8. Tetrahydrorhombifoline: EI-MS, RI as in [18]; CI-MS: 249 (100), 207 (20). – 9. Angustifoline: EI-MS, RI as in [17, 18]; CI-MS: 235 (100), 193 (30). – 10. Sparteine: EI-MS, RI as in [17–19]; CI-MS: 235 (100), 98 (10), 137 (15). – 11. 13-Cinnamoyloxy-lupanine: EI-MS, RI as in [18, 21]; CI-MS: 395 (100), 247 (80). – 12. 11,12-Dehydrosparteine: EI-MS, RI as in [18]. – 13. 17-Oxosparteine: EI-MS, RI as in [18, 19]; CI-MS: 249 (100), 220 (3), 98 (5). – 14. Aphylline: EI-MS, RI as in [18]; CI-MS: 249 (100), 220 (5), 136 (5), 84 (10). – 15. Epiaphylline: RI = 2058; EI and CI-MS as for aphylline. – 16. Epiaphyllidine: RI = 2042, EI-MS: 98 (100), 246 (55), 134 (18), 149 (18), 218 (10); CI-MS: 247 (100), 219 (10). – 17. Aphyllidine: RI = 2130, EI- and CI-MS as for epiaphyllidine. – 18. 10,17-Dioxosparteine: RI = 2340, EI-MS: 150 (100), 84 (50), 262 (50), 152 (36), 234 (10); CI-MS: 263 (100), 150 (6). – 19. α-Isosparteine: EI-MS, RI as in [18]; CI-MS as for sparteine. – 20. 17-Oxolupanine: EI-MS, RI as in [17, 18]; CI-MS: 263 (100), 151 (5), 235 (5). – 21. Albine: EI-MS, RI as in [9]; CI-MS: 233 (100), 191 (10). – 22. N-Methylalbine: EI-MS, RI as in [9]; CI-MS: 247 (100), 205 (10). – 23. Multiflorine: EI-MS, RI as in [9, 18]; CI-MS: 247 (100),

136 (5). – 24. A dehydrolupanine of unknown structure EI-MS: 150 (100), 136 (80), 246 (40), 134 (40), 84 (40); RI = 2340. – 25. 5,6-Dehydrolupanine: EI-MS, RI as in [18, 20]; CI-MS: 247 (100), 98 (5). – 26. Anagyrine: EI-MS, RI as in [18, 20]; CI-MS: 245 (100), 98 (20). – 27. Ammodendrine: EI-MS, RI as in [18]; CI-MS: 209 (100), 85 (20). – 28. Cytisine: EI-MS, RI as in [18, 20]; CI-MS: 191 (100), 147 (5). – 29. N-Methylcytisine: EI-MS, RI as in [18, 20]; CI-MS: 205 (100), 146 (5), 58 (8). – 30. Rhombifoline: EI-MS, RI as in [18]; CI-MS: 245 (100), 58 (75), 203 (30). – 31. Tinctorine: EI-MS, RI as in [18, 20]; CI-MS: 245 (100), 58 (75), 203 (40). – 32. 11-Allylcytisine: EI-MS, RI as in [18]; CI-MS: 231 (100), 189 (30). – 33. N-Formylcytisine: EI-MS, RI as in [18]; CI-MS: 219 (100), 189 (8). – 34. N-Acetylcytisine: EI-MS, RI as in [18]. – 35. Thermopsine: EI-MS, RI as in [18]; CI-MS: 245 (100), 146 (5), 98 (5). – 36. 13-Hydroxyanagyrine: EI-MS, RI as in [18]; CI-MS: 261 (100), 243 (80). – 37. 13-Acetyloxy-anagyrine: EI-MS, RI as in [18, 20]; CI-MS: 303 (100), 243 (20).

Results and Discussion

1. The alkaloid patterns of developing seedlings

The seeds of lupins usually contain lupanine and 13-hydroxylupanine as the major alkaloids (Table I), with the exception of *L. luteus* and related species which accumulate lupinine and sparteine [1]. During germination and seedling development a rapid increase of esters of 13-hydroxylupanine, especially 13-tigloyloxy-lupanine can be observed (Fig. 1) which is concomitant to a decrease of free 13-hydroxylupanine. The enzyme which catalyzes the esterification, a tigloyl-CoA: 13-hydroxylupanine O-tigloyltransferase has recently been characterized [29]. The activity of this transferase shows an increase which parallels the increase of esteralkaloids.

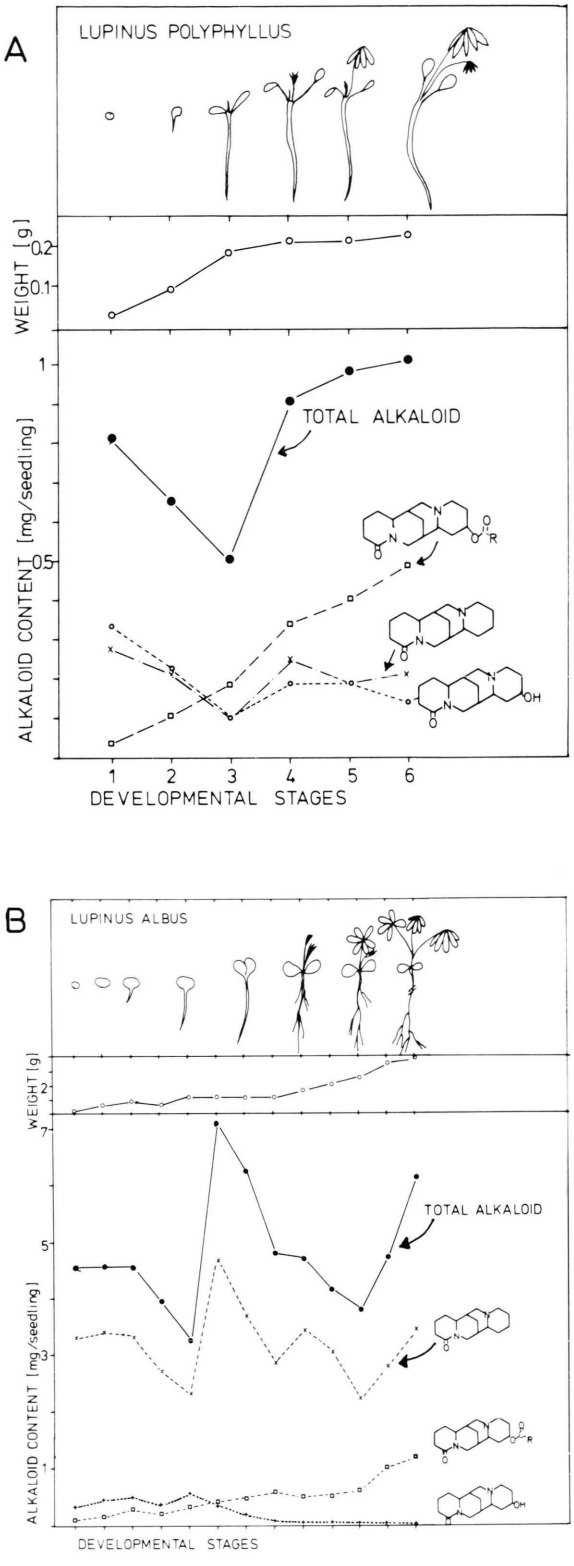
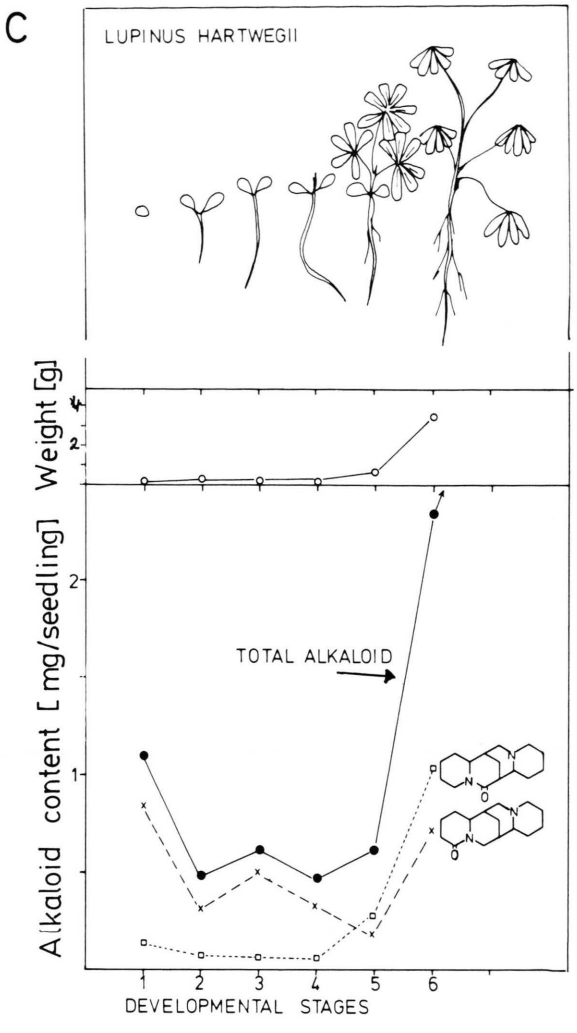
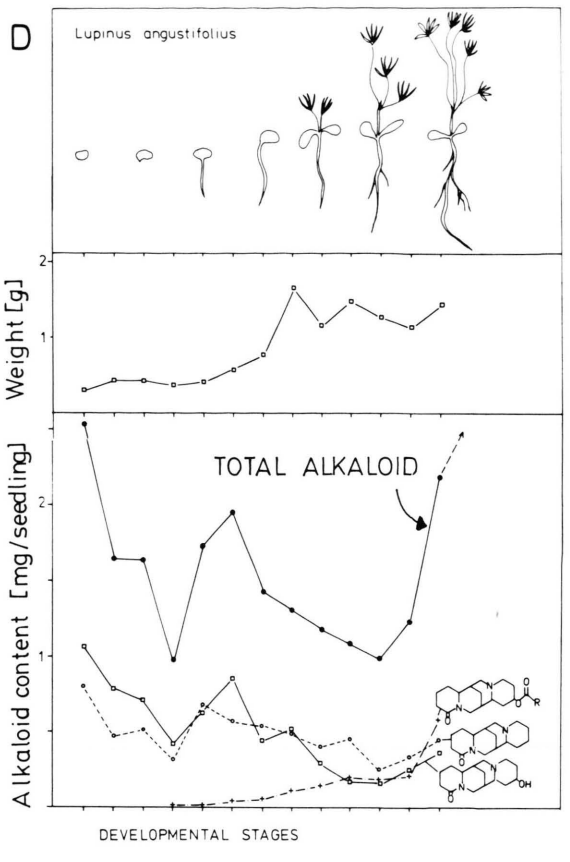
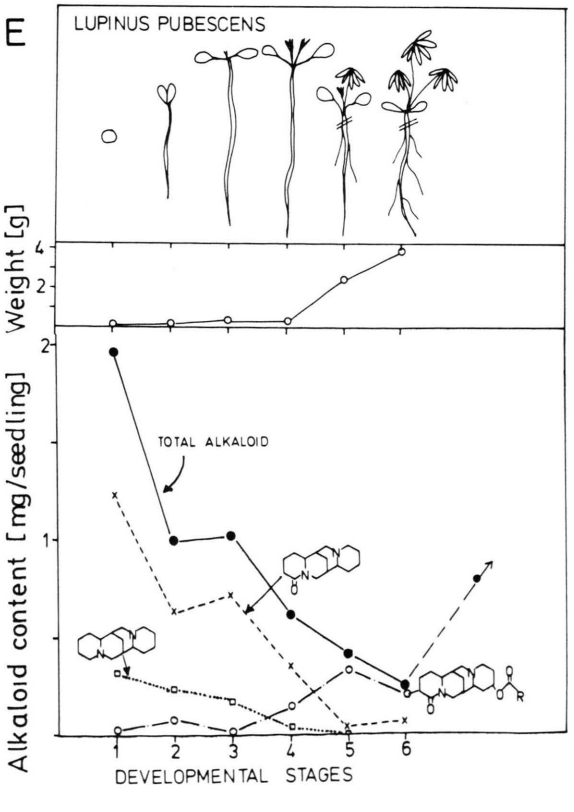
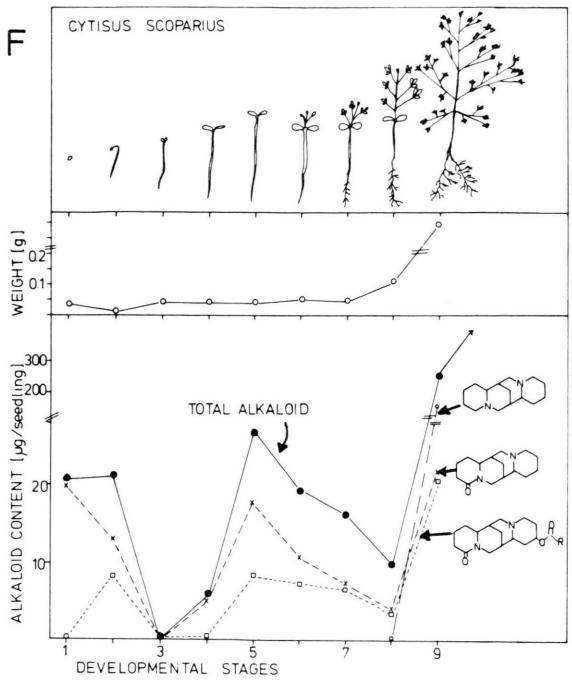


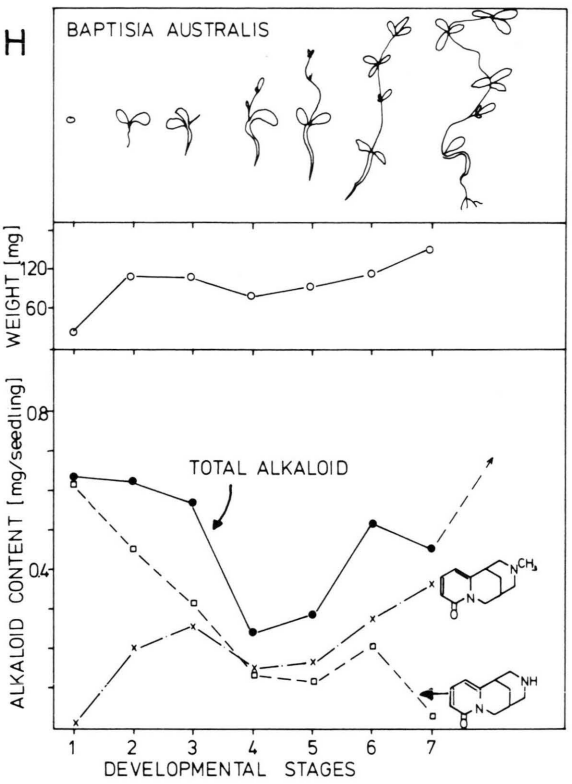
Fig. 1. Fluctuation of alkaloid patterns and total alkaloid during germination and seedling development.
The upper part of the graph illustrates schematically the developmental stages. The middle part gives data on growth as indicated by the fresh weight of individual plants. The lower part represent total alkaloid content and the alkaloid concentrations of the major alkaloids.
A. *L. polyphyllus*, B. *L. albus*, C. *L. hartwegii*, D. *L. angustifolius*, E. *L. pubescens*, F. *Cytisus scoparius*, G. *Spartium junceum*, H. *Baptisia australis*, I. *Laburnum anagyroides*.

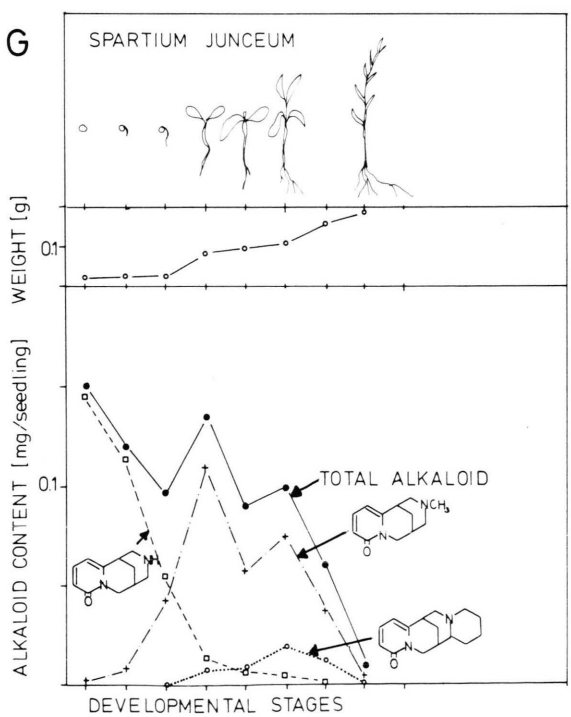

C
LUPINUS HARTWEGII
Weight [g]
Alkaloid content [mg/seedling]
TOTAL ALKALOID
DEVELOPMENTAL STAGES


D
Lupinus angustifolius
Weight [g]
Alkaloid content [mg/seedling]
TOTAL ALKALOID
DEVELOPMENTAL STAGES


E
LUPINUS PUBESCENS
Weight [g]
Alkaloid content [mg/seedling]
TOTAL ALKALOID
DEVELOPMENTAL STAGES


F
CYTISUS SCOPARIUS
WEIGHT [g]
0.2
0.1
ALKALOID CONTENT [µg/seedling]
300
200
20
10
TOTAL ALKALOID
1
3
5
7
9
DEVELOPMENTAL STAGES


H
BAPTISIA AUSTRALIS
WEIGHT [mg]
120
60
ALKALOID CONTENT [mg/seedling]
0.8
0.4
TOTAL ALKALOID
1
2
3
4
5
6
7
DEVELOPMENTAL STAGES


G
SPARTIUM JUNCEUM
WEIGHT [g]
0.1
ALKALOID CONTENT [mg/seedling]
0.1
TOTAL ALKALOID
DEVELOPMENTAL STAGES

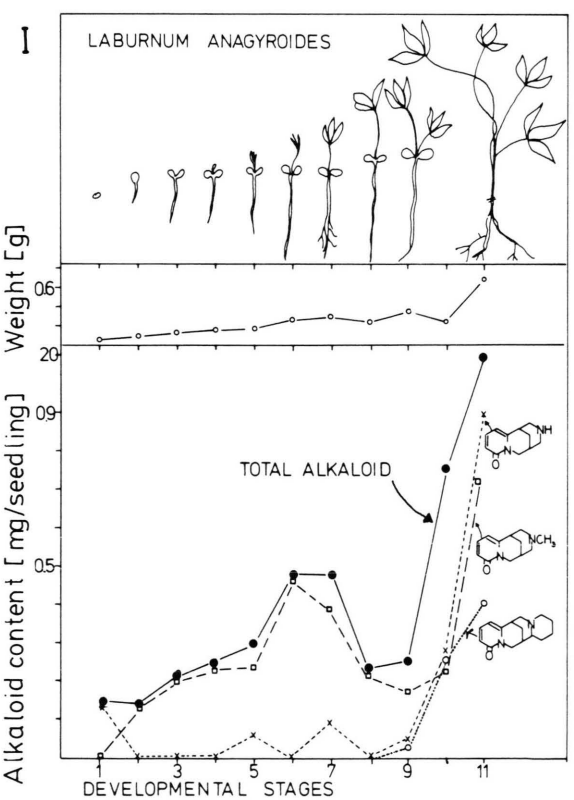

I
LABURNUM ANAGYROIDES
Weight [g]
0.6
Alkaloid content [mg/seedling]
2.0
0.9
0.5
TOTAL ALKALOID
1
3
5
7
9
11
DEVELOPMENTAL STAGES

Table I. Alkaloid pattern of leaves, seeds, and seedlings. a. Plants containing major alkaloids of the lupanine series. b. plants containing α-pyridone-type alkaloids. – = not recorded; + = trace amounts. Numbering of the alkaloids s. Material and Methods.

a.)

Species	Alkaloid content mg/g f.w.	Alkaloid abundance (%) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Lupinus albus																												
leaves	0.2	50	1.5	12	–	0.2	10	4	1	3	1	+	+	0.5	–	–	–	–	–	–	0.7	6	0.2	10	–	+	+	3
seeds	15	70	+	8		+	1	+	+	+	+	+	–	–	–	–	–	–	–	–	+	15	0.7	3	–	–	–	+
seedlings	2	59	+	1	–	0.5	21	2	+	3	–	+	–	+	–	–	–	–	–	–	0.4	9	2	3	–	–	–	–
L. polyphyllus																												
leaves	2	40	+	7	1	3	20	2	9	10	0.5	4	–	1	–	–	–	–	–	–	0.8	–	0.5	0.5	2	–	–	0.5
seeds	40	27	+	41	+	4	2	–	7	19	+	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–
seedlings	4	20	–	14	0.6	3	45	+	0.4	16	–	–	–	1	–	–	–	–	–	–	–	–	–	–	+	–	–	–
L. angustifolius																												
leaves	0.5	38	+	18	–	+	4.5	1	2	31	–	2	–	–	–	–	–	–	–	–	0.4	–	–	–	–	–	–	–
seeds	21	33	+	44	–	–	–	–	1.8	22	+	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–
seedlings	1.6	21	+	17	–	+	11	5	1.7	32	–	10	–	–	–	–	–	–	–	–	0.8	–	–	–	–	–	–	–
L. pubescens																												
seeds	33	66	0.4	–	13.6	1	+	–	0.8	–	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
seedlings	0.07	27	1	0.5	–	5	41	9	11	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
L. hartwegii																												
leaves	0.8	23	1.5	–	2.4	–	–	–	–	–	–	–	–	–	38	34	+	+	0.5		–	–	–	–	–	–	–	–
seeds	36	77	0.3	+	5.5	–	–	–	–	–	–	–	–	–	1.5	12	+	+	+		–	–	–	–	–	–	–	–
seedlings	3.2	31	1	2	15	–	+	–	+	–	–	–	–	–	14	28	+	2	+		–	–	–	–	–	–	–	–
C. scoparius																												
leaves	5	7	+	3	–	+	+	+	2	+	62	+	2	18	–	–	–	–	–	+	+	–	–	–	–	+	0.5	1
seeds	20	53	+	7	11	+	+	–	+	+	3	+	+	+	–	–	–	–	–	–	+	–	–	–	+	+	+	+
seedlings	0.02	80	–	10.5	–	+	3.7	+	0.4	–	–	+	–	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–

b. α-pyridone alkaloids

Species	Alkaloid content mg/g f.w.	Alkaloide abundance (%) 28	29	25	26	1	30	31	32	33	34	35	36	37	27	10	13	2
B. australis																		
leaves	0.8	13	74	0.5	6	0.5	1.5	1.5	+	+	+	+	+	+	+	1	+	+
seeds	34	90	5	0.7	1.4	+	+	+	+		–	–	0.7	–	–	–	–	+
seedlings	4.7	40	53	3.8	2	0.6	0.3	+	–	+	–	–	1.9	0.5	–	–	–	–
S. junceum																		
leaves	0.8	23	62	0.3	2.5	1.8	9	2	2	+	–	+	–	–	+	+	+	+
seeds	8	97	1.8	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
seedlings	1	7	58	2	22	4	+	–	–	–	–	–	2.0	–	0.4	–	–	–
L. anagyroides																		
leaves	0.2	65	17	0.1	8	2	+	+	–	–	–	7	–	–	+	+	+	+
seeds	3.3	98	+	+	+	–	–	–	–	–	–	–	–	–	+	–	–	–
seedlings	3	42	34	2	19	0.5	0.1	+	+	–	–	+	1	–	0.5	+	–	–

Cytisus scoparius plants contain sparteine and 17-oxosparteine as major alkaloids in the aerial green parts. In the seeds and in cell suspension cultures lupanine figures as the main alkaloid [19]. During the initial stages of seedling development the alkaloid patterns of broom seedlings are similar to those of lupins in that they form lupanine and 13-hydroxylupanine esters (Fig. 1). Only in the later growth stages, when the plants are higher than 30 cm, the formation of sparteine sets in (Fig. 1). Cell cultures of this species show somewhat similar behaviour: undifferentiated cultures accumulate lupanine, whereas differentiated cultures form sparteine [19].

The species which accumulate α-pyridone alkaloids (Table Ib), *i.e. Baptisia australis, Spartium junceum,* and *Laburnum anagyroides,* contain cytisine as the major seed alkaloid (Table I). During germination cytisine disappears rapidly being obviously transformed into N-methylcytisine (Fig. 1). This explanation could be confirmed by recent enzymatic experiments [22]: We could characterize a specific S-adenosyl-L-methionine: cytisine N-methyltransferase from *Laburnum* seedlings and cell cultures, which is most active during the initial stages of germination [22].

Most other changes (Table I) in alkaloid patterns usually concern minor alkaloids which are thought to be derived from lupanine [23].

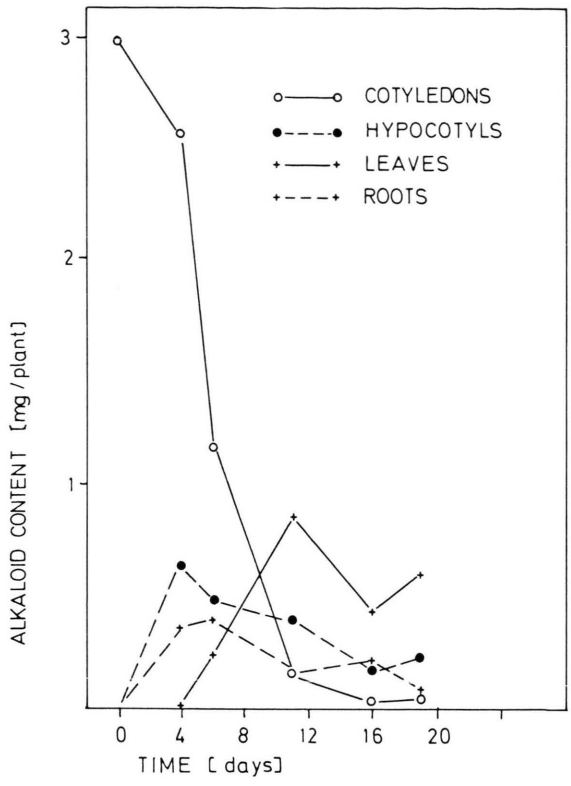


Fig. 2. Total alkaloids during germination of *Lupinus albus*. Alkaloid content was evaluated separately for cotyledons, hypocotyls, roots, and leaves.

2. *Alkaloids as a nitrogen source for seedlings*

The seeds of quinolizidine alkaloid accumulating plant species are generally rich in alkaloids. As a trend we could observe that seeds of species which produce few and big seeds contain an alkaloid amount of up to 5% (equivalent to 200 mmol/kg) whereas species with many small seeds usually had rather low alkaloid values [4]. It should be recalled that those legume species which do not accumulate QA in their seeds, usually store other nitrogenous natural products such as non-protein amino acids, lectins or protease inhibitors [14, 24].

The alkaloid contents of seeds or seedlings respectively decreased by 20 to 100% during germination and the initial seedling stages in nearly all the species studied (Fig. 1, 2). During the early developmental stages we could also observe that the alkaloids were transported from the cotyledons to roots and hypocotyls (Fig. 2). After 2 weeks the cotyledons were almost devoid of alkaloid. In *L. albus, L. angustifolius* and *C. scoparius* the overall alkaloid content increased when cotyledons were fully expanded and green (Fig. 1) which was probably due to *de novo* synthesis. When the seedlings had developed functional leaves, we observed substantial and continuous alkaloid formation (Fig. 1) which reached a maximum at the time of flowering and fruit maturation (3, 26]. How can we explain this observation? *L. albus* seeds loose about 2% alkaloid during imbibition and another 2% during the following stages by secretion of the alkaloids via the rhizosphere [25]. Therefore, it is unlikely that the decrease is due to alkaloid loss into the environment. We assume that the QA are degraded and that their nitrogen is used by the growing seedling. It has been established that the other nitrogenous compounds of legumes, *e.g.* non-protein amino acids, are first of all chemical defense compounds but that they also serve as nitrogen storage compounds, which are mobilized during germination and seedling development [14, 24], since

nitrogen is a limiting nutrient for seedlings. Do alkaloids constitute a relevant factor in this context? Seeds of *L. albus* contain approx. 30% protein and 5% alkaloid. Since protein contains approx. 16% nitrogen and QA approx. 11% nitrogen, we can calculate that QA contribute approx. 10% to the overall nitrogen stored in *L. albus* seeds. Therefore, a minor role of QA could be nitrogen storage.

3. Alkaloids as the sole nitrogen source for lupin cell cultures

If QA, such as sparteine or lupanine were added to culture media, the growth of cell suspension cultures of *Lupinus polyphyllus* and even those of QA-free species such as *Conium maculatum* is almost unimpaired (Table II). Obviously QA are not very toxic for cultured cells even at rather high concentrations such as 5 mM, whereas these concentrations exhibit significant repellent, inhibitory or toxic effects on microorganisms and animals [2–4].

We have shown in previous experiments that QA are taken up by the cells and are usually degraded within 48 h [8, 15, 23]. It is usually assumed that nitrate and/or ammonium salts are essential for the survival and growth of cultured plant cells. Cells of *Nicotiana* (XD cells) are able however to exploit urea and intermediates of purine metabolism as sole nitrogen sources [27]. XD cells even grow on barbituric acid as a synthetic nitrogen source [27]. These examples indicate that the metabolic capacities of cultured plant cells are much wider than generally anticipated. In a series of experiments we therefore tried to grow cell cultures of lupins on nutrient agar which contained sparteine as sole nitrogen source. If these experiments were successful it would show that lupin cells can use the nitrogen stored in the alkaloid molecule.

Suspension cultured photomixotrophic cells of *L. polyphyllus, Cytisus scoparius, C. purpureus,* and *L. hartwegii* were transferred to agar media devoid of any nitrogen source (control) or to media containing either 1, 5, or 10 mmol/l sparteine (Table III). While most cells and cell aggregates turned brown and died within 2–6 weeks on the control agar, 90–100% of all cell colonies remained green and even started to grow on agar containing 5 mmol/l sparteine (Table III). Growth of these cells on sparteine was very slow but some cultures survived up to 6–9 months with a transfer to fresh medium every 2 months. When these cultures were transferred back to agar with ammonium nitrate as nitrogen source, they showed normal growth after an initial lag period. Even if these experiments did not lead to cultures with rapid growth on alkaloid-medium, as was the case with the *Nicotiana* cells on urea media [27], these experiments nevertheless provide evidence that the cells are capable to remobilize the nitrogen stored in the sparteine molecule.

Table II. Growth of cell suspension cultures of *Lupinus polyphyllus* and of *Conium maculatum* in media containing sparteine. About 2 g (fresh weight) were transferred to 20 ml cell culture medium in 100 ml Erlenmeyer flasks. Data represent mean values of 2 replicates and are expressed as mg dry weight/flask.

Treatment	Time of incubation [days] 0	3	6	10
Conium maculatum				
control	32	85	134	274
+1 mM sparteine	32	93	158	277
+5 mM sparteine	32	97	155	335
Lupinus polyphyllus				
Strain I				
control	142	193	313	555
+1 mM sparteine	142	188	314	510
+5 mM sparteine	142	204	310	453
Strain II				
control	90	179	442	595
+1 mM sparteine	90	155	339	588
+5 mM sparteine	90	156	299	546

Table III. Survival of cultured cells on agar media containing sparteine as the sole nitrogen source.
Photomixotrophic, green cell aggregates were transferred from cell suspension cultures onto agar media (5 plates each). The number of green colonies after 6 weeks of cultivation was taken as a measure of cell survival. (Dead cells turned brown under the conditions studied).

Cell culture	Alkaloid concentration [mmol/l]	% living colonies
Cytisus scoparius	0	20
	1	52
	10	90
Lupinus polyphyllus	0	60
	1	70
	10	100
Cytisus purpureus	5	90
Lupinus hartwegii	5	100

According to preliminary experiments, the degradation of QA is mediated by a set of oxidative and hydrolytic enzymes [16, 28] which are presently studied in our laboratory.

QA will inhibit the growth of most microorganisms and deter the feeding of herbivores on the attractive and nutrient rich lupin seedlings at the QA concentration present in the seeds and the initial seedling stages (s. Table I). From the results reported in this communication it can be concluded that QA are definitely not inert end products of metabolism but dynamic metabolites whose metabolism was optimized during evolution. We can classify lupin alkaloids therefore to be toxic, but biodegradable chemical defense substances with minor roles as nitrogen transport and nitrogen storage compounds.

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