FATE OF QUINOLIZIDINE ALKALOIDS THROUGH THREE TROPHIC LEVELS: *Laburnum anagyroides* (Leguminosae) AND ASSOCIATED ORGANISMS

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Abstract—The quinolizidine alkaloids (QA) of golden rain, *Laburnum anagyroides*, and those of phytophagous insects associated with the plant, as well as of parasitoids of the latter, were analyzed by capillary GLC and GLC-MS. The alkaloid content in samples of vegetative plant parts was high at the beginning of the season, then decreased, while that of reproductive organs was high throughout flowering, pod formation, and maturation. The analyses showed that the QA of the plant passed through two higher trophic levels (herbivorous insects and their parasitoids) and that the alkaloid pattern changed little during the passage. The alkaloids were present in two phytophagous insect species associated with golden rain: the predispersal seed predator, *Bruchidius villosus* [5–13 µg/g fresh weight (fw)], and *Aphis cytisorum* (182–1012 µg/g fw), an aphid that feeds on shoots, leaves, and inflorescences. Braconid and chalcidoid parasitoids emerging from the bruchid host also contained alkaloids (1.3–3 µg/g fw), as did three foraging ant species, *Lasius niger*, *Formica rufibarbis*, and *F. cunicularia* (45 µg/g fw), that visited the aphid colonies or honeydew-covered leaves of aphid-infested plants. The hypothesis that developing bruchid larvae and/or the plant "manipulate" QA supply to infested seeds was not supported, because QA content of leftover endosperm in seeds after bruchid development was similar to that of uninfested seeds. The frass of developing bruchid larvae was rich in QA (31 mg/g dry weight). While aphids sequestered, the bruchid larvae took up and eliminated QA with the frass without chemical transformation.

Key Words—*Laburnum anagyroides*, *Robinia pseudacacia*, *Aphis cytiso-*

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rum, Homoptera, Aphididae, *Bruchidius villosus*, Coleoptera, Bruchidae, *Lasius niger*, *Formica* species, Hymenoptera, Formicidae, *Triaspis thoracicus*, Braconidae, Chalcidoidea, quinolizidine alkaloids.

INTRODUCTION

Evidence is accumulating that interactions at three trophic levels can be mediated and/or influenced by chemicals originating from only one member of the food web, the plant (Price et al., 1980). For example, the development time of the parasitoid *Hyposoter exiguae* was prolonged in its host, *Heliothis zea*, and the percentage of adult survivorship decreased, if α-tomatine, the naturally occurring steroid glycoside in tomatoes, was present in the host's diet to the extent of 0.3–0.5% (Campbell and Duffey 1979, 1981). Similarly, the percentage of emerging *Apanteles congregatus* parasitoids from the host *Manduca sexta* was significantly lower in comparison with the control when they developed in a host reared on a diet containing a higher level of nicotine (Thurston and Fox, 1972). Benn et al. (1979) found that pyrrolizidine alkaloids were present in the parasitoid *Microplitis* emerging from the insect host *Nyctemera* living on *Senecio spathulatus* plants. However, the insect hosts were not affected by the chemicals in the above studies.

Quinolizidine alkaloids (QA) are characteristic secondary metabolites of the phylogenetically primitive tribes of the family Leguminosae (Kinghorn and Balandrin, 1984) and are especially abundant in the members of the tribe Genisteae (Waller and Nowacki, 1978). They are assumed to play an important role in plant defense against nonadapted phytophages (Waller and Nowacki, 1978; Wink, 1984c, 1985, 1988), but also constitute a nitrogen source for the plant seedlings (Nowotnowna, 1928, cited in Waller and Nowacki, 1978; Wink, 1985; Wink and Witte, 1985a). Phytophagous insects, such as aphids, acquire defense against generalist predators by sequestering QA (Wink and Römer, 1986). QA also have bacterio-and fungistatic properties (Wink, 1984a; Tyski et al., 1988) besides being highly toxic to most vertebrates and inhibitory for the development of other plant species (Pöhm, 1966; Waller and Nowacki, 1978; Wink, 1983).

The primary aim of the present paper was to follow the fate of QA, both qualitatively and quantitatively, through a complex multispecies food web from the primary producer (*Laburnum anagyroides*) to the first (herbivorous) and second (carnivorous) consumer levels, as well as to scavengers (aphid-visiting ant species). For this purpose, we sampled and analyzed plant parts, and insect herbivores and organisms associated with them (parasitoids and ant species). We also were interested in the question of whether QA taken up by the bruchid seed predator would be found changed in a quantitative and compositional fashion when eliminated.

For some time it also has been known that some legume species are able to selectively abort seeds and/or pods containing seed predator larvae. Such and other "manipulations" by the plant can affect transport of nutrients and non-nutrients into the seeds (Stephenson, 1981). As, in the present plant seed predator system, egg-laying by bruchid females occurs on green pods and larvae develop in the ovules, the plant, by means of active defense, may alter the chemical environment within the seeds. Therefore, we also examined whether infested seeds contained a higher level of QA than uninfested ones.

METHODS AND MATERIALS

Host Plant. Laburnum anagyroides Medik (Leguminosae) is an endemic plant species in central and southern Europe (Soó, 1966). The tree is also widely grown as an ornamental in parks and gardens throughout Europe. Yellow flowers bloom within inflorescences through May and June. Pods containing one to nine seeds appear in June, grow to 2–8 cm in length, and harden by the end of August. Pods are dehiscent and remain on the plant. Many pods open in late September; however, most of them open only during the next spring. Some seeds fall out of the pod during the following spring, but many remain in the pod for a year or longer. Seeds are dark brown and 2–4 mm long.

Insect Herbivores. Aphis cytisorum Hart. (Homoptera: Aphididae) is a dark grey aphid with a waxy coating covering the body. Aphids hatch from overwintering eggs placed on the plant's trunk close to the soil surface. They move up the plant and form colonies on distal parts of new shoots, inflorescence axes, and leaves' lower surfaces. Individual aphid colonies are distinct; however, if the level of infestation is high, they later coalesce. Honeydew is produced in large amounts and is collected by several ant species.

The adults of the predispersal seed predator, *Bruchidius villosus* Fabr. (Coleoptera: Bruchidae), emerge from seeds of *L. anagyroides* and probably other overwintering sites such as under tree bark, although this is not well established. Females lay eggs on the surface of immature pods. Larvae enter through the pod wall and feed on developing seeds. By mid-August, larvae complete development and pupate, and adults emerge from open pods at the end of August or overwinter within seeds of closed pods on the plant.

Parasitoids. B. villosus is parasitized by chalcidoid and braconid species that emerge either in August–September or overwinter as larvae or adults inside the seeds and emerge the following May–June. (See more details in the Results section.)

Sampling Sites and Preparation of Plant and Insect Material for Chemical Analysis. Because *L. anagyroides* generally occurs as sporadically scattered individual trees and only rarely forms larger, homogeneous stands, both insect

and plant materials came from several collection sites in Hungary. Individual trees of (1) 2–3 m height and max. 10-cm trunk diameter, for which the local climatic and soil conditions varied greatly; and (2) one homogeneous stand (ca. 20 trees approx. 10–15 years old, growing on a long-abandoned damp area, on clay soil) were sampled for green plant parts, aphids, and ant species during 1987 (see Table 1 for dates of sampling).

TABLE 1. QUALITATIVE QA COMPOSITION OF *L. anagyroides* PARTS

	Composition (% of total QA)			
Date (1987)/sample	1[a]	2	3	4
May 27				
Leaf petioles and shoots	13.7	75.9	5.7	1.0
Leaf laminas	56.2	38.4	0.2	0.6
Inflorescence axis and flower pedicels	16.3	82	1.1	0.4
Flowers (sine pedicels)	14.9	85.1		
Very young pods[b]	30.6	67.6	0.7	0.3
June 8				
Inflorescence axis and flower pedicels	9.7	85	2.4	1.7
Half-grown pods I[c]	27.3	69.8	2.6	0.2
Half-grown pods II[d]	26.1	73.6		
July 22				
Leaf petioles and laminas	4.6	95		
Inflorescence axis and pod pedicels	1.0	89.9	2.3	3.8
Fully grown pods	1.3	97.7	0.7	
September 2–4				
Leaf petioles	6.3	88	5.2	1.0
Leaf laminas	11.8	88.2		
Mature dry pod wall (sine seeds)	4.1	12.7	74.4	8.1
1987 from 4 localities	6.9	15.1	76.7	1.8
1989	3.1[e]	95.5		
	(1.3)	(1.3)		
Leftover endosperm after bruchid development	4.5	94		
Endosperm (control)	3.4	95		
Testa of bruchid-infested seeds	1.5	97		
Testa (control)	5.4	93		

[a] 1 = *N*-Methylcytisine; 2 = cytisine; 3 = 5,6-dehydrolupanine; 4 = anagyrine.
[b] 0.5–1.5 cm long.
[c] "Small": 2–5 cm long.
[d] "Big": 4–6 cm long.
[e] Mean (SD).

For alkaloid analyses of green plant parts, lyophilized samples were used. One 6- to 60-g fresh weight sample of living plant parts, pooled from one to two trees for each sampling date and plant part (as indicated in Table 1) were removed and immediately put into a cool box, transferred to the laboratory, and stored at −60°C until further processing. The deep-frozen samples were crushed in a mortar filled with liquid N_2, put into a lyophilizer (Edwards, Great Britain, model EF 03), freeze-dried, ground, placed in vials, and finally placed back into the lyophilizer for sealing under vacuum. The samples were 2–9 g dry weight (dw) after lyophilization. In addition to the above, dry pods (without seeds), and whole mature seeds (4–5 g each sample; one from 1987 and several from 1989), testa (516 mg), and endosperm (1 g) of mature, dry intact seeds, as well as testa (184 mg) and leftover endosperm of seeds (447 mg) in which the predispersal seed predator bruchid had completed development, were collected and analyzed.'

Aphids were collected from infested trees by brushing them into a container, and ants from aphid colonies and honeydew-covered leaves were collected with an aspirator. There were three samples of *A. cytisorum* collected at three points of time in 1987 (see Table 2), each containing approximately 4900, 7500 and 14,000 aphids, and two samples of ants, one a mixed sample of *Formica rufibarbis* F. and *F. cunicularia* Latr. and the other a homogeneous sample of 444 *Lasius niger* L. (Hymenoptera: Formicidae).

On September 2, 1987, a large sample of pods, with even mature pods still closed, were collected for obtaining bruchid adults and parasitoids for chemical analysis. The seeds from one batch of pods were removed and placed, at 25–26°C, under a photoperiod of 20:4 hr (light–dark). The containers were covered with linen cloth and humidified with wet cotton until both bruchids and parasitoids emerged from the seeds.

Emerging insects were collected daily and stored at −20°C in 70% EtOH. For alkaloid analysis, the following were used: (1) two samples of *B. villosus* containing 357 and 520 beetles, respectively; (2) three samples of parasitoids, one of 43 braconids, one of 64 pteromalids, and a third of 82 specimens of about three species of parasitoids. Parasitoids emerged from the same seed samples from which the bruchids originated.

Following the emergence of bruchids and parasitoids, one sample of empty cocoons (94 mg) of the braconid parasitoid, and one sample of bruchid larval frass together with pupation chambers of larvae lined with cemented fecal material (134 mg) were collected from infested seeds and used for analysis.

Chemical Analysis of Plant and Insect Material. Plant samples (500 mg) were homogenized in 15 ml 0.5 M HCl and left standing for 1 hr. The homogenates were made alkaline by adding 6 N NaOH and applied to standard Chem-Elute columns for solid-phase extraction. After elution with methylene chloride, the solvent was evaporated in vacuo.

TABLE 2. QUANTITATIVE AND QUALITATIVE QA COMPOSITION OF INSECTS ASSOCIATED WITH *L. anagyroides*

Sample/date	Alkaloid content (µg/g)[a]	Alkaloids (% of total QA)			
		1[b]	2	3	4
A. cytisorum					
June 16, 1987	182	5.6	92.5	1.7	
July 5, 1987	450	4.6	91	4.2	
July 29, 1987	1,012	3.2	94	2.7	0.4
B. Villosus					
Sept. 9–15, 1987	13		100		
Sept. 18, 1987	5		100		
Larval frass	31,276[c]	0.8	98		
Lasius niger					
June 16, 1987	45	2.5	95	1.2	1.2
Formica rufibarbis and *F. cunicularia*					
June 16–July 5, 1987	d	1.9	91	6.5	0.3
Triaspis thoracicus					
Sept. 8–17, 1987	3		100		
Triaspis cocoons	1,617[c]		99.5		
Parasitoids I[e]					
Sept. 10, 1987–Jan. 25, 1988	1.3		100		
Parasitoids II[f]					
Sept. 18, 1987–Jan. 11, 1988	d	40	60		

[a] Measured on a fresh weight basis.
[b] 1 = *N*-Methylcytisine; 2 = cytisine; 3 = 5,6-dehydrolupanine; 4 = anagyrine.
[c] Measured on a dry weight basis.
[d] Due to the lack of data on body weights, alkaloid contents per gram body weight could not be calculated; however, the samples contained 76 and 5 µg alkaloids, respectively.
[e] Pure sample of one pteromalid species.
[f] Mixed sample of a minimum of three eulophid and pteromalid species.

Alkaloid extracts were analyzed by TLC (cyclohexane–diethylamine, 7:3) and capillary GLC: Perkin Elmer GLC 8500 or Chrompack CP438A; DB-1 column (30 m × 0.3 mm); oven: 150°C, 2 min isothermal, to 320°C with 20°C/min; injector: 250°C; detector: 320°C; carrier gas: helium, 92 kPa. Quantification was carried out with cytisine as an external standard using a nitrogen-specific detector. GLC-MS measurements were performed as described in Wink et al. (1983). Alkaloids were identified according to their specific

Kovats retention indices and mass spectra and by using alkaloid standards (for details see Wink et al., 1983).

The QA contents in plant materials were determined on a dry weight (dw) basis, those of insects on a fresh weight (fw) basis. The QA contents of bruchid adults, measured on a fresh weight basis, were converted into dry weight to compare with QA content of larval feces that was measured on a dry weight basis. For the conversion we assumed that QA content was 10 times as high in dry materials.
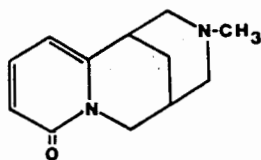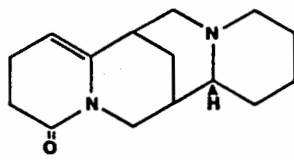
RESULTS

Parasitoids. There was one braconid species, *Triaspis thoracicus* Curt. (Hymenoptera), present in the samples. Among the chalcidoid species, pteromalids, eulophids, and eupelmids were represented, and the presence of a *Tetrastichus* sp. (Hymenoptera: Eulophidae) was confirmed. In case of a *L. anagyroides* seed sample (containing approximately 5000 seeds), pteromalids and braconids emerged from ca. 18% of the bruchid-infested seeds.

Ants. At least three ant species (*Lasius niger, Formica rufibarbis,* and *F. cunicularia,* roughly in a 15 : 1 : 2 ratio) regularly visited the aphid colonies or, in the case of high aphid infestation, only the twigs and the leaves that were covered with honeydew.
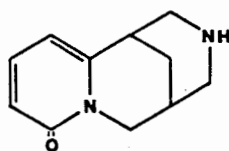
Alkaloid Analysis: Qualitative Aspects. QA were detected in all components of food chain examined. Both the plant and insect samples showed similar alkaloid patterns (Tables 1 and 2). There were four quinolizidine alkaloids identified by TLC, capillary GLC, and GLC-MS: cytisine, *N*-methylcytisine, 5,6-dehydrolupanine, and anagyrine (Figure 1).

As for seasonal change in alkaloid composition, cytisine remained the major component in almost all plant samples. There was not much change in minor (5,6-dehydrolupanine and anagyrine) alkaloids either. The only substantial variation was shown in *N*-methylcytisine. An unusual pattern was detected in mature seeds and pod walls (without seeds) collected in 1987. Here, the cytisine content was very low, while the level of 5,6-dehydrolupanine was high. In the 1989 seed samples, cytisine was the main alkaloid (95.5 $\pm$ 1.3%, mean $\pm$ SD, $N = 4$), and *N*-methylcytisine was present in 3.1 $\pm$ 1.3% ($N = 4$). In comparison with alkaloids in the endosperm of seeds, *N*-methylcytisine made up a smaller percentage of two alkaloids found in the frass of *B. villosus* (Tables 1 and 2).
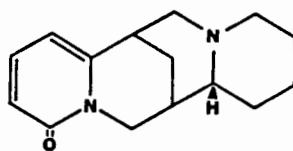
Bruchidius villosus beetles, *T. thoracicus,* and one pteromalid parasitoid sample contained predominantly cytisine; the other alkaloids were not present or occurred at levels too low to detect. In parasitoid sample II (Table 2), however, in addition to cytisine, a substantial proportion of *N*-methylcytisine was

Fig. 1. Structural formulas of the quinolizidine alkaloids found in the samples.

present also. Larval feces of the bruchid and the cocoons of the braconid parasitoid were low in N-methylcytisine and rich in cytisine (Table 2).

Quantitative Aspects. Individual plant parts (on a microgram per gram dry weight basis) showed a seasonal trend in total alkaloid contents (Figure 2). In May, much of the alkaloid content was found in the reproductive organs, specifically in inflorescence axes and individual flower pedicels. Since flowering and pod formation is continuous within a given inflorescence and within a time period, the very young pods already present at peak flowering also contained a relatively very high amount of QA. Gradually, stems, leaves, and inflorescence axis and/or flower pedicels lost their QA contents by September, while developing pods maintained some (ca. 1 mg total QA/g dry plant material) until ripening. Mature seeds were especially rich in QA (Figure 2). In four samples of such seeds (collected at four different localities in 1989), QA content ranged from 9.075 to 23.161 mg/g dw. Of the seed parts (on a microgram per gram dry weight basis), endosperm often contained four to five times as much QA as seed testa (Figure 3).

Alkaloid contents of bruchids (determined on a fresh weight basis) were much lower than either those in fully grown green pods, mature dry seeds, or endosperm (Table 2, Figure 3). On the other hand, in the aphids, the alkaloid levels were higher than in the plant (Table 2) and were in accordance with the
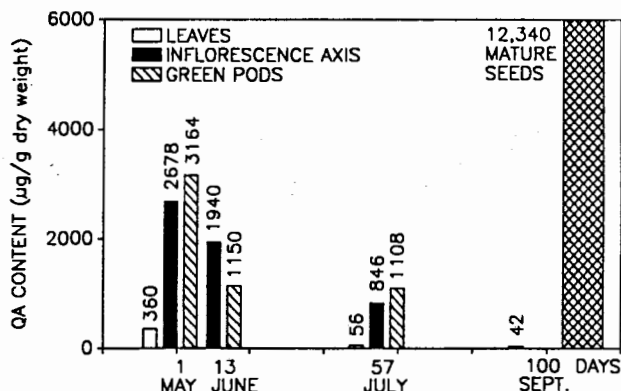
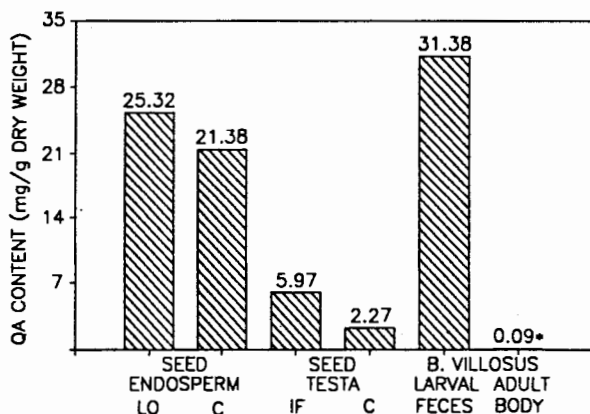FIG. 2. QA content of various parts of *L. anagyroides* between May 27 and September 2.

FIG. 3. QA contents of control and seed predator-infested *L. anagyroides* seed parts, as well as larval feces and bruchid adults. LO = leftover (i.e., bruchid-infested) endosperms, IF = infested, C = control. Asterisk denotes that the QA contents of adult bruchids were measured on a fresh weight basis, but were converted into dry weight values (see Methods and Materials for details).

seasonal change in the plant's QA content. Although the contents of QA in aphids were measured on a fresh weight basis, a cautious estimate on a dry weight basis (see Methods and Materials) would yield values 10 times as high as those found in the corresponding plant tissues. The most frequently visiting ant species, *L. niger*, weighed ca. 2 mg fw/individual and contained ca. 0.1

μg/ant of alkaloids. Alkaloid contents of parasitoids were much lower than those either in plant parts or in the host, but still detectable (Table 2).

QA content of endosperm of bruchid-infested and uninfested seeds were in the same range. The larval frass had a very high QA content (Figure 3 and Table 2). The peak oviposition period of bruchids (July; Szentesi, unpublished observation) coincided with a relatively low alkaloid content in the pod (a third to half of that present at early pod development).

DISCUSSION

Analysis of the quinolizidine alkaloid content of *L. anagyroides* and of associated insects showed that this major group of secondary plant metabolites passed through several trophic levels almost unchanged in terms of composition. One or more of the plant alkaloids appeared in two herbivores (an aphid and a bruchid), in various parasitoids, and in three ant species. This is remarkable if one considers the diversity and host specificity of participating organisms (see a more detailed discussion of this later).

Typical components of the QA group are cytisine (found in more than 79 plant species), *N*-methylcytisine, and anagyrine (Figure 1), constituting from trace quantities to 98% of total alkaloid content of a plant species (Mears and Mabry, 1971). Main sites of accumulation are the epidermal layers (Wink et al., 1984) and the reproductive organs, e.g., fruits and/or seeds (Wink, 1987; and present study).

Host Plant. In *L. anagyroides*, fresh leaves and stems contain about 150 μg/g fw cytisine (Wink, 1984c), and in both seeds and pods cytisine is the major alkaloid (Wink, 1984d). The QA level changed in different plant parts during the season. First young shoots, then inflorescences, and finally developing and mature pods and/or seeds contained the highest amount of QA (Figure 2). As reported also for other leguminous species (Waller and Nowacki, 1978; Wink and Hartmann, 1981), we found that *L. anagyroides* mobilized QA out of leaves and into seeds by the end of the season, showing that such compounds were not simply waste products. These findings are in agreement with the results of Greinwald et al. (1990) on *L. watereri* (Kirchn.) Dipp., a hybrid of *L. alpinum* and *L. anagyroides*. However, there were unusually high 5,6-dehydrolupanine and low cytisine levels in the 1987 pod wall and mature seed samples. The cause of these lower levels is unclear.

Insect Herbivores. Both *A. cytisorum* and *B. villosus* took up QA from *L. anagyroides*. The quantity present in the body, however, differed considerably between the two species (Table 2). *A. cytisorum* accumulated alkaloids against a concentration gradient. On two other legume species, *Petteria ramentacea* (Wink and Witte, 1985b) and *Cytisus scoparius* (Wink et al., 1982), *A. cyti-*

sorum also showed a higher level of QA content in the body than found in particular plant parts. The quantities of QA determined in aphid samples taken at three different points of time (Table 2) showed a seasonal change similar to that found in the plant. One of the reasons for this is that the aphids followed fresh tissue growth from shoot to flower axis to pod. Like many aphid species, *A. cytisorum* also taps the phloem sap, where the QA are reported to be transported in *Cytisus* and *Lupinus* plant species (Wink and Witte, 1984).

In the case of the bruchid species, the only stage feeding on the plant is the larva. Therefore, the QA uptake and quantity refer to this stage, although these were determined in the adults. The alkaloids present in the larvae eventually pass through the pupal stage to reach the adults. We think that the amount of QA taken up by a bruchid larva at any time during its development must have been in the range (ca. 1 mg/g dw) found in the plant tissue in which it developed in July. (In contrast, by the time the larvae completed development and adults emerged, there was a 10-fold increase of QA content in mature seeds in comparison with that in green pods.)

There was a high amount of QA continuously excreted by the bruchid larval feces: ca. 30 times as high as the quantity found in pods, and 200–600 times as high as the amount found in beetles (the latter was measured by a fresh weight basis and converted to dry weight). This reveals a very effective QA-eliminating process in the larvae. We assumed that they either continuously and quickly excreted the QA taken up while feeding, or the QA were degraded metabolically. The first mechanism seemed to be operating, although some changes in the qualitative QA pattern did occur. The fact that the feces were enriched in QA was the result of nutrients removal, and not the consequence of increasing QA content in the plant. We assume that, through N-demethylation reaction in the gut, some metabolic transformations also occur in the bruchid larva.

In connection with the described quantitative relationships, we believe that the possibility of "manipulating" QA content in the bruchid-infested seeds of *L. anagyroides* could exist both on the plant's and the seed predator's side. Janzen (1976) and Stephenson (1981) listed cases when fruits with bruchid larvae inside were subsequently aborted, although no proximal causes of this were known. In the case of *Laburnum/Bruchidius*, we considered the following possibilities: (1) The plant responded to the presence of bruchid larvae by delaying or hindering the maturation of seeds by not transporting all the nutrients, but increasing transport of the QA into the seeds. (2) Similarly, bruchid larvae could have avoided much of the QA transported to the seeds by either (a) influencing the transport itself or (b) completing development by the time of peak QA build-up (early to mid-August). The first hypothesis could be rejected with the present bruchid–plant relationship because infested and control seed endosperms contained the same quantities of QA (Figure 3); that is, the plant did not "manip-

ulate'' seed development. If abortion of bruchid-infested seeds occurred at all with *Laburnum*, it must have taken place at an earlier phase of seed development. As for the second hypothesis, assumption "a" is a possibility; however, we do not have evidence for it. Assumption "b" seems to be a realistic one, although it also implies that a high QA content is not preferred by the bruchid larvae.

As for the qualitative composition: the composition found in the aphids was similar to that demonstrated by the chemical analyses in the hostplant *L. anagyroides* (Tables 1 and 2). In other studies, the similarity was only partial. For example, although the most abundant alkaloid in *C. scoparius* is sparteine, 17-oxosparteine (derived from sparteine) was the most abundant alkaloid in aphids feeding on this plant (Wink et al., 1982). In *A. cytisorum* feeding on *Petteria ramentacea*, there was only one alkaloid, cytisine, found in the aphid, out of six occurring in the plant (Wink and Witte, 1985b).

The green *Laburnum* fruit varies more in QA composition during the time of bruchid larval development than does the mature seed or the bruchid adult. Although three components of the QA assemblage were present in fully grown pods, only two of them were found in larval frass, and one (cytisine) in the adult bruchid (Tables 1 and 2). Obviously, the plant gives the base for changes in the proportion of chemicals found in the herbivore feeding on it. For instance, more recently, Greinwald et al. (1990) identified 17 different QA from pods of *L. watereri*, of which 15 were not present in seeds.

We assume that both the aphid and the bruchid are likely to face much inter- and intraspecific chemical diversity in plant hosts. Besides the host plant, *L. anagyroides*, known to us, both insect species are reported from six other QA-containing plant species, and from a species, *Robinia pseudacacia*, devoid of QA (Krécsy, 1886; Kiss, 1895; Vadas, 1911; Escherich, 1923; Wahl, 1925; Zacher, 1936; Fischer, 1938; Hoffmann, 1945; Börner, 1952; F.P. Müller, personal communication). The main constituents of the latter are flavonoids and the nonprotein amino acid, canavanine (Bell, 1971). With some exceptions (see Wink, 1984b; Wink and Witte, 1984; Greinwald et al., 1990), few quantitative data are available on the seasonal, diurnal, or within-plant distribution of QA.

Herbivore infestation level and QA content of the host plant may be convergent. For example, the feeding sites of the legume-specialist aphid, *Acyrthosiphon spartii*, on the plant *Sarothamnus scoparius* were correlated with the highest concentration of the major alkaloid, sparteine (Smith, 1966). In contrast, the population density of a generalist aphid, *Macrosiphum euphorbiae*, decreased with increasing alkaloid level in *L. angustifolius* and *Cytisus scoparius*, respectively (Brusse, 1962; Wink et al., 1982). Possibly the within-plant density of a generalist insect species is inversely proportional, while that of an adapted specialist is directly proportional, to the alkaloid content of the plant.

Parasitoids. There was a decrease in QA content at the third trophic level

(Table 2), suggesting a limited. but still significant. passage of alkaloids. Similar passage of plant allelochemicals to parasitoids through an insect herbivore have been demonstrated under experimental conditions by Thurston and Fox (1972). Campbell and Duffey (1979. 1981). Thorpe and Barbosa (1986). and Barbosa et al. (1986). Benn et al. (1979) found about 300 μg alkaloid/larva in the lepidopterous host *Nyctemera* collected on *Senecio* plants in the field and stated that the alkaloids were present in the braconid parasitoids too. although no data were given. Despite the low level of QA detected in parasitoids in the present study, it is noteworthy that a substantial amount of cytisine was passed also to the pupal chamber wall. i.e., the cocoon. spun by the braconid parasitoid larvae (Table 2). The composition of QA found in the parasitoid samples corresponded to that present in adult bruchids with one exception (parasitoid sample II, Table 2) where *N*-methylcytisine occurred in large amounts.

Among the species found. *Tetrastichus* is reported both as a primary parasitoid (on the herbivore. *B. villosus*) and a secondary one (on the braconid. *T. thoracicus*), that is a (hyper)parasitoid (Medvedeva. 1978). or "metaparasite" sensu Steffan (1981), which may further complicate the possible routes of passage of alkaloids throughout the foodweb.

The parasitoid species we studied live in various bruchid species; e.g.. *T. thoracicus* parasitizes a number of other unrelated bruchid species (*Bruchus atomarius*, *B. lentis*. and *B. rufimanus*) (Luca. 1970. 1977). These species, in turn. inhabit phytochemically diverse host plants.

Ants. The alkaloid analyses demonstrated (Table 2) that ants gather a substantial amount of QA from honeydew. both directly from tending aphids and from leaf surfaces. We think that in the latter case. UV radiation may decrease the compounds' stability. QA and/or the breakdown products are probably taken back to the nests where their fate is not known.

QA Along the Food Web. It is believed that peak QA quantities in plant parts throughout the season serve plant defense purposes (Waller and Nowacki. 1978; Wink. 1984c, 1985: Wink and Hartmann,. 1981). Indeed. QA have important effects on the behavior and physiology of nonadapted herbivores. Their feeding-deterrent activity was demonstrated on the tortricid *Choristoneura fumiferana* (Bentley et al., 1984), on the pea aphid *Acyrthosiphon pisum* (Dreyer et al., 1985), and even on mollusks (Wink, 1984c).

The QA found in *L. anagyroides* have been sequestered by the aphids and excreted without much change by other members of the food web investigated. This suggests that QA might not play a primary role in the lives of the latter species. We do not know whether *A. cytisorum* on *L. anagyroides* enjoys any protection from specialized parasitoids or predators. However. evidence indicates that QA-containing aphids can gain protection from generalist predators. Wink and Römer (1986) and Gruppe and Römer (1988) demonstrated that two predators, *Carabus problematicus* and first-instar larvae of *Coccinella septem-*

punctata, were narcotized (temporarily immobilized) and killed, respectively, after feeding on *Macrosiphum albifrons* colonies grown (and containing 1.3 mg/g fw alkaloids) on a "bitter" *Lupinus* variety, whereas they preferentially consumed the aphids developing on a "sweet," i.e., alkaloid-free, lupine without exhibiting adverse symptoms.

In summary, all trophic levels studied tolerated the presence or absence of QA. This ability may prove to be a good strategy in a varying environment, variability meaning that both within- and among-plant species show chemical diversity.

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