Oecologia (1992) 92:257–265

Oecologia

Effects of pyrrolizidine alkaloids and sesquiterpenes on snail feeding

B. Speiser[1], J. Harmatha[2], and M. Rowell-Rahier[1]

[1] Zoologisches Institut der Universität, Rheinsprung 9, CH-4051 Basel, Switzerland
[2] Czechoslovak Academy of Sciences, Institute of Organic Chemistry and Biochemistry, 16610 Prague, Czechoslovakia

Received February 18, 1992 / Accepted in revised form May 19, 1992.

Summary. We determined in the laboratory the feeding response of two populations of the generalist herbivorous snail *Arianta arbustorum* (Helicidae) towards the composite *Adenostyles alliariae* and towards various allelochemicals. These were: a pyrrolizidine alkaloid (PA) extract of *Adenostyles* leaves; senecionine (a PA present in *Adenostyles*); retrorsine (a PA not present in *Adenostyles*) and two sesquiterpene (ST) fractions from *Adenostyles*: a mixture of the STs adenostylone and neoadenostylone, and deacyladenostylone. Tertiary PAs and PA N-oxides were tested separately. For each allelochemical, we tested whether it was deterrent or whether it induced changes of feeding behaviour (i.e. whether it had pre- or postingestive effects), and whether the effects were more pronounced with younger (smaller) snails. The tertiary PA extract from *Adenostyles* was deterrent, especially for young snails, but did not induce changes of feeding behaviour. Tertiary PA senecionine was deterrent for young snails only and induced changes of feeding behaviour. Also, consumption of untreated *Petasites* was higher after this treatment. Tertiary PA retrorsine was not deterrent, but induced changes of feeding behaviour. The PA N-oxides showed no activity against the snails. The mixture of adenostylone and neoadenostylone was deterrent and induced feeding aversions. Deacyladenostylone was highly deterrent, but did not induce changes of feeding behaviour. At the Jura site, PA content of *Adenostyles* was lower than at the Black Forest site. The snails from Jura consumed much less *Adenostyles* than the snails from Black Forest, and also ate a little less of the treated leaf discs. The PAs which are encountered by the snails in their natural food plants (PA extract and senecionine) were more deterrent than retrorsine (a novel compound). This suggests that the snails have mechanisms for the rejection of allelochemicals which they encounter in their natural food plants, but not for novel allelochemicals. The results suggest two hypotheses regarding the function of the allelochemicals in *Adenostyles*: (1) The allelochemicals act mainly on very young snails. (2) PAs render *Adenostyles* toxic, while STs act as feeding deterrents.

Correspondence to: B. Speiser

Key words: Adenostyles – Arianta – Feeding deterrency – Pyrrolizidine alkaloids – Furanoeremophilane sesquiterpenes

It is generally thought that secondary plant compounds protect plants against herbivores or pathogens. In most plants, however, we do not know which allelochemical protects the plant against which herbivore or pathogen. If a plant contains several allelochemicals (which is often the case), we do not even know whether all the compounds are needed to protect it from any one herbivore, or whether each of the different allelochemicals is active against a separate group of herbivores. The relative contributions of generalist and specialist herbivores to the total damage experienced by a given plant is seldom known. The few existing studies indicate that generalists may be important herbivores (Edwards and Gillman 1987; p. 304; Raffaelli and Mordue 1990).

Plant defenses can have pre- and/or postingestive effects on herbivores (Berenbaum 1986): (1) they may deter them from feeding, and (2) they may be toxic. These two categories of effects are not independent, because many herbivores can learn to avoid toxic plants, or have evolved feeding aversions against toxic plants. However, herbivores may also increase their food consumption to compensate for the negative effects of toxins (Simpson and Simpson 1990). In snails and slugs, learning of food aversion has been amply demonstrated (Gelperin 1975; Sahley et al. 1981a, b; Whelan 1982; Balaban et al. 1987; Delaney and Gelperin 1986; Lee and Chang 1986). Postingestive toxic effects and subsequent aversion could be especially important in mobile herbivores which are able to change food plants frequently in the field, as do snails. We examined whether *Adenostyles alliariae* (Asteraceae) has either preingestive (=deterrent) or postingestive effects on a herbivorous land snail (*Arianta arbustorum*, Helicidae). Postingestive effects were measured indirectly via induced changes of feeding behaviour during or after the experimental treatments.

Juvenile animals are often more sensitive to toxins than adults (e.g. Sharrow et al. 1988). Age-dependent effects

could arise because of greater physiological sensitivity towards the compound of younger snails (many toxins are known to affect developmental processes, e.g. Nawrot et al. 1986; Robert et al. 1987). Also, older snails have had more time to adjust their production of detoxification enzymes (Brattsten 1988), and their gut symbionts might be better adapted to the food (Charrier 1990). We therefore investigated whether younger snails were affected more strongly by the allelochemicals than older snails.

In this study, we investigate the effect of several allelochemicals found in the composite *Adenostyles alliariae* (henceforth referred to as *Adenostyles*) on one important generalist herbivore, the snail *Arianta arbustorum* (henceforth *Arianta*). By "allelochemicals", we mean secondary plant compounds or mixtures thereof, regardless of their chemical purity. We have shown earlier (Speiser and Rowell-Rahier 1991) that *Arianta* eats *Adenostyles* in the wild, but with a marked seasonal pattern: In spring, *Adenostyles* makes up only 3% of the total diet, but this percentage increases to over 60% in autumn. Since the pyrrolizidine alkaloid (PA) content of *Adenostyles* leaves decreases drastically from spring to autumn, there is a negative correlation between PA content and the amount eaten by snails. Here, we test whether this relationship is one of causality or not. Alternatively, the negative correlation between herbivory by snails and the PA content of *Adenostyles* leaves might be caused by other secondary metabolites having the same temporal variation as the PAs. As well as PAs, *Adenostyles* contains sesquiterpenes (STs).

In the guts of mammals, N-oxides are reduced to tertiary PAs, while the opposite reaction takes place in the liver. The two forms are therefore metabolically interconvertable (Mattocks 1986, p. 212). Because of their different polarities, tertiary PAs and their N-oxides may be detected or taken up in different quantities. Many ecological studies on the effects of PAs have been made with tertiary PAs. However, PAs are transported and stored in plants as N-oxides (Hartmann et al. 1989). We have tested the tertiary form and the N-oxide of PAs separately.

As a first step, we measured the snails' consumption of *Adenostyles* in the laboratory. Secondly, we prepared a PA extract from *Adenostyles* leaves and applied it to leaves of *Petasites hybridus* (henceforth *Petasites*), a closely related species. The leaves of *Petasites* contain no PAs. Thirdly, we tested separately different allelochemicals which are characteristic of *Adenostyles* or chemically closely related to them. Since snails that live in an area where *Adenostyles* occurs abundantly might be adapted to its specific secondary compounds, we have tested both a PA which is present in *Adenostyles* in small quantities (senecionine), and another similar PA which is not present in *Adenostyles* (retrorsine). Additionally we tested different STs which are chemically unrelated to the PAs but also characteristic of *Adenostyles*.

Materials and methods

Sites and snail collections

We used snails collected at two different sites: "Jura": in the beech forest on the Northern border of Hofstettermatte, 10 km south of Basel (Switzerland), at an altitude of 780 m; and "Black Forest": in the bog north of the Zastler nature reserve near Feldberg (Germany), 50 km north of Basel, at an altitude of 1080 m. At both sites, *Adenostyles* plants occur, but at the Jura site it is the dominant herbaceous plant species, while at the other site several other plant species occur in high abundance. At the site Jura, the field season starts about 1 month earlier than at the Black Forest site.

At the Jura site, 32 snails were collected on 11 and 13 May 1990 for the *Adenostyles* experiment. These snails had shell diameters of 14–25 mm. Another 87 snails (size: 10–25 mm) were collected at this site on 11 May 1991 for the allelochemicals experiments. At the Black Forest site, 26 snails (size: 17–20 mm) were collected on 15 May 1990 for the *Adenostyles* experiment. On 19 June and 17 July 1991, 145 snails were collected at Black Forest for the allelochemicals experiments. Of these, 86 snails (size: 13–23 mm) were used to test the PAs, and 59 snails (size: 10–14 mm) were used to test the STs.

Plants

Adenostyles alliariae (Asteraceae) is common in moist subalpine and alpine habitats of Central Europe. The genus belongs to the subtribe Senecioneae (Toman et al. 1968), which is well known for its pyrrolizidine alkaloids (PAs; often called "Senecio alkaloids"). *Adenostyles* contains seneciphylline as the major PA (over 75% of total PAs) along with several other minor PA components. The PAs are present in the plant as N-oxides (Rowell-Rahier et al. 1991). It also contains the sesquiterpenes (STs) adenostylone, neoadenostylone and deacyladenostylone (Harmatha et al. 1969; Samek et al. 1969).

Analysis of pyrrolizidine alkaloids

The PA content of *Adenostyles* leaves in both snail habitats was measured throughout the season with thin layer chromatography (Mattocks 1967, 1986). Only seneciphylline (the major PA component) was quantified. For each analysis, a sample of 10–30 different plants was used, as described by Speiser and Rowell-Rahier (1991). The results obtained with this analytical method are comparable to those obtained by Rowell-Rahier et al. (1991) with gas chromatography-mass spectrometry (GC-MS).

General experimental procedures

Snails were kept individually in plastic containers with moist soil to which some calcium carbonate had been added. The containers were placed in a temperature-controlled chamber at 18° C, under a light regime of 18 h light/6 h darkness. To standardize the snails' hunger in the feeding experiments, they were subjected to the following pretreatment. Two days before the experiment, each snail was given one "large disc" (diameter 4 cm) of lettuce. One day before the experiment, any remaining lettuce was removed and the snails starved until the experiment began. According to the experimental protocols, snails were given standardized amounts of food (see below) and allowed to feed for 24 h. After this period, the surface of the uneaten leaves was measured with a video camera and an image analysis system, and the area consumed was calculated. In consecutive experiments, snails were assigned to different treatment groups.

A "no choice" experimental approach was used in these experiments (see Speiser and Rowell-Rahier 1991, for a justification of this method). We measured consumption of different foods over a 24-h feeding period and repeated this for two consecutive days. The first day gives primarily a measure of feeding deterrency. Toxicity is more difficult to measure directly in snails. However, induced changes of feeding behaviour during or after the experimental treatments might be indicators of toxicity. First, we tested whether consumption of the treated discs varied between days, as a measure of induced changes of feeding behaviour during the experimental treatment. Secondly, we compared the consumption of untreated

Petasites before and after each experimental treatment as a measure of changed feeding behaviour after the experimental treatment.

Experiment on the consumption of Adenostyles

After pretreatment (see above), the snails were split into two groups: the snails in the experimental group were fed only *Adenostyles* throughout the experiment, while the snails in the control group were fed only lettuce. Each snail was presented daily with two large leaf discs. This was repeated during at most 5 consecutive days. The *Adenostyles* was picked daily at the Jura site.

Experiments on the effects of allelochemicals

The following allelochemicals were tested.

1. PA extract. We prepared two *Adenostyles* leaf extracts, one which contained the PAs in the tertiary form, and one which contained the PAs as N-oxides. The extracts were prepared as follows: 700 g of frozen *Adenostyles* leaves were crushed in 3 l of 0.5 M sulfuric acid and extracted for 24 h. After filtration, equal amounts of this PA extract were divided into two fractions. To the fraction intended for extraction of tertiary PAs, several grams of zinc dust were added for approximately 3 h. Ammonia was added until the pH was >10, and the PAs extracted with dichloromethane. When all solvents were evaporated, we obtained 130 mg of a crystalline substance. Analysis by GC-MS showed that the tertiary PA extract contained 69% seneciphylline, 7% senecionine, 5% spartioidine and platyphylline, 5% acetylseneciphylline and traces of integerrimine (data provided by T. Hartmann, Braunschweig). Thus the 50 µg of tertiary PA extract that were applied correspond to 43 µg of mixture of tertiary PAs in similar proportions to those found in *Adenostyles* leaves (see Rowell-Rahier et al. 1991).

To the fraction intended for extraction of PA N-oxides, no zinc dust was added. The extraction procedures were the same as for the tertiary PAs, but since N-oxides are less readily extracted with dichloromethane than tertiary PAs, the aqueous phase was additionally extracted with ethyl acetate, and all solvents were evaporated. From the N-oxide fraction, we obtained 53 mg of a viscous brown liquid. In the N-oxide extract, the same PAs were present in almost identical proportions as in the tertiary PA extract. They were all in the N-oxide form, except seneciphylline which was partly present in the tertiary form, and acetylseneciphylline which was only present as tertiary PA. Thus, the applied 50 µg of N-oxide extract correspond to 43 µg of mixture of PAs (mostly PA N-oxides). Because of the small quantity extracted, the N-oxide extract could only be tested with the snails from Jura. The procedure used to extract the PAs should not extract STs.

2. Senecionine. Senecionine was isolated and crystallized from *Senecio vernalis* according to Hartmann and Zimmer (1986) and provided by T. Hartmann. The N-oxide was prepared by chemical oxidation of the pure tertiary PA. Both samples contained approx. 7% seneciphylline. The quantity of tertiary PA senecionine available was only enough to test the snails from the Jura population.

3. Retrorsine. Retrorsine and its N-oxide were obtained from the Sigma Corp. The purity of these compounds was >99% (manufacturer's certificate of analysis).

4. Mixture of adenostylone and neoadenostylone (neo- /adenostylone mixture). These STs were isolated from *Adenostyles* by J. Harmatha. The method is described in Harmatha et al. (1969).

5. Deacyladenostylone. This ST was also isolated from *Adenostyles* by J. Harmatha and is described in Harmatha et al. (1969) as 6-hydroxy-9-oxo-$\Delta^{10(1)}$-furoeremophilane. It was also prepared by deacylation (alkaline hydrolysis) of adenostylone and/or neoadenostylone.

To test the allelochemicals, we used *Petasites hybridus* (Asteraceae: Senecioneae) as a neutral acceptable substrate. PAs have been found in the flowers and stems of *Petasites* (Lüthy et al. 1983), but we did not find detectable quantities in the leaves. Several STs have been found in *Petasites* (Novotny et al. 1969; Neuenschwander et al. 1979a, b). The solution of allelochemical tested was painted on the *Petasites* discs which were left to dry at room temperature a few minutes until the solvent had evaporated. The concentration of the allelochemicals on the disc was always 50 µg/cm^2. This is the average PA concentration of *Adenostyles* leaves in April. All the PAs including the PA extract were dissolved in 40% ethanol, and the STs in methanol. The control discs were treated with the solvent matching the allelochemical tested.

We ensured that the snails were never offered less food than what they could eat within the experimental period. At the same time, the size of the discs was kept as small as possible in order to save allelochemicals and to improve the accuracy of the area measurements. The snails' food rations were therefore adjusted to snail size by means of a preliminary experiment which established the relationship between food intake and shell diameter. Snails with shells <13 mm received a small leaf disc (2.5 cm diameter $=4.91$ cm^2 area), snails (13–18) mm received a large leaf disc (4 cm diameter $=12.55$ cm^2 area) and snails ≥ 18 mm received two large leaf discs ($=25.1$ cm^2 area).

The design of the allelochemicals experiments is illustrated schematically in Fig. 1. For the last experiment in a series, changed feeding behaviour after the experimental treatment could not be estimated. With the Jura snails, the allelochemicals were tested in the following order: PA extract, senecionine, STs, retrorsine. With the Black Forest snails, retrorsine was tested first, while tertiary PA extract and senecionine N-oxide were tested second. The STs were tested in a separate series.

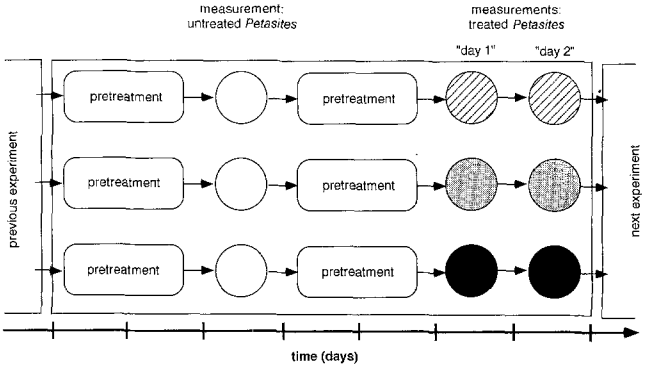

Fig. 1. Design of the allelochemicals experiments. Each snail was assigned to one of the three treatment groups shown. These three groups differed only on "*day 1*" and "*day 2*". The pretreatment (*rectangles*) consisted of a ration of lettuce on the first day, followed by a starvation day. Food consumption was measured on those days on which *Petasites* was offered (*circles*). The two allelochemicals tested were either a tertiary alkaloid and its N-oxide, or the two STs. In the next experiment, the snails were redistributed randomly among the three treatment groups. Relative consumption was calculated by dividing consumption on day 1 (or 2) by the same snail's consumption of untreated *Petasites* earlier in the experiment. Feeding deterrency of the allelochemicals was determined by comparing relative consumption of an allelochemical on day 1 with relative consumption of the solvent control (*vertical comparison*). Induced changes of feeding behaviour were determined by comparing the performance of individual snails on day 1 and on day 2 (*horizontal comparison*)

Data analysis

There were two sources of variation which could have obscured the treatment effects. (1) Individual snails consumed very different amounts of food (partly because of different body sizes). (2) On each day, all leaf discs were cut from a single, large *Petasites* leaf. Possibly because of qualitative differences between the individual *Petasites* leaves used, the average amounts consumed differed greatly between days. To correct for differences between individual snails, relative consumption was calculated by dividing the amounts eaten on day 1 (or day 2) by the amounts of the untreated control eaten. To correct for differences between *Petasites* leaves, a correction factor was determined for each day, so that the average relative consumption of solvent control equalled 1.0 on all days. For determination of changed feeding behaviour after the experimental treatments, the consumption of untreated *Petasites* was adjusted so that the average for all snails equalled 1.0 on each day.

To determine whether deterrency was significantly age-dependent, we regressed the relative consumption on day 1 against shell diameter. The slope of this regression should be zero if there is no effect of age. To obtain a more comprehensive measure of this effect, we compared the consumption of the largest snails with that of the smallest snails. This ratio was calculated as follows: average consumption of (snails ≥ 20 mm)/(snails < 15 mm).

The lettuce ration offered in the pretreatment of the following experiment served as a control for any toxic effects of the previous experiment. If a snail consumed less than 2 cm^2 of lettuce, it was excluded from data analysis. Several snails were excluded for this reason. These were, however, equally distributed over the treatment groups, suggesting that the reason why they stopped feeding was unrelated to the experimental procedures.

Results

Pyrrolizidine alkaloid content of Adenostyles *in the field*

At both sites, the PA content of *Adenostyles* leaves decreased over the season (Fig. 2). The PA content of Black Forest leaves was consistently higher than of those from Jura. The PA content of *Adenostyles* was similar at both sites when the snails tested with the allelochemicals were collected.

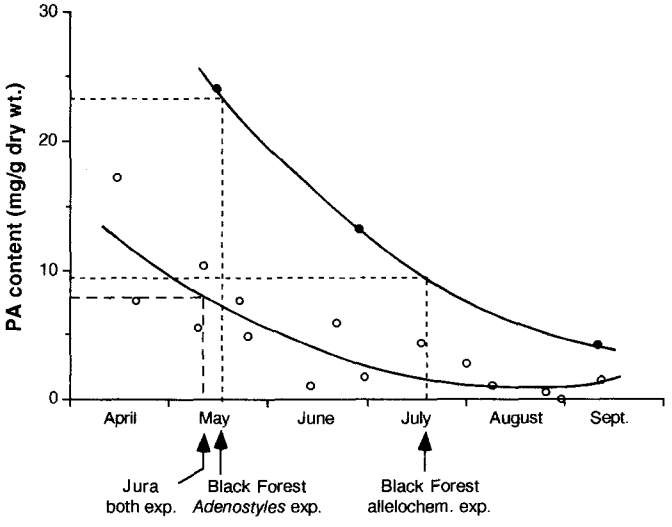

Fig. 2. PA content (seneciphylline N-oxide) in the leaves of *Adenostyles alliariae* over the season at both study sites (*open circles*: Jura; *solid circles*: Black Forest). The time of collection of snails for the experiments is indicated

Consumption of Adenostyles *and lettuce in the laboratory*

Lettuce consumption was relatively constant over time and similar for both snail populations (Fig. 3). The Jura snails ate on average 19.2 cm^2 lettuce per day, while the Black Forest snails ate 23.1 cm^2. The lettuce consumption of the two populations was different only on day 2 (Mann-Whitney U-test, $P=0.024$). In the Jura snails, lettuce consumption decreased significantly from day 1 to day 3, while the Black Forest snails' lettuce consumption did not change in the course of the experiment (Spearman rank correlation coefficient, Jura: $\rho=-0.31$, $P=0.04$; Black Forest: $\rho=-0.17$, $P>0.18$).

By contrast, *Adenostyles* consumption of the two populations was very different. The Jura snails ate 0.08 cm^2 on average, while the Black Forest snails ate 1.95 cm^2 (Mann-Whitney U-test: $P<0.0001$). In the Jura snails, *Adenostyles* consumption did not differ among days (Kruskal-Wallis test, $P>0.3$). Because the Jura snails consumed so little *Adenostyles*, the experiment was stopped after 3 days. In the Black Forest snails, *Adenostyles* consumption was significantly higher on day 1 than on all the other days (Mann-Whitney U-test, $P<0.0001$); from day 2 to day 5, there was no further change in *Adenostyles* consumption (Spearman rank correlation coefficient, $P>0.9$).

In both snail populations and on all days, lettuce consumption was much higher than *Adenostyles* consumption (Mann-Whitney U-tests, in all cases $P<0.0001$).

Deterrency of tested allelochemicals

The mean relative amounts eaten of leaves coated with different allelochemicals are shown in Fig. 4, and the statistical significance of the deterrency test in Table 1. These figures are averages for all snails tested (size 10–25 mm). The tertiary PA extract was deterrent for the Jura snails. The neo- /adenostylone mixture was deterrent for the Jura snails, and deacyladenostylone was highly deterrent for both snail populations.

Table 2 indicates which allelochemicals showed age-dependent deterrency. Because relative consumption was used for these calculations, the age effect does not merely reflect the fact that older snails eat more than younger

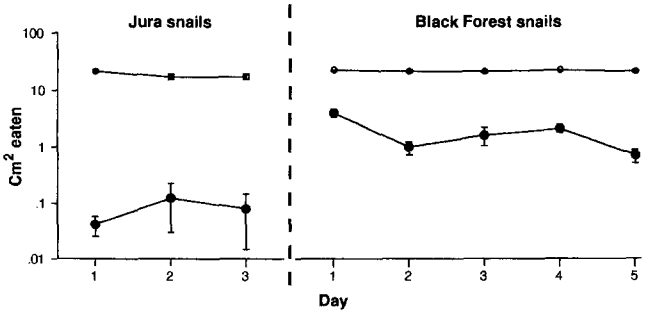

Fig. 3. Consumption of *Adenostyles alliariae* (*large dots*) and lettuce (*small dots*) in the laboratory. *Error bars* indicate 1 SE (the error bars for lettuce consumption are very small and almost invisible on the graph)

snails. With one exception, the relative consumption of the solvent controls was independent of the snails' age. Age-dependent deterrency was observed for the tertiary PA extract in both snail populations, and for tertiary PA senecionine, in the Jura snails. In these cases, the relative consumption of the largest snails was up to twice the relative consumption of the smallest snails. For retrorsine, tested on the Black Forest snails, there was a significant negative correlation between snail size and relative consumption of the solvent control. For tertiary PA retrorsine, the correlation is less negative than (but not significantly different from) the solvent control. The STs showed no age-dependent deterrency.

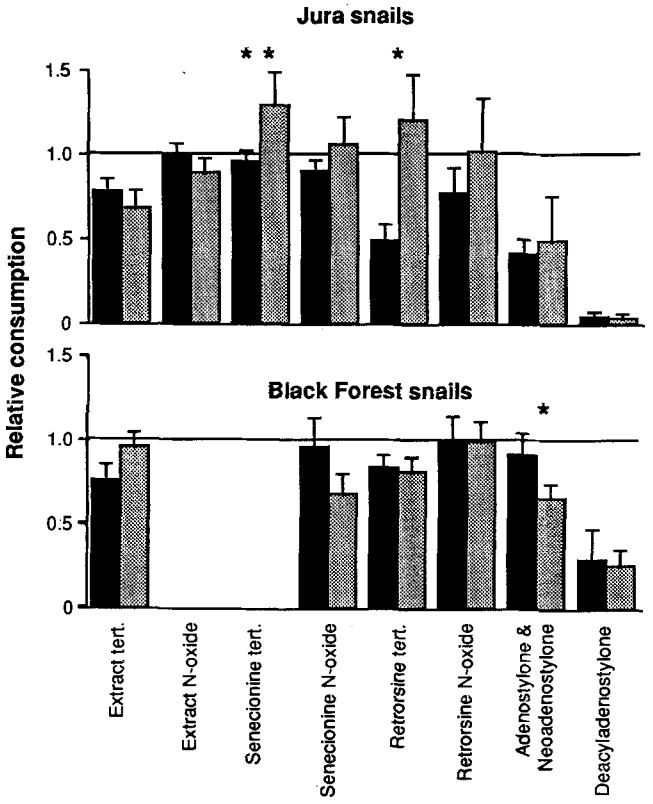

Fig. 4. Relative consumption of leaf discs of *Petasites hybridus*, treated with allelochemicals, in the laboratory (*solid bars*: day 1; *shaded bars*, day 2). Data are corrected for differences between leaves, so the average consumption of solvent control equals 1.0 (represented by the *lines*). *Error bars* indicate 1 SE. The *asterisks* indicate significant differences between day 1 and day 2 (symbols as in Table 1)

Induced changes of feeding behaviour

In tertiary PA senecionine and in tertiary PA retrorsine, tested with the Jura snails, consumption was significantly higher on day 2 than on day 1 (Fig. 4). In the neo- /adenostylone mixture, tested with the Black Forest snails, consumption was significantly lower on day 2 than on day 1.

Table 1. Deterrency of the allelochemicals tested

Allelochemical	Jura snails		Black Forest snails	
	P	n	*P*	n
Tertiary PA extract	*	26	ns	26
N-oxide extract	ns	27	–	–
Tertiary PA senecionine	ns	27	–	–
Senecionine N-oxide	ns	25	ns	25
Tertiary PA retrorsine	ns	22	ns	29
Retrorsine N-oxide	ns	21	ns	29
Neo- /adenostylone	*	16	ns	25
Deacyladenostylone	* * *	14	* *	9

The significance (two-tailed *P* values) of the test for deterrency is given, together with the number of snails tested. Deterrency was calculated by comparing the relative consumption on the first experimental day of each allelochemical with consumption of the solvent controls (relative consumption = consumption of treated food/consumption of untreated food by the same snail). Significance was determined with Mann-Whitney *U*-tests
–, not determined; ns, not significant; * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 2. Age-dependent deterrency of the allelochemicals

Allelochemical	Jura snails			Black Forest snails		
	P	r^2	Ratio	*P*	r^2	Ratio
Solvent control	ns			ns		
Tertiary PA extract	**	0.228	1.80	**	0.245	2.00
N-oxide extract	ns			–		
Solvent control	ns			ns		
Tertiary PA senecionine	**	0.226	1.56	–		
Senecionine N-oxide	ns			ns		
Solvent control	ns			** [a]	0.210	0.67
Tertiary PA retrorsine	ns			* [a]	0.106	0.68
Retrorsine N-oxide	ns			ns		
Solvent control	ns			ns		
Neo- /adenostylone	ns			ns		
Deacyladenostylone	ns			ns		

Age-dependence was determined by regressing relative consumption against snail size. Where the regression was significant, both the r^2 and the "ratio" are given. The ratio is calculated as average consumption of (snails ≥ 20 mm)/(snails <15 mm). When the ratio is >1, the allelochemical was more deterrent for younger snails. Symbols and sample sizes as in Table 1.
[a] The two slopes of the regression of tertiary PA retrorsine and the solvent control against snail size are not significantly different from each other

Table 3. Changed feeding behaviour after treatment with allelochemicals

Allelochemical	Difference (±SE)		Significance
Tertiary PA extract	+0.032	(±0.131)	ns
N-oxide extract	+0.009	(±0.123)	ns
Tertiary PA senecionine	−0.531	(±0.218)	*
Senecionine N-oxide	−0.257	(±0.191)	ns
Tertiary PA retrorsine	−0.053	(±0.090)	ns
Retrorsine N-oxide	+0.038	(±0.092)	ns
Neo- /adenostylone	+0.591	(±0.177)	ns
Deacyladenostylone	+0.433	(±0.184)	ns

Differences in consumption of untreated *Petasites* before and after the treatment with an allelochemical (±SE). Positive differences indicate that more was eaten before the treatment and vice versa. The differences were calculated with the data corrected for individual leaves. For each allelochemical, we determined whether the mean difference was significantly different from zero with one-group *t*-tests. For the solvent controls, the difference was only significant in the ST experiment. In this case, significance was determined by testing the mean of the treatments against the mean of the solvent controls, rather than against zero. Changed feeding behaviour after the experimental treatment was determined only with the Jura snails for all allelochemicals except retrorsine, for which it was only determined with the Black Forest snails, because for the last experiment in a series, this comparison could not be made. Symbols as in Table 1

In the solvent controls, no significant difference between days was observed.

To see whether there were also age-dependent induced changes of feeding behaviour, we recalculated the tests, using only the data for juvenile snails (juvenile snails have not yet formed a lip at the shell aperture): the pattern was identical.

Increased feeding after treatment with allelochemicals

The amounts of untreated *Petasites* eaten after treatment with tertiary PA senecionine were larger than those eaten before (tested on the Jura snails; see Table 3), thus possibly indicating a process similar to compensatory feeding (see Discussion).

To see whether there were also age-dependent changes of feeding behaviour, we recalculated the tests, using only the data for juvenile snails. The pattern was identical with one exception: tertiary PA retrorsine changed the feeding behaviour of the juvenile snails at $P<0.01$.

Discussion

Feeding deterrents act on herbivores when they contact chemoreceptors (preingestive effect; Berenbaum 1986), while toxins act after they have been eaten (postingestive effect). Changes in meal size over time can be interpreted as a consequence of post-ingestive effects (Champagne and Bernays 1991). Snails and slugs have been shown to alter their feeding behaviour in response to food quality (Gelperin 1975; Sahley et al. 1981a, b; Whelan 1982; Balaban et al. 1987; Delaney and Gelperin 1986; Lee and Chang 1986). The comparison of consumption of a food when it is first encountered in the experiment (on day 1) with the consumption when the snail has experience of it (on day 2), should indicate differences between pre- and postingestive effects. If the snails ate more of a food on the second day, this food may be harmless to the snails, or it may cause compensatory feeding (Simpson and Simpson 1990). Also, if more of the untreated *Petasites* was consumed after consumption of an allelochemical than before, a physiological process similar to compensatory feeding might have occurred.

Comparison of populations

With our experimental design, we tried to minimize the variability among individual snails. More comprehensive analyses of individual variability (using snails from more populations) are currently under way, so this topic will not be further addressed here.

At the Black Forest site, the PA content of *Adenostyles* leaves was always higher than at the Jura site. At the time of collection of the snails for the *Adenostyles* consumption experiment, the PA content of *Adenostyles* at Black Forest was more than twice the PA content at Jura. In this experiment, the Black Forest snails consumed far more *Adenostyles* than the Jura snails. However, they consumed only slightly more lettuce than the Jura snails. The difference in *Adenostyles* consumption is therefore not simply a difference in feeding activity or metabolic rate. These data suggest that snails occurring in a habitat with PA-rich food plants can eat more PAs in the laboratory than can other snails occurring in habitats with plants poorer in PAs, suggesting either phenotypic plasticity or genetic adaptation. The most probable mechanism of phenotypic plasticity with respect to toxic foods is induction of detoxification enzymes (Brattsten 1988).

In the experiments with allelochemicals, the Black Forest snails consumed more of the tertiary PA extract and the neo- /adenostylone mixture than the Jura snails, but the difference between the snail populations was less pronounced than in the *Adenostyles* experiment. The snails for the allelochemicals experiments were collected earlier at the Jura than at the Black Forest site, and the PA content of *Adenostyles* was similar for both sites at the respective collection dates. Thus the differences between snail populations cannot be explained exclusively by the PA content of *Adenostyles* at the time of snail collection. This suggests that snails might be in part adapted to the chemistry of the food plants of their habitat. Genetic differentiation has been demonstrated for populations of *Arianta arbustorum* that are much closer together than Jura and Black Forest (Arter 1990). To what extent the observed differences between snail populations are genetically based cannot be determined from our data. Because the difference between the two populations was more pronounced in the *Adenostyles* consumption experiment, in which the snails had been collected at a time when PA content of *Adenostyles* in the field was very different (see above), it seems that snails may adjust their digestive physiology to the seasonal changes in toxin content of their food plants (e.g. via induced mixed function oxidases).

Consumption of Adenostyles *compared with other foods*

In the laboratory, *Adenostyles* is only eaten in small quantities, compared with lettuce or *Petasites*. In the experiments with allelochemicals, the absolute amounts of treated *Petasites* eaten were larger than the amounts of untreated *Adenostyles* eaten in the *Adenostyles* consumption experiment in May. The PA concentration in the *Adenostyles* used was lower than that applied in the allelochemicals experiment, since the *Petasites* discs were treated with 15 mg/g, which is the PA concentration of *Adenostyles* in April. Several hypotheses might explain these differences. (1) *Petasites* might contain substances providing positive feeding stimuli. (2) Allelochemicals applied only to the leaf surface might not achieve the same effect as the naturally occurring allelochemicals. (3) Feeding aversions may be acquired more easily if a food is not only toxic, but also has a distinctive taste (Sahley et al. 1981b; Bernays and Bright 1991). (4) Possibly several *Adenostyles* secondary compounds interact to achieve the observed effect. (5) *Petasites* leaves might be less nutritious per unit area, thus consuming a larger area may provide the same amount of nutrients.

Effect of allelochemicals

Compared with the STs, the PAs were not very strong deterrents. In earlier experiments, we were unable to demonstrate an effect of monocrotaline (another PA) on snail feeding (unpublished data). In tertiary PA senecionine and retrorsine, compensatory feeding as a consequence of mild toxicity may have occurred (e.g. Simpson and Simpson 1990). There are no data in the literature on the effect of PAs on gastropods, and only scattered data on the effect on insects. Masters (1991) found that tertiary PAs in floral nectar are deterrent for generalist butterfly species, but not for specialists. Tertiary PAs (including senecionine) are deterrent for *Locusta migratoria* (Bernays and Chapman 1977). In contrast, PAs (mostly tertiary) are not very deterrent for spruce budworm (Bentley et al. 1984), and are attractive for the pyrgomorphid grasshopper *Zonocercus* (Bernays et al. 1977). For pea aphid, neither tertiary PAs nor PA N-oxides are very strong deterrents (Dreyer et al. 1985). *Oreina cacaliae* and *O. speciosissima* (Chrysomelidae), specialized feeders on *Adenostyles* and *Petasites*, were not deterred at all by either tertiary PA monocrotaline or monocrotaline N-oxide (Rowell-Rahier et al. 1991). The toxicity of PAs for mammals, by contrast, is well studied. PAs are highly toxic for many mammal species, especially at chronic, low doses. Feeding deterrency of PAs to mammals has not been reported (Mattocks 1986; Glendinning et al. 1990).

The STs were strongly deterrent. Induced changes of feeding behaviour during or after the experimental treatments were observed in 1 out of 6 tests with STs, compared to 3 out of 8 tests with tertiary PAs (omitting the N-oxides which are probably transformed into tertiary PAs in the body). Thus it seems that in our study the STs globally had less postingestive effect than the PAs. STs are deterrent for land snails (Baig et al. 1989). Many STs are toxic to aquatic snails (Marchant et al. 1984; Barros et al. 1985; Guerrero et al. 1988; Cruz Reyes et al. 1989; Alarcon et al. 1990; Wurzel et al. 1990; Jansen and De Groot 1991). Both feeding deterrency and toxicity of STs towards insects are well documented (e.g. Bernays and Chapman 1977; Harmatha and Nawrot 1984; Nawrot et al. 1984; Rees and Harborne 1985; Nawrot et al. 1986, 1987, 1991; Robert et al. 1987).

Of all the allelochemicals tested, deacyladenostylone was by far the most active feeding deterrent. Deacyladenostylone differs from adenostylone and neoadenostylone only by deacylation of their ester groups releasing a free activated hydroxyl group with changed configuration. There are indications that deacyladenostylone is formed in *Adenostyles*, via hydrolysis of adenostylone and/or neoadenostylone, as a response to herbivore or pathogen attack (J. Harmatha, pers. observ.). Experiments are planned to confirm this. A similar activation of functional groups is observed in the transformation products of stearoylvelutinal (another ST) in the mushroom *Lactarius vellereus*. In *L. vellereus*, deacylation of stearoylvelutinal is initiated after injury and the deacylation products velleral and isovelleral are formed within minutes. These compounds have much higher biological activities than their precursor substance and are therefore regarded as inducible defenses (Sterner et al. 1985).

Age-dependent deterrency of allelochemicals

The tertiary PA extract and tertiary PA senecionine had age-dependent effects, i.e. they were most deterrent for young snails. The snails we used in these experiments were all at least 1 year old and had a shell diameter of at least 10 mm. The maximum size of the snails was 25 mm. The age of the snails can only be estimated from shell size until maturation. Thus snails <22 mm were up to 2 years old and juvenile, while snails ≥ 22 mm were at least 3 years old and adult. It might be that the allelochemicals affect still younger snails yet more. These snails might then learn to avoid *Adenostyles*. This hypothesis would explain in parts why whole *Adenostyles* leaves are eaten much less than expected from the effects of the allelochemicals. The effect of PAs in the field is probably underestimated, because we did not use very young snails (<10 mm).

Effects of tertiary PAs compared with N-oxides

Direct and age-dependent deterrency as well as induced changes of feeding behaviour during or after the experimental treatment were observed with the tertiary forms of PAs, while PA N-oxides had no significant pre- or postingestive effects. In the literature, tertiary PAs have occasionally been reported to be more effective than N-oxides, but this is not well documented (Dreyer et al. 1985). Our evidence supports this.

Effect of novel pyrrolizidine alkaloids

Because the comparison of sites and snail populations suggests local adaptations to allelochemicals which the

snails regularly encounter in their food plants, it was interesting to see whether snails react differently to novel, but chemically related allelochemicals. From an evolutionary viewpoint, feeding deterrency is not necessarily expected for novel compounds, while both regularly encountered and novel compounds may be toxic.

Indeed, retrorsine, which is not encountered by the snails in nature, and senecionine, which is only encountered in small amounts, were not deterrent but induced changes of feeding behaviour, while the PA extract was deterrent but did not induce changes of feeding behaviour. The main component of the PA extract is seneciphylline. In our experiments, the PA extract was more deterrent than senecionine, while seneciphylline and senecionine are similarly deterrent for locusts (Bernays and Chapman 1977). This could be because the snails had developed feeding aversions against the specific mixture of PAs found in *Adenostyles*.

Conclusions

The present study indicates a causal relationship between allelochemical content of *Adenostyles* and snail feeding. The effect of both PAs and STs suggests the following hypotheses: (1) The allelochemicals act mainly on very young snails. (2) PAs render *Adenostyles* toxic, while STs act as feeding deterrents. (3) PAs might protect *Adenostyles* against vertebrate herbivores, while STs protect it against invertebrate herbivores.

Acknowledgements. We warmly thank T. Hartmann, A. Ehmke, L. Witte, and C. Theuring (Braunschweig) for providing the senecionine and for performing the GC-MS analyses of the PA extracts, D. Vonlanthen for help with the PA analyses, J. Bonato for help with the feeding experiments, H. Rowell, T. Hartmann, A. and B. Baur, N. Rank and B. Hägele for improving the manuscript with numerous comments, and the Schweizerischer Nationalfonds for financial support (grant # 31-26263.89 to MRR).

References

Alarcon MCBV, Lopes JLC, Herz W (1990) Glaucolide b, a molluscicidal sesquiterpene lactone, and other constituents of *Vernonia eremophila*. Planta Med 56: 271–273

Arter HE (1990) Spatial relationship and gene flow paths between populations of the alpine snail *Arianta arbustorum* (Pulmonata: Helicidae). Evol 44: 966–980

Baig MA, Banthorpe DV, Gutowski JA (1989) Accumulation in cultivars of *Santolina chamaecyparissus* of a rare sesquiterpene with gastropod-repellent activity. Fitoterapia 60: 373–375

Balaban PM, Vehovszky A, Maximova OA, Zakharov IS (1987) Effect of 5, 7-dihydroxytryptamine on the food-aversive conditioning in the snail *Helix lucorum* L. Brain Res 404: 201–210

Barros DAD, Lopes JLC, Vichnewski W, Lopes JNC, Kulanthaivel P, Herz W (1985) Sesquiterpene lactones in the molluscicidal extract of *Eremanthus glomerulatus*. Planta Med 0: 38–39

Bentley MD, Leonard DE, Stoddard WF, Zalkow LH (1984) Pyrrolizidine alkaloids as larval feeding deterrents for spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Ann Entomol Soc Am 77: 393–397

Berenbaum M (1986) Postingestive effects of phytochemicals on insects: on Paracelsus and plants. In: Miller JR, Miller TA (eds) Insect-Plant Interactions. Springer, Berlin Heidelberg New York, pp 121–153

Bernays EA, Bright KL (1991) Dietary mixing in grasshoppers: switching induced by nutritional imbalances in foods. Entomol Exp Appl 61: 247–253

Bernays EA, Chapman RF (1977) Deterrent chemicals as a basis of oligophagy in *Locusta migratoria* (L.). Ecol Entomol 2: 1–18

Bernays E, Edgar JA, Rothschild M (1977) Pyrrolizidine alkaloids sequestered and stored by the aposematic grasshopper *Zonocercus variegatus*. J Zool 182: 85–87

Brattsten LB (1988) Enzymic adaptations in leaf-feeding insects to host-plant allelochemicals. J Chem Ecol 14: 1919–1939

Champagne DE, Bernays EA (1991) Phytosterol unsuitability as a factor mediating food aversion learning in the grasshopper *Schistocerca americana*. Physiol Entomol 16: 391–400

Charrier M (1990) Evolution, during digestion, of the bacterial flora in the alimentary system of *Helix aspersa* (Gastropoda: Pulmonata): A scanning electron microscope study. J Moll Stud 56: 425–433

Cruz Reyes A, Chavarin C, Arias MPC, Taboada J, Jimenez EM (1989) The molluscicide activity of piquerol a isolated from *Piqueria trinervia* (Compositae) against eight species of pulmonate snails. Mem Inst Oswaldo Cruz Rio J 84: 35–40

Delaney K, Gelperin A (1986) Post-ingestive food-aversion learning to amino acid deficient diets by the terrestrial slug *Limax maximus*. J Comp Physiol A 159: 281–295

Dreyer DL, Jones KC, Molyneux RJ (1985) Feeding deterrency of some pyrrolizidine, indolizidine, and quinolizidine alkaloids towards pea aphid (*Acyrtosiphon pisum*) and evidence for phloem transport of indolizidine alkaloid swainsonine. J Chem Ecol 11: 1045–1051

Edwards PJ, Gillman MP (1987) Herbivores and plant succession. In: Gray AJ, Crawley MJ, Edwards PJ (eds) Colonization, succession and stability. Blackwell Sci Publ, Oxford, pp 295–314

Gelperin A (1975) Rapid food-aversion learning by a terrestrial mollusk. Science 189: 567–570

Glendinning JI, Brower LP, Montgomery CA (1990) Responses of three mouse species to deterrent chemicals in the monarch butterfly. I. Taste and toxicity tests using artificial diets laced with digitoxin or monocrotaline. Chemoecology 1: 114–123

Guerrero C, Campos G, Taboada J (1988) Chemical studies on *Eupatorium brevipes* and some biological activities of brevipenin. Rev Latinoam Quim 19: 147–149

Harmatha J, Nawrot J (1984) Comparison of the feeding deterrent activity of some sesquiterpene lactones and a lignan lactone towards selected insect storage pests. Biochem Syst Ecol 12: 95–98

Harmatha J, Samek Z, Novotny H, Herout V, Sorm F (1969) The structure of adenostylone, isoadenostylone and neoadenostylone – components of the rhizome of *Adenostyles alliariae* (Gouan) Kern. Coll Czech Chem Comm 34: 1739–1749

Hartmann T, Zimmer M (1986) Organ-specific distribution and accumulation of pyrrolizidine alkaloids during the life history of two annual *Senecio* species. J Plant Physiol 122: 67–80

Hartmann T, Ehmke A, Eilert U, Von Borstel K, Theuring C (1989) Sites of synthesis, translocation and accumulation of pyrrolizidine alkaloid N-Oxides in *Senecio vulgaris* L. Planta 177: 98–107

Jansen BJM, De Groot A (1991) The occurrence and biological activity of drimane sesquiterpenoids. Nat Prod Rep 8: 309–318

Lee JH, Chang JJ (1986) Learning of post-ingestive food-aversion by the land slug *Incilaria frühstorferi daiseniana* to amino acid deficient diets. Soc Neurosci Abstr 12: 39

Lüthy J, Zweifel U, Schmid P, Schlatter C (1983) Pyrrolizidin-Alkaloide in *Petasites hybridus* L. und *P. albus* L. Pharm Acta Helv 58: 98–100

Marchant YY, Balza F, Abeysekera BF, Towers GHN (1984) Molluscicidal activity of sesquiterpene lactones. Biochem Syst Ecol 12: 285–286

Masters AR (1991) Dual role of pyrrolizidine alkaloids in nectar. J Chem Ecol 17: 195–205

Mattocks AR (1967) Detection of pyrrolizidine alkaloids on thin-layer chromatograms. J Chromatogr 27: 505–508

Mattocks AR (1986) Chemistry and Toxicology of Pyrrolizidine Alkaloids. Academic Press, London

Nawrot J, Harmatha J, Novotny L (1984) Insect feeding deterrent activity of Bisabolangelone and of some sesquiterpenes of eremophilane type. Biochem Syst Ecol 12: 99–101

Nawrot J, Bloszyk E, Harmatha J, Novotny L, Drozdz B (1986) Action of antifeedants of plant origin on beetles infesting stored products. Acta Entomol Bohemoslov 83: 327–335

Nawrot J, Drozdz B, Holub M (1987) Feeding-deterrent activity of some natural sesquiterpene lactones for selected storage pests. Herba Pol 31: 209–214

Nawrot J, Koul O, Isman MB, Harmatha J (1991) Naturally occurring antifeedants effects on two polyphagous lepidopterans. J Appl Entomol 112: 194–201

Neuenschwander M, Neuenschwander A, Steinegger E (1979a) Struktur der Sesquiterpene von *Petasites hybridus* (L.) G.M. et SCH.: Petasol und Isopetasol-Abkömmlinge. Helv Chim Acta 62: 609–626

Neuenschwander M, Neuenschwander A, Steinegger E (1979b) Struktur der Sesquiterpene von *Petasites hybridus* (L.) G.M. et SCH. : Neopetasol-Abkömmlinge. Helv Chim Acta 62: 627–634

Novotny L, Samek Z, Harmatha J, Sorm F (1969) 9-Hydroxyfuroeremophilane, a further dominating component from light petroleum extract of *Petasites hybridus* (L.) Gaertn., Mey et Scherr rhizomes. Coll Czech Chem Comm 31: 336–339

Raffaelli D, Mordue AJ (1990) The relative importance of molluscs and insects as selective grazers of acyanogenic white clover (*Trifolium repens*). J Moll Stud 56: 37–46

Rees SB, Harborne JB (1985) The role of sesquiterpene lactones and phenolics in the chemical ecology of the chicory plant. Phytochemistry 24: 2225–2231

Robert PC, Blaisinger P, Bouchery Y, Simonis MT, Kienlen JC, Muckensturm B, Riss B, Pflieger D (1987) Influence de la bisabolangélone, un antiappétant sesquiterpénöide, sur le développement des chenilles de *Mythimna* (*Pseudaletia*) *unipuncta* Haw. (Lepidoptera, Noctuidae). Agronomie Paris 7: 167–174

Rowell-Rahier M, Witte L, Ehmke A, Hartmann T, Pasteels J (1991) Sequestration of plant pyrrolizidine alkaloids by chrysomelid beetles and selective transfer into the defensive secretions. Chemoecol 2: 41–48

Sahley C, Gelperin A, Rudy JW (1981a) One-trial associative learning modifies food odor preferences of a terrestrial mollusc. Proc Natl Acad Sci USA 78: 640–642

Sahley C, Rudy JW, Gelperin A (1981b) An analysis of associative learning in a terrestrial mollusc. J Comp Physiol A 144: 1–8

Samek Z, Harmatha J, Novotny L, Sorm F (1969) Absolute configuration of Adenostylone, Neoadenostylone and Isoadenostylone from *Adenostyles alliariae* and of Decompostin from *Cacalia decomposita*. Coll Czech Chem Comm 34: 2792–2808

Sharrow SH, Ueckert DN, Johnson AE (1988) Ecology and toxicology of *Senecio* species with special reference to *Senecio jacobaea* and *Senecio longilobus*. In: James LF, Ralphs MH, Nielsen DB (eds) The Ecology and Economic Impact of Poisonous Plants on Livestock Production. Westview Press, Boulder London, pp 181–196

Simpson SJ, Simpson CL (1990) The mechanisms of nutritional compensation by phytophagous insects. In: Bernays EA (ed) Insect-Plant Interactions, vol II. CRC Press, Boca Raton Ann Arbor Boston, pp 111–160

Speiser B, Rowell-Rahier M (1991) Effects of food availability, nutritional value, and alkaloids on food choice in the generalist herbivore *Arianta arbustorum* (Gastropoda: Helicidae). Oikos 62: 306–318

Sterner O, Bergman R, Kihlberg J, Wickberg B (1985) The sesquiterpenes of *Lactarius vellereus* and their role in a proposed chemical defense system. J Nat Prod 48: 279–288

Toman J, Harmatha J, Novotny L (1968) Verwandtschaftsbeziehungen der Gattung *Adenostyles* und die Berechtigung ihrer Einreihung in die Tribus Senecioneae. Preslia (Praha) 40: 122–132

Whelan RJ (1982) Response of slugs to unacceptable food items. J Appl Ecol 19: 79–87

Wurzel G, Becker H, Eicher T, Tiefensee K (1990) Molluscicidal properties of constituents from the liverwort *Ricciocarpos natans* and of synthetic lunularic acid derivatives. Planta Med 56: 444–445