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Sesquiterpenes from the *Senecioneae* and their effect on food choice of the specialised leaf beetles *Oreina cacaliae*, *Oreina speciosissima* and the generalist snail *Arianta arbustorum*

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Introduction

The occurrence of sesquiterpenes of the furanoeremophilane type (hereafter STs) in plants of the tribe Senecioneae (Asteraceae) has been known since the sixties (see Seaman, 1982 and references therein). They have been investigated in different plants of this tribe and there found to be reliable systematic traits (Toman *et al.*, 1968). Other major groups of secondary chemicals such as pyrrolizidine alkaloids and benzofurans also occur frequently in the Senecioneae.

STs such as bakkenolide A are effective feeding deterrents to stored product pests (Nawrot & Harmatha, 1994). However, when tested for toxicity and influence on insect larval development after external application to the abdomen, bakkenolide A had no effect (Nawrot & Harmatha, 1994). Attempts to link the observed deterrence of substances to their characteristic molecular structural properties have led to equivocal results. Thus Nawrot & Harmatha (1994) conclude that some substances are highly active only for certain animals, and that their structural properties cannot easily be related to each other.

To date only one study deals with STs and a herbivore which naturally feeds on Senecioneae plants. Speiser *et al.* (1992) tested the effect of a mixture of adenostylone and neoadenostylone (1:1) as well as deacyl-adenostylone on food consumption of the omnivorous snail *Arianta arbustorum*. Here we present data on the effect of STs and one benzofuran, both isolated from *Senecioneae*, on the food choice of herbivores which in nature frequently feed on these plants.

Material and methods

The STs isolated from *P. albus* were albopetasine and bakkenolide A, from *P. hybridus*, furanopetasine and isopetasine, and from *P. kablikianus*, kablicine and isopetasine (Novotny *et al.*, 1972). The STs derived from *A. alliariae* were among others a mixture of adenostylone and neoadenostylone (1:1) and deacyl-adenostylone (Harmatha *et al.*, 1969). Cacalol was isolated from *A. alpina* (Harmatha *et al.*, unpubl.). Bakkenolide A and the benzofurane euparin was isolated from *Homogyne alpina* (Harmatha *et al.*, 1976). All plant names correspond to the nomenclature of the Flora Europaea (Tutin *et al.*, 1976).

All animals were collected in the wild near Zastler (Black Forest, Germany, 1000 m above sea level). Our nomenclature for the beetles follows Bourdonné & Doguet (1991). Choice experiments were conducted with *Oreina cacaliae*, *O. speciosissima* (Coleoptera: Chrysomelidae: Chrysomelinae) and *Arianta arbustorum* (L.) (Gastropoda: Helicidae). *O. cacaliae* is specialised on *A. alliariae* and *Senecio fuchsii*. *O. speciosissima*, which is less specialised, feeds on *A. alliariae*, *S. fuchsii*, *P. albus* and *Doronicum austriacum*, but at the collecting site is mostly found on *P. albus*. *A. arbustorum* is an omnivorous snail that includes green plants, decaying plant material and fungi in its diet. It is the commonest helicid snail in central Europe and present in all habitats where the leaf beetles occur.

Beetles were kept individually in petri dishes lined with moist filter paper in an incubator at 17 ° C, and L14:D10 regime. In the experiment two leaf discs were presented to each beetle. The control disc was marked on the lower side with a water soluble ink and treated with methanol on the upper side. The ST dissolved in methanol was applied to the upper side of the treatment disc. The amount of applied substance corresponded to 1% of the disc's dry weight. STs from *Adenostyles* were tested on *Petasites* leaves and vice versa to avoid supplementing the experimental dose with native compound.

The snails were kept individually on $CaCO_3$-enriched soil in plastic containers of 9 cm diameter in an incubator at 17 °C, L14:D10 regime. They were fed regularly with fresh lettuce (*Lactuca sativa*). The choice experiments were conducted as described above using lettuce discs as the test substrate. In all cases the size of the discs presented and the duration of the test were such that the animals would generally eat half of the preferred food.

The experimental treatment of the beetles was as follows: Day 1: feeding of one untreated pre-experimental disc. Day 2: removal of the pre-experimental disc and insertion of the two experimental discs. Day 3: removal of the experimental discs and insertion of an untreated post-experimental disc. Finally on day 4 removal of the post-experimental disc. The experiment for the snails consisted of: (a) removing any food on day 1, (b) offering the treatment and control discs on day 2, (c) recovering the experimental discs and feeding with an untreated post experimental disc on day 3 and (d) visual inspection of the post-experimental disc on day 4. The surface area of the leaf discs recovered was measured digitally with the 'NIH Image' public domain software. The areas eaten from the control and treatment discs were then compared in a paired *t*-test.

To detect possible toxic effects of the substances presented, the amounts eaten of the pre- and post-experimental discs were compared by *t*-tests in *O. cacaliae* and *O. speciosissima*. In *A. arbustorum* individual snails which had eaten less than 50% of the post-experimental disc were excluded from the following experimental set until they showed normal feeding again.

Results and discussion

Figure 1 shows the results of the choice experiments. *O. cacaliae*, which is the most specialised species, ate less of discs treated with albopetasine and bakkenolide A, two STs which are derived from the less preferred host *P. albus*. None of the other STs (i.e. those derived from the preferred host *A. alliariae*, or the non-hosts *A. alpina* and *P. hybridus*) nor the benzofurane euparin had any effect on the feeding of *O. cacaliae*. Except in the group tested with cacalol, which reduced its consumption after the experiment ($t = 3.91$, $P < 0.001$), there was no difference in consumption before and after the experiment.

These results correspond well with the observed food-plant specialisation of *O. cacaliae*, which is rarely found on *P. albus* except early in the season when *P. albus* is more abundant than *A. alliariae*. In contrast, STs from *P. hybridus* had no effect on food consumption. This may be explained by the distribution of *O. cacaliae* which probably never encounters this plant in nature because *P. hybridus* is found at lower altitudes than the beetles. The post-experimental effect of cacalol might indicate long term effects; whether these are toxic or aversive is not revealed in a short-term choice experiment. In the field *O. cacaliae* is hardly ever found on *A. alpina*, which has cacalol in its leaves.

Food consumption of the more oligophagous leaf beetle *O. speciosissima* was not affected by the STs or the benzofurane euparin. Deacyl-adenostylone, derived from *A. alliariae*, may affect consumption ($t = 2.2$, $P = 0.053$). Interestingly, beetles which were collected near Zamecek (Northern Bohemia, Czech Rep.) and which live there exclusively on *S. fuchsii* (see Pysek, 1992) ate more of cacalol-treated leaf discs ($N = 9$, $t = 3.2$, $P = 0.013$). Consumption before and after the experiments did not change.

These results also correspond well with our field observations. *O. speciosissima* often occurs sympatrically with *O. cacaliae*, but despite being less specialised than *O. cacaliae* it is very often found only on *P. albus*. The absence of a strong effect of deacyl-adenostylone could be an experimental artefact, since the ST was tested on *P. albus*; which could provide feeding stimuli to the beetles, thus overriding the effects of deacyl-adenostylone. The preference for cacalol, of the beetles from Zamecek illustrates population differences within one species and demonstrates that otherwise neutral substances may be recruited for host recognition or feeding stimulation.

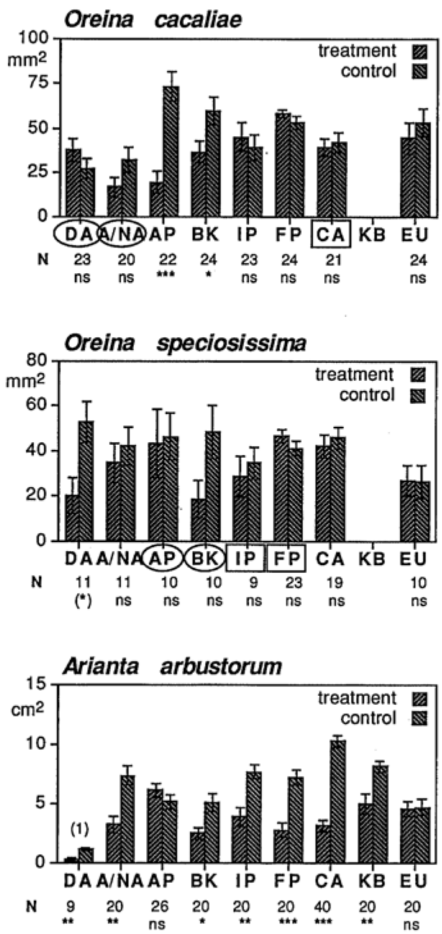

Figure 1. Area eaten from treated and untreated leaf discs. Means and standard errors are given. N is the number of individuals tested, *, **, * * * indicate significant differences with $P<0.05$, 0.01, 0.001 respectively, ns = not significant. DA: deacyl-adenostylone, A/NA: adenostylone + neoadenostylone 1:1, AP: albopetasine, BK: bakkenolide A, IP: isopetasine, FP: furanopetasine, CA: cacalol, KB: kablicine, EU: euparin. Circled compounds occur in the preferred host plant, compounds highlighted with squares are found in non-hosts of the same genus. (1) Data from Speiser *et al.* (1992).

All STs except albopetasine reduced food consumption by the generalist snail *A. arbustorum*. The benzofurane euparin had no effect. Deacyl-adenostylone was tested by Speiser *et al.* (1992) in a no-choice experiment, and found to reduce food consumption significantly.

In contrast to pyrrolizidine alkaloids, which were not very active in deterring snails (Speiser *et al.*, 1992), these results support the idea that STs could protect plant tissue from herbivory by snails. The finding that albopetasine is not deterrent accords with data showing that *P. albus* is an acceptable food for *A. arbustorum* throughout the season, whereas *A. alliariae* is only consumed in summer, and *A. alpina* could never be detected in the snails' faeces (Hägele, 1992).

A further complication of the interpretation of these results is, however, the fact that most of the compounds tested occur only in the rhizomes of the Senecioneae and are either not present in their leaves at all or only in very low quantities. So far only cacalol has been found in considerable amounts in the leaves of *A. alpina* (J. Harmatha, unpubl.). Petasine, another ST from *P. hybridus*, is also present in the leaves (E. Wildi, pers. comm.). Adenostylone and neoadenostylone have been found in low quantities in leaves of *A. alpina* and in trace amounts in leaves of *A. alliariae* (J. Harmatha, unpubl.). For *P. albus* we do not yet have exact data, but in all cases there was considerably more of these substances in the rhizomes or flower buds than in the leaves of the plants (J. Harmatha, unpubl.; E. Wildi, pers. comm.). This indicates that the valuable storage and reproductive tissues are well protected against soil dwelling herbivores, amongst which are certainly several snail and slug species. The role of STs in above ground herbivory remains to be elucidated. Especially the possibility of induction by feeding, rapid mobilisation from the rhizomes and long-term deterrency effects should be considered.

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