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Choice, performance and heritability of performance of specialist and generalist insect herbivores towards cacalol and seneciphylline, two allelochemicals of *Adenostyles alpina* (Asteraceae)

B. F. HÄGELE* & M. ROWELL-RAHIER†

**Zoologisches Institut der Universität Basel, Rheinsprung 9, CH-4051 Basel, Switzerland*
†Institut de Zoologie, Université de Neuchâtel, Rue Emile-Argand 11, CH-2007 Neuchâtel, Switzerland

Abstract

We compared the effects of a sesquiterpene (ST, cacalol) and a pyrrolizidine alkaloid (PA, seneciphylline), both occurring in *Adenostyles alliariae*, on food choice and performance of specialist and generalist insect herbivores which are all known to feed or live on *A. alliariae*. In choice experiments we investigated whether the compounds were preferred, deterrent or had no effect. All specialist species *Aglaostigma discolor* (Hymenoptera, Tenthredinidae), *Oreina cacaliae* (Coleoptera, Chrysomelidae) and *O. speciosissima* avoided feeding when confronted with the combination of compounds. Only larvae of *A. discolor* avoided the single ST treatment as well. Larvae of the generalist species *Callimorpha dominula* (Lepidoptera, Arctiidae), *Cylindrotoma distinctissima* (Diptera, Tipulidae) and *Miramella alpina* (Caelifera, Acrididae) generally avoided feeding from PA, ST and PAST treatments. The only exception were caterpillars of *C. dominula* which were indiscriminate towards PA when naive, and preferred to feed on the PA treatment when they had experienced the compound before.

Performance, measured as the growth of larvae on the different treatments in a no choice situation over a period of 10–17 days, was not different between treatments in the specialist leaf beetles *O. cacaliae* and *O. speciosissima*. Their avoidance of the combination treatment in the choice experiments had no obvious effect on growth when forced to feed from the treatment. In the generalist *C. dominula* only the high concentration combination treatment (PAST) reduced growth of the larvae due to decreased consumption. In *C. distinctissima* we found reduced growth in all treatments except one (PA3%). Poor growth performance in *C. distinctissima* was due to postingestive physiological effects of all treatments and additionally to consumption reduction in high-dose ST treatments.

Genetic variability (broad sense heritability) of growth performance metabolism varied in accordance with the specialization degree of the species. *O. cacaliae*, the most specialized species, had no significant heritability; *O. speciosissima*, the less specialized specialist, had a heritability of 0.46; *C. dominula*, the PA adapted generalist species, had a heritability of 0.64; *C. distinctissima*, the generalist with no apparent adaptations, had a heritability of 0.84.

Keywords: plant–insect interaction.

Correspondence: Martine Rahier, Institut de Zoologie, Université de Neuchâtel, Rue Emile-Argand 11, CH-2007 Neuchâtel, Switzerland.
Tel: +41 32 718 3137; fax: +41 32 718 3001;
e-mail: martine.rahier@zool.unine.ch

Introduction

Since Stahl's (1888) work on the protective properties of plant secondary compounds and the revival of this kind of research by Fraenkel (1959), secondary compounds are generally regarded as important defences against herbivores and disease agents of plants (Rausher, 1992). Insects are commonly regarded as important selective agents in the evolution of chemical defences (Ehrlich & Raven, 1964; Feeny, 1976; Rhoades & Cates, 1976; Berenbaum, 1983), although this has been disputed (Heikertinger, 1914; Jermy, 1976, 1984; Crawley, 1983) and the evidence for coevolved insect–plant systems is sparse (Strong *et al.*, 1984), but seems to be more common in plant–pathogen systems (Thompson, 1994).

The obvious differences in quantity and quality of plant secondary compounds between long-lived plants and annuals have led Feeny (1976) and Rhoades & Cates (1976) to the distinction between quantitative and qualitative defences. They proposed that long-lived plants which are more liable to attack by specialist and generalist herbivores should rely on quantitative defences, whereas plants which are likely to escape herbivory by specialist herbivores should rely on qualitative defences which would be active against the omnipresent generalists. These plant life histories, however, are two extremes of a continuum and the question arises as to which side the herbaceous perennials should be placed (Klinkhammer *et al.*, 1997). Also, differing dose–response characteristics of a given compound between various (generalist) herbivorous species may make it hard to differentiate whether a compound might act as a qualitative or quantitative defence.

Van der Meijden (1996) employed the generalist/specialist dichotomy to explain the peculiar pattern of alkaloid distribution in the biennial *Cynoglossum officinale* (Boraginaceae), where most plants have low to intermediate levels of alkaloids (van Dam *et al.*, 1995). He argued that the results would support the idea of simultaneous selection of generalist and specialist herbivores. High levels of chemical defence would successfully deter generalists, but would be counteracted by the attraction of specialists to the same compound, favouring selection for lower levels of chemical defence.

In this context, specialist and generalist herbivores are defined by their reaction to plant compounds. However, the diet breadth of herbivorous species is a continuum with both extremes (strict monophagy and wide polyphagy) being relatively rare and therefore with unclear consequences for the type of selection one might expect on levels of secondary compounds. For example, many species of the Arctiidae (Lepidoptera) which are polyphagous may utilize plant-derived alkaloids for their defence or as components of their pheromones, a highly evolved use which requires some sort of specialization, at least at some stage during the development. Other species might be facultative specialists where individuals spend their entire development on a given host, but various unrelated host plants are recorded for the species. Specialists, on the other hand, might not utilize a specific component as a host finding cue, so there might be no selection against high concentrations of this compound by them.

The idea that the joint actions of several compounds might be an important aspect in plant defence against herbivores is generally acknowleged (Bernays & Chapman, 1994; Schoonhoven *et al.*, 1998) but has not been scrutinized extensively (Adams & Bernays, 1978; Berenbaum & Neal, 1985; Berenbaum, 1986; Stamp & Osier, 1998). However, it is probably a common phenomenon that feeding deterrent activity exists in several fractions of whole plant extracts (Adams & Bernays, 1978; Woodhead & Bernays, 1978; Jermy *et al.*, 1981; B. Hägele unpublished results). In principle, joint effects of compounds might be additive (Woodhead & Bernays, 1978), synergistic or complementary in the way that they act on different (classes) of herbivores.

In this study we present data on the effects of cacalol (sesquiterpene) and seneciphylline (pyrrolizidine alkaloid) on specialist and generalist insect herbivores which naturally feed on plants containing at least one of those compounds.

The plants belong to the tribe Senecioneae (Asteraceae) in which sesquiterpenes (STs) of the furoeremophilane type and the pyrrolizidine alkaloids (PAs) are characteristic compounds (Toman *et al.*, 1968; Seaman, 1982; Mattocks, 1986). Both STs and PAs are present in *Adenostyles alliariae* and *A. alpina*, but leaf tissue STs, apart from trace amounts, are only found in *A. alpina* (J. Harmatha, unpublished results; Pasteels *et al.*, 1995; Hägele & Rowell-Rahier, 1999). The ST cacalol has phytotoxic activity (Aguilar-Martinez *et al.*, 1996) and is also a feeding deterrent to the snail *Arianta arbustorum* (Hägele *et al.*, 1996, 1998). Seneciphylline is the main PA of *A. alpina* and *A. alliariae*, where it represents up to 95% of the total amount of detected PAs (Pasteels *et al.*, 1995; Hägele, 1996; Hägele & Rowell-Rahier, 1999). It has been found to be a feeding deterrent to aphids (Dreyer *et al.*, 1985) and is toxic to liver and lung function in mammals (Mattocks, 1986).

From the insect herbivores which we observed feeding on *A. alliariae* (Table 1) we chose three specialists and three generalists to study their choice and performance when they were confronted with the two compounds. Two of the three specialists *Oreina cacaliae* and *O. speciosissima* are sister species (Dobler *et al.*, 1996) whereas all the other species are from different insect orders. Since most of the investigated species are phylogenetically distant, we cannot separate constraints imposed by phylogeny from effects attributable solely to host specialization or generalization. However, by investigating specialist and generalist insect species which are all part of a particular host plant–insect association, and studying their choice and performance

Table 1 Known specialist and generalist herbivores of *Adenostyles alliariae*.

Specialists	Generalists
Coleoptera, Chrysomelidae	Caelifera, Acrididae
Oreina cacaliae	*Miramella alpina*
Oreina intricata	Lepidoptera, Arctiidae
Oreina elongata	*Callimorpha dominula*
Longitarsus saturellus	Diptera, Tipulidae
Curculionidae	*Cylindrotoma distinctissima*
Otiorhynchus morio	Gastropoda, Helicidae
Hymenoptera, Tenthredinidae	*Arianta arbustorum*
Aglaostigma discolor	Arionidae
Lepidoptera	*Arion ater*
2 unidentified species	

when exposed to compounds found in their host plant, we are able to draw conclusions about physiological effects and their ecological and possible evolutionary consequences for this particular plant–insect association.

We specifically asked whether (1) larvae of both specialists and generalists would show different avoidance patterns towards the two allelochemicals, and whether (2) the allelochemicals, when fed to specialists and generalists, would have different performance consequences.

The outcome of our experiments will clarify, based on the choice and performance patterns between generalists and specialists, whether our system meets the prerequisites of van der Meijden's (1996) hypothesis about differential selection of generalists and specialists on any target plant. Furthermore, the assessment of the degree of genetic variability (heritability) for growth performance of specialists and generalists allows us to judge the evolutionary potential of the observed allelochemic–herbivore interactions in the examined populations.

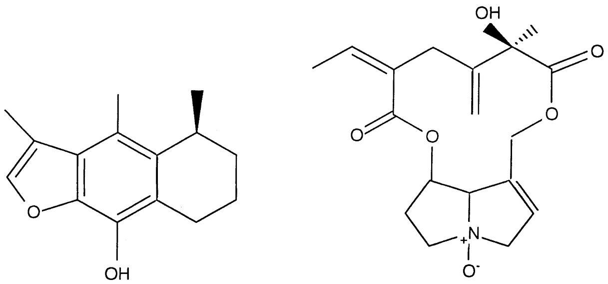
Materials and methods

Experimental treatments and compounds

We conducted choice experiments by offering two leaf discs to each larva. Because leaves of *Petasites fragrans* contain neither seneciphylline nor cacalol and were acceptable to all larvae we used them as experimental food. Leaf discs were cut out with a cork borer from a fresh leaf, and were treated with either a methanolic solution of the test substance (treatment disc) or with the solvent alone (control disc). The size of the leaf allowed for all discs of an experimental day to be cut out of the same leaf, which reduced variability between discs of the various treatments. The amount of the seneciphylline (PA) topically applied to the treatment discs was 3% of the disc's dry weight, cacalol (ST) was 1.5% and the combination treatment discs (PAST) received both substances (PA 3% + ST 1.5%). In leaves of *A. alliariae* seneciphylline represents about 3% of the dry weight, although individual plants show large differences in their PA content (Hägele, 1996; Hägele & Rowell-Rahier, 1999). In leaves of *A. alpina* mean seneciphylline concentration is about 0.3% of the leaf's dry weight and cacalol concentration about 0.02% of leaf dry weight. Again individual variation is high and values up to 0.2% cacalol g^{-1} dry weight could be detected in leaves of *A. alpina* (Hägele, 1996; Hägele & Rowell-Rahier, 1999). After evaporation of the solvent, one treatment and one control disc were placed into each experimental container. All larvae were kept individually in small Petri dishes (5.5 cm diameter) which had plaster bottoms lined with filter paper. Petri dishes were placed in an incubator set at 17 ± 2 °C, 16 h light. Choice experiments with *Miramella alpina* were conducted in larger containers (9 cm diameter, 6 cm height) and leaf discs were fixed with insect pins to the bottom of the container. After 16–20 h all disc rests were removed from the containers, glued on a white paper sheet and dried between the pages of a book. After drying we recorded the sheets with a video camera, stored the digitized images on a computer and calculated the area eaten from the measurement of the remaining surface area of the leaf disc.

In the growth experiments we placed every larva together with a single leaf disc of *P. fragrans* into an experimental container. Experimental groups were (1) a control group, which received only methanol-treated discs; (2) a PA 1% group, which received discs treated with PA corresponding to 1% of the discs' dry weight; (3) a PA 3% group; (4) an ST 0.5% group; (5) an ST 1.5% group; (6) a PAST 1 + 0.5% group; and (7) a PAST 3 + 1.5% group. We weighed each larva before we put it into its experimental container. The larvae were allowed to feed *ad libitum* from the leaf discs, which were exchanged twice a week. As a control for changes of the leaf material during the experiment, we kept the same number of leaf discs which were used in one treatment under the same conditions as in the experiment. They were also dried, stored and weighed to give the mean weight of leaf discs of every feeding. All remnants of the experimental discs were dried and stored. At the end of the experiment, after 10–17 days, we weighed all the larvae, and we calculated the amount of food consumed during the experiment by subtracting the weight of the pooled disc remnants from the mean weight of the leaf discs offered.

The ST cacalol (Fig. 1) was isolated from *Adenostyles alpina* leaves and rhizomes (J. Harmatha, unpublished results). The PA seneciphylline used in the experiments was bought from Carl Roth, Karlsruhe. Since in the plants seneciphylline occurs mainly in its N-oxide form (Fig. 1, Rowell-Rahier *et al.*, 1991), we N-oxidized it prior to use (Craig & Purushothaman, 1970).



Cacalol Seneciphylline N-oxide

Fig. 1 Structural formulae of cacalol and seneciphylline N-oxide. Both substances are present in leaves of *Adenostyles alpina*.

P. fragrans has large leaves so we could cut out of one single leaf all treatment and control discs needed for an experimental feeding. We collected the leaves as they were needed at the botanical garden in Brüglingen (Basel, Switzerland). All plant names given are from the *Flora Europaea* (Tutin *et al.*, 1976).

Specialist insect species

Aglaostigma discolor (Kluge) is a sawfly (Hymenoptera, Tenthredinidae) of the montane areas of middle Europe. We collected young larvae on *Adenostyles alliariae* and *Senecio fuchsii* in an open mixed forest at the northern slope of the Blauen (Swiss Jura mountains). Larvae have previously been reported to feed on *Tussilago farfara* (Hinz, 1984). Our feeding experiments confirmed this and revealed that larvae would also accept *Petasites albus, P. hybridus, A. alliariae, A. alpina* and *S. fuchsii* as food plants. All of these plants belong to the tribe Senecioneae (Asteraceae). Other plants from the habitat of the larvae were not accepted as food plants (B. Hägele, unpublished results). We used the larvae only in choice experiments because we could not obtain larvae of known relatedness through breeding.

Oreina cacaliae and *O. speciosissima* (Coleoptera, Chrysomelidae) are sister species (Dobler *et al.*, 1996). We collected gravid females near Zastler (Germany, Black Forest) early in the season. *O. cacaliae* is mostly found on *A. alliariae* and *S. fuchsii*, which contain PAs in their leaves, but also accepts *P. albus* which has no PAs in its leaves (Rowell-Rahier *et al.*, 1991). *O. speciosissima*, which often occurs sympatrically with *O. cacaliae*, is most often found on *P. albus* but also accepts *A. alliariae* and *S. fuchsii* as food plants. In the laboratory we kept females individually and collected larvae from mothers of both species as they were laid, thus keeping larval families separated from each other. We conducted choice experiments with *O. cacaliae* using three larvae from each of seven families (21 larvae in total) per experimental group. From *O. speciosissima* we chose four larvae from each of five families (20 larvae in total) for each experimental group. For growth experiments with *O. cacaliae* we chose three larvae from each of seven families and four larvae from each of two additional families (29 larvae in total) for every experimental group. From *O. speciosissima* we chose four larvae from each of five families (20 larvae in total) for every experimental group.

Generalist insects

We collected caterpillars of *Callimorpha dominula* L. (Lepidoptera, Arctiidae) near Hohwald (France, Vosgues) mostly on *A. alliariae* early in the season. We then reared the caterpillars to obtain moths, which we sexed, paired and allowed to lay eggs. We kept full sib families of newly hatched caterpillars in breeding boxes on the experimental food plant until they were used for the experiments. We chose four larvae from each of five families (20 larvae in total) for every experimental treatment in choice and growth experiments.

In a similar way we collected larvae of *Cylindrotoma distinctissima* Meigen (Diptera, Tipulidae) near Zastler (Germany, Black Forest) on *A. alliariae* and *P. albus*. We also reared all larvae to adulthood, paired adults individually and obtained full sib families of newly hatched larvae which we kept on the experimental food plant. We chose three larvae from each of seven families (21 larvae in total) for every experimental treatment in choice and growth experiments.

We collected penultimate instar nymphs of *Miramella alpina* Kollar (Caelifera, Acrididae) at the Chasseral range (Swiss Jura mountains) where they occur in the humid part of a pasture at 1350 m elevation. We used this species only in choice experiments since we could not breed it to obtain nymphs of known relatedness.

Data analysis

We compared the areas eaten of treatment and control discs from the choice experiments by paired *t*-tests. In the growth experiments, the final weight of larvae in the different experimental treatments was compared by two-way analysis of covariance (ANCOVA), taking the initial weight of the larvae as covariate and treatment and family as factors. In this way, the influence of weight differences at the beginning of the experiment is factored out from their effect on final weight, which is a more appropriate approach than calculating an ANOVA on ratio variables like growth rate (Raubenheimer & Simpson, 1992; Raubenheimer, 1995). Since the results obtained from this analysis include the (predigestive) effect of consumption as well as postingestive metabolic effects on final weight, we tried to further separate physiological effects of the treatments from consumption effects. This was done by calculating multiple regressions with initial weight and consumption as independent variables and final weight as the dependent variable. We then performed two-way ANOVAS taking the residual values for final weight, as obtained from the multiple regression equations, as the dependent variable and treatment and family as factors. However, factoring out the effects of consumption on the final weight of larvae is not equivalent with a strict partition between pre- and postingestive effects of diets on growth, since consumption itself may be influenced by postingestive feedbacks (Simpson & Simpson, 1990; Bowdan, 1995; Bernays & Weiss, 1996). Our method is therefore only suited to differentiate between postingestive (physiological) effects which influence growth, and effects (including postingestive ones) which influence growth via the reduction of food consumption.

We calculated the heritability of growth performance using the residuals from the multiple regression equations (Garland, 1988). Before calculations of ANOVAS, we inspected frequency distributions of the residuals for their accordance with normality.

By full sib comparison (Falconer, 1984; Garland, 1988) we calculated broad sense heritabilities as $h^2 = 2V_{\mathrm{Fam}}/(\mathrm{V}_{\mathrm{fam}} + \mathrm{V}_{\mathrm{err}})$ from variance components 'family' and 'error' of the two-way ANOVAS. Since we could not separate maternal and dominance effects from the overall genetic variance subsumed under the family variance V_{Fam}, this is an estimate of the upper limit of heritability rather than a precise measure of it (Falconer, 1984; Garland, 1988). On the other hand, if we assumed wrongly that no half sibs are present among families of *Oreina*, our estimation of heritability for those species would be too low. We obtained the variance components by the VARCOMP procedure with the restricted maximum likelihood method (REML) calculated with the SAS statistical package. The variance component of the treatment effect (fixed) was calculated using the formula given by Dagnelie (1975), and its adjacent multiplicator by equating observed and expected mean squares (Bulmer, 1980, p. 21). The approximate standard errors of heritabilities were calculated according to Falconer (1984, p. 242). Significant differences of heritabilities from zero were determined by *t*-tests.

For all mean square calculations we used type III sum of squares which tolerate unbalanced cell sizes. With Dunnett's test set to $P < 0.05$ we tested for differences between treatment groups and the control group in the case of significant treatment effects. We always regarded treatment as a fixed and family as a random factor.

Analyses were computed using StatView™ and SuperAnova™ by Abacus Concepts (1992, 1989) on a Macintosh computer and using SAS® (SAS Institute Inc., 1990) on a VAX 7620 workstation.

Results

Choice of specialists and generalists

The results of choice experiments are shown in Fig. 2. Larvae of *A. discolor* avoided feeding on ST- and PAST-treated leaf discs whereas they were indifferent towards PA-treated discs. Both *O. cacaliae* and *O. speciosissima* larvae avoided feeding on the PAST-treated leaf discs, whereas they were indifferent towards the PA- and ST-treated discs.

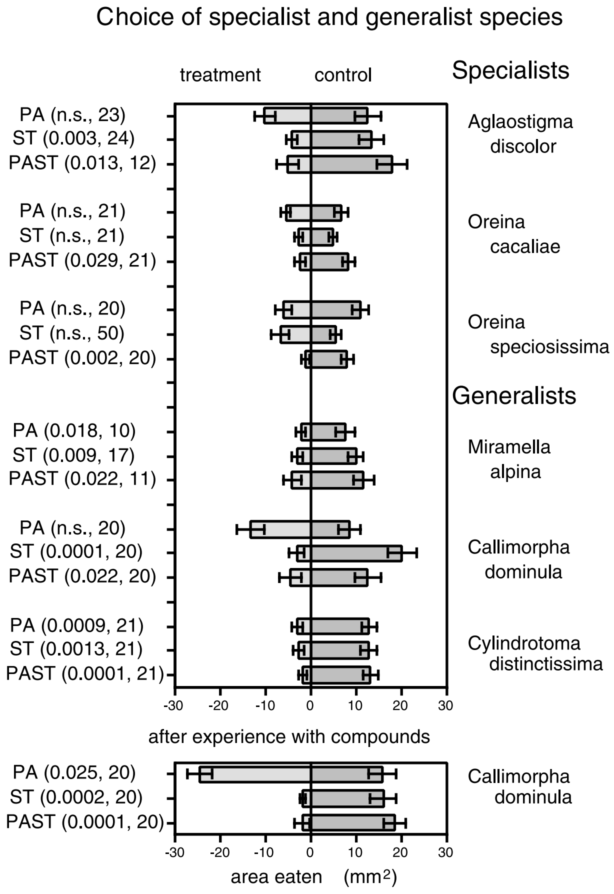


Fig. 2 Mean areas eaten (in mm^2) of compound- and solvent-treated leaf discs. Means and standard errors are shown. Treatments were PA (seneciphylline, 3% of discs dry weight), ST (cacalol, 1.5% of discs dry weight) and PAST (seneciphylline and cacalol, 3 + 1.5% of discs dry weight). *P*-values and the number of replicates are given in parentheses. Values for *M. alpina* were divided by 10.

Larvae of all generalist species preferred to feed from the untreated leaf discs. The only exception were naive *C. dominula* caterpillars which were indifferent towards PA. After they had been exposed to the substances for 10 days in the growth experiments, when tested again they even preferred to feed on the PA-treated leaf discs, whereas they still avoided feeding from ST- and PAST-treated discs (Fig. 2).

Performance of specialists and generalists

Because of excessive humidity in the experimental containers of *O. cacaliae*, we lost a relatively large amount

Table 2 Effects of treatment and family on final weight of larvae. Two-way ANCOVAS taking the initial weight of the larvae as the covariate were calculated. The results reflect the combined action of pre- and postingestive effects.

	Source of variation	d.f.	Mean squares	F	P
Oreina cacaliae					
final weight*	treatment	3	0.2451	1.242	0.3210
	family	8	0.2574	1.231	0.2980
	initial weight	1	1.7025	8.140	0.0060
	treat. × fam.	20	0.1974	0.944	0.5381
	error	58	0.2091		
Oreina speciosissima					
final weight†	treatment	6	0.3619	2.808	0.0325
	family	4	1.6487	16.98	0.0001
	initial weight	1	9.2958	95.69	0.0001
	treat. × fam.	24	0.1289	1.326	0.1693
	error	94	0.0971		
Callimorpha dominula					
final weight‡	treatment	6	0.3386	11.04	0.0001
	family	4	0.2367	5.521	0.0005
	initial weight	1	1.1097	25.89	0.0001
	treat. × fam.	24	0.0307	0.716	0.8248
	error	100	0.0429		
Cylindrotoma distinctissima					
final weight§	treatment	6	0.5417	18.80	0.0001
	family	6	0.1373	6.45	0.0001
	initial weight	1	1.2656	59.45	0.0001
	treat. × fam.	36	0.0288	1.353	0.1320
	error	80	0.0213		

*Due to the nonsignificance of the terms 'treat. × fam. × in. wt.' ($P = 0.5384$), 'treat. × in. wt' ($P = 0.8442$) and 'fam. × in. wt.' ($P = 0.5683$) the treatment, family and covariate effects could be calculated. †Due to the nonsignificance of the terms 'treat. × fam. × in. wt.' ($P = 0.2909$), 'treat. × in. wt' ($P = 0.3752$) and 'fam. × in. wt.' ($P = 0.1411$) the treatment, family and covariate effects could be calculated. ‡Due to the nonsignificance of the terms 'treat. × fam. × in. wt.' ($P = 0.6993$), 'treat. × in. wt' ($P = 0.1504$) and 'fam. × in. wt.' ($P = 0.5960$) the treatment, family and covariate effects could be calculated. §Due to the nonsignificance of the terms 'treat. × fam. × in. wt.' ($P = 0.5626$), 'treat. × in. wt' ($P = 0.7221$) and 'fam. × in. wt.' ($P = 0.1498$) the treatment, family and covariate effects could be calculated.

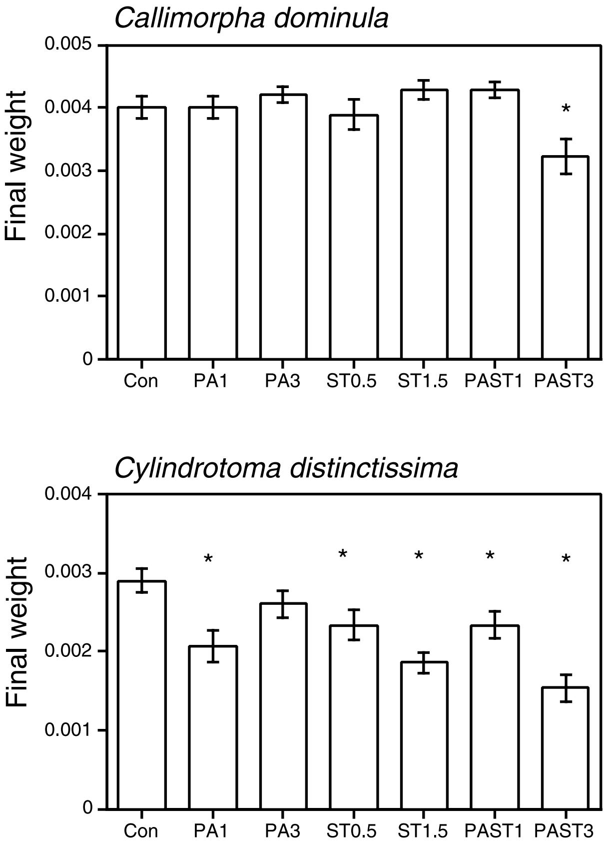


Fig. 3 Final weight (in g) reached in the experiment. Means and standard errors are shown. Physiological and behavioural (consumption) effects are jointly responsible for the final weight reached in the different experimental groups. Significant differences between treatment and control groups (Dunnett's test set to $P < 0.05\%$) are indicated by asterisks.

of larvae due to drowning in condensation droplets and obtained an unbalanced dataset. Therefore, we combined the qualitatively similar treatments (PA 1% with PA 3%, ST 0.5% with ST 1.5% and PAST 1 + 0.5% with PAST 3 + 1.5%) for the analyses of *O. cacaliae* data.

We found no effect of treatments on the final weight of *O. cacaliae* larvae (Table 2). In *O. speciosissima* we found an overall treatment effect, but none of the treatments differed significantly from the control group. There was also a significant family effect suggesting that different families had different growing capabilities independent of treatment and initial weight.

In both generalist species, treatment had a significant effect on growth of the larvae (Table 2). In *C. dominula* only the PAST 3 + 1.5% treatment reduced final weight of caterpillars as compared with the control group, whereas in *C. distinctissima* all treatments except PA 3% reduced the final weight of larvae compared with the control group (Fig. 3).

Initial weight and consumption could explain high variability in the final weight of the larvae. In *O. cacaliae* and *O. speciosissima* multiple regressions showed that both factors were significantly correlated to final weight and together they could explain 74% and 64% of the variability in final weight. In *C. dominula* initial weight and consumption together explained 78% of the variability in final weight and in *C. distinctissima* only consumption contributed significantly to the variation in final weight, explaining 73% of the variance.

Table 3 shows the results of two factor ANOVAS calculated on the residual values of final weight as obtained from the multiple regressions. Since the effects of initial weight and consumption are factored out by the multiple regression, these results should reflect all other (post-ingestive regulatory) effects of the treatments on the

Table 3 Results of the ANOVAS on final weight residuals as obtained from multiple regressions (see text). Expected mean squares and variance component estimates are shown.

	Source of variation	d.f.	Mean squares	*F*	*P*	Expected mean squares
Oreina cacaliae						
final weight residual	treatment	3	149.55	2.441	0.0737	$V_e + 2.12V_{txf} + 16.80V_t$
	family	8	79.21	1.290	0.2665	$V_e + 2.31V_{txf} + 8.01V_f$
	treat. × fam.	20	76.97	1.250	0.2470	$V_e + 2.57V_{txf}$
	error	59	61.42			V_e
Oreina speciosissima						
final weight residual	treatment	6	517.41	3.632	0.0104	$V_e + 3.67V_{txf} + 18.99V_t$
	family	4	622.87	10.09	0.0001	$V_e + 3.66V_{txf} + 25.65V_f$
	treat. × fam.	24	143.13	2.319	0.0021	$V_e + 3.69V_{txf}$
	error	95	61.71			V_e
Callimorpha dominula						
final weight residual	treatment	6	12.92	0.823	0.5632	$V_e + 3.86V_{txf} + 18.92V_t$
	family	4	153.58	14.43	0.0001	$V_e + 3.86V_{txf} + 27.01V_f$
	treat. × fam.	24	15.72	1.476	0.0932	$V_e + 3.88V_{txf}$
	error	101	10.64			V_e
Cylindrotoma distinctissima						
final weight residual	treatment	6	69.87	13.23	0.0001	$V_e + 2.59V_{txf} + 17.80V_t$
	family	6	102.70	12.05	0.0001	$V_e + 2.59V_{txf} + 18.13V_f$
	treat. × fam.	36	5.20	0.610	0.9496	$V_e + 2.65V_{txf}$
	error	82	8.52			V_e

Variance components as obtained by the restricted maximum likelihood procedure: *O. cacaliae* $V_e = 58.92$, $V_{txf} = 9.91$, $V_f = 3.28$, $V_t = 8.90$; *O. speciosissima* $V_e = 61.82$, $V_{txf} = 22.06$, $V_f = 18.34$, $V_t = 27.23$; *C. dominula* $V_e = 10.60$, $V_{txf} = 1.35$, $V_f = 4.94$, $V_t = 0.68$; *C. distinctissima* $V_e = 7.51$, $V_{txf} = 0$, $V_f = 5.49$, $V_t = 3.93$.

variability in final weights. In the specialist *O. cacaliae* we could detect no significant influence of chemical treatments and genetic background (families) on the physiology of weight gain performance.

In *O. speciosissima* the overall treatment effect was significant, but none of the chemical treatments differed significantly from the control treatment. The significant family effect means that different families had differing growing capabilities, independent of initial weight, consumption and treatment. Since the treatment–family interaction was significant as well, we conclude that certain families did well on specific chemical treatments whereas they performed relatively poorly on other chemical treatments (Fig. 4). For example, performance of family F2 was relatively uniform in the control, PA and PAST treatments whereas it was markedly different in the ST treatments. Families F1 and F3 had differing performances in the PAST 3 + 1.5% treatment, and the performance of the F8 control treatment group was different from all other treatment groups.

In the generalist *C. dominula* the treatment effect was not significant (Table 3), suggesting that the growth-reducing effect of the PAST 3 + 1.5% treatment had no postingestive basis and was due solely to reduced consumption on this diet (Table 4). The significant family effect, however, indicates that there are family-specific differences in postingestive processing of the different diets; families show an overall good or poor growth performance. However, the trend of a treatment–family interaction indicates that families might still differ in their postingestive ability to respond to certain treatments (Fig. 4). Family C, for instance, showed a deviation from the average performance on the PA 3% and the ST 1.5% treatment, and family E did well on the high-concentration combination treatment (PAST 3 + 1.5%).

In *C. distinctissima* postingestive metabolic effects were responsible for growth reduction in all treatment groups (Table 3); additionally, consumption was reduced on the ST 1.5% and PAST 3 + 1.5% treatment groups (Table 4). In the PA3% treatment group high consumption (highest of all treatment groups) could alleviate the postingestive metabolic effect on growth (Fig. 3). The highly significant family effect means that families had different overall postingestive physiologies, independent of the experimental treatments.

Heritabilities of performance residual character (postingestive physiology)

In *O. cacaliae* we could not detect a significant heritability for the performance residual character among the observed families (Table 5), which means that there is no detectable genetic variability among the tested families in their postingestive physiological capabilities. In *O. speciosissima*, however, a significant amount of genetic variability in postingestive physiological capability existed among the tested families, as indicated by the significant

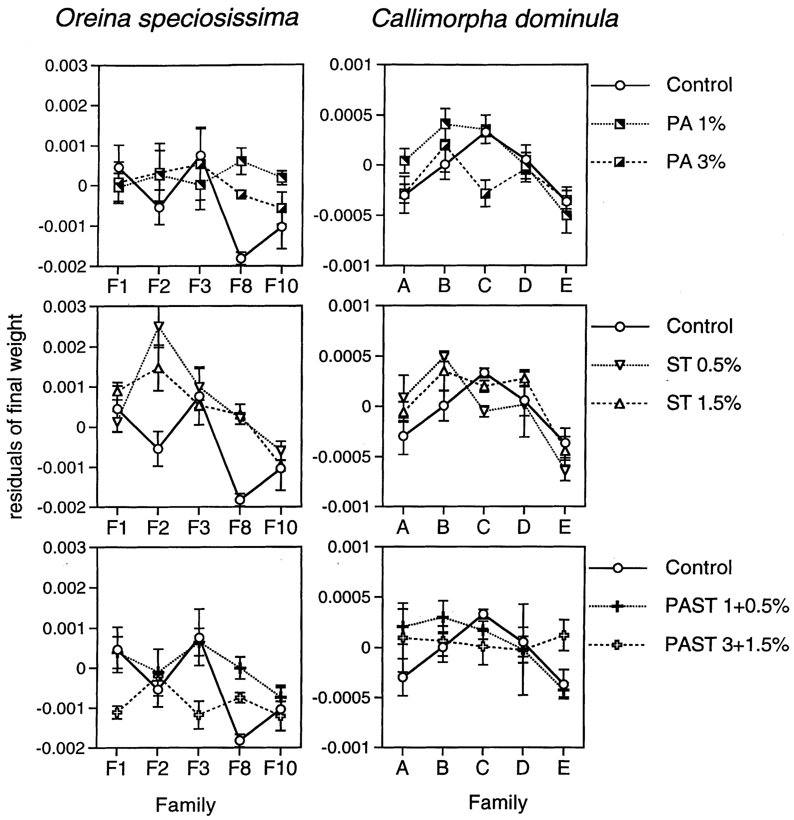


Fig. 4 Interaction plots (treatment–family interactions) of performance residual character analysis. Means and standard errors are shown.

broad sense heritability (Table 5). In addition, the significant treatment–family interaction indicates that family differences in performance on treatments has a genetic basis, a precondition for host specialization.

In the generalist *C. dominula* heritability of the performance residual character was significant and higher than in *O. speciosissima* (Table 5), indicating that a relatively large amount of genetic variability for postingestive performance physiology under the various treatments is present among the observed families. In *C. distinctissima* heritability of the performance residual character was significant and the highest of all species (Table 5), again indicating the presence of a large amount of genetic variability for postingestive performance physiology among families.

Discussion

Choice experiments

The expectation that specialist herbivores should be adapted to allelochemicals occurring in their host plants, and therefore show no deterrence towards them, is supported by the results of the choice experiments. The avoidance of ST by *A. discolor* does not contradict this, since although it accepted *A. alpina* (with cacalol in its leaves) in feeding trials, the larvae were collected from *A. alliariae* (which has no cacalol in its leaves) and hence might not be expected to be adapted to it. Under this aspect it is rather surprising that both *Oreina* species did not discriminate towards cacalol. Since cacalol and seneciphylline together could elicit avoidance in all specialists, this indicates that at least in the *Oreina* species both compounds act synergistically.

In the generalist species, the expectation that all compounds should act as deterrents was also met. The only exception was *C. dominula* caterpillars, which did not discriminate between PA-treated leaf discs and the control discs. Furthermore, after the larvae had eaten PA for 10 days, they clearly preferred the PA-treated discs over the control discs (Fig. 2). This finding is remarkable, since in most other studies on long-term effects of feeding deterrents habituation occurred, which was the result of reduced sensitivity of deterrence receptors (Städler & Hanson, 1978; Szentesi & Bernays, 1984). In order to perceive the difference between PA-treated and untreated leaves, *C. dominula* should possess some PA detection system (receptor?). However, only (strong) circumstantial evidence can be given to support this conclusion. This is the ability positively to discriminate between PA-treated and untreated food; the uptake and storage of large amounts of seneciphylline (Speiser, 1992); the presence of the insect PA callimorphine (Edgar *et al.*, 1980), which as in the closely related *Tyria*

Table 4 Effects of treatment on consumption of larvae. One-way ANCOVAS taking the initial weight of the larvae as the covariate were calculated.

	Source of variation	d.f.	Mean squares	*F*	*P*
Oreina cacaliae					
consumption†	treatment	6	0.2343	1.844	0.1004
	initial weight	1	1.2402	9.759	0.0025
	error	83	0.1271		
Oreina speciosissima					
consumption	treatment	6	0.0443	1.554	n.a.*
	initial weight	1	1.9922	69.914	n.a.*
	treat × in. wt.	6	0.0690	2.421	0.0303
	error	119	0.0285		
Callimorpha dominula					
consumption‡	treatment	6	0.7453	14.442	0.0001
	initial weight	1	1.7877	34.641	0.0001
	error	128	0.0516		
Cylindrotoma distinctissima					
consumption§	treatment	6	0.4478	16.177	0.0001
	initial weight	1	1.7807	64.329	0.0001
	error	122	0.0277		

*n.a. = not applicable. †Due to the nonsignificance of the term 'treat. × in. wt.' ($P = 0.8701$) the treatment and covariate effects could be calculated. ‡Due to the nonsignificance of the term 'treat. × in. wt.' ($P = 0.4469$) the treatment and covariate effects could be calculated. Consumption on PAST 3 + 1.5% was smaller than consumption of the control diet (Dunnett's test $P < 0.05$). §Due to the nonsignificance of the term 'treat. × in. wt.' ($P = 0.4774$) the treatment and covariate effects could be calculated. Consumption on ST 1.5% and PAST 3 + 1.5% was smaller than consumption of the control diet (Dunnett's test $P < 0.05$).

Table 5 Heritabilities of growth performance residual character of larvae of specialist and generalist species. Values for *O. cacaliae* were obtained from calculations with pooled data of qualitatively similar treatments (different concentrations of the same chemicals).

	h^2 (±SE)	*t*	d.f.	*P*
Oreina cacaliae (pooled)	0.10 (±0.14)	0.77	90	n.s.
Oreina speciosissima	0.46 (±0.23)	1.98	137	<0.05
Callimorpha dominula	0.64 (±0.27)	2.32	135	<0.03
Cylindrotoma distinctissima	0.84 (±0.32)	2.66	133	<0.01

jacobaeae L. should result from the sequestration of plant PAs (Aplin & Rothschild, 1972; Edgar *et al.*, 1980; Ehmke *et al.*, 1990), and therefore requires a highly specific set of sequestration enzymes. However, it is very likely that the caterpillars are adapted to plant PAs and make use of them in their own defence against predators.

Performance experiments

When the two specialist *Oreina* species were forced to feed on the treatment discs, we could not detect any effects on growth of the allelochemical treatments compared with the control treatment. This was even the case with the PAST 3 + 1.5% treatment, which was avoided by the larvae in the choice experiments. The synergistic effect of cacalol and seneciphylline on food choice had obviously no equivalent effect on growth performance when both allelochemicals could not be avoided. Since specialist species should be adapted to allelochemicals in their host plants, this result confirms the expectation that no adverse performance effects result from the exposure to the allelochemicals.

In the case of *O. cacaliae*, the mortality due to drowning in condensation droplets might have interfered with the results from the treatments. The loss of animals happened across all treatments, and since the larvae either became caught in a droplet or not, we also assume that there was no systematic influence of humidity on any of the treatments. Nevertheless, the results should be interpreted cautiously since an increased error variance might have obscured potentially significant effects. Bearing that in mind, the lack of any detectable genetic variability in the growth performance residual (post-ingestive physiology) character in *O. cacaliae* indicates that the ability to deal with allelochemicals is probably genetically fixed in this population which could be the result of past directional selection for detoxification efficiency. In *O. speciosissima*, however, we detected a significant amount of genetic variability in postingestive physiology of diet processing. Furthermore, we found a significant treatment–family interaction which indicates that there is not only genetic variability between families in their overall ability to process the allelochemical-treated diets but, in addition, there are differences between families in their ability to process specific allelochemicals. If performance on hosts containing either PA or ST is linked to a specific genotype, one prerequisite for host specialization would be met. On another occasion we also found indirect evidence for a genotype difference in choice behaviour against cacalol. This is the difference between beetles from the Zastler and Zamecek populations in their choice towards cacalol-treated leaf discs. Whereas Zastler beetles were indiscriminate towards cacalol-treated discs, beetles from Zamecek actually preferred cacalol-treated discs (Hägele *et al.*, 1996). Beetles of this population live exclusively on *Senecio fuchsii* in a deforested area of the Czech Republic (Pysek, 1992). Since there is no report about the occurrence of cacalol in *S. fuchsii*, this might be the result of cross-adaptation (Gould, 1983) to other sesquiterpenes occurring in their food plant (Seaman, 1982).

In the generalist *C. dominula*, performance on all treatments except PAST 3 + 1.5% was good. Genetic variability in the performance residual character (post-ingestive physiology) was higher than in *O. speciosissima* and there was at least a trend of a treatment–family interaction. However, we can only confidently conclude that there are family differences in performance, which is that families perform either well or poorly on all treatments. Since larval avoidance of cacalol was not matched by poor performance on the diet, this indicates that cacalol on its own acts merely as a deterrent, without affecting performance negatively when the larvae are forced to incorporate it. However, negative performance due to deterrence (lowered consumption) was achieved only when cacalol was combined with the high concentration of seneciphylline, indicating a synergistic effect of the two substances.

Performance effects in *C. distinctissima* were the most pronounced of all investigated species. Initially all compounds were deterrent, but only the high dosage of ST (alone or in combination with PA) had a lasting effect on consumption. Interestingly, all treatments had negative effects on the postingestive performance physiology and the effect was highly variable between families, which is reflected in the high heritability value. Pashley (1988) found heritabilities for performance on different diets of strains of *Spodoptera frugiperda* (a generalist Lepidoptera) to be between 0.67 and 0.91, which is in the same range as we found for our generalist species (Table 5). Singer *et al*. (1988) reported a heritability of 0.89 for oviposition preference in the oligophagous nymphalid moth *Euphydryas editha* and a preference/performance correlation of 32%.

Evolutionary significance

Do the chemicals tested have the potential to exert selective pressure on the tested specialists and generalists? As we have seen, there is no universal answer to this question. Each species has to be considered individually, and even within the obvious categories specialists and generalists, no generalization can be drawn.

O. cacaliae, the most advanced specialist of the investigated species, seems to be remarkably well adapted to the investigated compounds. Only the combination of both compounds at high concentrations led it to choose the alternative food. However, this rejection is unlikely to have evolutionary consequences, since we could not detect any differences in final weight between the larvae, nor was there significant genetic variability present which would allow selection to further improve metabolic efficiencies in handling these compounds. However, as for all quantitative genetic studies we have to caution that these conclusions are only valid for the investigated population of the species and that had we included more families into our study heritability values might have become significant.

O. speciosissima, the somewhat less specialized sister species, is also well adapted to the compounds, since none of the treatments influenced performance significantly different from the control treatment. However, metabolic efficiencies to handle the compounds varied between families so that selection by higher compound concentration should produce a metabolically well-adapted population. Moreover, the family–treatment interaction indicates that, given the circumstances, adaptive specialization, with subpopulations being adapted to one or the other compound, should be possible. However, as for all studies which are based on broad sense heritability and for which the precise amount of additive genetic variation (V_A) is therefore not known, this is only true if V_A makes up for most of the variation between families and nonadditive genetic variation is considerably smaller than V_A. Our heritability measure therefore has to be interpreted as the upper limit of heritability.

In the generalist moth *C. dominula*, only the combination and high concentration of both compounds might exert selection pressure on caterpillar performance. This effect is exclusively due to consumption reduction on this diet since there was no difference between metabolic efficiencies of the different diet treatments. In this respect *C. dominula* is more like the other specialists than the other generalist species. However, selection towards overall improved metabolic efficiencies is possible, since we found the high genetic variability within the population. The possibility of an adaptive specialization to PA remains open, since we could not detect a clear treatment–family interaction. This of course does not preclude behavioural specialization towards PAs, which seems very likely in the light of our findings that PA consumption is attractive after an initial experience (Fig. 2). In addition, the aposematic coloration of the larvae and the finding that they store PAs in their body suggests that larvae benefit directly from PA incorporation, which should favour a specialization on PA-containing plants.

In *C. distinctissima*, ST at high concentrations has the potential of being a selective agent to growth performance via consumption reduction. Furthermore, all treatments had negative effects on metabolic efficiencies. Since we found no treatment × family effect, specialization should be very unlikely to occur and selection on performance will be selection for overall performance improvement rather than for performance improvement towards a specific compound. The lack of significance of the treatment–family interaction indicates true generalism in *C. distinctissima* (Rossiter, 1987), whereas the interaction trend in *C. dominula* indicates that it might fall between a true and a composite generalist, with families expressing different levels of adaptation to specific compounds (Rossiter, 1987).

Overall, our results support the hypothesis that specialists and generalists are differentially affected by plant allelochemicals. This is most obvious in the difference in choice between treated and untreated leaf discs. However, consequences on growth performance of chemicals for generalist species are less clear-cut. In one case (*C. distinctissima*) the ingestion of each compound had significant performance consequences, whereas in *C. dominula* this was true only for the high-dose combination of both chemicals.

Genetic variation in metabolic efficiency of growth performance increased from specialist to generalist species. A host switch of the most specialized *O. cacaliae* might therefore be limited by the lack of genetic variation for performance on novel allelochemicals (Futuyma *et al.*, 1994; Futuyma *et al.*, 1995). This condition seems to be relaxed in the less specialized *O. speciosissima* (see above) and even more so in the two generalist species. So allelochemicals should not impose considerable performance constraints for specialization in the observed generalist species.

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