Ann. Zool. Fennici 29:273–278
Helsinki 23 December 1992

ISSN 0003-455X

Differences between species and instars of *Phratora* leaf beetles (Coleoptera, Chrysomelidae) in the probability of being preyed on

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Received 2 March 1992, accepted 6 July 1992

The defence against predators of two closely related leaf beetle species was compared. *Phratora vitellinae* (Linnaeus) is known to use plant precursors in producing defensive compounds but *P. polaris* (Sparre Schneider) obviously synthesizes its defensive compounds from the first on its own, which is known to be true for all the other *Phratora* species studied except *P. vitellinae*. In predation experiments *P. vitellinae* was significantly less eaten by spiders than *P. polaris*. First instar larvae of *P. polaris* were more susceptible to spiders than older larvae. This may be explained by instar-specific defensive behaviour of the larvae.

1. Introduction

Host plant chemistry can have a strong effect on the ability of leaf beetle larvae to repel predators (e.g. Price et al. 1980, Damman 1987, Pasteels et al. 1988). Some leaf beetles are able to sequester chemicals from their host plants, and to use the secondary, defensive chemicals of plants in their own defence (Pasteels et al. 1983b, Smiley et. al. 1985). However, there are differences in the ability to sequester defensive chemicals even among closely related species. *Phratora vitellinae* larvae secrete salicylaldehyde derived from salicin in their food, but other *Phratora* species studied up to now secrete chemicals that are most likely synthesized *de novo* (autogeneously) because their food plants do not produce obvious, direct precursors (Pasteels et al. 1982, 1984, 1990). It has been suggested that the autogeneously produced chemicals represent the ancestral defence in the genus *Phratora*, while the secretion of salicylaldehyde has followed a shift to food plants with a high salicylate content (Pasteels et al. 1983b, 1984).

When disturbed (e.g. by ants or spiders) *Phratora* larvae try to repel the enemy by droplets of secretion released from eversible glands in the thorax and abdomen. When there is no further danger, the droplets are drawn back into the larvae and they can be used again several times

(Pasteels et. al. 1988; Palokangas & Neuvonen, personal observations). Simultaneously with the droplet release the larvae erect their abdomena. This behaviour is impressive, especially when gregarious first instar larvae exhibit it synchronously.

The larval secretions are mainly volatile irritants (Pasteels et al. 1988). The efficacy of the secretions and their chemical components have generally been shown by testing the reactions of generalist predators, e.g. ants, to an artificial medium treated with the chemicals (Pasteels et al. 1983a). On the basis of this kind of information it is difficult to compare the efficacy of the defence of different chrysomelid species, or individuals of the same species feeding on different plant species. Knowledge about the relative efficacy of defences is, however, essential for understanding inter- and intraspecific variation in antipredator behaviour and its consequences to the population dynamics of leaf beetles.

Two closely related leaf beetles, *Phratora polaris* (Sparre Schneider) and *P. vitellinae* (Linnaeus), provide a good opportunity to study how differences in host preferences are reflected in predation. In northern Fennoscandia *P. polaris* is a common herbivore on mountain birch (*Betula pubescens* spp. *tortuosa*) (Tenow 1963, Nuorteva 1966, Koponen 1973) but it lives also on willows. In preference experiments *P. polaris* larvae chose mountain birch over *S. phylicifolia* (*S. borealis* and *P. tremula* were rejected), but in growth trials they performed equally well on mountain birch and *S. phylicifolia* (Palokangas et. al. 1992). In our study area *P. vitellinae* feeds on two plant species with high salicylate content (Julkunen-Tiitto 1989): aspen (*Populus tremula*) and a willow (*Salix borealis*) (Neuvonen & Palokangas, personal observation). This conforms to the observations from more southern areas (Rowell-Rahier 1984, Tahvanainen et al. 1985).

The aim of this study was to compare the outcomes of spider attacks on leaf beetle larvae. Specifically, we addressed the following questions:

1) Is survival higher for *P. vitellinae* larvae (feeding on willow or aspen foliage, with high content of salicylate) than for *P. polaris* larvae (feeding on birch foliage, with no salicin) when exposed to spider predation?
2) Are there differences between larval instars of *P. polaris* in the probalitity of being killed when exposed simultaneously to spider predation?

2. Material and methods

We did two laboratory experiments at the Kevo Subarctic Research Station (69°45′N, 27°01′E) in northern Finland during summer 1991. The larvae used in the experiments were collected near the station in June when they were neonate. *P. vitellinae* larvae were collected from *S. borealis* and aspen, and *P. polaris* larvae from mountain birches. We used both wolf spiders (Lycosidae: *Pardosa eiseni* (Thorell), *P. hyperborea* (Thorell), *P. lugubris* (Walckenaer) and *Alopecosa aculeata* (Clerck)) and crab spiders (Thomisidae: *Xysticus audax* Schrank and *X. obscurus* Collett) as predators. Wolf spiders hunt on the ground, and probably do not often (if at all) prey on *Phratora* larvae in the wild (except perhaps larvae fallen from foliage). Crab spiders are commonly found on the foliage of trees and bushes in the study area (Koponen 1977). We use spiders as models of generalist predators with probably no (Lycosidae) or little (Thomisidae) previous experience of *Phratora* larvae and their defences.

2.1. Comparison between *Phratora polaris* and *P. vitellinae*

Transparent plastic tubes (diam. 18 cm, height 50 cm) were used as an arena for spiders and leaf beetle larvae. We had 16 replicates, ten wolf spiders and six crab spiders. In each tube we had three twigs with leaves: birch, willow and aspen. The positions of twigs in the tubes were randomized. Three second instar larvae, which had been living on the same food plants, were placed on the leaves of each plant species, and a spider was released in the tube. The experiment lasted 24 hours, during which time we recorded the plant species on which or in the vicinity of which the spider stayed. At the end of the experiment we counted the number of living larvae on each host plant. The actual hunting was usually hard to notice, because it happens very quickly.

Also, spiders were aware of the observer and did not hunt when observed.

The number of larvae killed was log-transformed [log(n+1)] to satisfy the normality assumption of ANOVA, but in the figure untransformed values are presented. Differences among host plant species in the number of *Phratora* larvae killed and in the number of times the spider was on or close to the plant were tested with an ANOVA of treatments by subjects experimental design (Zar 1984:222–224). For within subject comparisons (in the present case leaf beetles on different host plants) the analysis considers the fact that the observations from each spider are not independent (actually our analysis is an extension of the paired t-test). The overall differences (among subject comparisons) between wolf and crab spiders (spider family) and those between spiders of different ages (juveniles vs. subadults and adults) were tested against the variation of individual spiders within family × age combinations. Interaction terms (plant species × spider family, and plant species × spider age) tested whether spiders of different family or age behaved differently with respect to host plant species. Comparisons among host plants were decomposed to orthogonal contrasts on the basis of *a priori* knowledge about chemical differences among plants: birch with no salicin was contrasted against aspen and *S. borealis* with a high salicylate content (Palo 1984, Julkunen-Tiitto 1989).

2.2. Comparison among different instars of *P. polaris*

We tested the efficacy of defence of the first, second and third instar larvae of *P. polaris* against the enemy. We had 16 petri dishes (diam. 18 cm), 13 with wolf spiders and three with crab spiders. In each petri dish we had one larva of each instar and a spider. After 24 hours we counted the number of beetle larvae in different instars killed by spiders. Since the set-up is a randomized block experimental design (individual spiders as "blocks") and the response variable is measured in dichotomous scale (dead vs. alive), the data was analysed with Cochran's Q test (Zar 1984:231–233).

3. Results

3.1. Predation of *Phratora* larvae on different host plants

During the experiment crab spiders killed about twice as many *Phratora* larvae as wolf spiders ($P < 0.02$; Table 1; Fig. 1). There were no significant differences in the number of larvae eaten by spiders of two age classes. Spiders ate significantly more *P. polaris* living on birch than *P. vitellinae* living on aspen or willow ($P < 0.001$; Table 1; Fig. 1). There were no significant inter-

Table 1. ANOVA table for the effects of spider family (wolf vs. crab spiders) and age, and host plant on the number of leaf beetle larvae killed, and on the time spent by spiders close to different plants ("site preference"). *df* = degrees of freedom, *MS* = mean square, *F* = *F*-ratio, *P* = corresponding α-risk level.

		Log (killed+1)			"Site preference"		
Source	*df*	*MS*	*F*	*P*	*MS*	*F*	*P*
Spider family (SF)	1	1.7038	9.24	0.0103	0.0500	0.01	0.9196
Spider age (SA)	1	0.2191	1.19	0.2970	0.0000	0.00	1.0000
SF×SA	1	0.0376	0.20	0.6594	2.0056	0.43	0.5261
Individuals (SF×SA)	12	0.1843			4.7037		
Plant species (P)	2	2.0294	10.22	0.0005	0.7125	0.12	0.8889
P×SF	2	0.0991	0.50	0.6126	32.7125	5.43	0.0107
P×SA	2	0.3277	1.65	0.2114	3.1458	0.52	0.5992
Error	26	0.1985			6. 0221		
Contrasts:							
Birch vs. (aspen & willow)	1	4.0580	20.45	0.0001	0.5063	0.08	0.7742
Aspen vs. willow	1	0.0009	0.00	0.9481	0.9188	0.15	0.6993

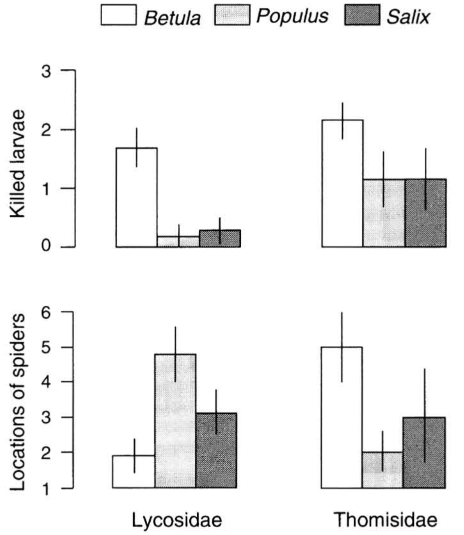

Fig. 1. Number of leaf beetle larvae killed (mean ± *SE*) by wolf (Lycosidae, $n = 10$) and crab (Thomisidae, $n = 6$) spiders in relation to the host plant of larvae, and number of times (mean ± *SE*) wolf and crab spiders were observed on or close to twigs of different plant species. *Betula* = mountain birch, *Populus* = aspen, *Salix* = a willow (*Salix borealis*). Larvae on birch belonged to the species *Phratora polaris*, and those on aspen and willow to *P. vitellinae*. Beetle larvae on different host plants were simultaneously exposed to spiders (one spider/cage).

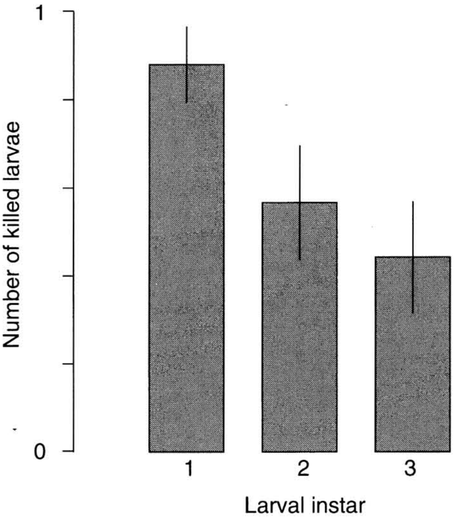

Fig. 2. Number (mean ± *SE*) of *P. polaris* larvae of different instars killed by spiders (wolf spiders 13, crab spiders 3).

actions between the host plant of the larvae and the family or age of spiders ($P > 0.20$), i.e. the spiders killed more *P. polaris* than *P. vitellinae* larvae irrespective of spider family or age.

There was no significant overall difference in the number of cases the spiders were observed close to different host plants. However, an interaction of spider family and plant species ($P < 0.02$) showed that wolf and crab spiders behaved differently in this respect. Wolf spiders were observed less often on or close to birch than aspen or willow, while the opposite was true for crab spiders (Fig. 1).

3.2. Predation risk in different instars of *Phratora polaris*

There was a significant overall difference in the proportion of *P. polaris* larvae in different instars killed by spiders ($Q = 7.8$, $P < 0.025$; Fig. 2). Spiders ate more first than second or third instar larvae ($Q = 5.0$, $P < 0.05$ and $Q = 7.0$, $P < 0.01$, for 1st vs. 2nd and 1st vs. 3rd instar). There was no significant difference between second and third instar larvae in the probability of being killed by spiders.

4. Discussion

4.1. Predation of *Phratora* larvae on different host plants

P. vitellinae larvae (feeding on foliage with a high content of phenolic glycosides) had significantly better chances of surviving than *P. polaris* (feeding on birch foliage) when the larvae were exposed simultaneously to spider predation. This corresponded to our subjective estimate of the efficacy of defence: the smell of the defensive droplets of *P. vitellinae* was distinctive and also the defensive droplets were larger than those of *P. polaris*.

At least in theory, there are also other possible explanations for the higher mortality of *P.*

polaris larvae than differences in the efficacy of defence. This kind of difference may have arisen if spiders had preferred to stay in the vicinity of birch twigs and so had more encounters with *P. polaris* larvae (without any differences in the outcome of these encounters). Our data does not support this hypothesis. Only crab spiders were observed more often associated with birch than with aspen or willow twigs but spiders belonging to both groups killed more larvae from birch than from aspen or willow.

P. vitellinae is a well known example of a herbivore that can derive its own defences from precursors in host foliage (Rowell-Rahier & Pasteels 1982, Pasteels et al. 1983b). Using a precursor minimizes synthesizing costs and the larvae profit also from utilizing the glucose present in the precursor (salicin) which may cover almost half of the larval requirement (Rowell-Rahier & Pasteels 1986).

Our results suggest that the defence of *P. vitellinae* is also more efficient against generalist predators (spiders) than that of *P. polaris* feeding on plants without salicin (e.g. mountain birch). However, it is possible that the responses of specialized predators to the defences of prey may depend on their past experience. For instance, female sawflies (*Tenthredo olivacea*) preying on larvae of *Plagiodera versicolora* and *P. vitellinae* in choice experiments were more deterred by the secretion with which they had no or less experience (Pasteels & Gregoire 1984). Considering the patchy occurrence of aspen and *Salix borealis* in our study area, it is, however, probable that a great majority of the potential predators of leaf beetle larvae in mountain birch forests have previous experiences of birch herbivores. In this kind of habitat the use of salicin as a precursor for defences (if possible) is surely benefical to insects, because it is an inexpensive way to acquire an effective deterrent against generalist arthropod predators.

4.2. Predation risk of different instars of *Phratora polaris*

We found that first instar *P. polaris* larvae were more susceptible to spiders than older larvae. In the wild, first instar *Phratora* larvae usually live in groups. An individual larva living in a small group may benefit from the defences of the other group members. Thus there may be only weak evolutionary pressure to develop a more effective defence individually in the first instar. Another explanation may simply be that it is physiologically impossible for newly hatched larvae to have a strong defence. It is necessary for neonate larvae to eat before they can produce defensive chemicals themselves. Neonate larvae have two functional pairs of defensive glands while older larvae have nine functional gland pairs (Pasteels et al. 1986). When the larvae grow up they start to disperse. Now, stronger individual defence is of high value. Considering resource allocation it is beneficial for the first instar larvae to direct their resources primarily towards growth and secondarily to defence. According to optimization theory larvae should optimize their defence rate according to costs and benefits.

Optimal foraging of the predator (Krebs 1989) can also affect the predation risk of different instars. Because of the low nutritional value of small first instar larvae, they may not have so high a risk of being eaten, hence the low defensive allocation by first instar larvae. As the larva grows, its nutritional value increases. Then it is necessary for the larva to invest more in defence, because predators will suffer higher energetic costs in capturing large larvae with a strong defence. Also, large larvae may be harder to digest because of the larger quantity of defensive chemicals (Pulliam 1975).

Acknowledgements. We thank Sinikka Hanhimäki, Kai Ruohomäki and three anonymous referees for valuable comments on a previous version of the manuscript. Stefan Schmidt kindly loaned us equipment and helped in finding willows (*S. borealis*) for the experiments. We are indepted to Seppo Koponen for identifying the spiders and Riitta Julkunen-Tiitto for clarifying the terminology of phenolic glycosides. The project is being supported by Finnish Academy.

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