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HOST PREFERENCE AND LARVAL PERFORMANCE OF THE SALICYLATE-USING LEAF BEETLE *PHRATORA VITELLINAE*

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Abstract. Larvae of *Phratora vitellinae* (Coleoptera: Chrysomelidae) convert salicyl glucosides from the host plant into a larval defensive secretion with salicylaldehyde. This secretion repels generalist predators. Willows vary greatly in the concentrations of salicyl glucosides in their leaves. One may predict that *P. vitellinae* prefers and survives better on plants that contain more salicyl glucosides. We determined the amount of larval secretion, host preference, larval growth, and larval survival of *P. vitellinae* on *Salix myrsinifolia, S. pentandra,* and *S. phylicifolia.* We also measured feeding rates of three natural predators on *P. vitellinae* larvae feeding on different hosts. *Salix pentandra* and *S. myrsinifolia* contained substantial amounts of salicyl glucosides, but *S. phylicifolia* contained very little of them. *Phratora vitellinae* larvae produced more secretion on *S. pentandra* than on *S. myrsinifolia.* They produced little secretion on *S. phylicifolia.* Adult beetles preferred *S. myrsinifolia* over *S. pentandra* and *S. pentandra* over *S. phylicifolia.* Larvae grew most rapidly on *S. myrsinifolia* and *S. pentandra.* Their growth was slowest on *S. phylicifolia.* The larval survival was similar on *S. myrsinifolia* and *S. phylicifolia,* but it was significantly lower on *S. pentandra.* The natural predators fed equally well on *P. vitellinae* feeding on *S. myrsinifolia* and *S. phylicifolia.* Thus, the host preference of *P. vitellinae* did not correspond to larval survival on these hosts, but rather to larval growth. Larval survival of *P. vitellinae* was not related to the amount of defensive secretion. Natural predators were not repelled by the host-derived defensive secretion. We discuss the implications of these findings for the evolution of host plant use in this herbivore.

Key words: host suitability; larval defensive secretion; larval performance; Phratora vitellinae; Salix; *trophic level interactions.*

INTRODUCTION

Herbivorous insects have diversified greatly during the last 60×10^6 yr. They now constitute a large proportion of terrestrial arthropods. However, most of them feed on only a few host plants (Strong et al. 1984). The high degree of specialization in insect herbivores has yet to be explained. The predation hypothesis proposes that herbivore specialization has been caused by natural selection imposed by predators and parasites. This may occur if specialist herbivores suffer less predation than do generalists (Bernays and Graham 1988). Many herbivores use host plant compounds for their own chemical defense (Duffey 1980, Brown 1984, Pasteels et al. 1988, Bowers 1990). One may predict that herbivores should specialize on plants that contain these compounds because natural enemies will be less successful at preying on them on those plants. Thus, herbivores may evolve a preference for hosts on which they find ''enemy-free space'' (Denno et al. 1990). Few studies have measured the effect of predators on host suitability to herbivores in a natural environment. Such studies are needed to determine whether the host preference of herbivores is related to their larval performance (Thompson 1988). Thus, the predation hypothesis represents a plausible but largely untested explanation for the evolution of herbivore specialization.

Natural enemies may influence the use of host plants by willow leaf beetles (*Chrysomela* spp., *Phratora vitellinae* L.) (Smiley et al. 1985, Pasteels et al. 1988, Denno et al. 1990). The larvae of these beetles convert salicyl glucosides (SGs) from the leaves of their host plants into a defensive secretion, which consists mostly of salicylaldehyde (Pasteels et al. 1983). This secretion is stored in gland reservoirs along the dorsal side of the thorax and abdomen. When larvae are disturbed, they expose large droplets of their secretion, which repels ants (Wallace and Blum 1969, Pasteels et al. 1983), ladybird beetles (Denno et al. 1990), and spiders (Palokangas and Neuvonen 1992). When beetle larvae feed on willows that contain no salicyl glucosides, they produce very little secretion (Smiley et al. 1985, Denno et al. 1990).

Several researchers have suggested that SG-using beetles like *P. vitellinae* have specialized on SG-rich willows because the larvae are better protected from natural enemies on them (Smiley et al. 1985, Pasteels

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et al. 1988, Denno et al. 1990, Pasteels and Rowell-Rahier 1992). Indeed, this plant–herbivore–predator interaction has been considered to be one of the few where the significance of plant compounds on all three trophic levels is well understood (Montllor and Bernays 1993). However, no studies have compared the larval performance of *P. vitellinae* on chemically variable willows in nature, and the effect of the secretion on natural predators has never been determined.

In this study, we compared the host preference and larval performance of *P. vitellinae* on three Finnish willow species. Two of these willows, *Salix pentandra* (L.) and *Salix myrsinifolia* (Salisb.), contain substantial amounts of the salicyl glucosides that are precursors of the larval defensive secretion. In contrast, *Salix phylicifolia* (L.) contains very little salicyl glucosides. We conducted detailed analyses of the secondary chemistry and nutritional characteristics of these three hosts. We also determined the feeding preference and quantified the amount of defensive secretion produced by *P. vitellinae* larvae on these hosts. To measure larval performance, we measured larval growth in the laboratory and the field. Additionally, we measured larval survival in nature from shortly after hatching until larvae left their host plants to pupate. As a part of the field experiment, we determined whether crawling arthropod predators affected larval survival by excluding them from one-half of the experimental branches. Because larvae of *P. vitellinae* use host plant salicyl glucosides for their secretion, while pupae and adults do not, our survival experiment covers the entire period where the predation hypothesis has been proposed to explain the host preference of this herbivore.

Finally, we compared the feeding rates of three natural enemies of *P. vitellinae* larvae on *S. myrsinifolia* and *S. phylicifolia.* For these tests, we chose predators that we had observed feeding on the beetle larvae at our field sites. Most previous studies of the repellence of the salicylaldehyde secretion have been conducted using generalist predators that were not known to feed on these beetle larvae in nature (Pasteels et al. 1983, Denno et al. 1990, Palokangas and Neuvonen 1992). We believe that our tests give a more realistic picture of the effectiveness of defensive secretions against the natural enemies that are likely to affect the host preference of *P. vitellinae.*

Materials and Methods

Life history and enemies of P. vitellinae

Salix myrsinifolia, S. pentandra, and *S. phylicifolia* are common and widespread in Scandinavia (Jalas and Suominen 1976). *Salix myrsinifolia* and *S. phylicifolia* often grow together along streamsides and lakes, are similar morphologically, and sometimes hybridize (Meikle 1992). *Salix pentandra* has a more erect growth form than the other two willows, with glabrous twigs and leaves (Meikle 1984). *Salix myrsinifolia* is a preferred host for *P. vitellinae* (Rowell-Rahier 1984*a, b,* Tahvanainen et al. 1985), but the beetles never occur on *S. phylicifolia. Phratora vitellinae* occurs in lower abundances on *S. pentandra* than on *S. myrsinifolia.*

In Finland, *P. vitellinae* is univoltine (Kanervo 1939). Overwintering adults emerge from diapause in late May/early June and lay eggs for 3–4 wk. The eggs hatch in 5–8 d. Larval development is completed within 14–21 d. The mature third-instar larvae crawl to the base of the plant to pupate in the soil (Görnandt 1955). During our field studies in 1993, an exceptionally cold and rainy spring delayed the beetles' emergence by 1 mo.

Little is known about the natural enemies of *P. vitellinae.* The pentatomid bug *Rhacognathus punctatus* L. was observed feeding on larvae in central Europe (Görnandt 1955). A trap-nesting wasp, *Symmorphus bifasciatus* L. (Hymenoptera: Eumenidae), also preys on the larvae of *P. vitellinae* (Berland 1928, Blüthgen 1961). Wasps in the genus *Symmorphus* specialize on larvae of *Phratora* and on other chrysomelid beetles (Cumming 1989).

Field sites

We studied two *P. vitellinae* populations near Joensuu, Finland (62°40′ N, 29°45′ E) along a canal (Höytiäinen Canal) and in an abandoned field (Old Field site). Along a 1-km transect at the Höytiäinen Canal, we chose 19 pairs of *Salix myrsinifolia* and *S. phylicifolia* clones. The Höytiäinen Canal is an exposed area, where the soil is relatively dry and sandy. *Phratora vitellinae* had been abundant at this site for several years before the study was conducted. At the Old Field site, *Salix myrsinifolia, S. pentandra,* and *S. phylicifolia* grew together along abandoned drainage ditches. This moist field had not been cultivated for 3–5 yr. A few *P. vitellinae* individuals occurred there. We chose 15 triplets with one clone per species. We will refer to pairs and triplets as blocks.

Plant characteristics

Leaf phenolics.—To determine concentrations of salicyl glucosides (SG) and other leaf phenolics, we conducted high-pressure liquid chromatography (HPLC) of fresh leaves. Samples were collected on 21–23 July 1993. We pooled three leaves from three shoots per plant. Leaves were cut into 1-cm^2 squares (without the midvein). One square was used for chemical analysis and the other to determine water content. Leaf extractions were conducted as described in Julkunen-Tiitto et al. (1993). For several samples, the identity of compounds was verified with a coupled gas chromatography mass spectrophotometer (GCMS, Hewlett-Packard quadropole type model 5890/5971, Julkunen-Tiitto et al. 1996). The HPLC-spectral comparison and the GC/MS fragmentation patterns of the unknown compounds in *S. pentandra* (which were compared to standard salicyl glucosides) indicated that these compounds

TABLE 1. Results of HPLC analyses of plant phenolics.

	Höytiänen Canal				Old Field site					
	myr (N = 20)		*phy* (N = 19)		*myr* (N = 15)		*pen* (N = 15)		*phy* (N = 15)	
Plant phenolics	Mean	(1 SD)	Mean	(1 SD)	Mean	(1 SD)	Mean	(1 SD)	Mean	(1 SD)
Salicyl glucosides										
Salicin	3.5	(1.3)	0.2	(0.4)	2.5	(1.1)	0.7	(0.5)	0.1	(0.2)
SAL1	2.2	(1.0)	⋯	⋯	1.3	(0.4)	⋯	⋯	⋯	⋯
Salicortin	61.0	(10.7)	⋯	⋯	52.9	(8.1)	⋯	⋯	⋯	⋯
O-acetylsalicin	⋯	⋯	⋯	⋯	⋯	⋯	2.1	(1.1)	⋯	⋯
O-acetylsalicortin	⋯	⋯	⋯	⋯	⋯	⋯	22.7	(12.0)	⋯	⋯
O-ACSAL1	⋯	⋯	⋯	⋯	⋯	⋯	42.7	(26.8)	⋯	⋯
Other phenolics										
(+)-catechin	0.7	(0.7)	1.1	(0.8)	3.1	(1.5)	1.3	(0.8)	1.8	(1.2)
Gallocatechin	⋯	⋯	0.6	(0.2)	⋯	⋯	⋯	⋯	0.9	(0.5)
Ampelopsin	⋯	⋯	130.0	(40.2)	⋯	⋯	⋯	⋯	136.0	(38.1)
AMPEL1	⋯	—	4.0	(2.7)	⋯	⋯	⋯	⋯	3.6	(2.5)
Chlorogenic acid	10.0	(3.0)	⋯	⋯	(12.0)	(2.4)	18.4	(5.1)	⋯	⋯
Cinnamic acid	—	⋯	⋯	⋯	⋯	⋯	12.9	(9.6)	⋯	⋯

Notes: All values are expressed as mg/g per leaf. Means per species and locality are given (with one standard deviation given in parentheses), and sample sizes are indicated at the top of each column. The willow species are labeled as follows: *myr* = *S. myrsinifolia*, *pen* = *S. pentandra*, *phy* = *S. phylicifolia.* The structure of compounds in uppercase is unknown.

were O-acetylsalicylate derivatives. One compound (Ampell on Table 1) in *S. phylicifolia* gave a similar HPLC spectrum as authentic ampelopsin. It was thus considered to be an ampelopsin derivative.

Leaf toughness and shoot length.—We collected two shoots per plant on 23 July at the Höytiäinen Canal and on 29 July at the Old Field site. To measure leaf toughness, we punctured the first fully expanded leaf with a hand-held penetrometer (area 5.5 mm^2) three times between the margin and midvein. Before analysis, we converted the values to kilopascals per square millimeter and took the average three punctures (Kearsley and Whitham 1989). We measured shoot length from the base to the terminal bud. The larval performance of some willow-feeding herbivores is positively related to shoot length (Price et al. 1987*a*, *b*).

Nitrogen content.—For this analysis, we used the same shoots that had been collected for the leaf toughness measurements. Shoots were initially air dried at room temperature. We dried them for 48 h at 70°C before chemical analysis. We used a high speed mill to grind leaves (centrifugal mill type ZMI, Retsch RS1 Schwingscheibenmühle, Haan, Germany). Chemical analysis was conducted with a CHN analyzer (Leco CHNS 932) at the Laboratory for Organic Chemistry at the Swiss Federal Institute of Technology Zürich.

Analysis.—We tested for species differences in plant characteristics with two-way ANOVAs, where species was considered a fixed effect and blocks were considered random effects. When necessary, we used quasi F ratios calculated by the SAS program for significance testing (Kirk 1982). We also estimated the proportion of the variation explained by the effects using PROC VARCOMP (restricted maximum likelihood option). The SAS program was used in all analyses (SAS 1988).

We also conducted correlation analyses. The total of SG and non-SG phenolics was compared to nitrogen content, water content, average shoot length, and leaf toughness. To reduce mean/variance relationships, we log-transformed the amounts of the phenolics and shoot lengths before analysis. For the correlation analysis, each species was analyzed separately, and we used the sequential Bonferroni procedure to adjust significance levels, based on fifteen comparisons made per species and locality (Rice 1989).

Host preference

We determined the feeding preference of *P. vitellinae* adults in laboratory choice tests. Adults were collected on *S. myrsinifolia* at the Höytiäinen Canal. Each beetle was offered foliage from *S. myrsinifolia*, *S. pentandra*, and *S. phylicifolia.* The foliage was obtained from five clones per host species. The tests were conducted on 6–7 July 1993. We placed three leaves (one leaf from each willow species) onto moist paper towel and covered them with a plexiglas plate. This plate contained three holes (each 16 mm in diameter) that exposed an equal amount of each leaf (Kolehmainen et al. 1995). The bottom of a petri dish (9 cm diameter) was placed upside down onto each plate and one beetle was placed inside. After 24 h, leaves were replaced by new ones from different clones, and the positions of the leaves were changed. The choice test was completed after the second 24-h trial. These choice tests were conducted in a temperature chamber kept at 20°C (14:10 L:D) at the University of Joensuu in Finland. To measure leaf areas eaten by beetles, we used an image analysis system with a Macintosh computer and the NIH Image program (available by anonymous ftp).[4]

We analyzed the preference data as a randomized

[4] URL = zippy.nimh.nih.gov

blocks design. First we took averages of the area fed in the two feeding trials and log-transformed the data. Then we conducted an ANOVA, where each beetle represented a block and the host was the treatment. This design represents an extension of the paired comparisons *t* test to three treatments (Sokal and Rohlf 1981). It is thus appropriate for feeding choice tests, where the amount fed on each host species depends partly on the amount fed on other species.

Larval secretion

To measure the amount of larval secretion produced on the three hosts, we collected third instar *P. vitellinae* larvae at the Höytiäinen Canal on 2 August 1993 and placed them individually on leaves of five plants per willow species. After 5 d, we measured the size of their secretion droplets. Under a dissecting scope, we prodded larvae at the anterior end until they displayed their secretion glands. While the droplets were exposed, we made a computer image of them. To determine the volume of droplets, we measured their diameters along the major and minor axes. We considered the droplets to be ellipsoid forms and calculated their volume using the following equation: $(4/3)\pi abc$, where a = minor radius, b = major radius, and c = the average of a and b. Before analysis, we obtained the sum of the volumes of the individual droplets for each larva and log-transformed this value. We analyzed the data with nested ANCOVAs, using larval body length as a covariate.

Larval growth in the laboratory

We collected early second-instar *P. vitellinae* larvae from *S. myrsinifolia* at the Höytiäinen Canal and reared them on the same willow species in the laboratory for 1 d. Then we weighed them to the nearest 0.01 mg and placed them singly onto a leaf in a vial of fresh water. Two larvae were used per plant, for a total of 170 larvae. Larval growth was tested on the same willow clones that were used for the survival experiments in nature. Petri dishes were arranged in randomized positions in a growth chamber (20°C, photoperiod 14:10 L:D). We re-weighed them 48 h after beginning the experiment. If a leaf started to dry by the end of the experiment (17 cases), we dropped it from analysis. Larvae that died (21 cases), consumed <10 mm^2 (three cases), or that had been observed off their experimental leaves were omitted.

We calculated relative larval growth by the following equation: $[\ln(m_f) - \ln(m_i)]/[t_f - t_i]$, where m_i was the initial mass, m_f the final mass, and $[t_f - t_i]$ was the time between the measurements. We used image analysis to measure leaf area eaten by larvae. To compare larval growth and area eaten on each willow species, we conducted two-way ANOVAs, which included species, blocks, and a block-by-species interaction. To avoid statistical problems that occur with missing cells in ANOVA (Shaw and Mitchell-Olds 1993), we dropped blocks from the analysis where both observations from one plant had been omitted.

We conducted multiple regressions of host plant characteristics vs. larval growth. The six independent variables were total SGs, non-SG phenolics, nitrogen content, water content, leaf toughness, and shoot length. The regressions were run separately per host species. To select the best regression model, we compared coefficients of determination (r^2) for all possible combinations of the independent variables (SAS PROC REG, RSQUARE option). We tested the significance of the partial *F* values to determine how many variables to include in the model [cf. Kleinbaum et al. 1988: 132–136].

Larval survival in nature

To determine larval survival on each host under natural conditions, we conducted a survival experiment at both localities. We measured survival and developmental rate of larvae from the first until the late third instar. The same willow clones were used as in the laboratory studies. In the field experiment, we measured survival on four branches per willow clone. To two branches, we added a sticky resin that excludes crawling arthropod predators such as ants. This partial predator exclusion determined whether crawling predators were important sources of mortality to the beetles.

Before the experiments, we collected 50–70 groups of young larvae from >20 *S. myrsinifolia* clones at the Höytiäinen Canal. Most of these groups consisted of newly hatched first-instar larvae from one egg clutch. We placed groups of larvae onto branches in their original group sizes (306 groups of 4–7 larvae and 25 groups outside this range). In all, we measured the survival of 1739 beetle larvae on 83 willow clones. Preliminary ANCOVAs showed that there was no relationship between initial group size and larval survival (Höytiäinen Canal $F_{1,74} = 1.4$, $P > 0.23$, Old Field site $F_{1,89} = 0.0$, $P > 0.99$).

Over the next 3 wk, we counted surviving larvae and recorded their larval instar. At the Höytiäinen Canal, larvae were placed onto leaves on 21 July, and survivors were counted seven times (days 3, 5, 8, 10, 13, 17, 22). At the Old Field site, we began the experiment on 28 July 1994, and we counted survivors six times (days 2, 4, 6, 12, 16, 21). Larvae do not naturally disperse from their natal host plant until just before pupation (Görnandt 1955). Thus, we considered the disappearance of a larva to be a mortality event.

In the late third instar, *P. vitellinae* larvae crawl to the ground to pupate. On day 13 at the Höytiäinen Canal and day 16 at the Old Field site, we observed the first large third-instar larvae on the predator exclusion branches just above the band of resin. These were signs that some larvae had completed their development and were beginning to leave the plants. We omitted these and the subsequent counts from the survival analysis.

TABLE 2. Analyses of plant nutritional characteristics and larval growth of *P. vitellinae.*

Locality and host species	Nitrogen content (mg/g dry mass)			Water content (100 × dry mass/wet mass)			Leaf toughness (kPa)		
	N	Mean	(1 SD)	*N*	Mean	(1 SD)	*N*	Mean	(1 SD)
Höytiänen Canal									
S. myrsinifolia	19	2.12[a]	(0.35)	19	62.4[a]	(2.7)	19	267[a]	(77)
S. phylicifolia	19	1.76[b]	(0.31)	19	57.4[b]	(3.0)	19	285[a]	(96)
Old Field site									
S. myrsinifolia	15	1.80[a]	(0.28)	15	60.0[a]	(3.5)	15	261[b]	(44)
S. pentandra	15	1.47[b]	(0.28)	15	56.5[b]	(2.7)	15	347[a]	(100)
S. phylicifolia	15	1.95[a]	(0.28)	15	59.3[a]	(2.4)	15	250[b]	(112)

Notes: Means and standard deviations (given in parentheses) per species and locality are given. Different superscripts indicate significantly different means. For the Höytiänen Canal, the significance of species differences was determined by ANOVA (Table 3), but for the Old Field site, Student-Newman-Keuls multiple-comparisons tests were used.

Analysis of larval survival.—We analyzed larval survival using survival curves and an index of average survivorship. The index determines the area beneath the survival curve and divides it by the total area if all initial larvae had survived to the last count (Breden and Wade 1987, Rank 1994). It ranges between zero and one. The advantages of this survival index are that it measures mortality over the entire experiment and that its values conform closely to assumptions for parametric statistical analysis. A potential disadvantage is that disparate survival curves could produce a similar value for average survivorship. For example, high early mortality and low late mortality could produce a similar value as low early mortality and high late mortality. The survival curves that we obtained on each host species correspond well to the average survivorship (see *Results*, including Figs. 4 and 5). We therefore used average survivorship in all statistical analyses. The ANOVAs included host species, blocks, the partial exclusion treatment, and all possible interactions.

Larval development in nature

Because we had recorded instars of larvae in the survival counts, we also determined their developmental rates on each willow in the field. We calculated the average instar per branch on each date by multiplying the number of larvae in each instar by their instar (first instar = 1, second = 2, or third = 3), and dividing the sum by the number of larvae on the branch. The developmental rate was the slope of the linear regression of the number of days until a count (log-transformed) vs. average instar at that count. We did not calculate the slope for branches where no larva survived to the last two counts. The log-transformed slopes were used as the dependent variable in an ANOVA, which included the same factors as those used in the survival analysis.

Predator observations in the field

We noted any observations of predation on experimental larvae or other *P. vitellinae* larvae at our field sites. In addition, we censused predatory arthropods on experimental branches. The censuses lasted 3 min per branch. At the Höytiäinen Canal, we censused half of the plants on 22 July and the other half on 27 July. At the Old Field site, we made one census on 1 August.

Predator feeding tests

In the summer of 1995, we compared feeding rates of three natural enemies of *P. vitellinae* on chemically defended (reared on *S. myrsinifolia*) vs. undefended beetle larvae (reared on *S. phylicifolia*). These no-choice tests were conducted in 12.5-cm petri dishes. Before each test, the larval secretion was removed and the beetle larvae fed for 24 h on either host. During this period, larvae feeding on *S. myrsinifolia* replenished their secretion, while those feeding on *S. phylicifolia* produced very little secretion. All predators were starved for 24 h before the feeding test.

In the first test, we compared the feeding rate of 27 medium-sized larvae of *P. nigritarsis* Zett. (Diptera: Syrphidae) on two kinds of *P. vitellinae* larvae. Because fly larvae were rare at the Höytiäinen Canal in 1995, we collected them from a nearby population feeding on larvae of the alder leaf beetle *Linaeidea aenea* L. The alder beetle larvae produce a defensive secretion containing cyclopentanoid monoterpenes (Sugawara et al. 1979). During the test, each fly larva was offered five second instar beetle larvae. The number of beetle larvae eaten was measured 10 times over 26 h. We converted the values into the index of average survivorship.

In the second test, we compared feeding rates of 26 *Anthocoris nemorum* L. (Hemiptera: Anthocoridae) adults on larvae feeding on *S. myrsinifolia* or *S. phylicifolia*. Each bug was offered a group of five second-instar beetle larvae feeding on either host. The *A. nemorum* adults had been collected at the Old Field site. We counted the number of surviving beetle larvae after 24 h. In the third test, we compared feeding rates of 24 second-instar *Rhacognathus punctatus* nymphs on 10 *P. vitellinae* larvae feeding on *S. myrsinifolia* or *S.*

TABLE 2. Extended.

Shoot length (cm)			Area eaten (mm^2)			Larval growth rate ($ln(mg_f \cdot mg_i^{-1}) \cdot d^{-1}$)		
N	Mean	(1 SD)	*N*	Mean	(1 SD)	*N*	Mean	(1 SD)
19	9.0	(3.0)[b]	9	38.4	(10.0)[a]	9	0.392	(0.050)[a]
19	13.2	(5.2)[a]	9	37.6	(20.4)[a]	9	0.196	(0.086)[b]
15	7.9	(3.2)[a]	9	32.3	(11.6)[b]	9	0.391	(0.037)[a]
15	9.1	(2.7)[a]	9	47.5	(15.5)[a]	9	0.340	(0.044)[a]
15	11.8	(4.5)[a]	9	28.0	(10.5)[b]	9	0.189	(0.093)[b]

phylicifolia. The *R. punctatus* nymphs had been collected at the Höytiäinen Canal. We counted the number of surviving beetle larvae after 12 and after 24 h. Because the bugs had eaten all *P. vitellinae* by the end of the experiment, we analyzed the proportion surviving after 12 h.

RESULTS

Plant characteristics

Leaf chemistry.—Leaves of the three willow species differed substantially in their phenolic concentrations (Table 1). *Salix myrsinifolia* contained salicortin, salicin, and a salicin derivative. *Salix pentandra* contained high concentrations of O-acetyl salicyl glucosides, but only small amounts of salicin. The total amount of SGs did not differ between *S. pentandra* and *S. myrsinifolia* ($F_{1,14} = 0.05$, $P > 0.8$). *Salix phylicifolia* contained little or no salicin, and no other SGs were present in its leaves. However, it contained moderate amounts of (+)-catechin. It was the only species with gallocatechin and ampelopsin (Table 1). Ampelopsin is a dihydroflavonol that also occurs in the central European willow *Salix hegetschweileri* (Shao 1991, Meier et al. 1992).

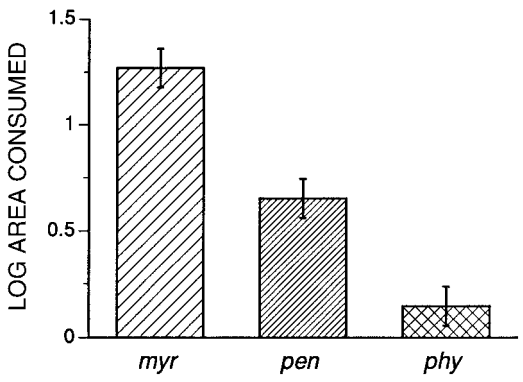

FIG. 1. The feeding preference of *P. vitellinae* adults among *S. myrsinifolia* (*myr*), *S. pentandra* (*pen*), and *S. phylicifolia* (*phy*). All three species were offered simultaneously to the beetles. Values represent log-transformed means of the areas consumed (measured in square millimeters) over two feeding trials ($N = 20$ beetles). Least squares means and their standard errors (±1 SE) are shown.

Plant nutritive quality.—The willow species differed in their nitrogen and water contents (Tables 2 and 3). At the Höytiäinen Canal, *S. myrsinifolia* leaves contained more nitrogen and water than did *S. phylicifolia*, but the shoots of *S. myrsinifolia* were shorter. The two species did not differ in leaf toughness (Tables 2 and 3). At the Old Field site, *S. myrsinifolia* and *S. phylicifolia* contained similar amounts of nitrogen and water. *Salix pentandra* leaves contained less nitrogen and water than did the other two species. Leaf toughness of *S. pentandra* was greater than that of *S. myrsinifolia* or *S. phylicifolia* (Table 2).

Correlations among plant characteristics.—Leaf water and nitrogen contents were always positively correlated. For *S. myrsinifolia* and *S. phylicifolia*, these correlations were highly significant (Table 4). For *S. myrsinifolia*, the SGs were negatively correlated with other phenolics.

Host preference

The *P. vitellinae* adults preferred to feed on *S. myrsinifolia* over *S. pentandra* or *S. phylicifolia* ($F_{2,38} = 38.0$, $P < 0.001$). Very little feeding occurred on *S. phylicifolia* (Fig. 1). Three of the twenty beetles preferred *S. pentandra* over *S. myrsinifolia*, but none preferred *S. phylicifolia*.

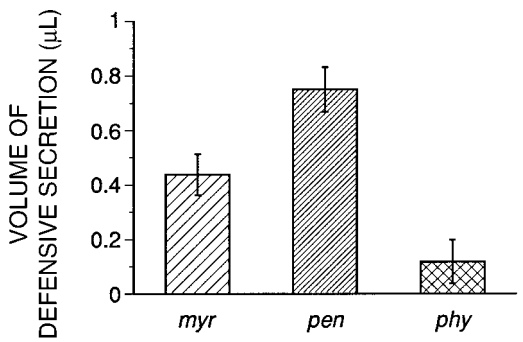

FIG. 2. The volume of larval secretion and the number of droplets produced by third-instar *P. vitellinae* larvae after feeding on *S. myrsinifolia*, *S. pentandra*, and *S. phylicifolia* ($N = 15$ larvae feeding on different host clones). Least squares means and their standard errors (±1 SE) are shown.

TABLE 3. ANOVAs of the results shown in Table 2. The shoot lengths and areas eaten were log-transformed before analysis.

Effect	Höytiänen Canal df	MS	*F*	% of the variation	Old Field site df	MS	*F*	% of the variation
A) Plant nutritional characteristics								
Nitrogen content								
Species	1	1.213	21.4***	35.7	2	0.900	18.1***	41.9
Block	18	0.162	2.9*	30.9	14	0.137	2.8*	21.4
Within cells	18	0.057		33.3	28	0.0497		36.7
Water content								
Species	1	237.7	55.1***	59.7	2	50.5	13.5***	26.8
Block	18	12.3	2.8*	19.3	14	18.0	4.8***	40.9
Within cells	18	4.3		21.0	28	3.75		32.3
Leaf toughness								
Species	1	11 894	1.3	0.7	2	83 797	6.3**	17.3
Block	18	17 571	1.9	19.7	14	22 581	1.7	11.4
Species × Block	18	9 055	1.1	4.1	28	13 353	2.2**	27.3
Within cells	38	8 170		75.5	45	5954		44.0
Shoot length								
Species	1	0.2953	5.4*	10.2	2	0.1966	2.7	5.7
Block	18	0.0829	1.5	11.2	14	0.0595	0.8	0.0
Species × Block	18	0.0551	1.3	10.2	28	0.0729	1.0	0.0
Within cells	38	0.0424		68.4	45	0.0707		94.3
B) Area eaten and larval growth								
Area eaten								
Species	1	0.0117	0.2	0	2	0.1623	5.9**	25.5
Block	8	0.0378	0.6	0	8	0.0388	1.4	3.2
Species × Block	8	0.0610	5.5*	67.6	16	0.0269	0.7	0.0
Within cells	7	0.0110		32.4	12	0.0367		71.3
Larval growth								
Species	1	0.215	54.7***	79.8	2	0.137	31.4***	58.7
Block	8	0.008	2.0	6.7	8	0.007	1.6	0.0
Species × Block	8	0.004	1.6	0.9	16	0.004	0.5	0.0
Within cells	7	0.003		12.6	12	0.008		41.3

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Larval secretion

The *P. vitellinae* larvae produced more secretion on *S. pentandra* than on *S. myrsinifolia* ($F_{2,19} = 8.1$, $P = 0.003$). They produced little secretion on *S. phylicifolia* (Fig. 2). There was no between-plant variation in the volume of secretion ($F_{11,14} = 1.4$, $P = 0.3$). There was no significant relationship between volume of secretion and larval body length ($F_{1,14} = 3.7$, $P = 0.08$).

Larval growth in the laboratory

Larval growth was greatest on *S. myrsinifolia,* intermediate on *S. pentandra,* and lowest on *S. phylicifolia* (Fig. 3, Table 2). The area eaten was greater on *S. pentandra* than on the other two species (Tables 2 and 3). This suggests that feeding efficiency was greater on *S. myrsinifolia* than on *S. pentandra.*

Plant characteristics vs. larval growth.—For *S. myr-*

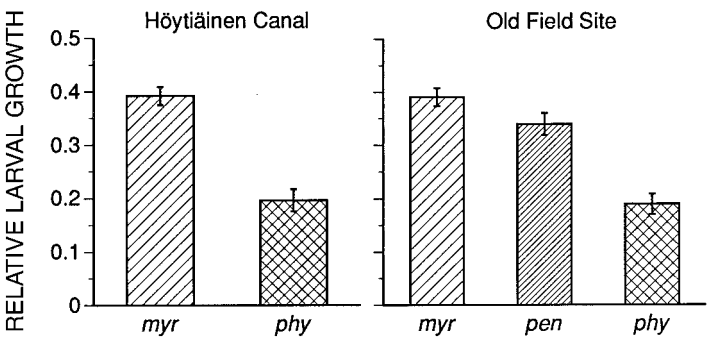

FIG. 3. The growth of *P. vitellinae* larvae on three host species. The data from each locality are shown separately. See Results for an explanation of how relative growth was calculated. Error bars represent ±1 SE.

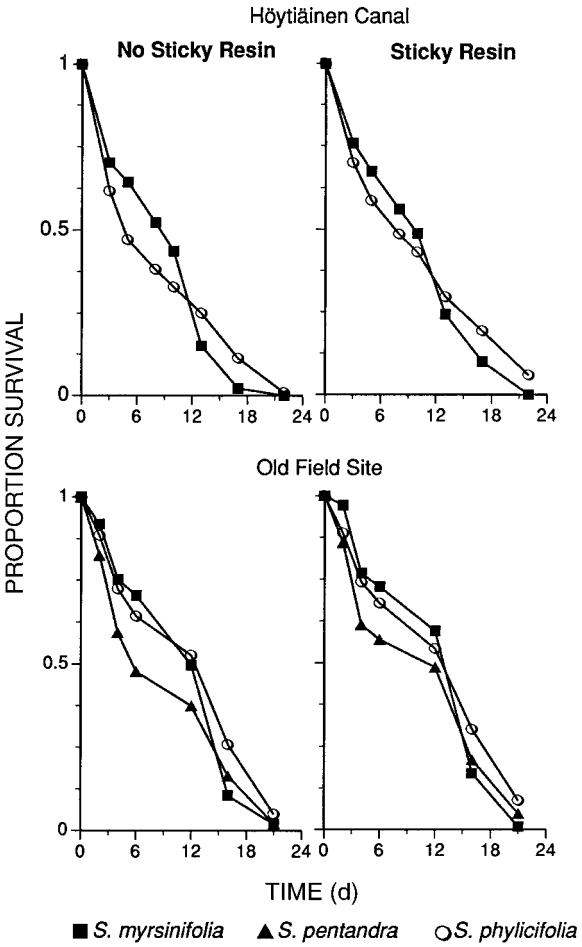

FIG. 4. The proportion survival of *P. vitellinae* larvae on three host species in nature. Data show the entire period of the experiment. Many larvae had reached the late third instar by days 10–12. The decline in numbers after this point was partly caused by emigration of late third-instar larvae for pupation.

sinifolia, larval growth was related positively to leaf water content ($m = 0.006$, $t = 2.9$, $P = 0.006$) and negatively to leaf toughness ($m = -0.0003$, $t = -2.7$, $P < 0.015$). The multiple regression with water content and leaf toughness explained significantly more variation ($r^2 = 0.35$, partial $F_{1,30} = 5.5$, $P < 0.03$) than a model with water content alone. For *S. pentandra,* larval growth was positively related to SG content ($m = 0.122$, $t = 3.0$, $P = 0.01$), but no other plant characteristics were related to growth ($n = 13$, $P > 0.14$ for all comparisons). No plant characteristic was related to larval growth in *S. phylicifolia* ($n = 22$, $P > 0.20$ for all comparisons).

Larval survival in nature

Survival of *P. vitellinae* larvae was similar on both host species at the Höytiäinen Canal (Fig. 4). Mortality was slightly higher during the first 2 d, when most larvae were in the first instar, than during following counts (see Fig. 6). Mortality was slightly higher on branches without sticky resin. At the Old Field site, survival was similar on *S. myrsinifolia* and *S. phylicifolia,* and it was lowest on *S. pentandra* (Fig. 4). Mortality was highest during the first 4 d of the experiment, when most larvae were in the first and early second instars (Fig. 6). Larval survival on branches with sticky resin was similar to that on branches with no resin (Fig. 4).

Average survivorship reflected these patterns closely. At both localities, survivorship differed little between *S. myrsinifolia* and *S. phylicifolia* (Fig. 5). There was, therefore, no significant species effect at the Höytiäinen Canal (Table 5). At the Old Field site, survivorship was significantly lower on *S. pentandra* than on *S. myrsinifolia* or *S. phylicifolia* (Fig. 5, Table 5). The sticky resin treatment had no effect on survivorship in either locality (Table 5).

Larval development in nature

Phratora vitellinae larvae were able to complete their development successfully on all three host species in nature (Fig. 6). On most of the counts, a majority of larvae were in the same instar on each host. However, larvae developed more rapidly on *S. myrsinifolia* than on *S. pentandra* or *S. phylicifolia* (Figs. 6 and 7, Table 5). Development was also more rapid at the Höytiäinen Canal than the Old Field site. Differences between *S. myrsinifolia* and *S. phylicifolia* in develop-

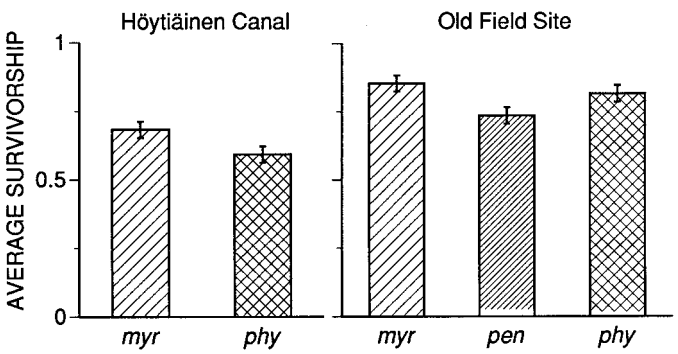

FIG. 5. The average larval survivorship of *P. vitellinae* on three host species in nature. The values for the dependent variable were based on a calculation of the area beneath the mortality curve (*Materials and methods: Analysis of larval survival*). Error bars represent ±1 SE.

TABLE 4. Correlations between plant characteristics.

Plant characteristics	Salicyl glucosides	Other phenolics	Nitrogen content	Water content	Leaf toughness	Shoot length
S. myrsinifolia ($N = 35$)						
Salicyl glucosides	⋯					
Other phenolics	−0.49*	⋯				
Nitrogen content	0.31	−0.21	⋯			
Water content	0.45†	−0.18	0.60**	⋯		
Leaf toughness	0.32	−0.22	−0.20	−0.04	⋯	
Shoot length	0.26	−0.29	0.28	0.27	0.11	⋯
S. pentandra ($N = 15$)						
Salicyl glucosides	⋯					
Other phenolics	−0.10	⋯				
Nitrogen content	0.47	−0.03	⋯			
Water content	0.26	−0.32	0.70†	⋯		
Leaf toughness	−0.44	0.00	−0.28	−0.21	⋯	
Shoot length	0.09	−0.17	0.12	−0.09	0.62	⋯
S. phylicifolia ($N = 34$)						
Salicyl glucosides	⋯					
Other phenolics	−0.24	⋯				
Nitrogen content	−0.20	−0.07	⋯			
Water content	−0.15	0.01	0.71**	⋯		
Leaf toughness	−0.01	−0.12	−0.39	−0.46†	⋯	
Shoot length	0.00	0.13	0.13	−0.10	0.10	⋯

Notes: The probabilities were adjusted by the sequential Bonferroni method, based on 15 comparisons made per species. The salicyl glucosides, phenolics, and shoot lengths were log-transformed before analysis.
† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$.

mental rates were most pronounced at the Höytiäinen Canal (Fig. 6).

Predator observations in the field

We observed 20 instances of predation in the field during our survival experiments in 1993. All these observations occurred on our experimental willows. At the Höytiäinen Canal, we found three beetle clutches with eggs of the predatory syrphid fly, *Parasyrphus nigritarsis.* When the fly eggs hatched, they consumed the beetle eggs. An additional 10 *P. nigritarsis* larvae were found feeding on *P. vitellinae* on other willows. All these *P. nigritarsis* individuals were found on *S. myrsinifolia,* but none occurred on our experimental willows. In laboratory tests, we found that *P. nigritarsis* larvae readily consumed *P. vitellinae* larvae of all stages. They also consumed other leaf beetle larvae but never accepted willow feeding aphids.

TABLE 5. ANOVAs of larval survivorship and development of *P. vitellinae* on three host species in the field. The survivorship was measured until day 10 at the Höytiänen Canal and until day 12 at the Old Field site.

	Höytiänen Canal				Old Field site			
Effect	df	MS	*F*	% of the variation	df	MS	*F*	% of the variation
Larval survival								
Species (S)	1	1.68	1.9	5.1	2	1.364	3.8*	5.8
Block (B)	18	1.08	0.7	⋯	14	0.387	1.5	1.6
Treatment (T)	1	0.83	1.6	0.9	1	0.391	2.7	⋯
S × B	18	0.870	2.6*	20.8	28	0.362	1.5	1.6
S × T	1	0.139	0.4	⋯	2	0.243	0.2	11.4
B × T	18	0.537	1.6	4.9	14	0.145	0.6	0.6
S × B × T	18	0.326	1.1	6.8	28	0.301	1.2	⋯
Within cells	75	0.294		61.4	90	0.243		80.6
Larval development								
Species	1	2.68	13.6**	29.2	2	0.198	3.6*	2.4
Block	15	0.144	0.7	⋯	10	0.134	1.9	6.0
Treatment	1	0.231	2.7	0.8	1	0.000	0.0	⋯
S × B	15	0.199	2.3	16.5	20	0.054	1.1	⋯
S × T	1	0.153	1.8	0.1	2	0.140	2.8	0.5
B × T	15	0.085	1.0	⋯	10	0.654	1.3	⋯
S × B × T	15	0.085	0.9	⋯	20	0.049	0.5	⋯
Within cells	48	0.091		53.3	59	0.098		91.0

* $P < 0.05$, ** $P < 0.01$.

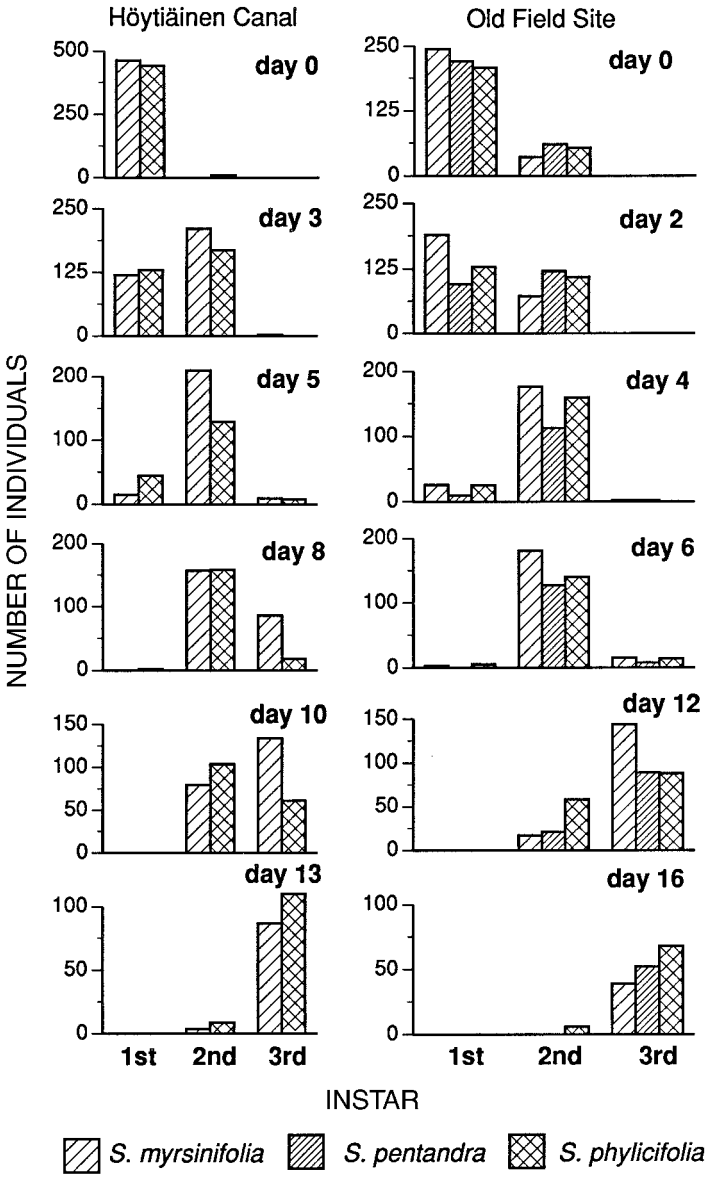

FIG. 6. The numbers of larvae in each instar during the first 13–16 d of the survival experiments. Values for each host species are shown separately.

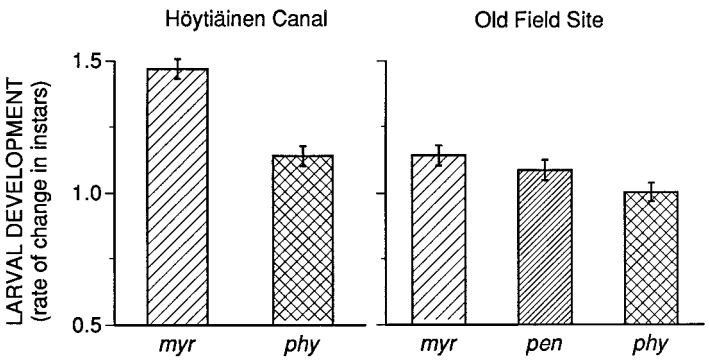

FIG. 7. The larval development of *P. vitellinae* on three host species. The values for the dependent variable were obtained from linear regressions of the average instar on each day vs. the number of days since beginning the experiment. Error bars represent ±1 SE.

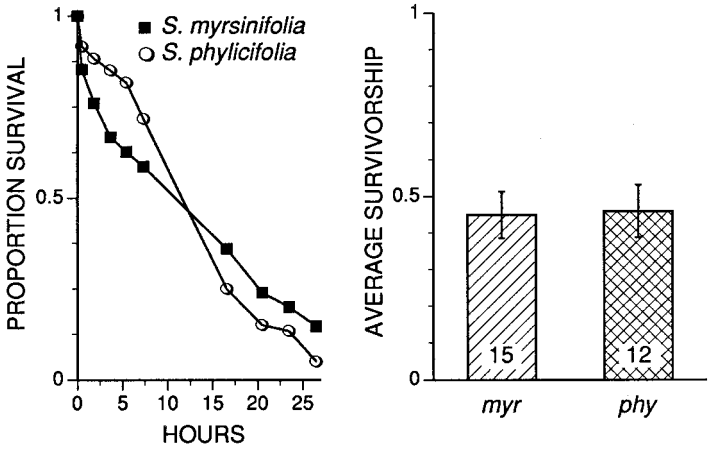

FIG. 8. Feeding rate of *P. nigritarsis* on *P. vitellinae* larvae feeding on *S. myrsinifolia* vs. *S. phylicifolia.* This was a no-choice test. The number of predator individuals is indicated. Five second-instar beetle larvae were offered to each fly larva. Left: The proportion of beetle larvae surviving over time. Right: The average survivorship of beetle larvae, calculated from the data shown on the survival curve. Error bars represent ±1 SE.

At the Old Field site, we observed six adults of the predatory bug, *Anthocoris nemorum* L., feeding on first- and second-instar *P. vitellinae* larvae. Four of these were observed on *S. myrsinifolia,* one on *S. pentandra,* and one on *S. phylicifolia.* We also observed a lacewing larva (Neuroptera: Chrysopidae) feeding on a second-instar *P. vitellinae* larva on *S. pentandra.* We did not observe ants or ladybird beetle larvae feeding on *P. vitellinae,* nor did we observe any aggressive behavior of ants towards beetle larvae.

During our predator censuses of the experimental plants, we found a similar overall predator abundance at the two localities (0.07 predators/branch at the Höytiäinen Canal [excluding mites that were too small to eat beetle larvae], vs. 0.06 predators/branch at the Old Field site). The willow species differed little in predator abundance. Some ants and coccinellids were also present. At the Old Field site, the most common predators were bugs, usually *A. nemorum* adults. Ants and chrysopid larvae were also present. No ants were observed on experimental branches that had been isolated with sticky resin.

Predator feeding tests

The *P. nigritarsis* larvae consumed all beetle larvae that had been offered to them within 32 h. The average survivorship of beetle larvae was equal on both host species ($F_{1,25} = 0.01$, $P = 0.91$). During the first 12 h of the test, *P. nigritarsis* larvae consumed more beetle larvae feeding on *S. myrsinifolia* than larvae feeding on *S. phylicifolia.* This pattern was reversed during the second period (Fig. 8).

The *A. nemorum* adults also fed readily on beetle larvae. Although there was a slight trend for higher beetle survival on the SG-poor *S. phylicifolia* (Fig. 9), the difference in survival was not statistically significant ($F_{1,24} = 1.3$, $P = 0.27$). Most beetle larvae were consumed within 24 h. The *Rhacognathus punctatus* nymphs consumed all first-instar *P. vitellinae* larvae within 24 h, and the proportion of the *P. vitellinae* that survived the first 12 h was roughly equal on both willow species (Fig. 9, $F_{1,22} = 0.6$, $P = 0.45$).

DISCUSSION

In this study, we found that *P. vitellinae* adults preferred two SG-rich willow species (*S. myrsinifolia* and

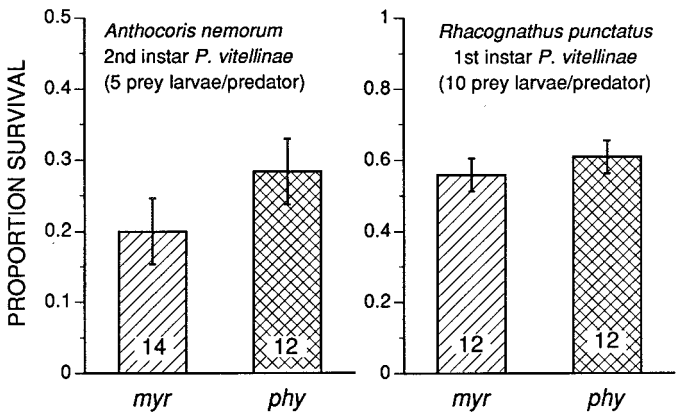

FIG. 9. Feeding rate of two predatory bugs on *P. vitellinae* larvae feeding on *S. myrsinifolia* vs. *S. phylicifolia.* This was also a no-choice test. The proportion survival of the beetle larvae is shown in both graphs. Error bars represent ±1 SE.

S. pentandra) over the SG-poor *S. phylicifolia.* We also found that beetle larvae grew faster on the beetle's preferred host, *S. myrsinifolia,* than on *S. pentandra,* and that they grew much more slowly on *S. phylicifolia.* The larvae did not survive better on *S. myrsinifolia* than on *S. phylicifolia* in nature, and their survival was lowest on *S. pentandra.* In addition, the natural predators of *P. vitellinae* larvae fed equally well on beetle larvae feeding on *S. myrsinifolia* and *S. phylicifolia.* These results suggest that *P. vitellinae* has not specialized on willows where it obtains enemy-free space. Rather, it prefers the SG-rich host species, which are the most suitable for larval growth. It also prefers the SG-rich host where the larvae survived best (*S. myrsinifolia*) over the other SG-rich host (*S. pentandra*).

The feeding preference for SG-rich willows observed in this study corresponds with other studies of the host selection of *P. vitellinae* (Rowell-Rahier 1984*b*, Tahvanainen et al. 1985, Denno et al. 1990). Like other SG-using beetles (Matsuda and Matsuo 1985, Rank 1992), *P. vitellinae* is stimulated to feed by salicyl glucosides (Tahvanainen et al. 1985, Soetens and Pasteels 1994, Kolehmainen et al. 1995). However, Kolehmainen et al. (1995) showed that salicyl glucosides from *S. myrsinifolia* stimulated adult feeding more than those from *S. pentandra.*

Larval growth in the laboratory was related to developmental rate in the field. Neither measure of larval performance was related to larval survival in nature. Both survival and growth should be important determinants of host suitability to herbivores. Larval growth may be important for two reasons. First, rapidly developing larvae are exposed to predators for a shorter period. Second, for many herbivores including leaf beetles, larval development is closely related to fecundity (Baur and Rank 1996). The more rapid growth we observed on *S. myrsinifolia* and *S. pentandra* probably indicates that fecundity would also be greater on these two hosts than on *S. phylicifolia.*

Thus the host preference of *P. vitellinae* among these willow species appears to be adaptive. The preferred host, *S. myrsinifolia,* was the only one where both growth and survival were high. On *S. phylicifolia,* survival was high while growth was low, while on *S. pentandra,* survival was low while growth was high. If the fecundity difference outweighs the difference in survival, or if fecundity influences herbivore fitness more strongly than survival, then *S. pentandra* may be a more suitable host than *S. phylicifolia* despite the lower survival of *P. vitellinae* on it. An alternative hypothesis is that the use of *S. pentandra* is an artifact of its preference for hosts containing salicyl glucosides. We do not favor this hypothesis, because Kohlemainen et al. (1995) have shown that *P. vitellinae* adults prefer the salicyl glucosides found in *S. myrsinifolia* over those found in *S. pentandra.* Thus through laboratory and field experimentation, we have detected a relationship between the host preference and the larval performance of this beetle that would not have been observed without both kinds of data.

There was no effect of predator exclusion (sticky resin) on larval survival of *P. vitellinae,* despite the fact that the resin posed a clear barrier to insect movement. No ants were observed on branches that had been isolated with resin, and its presence hindered beetle larvae from leaving the host plant to pupate. The lack of a difference in larval mortality between predator exclusion and control branches suggests that crawling predators, such as ants, had a minimal effect on larval survival. Many previous studies of the effectiveness of the larval defensive secretion used ants as predators (Wallace and Blum 1969, Matsuda and Sugawara 1980, Pasteels et al. 1983, Pasteels et al. 1988, Kearsley and Whitham 1992). However, ants were not mentioned as larval predators in field studies of natural enemies of four different SG-using beetles (Devantoy 1948, Smereka 1965, Burkot and Benjamin 1979, Rank 1994).

We found that the natural predators of *P. vitellinae* were successful at attacking chemically defended larvae on an SG-rich willow. Their feeding rates were not significantly different on beetle larvae on *S. myrsinifolia* and on *S. phylicifolia.* Some predators, such as *Parasyrphus nigritarsis,* appear to specialize on leaf beetles with external defensive glands. Instead of being repelled by the defensive secretion, *P. nigritarsis* larvae are attracted to it (Rank et al. 1996). On the other hand, the secretion was also ineffective against two generalist bug predators, *Anthocoris nemorum* and *Rhacognathus punctatus.* These predators are common, occur over a broad geographic range, and feed on many arthropod species (Hill 1957, Lange 1987, Dolling 1991). They probably avoid direct contact with the defensive secretions of leaf beetles by puncturing them with their beaks away from defensive secretion glands. Olmstead and Denno (1993) found that hemipteran predators with similar beaks were effective at circumventing defensive shields of tortoise beetle larvae.

Symmorphus and *Parasyrphus* species are associated with natural populations of SG-using beetles throughout the Northern Hemisphere. Hemipteran bugs and certain coccinellid beetles also frequently feed on the beetle larvae. Most enemies of SG-using beetles also feed on larvae of related leaf beetles that possess external defensive glands but do not obtain their chemical defense from the host plant (Rank et al. 1996). However, they avoid other more distantly related leaf beetle larvae (family Galerucinae) that contain noxious compounds in their hemolymph (Baur and Rank 1996). Thus it appears that SG-using leaf beetles and their relatives may be more palatable to a broad range of predators than are many other chrysomelids.

The relationships between SG-using leaf beetles and their specialist predators (*Symmorphus* sp., *Parasyrphus* sp.) probably predate the herbivores' use of host plant salicyl glucosides for their defensive secretion (Cumming 1989, Rotheray and Gilbert 1989). It ap-

pears that the beetles' most important predators have adapted to the chemical defense of their prey, just as beetles have adapted to repellent compounds in their host plants. These relationships between willow leaf beetles and their specialist predators, which have only been discovered through careful field observations, reveal distinct parallels to the relationships between toxic host plants and the herbivores that evolved to overcome the plant defenses.

The present study parallels another study of larval survival in an SG-using leaf beetle. The Californian SG-using leaf beetle *Chrysomela aeneicollis* preferred SG-rich willows over SG-poor ones (Rank 1992), but larvae did not usually survive longer on those willows in nature (Rank 1994). In addition, larval secretions of *C. aeneicollis* had no effect on a *Parasyrphus* species that was one of its most abundant natural predators (Rank and Smiley 1994). The larval defensive secretion had little effect on another important predator of *C. aeneicollis,* the trap nesting wasp *Symmorphus cristatus.* Despite differences between the predator communities of *C. aeneicollis* and *P. vitellinae,* neither study supported the predictions of the predation hypothesis for SG-using leaf beetles. It appears that the larval defensive secretion is less effective against natural enemies than has been supposed previously.

In summary, we found that larval survival of *P. vitellinae* was related to plant factors other than the SG chemistry of their hosts. The antipredator benefits of the larval defensive secretion were small when the feeding behavior of natural predators was tested. The predation hypothesis is probably insufficient to explain host preferences of *P. vitellinae.* Our results emphasize the importance of testing adaptational hypotheses under natural conditions. The predation hypothesis was based on a plausible, but untested adaptive scenario for the function of this defensive secretion. Other hypotheses about its adaptive significance need to be examined more closely. For example, its repellence to conspecific females may make them avoid host plants with high densities of larvae in nature (Hilker 1989). This would ensure that their offspring do not experience overcrowding during larval development.

Acknowledgments

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