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## Host-plant effects on larval survival of a salicin-using leaf beetle *Chrysomela aeneicollis* Schaeffer (Coleoptera: Chrysomelidae)

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**Abstract** Several species of willow leaf beetles use host-plant salicin to produce a defensive secretion that consists of salicylaldehyde. Generalist arthropod predators such as ants, ladybird beetles, and spiders are repelled by this secretion. The beetle larvae produce very little secretion when they feed on willows that lack salicylates, and salicin-using beetles prefer salicylate-rich willows over salicylate-poor ones. This preference may exist because the larvae are better defended against natural enemies on salicylate-rich willows. If this is true, the larvae should survive longer on those willows in nature. However, this prediction has not been tested. I determined the larval growth and survival of *Chrysomela aeneicollis* (Coleoptera: Chrysomelidae) on five willow species (*Salix boothi*, *S. drummondiana*, *S. geyeriana*, *S. lutea*, and *S. orestera*). These species differed in their salicylate chemistries and in leaf toughness but not in water content. The water content varied among the individual plants. Larval growth of *C. aeneicollis* did not differ among the five species in the laboratory, but it varied among the individual plants and it was related to the water content. In the field, *C. aeneicollis* larvae developed equally rapidly on the salicylate-poor *S. lutea* and on the salicylate-rich *S. orestera*. Larval survival was greater on *S. orestera* than on *S. lutea* in one year (1986), but there was no difference between them during three succeeding years. In another survivorship experiment, larval survival was low on the medium-salicylate *S. geyeriana*, but high on the salicylate-poor *S. boothi*

and on *S. orestera*. Larval survival in the field was related to the larval growth and water content that had been previously measured in the laboratory. These results showed that the predicted relationship between the host plant chemistry and larval survival did not usually exist for *C. aeneicollis*. One possible reason for this was that the most important natural enemies were specialist predators that were unaffected by the host-derived defensive secretion. One specialist predator, *Symmorphus cristatus* (Hymenoptera: Eumenidae), probably caused much of the mortality observed in this study. I discuss the importance of other specialist predators to salicin-using leaf beetles.

**Key words** *Chrysomela/Salix* interaction  
Larval performance · Specialist predators · Predation  
Larval survivorship

### Introduction

Insect herbivores should prefer the host plants that are most suitable for their survival and reproduction in nature (Futuyma and Moreno 1988; Jaenike 1990; Via 1990). The host plant can affect herbivore fitness directly or indirectly by affecting the success of other species that interact with the herbivore (Price et al. 1980; Thompson 1988; Jaenike 1990). These indirect effects may be caused by plant effects on pathogens (Hunter and Price 1992), other herbivores (Hunter 1990; Strauss 1991), predators (Smiley 1978; Price et al. 1980; Bernays and Graham 1988; Bernays 1988), or parasitoids (Vet and Dicke 1992; English-Loeb et al. 1993). Although many studies have investigated the relationship between host plant preference and larval performance (Chew 1980; Rausher 1980; Holdren and Ehrlich 1982; Roininen and Tahvanainen 1989; Dodge et al. 1990; Fox 1993), few have been conducted under natural conditions (Damman 1987; Lawrence 1990; English-Loeb et al. 1993; Hanks et al. 1993). This is unfortunate because host suitability in nature is not always related to suit-

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ability in the laboratory (Price et al. 1980; Thompson 1988; Jaenike 1990).

Larvae of the willow leaf beetle *Chrysomela aeneicollis* Schaeffer produce a defensive secretion, salicylaldehyde, which they derive from salicin in the leaves of their host plants (Pasteels et al. 1983). This secretion probably evolved from the autogenous defensive secretions produced by related chrysomelid beetles (Pasteels et al. 1982, 1984). Salicin-using beetles depend on the host plant for the secretion, but many willow species contain little salicin or other salicylates (Palo 1984; Julkunen-Tiitto 1986; Meier 1988). The beetle larvae produce little or no defensive secretion when they feed on salicylate-poor willows (Rowell-Rahier and Pasteels 1982; Pasteels et al. 1983; Denno et al. 1990). Like other salicin-using leaf beetles (Rowell-Rahier 1984; Tahvanainen et al. 1985; Denno et al. 1990), *C. aeneicollis* prefers salicylate-rich willows over salicylate-poor ones (Rank 1992).

Several researchers have suggested that the beetles' preference for salicylate-rich willows exists because the larvae can defend themselves better against natural enemies on them (Smiley et al. 1985; Pasteels et al. 1988; Denno et al. 1990). Predatory arthropods such as ants (Wallace and Blum 1969; Pasteels et al. 1983, 1988), ladybird beetles (Denno et al. 1990; Smiley 1991), and spiders (Palokangas and Neuvonen 1992), are deterred by the salicylaldehyde secretion in the laboratory. Additionally, Smiley et al. (1985) found that the survival of third-instar *C. aeneicollis* larvae was greater on two salicylate-rich willow clones than on two-salicylate poor ones in nature. However, apart from this short-term experiment, no studies have compared the larval survival of salicin-using leaf beetles on willows that differ in their salicylate chemistries.

The objective of this study was to determine whether *C. aeneicollis* larval performance was greater on salicylate-rich willows under field conditions. I asked several questions: (1) How do the leaves of five potential host species vary in salicylate content, water content, and leaf toughness? (2) How does this variation affect *C. aeneicollis* larval growth in the laboratory? (3) Does larval survival in nature differ between a salicylate-rich species and a salicylate-poor one? (4) Does larval survival differ among three willow species with different salicylate chemistries? (5) Is larval survivorship related to larval growth in the laboratory? Additionally, I determined the abundance of *C. aeneicollis* adults at the field sites in the spring of 1987 and 1988. Finally, I recorded any observations of predation on *C. aeneicollis* eggs and larvae.

## Natural history and the system

*Chrysomela aeneicollis* occurs throughout western North America (Brown 1956). In the Sierra Nevada mountains of California, it feeds on several willow species and on quaking aspen (*Populus tremuloides* Michx.). The host species occur together in the wild, but they

have different elevational ranges (Smiley and Rank 1991). I studied *C. aeneicollis* populations at two sites along Big Pine Creek (37°7'N, 118°29'W), the Cabin Bog (2840 m) and the Falls Site (2940 m). *Salix orestera* Schneid. and *S. lutea* Nutt. are found in the Cabin Bog. At the Falls Site, *S. boothi* Dorn, *S. drummondiana* Barratt, *S. geeyeriana* Anderss., and *S. orestera* are common, but *S. lutea* is rare (Smiley and Rank 1991). The Cabin Bog consists of a shaded wet habitat while the Falls Site has sandy areas, pine woodlands, and open bogs.

A gas chromatography analysis of the willow leaves found that they differed greatly in their salicylate chemistries. *Salix orestera* had high levels of three salicylates: tremulacin, salicortin, and salicin. *Salix geeyeriana*, *S. boothi*, and *S. drummondiana* had moderate to low concentrations of salicortin and salicin. *Salix lutea* contained no detectable salicylates (R. Julkunen-Tiitto, personal communication). The concentrations of salicin and salicortin were correlated in the four species that contained salicylates ( $r=0.87$ ,  $n=8$ ,  $P=0.005$ , log-transformed data), and the salicin content was correlated with the total salicylate content ( $r=0.89$ ,  $n=8$ ,  $P=0.003$ ).

## Natural enemies

Predators of *C. aeneicollis* eggs and larvae included mites, spiders, lacewings (Neuroptera: Chrysopidae), and bugs (Hemiptera: Pentatomidae). Parasitism was rare, but two parasitoids were present: a fly (Diptera: Tachinidae) and *Schizonotus latus* Walker (Hymenoptera: Pteromalidae). The most common predators were a specialist fly larva and a trap nesting wasp (Smiley and Rank 1986). *Parasyrphus melanderi* Curran (Diptera: Syrphidae) oviposits on *C. aeneicollis* clutches. The fly larvae feed exclusively on *C. aeneicollis* eggs and larvae (Rank and Smiley in press). *Symmorphus cristatus* Saussure (Hymenoptera: Eumenidae) provisions its nests with *C. aeneicollis* individuals. This wasp feeds on other *Chrysomela* species elsewhere in North America (Smereka 1965; Krombein 1967).

Lady-bird beetles (Coleoptera: Coccinellidae) and ants were never observed feeding on *C. aeneicollis*. Lady-bird beetles feed on *Chrysomela* species elsewhere (Burkot and Benjamin 1979; Penev and Ovcharov 1992), but they were rare in Big Pine Creek. Ants were present at the study sites, but they were rarely found on the willow foliage. Smereka (1965) reported that ants consumed the eggs but not the larvae of *Chrysomela crotchii* Brown in Ontario.

## Materials and methods

### Plant characteristics

#### *Leaf toughness and water content*

I collected leaf samples from *S. boothi*, *S. drummondiana*, *S. geeyeriana*, *S. lutea*, and *S. orestera* in July 1989 (at least six clones per

species). For all plant measurements, I used the first fully expanded leaf on the shoot. The first expanded leaves are similar to the basal leaves in these montane willows, because the shoots grow rapidly during the spring and slowly over the summer. To determine the leaf toughness, I punctured the leaf three times between the leaf margin and the mid-vein with a hand-held penetrometer (area 5.5 mm<sup>2</sup>). I subsequently converted the values to kilopascals per square millimeter (Kearsley and Whitham 1989). To determine the water content, I measured the fresh and dry mass of three leaves per plant.

#### Salicylate index

To obtain an index of the leaf salicylates, I conducted thin-layer chromatography of leaf samples in water extracts. The samples were collected on July 10–15 and on August 16–18 1989. They were left to dry at room temperature. For the analysis, I included leaves from three shoots per plant (19–21 mg cut into 1-mm strips). With an image analysis system (JAVA, Jandel Scientific) I compared the intensities of the sample spots to salicin standards run on the same plate. This salicylate index reflects the amount of salicin and other salicylates that have degraded into salicin (Lindroth and Pajutee 1987; Julkunen-Tiitto and Tahvanainen 1989). Although the salicylate index does not distinguish between salicin and other salicylates, its values were highly correlated with the salicin content obtained by gas-liquid chromatography analysis of the same plants (Spearman's  $r=0.89$ ,  $n=10$ ,  $P=0.0006$ ), and to the total salicylates (Spearman's  $r=0.92$ ,  $n=10$ ,  $P=0.0001$ ).

#### Larval growth

##### Laboratory growth experiment

I obtained first-instar *Chrysomela aeneicollis* larvae from a population along Bishop Creek (37°11'N, 118°32'W), and I reared them on *Salix lasiolepis* until they molted. Then I weighed them and placed each one onto a leaf kept in a vial of fresh water in a petri dish. The dishes were arranged in randomized positions in a Percival incubator (20°C, photo period 14:10 L:D). At 8–14 h intervals, I recorded whether the larvae were still on their leaves and I re-weighed them 48 h after the initial weighing. To determine the relative growth rate ( $r$ /day), I calculated  $[\ln(m_f) - \ln(m_i)] / [t_f - t_i]$ , where  $m_i$  was the initial mass,  $m_f$  the final mass, and  $[t_f - t_i]$  was the interval between the two measurements. Any larva that had been observed away from its leaf was omitted from the analysis. Most of these larvae appeared to be in poor condition. After the growth experiment, I reared the larvae on the same host species to determine their survival in the laboratory.

##### Larval development in the field

I placed first-instar larvae on four *S. lutea* and four *S. orestera* clones, and I recorded the developmental stages of the survivors over the summer. These data were taken during the survival experiment described below. The stages included the three larval instars, the prepupal third-instar larvae attached to the leaves, the pupae, or the pupal skins that indicated that an adult had emerged. To determine the developmental rates of the larvae, I conducted frequency tests of the number of individuals in each stage on (1) the two host species, and (2) among the plants within each species. For most frequency tests, the probabilities were based on  $G$ -tests, but when any expected values were less than 4, exact probabilities were calculated. (There were two cases where  $G$ -tests were used when an expected value was 4 because the exact probability could not be computed.) The significance values were adjusted by the number of tests made within each year, using the sequential Bonferroni method (Rice 1989).

#### Larval survivorship in nature

##### Survival over the summer on *S. lutea* and *S. orestera*

I compared larval survival on four *S. orestera* and four *S. lutea* individuals growing wild in the Cabin Bog. Seven of the eight plants were used in all three years (one *S. lutea* individual was eliminated and a different one was added in 1987). To isolate the branches, I clipped the surrounding foliage and added Tree Tanglefoot, a sticky resin that *C. aeneicollis* larvae do not cross, to the base. I added newly hatched larvae to each branch in several group sizes (2, 5, 10, and 20 larvae). There were three replicates of each group size per plant. Any larvae feeding on other branches were left undisturbed. I counted the number of survivors 5 times in 1986 (days 5, 21, 33, 44), 4 times in 1987 (days 2, 10, 13, 31), and 11 times in 1988 (days 1, 3, 4, 5, 6, 9, 12, 21, 23, 25, 46). Because the larvae normally do not fall off the plant (Smiley and Rank 1986), I considered their disappearance a result of mortality. Although this method excluded crawling predators, including *Parasyrphus melanderi*, the larvae were still exposed to all the flying predators and to spiders.

I used an index of average survivorship to analyze these data (Breden and Wade 1987, 1989). The average survivorship equalled the product of the average number of larvae found on two successive counts and the number of days between those counts, summed over the entire experiment and divided by the product of the initial number of larvae and the total number of days in the experiment. This survivorship index measures the mortality over the entire experiment rather than the proportion surviving between any two intervals. It conforms better to assumptions of the ANOVA than the proportion survival (Breden and Wade 1987). I analyzed the 1986 survivorship experiment separately from the experiments conducted in 1987 and 1988, because the experimental design would have been too unbalanced if all three years had been combined [one tree was switched and a new group-size treatment level (10 larvae) was added in 1987]. I considered the plants as random factors in the ANOVA, but the host species, group sizes, and years were classified as fixed factors. The values were weighted by the initial group size to correct for the inequality of variances among the group sizes (Neter et al. 1985).

##### Survival of third-instar larvae on *S. lutea* and *S. orestera* in 1989

I chose 12 pairs of willows consisting of one *S. lutea* and *S. orestera* clone growing within 2 m of each other, and I isolated two branches per plant. Then I added five third-instar larvae to each branch. The survivors were counted on days 2, 3, 4, and 5. Thus, in this experiment, more plants were included than in the earlier comparisons (24 versus 8 individuals), and a block design was used. In the analysis, I considered the host species a fixed effect and the pairs of willows a random effect.

##### Survival on *S. boothi*, *S. geyeriana*, and *S. orestera* in 1989

I selected willows in three species pairs: *S. boothi* versus *S. geyeriana*, *S. boothi* versus *S. orestera* and *S. geyeriana* versus *S. orestera*. Five third-instar larvae were added to each branch, and I counted the number of survivors four times (days 1, 2, 3, 5). This experiment ran simultaneously with the comparison between *S. lutea* and *S. orestera* at the Cabin Bog for 3 of the 5 days. The ANOVA tested for differences among the three species-pairs, whether survival was generally higher on the species with more salicylates (salicylate-rank effect), and whether the difference between the higher and lower salicylate species depended on the species pair (salicylate-rank by species pair interaction).

Using the data from both localities and all four species, I conducted a multiple regression analysis of water content, leaf toughness, larval growth, and the salicylate index (August measurement) on larval survivorship. If both plants from a pair had been

**Table 1** Salicylate indices, leaf water content, leaf toughness, and *Chrysomela aeneicollis* larval growth on five willow species. Data are means and SDs. Means were compared using Student-New-

man-Keuls tests; the same superscripts indicate means that are not significantly different. For the salicylate index, the tests were conducted on the log-transformed values

Species	Salicylate index						Water content			Leaf toughness			Larval growth rate		
	10–15 July			16–18 August			(100 × wet mass/dry mass)			(kilopascals)			(ln(mg <sub>f</sub> · mg <sub>i</sub> <sup>-1</sup> ) · d <sup>-1</sup> )		
	(mg/g dry mass)														
n	Mean	(SD)	n	Mean	(SD)	n	Mean	(SD)	n	Mean	(SD)	n	Mean	(SD)	
<i>Salix boothii</i>	26	1.45	(1.90) <sup>c</sup>	25	1.24	(1.12) <sup>c</sup>	7	56.7	(5.06) <sup>a</sup>	7	373	(53.2) <sup>ab</sup>	6	0.442	(0.027) <sup>a</sup>
<i>S. drummondiana</i>	9	0.23	(0.19) <sup>d</sup>	10	1.10	(0.71) <sup>c</sup>	7	60.6	(6.05) <sup>a</sup>	7	293	(86.6) <sup>b</sup>	7	0.424	(0.070) <sup>a</sup>
<i>S. geyeriana</i>	30	3.29	(2.13) <sup>b</sup>	28	2.90	(1.90) <sup>b</sup>	9	54.8	(6.55) <sup>a</sup>	9	294	(131.1) <sup>b</sup>	7	0.411	(0.071) <sup>a</sup>
<i>S. lutea</i>	12	0.18	(0.13) <sup>d</sup>	12	0.18	(0.08) <sup>d</sup>	7	61.9	(3.29) <sup>a</sup>	7	444	(88.2) <sup>a</sup>	6	0.413	(0.040) <sup>a</sup>
<i>S. orestera</i>	37	5.00	(1.64) <sup>a</sup>	37	5.84	(1.83) <sup>a</sup>	14	59.0	(5.11) <sup>a</sup>	14	381	(110.3) <sup>ab</sup>	14	0.417	(0.070) <sup>a</sup>

included in the growth experiment, I deleted one of them. *Salix orestera* individuals were preferentially deleted to obtain a more balanced sample.

#### Beetle abundance

I counted the adult beetles on 39 willows in 1987 and 1988. For each plant, I recorded the number of beetles observed over two minutes (Rank 1992). The willows grew from 2800 to 3220 m along Big Pine Creek, and the beetles were censused at five localities: the Cabin Site (*Salix lemmoni* Bebb. above the bog and *S. lutea*, and *S. orestera* in the bog), the Falls Site (*S. boothii*, *S. geyeriana*, and *S. orestera*), three sites along the Big Pine lakes (*S. boothii*, *S. orestera*), and an Upper Site (*S. orestera*). The first counts were made on 13 June 1987, and the second counts were made on 22 June 1988, except for six willows at the Falls Site that were sampled 5 days later. In both years, this period represented the early part of the growing season.

All statistical analyses in this study were conducted with SAS (SAS 1988). Before parametric analyses of the salicylate index values and the beetle counts, they were log-transformed. When no error term was appropriate for significance testing in the ANOVAs, quasi *F*-ratios were used (Kirk 1982). The VARCOMP procedure (restricted maximum likelihood option) in SAS was used to estimate the proportion of the variance explained by the random effects in the ANOVAs. I conducted the laboratory experiments (except for the plant chemistry) at the White Mountain Research Station. Voucher specimens of the willows were deposited at the Big Creek Reserve of the University of California.

## Results

### Leaf characteristics

The salicylate index and leaf toughness differed significantly among the willow species, but they did not differ in water content (Tables 1 and 2). *Salix orestera* had the highest salicylate index values, followed by *S. geyeriana*, *S. boothii*, *S. drummondiana*, and *S. lutea*. Except for *S. drummondiana*, the salicylate index values did not change much between the two sampling dates (Table 1). The leaves of *S. lutea* were significantly tougher than those of *S. geyeriana* or *S. drummondiana*, but *S. boothii* and *S. orestera* leaves were intermediate in toughness (Table 1).

Significant variation among the plants existed for all three characteristics. For the water content and leaf toughness, the among-plant differences accounted for most of the explained variation (Table 2). None of the plant characteristics were significantly correlated with one another (Table 3).

### Larval growth

#### Laboratory growth experiment

Larval growth did not differ among the species, but it varied significantly among the individual plants (Tables 1 and 2). These among-plant differences accounted for virtually all the explained variation in larval growth (Table 2). The water content was significantly related to larval growth, but the toughness and the salicylate-index were not (Table 3). When all three plant characteristics were included in a multiple regression, the water content was the only significant factor. Only 5% more of the variation was explained by all three variables than by water content alone. The larvae did not tend to leave the foliage of any species more than the others (Fisher's exact test,  $P=0.6$ ). There were no differences among the species in the larval survival to pupation (Fisher's exact test,  $P>0.75$ ).

#### Larval development in the field

*Chrysomela aeneicollis* larvae tended to develop more rapidly on *S. orestera* than on *S. lutea* (Fig. 1), but this was usually not significant (Table 4). Nevertheless, the larval development usually differed among the plants (Table 4). The years also differed. For example, larval development was slow during 1987. On day 13 of that year, all the larvae were still in the first or second instar, while on day 12 in 1988, most were in the second or third instar (Fig. 1). This probably resulted from a freeze in July 1987. The temperatures were unusually low at the field site for several days (minimum  $-5.5^{\circ}\text{C}$ ). The

**Table 2** ANOVAs of the characteristics of the willow leaves and *C. aeneicollis* larval growth

Factor	df	MS	F	P	Explained variation
<i>Salicylate index</i>					
Date	1	2.11	0.6	0.49	—
Species	4	209.60	33.8	0.0001	61.8%
Plant (species)	113	3.60	2.3	0.0001	14.4%
Date X species	4	4.21	2.7	0.04	1.6%
Within cells	104	1.58			
<i>Water content</i>					
Species	4	0.1861	2.2	0.09	7.4%
Plant (species)	39	0.0085	2.3	0.0005	29.0%
Within cells	87	0.0036			
<i>Leaf toughness</i>					
Species	4	181921.3	3.0	0.03	11.2%
Plant (species)	39	58728.9	2.8	0.001	34.9%
Leaf (plant)	42	21023.8	2.0	0.001	0.5%
Within cells	165	10489.2			
<i>Larval growth rate</i>					
Species	4	0.0020	0.3	0.91	0.0%
Plant (species)	35	0.0054	2.0	0.04	38.6%
Within cells	24	0.0027			

**Table 3** Correlations among leaf characteristics and *C. aeneicollis* larval growth

Variable	Water content		Leaf toughness		Salicylate index	
	n	r	n	r	n	r
Leaf toughness	44	0.03				
Salicylate index	42	-0.22	42	-0.04		
Larval growth rate	40	0.44**	40	-0.12	38	-0.01

\*\* P < 0.01

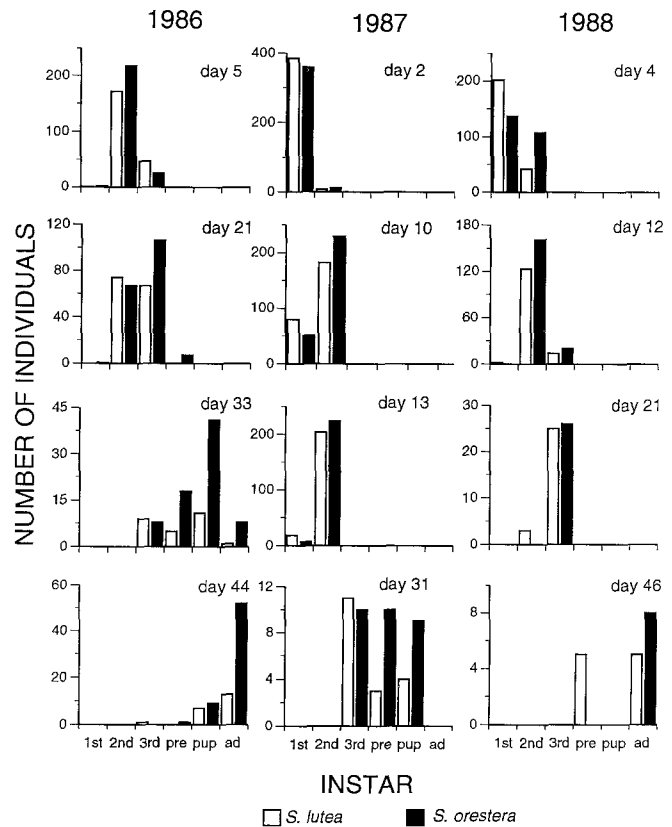
carcasses of several larvae that had been killed by the frost were found on the leaves.

**Larval survivorship in nature**

*Larval survival on S. orestera and S. lutea*

In the first year of the experiment (1986) the larval survival was considerably higher on *S. orestera* than on *S. lutea* (Fig. 2). Larvae that were initially in the larger groups (20 individuals) survived longer than those in the smaller groups (Fig. 2). The species and group size effects were statistically significant (Table 5).

During the following two years, the difference between the species was small, and there was no effect of the initial group size (Fig. 2). Survival was very similar on both species in 1987 (Fig. 2, Table 6). In 1988, it was greater on *S. orestera* than on *S. lutea* during the early and late summer (Fig. 2), but during the middle of the summer it was greater on *S. lutea* (Table 6). Larval survival declined from 1986 to 1988 (Fig. 2). In all three



**Fig. 1** Larval development on *Salix orestera* (solid columns) and *S. lutea* (open columns) at the Cabin Bog from 1986 to 1988. The number of individuals in each stage is plotted on the y axis. Stages were classified into one of the three larval instars, the prepupal stage, pupae, or adults which had emerged

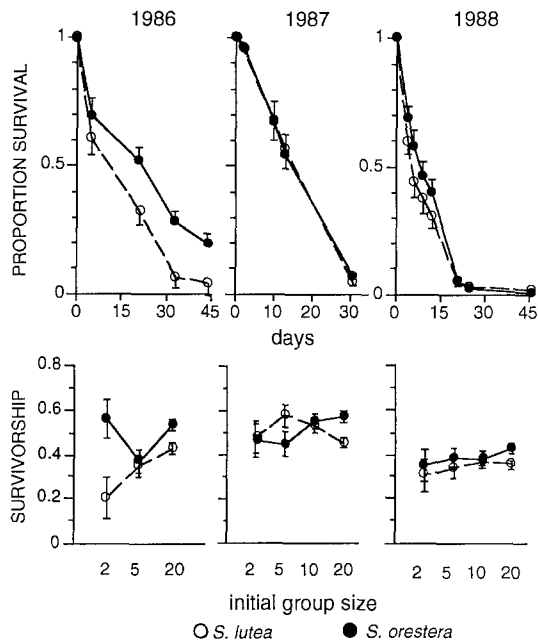
**Table 4** Frequency tests of *C. aeneicollis* developmental rates. The tests were made on the frequency differences between the species and among the plants within each species. The significances were determined by *G* tests unless otherwise noted. Sequential Bonferroni adjustments of the significance levels were made for the number of tests conducted each year (Rice 1989)

Days from start of experiment	Between species	Among plants in <i>S. lutea</i>	Among plants in <i>S. orestera</i>
<b>1986</b>			
5	* a, b	***	*** a
21	n.s.	n.s.	*** a
33	n.s.	n.s.	* b
44	n.s.	n.s.	n.s.
<b>1987</b>			
2	n.s.	*** b	*
10	**	**** b	**
13	*	**** b	n.s.
31	n.s.	** b	n.s.
<b>1988</b>			
4	****	****	****
12	n.s.	** a, b	** a, b
21	n.s.	n.s.	—
46	n.s.	n.s.	—

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$

<sup>a</sup> Developmental stages were pooled to avoid low expected frequencies

<sup>b</sup> Exact probability used



**Fig. 2** Larval survival on *S. orestera* (solid spots) and *S. lutea* (open circles) at the Cabin Bog from 1986–1988. The top graphs show the proportional survival over the summer, and the bottom graphs show average survivorship (see text) plotted against the initial group size and host species. The least-squares means and their SEs are shown

**Table 5** ANOVA of larval survivorship on *S. lutea* and *S. orestera* from 1986 to 1988 at the Cabin Bog

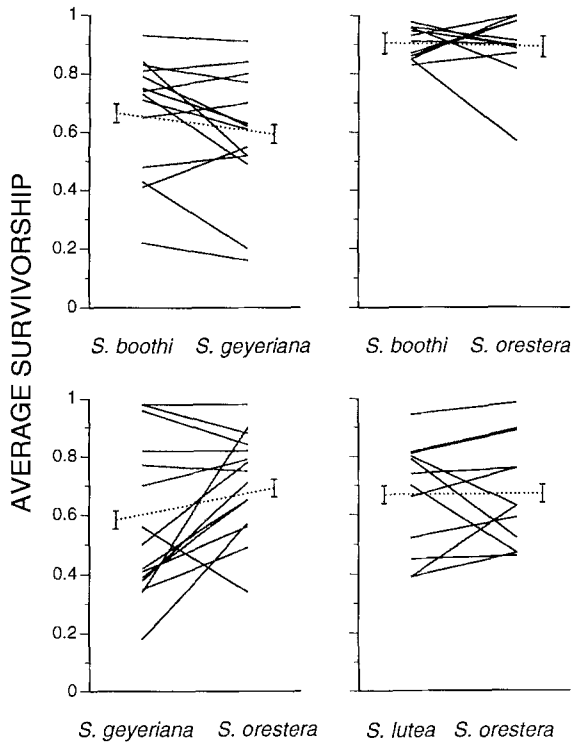
Factor	df	MS	F	P
<b>1986 experiment</b>				
Species	1	1.840	8.0	0.03
Plant (species)	6	0.230	1.3	0.31
Group size	2	0.914	4.9	0.03
Species × Group size	2	0.463	2.5	0.12
Plant (species) × Group size	12	0.185	1.0	0.43
Within cells	47	0.177		
<b>1987 and 1988 experiment</b>				
Year	1	4.679	36.6	0.0001
Species	1	0.116	0.3	0.59
Plant (species)	6	0.368	2.8	0.02
Group size	3	0.103	0.8	0.53
Species × Group size	3	0.350	2.6	0.08
Plant (species) × Group size	18	0.135	1.1	0.40
Year × Species	1	0.130	1.0	0.31
Year × Plant (species)	6	0.295	2.3	0.04
Year × Group size	3	0.053	0.4	0.74
Year × Species × Group size	3	0.171	1.2	0.26
Within cells	140	0.128		

**Table 6** Table of *C. aeneicollis* larval survival on *S. lutea* and *S. orestera* over the three summers

Days	<i>S. lutea</i>		<i>S. orestera</i>	
	Number of larvae	Survival from: Day 0 % Last count %	Number of larvae	Survival from: Day 0 % Last count %
<b>1986</b>				
0	327		320	
5	220	67.3	245	76.6
21	141	43.1 64.1	181	56.6 73.9
33	26	8.0 18.4	75	23.4 41.4
44	21	6.4 80.8	62	19.4 82.7
<b>1987</b>				
0	413		389	
2	392	94.9	370	95.1
10	262	63.4 66.8	281	72.2 75.9
13	223	54.0 85.1	231	59.4 82.2
31	18	4.4 8.1	29	7.4 12.6
<b>1988</b>				
0	443		429	
4	244	55.1 55.1	245	57.1 57.1
12	139	31.4 57.0	181	42.2 73.9
21	28	6.3 20.1	26	6.1 14.4
46	10	2.3 35.6	8	1.9 30.8

summers, the survival was lowest when most of the larvae were in the third instar (Fig. 1, Table 6). The analysis of variance showed that the decline in survival between 1987 and 1988 was significant, and that the among-plant variation and the year-by-plant interaction were also significant (Table 5).

The survivorship of the third-instar larvae was nearly identical on *S. orestera* and *S. lutea* in 1989 (Fig. 3). For most of the plant pairs, it was somewhat greater on *S.*



**Fig. 3** Larval survivorship at both sites in 1989. The Cabin Bog comparison between *S. orestera* and *S. lutea* is shown at lower right; the other graphs show the three comparisons between *S. boothi*, *S. geyeriana*, and *S. orestera* from the Falls Site. The solid lines connect the plant means in each pair, and the dashed lines connect the species means (with their SEs). The slope of the solid lines indicates higher survival on one of the plants, while the position of the lines along the y axis indicates differences among the pairs

*orestera* than on *S. lutea*, but it was higher on *S. lutea* for three pairs. When the survivorship was high on one of the plants in a pair, it was usually high on the other plant (Fig. 3). This relationship was represented by the block effect in the ANOVA, which was highly significant and explained 51% of the variation (Table 7). The block-by salicylate rank interaction was not significant (Table 7).

*Larval survival on S. boothi, S. geyeriana and S. orestera*

The survivorship of the third-instar larvae was high on the salicylate-rich *S. orestera* and on the salicylate-poor *S. boothi*. However, survivorship was low on the medium-salicylate *S. geyeriana* (Fig. 3). This pattern was consistent among the species comparisons. For example, survivorship was high on both species in the *S. boothi* versus *S. orestera* species pair, greater on *S. orestera* than *S. geyeriana*, and greater on *S. boothi* than on *S. geyeriana* (Fig. 3). The results of the ANOVA indicated that the species pair difference, the salicylate-rank by species pair interaction, and the block effect were signifi-

**Table 7** ANOVAs of the 1989 larval survivorship experiment on four willow species. The results from the Cabin Bog include the *S. lutea* versus *S. orestera* species pair, while the results from the Falls Site include three species comparisons (see text)

Factor	df	MS	F	P
<i>Cabin Bog</i>				
Salicylate rank	1	0.002	0.0	0.92
Block	11	0.115	5.1	0.006
Block × Salicylate rank	11	0.023	1.1	0.42
Within cells	24	0.021		
<i>Falls Site</i>				
Salicylate rank	1	0.003	0.2	0.73
Species pair	2	1.120	8.8	0.0007
Block (species pair)	37	0.127	4.5	0.0001
Salicylate rank × species pair	2	0.123	4.3	0.02
Salicylate rank × Block (species pair)	37	0.028	1.2	0.25
Within cells	80	0.024		

cant (Table 7). The significant block differences, accounting for 49% of the total variation, were consistent with the results from the *S. orestera* versus *S. lutea* comparison (see above).

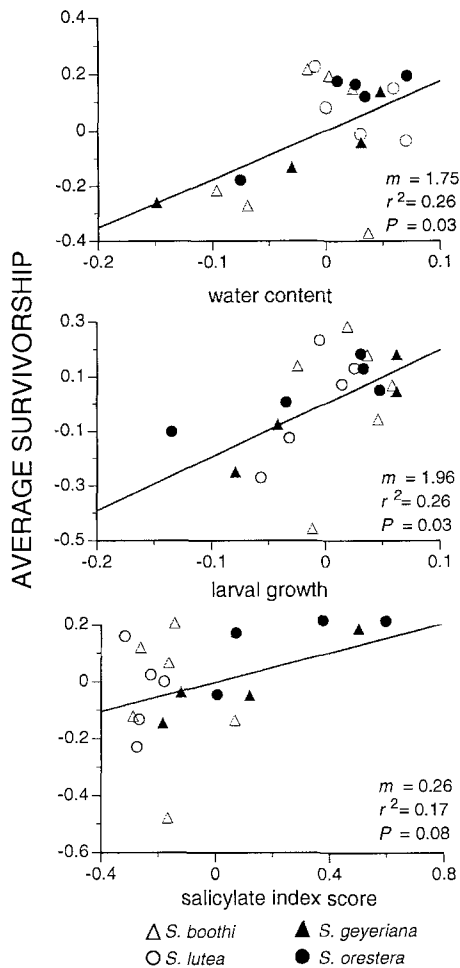
Two of the four plant variables that had been measured in the laboratory, the water content and larval growth, were significantly related to larval survivorship in the field. In the original multiple regression with all four variables, 51% of the variation in survivorship was explained. However, the leaf toughness was not significant, and in a reduced model without leaf toughness, nearly the same proportion of the variation (48%) was explained (Fig. 4). The contribution of the salicylate index was only marginally significant ( $P < 0.1$ ; Fig. 4).

*Beetle abundance*

The abundance of the adults declined at the lower elevations between 1987 and 1988, but it increased at the Upper Site. On average, 40% fewer beetles were observed on the willows in 1988 than in 1987 ( $x=5.8$  beetles per plant, SD 6.6 versus  $x=10.2$ , SD 13.7). This decline occurred on 23 willows, and it was highly significant (Wilcoxon signed-ranks test,  $n=39$ ,  $P < 0.001$ ). The abundance increased, however, for 12 of 13 willows at the Upper Site. The adults were 6 times more abundant there in 1988 than they had been in 1987 ( $x=4.0$ , SD 2.7 versus  $x=0.6$ , SD 1.7). The tendency for the abundance to increase (versus decline) differed significantly among the localities (Fishers exact test,  $P=0.00002$ ), but the average abundance over the two years declined with increasing elevation ( $y = -0.0011X + 4.2$ ,  $r = -0.45$ ,  $P=0.0007$ ).

*Predator observations*

During this study, I observed 46 predators feeding on *C. aeneicollis*. Mites (14), *Parasyrphus melanderi* larvae (7),



**Fig. 4** Multiple regression of water content, larval growth, and salicylate content on larval survivorship. Each point represents a plant average. The slope coefficients ( $m$ ), the squared partial-correlation coefficients ( $r^2$ ), and the  $P$  values are given for each independent variable. Open triangles *S. boothi*, open circles *S. lutea*, solid triangles *S. geeyeriana*, solid circles *S. orestera*

hemipterans (2), and a *C. aeneicollis* larva consumed *C. aeneicollis* eggs. *Parasyrphus melanderi* larvae (9), mites (2), and a hemipteran fed on first-instar larvae. A spider was observed consuming second-instar larvae. Larvae of *P. melanderi* commonly fed on the second- and third-instar *C. aeneicollis* larvae.

I observed *Symmorphus cristatus* females for 30 min at a dead tree with several wasp nests. During this period, six of them placed a third-instar *C. aeneicollis* larvae into their nests. Many more wasps alighted on the tree (57 were observed in the first 10 min). Several of these trees with wasp nests were present at the Falls Site and at the Cabin Bog. On 25 July 1988, I placed 11 artificial trap-nests at the field sites (one nest hole per trap). Within 29 days, 9 of them had been filled with 89 third-instar *C. aeneicollis* larvae (not counting the remains of the other beetle larvae that had already been consumed). Additionally, I found 30 wasp larvae in these traps. I never witnessed birds feeding on *C. aeneicollis* larvae. Nevertheless, in July 1989, I observed an acorn wood-

pecker (*Melanerpes formicivorus*) removing beetle larvae from a *S. cristatus* nest and eating them.

The only parasitoid found during this study was a tachinid fly. On the last count in the 1986 survivorship experiment, one larva on *S. lutea* had been parasitized by this fly, and three parasitized larvae were found on *S. orestera*. No parasitized larvae were found in 1987, but one was found on *S. orestera* in 1988. The parasitized larvae were also taken by other predators. Two of the *C. aeneicollis* larvae in the *S. cristatus* wasp traps had been parasitized by the fly. Because of this predation, the actual parasitism rates were underestimated.

## Discussion

*Chrysomela aeneicollis* larval growth was not related to the salicylate chemistry of their host plants. Larval growth did not differ among the five willow species in the laboratory, and larval development was similar on the salicylate-poor *S. lutea* and the salicylate-rich *S. orestera* in the field. This absence of a difference among the species existed despite the beetles' preference for salicylate-rich willows (Rank 1992). Other studies on salicin-using beetles have also found no consistent relationship between the host plant salicylates and larval growth in the laboratory (Horton 1989; Denno et al. 1990; Dodge et al. 1990).

For *C. aeneicollis*, the among-plant variation was the main factor influencing larval growth in the laboratory and in the field. This variation was partly accounted for by the among-plant differences in water content. Leaf moisture can directly affect herbivore performance (Reese and Beck 1978; Holtzer et al. 1988; Horton 1989; McQuate and Connor 1990), or it may indirectly affect performance through correlations with other nutritional factors (Mattson and Scriber 1987). Scriber (1977) found that the larval growth of a butterfly was twice as rapid when the water content of the leaves was 60% than when it was 50%. Of the 44 willows in the present study, 35 had water contents below 60%, which is unusually low for woody plants (Mattson and Scriber 1987). This may explain the observed relationship between the water content, larval growth in the laboratory, and larval survival in the field (1989 experiment).

*Chrysomela aeneicollis* larvae did not always survive longer on the salicylate-rich willows in nature. Of the four years of *S. orestera* versus *S. lutea* survival comparisons, only one of them indicated significantly greater survival on the salicylate-rich *S. orestera*. Additionally, in the three-species comparison at the Falls Site, survival was lowest on the medium-salicylate willow (*S. geeyeriana*) rather than on the salicylate-poor one (*S. boothi*). The results from the Falls Site may be partly explained by differences in plant quality that affected larval performance differently in the field than in the laboratory. However, this does not explain the change in larval survival on *S. lutea* versus *S. orestera* after 1986. This change was probably a consequence of a

change in the factors influencing larval mortality in *C. aeneicollis*.

The drought that affected California in the late 1980s may have affected the outcome of the survival experiments. During this drought, several shrub species in the nearby Owens Valley experienced severe water stress (Manning 1991). In Big Pine Creek, the willow leaves emerged several weeks later than normal, resulting in unusually short growing seasons from 1987–1989 (personal observation). The drought probably changed the water balance of the host plants. Other studies have demonstrated that low host plant moisture caused a reduction in herbivore survival (Craig et al. 1988; Hanks and Denno 1993). The decline in *C. aeneicollis* adult abundance at the lower elevations may also have been partly caused by the drought. Low levels of precipitation are known to affect herbivore populations negatively (Richards and Waloff 1961; Price and Clancy 1986).

The specialist wasp *Symmorphus cristatus* probably caused much of the larval mortality during this study. This wasp was abundant in Big Pine Creek throughout the 1980s (Smiley and Rank 1986), and it was the most common predator observed at the field sites. Additionally, the wasps collected third-instar beetle larvae for their nests, which corresponded with the period of high *C. aeneicollis* larval mortality. *Symmorphus* species feed on several related genera of chemically defended chrysomelids (Berland 1928; Krombein 1967; Iwata 1976; Richards 1980; Yamane 1990), suggesting that their association with leaf beetles is an evolutionarily old one. One might expect that they have adapted to circumvent the beetles' defensive secretion. Indeed, *S. cristatus* wipes off the defensive secretions of the beetle larvae before placing them into its nest (Smiley and Rank 1986). The salicylaldehyde defensive secretion appears to have little effect on *Symmorphus* predation on other *Chrysomela* species (Fabre 1891; Devantoy 1948).

The decline in *C. aeneicollis* abundance may have been caused by an increase in *S. cristatus* predation. This decline was part of a longer-term pattern. From 1984 to 1986, the beetle populations were dense on *S. orestera* in the Cabin Bog and on *S. lemmoni* on the drier slopes surrounding it. However, by 1988 they were virtually absent from the slopes and rare in the bog. By 1993, all the study populations were extinct except for the willows at the Upper Site (J. Smiley, personal communication). This decrease of the beetle populations in habitats where they were previously abundant probably led to increased predation on the remaining individuals. This was the case for *Parasyrphus melanderi*. The number of *P. melanderi* eggs per *C. aeneicollis* clutch (on *S. orestera* at the Falls Site) increased by a factor of 10 between 1988 and 1989. On some plants in 1989, every *C. aeneicollis* egg clutch contained at least one *P. melanderi* egg (Rank and Smiley in press). It is possible that *S. cristatus* responded in a similar way. The decrease in larval survival over the three years from 1986 to 1988 also suggests that predation by *S. cristatus* increased.

Other salicin-using leaf beetles also suffer high levels of mortality through specialist predators. *Parasyrphus* species were reported on *Chrysomela crotchii* in Manitoba (Elliott and Wong 1966), and on *Melasoma vigintipunctata* L. in Switzerland (Schneider 1953). A syrphid larva (probably a *Parasyrphus* species), feeds only on *Phratora vitellinae* eggs and larvae in eastern Finland (personal observation). *Symmorphus* species were recorded as major predators on *C. crotchii* in Ontario (Smereka 1965), and *Melasoma populi* L. in France (Devantoy 1948), and they also feed on *P. vitellinae* (Berland 1928; Richards 1980). The larvae of salicin-using leaf beetles may also suffer high mortality from parasitoids. For example, *Meigenia bisignata* Mg. (Diptera: Tachinidae) parasitized 50% of the larvae of *M. populi* in France (Devantoy 1948). Finally, specialist predators have been shown to be important sources of mortality to other chemically defended leaf beetles that do not use salicin. Kanervo (1946) reported that *Parasyrphus nigritarsus* Zett., several species of ladybird beetles, and hemipteran bugs caused most of the mortality to the alder leaf beetle (*Linnaeidea aenea* L. in southern Finland. This beetle has similar defensive glands to *C. aeneicollis*. These species have been recorded as enemies of salicin-using leaf beetles elsewhere in Europe (Devantoy 1948; Jolivet 1950; Herting 1973), and several of them are chrysomelid specialists.

On the other hand, generalist predators are important mortality factors to some salicin-using leaf beetles. Burkot and Benjamin (1979) found that the most important predator on *Chrysomela scripta* Fab. in Wisconsin was a generalist ladybird beetle, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). Additionally, the generalist ladybird beetle *Adalia bipunctata* L. was considered to be the most important predator on *M. vigintipunctata* in Bulgaria (Penev and Ovcharov 1992). Several studies of predation on other chemically defended leaf beetles found that generalist predators were the most important ones (Dempster 1960; Richards and Waloff 1961; Eickwort 1977; Wade and Breden 1986).

Specialist predators should have a different impact on plant-herbivore interactions than generalists. Generalist predators may be less successful at obtaining their herbivorous prey on certain host plants (Bernays and Graham 1988; Bernays 1988). This would evolutionarily favor herbivore specialization on the plants where it escapes predation. On the other hand, specialist predators may favor a broader host range because they have adapted to the herbivore's host-based antipredator mechanisms (Jaenike 1990). For example, the *C. aeneicollis* larval defensive secretion has little effect on *P. melanderi* (Rank and Smiley in press). This predator did not avoid chemically defended *C. aeneicollis* larvae in laboratory choice tests, and it was more abundant on beetle clutches on *S. orestera* rather than on the medium-salicylate *S. geyeriana* in the field. Natural selection by this predator may favor a broader diet in *C. aeneicollis*.

Thus, to understand the role of predators in deter-

mining host suitability to salicin-using leaf beetles, more information is needed about the relative importance of generalist versus specialist predation. One possibility is that generalist predators are more important when the beetle populations are small and widely dispersed, and that specialists are more important when the beetles occur at high densities. The predominant mortality factors on several forest herbivores have been shown to differ between high and low density populations (Berryman 1987). However, little is known about the population dynamics of willow leaf beetles. For example, no one has determined the proportion of the individuals that live in high density populations, and no studies have compared the natural enemies at low versus high herbivore densities.

In conclusion, the predicted relationship between the host plant chemistry and the larval survival did not exist for *C. aeneicollis*. Several specialist predators appear to be unaffected by the host-derived defensive secretion, and larval survival was not usually greater on the salicylate-rich species. On the other hand, because there was a difference in one year, natural selection could still maintain the beetle's preference for salicylate-rich willows. These results emphasize the importance of testing the benefits of anti-predator adaptations under natural conditions. Additionally, they highlight the need for caution in basing generalizations about ecological processes on research conducted in a single year at a single locality. Finally, field studies are required to understand the role of host plant chemistry in the interactions between herbivores and their predators.

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