

# Benefits of larval group feeding by *Chrysolina aurichalcea asclepiadis* on *Vincetoxicum*: improved host location or feeding facilitation?

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## Abstract

Several studies have shown that group feeding by immature insects enhances larval performance through feeding facilitation. However, the benefits of this behavior have not been studied for species that hatch off from their host plant. For these taxa, group feeding may enhance recruitment of newly emerging larvae and may improve larval performance through feeding facilitation. I conducted laboratory experiments with the European chrysomelid *Chrysolina aurichalcea asclepiadis* (Villa) (Coleoptera: Chrysomelidae: Chrysomelinae), whose larvae hatch on the ground near their host plant [*Vincetoxicum* (Apocynaceae)] and feed in groups during early instars, to determine whether cues associated with larval feeding enhance neonate establishment and determine whether feeding in groups improves larval performance. Neonates located shoots infested with larvae more often than when confined to shoots with leaves that were undamaged or mechanically damaged. When provided a choice, neonates preferred shoots infested with 10 larvae to non-infested shoots and shoots with one or five larvae. In the larval performance test, group feeding did not improve survival, but larvae completed larval development about 2 days faster when larvae were raised in groups of 10 compared to isolated larvae. However, larval group size negatively affected the size of emerging adults. The results of this study indicate that the main benefit of group feeding is to young *C. a. asclepiadis* larvae and this benefit diminishes over development due to competition of mature larvae.

## Introduction

Gregarious feeding, a trait exhibited by many immature herbivorous insects, has evolved to mitigate constraints on development imposed by plant defense, natural enemy pressure, and competition (Grégoire, 1988; Damman, 1994; Hunter, 2000). The selective advantage of this behavior has been evaluated mainly with Lepidoptera and Symphyta (Hunter, 2000). Gregarious larval behavior has been studied to a lesser extent in the Chrysomelidae (Grégoire, 1988) but interest in this is increasing (Chang & Morimoto, 1988; Chattopadhyay & Sukul, 1994; Damman, 1994; Crowe, 1995a, b; Olckers, 1998; Nahrung et al., 2001; Chaboo, 2004; Zvereva & Rank, 2004; Costa et al., 2007). Group feeding may result in improved neonate establish-

ment and foraging efficiency (feeding facilitation), improved protection from natural enemies, and for some species improved thermoregulation (Ghent, 1960; Chang & Morimoto, 1988; Grégoire, 1988; Stamp & Bowers, 1990; Crowe, 1995b; Benrey & Denno, 1997; Fordyce, 2003).

Major benefits of larval group feeding to chrysomelids are enhanced establishment of early instars (Wade & Breden, 1986; Chang & Morimoto, 1988; Nahrung et al., 2001) and faster growth (Breden & Wade, 1987; Costa et al., 2007). For some species, faster larval growth produces larger adults that are more fecund (Breden & Wade, 1987; Costa et al., 2007). Moreover, faster growth may decrease exposure to natural enemies (Benrey & Denno, 1997), but this hypothesis has not been tested for chrysomelids. Despite the benefits of group feeding on larval performance this behavior subjects later instars to strong intraspecific competition (Matsumoto, 1990; Le Masurier, 1994; Fordyce, 2003), which can decrease adult weight,

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and in some instances, increase exposure to natural enemies (Damman, 1994). Competition is thought to be one reason for the shift from gregarious to solitary feeding as larvae develop (Breden & Wade, 1987; Fordyce, 2003). Because the costs and benefits of group feeding change over ontogeny for some species, assessments of this behavior need to be evaluated at specific developmental stages (Reader & Hochuli, 2003).

Larval feeding aggregations are maintained by trail following (Roessingh, 1989; Fitzgerald, 1993), physical contact (Grégoire, 1988), and abdomen tapping (Carne, 1962; Cocroft, 2001). Some larval chrysomelids also utilize short-distance cues associated with conspecifics or host plants to maintain feeding groups (Grégoire, 1988; Crowe, 1995a). All of these studied examples consider taxa with larvae that hatch directly on their host plant. These species typically form feeding groups immediately as they hatch from eggs that are laid in clusters. However, for species hatching near their host, semiochemical cues associated with conspecific feeding or the host plant may enhance larval recruitment to the host that create and maintain aggregations. The role of these cues in larval host location has not been studied, but the importance of host plant volatiles, plant waxes, conspecific feeding, and pheromones in directing host location of adult chrysomelids is well established (Peng & Weiss, 1992; Müller & Hilker, 2001; Peacock et al., 2001; Cossé et al., 2005).

Gregariously feeding chrysomelids are common on herbaceous plants, but most published studies address taxa feeding on woody perennials (Grégoire, 1988). Herbaceous and woody plants typically differ in their exposure period to herbivores and level of attack, quality of plant tissue for herbivore development (Mattson, 1980; Scriber & Slansky, 1981), and in some cases, resistance strategies against herbivore attack (Coley et al., 1985; Stamp, 2003). Thus the benefits of group feeding may differ for their herbivores. Two published studies have examined group feeding by chrysomelids on herbaceous plants (Damman, 1994; Costa et al., 2007). Publications on gregarious Lepidoptera also focus primarily on woody perennials (Hunter, 2000). Among these studies, the benefits of communal feeding are generally similar on woody and herbaceous plants (i.e., feeding facilitation and natural enemy protection). The most striking difference is that aggregations on herbaceous species commonly consume all host resources, forcing larvae to disperse (Matsumoto, 1990; Damman, 1994; Le Masurier, 1994). Dispersal from natal hosts greatly increases mortality risks, especially at low plant densities (Le Masurier, 1994).

*Chrysolina aurichalcea asclepiadis* (Villa) (Coleoptera: Chrysomelidae: Chrysomelinae) is a univoltine leaf beetle that feeds on the perennial herb *Vincetoxicum hirundinaria*

(Apocynaceae) in the Western Alps (Weed & Gassmann, 2007). Eggs of this species overwinter in the leaf litter and larvae hatch in early spring, crawl onto plants, and feed within developing leaf buds at least during early instars (Weed, 2010). The importance of feeding in groups became obvious while evaluating a Swiss population of *C. a. asclepiadis* as a potential biocontrol agent of *Vincetoxicum nigrum* (L.) Moench and *Vincetoxicum rossicum* (Kleop.) Barb. in North America. During host range testing larvae raised singly took 1–2 days longer to initiate feeding on *Vincetoxicum* compared to larvae raised in groups. In addition, I also observed larval development on *Artemisia absinthium* L. and *Tanacetum vulgare* L. (both Asteraceae) but only when these species were presented to grouped larvae (Weed, 2010). These tests also concluded that beetle growth and reproduction is better on *Vincetoxicum* than on *A. absinthium* and *T. vulgare*, and better on *V. nigrum* than on *V. rossicum* and *V. hirundinaria* (Weed, 2010). Only *V. hirundinaria*, *A. absinthium*, and *T. vulgare* are available to *C. a. asclepiadis* in southern Switzerland but field use of these hosts has never been studied. Apparently other subspecies prefer *Artemisia* over *V. hirundinaria* in Germany (H Kippendorf, pers. comm.).

These observations during host range testing provided the incentive to evaluate the benefits of group feeding to *C. a. asclepiadis*. The first objective of this study was to monitor group size formation and evaluate whether cues associated with larval feeding attract other larvae. If these cues are attractants to larvae searching for a host plant it is possible that feeding in groups could enhance larval recruitment. The second objective was to determine whether group feeding improves larval performance and evaluate at which stages of larval development group feeding is beneficial.

## Materials and methods

### Study organisms

The colony of *C. a. asclepiadis* originated from adults collected on *V. hirundinaria* in September 2006 in Anzonic, Ticino, Switzerland (46°26.016'N, 08°51.537'E; 892 m a.s.l.). They were brought to the University of Rhode Island quarantine laboratory in Kingston, RI, USA. The effects of group feeding were assessed individually on *V. nigrum* and *V. rossicum* with neonates obtained from bulk egg collections taken from the F<sub>1</sub> generation of adults. Plants originated from rootstocks of *V. rossicum* collected in Cumberland, RI, and Groton, CT, USA, and *V. nigrum* collected in Charlestown, RI, in 2006. Rootstocks were transplanted into 2.8-l pots with Metro-mix 510 (Sun Gro Horticulture, Bellevue, WA, USA) and pots were held outdoors. Newly emerging shoots were cut just above the

rootstock when needed for experiments that were conducted from late May to early June 2008. To avoid exposing larvae to shoots potentially induced with allelochemicals, I never cut shoots more than once from an individual plant which required cuttings from multiple plants over the course of the experiment.

#### Neonate behavior

**Larval group size formation.** Neonates were released into small arenas with shoots of *V. nigrum* to estimate the size of larval feeding groups. Four small equidistant holes were made in the corners of a 0.6-l arena (14 × 14 × 6 cm) and a water pic was affixed under each hole. Each pic was filled with water and a 10- to 15-cm shoot of *V. nigrum* was placed into each pic. Twenty neonate larvae (1–2 days old) were released into the center of an arena that was covered with a clear lid. The larvae per shoot were counted after 2 h and the experiment was replicated 10 times. The experiment was conducted under fluorescent lights (L16:D8 photoperiod) at ambient room temperature averaging  $24.1 \pm 1.1$  °C and  $33.0 \pm 6.5\%$  r.h. (mean ± SD). Over the course of the experiment 30 larvae (15%) did not make a choice after 2 h. The distribution of *C. a. asclepiadis* group sizes formed on all shoots (n = 40) was tested for randomness by comparison to a Poisson distribution, and the degree of aggregation was calculated by the index of patchiness (IP) (Lloyd, 1967).

**Larval group size preference.** If neonates form natural feeding groups after emergence, it is possible that there are visual or chemical cues responsible for directing this behavior (Crowe, 1995a; Peacock et al., 2001). Two experiments were set up to assess the importance of conspecific or host-derived cues in neonate establishment. In the first experiment, neonate attraction to undamaged, larval infested, and mechanically damaged shoots was evaluated under no-choice conditions. Sixty individual shoots of *V. nigrum* were placed separately in jars (473 ml) fitted with water pics and subjected to one of three treatments: (1) a shoot was infested with five larvae, (2) a shoot was damaged along the leaf margin using scissors (removal of 5–10% leaf area), or (3) shoots were left undamaged. Two days following the treatments a single neonate was gently placed in the bottom of each jar using a fine-tip brush. After 2 h the location of the larvae was recorded and if the neonate ascended the shoot after 2 h the trial was scored a success. Twenty larvae were exposed to each treatment over the entire experiment and the proportion of larvae successfully locating each substrate was calculated.

A second experiment was conducted to determine whether individual larvae discriminate between damaged and undamaged leaves and among increasing densities of

conspecifics under multiple-choice conditions. Two days prior to the experiment, individual shoots (10 per treatment) of *V. nigrum* were infested with one, five, and 10 neonates each and kept in jars as described above. In setting up the multiple-choice arena, each infested shoot was carefully removed from the individual cups and randomly placed into the corners of the same four-choice arenas as described in the group size formation study. A shoot without damage placed in the remaining corner served as the control. Thus the four corners of each arena had shoots randomly arranged with zero, one, five, and 10 larvae (subsequently referred to as 0L, 1L, 5L, and 10L). One newly emerged larva (2 days old) was released into the center of each arena and its location was recorded after 2 h. Five arenas were monitored at a time and the experiment was repeated 68 times. The locations of all shoots were rearranged within each arena after every five tests and arenas were cleaned between tests. During the experiment only four of the 68 larvae failed to ascend a treatment shoot after 2 h and these were excluded from the analysis.

Both experiments were conducted under the same conditions as the above study. The experimental larva was easily distinguished in these experiments from the previously established larvae because it was not distended from feeding. Larval choice was compared among substrates in both experiments using a  $\chi^2$  goodness-of-fit test.

#### Effect of group size on larval performance

The effect of group size on larval development of *C. a. asclepiadis* was evaluated by raising larvae individually (1L), or in groups of five (5L), or 10 (10L) on *V. nigrum* and *V. rossicum*. Newly emerged neonates were transferred from overwintering dishes onto freshly cut shoots of *V. nigrum* or *V. rossicum* held in 473 ml jars fitted with water pics as described above. Jars with ventilated clear lids were held under L16:D8 h photoperiod and at ambient room conditions which averaged  $24.3 \pm 2.1$  °C and  $33.1 \pm 8.5\%$  r.h. (mean ± SD) over the course of the experiment. Fifteen jars with individual larvae (1L) and 10 jars each of the 5L and 10L larval groups were monitored on each plant species. Larvae were checked daily to record developmental status. Over the experiment, survivorship and developmental time to the L3, L4, prepupal, and adult stages were monitored to determine whether benefits of group feeding changed during development. At 9 days of development, 10 randomly selected individuals from each group size were weighed. During the L4 stage, 2 cm of moist, sterilized vermiculite was added into the containers as a pupation substrate and teneral adults were weighed and sexed on emergence.

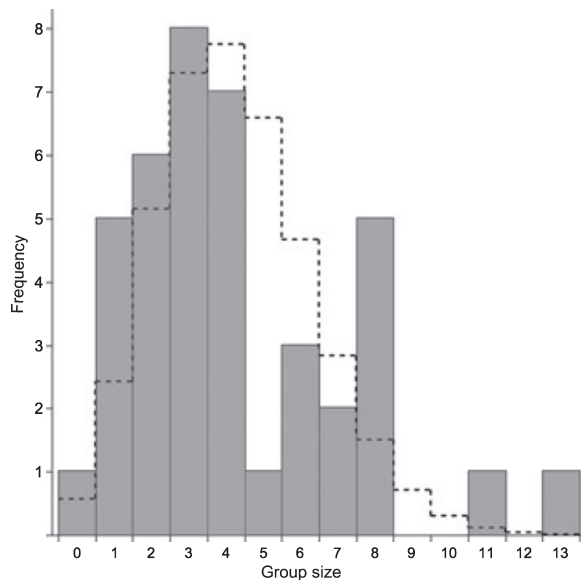
The effects of larval group size and host plant on *C. a. asclepiadis* survival and developmental time to each

life stage (L3, L4, pupal, and adult) was analyzed as a repeated measures experiment using Proc MIXED in SAS (version 9.2) (SAS Institute, Cary, NC, USA). In the analysis of development time, I used the mean value of each trial for group sizes of 5L and 10L. Two-way ANOVA was used to compare the effects of group size and host plant on larval weight measured at 9 days of development. The effect of group size, host plant, and gender on adult weight was analyzed using a three-way ANOVA. Data were transformed to meet assumptions of statistical methods and all analyses were followed by a Tukey's HSD test to compare means when appropriate. Because it was not possible to sex larvae prior to experiments a post-hoc analysis was conducted to examine whether the sex ratio of beetles differed among treatments. In this analysis the proportion of males was compared among group sizes and between host plants using separate Kruskal–Wallis tests. All statistical analyses were performed using SAS (version 9.2).

## Results

### Neonate behavior

The distribution of group sizes differed significantly from a Poisson distribution ( $\chi^2 = 96.7$ ,  $P < 0.0001$ ;  $n = 40$ ) (Figure 1), indicating that *C. a. asclepiadis* larval distribution was not random on the test shoots. Larvae aggregated on shoots (IP = 1.24), with group sizes following a lognormal



**Figure 1** Expected (dashed line) and observed (gray bars) frequency of group sizes formed on each shoot of *Vincetoxicum nigrum* by *Chrysolina aurichalcea asclepiadis* neonates. The mean group size formed over the experiment was  $4.25 \pm 1.19$  larvae ( $\pm$  95% confidence interval;  $n = 40$ ).

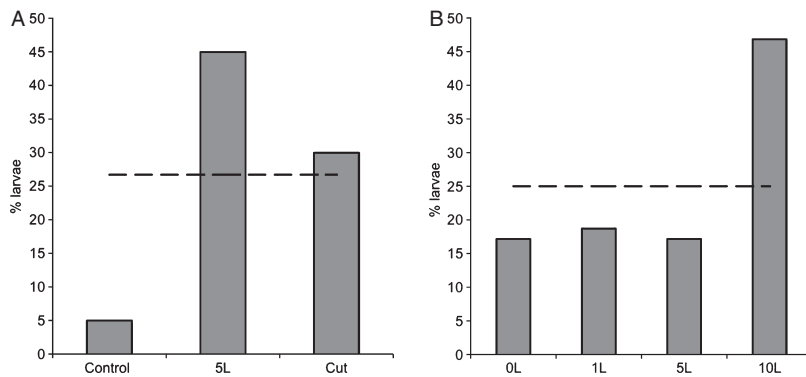
distribution (Shapiro Wilk:  $W = 0.97$ ,  $P = 0.30$ ;  $n = 40$ ). Group sizes of 2–4 larvae per shoot were most frequent (Figure 1). In the second set of experiments, the number of shoots successfully located by larvae differed among substrates under no-choice conditions ( $\chi^2 = 8.44$ , d.f. = 2,  $P = 0.015$ ) (Figure 2A). Only one larva (of 20) ascended the undamaged shoot and it did not begin feeding within 2 h. Nearly half of the larvae ascended the shoot infested with 5L (Figure 2A) and 70% began feeding. Six neonates ascended the mechanically damaged shoot (Figure 2A), but none began feeding during the 2-h period. In the multiple-choice test, neonates discriminated among individual group sizes ( $\chi^2 = 16.38$ , d.f. = 3,  $P = 0.0009$ ) preferring the shoot infested with 10L (30 larvae) (Figure 2B). Neonates were equally attracted to undamaged shoots and shoots infested with 1L and 5L (Figure 2B).

### Effect of group size on larval performance

Larval survival was similar between *V. nigrum* and *V. rossicum* and among larval group sizes but varied among developmental stages (Table 1). Survival decreased slightly over larval development but only significantly after pupation (Figure 3). Larval developmental time varied between host plants and among group sizes and developmental stages (Table 1). Developmental time varied between hosts at each developmental stage (Table 1) where larvae completed development to each life stage faster on *V. nigrum* than *V. rossicum* (Figure 4A). Developmental time also varied among group sizes at each developmental stage (Table 1). Larvae feeding in groups of 10 completed development to all life stages except the adult roughly 2 days faster than individual larvae (Figure 4B). Developmental times of the 5L and 10L and 1L and 5L group sizes were similar (Figure 4B). Development time also varied significantly between host plants for each group size but the three-way interaction of group size, host plant, and developmental stage was not significant (Table 1).

Larval weight at 9 days of development varied between host plants and among group sizes (Table 1). Larvae raised on *V. nigrum* were heavier than those on *V. rossicum* (Figure 5A) and larvae raised in groups of 10 were significantly heavier than larvae raised individually (Figure 5B). Larval group size and gender significantly affected the weight of emerging adults, but adult weight was similar between host plants (Table 1). Females were heavier than males and larvae raised individually produced heavier adults than larvae in the 10L group size (Figure 6). Adults from the 5L group size were similar in size to the 1L and 10L group sizes (Figure 6).

The post-hoc analysis concluded that the proportion of males was equally distributed between host plants ( $H = 3.06$ , d.f. = 1,  $P = 0.08$ ) and group sizes ( $H = 0.0435$ ,



**Figure 2** Percentage of larvae that selected each substrate in (A) no-choice and (B) multiple-choice tests. Dashed lines represent the expected percentage in each test.

Variable	Source	d.f.	F	P
Survival (%)	Host (H)	1,137	1.96	0.16
	Group size (GS)	2,39	0.35	0.71
	Developmental stage (DS)	3,202	18.08	<0.0001
	H*GS	2,137	2.41	0.094
	H*DS	3,208	1.04	0.38
	GS*DS	6,206	1.03	0.40
	H*GS*DS	6,212	0.97	0.45
Developmental time (days)	Host (H)	1,147	91.99	<0.0001
	Group size (GS)	2,47	7.36	0.0016
	Developmental stage (DS)	3,201	5357.51	<0.0001
	H*GS	2,147	3.09	0.049
	H*DS	3,206	6.79	0.0002
	GS*DS	6,204	4.10	0.0007
Larval weight at 9 days (mg)	Host (H)	1	21.29	<0.0001
	Group size (GS)	2	7.61	0.0012
	H*GS	2	0.47	0.63
	Error	59		
	Adult weight (mg)	Host	1	0.93
	Group size	2	3.93	0.021
	Gender	1	385.91	<0.0001
	Error	262		

**Table 1** Analysis of variance for effects of host and group size on survival, developmental time, larval weight, and adult weight of *Chrysolina aurichalcea asclepiadis* fed *Vincetoxicum nigrum* and *V. rossicum*

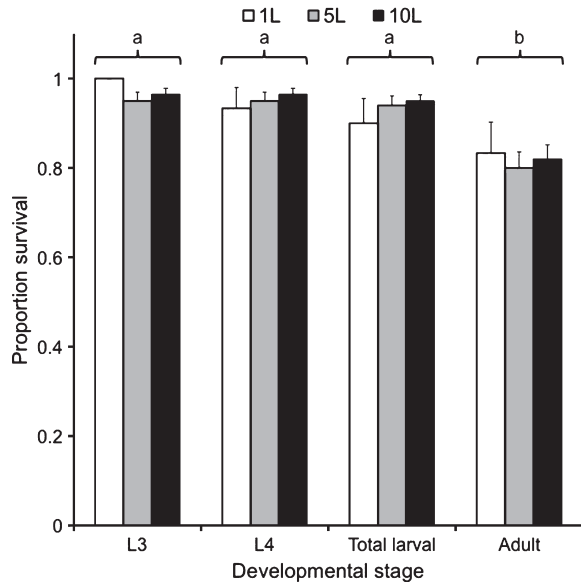
d.f. = 2,  $P = 0.98$ ), indicating that variation in adult weight was attributed mainly to the treatments groups and not gender. At the end of the experiment,  $50.8 \pm 4.4\%$  (mean  $\pm$  SE,  $n = 66$ ) of beetles were male.

## Discussion

The goal of this study was to test the hypothesis that larval group feeding enhances host location and performance of *C. a. asclepiadis*. My experiments demonstrated that cues associated with larval feeding attract neonates and for the first time group feeding was shown to enhance host location of larvae which hatch off from their host plant. Unlike

other gregarious species that form large, tightly aggregated feeding groups (Breden & Wade, 1987; Vasconcellos-Neto & Jolivet, 1994), young larvae of *C. a. asclepiadis* typically form loose feeding groups within the apical buds of expanding *Vincetoxicum* shoots. The effect of group feeding on larval performance was small; larvae feeding in groups completed larval development quicker than isolated larvae.

Some immature insects maintain feeding aggregations by short-distance cues associated with conspecifics or host plants (Grégoire, 1988; Crowe, 1995a). It is clear that these cues are also very important attractants for *C. a. asclepiadis*. This was very apparent in the no-choice test where

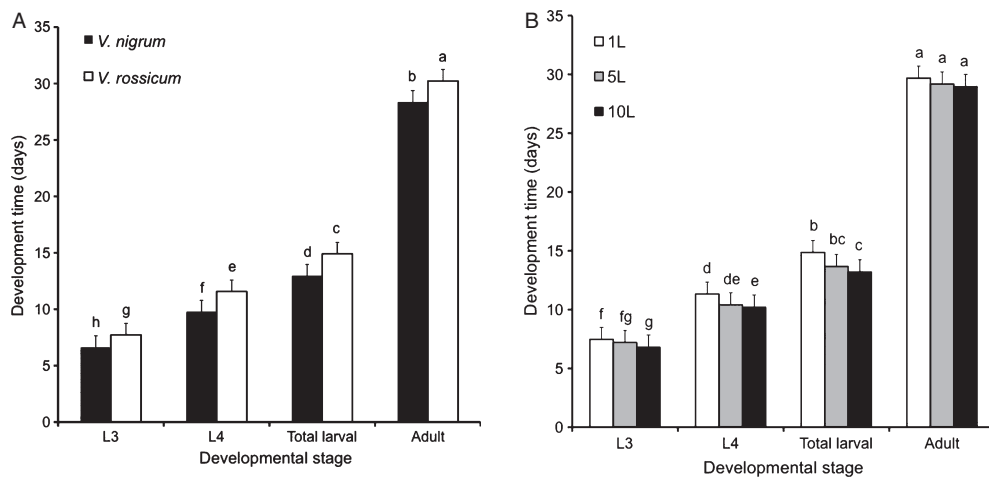


**Figure 3** Mean (+ SE) larval survival of *Chrysolina aurichalcea asclepiadis* group sizes [one (1L), five (5L), and 10 (10L) larvae] at each developmental stage. Different letters above bars indicate significant differences among developmental stages (Tukey's HSD test:  $P < 0.05$ ).

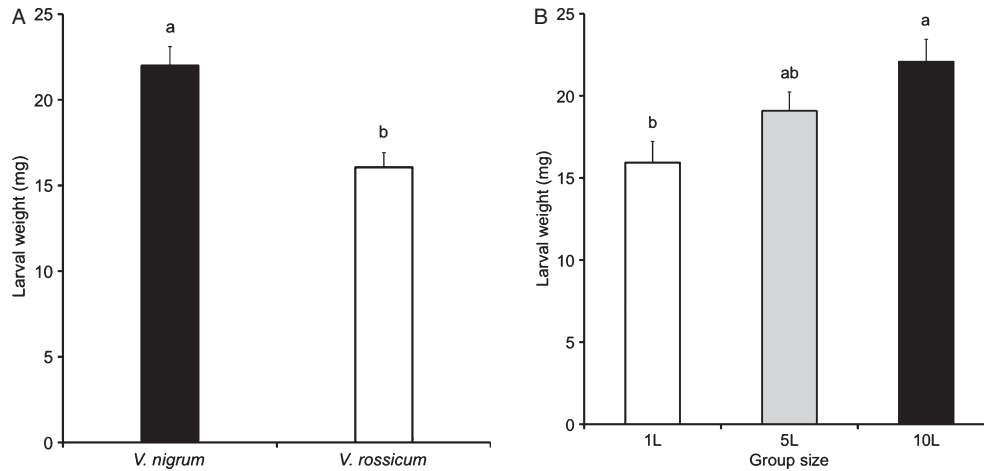
only one larva (of 20) ascended an undamaged shoot after 2 h despite larvae always being in close proximity (within 6 cm) to shoots. Moreover, when given a choice, neonates preferred shoots infested with larvae to undamaged shoots. However, it remains unclear whether the individual cues originating from the host or conspecifics or their combina-

tion are more attractive to *C. a. asclepiadis*. Larvae of the willow feeder *P. versicolora* utilize secretions or feces produced by siblings to reestablish feeding groups larvae, instead of tactile or visual cues (Crowe, 1995a). My study suggests that *C. a. asclepiadis* neonates are responding to similar cues as *P. versicolora* and these cues likely enhance host location and maintain feeding groups (Grégoire, 1988; Crowe, 1995a). It is also possible that visual or tactile cues may affect larval behavior.

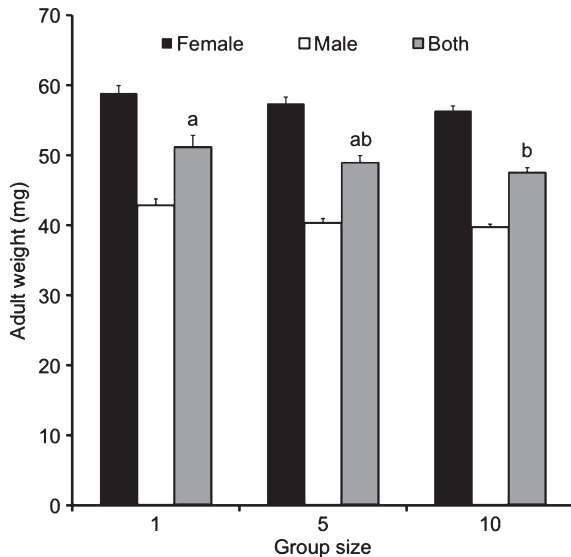
For many chrysolids feeding in groups enhances survivorship and larval growth by feeding facilitation (Breden & Wade, 1987; Chang & Morimoto, 1988; Grégoire, 1988; Nahrung et al., 2001; Costa et al., 2007). One type of facilitation is provided by the mechanical action of feeding by previous larvae that create feeding sites for other individuals (Nahrung et al., 2001). Another form of facilitation is enhancement of host plant quality or overcoming host defense (Denno & Benrey, 1997; Fordyce, 2003). For example, aggregative feeding by some Lepidoptera either induces a nutrient response in the plant or reduces the ability of plants to defend themselves against herbivores (Denno & Benrey, 1997; Fordyce, 2003). In this study, solitary larvae did not suffer greater mortality compared to grouped larvae, but larval development was faster (by about 2 days) for larvae in groups of 10. This effect on larval growth became evident early in development (to L3 stage) and continued until pupation. This is evidence that group feeding has a small positive effect on larval growth, which could be a result of feeding facilitation. However, facilitation was not by means of manipulating host plant suitability because excised shoots were used in the experiment.



**Figure 4** Effect of (A) host plant (*Vincetoxicum nigrum* and *V. rossicum*) and (B) group size [one (1L), five (5L), and 10 (10L) larvae] on mean (+ SE) development time to life stages of *Chrysolina aurichalcea asclepiadis*. Different letters above bars indicate significant differences (Tukey's HSD test:  $P < 0.05$ ).



**Figure 5** Effect of (A) host plant (*Vincetoxicum nigrum* and *V. rossicum*) and (B) group size (one, five, or 10 larvae) on mean (+ SE) larval weight of *Chrysolina aurichalcea asclepiadis* at 9 days. Different letters above bars indicate significant differences (Tukey's HSD test:  $P < 0.05$ ).



**Figure 6** Effect of group size (one, five, or 10 larvae) and gender on mean (+ SE) adult weight of *Chrysolina aurichalcea asclepiadis*. Different letters above bars indicate significant differences among group sizes (Tukey's HSD test:  $P < 0.05$ ).

Group feeding commonly improves larval growth during early instars (Breden & Wade, 1987; Chang & Morimoto, 1988) but is disadvantageous to later instars because of intense competition for food (Reader & Hochuli, 2003). To balance this trade-off many species that aggregate as young larvae become solitary over development (Matsumoto, 1990; Le Masurier, 1994; Fordyce, 2003). The results of this study indicate that a similar trade-off may occur during development of *C. a. asclepiadis*. Group feeding

positively affected weight gain during early larval development at least until 9 days. However, at the end of the experiment, teneral adult size was negatively affected by group feeding; larvae raised singly produced adults that were on average heavier than those from grouped larvae. This conclusion was not affected by gender-related variation in weight because sex ratios were equal among group sizes. Therefore, it appears that the shift from group to solitary feeding by *C. a. asclepiadis* during the L3 stage is a response to increased resource competition (Reader & Hochuli, 2003).

The effects of increasing larval group size on larval performance were generally similar between *V. nigrum* and *V. rossicum* despite the fact that leaf nitrogen, phosphorus, and water content tend to be greater in *V. nigrum* than *V. rossicum* (AS Weed, unpubl.). The measured differences in leaf quality possibly explain the faster development and weight gain on *V. nigrum* (Scriber & Slansky, 1981) but appear to be too subtle to cause species-specific benefits from group feeding as for the leaf miner *Microrhopala vitta* (Fabricius) on *Solidago* spp. (Damman, 1994).

The larval behavior experiments raise an important question regarding the approach used in the larval performance test. At the beginning of the larval performance test, I placed larvae directly on the host leaves instead of forcing them to initially locate their host plant. Given that group feeding was shown to enhance host location of neonates in the other experiments, if larvae were forced to locate the leaves in this test it is possible a greater proportion of larvae would have failed or taken longer to establish. By excluding what is arguably the most important benefit of group feeding to this species – host location – I may have

diminished the magnitude of the group size effect on larval performance.

For many aggregative species group feeding has evolved in response to natural enemy pressure (Hunter, 2000). In many of these species, feeding groups of young larvae actively defend themselves from predators through coordinated movements or secreting plant-derived compounds (Ghent, 1960; Grégoire, 1988; Vasconcellos-Neto & Jolivet, 1994). *Chrysolina aurichalcea asclepiadis*, like other species of *Chrysolina*, is likely protected by chemicals sequestered from its host plant (e.g., Fields et al., 1990), which it regurgitates on disturbance (AS Weed, unpubl.) as defensive behavior similar to *P. versicolora* (Crowe, 1995b). A group of larvae may be able to defend themselves from a predator more effectively than isolated larvae, or by enhancing larval growth group feeding may decrease exposure to natural enemies (Benrey & Denno, 1997). Alternatively, larvae feeding in groups may be more attractive to predators or parasitoids. I could not locate any reports of natural enemies of this species so benefits of group feeding related to natural enemy pressure are uncertain.

While most studies have evaluated chrysomelids that form larval aggregations on woody plants (e.g., Breden & Wade, 1987), this study and two others (Damman, 1994; Costa et al., 2007) have shown that leaf beetles also benefit from feeding in groups on herbaceous species. Both plant growth forms differ in traits affecting herbivore development, such as host plant quality and resistance strategies (Mattson, 1980; Scriber & Slansky, 1981; Coley et al., 1985; Stamp, 2003), which may affect the benefits of group feeding for their herbivores. The few published studies with Lepidoptera suggest that herbaceous plants are commonly defoliated by aggregative feeding species resulting in intense resource competition leading to dispersal that increases predation risk (Matsumoto, 1990; Le Masurier, 1994). A similar finding was also reported for the chrysomelid *M. vitta* on *Solidago* spp. (Damman, 1994). Thus it appears that, in general, the benefits of group feeding are similar on both plant growth forms (i.e., feeding facilitation and natural enemy protection), but differences in plant architecture and resource quality and availability (i.e., quantity of and distance between plant resources) characteristic of each growth form may present differing trade-offs to aggregative feeders. However, comparative studies are needed to test this hypothesis.

It is not surprising that group feeding by *Chrysolina* has gone unreported until now because early instars of *C. a. asclepiadis* and other species such as *C. aurichalcea* (Mannerheim), *C. hyperici* (Foster), and *C. quadrigemina* (Suffrian) are concealed within terminal leaf buds (Fields et al., 1990; Hayashi et al., 1994). A particularly novel

aspect of this study was that grouped larvae were shown to enhance host location. Additionally, this is the first study to examine the benefits of this behavior for a species that hatches off from its host plant and forms loose feeding groups. The majority of published studies have considered large, tightly aggregated species that feed on woody species. Despite these subtle differences in life history strategies, group-feeding behavior provides similar benefits to these taxa and also subjects them to a similar set of trade-offs during development. My experiments all suggest that group feeding is generally beneficial to young larvae of *C. a. asclepiadis* but additional research is needed to evaluate how the interactive effects of natural enemy pressure, competition, and host plant response to herbivory affect the benefits of this behavior to this beetle.

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### References

- Benrey B & Denno RF (1997) The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. *Ecology* 78: 987–999.
- Breden F & Wade MJ (1987) An experimental study of the effect of group size on larval growth and survivorship in the imported willow leaf beetle, *Plagioderia versicolora* (Coleoptera: Chrysomelidae). *Environmental Entomology* 16: 1082–1086.
- Carne PB (1962) The characteristics and behaviour of the sawfly *Perga affinis affinis* (Hymenoptera). *Australian Journal of Zoology* 10: 1–34.
- Chaboo CS (2004) Natural history observations in *Eurypepla calochroma* Blake (Chrysomelidae: Cassidinae: Physonotini). *Coleopterists Bulletin* 58: 142–143.
- Chang KS & Morimoto N (1988) Life table studies of the walnut leaf beetle, *Gastrolina depressa* (Coleoptera: Chrysomelidae), with special attention to aggregation. *Population Ecology* 30: 297–313.
- Chattopadhyay AK & Sukul N (1994) Anti-predator strategy of larval aggregation pattern in *Aspidomorpha miliaris* (Chrysomelidae; Coleoptera). *Entomon* 19: 125–130.
- Cocroft RB (2001) Vibrational communication and the ecology of group-living, herbivorous insects. *Integrative and Comparative Biology* 41: 1215–1221.
- Coley PD, Bryant JP & Chapin FS (1985) Resource availability and plant antiherbivore defense. *Science* 230: 895–899.



- Cossé AA, Bartelt RJ, Zilkowski BW, Bean DB & Petroski RJ (2005) The aggregation pheromone of *Diorhabda elongata*, a biological control agent of saltcedar (*Tamarix* spp.): identification of two behaviorally active components. *Journal of Chemical Ecology* 31: 657–670.
- Costa JF, Cosio W & Gianoli E (2007) Group size in a gregarious tortoise beetle: patterns of oviposition vs. larval behaviour. *Entomologia Experimentalis et Applicata* 125: 165–169.
- Crowe ML (1995a) Daytime mechanisms of reaggregation in imported willow leaf beetle, *Plagiodera versicolora*, larvae (Coleoptera: Chrysomelidae). *Animal Behaviour* 50: 259–266.
- Crowe ML (1995b) The effect of season and group size on survivorship and larval growth in *Plagiodera versicolora*. *Ecological Entomology* 20: 27–32.
- Damman H (1994) Defence and development in a gregarious leaf-mining beetle. *Ecological Entomology* 19: 335–343.
- Denno RF & Benrey B (1997) Aggregation facilitates larval growth in the neotropical nymphalid butterfly *Chlosyne janais*. *Ecological Entomology* 22: 133–141.
- Fields PG, Arnason JT & Philogene BJR (1990) Behavioural and physical adaptations of three insects that feed on the phototoxic plant *Hypericum perforatum*. *Canadian Journal of Zoology* 68: 339–346.
- Fitzgerald TD (1993) Trail following and recruitment: response of eastern tent caterpillar *Malacosoma americanum* to 5 $\beta$ -cholestane-3, 24-dione and 5 $\beta$ -cholestan-3-one. *Journal of Chemical Ecology* 19: 449–457.
- Fordyce JA (2003) Aggregative feeding of pipevine swallowtail larvae enhances hostplant suitability. *Oecologia* 135: 250–257.
- Ghent AW (1960) A study of the group-feeding behaviour of larvae of the jack pine sawfly, *Neodiprion pratti banksianae* Roh. *Behaviour* 16: 110–148.
- Grégoire J-C (1988) Larval gregariousness in the Chrysomelidae. *Biology of Chrysomelidae* (ed. by P Jolivet, E Petitpierre & TH Hsiao), pp. 253–260. Kluwer Academic Publishers, Boston, MA, USA.
- Hayashi Y, Fujiyama S & Suekuni J (1994) Life-cycle synchronization in *Chrysolina aurichalcea* (Coleoptera: Chrysomelidae) to its host *Artemisia princeps*: effects of host-leaf age on survival. *Applied Entomology and Zoology* 29: 149–155.
- Hunter AF (2000) Gregariousness and repellent defences in the survival of phytophagous insects. *Oikos* 91: 213–224.
- Le Masurier A (1994) Costs and benefits of egg clustering in *Pieris brassicae*. *Journal of Animal Ecology* 63: 677–685.
- Lloyd M (1967) 'Mean crowding'. *Journal of Animal Ecology* 36: 1–30.
- Matsumoto K (1990) Population dynamics of *Luehdorfia japonica* Leech (Lepidoptera: Papilionidae). II. Patterns of mortality in immatures in relation to egg cluster size. *Researches on Population Ecology* 32: 173–188.
- Mattson WJ, Jr (1980) Herbivory in relation to plant nitrogen content. *Annual Reviews in Ecology and Systematics* 11: 119–161.
- Müller C & Hilker M (2001) Host finding and oviposition behavior in a chrysomelid specialist—the importance of host plant surface waxes. *Journal of Chemical Ecology* 27: 985–994.
- Nahrung HF, Dunstan PK & Allen GR (2001) Larval gregariousness and neonate establishment of the eucalypt-feeding beetle *Chrysophtharta agricola* (Coleoptera: Chrysomelidae: Paropsini). *Oikos* 94: 358–364.
- Olckers T (1998) Biology and host range of *Platyphora semiviridis*, a leaf beetle evaluated as a potential biological control agent for *Solanum mauritianum* in South Africa. *BioControl* 43: 225–239.
- Peacock L, Lewis M & Herrick S (2001) Factors influencing the aggregative response of the blue willow beetle, *Phratora vulgatissima*. *Entomologia Experimentalis et Applicata* 98: 195–201.
- Peng C & Weiss MJ (1992) Evidence of an aggregation pheromone in the flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae). *Journal of Chemical Ecology* 18: 875–884.
- Reader T & Hochuli DF (2003) Understanding gregariousness in a larval lepidopteran: the roles of host plant, predation, and microclimate. *Ecological Entomology* 28: 729–737.
- Roessingh P (1989) The trail following behaviour of *Yponomeuta cagnagellus*. *Entomologia Experimentalis et Applicata* 51: 49–57.
- Scriber J & Slansky F, Jr (1981) The nutritional ecology of immature insects. *Annual Review of Entomology* 26: 183–211.
- Stamp N (2003) Out of the quagmire of plant defense hypotheses. *Quarterly Review of Biology* 78: 23–55.
- Stamp NE & Bowers MD (1990) Variation in food quality and temperature constrain foraging of gregarious caterpillars. *Ecology* 71: 1031–1039.
- Vasconcellos-Neto J & Jolivet PH (1994) Cycloalexy among chrysomelid larvae. *Novel Aspects of the Biology of Chrysomelidae* (ed. by PH Jolivet, ML Cox & E Petitpierre), pp. 303–309. Kluwer Academic Publishers, Boston, MA, USA.
- Wade MJ & Breden F (1986) Life history of natural populations of the imported willow leaf beetle, *Plagiodera versicolora* (Coleoptera: Chrysomelidae). *Annals of the Entomological Society of America* 79: 73–79.
- Weed AS (2010) Biology and Ecology of European Natural Enemies of Swallow-worts (*Vincetoxicum*) and the Potential for Biological Control. PhD Dissertation, Department of Plant Sciences, University of Rhode Island, Kingston, RI, USA.
- Weed AS & Gassmann A (2007) Evaluating the Potential for Biological Control of Swallow-worts, *Vincetoxicum nigrum* and *V. rossicum*. Unpublished 2006 Annual Report, CABI EU, Switzerland.
- Zvereva EL & Rank NE (2004) Fly parasitoid *Megaselia opacicornis* uses defensive secretions of the leaf beetle *Chrysomela lapponica* to locate its host. *Oecologia* 140: 516–522.