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Cornicle secretions by *Aphis fabae* (Hemiptera: Aphididae) result in age-dependent costs and improved host suitability for *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Braconidae)

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Abstract

We examined the life history consequences of cornicle secretion by *Aphis fabae* Scopoli in second and fourth instars, and its effects on host suitability for its parasitoid, *Lysiphlebus fabarum* (Marshall). Cornicle secretion did not affect aphid fecundity, but secretion in the second instar enhanced life table parameters, whereas secretion in the fourth instar affected them negatively, suggesting a higher cost of secretion in later instars. Secretion in either instar improved host suitability for *L. fabarum*. Although control and treated aphids were parasitized at similar rates, and with similar success, wasps developed faster and emerged as larger adults in aphids that had secreted, regardless of instar. Transgenerational effects were also evident. Progeny emergence was higher when parental wasps developed in fourth instars than in seconds, whether aphids secreted or not, and progeny were larger when parental hosts secreted in the second instar, but not in the fourth. Secreting fourth instars were preferred to controls by *L. fabarum* females in choice tests, but not secreting second instars, and fourth-instar secretion improved wasp emergence. When control aphids were attacked, second instars were more likely to secrete than fourth instars, whereas the latter were more likely to kick the parasitoid. Cornicle secretion reduced the probability of subsequent secretion events and the frequency of other aphid defensive behaviors, indicating energetic tradeoffs among defensive tactics. Overall, our results revealed that cornicle secretion by immature *A. fabae* exacts both physiological and behavioral costs and results in improved host suitability for its parasitoid.

Keywords: body size, development, life table, *Lysiphlebus fabarum*, parasitism

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Introduction

Aphid defensive behavior is one of the most important selective forces driving the evolution of foraging behavior in aphid parasitoids (Völkl & Mackauer, 2000). Pea aphids, *Acyrthosiphum pisum* Harris, may escape by dropping quickly from the plant (Chau & Mackauer, 1997; Villagra *et al.*, 2002), whereas other aphids may simply walk away (Weisser, 1994). However, some aphid species attempt to defend themselves *in situ*, thus avoiding the risks associated with host plant abandonment (Dill *et al.*, 1990), and differences in defensive tactics among aphid species faced with parasitoid attack can be pronounced. Whereas pea aphids drop from the plant when attacked regardless of parasitoid identity, *Myzus persicae*

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(Sulzer) is driven off the plant by *Aphidius ervi* Halliday, but is more likely to respond to attacks by *Aphidius colemani* Viereck with kicking and cornicle secretion (Ingerslew & Finke, 2017). *Aphis fabae* Scopoli displays a suite of defensive behaviors that can all be accomplished without withdrawal of the stylet from plant tissues. These include kicking, secreting from the cornicles, and raising and swiveling the body in circles around the point of attachment to the plant, a behavior that can serve to smear the attacker with cornicle secretions (Rasekh *et al.*, 2010*a*). However, because cornicle droplets usually contain alarm pheromones that serve to alert conspecifics (Mondor & Roitberg, 2003; Eichele *et al.*, 2016), the purpose of their secretion in particular contexts can be ambiguous, especially since some aphids can modulate pheromone content in secretions in a context-dependent manner (Joachim *et al.*, 2013).

Aphid cornicle secretions are composed largely of triglycerides and function as a fast-drying liquid wax with strongly adhesive properties (Callow *et al.*, 1973), although more recent analyses have revealed greater chemical complexity (Alfaress *et al.*, 2016). Droplets of cornicle secretion can permanently seal the mouthparts of smaller predators or otherwise ensnare them (Butler & O'Neil, 2006; Barry & Ohno, 2016), foul parasitoid antennae and force wasps to engage in an extended period of grooming, and even result in parasitoid death when wasps become permanently stuck to the aphid (Rasekh *et al.*, 2010*a*). However, the secretion of cornicle droplets can result in various fitness costs for the aphid. Subsequent development and reproduction may be impaired (Mondor & Roitberg, 2003; Moayeri *et al.*, 2012), and the alarm pheromones present in secretions may also act as kairomones that attract parasitoids (Micha & Wyss, 1996) and predators (Acar *et al.*, 2001; Verheggen *et al.*, 2008).

Although the fitness costs and benefits of cornicle secretions for aphids have received research attention, the fitness consequences for parasitoid wasps developing in such aphids has yet to be explored. *Lysiphlebus fabarum* (Marshall) (Braconidae: Aphidiinae) is the primary parasitoid attacking *A. fabae* throughout much of its range, including Iran (Talebi *et al.*, 2009). It attacks more than 70 species of aphids in agricultural and horticultural crops (Stary, 1986; Völkl, 1992) and occurs in sexual and asexual strains (Rakhshani *et al.*, 2005; Rasekh *et al.*, 2011), although the former appear to be more widely distributed. In the present study, we compared the developmental and life history consequences of cornicle secretion for *A. fabae*, with secretion occurring in the second or fourth instar. We also tested the acceptability of secreting aphids as hosts for *L. fabarum*, and whether cornicle secretion affected subsequent wasp–aphid interactions or the frequency of different aphid defensive behaviors. We hypothesized (1) that cornicle secretion would impose costs on developing aphids, and that these costs would scale inversely with the age of the aphid at secretion; (2) that cornicle secretion would diminish the probability of subsequent secretion by the aphid; (3) that cornicle secretion would increase the subsequent use of alternative defensive behaviors; and (4) that secretion would diminish host resistance to parasitism, which would be reflected in increased suitability for parasitoid development. We also tested whether development in secreting aphids would influence parental effects and thus the fitness of the subsequent wasp generation.

Materials and methods

Insect colonies

A stock colony of black bean aphids, *A. fabae*, was established from material collected in bean fields in Khuzestan Province (31°19′N, 48°41′E), Iran, in spring 2016, and mummies of the sexual strain of *L. fabarum* were obtained from these samples. The stock colony of *A. fabae* and the parasitoid wasp were maintained on potted broad bean, *Vicia fabae*, grown in pots filled with fertilized sawdust. All insects and experiments were maintained in growth chambers under the same environmental conditions: 21 ± 1°C, 65–75% RH, and a 16:8 (L:D) photoperiod.

To obtain synchronous cohorts of aphids for parasitism, broad bean shoots were each infested with 50 adult aphids from the stock colony. The cut stem of the bean shoot was immersed in a small vial of fertilized water (N:P:K = 20:20:20) to maintain turgor and placed in a ventilated plastic cylinder (8.0 cm diameter × 15.0 cm height) under the same physical conditions as the stock colony. The adult aphids were removed after 12 h, and the nymphs were left *in situ* to develop to the desired instar. Second- and fourth-instar *A. fabae* were 2.0 ± 0.4 and 3.5 ± 0.4 days old, respectively, at time of exposure to wasps. Second and fourth instars were selected for experimentation because they represent either end of the range of life stages suitable for parasitism by *L. fabarum*, both first instars and adults being outside the range of suitability, largely because of their small and large sizes, respectively (Ameri *et al.*, 2014).

In order to produce synchronous cohorts of parasitoid wasps, two-day-old *L. fabarum* females, without prior exposure to aphids, were introduced to an aphid cohort in a 1:5 ratio (one wasp for each five aphids) in plastic cylinders (as above). After 6 h, the wasps were removed and the parasitized aphids were reared on potted bean seedlings until mummies formed. These mummies were transferred into ventilated Petri dishes (3.5 cm diameter × 1 cm) until emergence of wasps, whereupon each wasp was provisioned with droplets of honey (diluted 50% in distilled water) on a strip of wax paper and water on a cotton ball. The water was refreshed daily, and the diluted honey every second day.

Bean leaves were collected from greenhouse plants and placed abaxial side on a layer of agar (1.5%) in a glass Petri dish (9.0 cm diameter). The desired number of *A. fabae* nymphs were then introduced to the Petri dish and allowed a period of 2 h to settle on the leaf before they were presented to parasitoids in experiments.

Elicitation of cornicle secretions

Within 12 ± 3 h of molting to the desired instar, aphids were stimulated to secrete cornicle droplets by gently stroking the anterior portion of the thorax with a fine brush. This was continued until it resulted in the secretion of visible cornicle droplets from both cornicles, as described by Mondor & Roitberg (2003). These are referred to henceforth as 'treated aphids' and were used in experiments within 30 ± 10 min of cornicle secretion, and before any subsequent molt.

Aphid development and reproduction

A series of second and fourth instar aphids (n = 25 of each) were stimulated to secrete cornicle droplets and then isolated in glass Petri dishes (9.0 cm diameter) on a broad bean leaf.

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Each aphid was observed every 12 h to determine the developmental time of all instars. Once aphids molted to the adult stage, each was transferred to a broad bean shoot so that the number of nymphs could be counted and removed daily; data collection continued until death. After death, the hind tibia of each adult aphid was photographed under a stereomicroscope equipped with a digital camera (Nikon Coolpix S10; Nikon Corporation, Tokyo, Japan) attached to a binocular microscope at 100× magnification and the hind tibia length (HTL) measured with a precision of 0.003 mm.

Development of L. fabarum *in secreting aphids*

Synchronous cohorts of aphids were reared to the second instar and a subset were stimulated to secrete droplets from both cornicles as in the previous experiment. Leaves of broad bean, each infested with 25 treated or control aphids (n = 18 in both cases), were placed in glass Petri dishes (9.0 cm diameter). A single, mated, two-day-old *L. fabarum* female was then released into each dish and observed continuously under a stereomicroscope until she attacked (probed with the ovipositor) a total of 20 aphids. Each aphid attacked was removed and placed on an excised bean shoot in a mini-cage sitting on a small container of water. Any control aphids which secreted cornicle droplets during wasp encounters were replaced with non-secreting aphids. After 3 days of rearing, 10 aphids from each replicate were dissected to assess their parasitism status and record numbers of larvae in each. The remaining aphids were reared to emergence of wasps. We recorded wasp developmental times, percentage of aphids parasitized, percentage of mummies emerging, and sex ratio. The HTL of wasps were also measured as in the previous experiment. In order to assess wasp fitness, wasps from each treatment were paired (n = 14 in both cases) on their second day of life and each female was then provided with 20 s instar aphids on a bean leaf in a Petri dish for 120 min. This combination of aphid number and exposure period was selected to maximize the resolution of differences between treatments based on our knowledge of how many aphids can be parasitized per female, per unit of time. The aphids from each replicate were then reared (as above) to emergence of wasps and their life history data recorded. The HTL of wasps was again measured. The entire experiment was repeated using fourth-instar *A. fabae* as hosts.

Acceptability of secreting aphids as hosts for L. fabarum*: a choice test*

A preliminary experiment, conducted separately with both second- and fourth-instar aphids, tested whether amputation of an aphid antenna would affect its acceptability as a host for *L. fabarum*, with the aim of using antennectomy as a tool for marking secreting vs. non-secreting aphids in a choice test. A single antenna on each aphid was snipped at its base with fine scissors and the aphid was allowed 2 h to recover prior to testing. Mated females of *L. fabarum* (n = 10) were each provided a choice of ten antennectomized and ten control aphids on a bean leaf in a Petri dish (9.0 cm diameter) for a period of 2 h. The aphids were then reared to mummification in order to determine the percentage parasitized. There was no significant preference expressed for antennectomized vs. control aphids either as second instars (t = 0.43, P = 0.681), or as fourth instars (t = 0.23, P = 0.826), so we were able to employ antennectomy as a means of marking aphids in choice tests without biasing results.

For the choice test, synchronous cohorts of aphids were prepared and a subset treated to induce to cornicle secretions (as above). Mated, two-day-old female *L. fabarum* (n = 18) were then released singly into glass Petri dishes (as above) containing a broad bean leaf infested with ten treated and ten control *A. fabae*, which were labeled by snipping one antenna (control aphids were snipped in half the replicates, and treated aphids in the other half). After 2 h of foraging, female wasps were removed, the aphids of each replicate were separated by treatment, and each type placed on their own excised bean shoot in a mini-cage for rearing. The percentage of aphids parasitized, percent emergence, and sex ratio were recorded for each treatment group in each replicate. The experiment was repeated with fourth-instar aphids.

Cornicle secretion and subsequent wasp–aphid interactions

Synchronous cohorts of second- and fourth-instar *A. fabae* were produced, as described in the previous experiment. Bean leaves were infested with either 20 treated aphids (n = 16) or 20 control aphids (n = 16) and each placed in a glass Petri dish (2.5 cm diameter). Mated, two-day-old *L. fabarum* females were then singly released into each dish. Once a female encountered the first aphid, the lid of the arena was removed and she was observed continuously under a stereomicroscope for 30 min, during which time the onset and duration of all distinguishable behavioral events were tallied. Wasp behavior was categorized according to the scheme of Rasekh *et al.* (2010*b*): latent period (time from introduction to patch until first aphid attack), resting, searching, host antennation, abdominal bending (in preparation for probing), grooming, probing aphids with the ovipositor, number of aphids encountered, number of aphids probed, and number of honeydew droplets consumed. Previous work has shown that *L. fabarum* females elicit honeydew secretion by *A. fabae* and consume droplets directly from the aphid's anus (Rasekh *et al.*, 2010*a*). Aphid defensive behaviors tallied included kicking, escaping an ovipositor probe, secreting cornicle droplets, and raising and swiveling the body around the point of stylet insertion.

Calculations of life tables parameters

Because aphid generation time is so short, mothers can continue to reproduce long after their daughters become reproductive, albeit at low to insignificant rates. Thus, estimates of fecundity used in life table calculations for a particular treatments are best tallied for a period equal to the developmental time in that treatment, plus 1 day to allow for adult maturation (Bayoumy *et al.*, 2015), otherwise use of lifetime fecundities will lead to underestimates or r_m. Thus, age-specific survival (l_x) and fecundity (m_x) were calculated for individual aphids using data from the first 7 days of adult life. Net reproductive rate, R_0, was defined as the product of age-specific survival and age-specific fecundity ($R_0 = \Sigma l_x m_x$), where l_x is the proportion of females alive on a given day, and m_x is the mean number of female births on that day (Southwood & Henderson, 2000). The mean generation time [$GT = (\Sigma l_x m_x x)/(\Sigma l_x m_x)$], intrinsic rate of natural increase ($r_m = \text{Ln } R_0/T$), finite rate of increase ($\lambda = e^{rm}$) were estimated according to Carey (1993) using the program MicroSoft Excel (2003). The population doubling time ($DT = \text{Ln } 2/r_m$) was calculated according to Mackauer (1983).

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Table 1. Mean (±SE) population growth parameters for *Aphis fabae* which secreted cornicle droplets in either the second or fourth instar (treated) compared with non-secreting aphids (control).

Parameter	Treated	Control	*F*	*P*
Second instars (df = 1,48)				
R_0	32.90 ± 0.07b	35.61 ± 0.11a	480.8	<0.001
r_m	0.513 ± 0.002a	0.486 ± 0.001b	1738.4	<0.001
GT	7.31 ± 0.005b	7.86 ± 0.004a	7763.5	<0.001
λ	1.67 ± 0.001a	1.62 ± 0.001b	1734.2	<0.001
DT	1.35 ± 0.001b	1.43 ± 0.001a	1741.0	<0.001
Fourth instars (df = 1,60)				
R_0	35.28 ± 0.033b	35.87 ± 0.034a	148.9	<0.001
r_m	0.392 ± 0.000b	0.429 ± 0.000a	48,978.5	<0.001
GT	9.10 ± 0.002a	8.12 ± 0.23b	17.6	<0.001
λ	1.48 ± 0.000a	1.53 ± 0.000b	48,412.7	<0.001
DT	1.77 ± 0.001a	1.62 ± 0.001b	50,913.2	<0.001

Values bearing the different letters were significantly different between treatments (one-way ANOVA, $\alpha = 0.05$).

Table 2. Mean (±SE) percentage of aphids mummified, percentage of mummies emerging, and sex ratio when mated *Lysiphlebus fabarum* females were individually provided with 20 *Aphis fabae* nymphs that had secreted cornicle droplets in either the second or fourth instar (treated), or non-secreting aphids (control).

	Treated	Control	*G*	df	*P*
			Second instars		
Percent mummified	76.0 ± 2.4a	77.0 ± 2.3a	0.19	1,30	0.666
Percent emerging	80.0 ± 3.7a	82.0 ± 2.9a	0.03	1,30	0.864
Sex ratio (% female)	53.5 ± 3.4a	52.1 ± 4.0a	0.08	1,30	0.779
			Fourth instars		
Percent mummified	80.0 ± 1.9a	83.0 ± 1.3a	1.33	1,34	0.257
Percent emerging	86.0 ± 2.2a	87.0 ± 1.6a	0.02	1,34	0.902
Sex ratio (% female)	62.5 ± 3.3a	58.4 ± 1.7a	1.32	1,34	0.258

Values bearing the same letter were not significantly different between treatments (*G* test, $\alpha = 0.5$).

Statistical analysis

A factorial two-way analysis of variance (ANOVA) was used to analyze biological data with 'treatment' and 'aphid instar' as independent fixed factors (SPSS, 1998). Percent aphids mummified, percent mummies emerging, and sex ratio data were analyzed by *G*-test using GLM with a binomial error distribution (Crawley, 1993). A χ^2 test was used to test for differences in numbers of aphids superparasitized. Data from choice tests were analyzed using a paired *t*-test (two-tailed). Since data generated by wasp–aphid interactions are either not normally distributed or are otherwise unsuited to parametric analysis, these data were analyzed by Mann–Whitney *U*-test (SPSS, 1998).

Results

Aphid development and reproduction

Cornicle secretions in the second instar decreased adult body size, as reflected in a mean (±SE) HTL of 2.19 ± 0.04 mm for treated aphids, compared with 2.33 ± 0.04 mm for control aphids ($F_{1,46} = 4.83$, $P = 0.033$), but secretion in the fourth instar did not reduce body size relative to controls (2.23 ± 0.03 vs. 2.22 ± 0.04 mm, $F_{1,58} = 0.004$, $P = 0.949$). Aphids secreting in the second instar molted to the third instar in 1.4 ± 0.05 days, faster than the 1.7 ± 0.1 days required for control aphids ($F_{1,45} = 5.56$, $P = 0.023$), but their total period of nymphal development did not differ ($F_{1,45} = 0.28$, $P = 0.645$), nor did the adult pre-reproductive period ($F_{1,45} = 0.11$, $P = 0.737$). There was no significant effect of treatment on the 7-day fecundity of aphids, whether aphids secreted droplets in the second instar ($F_{1,46} = 0.63$, $P = 0.432$), or in the fourth ($F_{1,46} = 0.63$, $P = 0.432$).

Analysis of population growth parameters suggested fitness benefits resulting from *A. fabae* cornicle secretion in the second instar, reflected in higher intrinsic rate of increase (r_m), shorter generation time (GT), and higher finite rate of increase (λ), although the net reproductive rate (R_0) was lower (table 1). These results were reversed for fourth instars, indicating costs for cornicle secretion in this life stage, and supporting our first hypothesis.

Development of L. fabarum *in secreting aphids*

Dissection of parasitized aphids revealed that there was no effect of treatment on the percentage of aphids parasitized, the percentage of mummies emerging, or the sex ratio, whether host aphids secreted cornicle droplets in the second or fourth instar (table 2). Similarly, there was no effect of treatment on the number of aphids superparasitized, whether treatment occurred in the second instar ($\chi^2 = 1.99$, $P = 0.158$) or in the fourth instar ($\chi^2 = 0.235$, $P = 0.628$). The two-way ANOVA of male developmental time revealed significant main effects of treatment ($F_{1,269} = 121.33$, $P < 0.001$) and aphid instar

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Table 3. Mean (±SE) developmental times and hind tibia lengths (HTL) of *Lysiphlebus fabarum* developing in *Aphis fabae* nymphs that secreted cornicle droplets in either the second or fourth instar (treated), or in non-secreting aphids (control).

	Treated	Control
Second instars		
Male development (d)	10.0 ± 0.05Ab	10.3 ± 0.03Aa
Female development (d)	9.9 ± 0.03Ab	10.1 ± 0.02Aa
Male HTL (mm)	0.49 ± 0.007Aa	0.46 ± 0.006Ab
Female HTL (mm)	0.49 ± 0.006Aa	0.47 ± 0.006Ab
Fourth instars		
Male development (d)	9.7 ± 0.04Bb	10.2 ± 0.02Ba
Female development (d)	9.6 ± 0.03Bb	10.1 ± 0.02Aa
Male HTL (mm)	0.51 ± 0.004Aa	0.47 ± 0.004Ab
Female HTL (mm)	0.50 ± 0.004Aa	0.48 ± 0.004Ab

Values bearing the same upper case letters were not significantly different between aphid instars within a treatment; values bearing the same lower case letters were not significantly different between treatments within an aphid instar (two-way ANOVA, $\alpha = 0.05$).

($F_{1,269} = 37.63$, $P < 0.001$), and a significant interaction between these factors ($F_{1,269} = 11.31$, $P = 0.001$) and the same was true for female developmental time ($F_{1,375} = 121.89$, $P < 0.001$; $F_{1,375} = 27.82$, $P < 0.001$; and $F_{1,375} = 14.90$, $P < 0.001$, respectively). The two-way ANOVA of male HTL revealed significant main effects of treatment ($F_{1,133} = 16.11$, $P < 0.001$), but not aphid instar ($F_{1,133} = 0.75$, $P = 0.387$), with no significant interaction between these factors ($F_{1,133} = 0.52$, $P = 0.820$). For female HTL, the main effect of treatment was significant ($F_{1,147} = 6.51$, $P = 0.010$), but not aphid instar ($F_{1,147} = 2.60$, $P = 0.110$), nor the interaction term ($F_{1,147} = 0.10$, $P = 0.757$). Developmental times were shorter, and HTLs longer, when either male or female *L. fabarum* developed in treated as opposed to control aphids, and this was true regardless of whether secretion occurred in the second or fourth instar, supporting our fourth hypothesis (table 3).

Development of L. fabarum *when parental wasps developed in secreting aphids*

When the treatment wasps obtained in the preceding experiment were paired, and the females provided with second-instar *A. fabae*, they mummified similar numbers of aphids as control wasps, and the mummies had similar emergence and sex ratios, whether their parents had emerged from host aphids that secreted cornicle droplets in either the second or fourth instar (table 4). Although there was no significant effect of host instar on the percentage of aphids mummified in either treated ($G_{1,30} = 0.014$, $P = 0.907$) or control ($G_{1,30} = 0.522$, $P = 0.476$) treatments, significantly more mummies emerged when parental wasps emerged from host aphids parasitized in the fourth instar than when they emerged from those parasitized in the second instar, and this was true for both secreting ($G_{1,30} = 3.481$, $P = 0.042$) and non-secreting ($G_{1,30} = 6.868$, $P = 0.014$) parental hosts. Sex ratio did not vary as a function of parental host instar for either treatment ($G_{1,30} = 1.050$, $P = 0.314$) or control ($G_{1,30} = 0.336$, $P = 0.567$) groups. Although developmental times were not affected by parental treatment, both male and female wasps had larger body size, as determined by HTL, when their parents emerged from host aphids secreting cornicle droplets in the second instar, but not when they secreted in the fourth instar (table 5).

Acceptability of secreting aphids as hosts for L. fabarum *in choice tests*

When females of *L. fabarum* were provided a choice between 10 s instar *A. fabae* nymphs that had secreted cornicle droplets and ten control nymphs that had not, females expressed a significant preference for aphids that had secreted cornicle droplets in the fourth instar ($t = 3.65$, $P = 0.002$), but not when they secreted in the second instar ($t = 0.54$, $P = 0.594$).

Cornicle secretion in the second instar did not improve emergence of mummies, but secretion in the fourth instar did (table 6). However, secretion in the second instar increased the proportion of female progeny, with a similar, if not quite significant, effect in the fourth instar. Secretion in the fourth instar increased the percentage of mummies emerging compared with the treatment in the second instar ($G_{1,34} = 10.917$, $P = 0.002$), but instar had no effect on the emergence of control wasps ($G_{1,34} = 0.228$, $P = 0.636$), and there was no significant effect of instar on sex ratio (treatment: $G_{1,34} = 0.036$, $P = 0.85$; control: $G_{1,34} = 0.254$, $P = 0.618$).

Cornicle secretion and subsequent wasp–aphid interactions

When females foraged for *A. fabae* nymphs that had secreted cornicle droplets in the second instar, they attacked the first aphid more quickly than when control aphids were provided, but this was not true of aphids that secreted in the fourth instar (table 7). A two-way ANOVA revealed significant main effects of treatment ($F_{1,60} = 35.30$, $P < 0.001$) and instar ($F_{1,60} = 29.19$, $P < 0.001$) on the number of aphid-kicking events, but no significant interaction between these factors ($F_{1,60} = 1.60$, $P = 0.212$). There were significant main effects of treatment ($F_{1,60} = 13.60$, $P < 0.001$), but not instar ($F_{1,60} = 1.97$, $P = 0.165$) on the number of aphid escapes following a probe with the ovipositor, and there was a significant interaction between them ($F_{1,60} = 5.21$, $P = 0.026$). Both treatment ($F_{1,60} = 68.89$, $P < 0.001$) and instar ($F_{1,60} = 38.861$, $P < 0.001$) affected the number of cornicle secretion events, and the interaction between these factors was also significant ($F_{1,60} = 33.39$, $P < 0.001$). Finally, treatment ($F_{1,60} = 19.64$, $P < 0.001$) and instar ($F_{1,60} = 4.91$, $P = 0.031$) both affected the number of body-swiveling events, but without a significant interaction ($F_{1,60} = 0.40$, $P = 0.529$).

Among both control and treatment aphids, fourth instars tended to use kicking and body swiveling more than second instars, and cornicle secretion less often. Fourth-instar controls also tended to escape following a probe with the ovipositor more often than did second-instar controls, but this was not true for treatment aphids. Aphids that secreted cornicle droplets prior to testing were less likely to do so when subsequently attacked by an *L. fabarum* female, regardless of instar, supporting our second hypothesis. However, secreting aphids also diminished their subsequent use of behavioral defenses such as kicking and body swiveling, which contradicted our third hypothesis (table 8).

Discussion

Our results revealed that cornicle secretion by immature *A. fabae* had developmental and life history impacts that depended on the age of the aphid at the time of secretion, confirming our first hypothesis. On balance, aphid fitness was increased by cornicle secretion in the second instar, but reduced by secretion in the fourth. Secretion in the second instar accelerated development, but reduced subsequent adult body

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Table 4. Mean (±SE) percentage of aphids mummified, percentage of mummies emerging, and sex ratio when mated *Lysiphlebus fabarum* females were individually provided with 20 *Aphis fabae* nymphs.

	Treated	Control	*G*	df	*P*
			Second instars		
Percent aphids mummified	69.0 ± 4.9Aa	58.0 ± 4.7Aa	2.40	1,26	0.121
Percent mummies emerging	68.0 ± 4.1Ba	65.0 ± 4.3Ba	0.19	1,26	0.663
Sex ratio (% female)	58.3 ± 4.1Aa	54.7 ± 4.1Aa	0.00	1,26	0.957
			Fourth instars		
Percent aphids mummified	68.0 ± 3.5Aa	63.0 ± 3.8Aa	1.01	1,34	0.322
Percent mummies emerging	91.0 ± 1.5Aa	90.0 ± 1.7Aa	0.25	1,34	0.620
Sex ratio (% female)	60.2 ± 2.6Aa	52.0 ± 3.6Aa	3.18	1,34	0.083

Parental wasps had developed either in aphids that secreted cornicle droplets in either the second or fourth instar (treated), or in non-secreting aphids (control). Values bearing the same lower case letter were not significantly different between treatments within an aphid instar; values bearing the same upper case letters were not significantly different between aphid instars within a treatment (*G* test, $\alpha = 0.5$).

Table 5. Mean (±SE) developmental times and hind tibia lengths (HTL) of *Lysiphlebus fabarum* developing in *Aphis fabae* nymphs.

	Treated	Control	*F*	df	*P*
			Second instars		
Male development time (d)	10.3 ± 0.06a	10.3 ± 0.06a	0.28	1102	0.597
Female development time (d)	10.2 ± 0.04a	10.3 ± 0.04a	1.18	1145	0.279
Male HTL (mm)	0.46 ± 0.004a	0.44 ± 0.006b	5.88	1102	0.017
Female HTL (mm)	0.46 ± 0.004a	0.44 ± 0.005b	4.17	1145	0.043
			Second instars		
Male development time (d)	10.3 ± 0.02a	10.3 ± 0.03a	0.36	1186	0.550
Female development time (d)	10.2 ± 0.02a	10.2 ± 0.03a	0.32	1243	0.074
Male HTL (mm)	0.45 ± 0.004a	0.44 ± 0.003a	3.51	1186	0.062
Female HTL (mm)	0.46 ± 0.004a	0.45 ± 0.003a	1.38	1243	0.242

Parental wasps had developed either in aphids that had secreted cornicle droplets in either the second or fourth instar (treated), or in non-secreting aphids (control). Values bearing the same letter were not significantly different between treatments (one-way ANOVA, $\alpha = 0.05$).

Table 6. Mean (±SE) percentage of mummies emerging, and sex ratio when mated *Lysiphlebus fabarum* females ($n = 18$ per treatment) were given a choice of ten *Aphis fabae* nymphs that had secreted cornicle droplets in either the second or fourth instar (treated), or in non-secreting aphids (control).

	Treated	Control	*G*	*P*
		Second instars		
Percent emerging	81.0 ± 2.5Ba	82.0 ± 4.5Aa	0.19	0.667
Sex ratio (% female)	69.8 ± 2.4Aa	55.1 ± 5.5Ab	6.40	0.016
		Fourth instars		
Percent emerging	93.0 ± 1.9Aa	86.0 ± 2.9Ab	6.44	0.016
Sex ratio (% female)	71.9 ± 4.1Aa	59.9 ± 3.9Aa	2.71	0.109

Values bearing the same upper case letter were not significantly different between instars; those bearing the same lower case letter were not significantly different between treatments (*G* test, $\alpha = 0.5$).

size; although it did not affect fecundity, it increased r_m and λ, while reducing GT and DT (table 1). Secretions in the fourth instar did not reduce adult body size or fecundity, but effects on life table parameters were negative rather than positive. Second-instar control aphids were also more than five times as likely to secrete cornicle droplets upon encounters with *L. fabarum* females as were fourth-instar control aphids (table 8), a result consistent with a higher cost of secretion in the fourth instar. A possible explanation is that second instars simply have more developmental time remaining in which to compensate for the cost of secretion, possibly by increasing their feeding rate. Cornicle secretion events also reduced the probability of subsequent secretion, and the subsequent use of defensive behaviors such as kicking or body swiveling, regardless of the instar in which secretion occurred. These changes in behavior likely reflect tradeoffs between various defensive tactics that derive from their respective costs within a limited energy budget for defense. However, aphids that secreted in the second instar became less likely to escape parasitoid attack following a probe with the ovipositor, an effect not seen in aphids that secreted in the fourth instar. The effectiveness of behaviors such as kicking and body swiveling is likely

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Table 7. Behavioral data for *Lysiphlebus fabarum* females foraging among 20 *Aphis fabae* nymphs (on a bean leaf disk) that had either secreted cornicle droplets in either the second or fourth instar (treated), or non-secreting aphids (control).

Behavior	Treated	Control	*U*	*P*
	Second instars			
Latent period to first attack (min)	1.44 ± 0.17b	2.12 ± 0.25a	75.5	0.047
Resting time (min)	0.60 ± 0.12b	1.55 ± 0.93a	85.0	0.110
Searching time (min)	5.97 ± 0.37a	7.24 ± 0.60a	90.5	0.160
Host antennation time (min)	3.29 ± 0.59a	1.61 ± 0.40a	87.0	0.122
Abdominal bending time (min)	4.26 ± 0.37a	4.79 ± 0.45a	103.5	0.361
Probing time (min)	14.95 ± 0.66a	9.30 ± 0.63b	16.0	<0.001
Grooming time (min)	5.18 ± 1171a	0.78 ± 0.22b	35.0	<0.001
No. aphid encounters	34.18 ± 1.31a	33.31 ± 2.04a	108.0	0.468
No. aphids probed	12.25 ± 1.01a	18.11 ± 0.89a	79.5	0.067
No. honeydew droplets consumed	0.38 ± 0.201b	1.25 ± 0.28a	67.0	0.021
	Fourth instars			
Latent period to first attack (min)	1.39 ± 0.17a	1.85 ± 0.20a	83.0	0.094
Resting time (min)	0.36 ± 0.07a	0.80 ± 0.13a	59.0	0.008
Searching time (min)	4.90 ± 0.24a	5.44 ± 0.42a	106.5	0.423
Host antennation time (min)	3.76 ± 0.36a	3.22 ± 0.23a	87.0	0.128
Abdominal bending time (min)	4.70 ± 0.35a	4.79 ± 0.32a	123.0	0.867
Probing time (min)	16.74 ± 0.68a	12.82 ± 0.42b	25.0	<0.001
Grooming time (min)	2.27 ± 0.51a	0.25 ± 0.11b	25.0	<0.001
No. aphid encounters	28.50 ± 1.67a	28.81 ± 1.25a	119.0	0.752
No. aphids probed	9.93 ± 0.52a	9.81 ± 0.34a	126.0	0.956
No. honeydew droplets consumed	0.06 ± 0.06a	0.18 ± 0.10a	112.0	0.564

Values bearing the same letter were not significantly different between treatments (Mann–Whitney *U*-test, $\alpha = 0.05$).

Table 8. Frequencies of different defensive behaviors displayed by *Aphis fabae* nymphs (on a bean leaf disk) in response to attacks from *Lysiphlebus fabarum* females ($n = 16$ replicates per treatment, 20 aphids per female, for 30 min).

Behavior	Treated	Control	*F*	*P*
	Second instars			
No. kicking events	5.87 ± 1.02Bb	16.75 ± 1.32Ba	42.32	<0.001
No. escapes following a probe	1.12 ± 0.22Ab	3.25 ± 0.58Aa	11.69	0.002
No. cornicle secretions	0.06 ± 0.06Ab	4.25 ± 0.56Aa	57.80	<0.001
No. body swiveling events	3.62 ± 0.58Bb	6.62 ± 0.62Aa	12.31	0.001
	Fourth instars			
No. kicking events	15.93 ± 1.63Ab	23.00 ± 1.90Aa	7.89	0.009
No. escapes following a probe	1.43 ± 0.22Aa	1.93 ± 0.26Aa	2.08	0.160
No. cornicle secretions	0.00 ± 0.00Ab	0.75 ± 0.19Ba	16.30	<0.001
No. body swiveling events	5.31 ± 0.54Ab	7.56 ± 0.61Aa	7.50	0.010

Aphids had either secreted cornicle droplets in either the second or fourth instar (treated), or were non-secreting aphids (control). Values bearing the same lower case letters were not significantly different between treatments within an aphid instar; values bearing the same upper case letters were not significantly different between aphid instars within a treatment (one-way ANOVA, $\alpha = 0.05$).

to scale with body size, and they are probably less costly than cornicle secretion, whereas cornicle secretion is probably a more effective defense for early instars, given their small size.

The cornicle secretions of *A. fabae* contain many volatile compounds, some of which have demonstrated kairomonal activity for foraging syrphid flies (Shonouda *et al.*, 1998), and some of which likely serve as alarm pheromones. Immature *A. pisum* Harris have been shown to secrete larger quantities of the alarm pheromone (*E*)-β-farnesene than reproductive adults, ostensibly because smaller aphids are more vulnerable to predation and parasitism (Mondor *et al.*, 2000). Moayeri *et al.* (2014) showed that experienced females of *Diaeretiella rapae* (McIntosh) orient to volatiles present in the cornicle secretions of their host, *Brevicoryne brassicae* (L.). Thus, the presence of alarm pheromone in cornicle droplets may have contributed to some of our results in these experiments. Mondor & Roitberg (2003) measured age-dependent fitness consequences of cornicle secretion in *A. pisum* and found a net benefit to fitness if secretion occurred in the third instar, a net cost if it occurred in the fourth instar, and no net impact if it occurred in the adult stage. These results are quite consistent with our own, in that secretion in a later immature stage had a more negative impact on aphid fitness than did secretion in an earlier stage.

Although cornicle secretion by *A. fabae* did not affect susceptibility to parasitism by *L. fabarum*, both male and female wasps developed faster and were larger when they emerged from secreting aphids, whether secretion occurred in the second or fourth instars (table 3). It is notable that female wasps parasitized significantly more secreting second instars

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than controls in the choice experiment, and also allocated more female eggs to secreting fourth instars than to controls (table 6). This suggests that females were sensitive to the increased suitability of secreting aphids and allocated proportionately more daughters to them, as predicted by sex allocation theory (Charnov *et al.*, 1981). Cornicle secretions have been previously shown to stimulate attacks by aphidiine parasitoids (Battaglia *et al.*, 2000), but the present results illustrate another tradeoff for the aphid: although secretion may increase the probability an *A. fabae* nymph escapes attack by *L. fabarum*, it results in improved host quality for parasitoid development when attacks are successful.

Host resources for parasitoid development are finite and parasitoid fitness is closely linked to host quality (Vinson & Iwantsch, 1980; Slansky, 1986; Sequeira & Mackauer, 1992). However, because the hosts of koinobiont parasitoids continue to feed and grow after parasitism, host quality is a dynamic, rather than static, property for aphidiine wasps, generating greater complexity (Harvey, 2005). Host insects express varying levels of immunological response to parasitism (Salt, 1941; Godfray, 1994) and parasitoids may require complex physiological mechanisms and a high level of host adaptation to overcome them (Carton *et al.*, 2008). Thus, reduced immunological resistance to parasitism is one possible mechanism by which cornicle secretion could increase host suitability for *L. fabarum*.

Interestingly, when parental wasps developed in aphids that had secreted cornicle droplets in the second instar, they gave rise to larger progeny as measured by HTL, a result that would suggest beneficial parental effects are yet another benefit of wasp development in secreting aphids (table 5). However, a larger proportion of mummies emerged when their parents began development in fourth-instar aphids compared with second-instar aphids, regardless of secretion status (table 4), a result that would suggest higher suitability of fourth instars, seemingly in contradiction to the life table data which indicate faster population growth in second-instar hosts.

Females *L. fabarum* seemed able to detect and respond to aphid cornicle secretion status, and this affected their foraging behavior. Females were faster to attack their first aphid in patches of aphids that secreted in the second instar, although this effect was not significant for aphids secreting in the fourth instar (table 7). Overall foraging efficiency appeared unaffected by host aphid secretion, but wasps spent more time probing aphids and grooming in arenas of treated aphids than in arenas of control aphids. They also spent less time resting and consuming honeydew droplets in the case of aphids that secreted in the second instar. Control aphids exhibited more frequent defensive behaviors than secreting aphids, including kicking, secreting cornicle droplets, and raising and swiveling the body (table 8), which may have caused wasps to spend more time grooming in control arenas. The greater reliance on kicking and body swiveling by fourth instars compared with second instars (table 7) probably reflects the greater effectiveness of these behaviors, and the higher costs of cornicle secretion, in later instars. These results reveal that cornicle secretion by *A. fabae* exacts a cost in the form of reduced capacity for subsequent defensive behavior, regardless of the instar in which it occurs, and that *L. fabarum* females adjust their foraging behavior to deal with the higher costs of handling non-secreting aphids that retain a higher defensive capability.

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