Defensive Behavior in Primary- and Secondary-Host Generations of the Soldier-Producing Aphid, *Pemphigus bursarius* (Hemiptera: Aphididae)

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The genus Pemphigus *comprises several species that produce soldiers (defensive morphs) in galls on the primary host. At the moment, it is unclear if host-alternating species also produce defenders on their secondary host. We therefore examined how* P. bursarius *morphs of the secondary host generations respond to predators to test whether they show defensive behavior. We further examined how this response compares with the antipredator behavior of soldiers in* P. bursarius *and* P. spyrothecae *occurring on the primary host. We performed two manipulative experiments using two predatory species to quantify the behavior of the different morphs in response to predators. In both experiments, secondary host morphs of* P. bursarius *showed no attacking behavior and antipredator behavior in these morphs was limited to escaping natural enemies by walking away. In contrast, the first instars of the primary host generations in both* Pemphigus *species showed attacking behavior and were capable of killing the predators.*

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INTRODUCTION

Aphid soldiers occur in approximately 60 gall-producing species of the subfamilies Hormaphidinae and Eriosomatinae (Hemiptera: Aphididae) (Aoki, 1977; Stern and Foster, 1996). Soldiers are capable of deterring or killing natural enemies and therefore may enhance persistence and reproductive output of a clone or colony of aphids (Foster, 1990; Foster and Rhoden, 1998; Rhoden and Foster, 2002). Some soldier morphs show clear morphological specialization for defence, such as frontal horns and enlarged, heavily sclerotized hind-legs (Stern and Foster, 1996). These "morphological weapons" are usually used to grasp and pierce the cuticle of an attacking natural enemy (Stern and Foster, 1996). The morphology of soldiers in other species is less specialized for defence and they are primarily defined as soldiers by their display of aggressive behavior against predators, which may involve the use of hind-legs, claws, and stylets (Stern and Foster, 1996).

Most species produce soldiers during the galling stage on the primary host, while only a few species produce soldiers on the secondary host. Aphids producing morphologically specialized soldiers on the secondary host are found in the Hormaphidinae and in the genus *Colophina* of the Eriosomatinae (Stern and Foster, 1996). Nevertheless, in many cases it is unclear whether species producing soldiers during the gall stage also produce soldiers on the secondary host. This is partly just a sampling bias, because in many soldier-producing species the secondary-host generations have not been tested for the occurrence of soldiers (this particularly true for species of the genus *Pemphigus*). In addition, soldiers are usually recognized as such by their specialized defensive morphology, yet soldiers need not necessarily show an extensive defensive morphology as mentioned earlier. A predominantly behavioral specialization for defense is more difficult to demonstrate and therefore the number of species producing soldiers on the secondary host may have been underestimated. So far, no studies have directly assessed the defense potential of secondary host generations that show no obvious defensive morphology but where soldiers are produced on the primary host.

The genus *Pemphigus* includes over 65 species, eight of which are known to produce soldiers during the galling phase of their life cycle (Aoki, 1980; Aoki and Kurosu, 1986, 1988; Foster, 1990; Moran, 1993; Rhoden and Foster, 2002). Behavioral data are lacking on one of the eight species (*P. monophagus*), but first instar virginoparae in the gall show the characteristic *Pemphigus* soldier morphology (Aoki and Kurosu, 1988). In the remaining species, soldiers are defined by their aggressive behavior and morphological specialization during the first nymphal instar (after which they develop into reproductive adults). However, recent results indicate

that later instars of five *Pemphigus* species also exhibit defensive behavior to some extent in the absence of the typical soldier morphology (Rhoden and Foster, 2002). This result stresses the need for more detailed observations on the antipredator behavior of aphid morphs lacking evident soldier morphology.

In this study, we performed quantitative experiments on the antipredator behavior in primary and secondary host generations of *Pemphigus bursarius* to address the question of whether secondary host morphs—with no apparent defensive morphology—show defensive behavior when confronted by a predator. Our objective was to quantify the differences in the antipredator behavior between morphs on the secondary host and the morphs of the primary host (soldiers) of *P. bursarius*. In this species, soldiers are morphologically specialized while morphs on the secondary host are not (Whitfield, 1998; C. Braendle, unpublished data). In addition, we compared the behavior of primary- and secondary-host generations of *P. bursarius* with the behavior of soldiers of *P. spyrothecae*, which show the most pronounced defensive behavior of all *Pemphigus* species (Rhoden and Foster, 2002).

Life Cycle and Soldier Production in *P. bursarius*

P. bursarius is a host-alternating species migrating between *Populus spp.* (primary host) and annual Compositae, such as *Cichorium, Lactuca*, or *Sonchus* (secondary host) (Dunn, 1959). In spring, the foundress induces a purse-shaped gall on the leaf petiole. The offspring (fundatrigeniae) are exclusively winged virginoparae, which leave the gall at maturity to colonize secondary host plants (Dunn, 1959; Grigarick and Lange, 1968; Phillips *et al.*, 1999). Once the winged migrants have settled on a secondary host plant their offspring will feed on the roots. These individuals are apterous and reproduce asexually throughout the summer. In early autumn, colonies begin to produce winged sexuparae, which fly back to the primary host where they give rise to the sexuals; however the secondary host colonies generally retain the potential to overwinter asexually in the soil by producing a proportion of nonmigrating virginoparae (Phillips *et al.*, 1999). The first instar fundatrigeniae (migrating virginoparae) exhibit soldier morphology typical for *Pemphigus* and they will readily attack predators. However, this morph also shows a lesser degree of defensive behavior during the second and third instar when no soldier morphology is apparent (Rhoden and Foster, 2002). There are no reports on the defensive behavior of secondary host morphs (wingless virginoparae and winged sexuparae), and these morphs do not show any soldier morphology as observed in *Pemphigus* soldiers (C. Braendle, unpublished data).

Life Cycle and Soldier Production in *P. spyrothecae*

Unlike most *Pemphigus* species, *P. spyrothecae* is not host-alternating and completes its entire life cycle on poplar (Foster, 1990). In spring, the foundress hatches from the overwintering egg and forms a spiral gall on the leaf petiole. The foundress offspring (fundatrigeniae) are wingless virginoparae and give birth to either winged sexuparae or wingless virginoparae. During late summer, the gall ostiole is formed and the adult sexuparous morphs fly from the gall to the bark of poplar trees where they give birth to sexual females and males. The morphs of all virginoparous generations in *P. spyrothecae* show a distinct soldier morphology and exhibit defensive behavior during the first instar (Aoki and Kurosu, 1986; Foster, 1990; Whitfield, 1998) and second instar (Rhoden and Foster, 2002). The sexuparae show no defensive morphology or behavior in any instar (Foster, 1990; Whitfield, 1998; Braendle, 1999).

METHODS

In early June 1999, we collected galls of *P. bursarius* and *P. spyrothecae* from mature Lombardy poplars (*Populus nigra* var. *italica*) in Cambridge (UK). Secondary host individuals of *P. bursarius* were obtained from a permanent laboratory culture maintained on lettuce (*Lactuca sativa*), reared under long-day conditions (LD 16:8 h) at approximately 20°C. As experimental predators, we used second-instar larvae of the two-spot ladybird *Adalia bipunctata* (Coleoptera: Coccinellidae) and third-instar larvae of the syrphid *Episyrphus balteatus* (Diptera: Syrphidae) (predators were derived from long-term laboratory cultures feeding on various aphid species). Both predators have been reported to prey on *P. spyrothecae* colonies as well as on *P. bursarius* primary and secondary host colonies (Dunn, 1960b; Foster, 1990; Braendle, 1999) and were therefore used in the experiments. We performed two experiments to test for the differences in the defensive behavior between the following morphs. (1) First- to fourth-instar virginoparae/sexuparae of *Pemphigus bursarius* (root generation, secondary host). Laboratory cultures of *P. bursarius* kept under long-day conditions (LD 16:8 h) contain virginoparae and sexuparae in a ratio of approximately 1:1 (Phillips *et al.*, 1999). The two morphs are morphologically very similar until the third instar, when wing pads are visible in the sexuparae whereas the virginoparae are apterous. For experiments involving first- and second-instar individuals, it was therefore not possible to determine the type of morph and it was assumed that an equal ratio of virginoparae and sexuparae was used (see above). (2) First-instar fundatrigeniae (soldiers) of

P. bursarius (gall generation, primary host). (3) First-instar fundatrigeniae (soldiers) of *P. spyrothecae* (gall generation, primary host).

Experiment 1: Attacking Propensity of Aphids
and Predator-Induced Mortality

We performed a modified experiment after Rhoden and Foster (2002) by constructing hemispherical arenas molded in blue Plasticene (1 cm in diameter, 1 cm deep) to observe the interactions between aphids and predators. For each of the three morphs, five first-instar nymphs were transferred into each arena ($N = 30$ per morph type). 15 minutes later, one second-instar two-spot ladybird larva was introduced in the arena. All experimental ladybird larvae had been starved for 24 h prior to the experiment to ensure that the larvae would forage for prey. After the introduction of the predator, the arena was covered with a microscope slide cover slip. The behavior of aphids and predator was then observed repeatedly over a period of 1.5 h. Every 10 min, it was noted whether aphids were attacking the ladybird larva and whether any aphids or the predator had been killed. A final observation was made after 24 h to count the number of killed aphids and killed predators. An aphid/predator was scored as killed when the animal was not moving in response to a gentle touch with a fine paint brush and showed a damaged cuticle (e.g. due to feeding or piercing of the predator or aphid). In a pilot assay prior to experiments, we found that aphids and ladybird larvae were capable of surviving in the experimental conditions (without food) for >30 h, i.e. a time period greater than the duration of the experimental period.

For the parametric test (ANOVA), the data were log-transformed to satisfy assumptions of homogeneity of variance and normality of error (Sokal and Rohlf, 1995).

Experiment 2: Quantifying the Behavior of Aphids

To quantify the behavior of morphs when exposed directly to a predator, we used an experimental procedure described by Rhoden and Foster (2002). In order to standardize the predator's behavior toward the aphids, a syrphid larva was restrained with a 0.5-mm diameter string tied about the middle of its body. For each experimental run, an aphid was picked up gently using a fine paintbrush, then placed on the back of the predator. The aphid's behavior on the predator was observed under a dissecting microscope for 1 min. The aphid responded in one of three ways: no reaction—the

aphid rested on the predator's back; walk away—the aphid left the predator; attack—the aphid attacked the predator by attempting to pierce the predator's cuticle with its stylets or claws. This experiment was also performed to examine the response of second- to fourth-instar secondary host morphs of *P. bursarius*. For each type of morph/instar, we carried out 30 independent runs, each time using a new syrphid larva.

RESULTS

Experiment 1: Attacking Propensity of Aphids and Predator-Induced Mortality

First-instar individuals of the secondary host generations of *P. bursarius* did not show any defensive behavior. Individuals were never observed to attack the ladybird larva. In contrast, the soldiers of *P. bursarius* and *P. spyrothecae* attacked the predator. The three morphs differed significantly in their attacking propensity within the first 90 min of predator exposure (χ^2 test, $\chi = 13.20$, df $= 2$, $P < 0.01$) (Fig. 1a). Within the same time
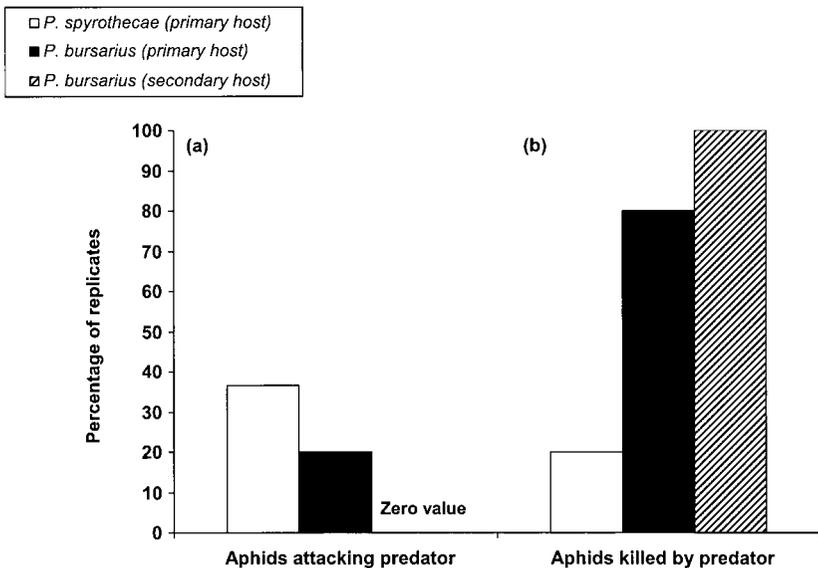

Fig. 1. Experiment 1. (a) Percentage of replicates that contained at least one aphid attacking the predator (within first 90 min after exposure) ($N = 30$ for each morph). (b) Percentage of replicates that contained at least one aphid killed by the predator (within first 90 min after exposure) ($N = 30$ for each morph).

period, morphs differed in their rate of survival (χ^2 test, $\chi = 37.14$, df $= 2$, $P < 0.001$). Soldiers of *P. spyrothecae* and *P. bursarius* showed a lower mortality than the secondary host morphs of *P. bursarius* (Fig. 1b).

After 24 h, at least one dead aphid was found in all replicates regardless of morph type or species, however the mean number of dead aphids per replicate differed significantly between morphs (ANOVA, $F_{2,87} = 42.48$, $P < 0.001$). In replicates (each containing five individuals) of *P. spyrothecae* soldiers, an average of 3.8 ± 0.35 individuals were found dead, in replicates of *P. bursarius* soldiers 4.83 ± 0.10, whereas none of the secondary host morphs of *P. bursarius* survived in any of the replicates. The proportion of replicates in which the predator was killed within 24 h differed significantly depending on the morph type (χ^2 test, $\chi = 16.41$, df $= 2$, $P < 0.001$). *Pemphigus spyrothecae* soldiers were more effective in killing the ladybird larvae than *P. bursarius* soldiers (35% [*P. spyrothecae*] and 10% [*P. bursarius*] of replicates contained a dead predator), whereas in replicates of the secondary host generations of *P. bursarius* all predators survived.

Experiment 2: Quantifying the Behavior of Aphids

The three morphs showed significant differences in their behavior (attack, walk away, or no reaction) in response to contact with the experimental predator (χ^2 test, $\chi = 31.10$, df $= 4$, $P < 0.001$) (Fig. 2). The soldiers of both species exhibited typical attacking behavior. *Pemphigus spyrothecae* soldiers were more prone to attack the predator than were *P. bursarius* soldiers. First-instar secondary host morphs of *P. bursarius* never attacked the syrphid larva (Fig. 2). In addition, the later instars of the secondary host generation of *P. bursarius* did not attack the predator, and there were no significant differences in the behavioral response between the different instars (χ^2 test, $\chi = 4.18$, df $= 3$, $P = $ NS).
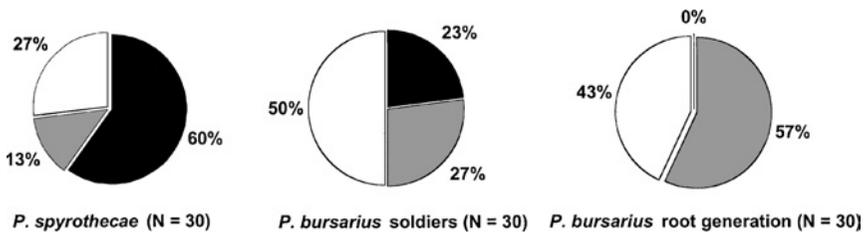

Fig. 2. Experiment 2. Behavior of different morphs in response to direct predator exposure. *Black section*: Percentage of aphids attacking the predator; *gray section*: percentage of aphids walking away from predator; *white section*: Percentage of aphids that showed no reaction.

DISCUSSION

The results indicate that secondary host morphs of *P. bursarius* lack defensive behavior. In two experiments, these morphs were never observed to attack the predator. In the first experiment, after 24 h, all individuals of this morph in all replicates were killed. In contrast, soldiers of both species were capable of defending themselves against the predator. In the first experiment, they succeeded in killing the predator: after 24 h, 35 and 10% of the ladybird larvae were killed by *P. spyrothecae* and *P. bursarius* soldiers respectively. Moreover, the soldiers of both species had a lower mortality rate throughout the experiment than the secondary host morphs of *P. bursarius*. The outcome of the second experiment indicates that the behavioral response of the secondary host morphs (first to fourth instar) is limited to escaping from the experimental predator by walking away. Conversely, soldiers of both species frequently attacked the syrphid larva. As in the first experiment, *P. spyrothecae* soldiers showed a higher propensity to attack the predator than did the soldiers of *P. bursarius*. This confirms the results of Rhoden and Foster (2002), who reported that *P. spyrothecae* soldiers are generally more aggressive than soldiers of the host-alternating *Pemphigus* species, including *P. bursarius*. The experiments presented here also corroborate previous studies showing that *Pemphigus* soldiers are capable of killing natural enemies (Foster, 1990; Foster and Rhoden, 1998; Rhoden and Foster, 2002). The differences in morphology between primary host soldiers and secondary host morphs of *P. bursarius* therefore appear to match the differences in the defensive behavior of morphs observed in the experiments.

Predation on colonies of *Pemphigus* species is common on both the primary and secondary host. On the primary host, predation events are largely restricted to the time period after the gall ostiole has formed (Dunn, 1960a,b; Braendle, 1999; Pike and Foster, 2004; Pike *et al.*, 2004). During this phase, predators may easily enter *Pemphigus* galls. Nevertheless, soldiers can limit their detrimental effects depending on the type of predator (Foster, 1990; Foster and Rhoden, 1998). In general, predator access to primary host colonies appears to be limited due to the habit of gall forming and the production of soldiers. Predators also attack secondary host colonies of *P. bursarius*. Both the immigrating fundatrigeniae and the root-dwelling generations may be preyed on by a number of insect predators (Dunn, 1960b). In contrast to primary host morphs, the secondary host morphs of *P. bursarius* exhibit no obvious means of defense although the root-dwelling habit may substantially decrease the spectrum of potential predators; however it has not been established to what extent the predation rates differ between primary and secondary host colonies.

Even though we assayed the antipredator behavior in response to a limited spectrum of predators (that were derived from laboratory cultures), it seems unlikely that secondary host morphs of *P. bursarius* would show defensive behavior when confronted by alternative predators occurring in their natural habitat. Defending aphids, such as *Pemphigus* soldiers, usually show defensive behavior in response to a large array of predators (including predators that do not occur in their natural habitat) and even artificial stimuli may elicit a defense response (Aoki and Kurosu, 1986; Foster, 1990; Rhoden and Foster, 2002).

Soldier production is very rare among aphids but many species display an array of alternative defense and escape strategies (Dixon, 1958; Rothschild *et al.*, 1970; Dahl, 1971; Malcolm, 1986; Witz, 1990; Gross, 1993; Smith, 1999; Weisser *et al.*, 1999). That the morphs of *Pemphigus* on the secondary host can show specialized behavior is shown by an intriguing suite of behaviors displayed by the first instars of *P. trehernei*. This species lives on the roots of a salt marsh plant and the first instars are specialized to disperse around the marsh by floating on the surface of the incoming tide (Foster, 1978; Foster and Treherne, 1978). Yet, it is not known whether the secondary hosts morphs of this, or any other, *Pemphigus* species have alternative ways of defending themselves against natural enemies.

ACKNOWLEDGMENTS

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