COLONY DEFENSE BY WINGPADDED NYMPHS IN *GRYLLOPROCIPHILUS IMBRICATOR* (HEMIPTERA: APHIDIDAE)

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ABSTRACT

Large, wax-covered colonies of the North American aphid *Grylloprociphilus imbricator* (Fitch) are known to last over several months on exposed twigs of American Beech (*Fagus grandifolia* Ehrhart). We hypothesized that the colonies could not persist for such a long period without defense against predators, and found that nymphs of the second generation attacked moth larvae that had been artificially introduced into the aphid colony. Nymphs of all four instars participated in the attack and stung the larvae with their stylets. Of 69 nymphs that attacked the larvae, 36 (52.2%) were 4th instar. Unlike older nymphs of other eriosomatines, wingpadded 4th-instar nymphs of *G. imbricator* were slender in shape with long legs, and actively walked around on the twig. This is the first report that wingpadded nymphs are the main defenders of an aphid colony.

Key Words: Defensive behavior, *Fagus*, soldier aphid, sociality, woolly beech aphid

RESUMEN

Se sabe que colonias grandes y lanudas del afido norteamericano *Grylloprociphilus imbricator* (Fitch) duran varios meses en ramitas expuestas de *Fagus grandifolia* Ehrhart. Formamos la hipótesis que las colonias no podrían durar por tanto tiempo sin defensa contra predadores, y encontramos que ninfas de segunda generación atacaron larvas de polilla que habían sido introducidas artificialmente a la colonia áfida. Ninfas de todos los cuatro instares participaron en el ataque y picaron las larvas con sus estiletes. De 96 ninfas que atacaron las larvas, 36 (52.2%) fueron 4to instar. A diferencia de esos otros eriosomatinos, ninfas de 4to instar "wingpadded" de *G. imbricator* fueron delgadas en forma con patas largas, y caminaron activamente en la ramita. Este es el primer reporte que ninfas "wingpadded" son los principales defendedores entre los áfidos.

Many aphid species of the subfamilies Eriosomatinae (formerly known as Pemphiginae (Blackman & Eastop 2000)) and Hormaphidinae are known to produce sterile or non-sterile defenders that attack predators, particularly in those species that form long-lived galls or large, exposed colonies (e.g., Aoki 1977; see review by Stern & Foster 1996). The defenders, or individuals that play a defensive role, are usually 1st- or 2nd-instar nymphs. Wingpadded 3rd- or 4th-instar nymphs, which are of course larger than younger nymphs but are usually obese, short-legged and sluggish, so far have not been reported to function as defenders. Exceptions are *Epipemphigus niisimae* (Matsumura) (Aoki et al. 1996) and *Dinipponaphis autumna* (Monzen) (Aoki et al. 1999), gall-forming aphids with small colony sizes. Although their wingpadded nymphs attacked artificially introduced insects, they functioned as auxiliary defenders at best (Aoki et al. 1999).

The woolly beech aphid, *Grylloprociphilus imbricator* (Fitch) (Eriosomatinae), forms large colonies on twigs of American Beech (*Fagus grandifolia* Ehrhart), its primary host, in North America (Hottes & Frison 1931; Smith 1974, Blackman & Eastop 1994). Colonies may reach 120-150 cm in length (Smith 1974). The single fundatrices or their offspring were found on beech from April to November in North Carolina (Smith & Denmark 1984). All the second-generation aphids become winged adult females, which migrate to the secondary host, baldcypress (*Taxodium distichum* (L.)), from the middle of June to the latter half of October or perhaps even to the end of November (Smith 1974; Smith & Denmark 1984). We questioned whether the colonies could last for so long without defense against predators, and found that nymphs of *G. imbricator* indeed attacked artificially introduced insects. In this species, nymphs of all four instars played a defensive role, but 4th-instar nymphs were the main defenders, as described below.

MATERIALS AND METHODS

Wax-covered colonies of *G. imbricator* were found on twigs of a few beech trees (*Fagus grandifolia*) in the Nichols Arboretum of the Univer-

sity of Michigan, Ann Arbor, Michigan, USA, on July 13 and 14, 2000. We chose one colony (accession no. 00168) for the experiment to determine whether nymphs might attack predators. Tortricid larvae (approximately 4-11 mm long) were collected from nearby shrubs of *Hamamelis virginiana* L., and individually placed on the colony to test for a defensive response. Although tortricid larvae are not natural predators of *G. imbricator*, in our extensive experience with defensive aphids we have found that moth larvae will readily elicit an attack response by aphids, if the aphids attack actual predators (see, e.g., Aoki 1977; Aoki & Kurosu 1986; Foster & Rhoden 1998). This is fortunate from a practical standpoint, because moth larvae are much more conveniently collected at a field site than are aphid predators such as syrphid or coccinellid larvae.

If an introduced larva fell off the colony due to attack by aphids within three minutes, we caught the larva in a small paper box and deposited the larva and the attached aphids into a vial of 80% ethanol. If a larva did not fall, we picked up the larva three minutes after introduction and deposited the larva and the attached aphids into a vial of 80% ethanol. The experiment was repeated ten times. After all trials, the entire colony was collected and preserved in 80% ethanol. The aphids were later examined and identified in the laboratory. Another entire colony (accession no. 00169) was also preserved in 80% ethanol. We also placed another tortricid larva on a third colony of *G. imbricator* to take photographs of attacking aphids. We did not replicate this experiment across colonies for practical reasons, due to the large size of the colonies (several thousand aphids, see Results). If trials had been replicated across multiple colonies, this would have necessitated collecting, counting, and sorting many thousands of aphids, and would have decimated the aphid population in the arboretum. We are confident that the defensive behavior observed should be consistent across colonies, due to our experience with other defensive aphids, and because the same behavior was elicited in the third colony assayed for photographs.

In the laboratory, all aphids from the two colonies (nos. 00168 & 00169) were detached from twigs, and the total number of aphids and 4th-instar nymphs was counted under a dissecting microscope. Many aphids, including nymphs that had attacked the larvae, were boiled in 10% KOH solution, stained with acid fuchsin or Evans' blue, and mounted in balsam. The slide-mounted specimens were examined under a light microscope to determine the instar of each nymph, and whether any dimorphism occurred within an instar. Because 1st-, 2nd- and 3rd-instar nymphs could be distinguished from each other only in slide-mounted specimens under a compound microscope, we could not categorize the entire colony according to each nymphal instar (as it is impractical to slide-mount thousands of aphids).

Results and Discussion

Colony Structure

The colony (no. 00168) used for the experiment contained a single fundatrix and a total of 4,218 nymphs of the 2nd generation, of which 479 (12.8%) were 4th-instar nymphs. Another colony (no. 00169) contained a single fundatrix, two winged adults, and 7,437 nymphs, of which 1,143 (15.4%) were 4th instar. No wingless adults (except the fundatrix) were found in the samples, which indicates that the individual fundatrices produced thousands of nymphs. The fundatrices were very large (approximately 5-6 mm long) and superficially resembled a termite queen (Fig. 1). Each fundatrix was hidden under a layer of nymphs, which we had to remove to observe her. No winged adults were contained in colony 00168. Because this colony contained cast-off skins of wingpadded 4th-instar nymphs, it is certain that the colony had already produced some winged adults. A number of winged adults were found in other colonies.

No ants were observed visiting the two experimental colonies, or five other colonies that we were able to examine at close range. When we lightly touched a twig on which a colony was formed, some nymphs produced honeydew, but not many droplets of honeydew fell from the colony, contrary to the usual observation of aphids. (Hottes & Frison (1931) mentioned that *G. imbricator* often "produces so much honey-dew that the ground beneath the infestation becomes discolored." Thus, if ants were present, they were not effectively removing the honeydew from the observed colonies.) Put together, these observations strongly suggest that *G. imbricator* does not depend on defense by ants on the primary host.

We found no within-instar dimorphism in the 2nd generation of *G. imbricator*. This indicates that no morphologically distinct soldier caste is produced in the species.

Defensive Behavior

All ten tortricid larvae introduced onto the colony were attacked by nymphs of *G. imbricator* (Fig. 2). Eight were attacked almost immediately after introduction, and the other two were attacked 14 and 39 seconds after introduction. The attacked larvae responded by wriggling, and four fell off the colony within three minutes (27-128 seconds after introduction). A total of 69 nymphs attacked the ten tortricid larvae, and 47 of them did not detach themselves from the larvae even after being deposited in ethanol. We ascertained under a dissecting microscope that many of them, including nymphs

Fig. 1. A fundatrix of *Grylloprociphilus imbricator* with a few nymphs on her body. The fundatrix was removed from the colony and place on a leaf.

Fig. 2. Six 4th-instar nymphs and several younger nymphs of *Grylloprociphilus imbricator* attacking a tortricid larva. The larva was removed from the colony and placed on a leaf.

Fig. 3. Part of a colony of *Grylloprociphilus imbricator* formed on a twig of *Fagus grandifolia*. Some 4th-instar nymphs were excitedly walking around while raising their abdomens covered with wax filaments.

of all four instars, stung the larvae with their stylets. We also confirmed the stinging behavior by placing some aphids on our hands. At least one 4th-instar nymph and one 3rd-instar nymph stung our skin and caused minor irritation.

Wingpadded nymphs, especially 4th-instar nymphs, were very active. When the colony was disturbed, many 4th-instar nymphs raised the tips of their abdomens covered with woolly wax and a few long wax filaments (Fig. 3), and walked around while waving their abdomens back and forth. Stationary (and probably feeding) nymphs also waved the tips of their abdomens in the same way. Unlike other aphids, including *Pseudoregma* (Aoki et al. 1981, Sakata & Ito 1991), they did not lift their hind legs or wave them.

Of the 69 nymphs that attacked the introduced larvae, 36 (52.2%), 4 (5.8%), 3 (4.3%) and 26 (37.7%) were 4th-, 3rd-, 2nd- and 1st-instar nymphs, respectively. These figures indicate that 4th-instar nymphs are more likely to attack introduced larvae than are non-4th-instar nymphs (test of proportions; 36/479 vs. 33/3739, $Z = 10.58$, $P < 0.001$). In eight of the ten trials, at least one 4th-instar nymph attacked the introduced larva. We observed that 4th-instar nymphs of *G. imbricator* have a slender body and long legs (Figs. 2 & 3), which look quite different from those of other eriosomatines, and probably account for their comparatively greater mobility. On the other hand, 4th-instar nymphs of *G. imbricator* become swollen just before molting into winged adults. These nymphs look more similar to 4th-instar nymphs of related species.

Defensive behavior, often exhibited by specialized defensive morphs, has evolved several times and in different generations in aphids (Stern & Foster 1996). This is the first report that wingpadded 4th-instar nymphs are the main defensive morph. As discussed earlier, in almost all aphid species, the main defenders are small 1st- or 2nd-instar nymphs. Some species, such as *Pseudoregma alexanderi* (Takahashi) and *Colophina monstrifica* Aoki, have acquired larger soldiers by enlarging their sterile 1st-instar nymphs (Aoki et al. 1981, Aoki 1983). In contrast, *G. imbricator* has acquired large defenders by modifying the behavior and morphology of its wingpadded 4th-instar nymphs.

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