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BEHAVIOR

Frequency Distribution of Larval Codling Moth, *Cydia pomonella* L., Aggregations on Trees in Unmanaged Apple Orchards of the Pacific Northwest

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ABSTRACT The codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), is a key pest of pome fruits in North America. After locating a pupation site, larvae spin a cocoon from which aggregation pheromone disseminates that attracts conspecific larvae. In two unmanaged apple orchards in Wenatchee and Yakima, Washington State, we systematically surveyed cracks and crevices of tree trunks for cocooning *C. pomonella* larvae. Aggregates of larvae were found significantly more often than solitary larvae. The number of cocooning larvae in aggregates (=group size) was inversely correlated with the frequency occurrence of that group size. Group size ranged between 2 and 20 cocoons. Height above ground had no effect on location of aggregates. In orchard 1, the cardinal direction of the tree trunk had no effect on location of aggregations, but in orchard 2, aggregations were located significantly most often on the south side of trunks. The mean ratio of males and females in aggregations was 1.08:1 and 1.04:1 in orchards 1 and 2, respectively. Moreover, the number of males in aggregates did not significantly differ from that of females. Our data support the conclusion that larvae seek pupation sites not by chance but in large part in response to pheromone signal and microhabitat cues. The probability of aggregates forming is likely proportional to the population density of *C. pomonella*.

KEY WORDS codling moth, *Cydia pomonella*, larval aggregation, pheromone, frequency distribution

In many insect taxa, individuals form groups or aggregations in response to signals or cues from conspecifics or resources (Prokopy and Roitberg 2001, Krause and Ruxton 2002) including potential mates, food, oviposition sites, or shelters. Individuals in aggregations benefit through reduced mortality by natural enemies, increased probability of finding food or mates, or improved ability to overcome plant defenses (Prokopy and Roitberg 2001).

In nonsocial holometabolous insects, phermonal signaling is common among adults but rare among larvae, or larvae and adults. Only a few such communication systems have been reported. The larval pheromone of the Indianmeal moth, *Plodia interpunctella*, induces oviposition by adult females, which results in larger larval populations that more efficiently use resource nutrients, reduce microbial growth on food, and generate a larger matting of silk as a means of protection from natural enemies (Phillips and Strand 1994, Prokopy and Roitberg 2001). At high larval densities and thus high concentrations of pheromone, however, the pheromone repels larvae, thereby regulating population density at a resource (Mossadegh 1980, Prokopy and Roitberg 2001). Larvae of the bark beetle, *Dendroctonus micans*, produce a pheromone that facilitates aggregative and efficient feeding, with aggregated individuals growing faster and larger than solitary ones (Storer et al. 1997).

Larvae of the codling moth, *Cydia pomonella* L., produce a pheromone during cocoon-spinning that attracts conspecific larvae to the site of pheromone release (Duthie et al. 2003; Jumean et al. 2004, 2005a, b, 2008). *C. pomonella* is the most important pest of apple fruits, other pome fruits, and nuts worldwide (Clausen 1978). In the western United States and British Columbia, Canada, it is the key pest of apple fruits (Judd et al. 1997, Calkins and Faust 2003). Damage is caused when first-instar larvae bore into apples, causing diagnostic “sting marks.” Larvae develop through five instars. Fully mature fifth-instar larvae exit the fruit either through the entrance tunnel or a new tunnel and traverse a host tree trunk in search for a suitable site in which to spin a cocoon and pupate. From the freshly spun cocoon of male and female larvae, the aggregation pheromone disseminates attracting male and female larvae (Duthie et al. 2003, Jumean et al. 2004, 2005a, b, 2008). Compared with solitary larvae, aggregated larvae are better protected from parasitism by the parasitic wasp *Mastrus ridibun-
Larvae respond to aggregation pheromone in the laboratory (Duthie et al. 2003, Juneau et al. 2005a) and to pheromone-baited cardboard bands in managed orchards (Juneau et al. 2007). However, the occurrence and characteristics of aggregations of *C. pomonella* larvae in orchard settings have not yet been intensely studied. Closing this knowledge gap would shed light on the significance of larval communication and aggregation and help design management tactics that target larvae in addition to adults. Here, we report group size, sex ratio, location, and frequency distribution of larval/pupal aggregates on trees in two unmanaged apple orchards.

**Materials and Methods**

**Survey Orchards.** Surveys were conducted in two 2.5-ha abandoned apple orchards (orchard 1: 47°26.286 N, 120°21.389 W; orchard 2: 46°58.279 N, 120°35.431 W) in, respectively, Wenatchee and Yakima, Washington State. Each orchard had moderate to high population densities of *C. pomonella*. Orchards were 25–40 yr old and planted at a density of 200 trees/ha, with a tree by row spacing of 3 by 5 (orchard 1) and 4 by 5 m (orchard 2). The apple variety in orchard 1 was Golden Delicious, and the varieties in orchard 2 were Golden Delicious and Red Delicious. Tree trunks in orchards 1 and 2 had a mean circumference at 50 cm above ground of 43 and 83 cm, respectively. The gross features of the bark (presence of numerous cracks, crevices, and fissures) and thus the quality of the microhabitat for *C. pomonella* larvae were comparable within and between orchards. Surveys were conducted between 20 and 27 October 2006, when aggregations of the overwintering generation were expected to be most prevalent.

**Sampling Protocol.** Selecting every other tree, we surveyed a total of 35 trees in orchard 1 and 25 trees in orchard 2 for the presence of *C. pomonella* larvae. We surveyed the lowest 1-m trunk section because our exploratory surveys of entire trees had yielded few larvae above this height. Three trees on either end of each row within blocks were excluded to minimize edge effects. Aggregation was defined as at least two occupied cocoons with at least one in physical contact with another. In sequence, we (1) searched the surface of a trunk and fissures on the surface for exposed and visible cocoons; (2) gently tore such cocoons apart with fine forceps to determine occupancy; (3) inspected the soil-tree interface for cocoons; (4) peeled off bark flakes with sturdy knives to expose larvae beneath; and (5) lifted or removed tightly attached sections of bark to expose cocooned larvae in such spaces. For each larva, we recorded (1) the sex (gonads are visible on the mid-dorsal surface of male larvae), (2) the sex and number of additional larvae; (3) cardinal direction; and (4) height above ground (in 10-cm partitions). All larvae were collected to avoid repeated recordings.

**Data Analyses.** For each orchard, the number of larvae cocooning singly or in aggregates was analyzed with the $\chi^2$ goodness-of-fit test with Yates correction for continuity. Differences in height or cardinal direction of pupation sites were analyzed with a $\chi^2$ goodness-of-fit test for multiple categories followed by subdivision of categories (Zar 1999). The mean sex ratio (male:female) of aggregations was determined by dividing the number of males with the total number of individuals in each aggregation, taking the mean of all aggregations and converting it to a ratio out of 1. The difference in numbers of females and males in aggregations was analyzed with a Wilcoxon signed rank paired-sample test (SAS version 9.1). The experimental error rate in all experiments was set at $\alpha = 0.05$.

**Results**

In both orchards, larvae were found to be cocooning singly or in aggregates of varying sizes (Fig. 1). The number of cocoons in aggregates (group size) was inversely correlated with the frequency occurrence of that group size (Fig. 1). Overall, aggregated specimens were found more often than solitary specimens (orchard 1: $\chi^2 = 18.14, P < 0.001, n = 127$; orchard 2: $\chi^2 = 31.20, P < 0.01, n = 195$; Fig. 2), but the number of solitary larvae encountered was greater than any other group size. Group size (excluding solitary larvae) ranged between 2 and 20 cocoons (mean = 3.33) in orchard 1 and between 2 and 16 (mean = 3.48) in orchard 2 (Table 1). In orchard 1, the cardinal direction of the tree trunk had no effect on location of aggregations ($\chi^2 = 5.60, P > 0.10, n = 76$), but in orchard 2, aggregations were located significantly most often on the south side of trunks ($\chi^2 = 3.94, P < 0.05, n = 108$; Table 1). Height above ground had no effect on location of aggregates in orchard 1 ($\chi^2 = 13.03, P > 0.05, n = 73$) or orchard 2 ($\chi^2 = 14.07, P > 0.05, n = 108$). The mean ratio of males and females in aggregations was 1.08:1 and 1.04:1 in orchards 1 and 2, respectively (Table 1). Moreover, the number of males in aggregates did not significantly differ from that of females (orchard 1: $P = 0.87, n = 34$; orchard 2: $P = 0.47, n = 47$).

**Discussion**

Our data showed that *C. pomonella* larval cocoon singly and in aggregates in the field (Fig. 1). However, the number of individuals cocooning with at least one other individual is greater than the number of individuals cocooning by themselves. These results contribute to our knowledge of cocooning behavior and dynamics of *C. pomonella* in the field. Moreover, these results seem to support the concept that larvae locate pupation sites in response to both pheromonal signal and microhabitat cues.

The search for pupation sites reportedly occurs in two distinct phases (Geier 1963). In the displacement phase, the very mobile larvae readily traverse tree trunks. This displacement phase is punctuated by an area-searching phase, often when larvae encounter a...
trunk section with suitable pupation sites (Geier 1963). In the area-searching phase, larvae typically stop and display characteristic head thrusts (Geier, 1963; Z.J., personal observation), possibly probing for the presence of aggregation pheromone at prospective pupation sites. With the active range of the pheromone exceeding 20 cm (Jumean et al. 2008), larvae are likely to detect airborne pheromone from cocoon-spinning conspecifics nearby. A combination of chemotokinetic and tactic responses (Jumean et al. 2008) might guide foraging larvae to the site(s) of pheromone release, resulting in aggregates of cocooning larvae.

This concept is equally applicable to female and male larvae, because they occurred in aggregation at a sex ratio of 1:1 (Table 1), and both female and male larvae produce and respond to aggregation pheromone (Jumean et al. 2004). The concept that aggregations may form in response to both pheromone and physical characteristics of potential pupation sites is supported by observations that at least 50% of sites, most of which were best suited to host a single larva, were unoccupied (Z.J., personal observation), suggesting that pupation sites are not a limiting resource. Interestingly, larvae were either absent from or present in numbers of at least two on each trunk surveyed.

The mean number of larvae in aggregates (3.33 and 3.48 in orchards 1 and 2, respectively) is a conservative estimate based on our strict protocol that larvae must be in physical contact with one another to be considered aggregated. There were numerous observations of cocoons or groups of cocoons that were separated by as little as 1 cm. When considering the proposed benefits (e.g., efficient location of pupation sites, expedient adult mating, reduced risk of parasitism) and costs (e.g., increased rate of location by parasitoids) (Jumean et al. 2009) of pheromone-based larval aggregation, insects occupying cocoons in close proximity to other cocoons would still experience the same

Fig. 1. Frequency distribution of C. pomonella larval aggregations in orchards 1 and 2. Shaded bars indicate the number of observations of a particular group size, whereas open bars indicate the number of specimens found in a group size. Total numbers of observations or specimens are given above bars. Bars with different letters within each observation group are significantly different by the χ² goodness-of-fit test for multiple categories followed by subdivision of categories.
selective pressures as insects occupying cocoons in physical contact with one another.

Neither height above ground nor cardinal direction had profound effects on selections of pupation sites (Table 1), suggesting that other physical parameters of prospective pupation sites provide stronger foraging cues. Thigmotactic stimuli associated with cracks and crevices may constitute such cues (Geier 1963) and may be critically important in selecting a pupation site. In our initial field survey, we quantified cocooning larvae on branches and trunks of trees. The typically smooth bark branches in the lower, middle, and upper canopy were nearly void of *C. pomonella* cocoons. Similarly, in two high-density planted orchards with smooth-bark trees, the three cocooned *C. pomonella* larvae that we located were in the duff layer of the soil at the base of trees, and no larvae were located within trees.

There is mounting evidence that aggregates of *C. pomonella* cocoons form, in part, in response to aggregation pheromone (Jumean et al. 2004, 2005a, b, 2007, 2008; this study). The probability of aggregates forming is likely directly proportional to the density of *C. pomonella* populations. At high population densities, many larvae may complete development on the same tree and on its trunk may aggregate in the same pupation site. If, as proposed by Duthie et al. (2003), adults eclosing from aggregate cocoons more readily acquire mates and produce more offspring than their counterparts eclosing from solitary pupae, aggregate cocoons aggravate the inefficiency of pheromone-based disorientation of adult males that is observed in orchards with high *C. pomonella* population densities (Barclay 1992, Barclay and Judd 1995). Even at low to moderate population levels and otherwise effective population control measures, mated females may originate from aggregate cocoons, and their offspring may generate fruit damage in localized “hot spots.”

With evidence that larval aggregations of *C. pomonella* can be frequent (this study) and that synthetic larval aggregation pheromone attracts fifth-instar larvae in the field (Jumean et al. 2007), an attract-and-kill formulation for larvae should be considered as a potential control tactic for *C. pomonella* in commercial apple orchards.

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**Table 1. Descriptive statistics of *C. pomonella* larval aggregations and observations of their location on tree trunks above ground and their cardinal direction**

<table>
<thead>
<tr>
<th>Descriptive statistics of aggregations</th>
<th>Orchard 1</th>
<th>Orchard 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range of group size</td>
<td>2–20</td>
<td>2–16</td>
</tr>
<tr>
<td>Mean of group size</td>
<td>3.33 ± 0.17</td>
<td>3.48 ± 0.19</td>
</tr>
<tr>
<td>Mean sex ratio (male:female) in group</td>
<td>1.081 ± 0.04</td>
<td>1.041 ± 0.03</td>
</tr>
<tr>
<td>Number of larval aggregations on tree trunks at specific height ranges above the soil line</td>
<td>Number of observations</td>
<td></td>
</tr>
<tr>
<td>1–10 cm</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>11–20 cm</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>21–30 cm</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>31–40 cm</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>41–50 cm</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>51–60 cm</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>61–70 cm</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>&gt;70 cm</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Cardinal direction of aggregation</td>
<td>Number of observations</td>
<td></td>
</tr>
<tr>
<td>North</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>South</td>
<td>17</td>
<td>37*</td>
</tr>
<tr>
<td>East</td>
<td>20</td>
<td>31</td>
</tr>
<tr>
<td>West</td>
<td>26</td>
<td>25</td>
</tr>
</tbody>
</table>

* Significant difference between categories within an orchard.
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