Attraction of male pear psylla, *Cacopsylla pyricola*, to female-infested pear shoots

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Abstract

Post-diapause winterform pear psylla, *Cacopsylla pyricola* ( Förster) (Homoptera: Psyllidae), exhibit a highly clumped distribution in late winter in pear orchards. The behaviors leading to clumped distributions in this species are unknown, but could include aggregation for mating activities. Choice tests and assays with an olfactometer were done to test whether male psylla of the overwintering morphotype are attracted to pear shoots infested by post-diapause females and to shoots previously occupied by females. Paired choice tests in small arenas showed that males accumulated on pear shoots currently occupied or previously occupied by females if those shoots were paired with un-infested shoots or shoots previously occupied only by males. Assays with an olfactometer showed that males were attracted to volatile odors from female-infested or previously infested shoots. The exact source of the attractants (i.e., the female psylla, the pear shoot, or a combination of these sources) remains to be determined.

Introduction

Pear psylla, *Cacopsylla pyricola* ( Förster) (Homoptera: Psyllidae), is one of the most important insect pests of commercial pears in North America and Europe. The species is seasonally dimorphic, producing a dark overwintering form (winterform) in late summer and autumn in response to shortening photoperiods (Oldfield, 1970), which is distinct from the small, lighter colored adult (summerform) that develops during the growing season. The winterform morphotype overwinters in a reproductive diapause both on the host plant and outside of the pear orchard, often on other fruit species (Horton et al., 1994). Re-entry into pear orchards following diapause occurs in late winter before the pear tree has begun to put on foliage (Horton et al., 1992). Very little mating occurs in diapausing insects, as shown by dissection of winterforms collected from the field throughout fall and winter (Krysan & Higbee, 1990) or by mating studies done in the laboratory (Krysan, 1990). Mating in the field by post-diapause winterforms begins as temperatures warm in mid-February, followed rapidly by ovarian maturation and egg-laying.

Management recommendations for pear psylla emphasize control of the overwintered generation or immature off-spring of the overwintered generation (Westigard & Zwick, 1972), as this prevents potentially severe problems later in the growing season. Thus, it is important to understand biology of the post-diapause winterform, and a great deal of research has been done addressing aspects of post-diapause development, dispersal, host plant colonization, and monitoring for this morphotype (Krysan & Higbee, 1990; Horton et al., 1992, 1998; Horton, 1999). Post-diapause winterforms are active in pear orchards well before foliage shows in the pear tree, and at this time exhibit a statistically clumped distribution among trees (Burts & Brunner, 1981). Visual examination of pear shoots in the field before the appearance of foliage has shown that winterforms also exhibit a highly clumped distribution among shoots within trees, with some shoots on a given tree hosting potentially large (e.g., >15 individuals) mixed-sex aggregations of psylla, while neighboring shoots are psylla-free (DR Horton, unpubl.). These observations suggest either that shoot quality varies within trees or that psylla are attracted to one another. The clumped distribution of overwintered psylla is not understood, but has ramifications for monitoring and for making decisions about the necessity for insecticide treatment.

Here, we explore whether post-diapause winterform *C. pyricola* attract conspecific psylla. We focus on the response by male psylla to female psylla, and test whether males are attracted to volatile chemicals associated with
infestation of shoots by females. Very little is known about the role of volatile chemicals affecting behavior of Psyllidae. A few studies have shown that host or non-host volatile chemicals affect host finding behavior in some psyllids (Moran & Brown, 1973; Lapis & Borden, 1993). Almost nothing is known about the possible role of volatile chemicals affecting mate location. Recently, Soroker et al. (2004) used olfactometer and electroantennogram methods to show that male summerforms of the pear psyllid, Cacopsylla bidens (Sulc), a close relative of C. pyricola, responded to volatile chemicals emitted from female-infested pear foliage. Objectives of our study are to test whether males of post-diapause winterform C. pyricola are attracted to odors of female-infested and previously infested pear shoots. We used choice tests conducted in small arenas to compare infested, previously infested, and clean pear shoots for attractiveness to male psylla. These choice tests were then followed with assays done using an olfactometer, to determine more specifically whether volatile chemicals were involved in affecting male behavior.

Materials and methods
Source of insects and plant material
Winterform psylla were collected from a commercial orchard in mid to late February 2003 and 2004. Psylla collected at this time of year have completed diapause, but require warming temperatures before mating and egg-laying commence (Krysan & Higbee, 1990; Horton et al., 1998). The insects were stored at 3 °C in 2 l plastic containers partially filled with slightly moistened tissue paper, until they could be assayed. The assays were done in late February and March both years. Pear shoots were collected in mid to late February from the same commercial orchard and were stored at 3 °C in moistened paper towels until use. We used reproductive shoots of first-year wood. At the time of collection from the field, bud-swelling had just begun. No green tissue was showing.

Choice test assays
We first tested whether males preferentially colonized pear shoots infested by post-diapause females or previously infested by post-diapause females, if the treatment shoot was paired with a clean (hereafter, control) shoot. Pear shoots were removed from 3 °C storage 24 h before the assay and rinsed thoroughly in tap water. The shoots were then placed with cut ends in tap water for 24 h at 20–25 °C in a greenhouse. After 24 h, we randomly selected shoots for use in the choice tests, cut the shoots to 12–15 cm in length, and placed them singly in 135 ml vented cages; shoot ends were kept in water. The shoots were then exposed to the desired treatment, depending upon the comparison to be tested (see following paragraph). To conduct the choice tests, control and treated shoots were paired 12 cm apart in 20 cm diameter × 8 cm tall clear plastic arenas. Shoot ends were forced through a square of cardboard (which functioned as the floor of the arena), taking care not to touch the shoot except at the cut end. The cut ends of the shoots were placed in water. Ten psylla of one sex were removed from 3 °C storage 24 h before the assay, and allowed to feed at 22–25 °C and a L16:D8 photoperiod on field-collected shoots. After the 24-h feeding period, the psylla were added to the arena for the choice tests. Location of the psylla (treatment shoot, control shoot, or arena wall) was determined 2 h later. Assays were done at room temperature (22–25 °C) under fluorescent lighting. After an assay had ended, the cardboard floors were discarded, and the arenas were washed in soapy water, rinsed thoroughly in tap water, and allowed to dry.

The following comparisons were made: (1) shoot infested by a female paired with a control shoot; choice test conducted using males. A shoot having a single female was paired with a control shoot in the arena. The female was allowed to settle for 24 h before conducting the assay; a 135-ml ventilated cage was used to prevent the female from moving onto the control shoot. After 24 h, the 135-ml cage was removed, taking care not to disturb the female, and 10 males were immediately added to the arena. Sample size was 20 replicates. (2) Shoot previously infested by females paired with a control shoot; choice test conducted using males. Five females were used to infest the treatment shoot 24 h preceding the assay. The females were removed after 24 h (taking care not to touch the shoot), and the shoot was paired with a control shoot. Males were added to the arena immediately after the shoots were paired. The assays were replicated 15 times. (3) Shoot previously infested by females paired with shoot previously infested by males; choice test conducted using males. Protocols were similar to (2), except that the control shoot received five males for 24 h; the treatment shoot received five females. Males and females were removed from the shoots immediately before the shoots were paired, and then 10 males were added to the arena. The assay was replicated 15 times. (4) Shoot previously infested by females paired with shoot previously infested by males; choice test conducted using females. Protocols were identical to (3), except that 10 females were assayed for choice.

Y-tube olfactometer
A Y-tube olfactometer was used to determine whether significant preferences shown in some of the choice test assays were due to response by psylla to volatile chemicals, rather than to settling on shoots due strictly to non-volatile cues on the shoot surface. For instance, male settling on a
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The shoot previously infested by females (as shown in choice tests; see below) could hypothetically have been due to unknown chemical changes within the shoot or on the shoot surface, to physical presence of eggs deposited by females during the 24-h infestation phase, or to volatile chemicals associated with female infestation. The olfactometer was constructed of a 2.5-cm diameter glass tube 27 cm in length, having two arms (at 135° to one another) each 7 cm in length (Figure 1). The arms were connected to treatment and control airflows, with the combined airflow vented out of the base of the Y-tube. Air (78% nitrogen and 21% oxygen) was metered through a carbon filter, distilled water, and 1 l glass jars containing treatment or control odor sources. The odor sources were connected to the ends of the arms of the Y-tube by 25 cm lengths of 2 mm diameter Teflon hose (Figure 1). Air was metered through each arm of the olfactometer at 50 ml min⁻¹. Pear shoots used in the assays were taken from 3°C storage, and moved to the greenhouse for 24 h (as in the choice test assays). After 24 h, shoots were randomly selected for the assays, cut to approximately 10 cm in length, and placed with cut ends in water in 35 ml glass vials; three shoots per vial were used. A group of three shoots then received one of several treatments (see below), before being placed in the 1 l glass jar attached to the Y-tube.

Three sets of assays were done to address three separate questions; all assays had similar protocols. Each assay was done to test response of male psylla to putative attractants. Females were not assayed, as the choice test done using females (see above; assay 4) failed to show any preferences by females for female- vs. male-infested shoots. For each of the three assays, a single replicate consisted of 10 males assayed for preference, one-at-a-time. Males were removed from storage and placed on field-collected shoots at room temperature and a L16:D8 photoperiod for 24 h before the assay. After the 24-h feeding period, they were removed from the shoots and placed in a 50-ml glass holding vial. A single male was then allowed to exit the holding vial and enter the stem end of the olfactometer without interference. The male was allowed 10 min to enter an arm of the Y-tube. If the male failed to enter an arm within 10 min, he was removed from the tube using a dry paint brush and discarded; data from discarded males were not used in the analyses. Choice was defined to have occurred once the male contacted the end of an arm (i.e., at the point of insertion for the Teflon hose). Once a male had made a choice, he was removed from the Y-tube and discarded. For a given replicate, five males were assayed, the arms of the olfactometer were rotated 180°, and the second group of five males was then assayed. Rate of air flow entering each arm was measured at the junctions of the Teflon hoses and Y-tube immediately before the first five males were assayed, between the first and second groups of five males, and at the end of the replicate, to confirm that the appropriate airflows were maintained over the course of the assay. Once 10 males had been assayed to produce a replicate, the olfactometer was dismantled and cleaned. Glassware was washed in hot soapy water, rinsed in water, rinsed in acetone and hexane, and then baked in an oven for 2 h at 150°C. Treatments were randomly assigned to the olfactometer arms between each replicate of 10 males.

The following comparisons were made: (1) Female-infested shoots vs. control shoots. One arm of the olfactometer was connected to a jar containing three shoots, infested 24 h earlier with 15 post-diapause females; the second arm was connected to three clean shoots. Control and infested shoots were held at 22–25°C and a L16:D8 photoperiod during the 24-h holding period. Sample size was 14 replicates (i.e., 140 males). (2) Shoots previously infested with females vs. control shoots. Three shoots were infested 24 h before the assay with 15 female psylla. The females were then removed immediately before the olfactometer tests, which included 12 replicates (120 males). (3) Female-infested shoots vs. shoots previously infested with females. This assay compared the two non-control treatments in (1) and (2). We had 12 replicates.

Statistical tests
Mean number of psylla choosing a treatment shoot in the choice tests was compared to mean number choosing the control shoot using paired sample t-tests. The analyses were done using PROC TTTEST in SAS (SAS Institute, 2001). The same test was used to compare mean numbers

**Figure 1** Schematic of Y-tube olfactometer. AT, air tank; CF, carbon filter; FM, flow meter; AH, air humidifier. Teflon hoses between odor sources and arms of olfactometer were 2 mm in diameter and 25 cm in length.
choosing one arm of the olfactometer vs. the opposite arm. The paired sample t-test assumes that the arithmetic differences between paired observations have a normal distribution; this assumption was tested using the Shapiro-Wilk statistic provided by PROC UNIVARIATE (SAS Institute, 2001). If the normality assumption was not met, we analyzed the data with a signed-ranks test using PROC UNIVARIATE (SAS Institute, 2001).

**Results**

**Choice test assays**

*Shoot currently infested by a female paired with a control shoot; choice test conducted using males.* Significantly more males settled on the shoot occupied by a female than on the female-free shoot (paired sample t-test: \( t = 2.24, \text{d.f.} = 19, P = 0.038 \); Figure 2); 62.2% of all males that settled on a shoot selected the shoot also occupied by the female.

*Shoot previously infested by females paired with a control shoot; choice test conducted using males.* Significantly more males settled on the shoot previously infested with females than the control shoot (signed ranks test: \( S = 33, P < 0.001 \); Figure 3A); 71.7% of males that settled on a shoot selected the shoot previously infested by the female.

*Shoot previously infested by females paired with shoot previously infested by males; choice test conducted using males.* Significantly more males settled on the shoot previously infested by females than the shoot previously infested by males ( paired sample t-test: \( t = 2.43, \text{d.f.} = 14, P = 0.029 \); Figure 3B); 65.6% of males that settled selected the shoot previously occupied by females. The results were statistically significant despite the final replicate in which all males settled on the shoot previously occupied by males (we may inadvertently have included a female in this final group of assayed males, although this is speculation).

*Shoot previously infested by females paired with shoot previously infested by males; choice test conducted using females.* Females showed no preference between shoots previously occupied by females and shoots previously occupied by males ( paired sample t-test: \( t = 0.4, \text{d.f.} = 14, P = 0.69 \); Figure 3C); 52.7% of
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the females that settled selected the shoot previously occupied by females.

Y-tube olfactometer

Female-infested shoots vs. control shoots. Significantly more males chose the arm connected to previously infested shoots than control shoots (t = 2.78, d.f. = 11, P = 0.018; Figure 4B); 69.2% of males that made a choice entered the arm of the olfactometer that was connected to the previously infested shoots.

Female-infested shoots vs. shoots previously infested with females. Significantly more males chose the arm connected to currently infested shoots than the arm connected to previously infested shoots (t = 2.22, d.f. = 11, P = 0.048; Figure 4C); 64.1% of males that made a choice entered the arm connected to currently infested shoots.

Discussion

Results of the choice test and olfactometer assays suggested the following: (1) males preferentially settled on shoots currently occupied (Figure 2) or previously occupied (Figure 3A,B) by females; and (2) volatile chemicals associated with shoots previously infested by females or currently infested by females attracted males (Figure 4A,B). The male response to infestation was apparently not due to a general attraction in males to the presence of conspecific psylla, but was due to the effects of infestation by conspecific females. That is, males were significantly more likely to settle on shoots previously occupied by females than shoots previously occupied by males (Figure 3B).

The types of cues that were responsible for prompting males to settle on infested or previously infested shoots in the choice tests are not known, but could include an accumulation of odors on the shoot surfaces associated with or emitted by the female, or to changes in surface chemistry of the shoots induced by the feeding or egg-laying activities of females. Studies with another pear psyllid suggested that volatile chemicals from females were attractive to male psyllids (Soroker et al., 2004). Whether volatile attractants from female *C. pyricola* might remain associated with pear shoots following removal of the female, and in that form prompt settling by males, is not known. Second, feeding by pear psylla may have affected attractiveness of shoots to males. It is known that infestation of pear trees by *C. pyricola* leads to changes in leaf chemistry, particularly in levels of phenolics in the leaves (Scutareanu et al., 1996, 1999). Whether feeding by *C. pyricola* on developing pear shoots before the appearance of foliage affects shoot chemistry is not known, nor is it known whether male pear psylla might use changes in chemistry caused by the feeding of females to judge the likelihood of a shoot being occupied by females.

Following the choice tests, an olfactometer was used to test whether volatile chemicals might be involved in

![Figure 4](https://example.com/figure4.png)

**Figure 4** Results of olfactometer trials. Number of *Cacopsylla pyricola* males choosing arm of olfactometer connected to treatment odor source (black fills) or control odor source (no fills); control includes clean shoots (panels A and B) or shoots previously infested by females (panel C). Each bar represents one replicate of 10 males; n = 14 or 12 replicates. White and black fill for a bar may often sum to fewer than 10 males, as some males in most replicates failed to choose an arm of the olfactometer within the 10 min limit. P-statistics are from paired sample t-tests (panels B and C) or signed-ranks test (panel A).
Influencing behavior of male psylla. The assays showed that males were attracted to volatile chemicals associated with shoots either currently infested with females or previously infested with females (Figure 4A,B). Again, as for the choice tests, the assays do not allow us to determine the specific source of the attractants. For example, infestation of pear trees by pear psylla not only affects internal leaf chemistry, as noted above, but also affects production of volatile chemicals (Scutareanu et al., 1997, 2003) in quantities sufficiently high to attract predators of pear psylla (Drukker et al., 2000). Whether pre-bloom shoots of pear respond to the feeding activities of psylla by producing volatile chemicals, which in turn may attract males from a distance, is not known. The egg-laying activities of females may also have led to attraction by males. Oviposition activities in some insects have been shown to prompt the production of volatile chemicals by the host plant (Hilker et al., 2002). Eggs of pear psylla are partially inserted into the pear shoot, and it is possible that the physical damage to the shoot caused by oviposition prompted the shoot to produce chemical volatiles. Males in other insect species are known to use plant-emitted volatiles to locate females for mating (Ruther et al., 2002; Tooker et al., 2002). It has yet to be determined whether egg-laying activities of female pear psylla lead to the release of volatiles by pear shoots, which in turn attract males from a distance.

Finally, the volatile chemicals prompting the behavioral responses by males may have been produced directly by the female psylla. That is, all of our results are consistent with the hypothesis that female C. pyricola emit odors that attract male psylla. As far as we have been able to determine, there is virtually nothing in the literature indicating that females in any species of Psyllidae emit sex pheromones. Very recently, Soroker et al. (2004) showed that male summerform pear psyllids (C. bidens) were attracted to female-infested pear foliage and to females in the absence of foliage. Volatile chemicals collected from female-infested pear trees were shown to elicit voltage responses by antennae of male pear psyllids (Soroker et al., 2004). The authors interpreted these results as evidence that females of C. bidens emit a volatile sex attractant. The study by Soroker et al. (2004) appears to be the first published report to suggest that a psyllid uses volatile cues to locate potential mates.

In summary, signals used by males in locating females are largely unknown and unstudied in the Psyllidae. The signals hypothetically could include visual (Krysan, 1990), acoustic (Campbell, 1964; Tishechkin, 1989; Percy, 2005), or olfactory cues, or some combination of the three. Substrate-borne acoustic signals and visual cues are both likely to be effective over relatively short distances, meaning that any long-distance communication between male and female pear psylla (if present) must require some other type of communication system, possibly including chemical communication. The chemical ecology of the Psyllidae is relatively poorly studied, particularly with respect to the role of olfaction and volatile chemicals in affecting psyllid behavior. Some Psyllidae are known to be attracted to volatile chemicals from host plants (Lapis & Borden, 1993) or to be deterred by non-host volatiles (Nehlin et al., 1994), suggesting that long-distance olfaction may play a role in host location. Sex pheromones have been described or inferred to occur for a number of Homoptera other than Psyllidae, including mealybugs, aphids, scale insects, and white flies (Doane, 1966; Yin & Maschwitz, 1983; Millar et al., 2002; Campbell et al., 2003), so perhaps it would not be unexpected to show that mate location in Psyllidae is mediated by sex pheromones. The studies done by Soroker et al. (2004) for C. bidens, in combination with results reported here for C. pyricola, suggest that additional research should be done that focuses on isolation and identification of volatile sex attractants in these two pear psyllids.

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