FINAL PROJECT REPORT
WTFRC Project Number: #PR-05-504

Project Title: Chemical ecology of pear psylla

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Dr. Victoria Soroker (Volcani Institute) – funded by BARD
Dr. Anat Zada (Volcani Institute) – funded by BARD
Bob Brown (WSU Master's candidate) – funded by USDA-ARS

Other funding Sources

Agency Name: USDA-CSREES-NRI
Amount awarded: $230,000 for FY 2006-2008; $120,000 to Wapato lab
Notes: Funding for GS-6 term technician (Horton's laboratory) and Dr. Jocelyn Millar (pheromone chemist at U.C. Riverside)

Agency Name: Binational Agricultural Research and Development (BARD)
Amount awarded: $273,000 for FY 2008-2010; $103,000 to Wapato lab
Notes: Partial funding for postdoctoral scientist Dr. Christelle Guédot (Landolt's laboratory), Dr. Victoria Soroker (Volcani Center), and Dr. Anat Zada (Volcani Center)

Budget History (WTFRC):

Item	**Year 1:** (2005)	**Year 2:** (2006)	**Year 3:** (2007)
Salaries	15,000	27,500	20,000
Benefits	4,500	8,250	6,000
Supplies		4,000	
Total	19,500	39,750	26,000

Significant Findings and Accomplishments

- Data obtained using WTFRC funding allowed us to apply for grants elsewhere. Outside funding has been obtained from two granting agencies, to supplement WTFRC funding:
    - $230,000 from USDA-CSREES-NRI used to fund technician (Horton's lab) and Dr. Jocelyn Millar, a pheromone chemist at University California, Riverside
    - $273,000 from Binational Agricultural Research and Development (BARD) used to partially fund a post-doctoral scientist (Dr. Christelle Guédot) in Landolt's lab, and two scientists at the Volcani Center in Israel. The Israeli scientists will work on the pheromone of a closely related pear psyllid.
- We brought in a Master's student candidate (Bob Brown) on USDA funds, who has developed a trap suitable for field-testing psylla attractants.
- Olfactometer assays were developed and used to define the life history traits in pear psylla that lead to maximal female attractiveness and optimal behavioral responses in males. It is necessary to have these behavioral data before we attempt to move into the chemistry portion of the project.
- We began attempts to isolate and identify chemical attractants from known attractive females. Two types of products were collected: surface extracts and headspace volatiles. One volatile apparently associated with female psylla but not male psylla was identified, and preliminary trials in the olfactometer were completed showing that males were indeed attracted to the compound.

Results and Discussion

OBJECTIVE: DEFINE LIFE HISTORY CHARACTERISTICS LEADING TO FEMALE ATTRACTIVENESS AND OPTIMAL MALE RESPONSE (HORTON, LANDOLT, GUÉDOT)

These trials were done using a Y-tube olfactometer (Fig. 1). Each comparison included a minimum of 10 replicates, with each replicate consisting of 10 males assayed one-at-a-time (i.e., a minimum of 100 males per test). Figure 2 shows typical results, in this case for an assay with summerforms in which we tested whether the host plant must be present to obtain attraction. Each bar is a single replicate of 10 males (assayed one-at-a-time). Figure 2A shows that most males chose the arm of the olfactometer connected to a female-infested seedling over an uninfested seedling. Figure 2B shows that females even in the absence of a seedling were attractive to males. Finally, Figure 2C shows that a female-infested seedling was no more attractive to males than an equivalent number of females in the absence of a seedling.

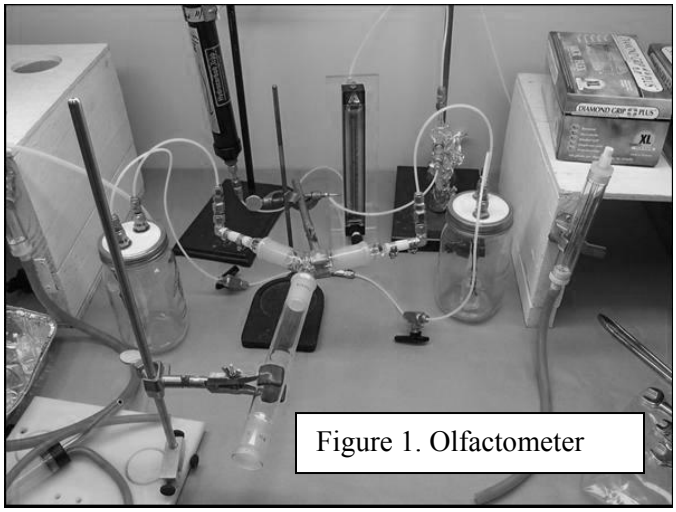


Figure 1. Olfactometer

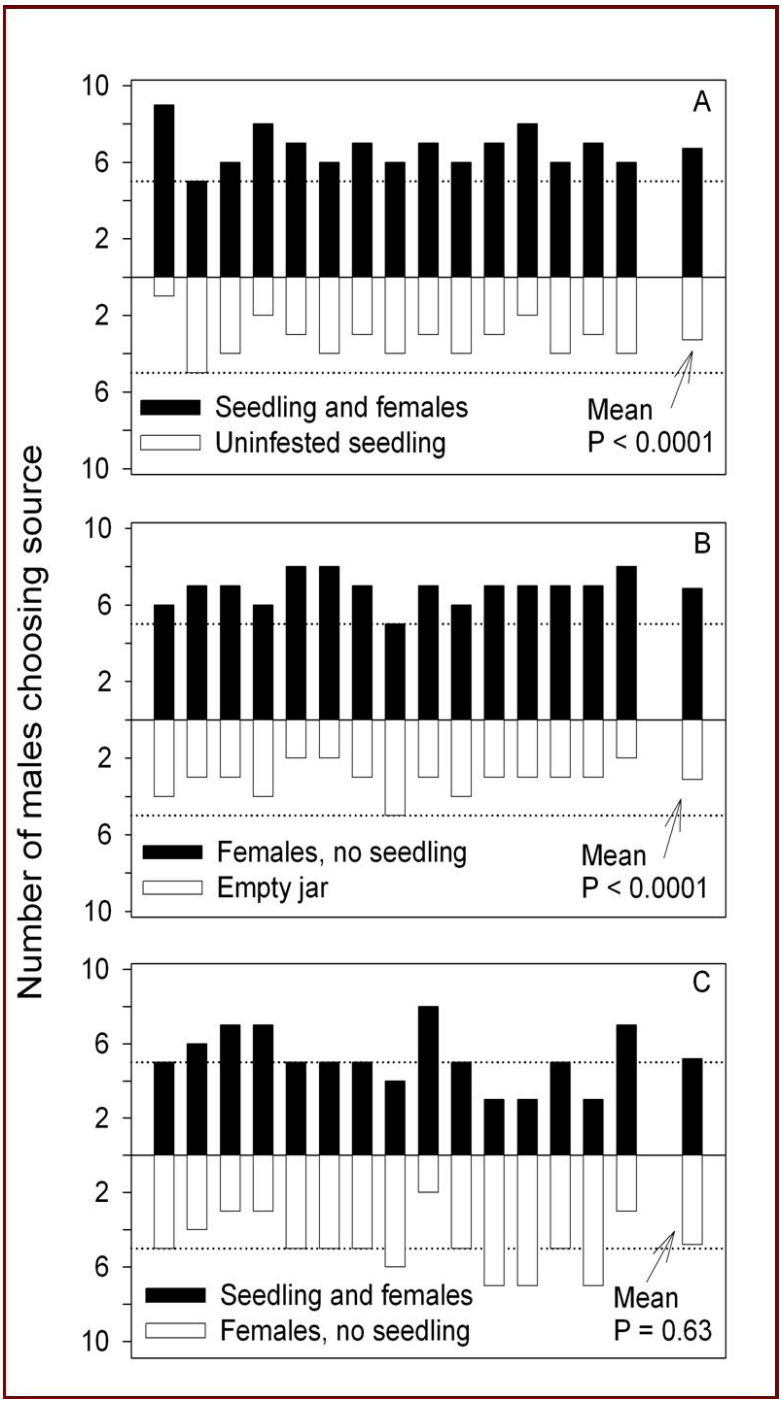


Figure 2. Numbers of males choosing arm of olfactometer attached to treatment or control odor sources. These assays tested whether presence of the host plant was necessary to prompt volatile production by females.

Rather than present a series of figures similar to Figure 2 to show the results of our olfactometer work, we summarize the results in tabular form (shown in Table 1).

Table 1. Summary of olfactometer results assessing the role of various life history factors affecting female attractiveness and male response. Underline indicates significant preference for that choice.

CHOICE 1 IN OLFACTOMETER	CHOICE 2 IN OLFACTOMETER
Summerforms	
Female-infested seedling	Uninfested seedling
8-10 day old females	2-5 day old females
Virgin females on seedling	Uninfested seedling
Mated females on seedling	Uninfested seedling
Virgin females on seedling	Mated females on seedling
Female-infested seedling	Uninfested seedling
Females, no seedling	Blank
Females, no seedling	Female-infested seedling
30 dead females	Blank
30 dead females	30 dead males
30 dead males	Blank
Winterforms	
Female-infested shoots	Uninfested shoots
Shoots previously infested (females)	Uninfested shoots
Female-infested shoots	Shoots previously infested (females)
Females exposed to long-days	Females kept at short-days
Females exposed to fenoxycarb	Control females
Shoots and diapause females	Uninfested shoots
Shoots and post-diapause females	Uninfested shoots

A final assay was done to determine when, seasonally, female winterforms begin to attract males in the olfactometer. Male and female winterforms were collected from the field at intervals between October and February, and assayed in the olfactometer. Females were not attractive to males until ovarian development and mating was seen in the field, beginning in early- to mid-February (Fig. 3). These results suggest that volatile production by female winterforms is closely associated with diapause status.

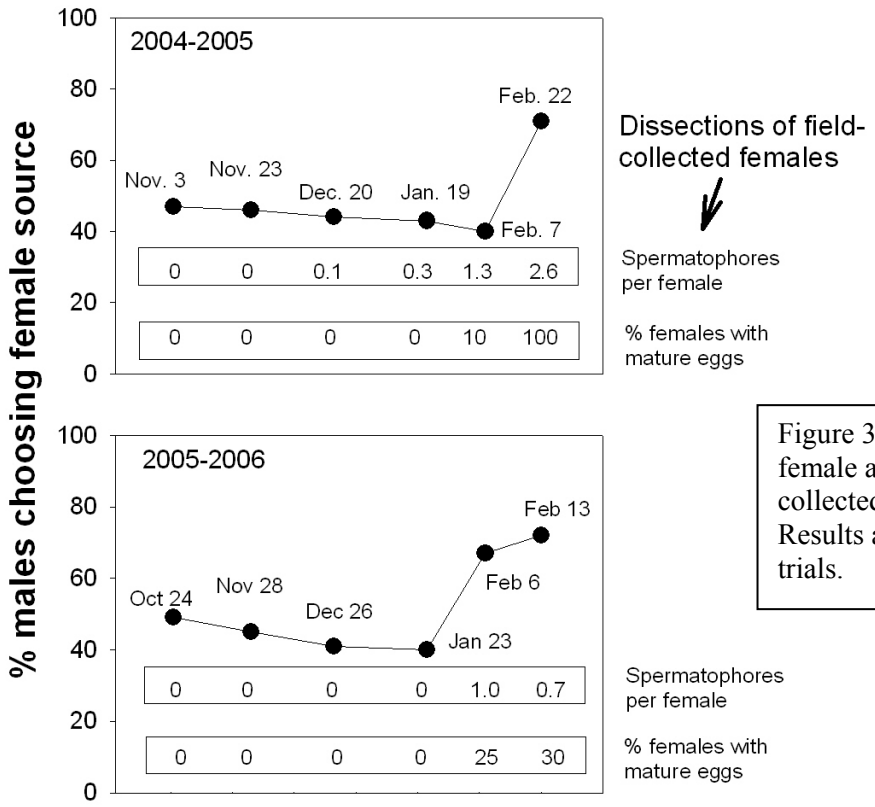


Figure 3. Seasonality of female attractiveness in field-collected winterforms. Results are from olfactometer trials.

Summary of olfactometer trials

- The results of the olfactometer trials indicated the following for summerforms:
  - ❖ both virgin and mated females attract males
  - ❖ older females are more attractive than very young females
  - ❖ the host plant does not have to be present for females to be attractive
  - ❖ even freshly killed females attract males

- The results for winterforms indicated the following:
  - ❖ shoots that had previously been occupied by females attract males in the olfactometer, suggesting residues left by females are both volatile and attractive
  - ❖ post-diapause females attract males, whereas diapausing females do not
  - ❖ onset of attractiveness (volatile production) coincides approximately with mating and ovarian development in the field

OBJECTIVE: DEVELOP FIELD-TRAPPING METHODS (BROWN, LANDOLT, HORTON)

Ultimate objectives of this project are to test candidate products in the field for their long-distance attractiveness to male pear psylla. This objective requires that we first develop a suitable trap. The trap shown in Figure 4 (developed by Bob Brown, WSU Master's candidate) is composed of tanglefoot-covered mesh, which is used to envelope a small organdy bag holding the source attractant. In testing this trap, we used live insects as our source of attractants. For both summerforms and winterforms, males were attracted to female-baited traps compared to male-baited or unbaited traps (Fig. 5). Conversely, females were distributed evenly among the three types of traps (Fig. 5). We conclude that this trap design will be suitable for testing synthetic attractants, once those products have been identified and synthesized.

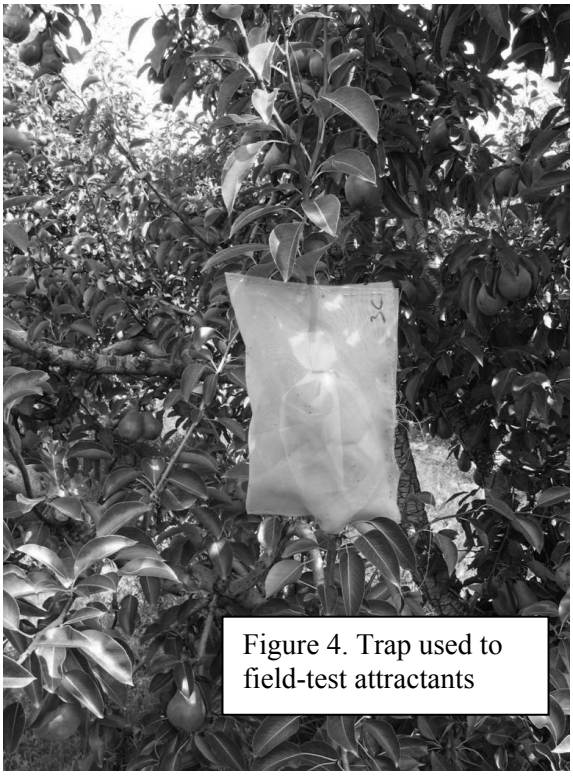
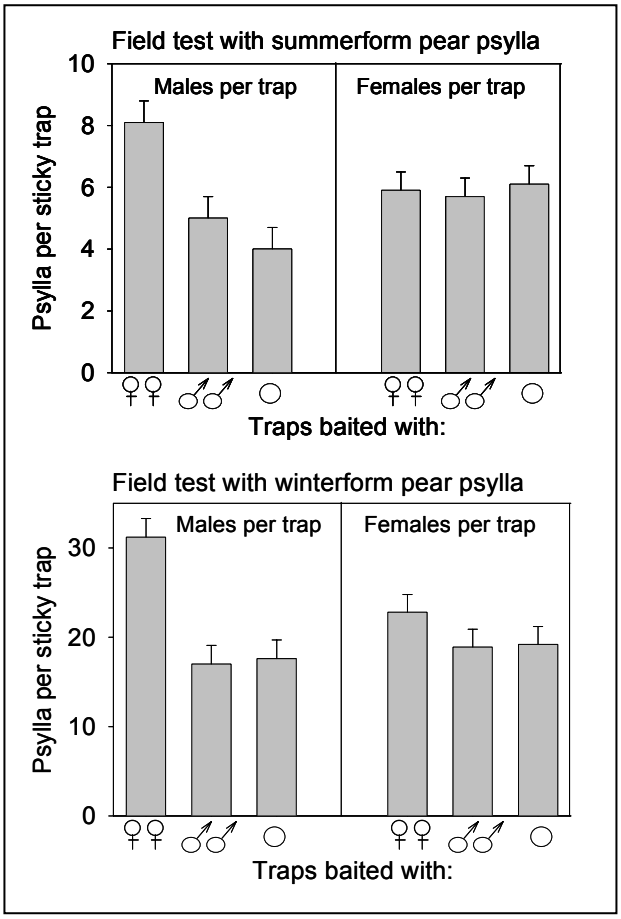


Figure 4. Trap used to field-test attractants

Figure 5. Field-test of trap, using live insects as attractants



OBJECTIVE: ISOLATE AND IDENTIFY THE ATTRACTANT (MILLAR, GUÉDOT, LANDOLT, HORTON)

Surface extracts of post-diapause winterforms were obtained by washing 500 live females in pentane. Fifty-female aliquots of the extract were then applied to filter paper disks, which were paired in the olfactometer against solvent-treated disks. The extract was attractive to post-diapause male winterforms (Fig. 6). Identification of the attractant has not yet been done.

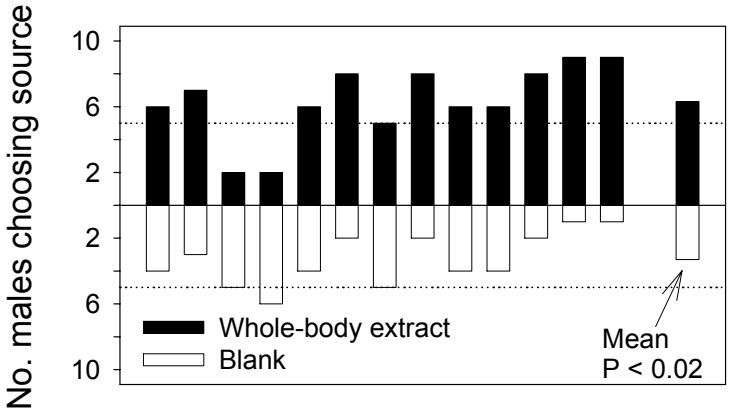


Figure 6. Attraction by males in olfactometer to filter paper disks treated with pentane (surface) extract of 50 female winterforms if paired with solvent control.

Head-space volatiles were collected by J. Millar from post-diapause female and male winterforms. The volatiles were adsorbed on an SPME fiber then desorbed directly into a gas chromatograph. GC-traces for female and male extracts were then compared (Fig. 7). Millar identified one peak in the trace that was found in the female-produced volatiles but not in the male-produced volatiles (shown by the arrow in Fig. 7). A synthetic formulation of the chemical was obtained. The formulation was then applied in solvent to filter paper disks, and compared in an olfactometer to solvent-treated disks. Summerform males were attracted to the compound (Fig. 8); winterform males, but not females, were also attracted to the compound (Fig. 9).

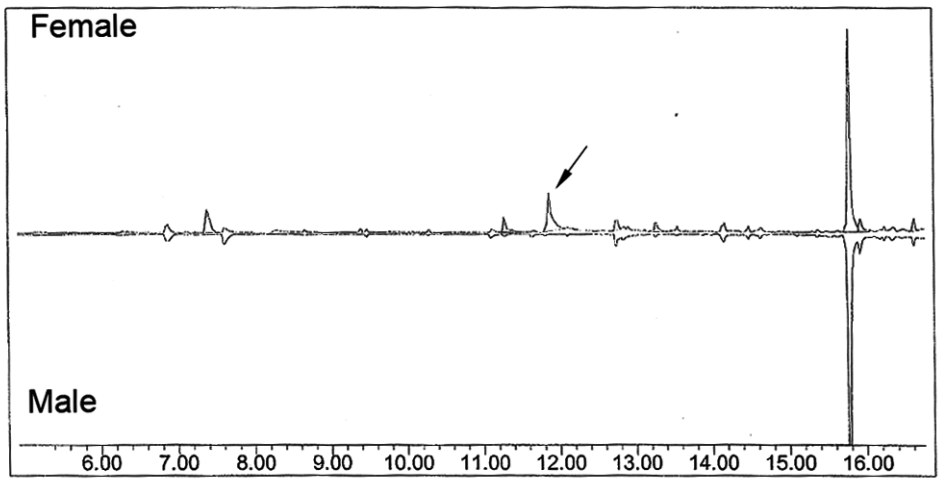


Figure 7. GC-traces from headspace volatiles

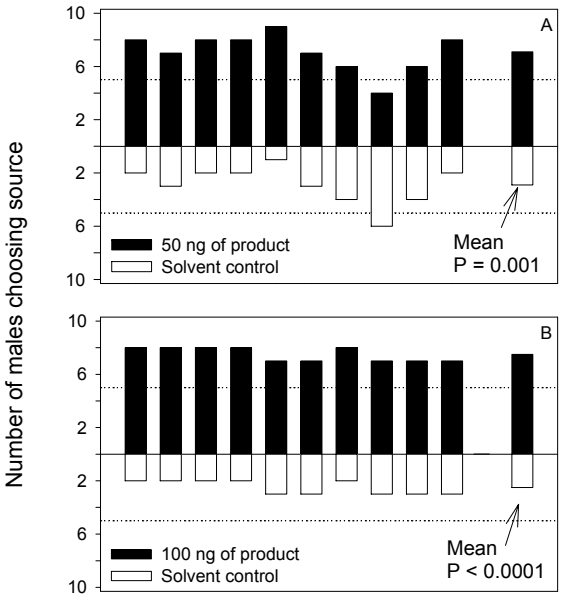


Figure 8. Preference by male summerforms for 50 or 100 ng of Millar product if paired against a solvent control

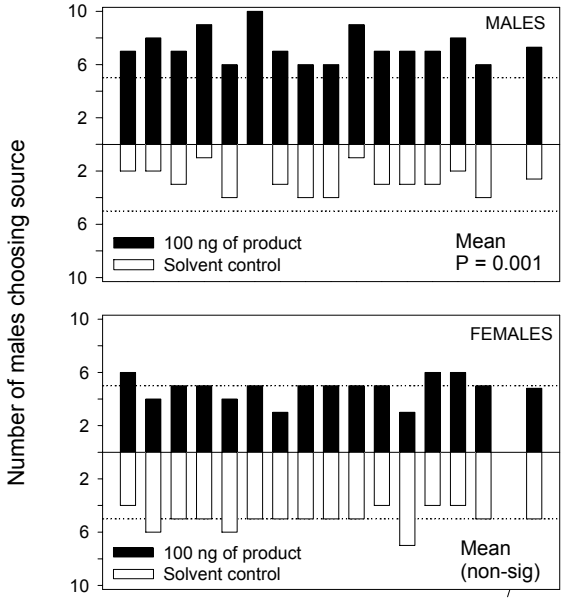


Figure 9. Preference by male or female winterforms for Millar product if product paired against solvent control

Presentations

Horton, D.R., C. Guédot and P. Landolt. 2006. Diapause affects attraction of male pear psylla to volatiles from females. 102nd Annual Meeting, Wash. State Hort. Assoc., Yakima, WA.

Guédot, C., D.R. Horton and P.J. Landolt. 2006. Chemical ecology of the sexual attractants in pear psylla, *Cacopsylla pyricola*. Entomological Society of America, Indianapolis, IN.

Brown, R., P.J. Landolt, D.R. Horton and R. Zack. 2007. Field demonstration of sex attraction in *Cacopsylla pyricola*. Pacific Branch, Entomological Society of America, Portland, OR.

Brown, R.L., P.J. Landolt, D.R. Horton and R.S. Zack. 2007. Sex attraction of the pear psylla, *Cacopsylla pyricola* (Hemiptera: Psyllidae) with a description of the diel periodicity of the attraction. Entomological Society of America. San Diego, CA.

Landolt, P., J. Millar, D. Horton and C. Guédot. 2007. Characterization and chemistry of sexual communication in pear psylla. Entomological Society of America, San Diego, CA.

Guédot, C., D.R. Horton, and P.J. Landolt. 2007. Attraction of male summerform pear psylla to volatiles from females: effects of female age, mating status, and presence of host plant. 103rd Annual Meeting, Washington State Horticultural Assoc., Wenatchee, WA.

Publications

Horton, D.R. and P.J. Landolt. 2007. Attraction of male pear psylla, *Cacopsylla pyricola*, to female-infested pear shoots. *Entomologia Experimentalis et Applicata* 123: 177-183.

Horton, D.R., C. Guédot, and P.J. Landolt. 2007. Diapause status of females affects attraction of male pear psylla, *Cacopsylla pyricola*, to volatiles from female-infested pear shoots. *Entomologia Experimentalis et Applicata* 123: 185-192.

Horton, D.R., C. Guédot, and P.J. Landolt. 2008. Attraction of male summerform pear psylla to volatiles from female psylla: effects of female age, mating status, and presence of host plant. *Canadian Entomologist* (in press).