

**FINAL PROJECT REPORT: PR-08-800**

**Project Title:** Volatile sex attractants in pear psylla

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**Other funding sources**

**Agency Name:** USDA-CSREES-NRI  
**Amount awarded:** \$233,473 (FY 2006-2008)  
**Personnel:** Horton, Guédot, Landolt, Millar

**Agency Name:** Binational Agricultural Research and Development Fund (BARD)  
**Amount awarded:** \$273,000 (FY 2008-2010)  
**Personnel:** Horton, Guédot, Landolt, Soroker, Zada

**Total Project Funding:** \$30,000

**Budget History:**

<b>Item</b>	<b>2008</b>	<b>2009</b>
<b>Salaries</b>	11,500	11,500
<b>Benefits</b>	3,500	3,500
<b>Total</b>	\$15,000	\$15,000

## OBJECTIVE:

Test compounds extracted and identified from cuticular washes of female psylla for attractiveness to male pear psylla

## SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS:

- Demonstrated that surface extracts from females are as attractive to males in olfactometer trials as an equivalent number of live females
- Used GC-MS to identify chemicals present in cuticular washes of diapausing and post-diapause male and female winterforms
- Synthesized one chemical (13-methylheptacosane) that was found to occur at levels 3-fold higher in post-diapause female winterforms than male winterforms
- Demonstrated attractiveness of 13-methylheptacosane to male winterform psylla in olfactometer trials and in field trials
- Identified 3 chemicals (including 13-methylheptacosane) that were found to occur at levels 2.7- to 8.6-fold higher in female summerforms than male summerforms

## RESULTS AND DISCUSSION:

***Olfactometer trials with cuticular extracts (winterform).*** Pentane extracts of post-diapause winterform female psylla were attractive to male psylla in olfactometer trials (**Figure 1: top chart**). Conversely, males were repelled by extracts of other males (**Figure 1: middle chart**), which appears to be the first example of male-male repellency in any psyllid species. The female extract was statistically as attractive to males as an equivalent number of live females (**Figure 1: bottom chart**).

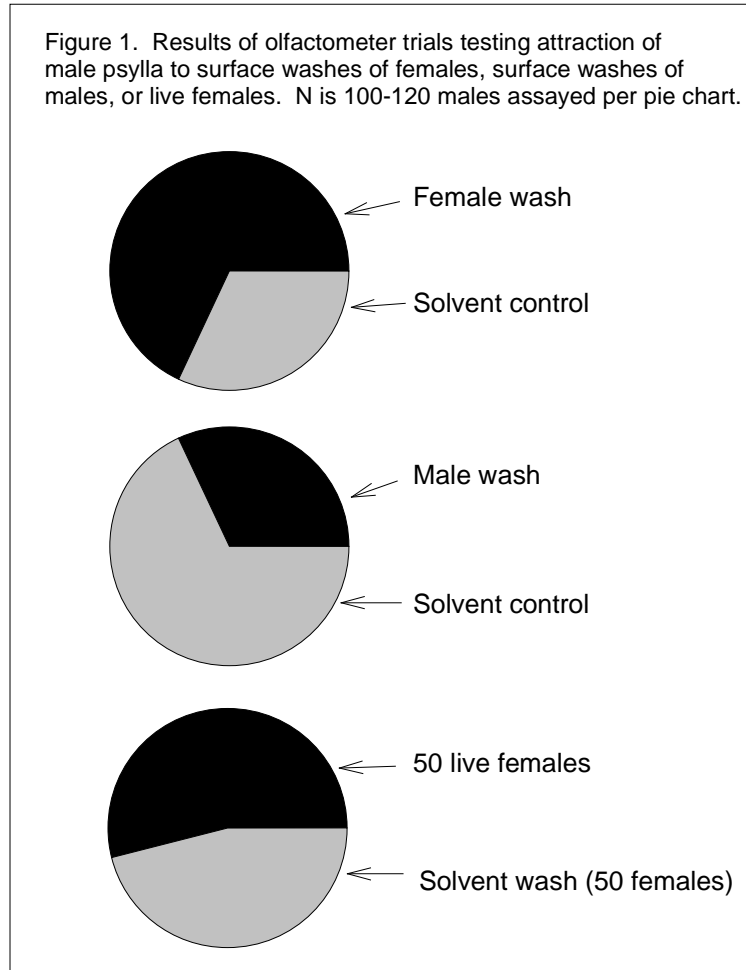
***GC-MS analysis of cuticular extracts from winterform psylla.*** A GC-MS analysis of cuticular extract from male and female diapausing and post-diapause winterform psylla was done (**Figure 2: upper panel – postdiapause psylla, lower panel – diapause psylla**). We identified many of the peaks in both traces (**Table 1**), and determined quantities of the chemicals in females and males (shown as female:male ratios in **Table 1**). One chemical (13-methylheptacosane) occurred at a level 3-fold higher in females than males for post-diapause winterforms, but was found to occur at similar levels between sexes in diapausing winterforms (**Table 1**).

***Olfactometer trials with post-diapause winterforms and 13-methylheptacosane.*** Olfactometer trials were conducted with post-diapause winterform psylla and 13-methylheptacosane (**Figure 3**). The chemical was synthesized by J. Millar, and forwarded to Wapato cooperators. Our first assay assessed attractiveness of the chemical (vs solvent blank) to male and female psylla. Male psylla (but not female psylla) were significantly attracted to the chemical (**Figure 3, top panel**). A second series of assays was then done to compare attractiveness of the synthesized 13-methylheptacosane versus the crude cuticular extract (**Figure 3, bottom panel**). Again, the synthesized chemical was attractive to male psylla when assayed against a solvent control. The crude extract was also attractive. Lastly, male psylla did not discriminate between 13-methylheptacosane and the crude extract, which suggests that the individual chemical was as attractive to male psylla as the full cuticular wash.

***Field trial with winterform psylla and 13-methylheptacosane.*** Field trials were done in late March and early April at the Moxee experimental farm. Sticky traps, composed of nylon mesh coated with tanglefoot (**Figure 4**), were used to assess attractiveness of 13-methylheptacosane to male and female winterform psylla. Gray septa were used to dispense 3 concentrations of the chemical (10, 100, or 1000  $\mu\text{g}$  of the product); solvent-loaded septa were used as controls. Each treatment was replicated 11 times. Numbers of males and females per trap were determined after 5 days in the field. There was marginally significant evidence that males preferentially accumulated on the 13-methylheptacosane traps during the late March test (**Figure 5, top panel**). More conclusively, our

early April trial provided very strong and highly significant evidence that males were attracted to the chemical (**Figure 5, bottom panel**). Females exhibited no obvious patterns in trap catch, other than some weak evidence for avoidance of higher concentrations of the chemical in the first trial.

***GC-MS analysis of cuticular extracts from summerform psylla.*** A GC-MS analysis of cuticular extracts from male and female summerform psylla identified 3 chemicals that occurred at higher levels in the female extract than the male extract (2.7- to 8.6-fold higher in females). The chemical with the largest female:male ratio was again 13-methylheptacosane (data not shown). Assays with these 3 chemicals are planned for 2010.



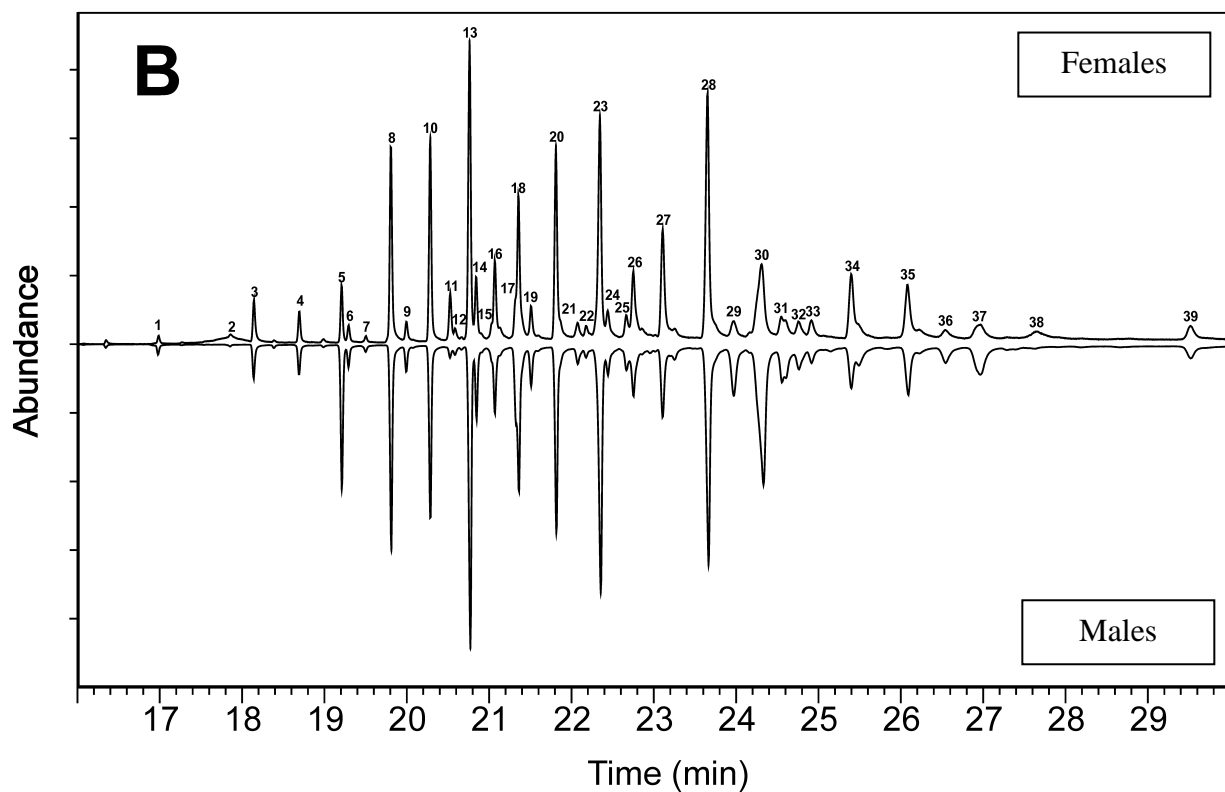
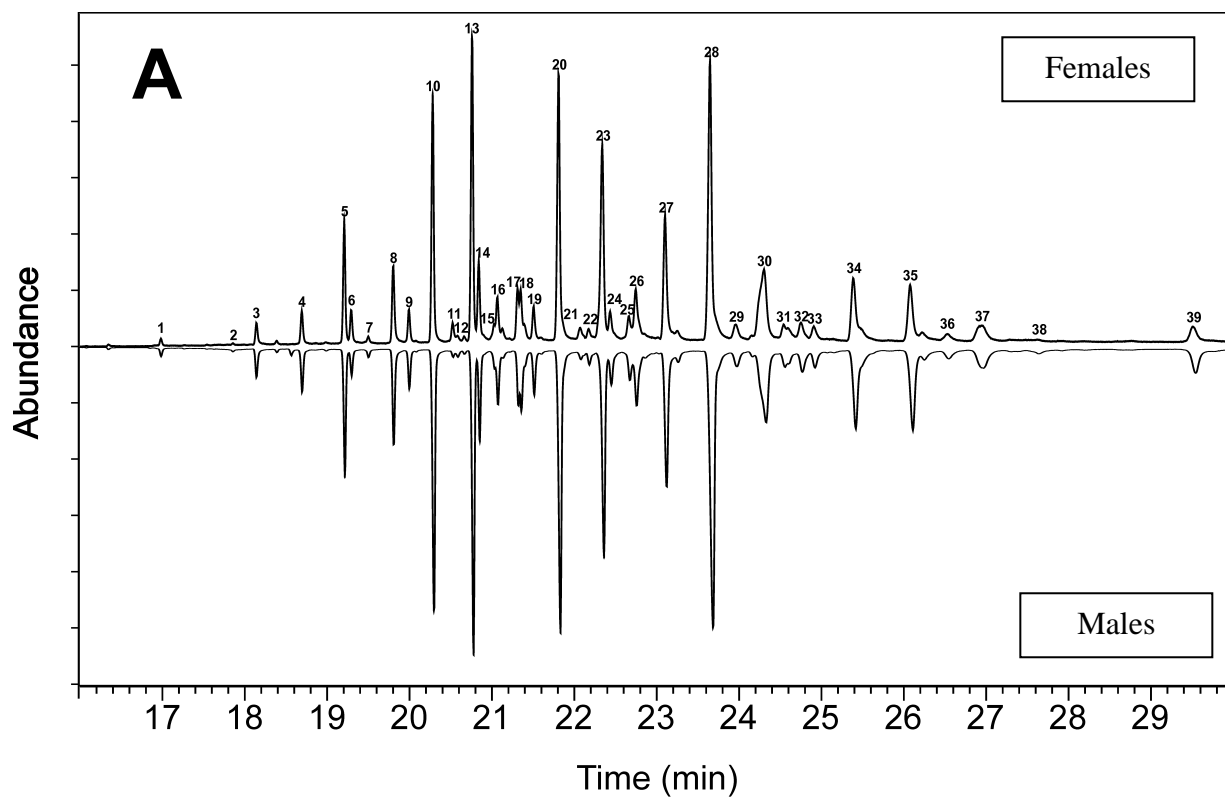


Figure 2. GC-MS traces of solvent extracts from diapausing female and male psylla (Panel A), and post-diapause female and male psylla (Panel B); in both panels, upper trace is female wash and lower trace is male wash. Peak #11 corresponds to 13-methylheptacosane.

**Table 1.** Identification of hydrocarbons and related compounds in cuticular extracts of winterform *Cacopsylla pyricola*. Bold font designates the 13-methylheptacosane.

Peak #	Retention time (min)	Identification	Ratio female/male	
			Diapause	Post-diap
1	16.99	tricosane	1.1	1.0
2	17.86	tetracosane	tr	tr
3	18.15	docosanal	1.1	1.0
4	18.70	pentacosane	1.1	1.0
5	19.21	2-methylpentacosane	1.2	0.6
6	19.36	3-methylpentacosane	1.3	1.1
7	19.51	hexacosane	1.0	tr
8	19.81	tetracosanal	1.2	0.8
9	20.00	2-methylhexacosane	1.0	1.0
10	20.29	heptacosane	1.1	1.2
<b>11</b>	<b>20.53</b>	<b>13-methylheptacosane</b>	<b>1.5</b>	<b>3.2</b>
12	20.59	pentacosanal	1.0	1.1
13	20.77	2-methylheptacosane	1.1	1.2
14	20.85	3-methylheptacosane	1.0	1.1
15	21.04	octacosane	1.0	1.2
16	21.07	unidentified	1.2	1.1
17	21.31	unidentified	1.1	0.9
18	21.36	hexacosanal	1.1	1.0
19	21.51	2-methyloctacosane	0.9	1.0
20	21.81	nonacosane	1.1	1.1
21	22.08	11-, 13-, and 15-methyl- nonacosane	1.1	1.2
22	22.18	heptacosanal	1.0	1.1
23	22.35	2-methylnonacosane	1.1	1.0
24	22.44	3-methylnonacosane	1.0	1.1
25	22.67	triacontane	1.0	1.1
26	22.75	unidentified	1.2	1.2
27	23.11	octacosanal	1.2	1.1
28	23.66	hentriacontane	1.1	1.1
29	23.97	11-, 13-, and 15-methyl- hentriacontane	1.0	0.8
30	24.31	11,15- and 13,17-dimethyl- hentriacontane	1.2	0.8
31	24.55	unidentified	1.0	1.0
32	24.61	unidentified	1.0	1.0
33	24.76	dotriacontane	1.0	1.1
34	25.40	triacontanal	1.1	1.1
35	26.08	trtriacontane	1.1	1.1
36	26.54	11-methyltrtriacontane	1.1	0.9
37	26.97	unidentified	1.3	0.9
38	27.66	tetratriacontane	1.0	1.0
39	29.52	pentatriacontane	1.0	1.0

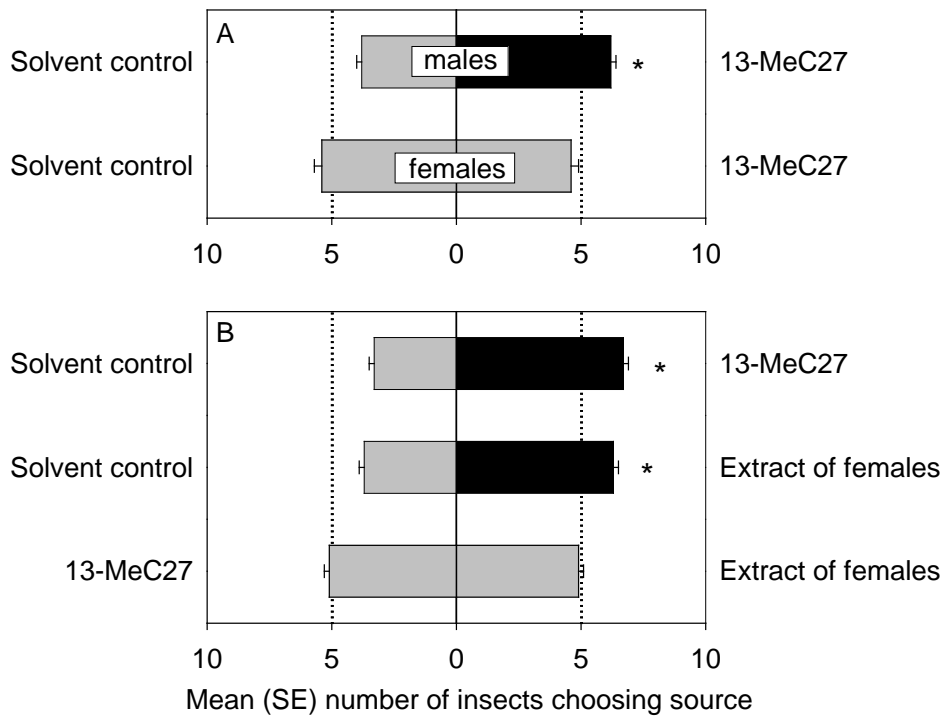


Figure 3. Olfactometer trials with 13-methylheptacosane

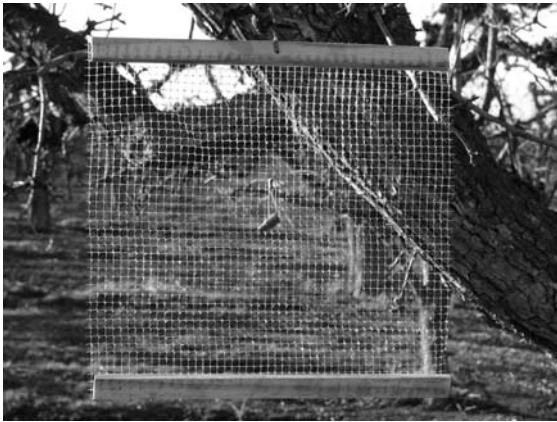


Figure 4. Trap design for field test

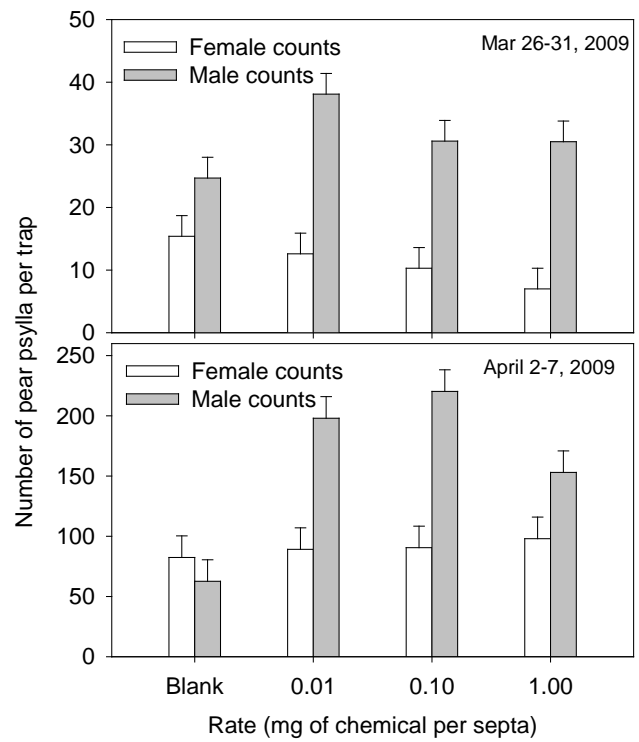


Figure 5. Results of field test

## EXECUTIVE SUMMARY

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**Outside Funding:** \$233,473 (NRI); \$273,000 (BARD)

**Total Project Funding:** \$30,000

### Budget History (Fresh Pear/Processed Pear Committees):

Item	Year 1: (2008)	Year2: (2009)
Salaries	\$11,500	\$11,500
Benefits	\$ 3,500	\$ 3,500
<b>Total</b>	\$15,000	\$15,000

## SUMMARY

A chemical (13-methylheptacosane) found to occur at higher levels in cuticular washes of post-diapause winterform female psylla than in washes from males was tested for attractiveness to male winterforms. Specific findings:

- Cuticular extracts from post-diapause winterform female psylla attracted males in olfactometer trials at a rate equivalent to attractiveness of an equivalent number of live females
- GC-MS analysis of extracts identified one chemical that occurs at a level several-fold higher in females than males (13-methylheptacosane)
- This chemical was synthesized by J. Millar, and then shown in laboratory and field trials to attract male winterform psylla
- The same chemical and two other compounds were identified in cuticular washes of summerforms at levels several-fold higher in females than males

Plans for 2010

- Assess in field and laboratory assays whether attractiveness of the 13-methylheptacosane compound to male psylla depends upon time of year
- Continue assays with summerforms and three identified female-specific compounds

## PUBLICATIONS

Guédot, C., D.R. Horton and P.J. Landolt. 2009. Attraction of male winterform pear psylla to female-produced volatiles and to female extracts and evidence of male-male repellency. *Entomologia Experimentalis et Applicata* 130: 191-197.

Guédot, C., J.G. Millar, D.R. Horton, and P.J. Landolt. 2010. Identification of a sex attractant pheromone for male winterform pear psylla, *Cacopsylla pyricola*. *Journal of Chemical Ecology* (in press).