

FINAL PROJECT REPORT

WTFRC Project Number: PR-10-100

Project Title: Development of field applications for a pear psylla sex attractant

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Cooperators: Jocelyn Millar, University of California, Riverside

Total Project Request: Year 1: \$20,000 Year 2: \$20,000

Other funding sources

Agency Name: Binational Agricultural Research and Development (BARD)
Amount awarded: \$280,000 (Oct 2011-Sept 2014); \$88,000 for the Horton lab.

Total Project Funding: \$40,000

Organization Name: USDA-ARS
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Contract Administrator: James Harris
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Item	2010	2011
Salaries	\$15,500	\$15,500
Benefits	\$ 4,500	\$ 4,500
Wages		
Benefits		
Equipment		
Supplies		
Travel		
Miscellaneous		
Total	\$20,000	\$20,000

OBJECTIVES

Our objectives were to:

1. **Winterform:** Conduct simultaneous field and laboratory assays with 13-methylheptacosane to assess whether attractiveness of the chemical to male psylla changes seasonally.
2. **Winterform:** Conduct field assays to optimize the use of 13-methylheptacosane as a male psylla attractant (dose response, trap design, release rate).
3. **Summerform:** Conduct laboratory assays to determine response of male summerform psylla to 13-methylheptacosane and to blends of this compound with 2-methylheptacosane and 3-methylheptacosane.

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS

- Proposal to BARD for the “Optimization and field-testing of synthetic sex attractants for two psyllid pests of pears”; \$280,000 for 3 years was awarded.

Objective 1:

- Determined that there is a seasonality in attraction of winterform males to 13-methylheptacosane (13-MeC27) both in the field and in laboratory bioassays which seems to coincide with females reaching reproductive maturity and being mated in the field.
- A seasonality in attraction of winterform males to live females was also observed in the laboratory and was delayed in comparison to male attraction to 13-MeC27 in the laboratory.

Objective 2:

- Traps baited with 100 ug and 1000 ug of 13-MeC27 consistently caught more males than the 10 ug, 1 ug or 0.1 ug doses or the control for all 3 dates (two conducted in the winter and one in late summer), although this trend was not statistically significant, probably due to the low densities of psylla present at the time and/or location.
- The clear screen trap caught significantly more winterform males and females than the clear panel, the yellowish mesh, or the commercial delta trap. However, the number of psylla caught was again low.
- The release rate in the laboratory was not conducted because Christelle Guédot was laid off for the second half of 2011.

Objective 3:

- Identified 3 chemicals that were predominant in summerform female extract compared to male extract (13-MeC27; 2-methylheptacosane, 2-MeC27; and 3-methylheptacosane, 3-MeC27).
- Demonstrated attraction of males but not females to 13-MeC27 and to a blend of 13-MeC27+2-MeC27+3-MeC27.
- Demonstrated that summerform males are as attracted to 13-MeC27 as to blend of chemicals, and that 13-MeC27 and the blend are as attractive to males as an extract of females.
- Demonstrated in the field that summerform males are attracted to traps baited with 13-MeC27 and to traps baited with the blend of chemicals compared to control traps.
- Demonstrated in the field that summerform females are not attracted by either 13-MeC27 or the blend of chemicals in either laboratory or field assays.

METHODS

Seasonality of attractiveness of 13-methylheptacosane. 13-MeC27 was loaded into gray rubber septa and pinned to the center of sticky traps composed of sections of nylon mesh and covered with tanglefoot. Simultaneously, psylla were collected from the orchard and immediately assayed in the olfactometer. We assessed male response to live females vs. a blank (2011 only) and to 13-MeC27 vs. a blank (2010 and 2011). The seasonality of attractiveness was assessed from end of January to early April 2010 and 2011.

Optimization of 13-methylheptacosane. We field tested different doses (0.1 to 1000ug) of 13-MeC27 on sticky traps (**Figure 1 C**) to assess the most efficient dose for optimum male capture. We also tested different trap designs (**Figure 1 (A)** clear panel, **(B)** clear screen, **(C)** yellowish mesh trap, and **(D)** commercial delta trap) with 13-MeC27 as the attractant, to determine the most efficient trap for male capture.

Summerform response to sex attractants. Chemical analyses of whole-body washes were conducted with a GC-MS to confirm the identity and quantify the chemicals predominant in female washes. Compounds of interest were tested in the olfactometer to assess male and female response to these chemicals. Because 13-MeC27 was already shown to be a sex pheromone attractant for winterform males and because it is also the compound most abundant in females compared to males in the summerform, we tested psylla response to 13-MeC27 alone and in combination with the other 2 compounds identified. We tested the effect of combining all 3 compounds in a blend to assess whether the addition of the other 2 compounds would enhance male response to the 13-MeC27. Assays were conducted in the laboratory with a Y-tube olfactometer and in the field using sticky traps.

RESULTS AND DISCUSSION

Seasonality of attractiveness of 13-methylheptacosane. Attraction by winterform males to 13-MeC27 occurred from early to late February in 2010 and was consistent between laboratory (**Figure 2**) and field (**Figure 3**) assays. Females were not attracted to 13-MeC27 baited traps in the field (not shown). Beginning in March, males were no longer attracted to 13-MeC27 in laboratory or field assays (**Figure 2 and 3**), coinciding with females reaching reproductive maturity in the field (**Figure 4** dashed line) and being mated in the field (**Figure 4**). Furthermore, males assayed to live females in olfactometer tests on March 2nd, 2010 were not attracted to females when paired with a blank. In 2011, attraction by winterform males to synthetic 13-MeC27 in the laboratory occurred from mid-February to late March (**Figure 5**). Attraction by males to live females in the laboratory occurred from late February to late March (**Figure 6**). The delay in the onset of male attraction to live females compared to the onset of male attraction to 13-MeC27 suggests that males might become responsive before females become attractive. At the end of March, males were no longer attracted to 13-MeC27 and to live females in the laboratory, coinciding with males no longer being attracted to 13-MeC27 in the field (**Figure 7**). Females were not attracted to 13-MeC27 baited traps in the field (not shown). Females reached reproductive maturity around mid-March with most females being mated in the field (**Figure 8**). In conclusion, the same trends were observed between 2010 and 2011 with a >2-week delay in 2011 probably due, at least in part, to the lower temperatures experienced in the winter of 2011 compared to 2010. This series of experiments will be conducted again in 2012 to confirm the trends.

Optimization of 13-methylheptacosane. The dose response experiment was conducted 3 times: in February, March and September 2011. However, due to low densities (< 2 males/trap) in February and September, only the data obtained in March 2011 is presented here (**Figure 9**). The traps baited with 100 ug and 1000 ug of 13-MeC27 consistently caught more males than the 10 ug, 1 ug, 0.1 ug

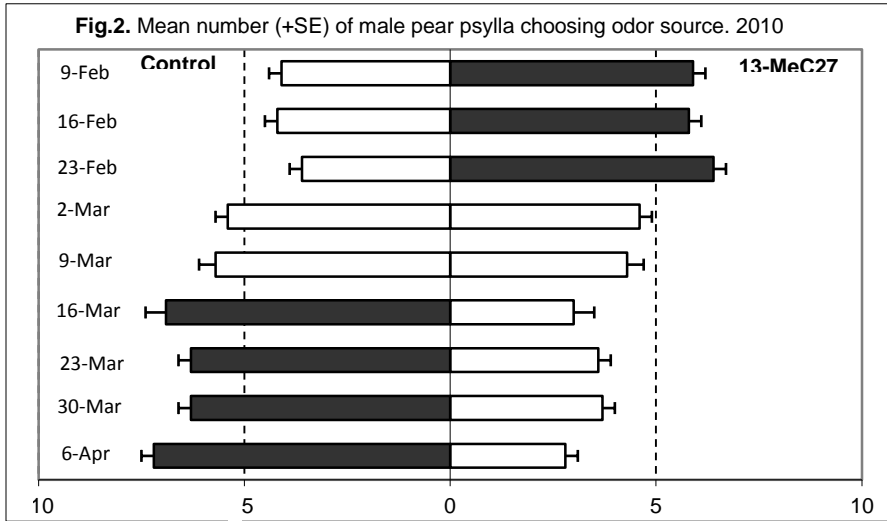
or the control for all 3 dates, although this trend was not statistically significant, probably due to the low densities of psylla present at the time of year and/or location (**Figure 9**). This experiment will be conducted again in March 2012 to confirm this trend. Regarding the trap design experiment, the clear screen trap (**Figure 1B**) caught significantly more winterform males and females than the clear panel, the yellowish mesh, or the commercial delta trap (**Figure 1C, D, and E** respectively). However, the number of psylla caught was again low (**Figure 10**) and we plan on running this experiment again in March 2012. The release rate of 13-MeC27 from gray rubber septa over time in the laboratory was not conducted because Christelle Guédot was laid off for the second half of 2011. We intend to run this experiment in 2012.

Summerform response to sex attractants. Chemical analyses of whole-body washes of summerform psylla revealed that 13-MeC27, 2-MeC27, and 3-MeC27 were found to be considerably more abundant in females than males. Females did not respond to either 13-MeC27 or to the blend of chemicals, i.e. 13-MeC27+2-MeC27+3-MeC27 (**Figure 11**). On the other hand, males were attracted to both 13-MeC27 and to the blend, with no statistical difference between 13-MeC27 and the blend when presented in pair (**Figure 12: upper panel A**; filled bars and asterisks indicate significant preference). We then compared male attraction to 13-MeC27 and the blend vs. an extract of females. Males did not show a preference for 13-MeC27 when paired with the extract of females. Similarly, males did not show a preference for the blend when paired with the extract of females (**Figure 12: middle panel B**). We also assessed the effect of chirality of 13-MeC27, i.e. (*R*)-13-MeC27 and (*S*)-13-MeC27 enantiomers, on male attraction. More males were attracted to the racemic blend containing both enantiomers than to the (*R*)-13-MeC27 or the (*S*)-13-MeC27 enantiomers (**Figure 12: lower panel C**). Males did not show a preference for either enantiomer when presented in pair. Finally, in field assays, more males were caught on traps baited with 13-MeC27 alone and on traps baited with the blend than on unbaited traps, with no significant difference in trap catches between 13-MeC27- and blend-baited traps (**Figure 13**). Females were not attracted to 13-MeC27- or blend-baited traps compared to the control traps (**Figure 13**). These results suggest that 13-MeC27 is also a sex attractant pheromone for pear psylla males of the summerform.

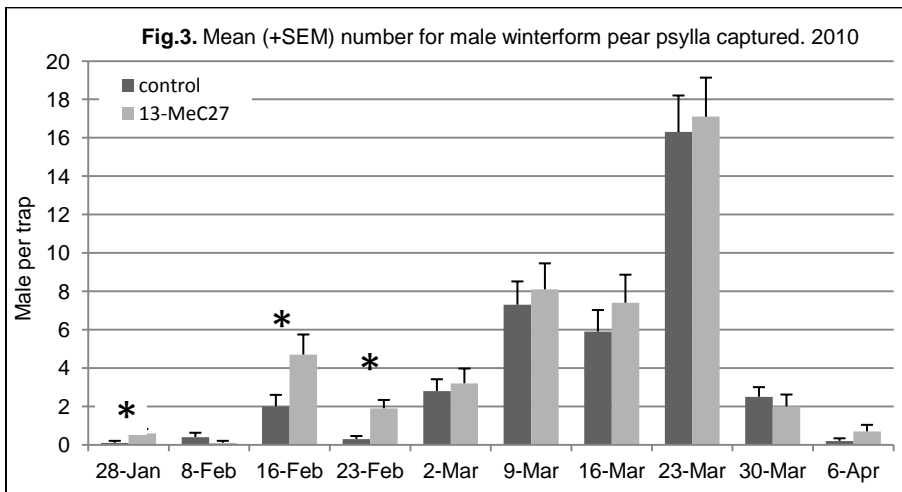
PUBLICATIONS

- Horton D.R., Guédot C., 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 pear psylla: effects of female age, mating status, and presence of host plant. *Canadian Entomologist* 140: 184-191.
- Guédot C., Horton D.R., and P.J. Landolt. 2009. Attraction of male winterform pear psylla to female-produced volatiles and to female cuticular extracts with evidence of male-male repellency. *Entomologia Experimentalis et applicata* 130: 191-197
- Guédot C., Millar J.G., Horton D.R., and P.J. Landolt. 2009. Identification of a sex attractant pheromone for male winterform pear psylla, *Cacopsylla pyricola*. *The Journal of Chemical Ecology* 35: 1437-1447
- Guédot C., Horton D.R., and Landolt P.J. 2011. Response of summerform pear psylla (Hemiptera: Psyllidae) to male- and female-produced odors. *Canadian Entomologist* (In press)

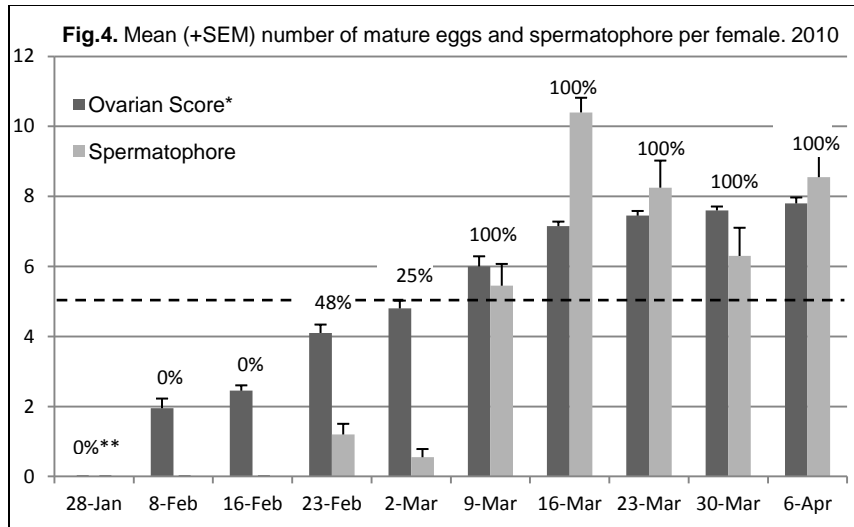
Fig.1. Traps used with gray rubber septa loaded with 100 ug of 13-methylheptacosane. (A) clear panel trap, (B) clear screen trap, (C) yellowish mesh trap, and (D) delta trap.



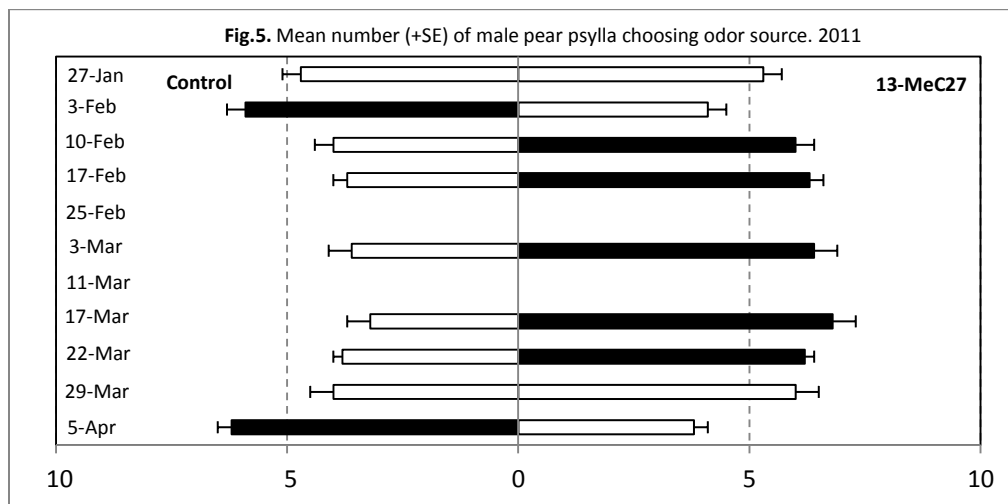
Black shading indicates significant preference for the odor source



* indicates significant preference

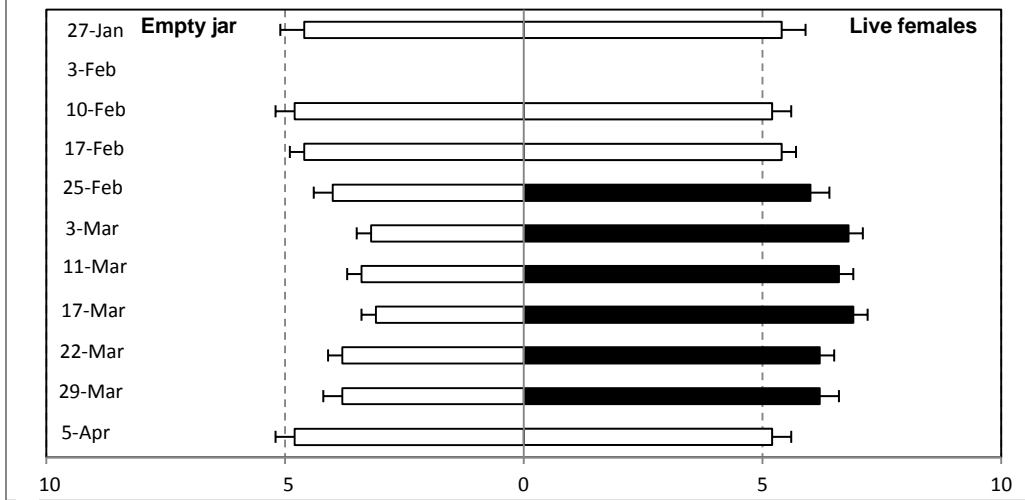


*Dashed line indicates the ovarian score (5) at which females have mature eggs (Krysan and Higbee 1990).
 **Percent mated females



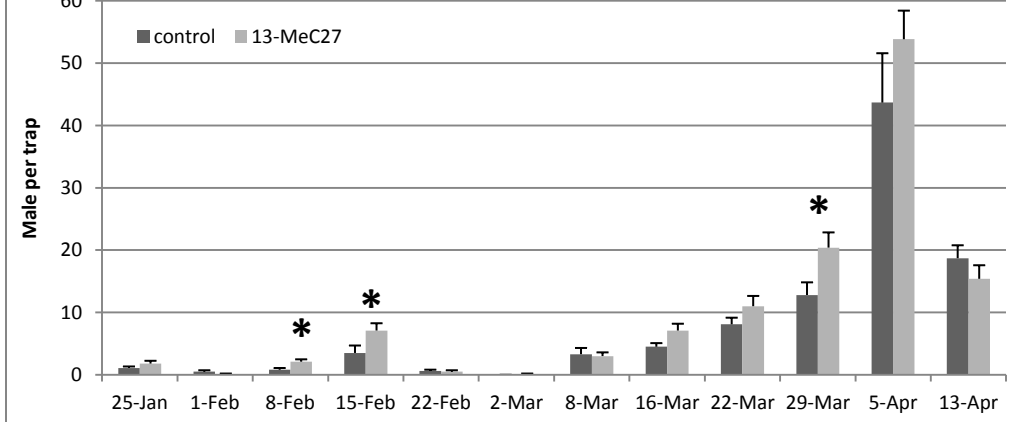
Black shading indicates significant preference for the odor source

Fig.6. Mean number (+SE) of male pear psylla choosing odor source. 2011



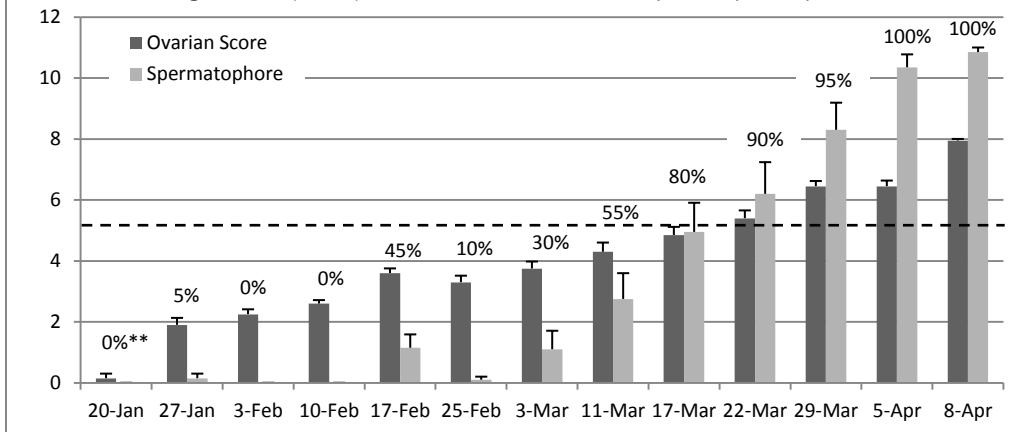
Black shading indicates significant preference for the odor source

Fig.7. Mean (+SEM) number for male winterform pear psylla captured. 2011



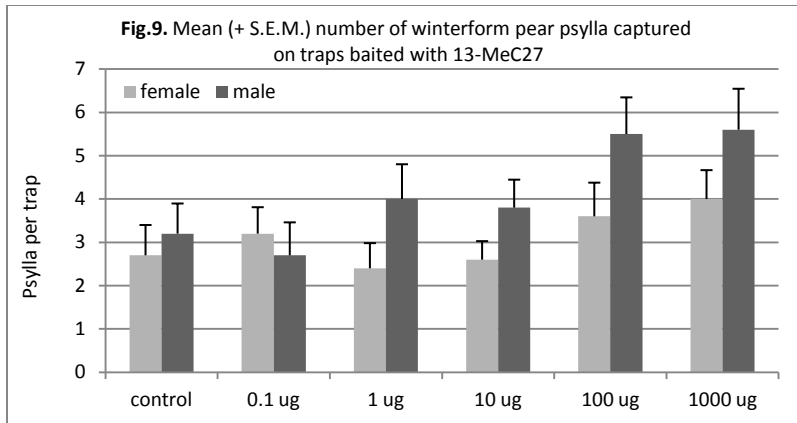
* indicates significant preference

Fig.8. Mean (+SEM) ovarian score and number of spermatophores per female. 2011

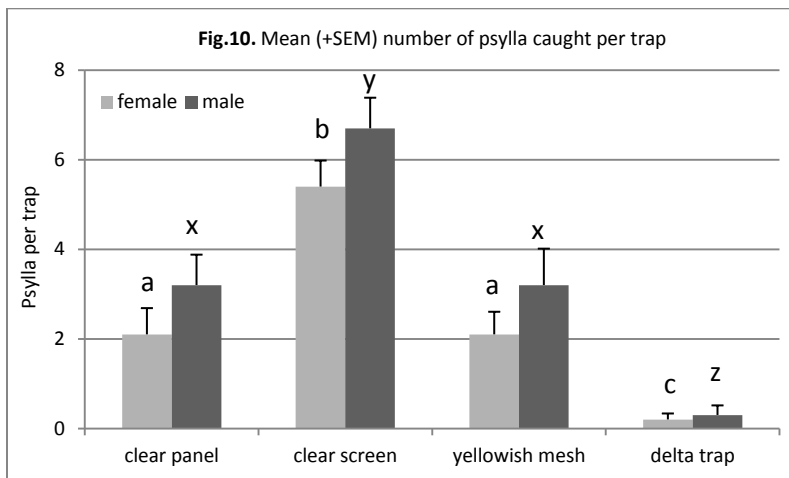


*Dashed line indicates the ovarian score (5) at which females have mature eggs (Krysan and Higbee 1990).

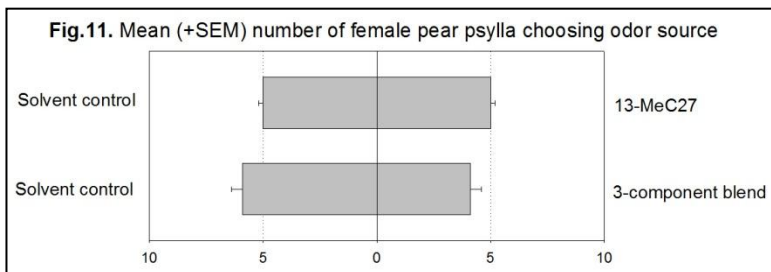
**Percent mated females.

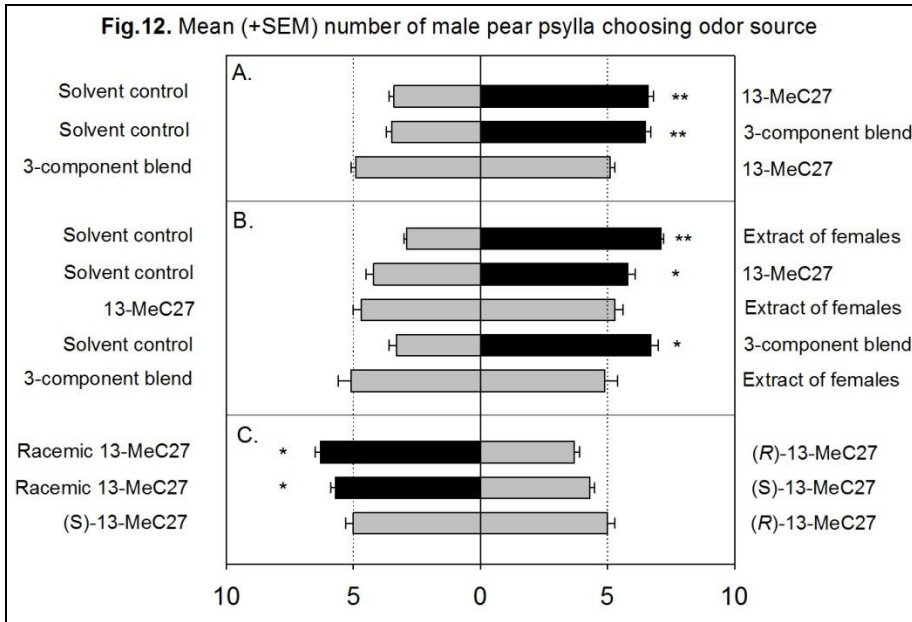


Traps baited with different amounts of 13-MeC27 dispensed from gray rubber septa ($n = 10$ traps per treatment).

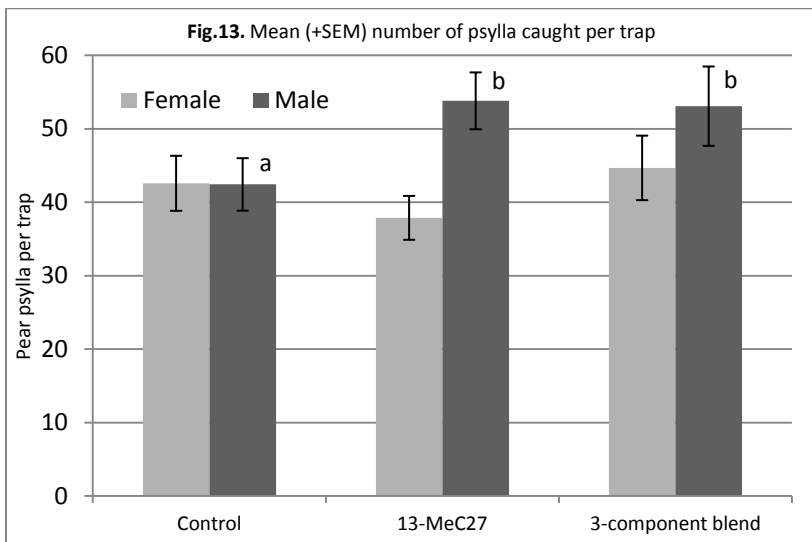


Different types of traps baited with 13-MeC27 (100 ug) dispensed from gray rubber septa ($n = 10$ traps per treatment). For male trap catches and female trap catches, treatments with different letters above them are significantly different (Tukey test, adjusted $P \leq 0.05$).





Black shading indicates significant preference for the odor source



Traps baited with 13-MeC27 (100 ug), or 3-component blend made of 13-MeC27 (100ug), 2-MeC27 (100ug), and 3-MeC27 (30ug), dispensed from gray rubber septa ($n = 10$ traps per treatment). For male trap catches, treatments with different letters above them are significantly different (Tukey test, adjusted $P \leq 0.05$).

FINAL PROJECT REPORT: EXECUTIVE SUMMARY

WTFRC Project Number: PR-10-100

Project Title: Development of field applications for a pear psylla sex attractant
PIs: Christelle Guédot, David Horton, and Peter Landolt
Organization: USDA-ARS
Email/Telephone: David.Horton@ars.usda.gov (509) 454-5639
Address: 5230 Konnowac Pass Road, Wapato, WA 98951

Outside Funding: \$280,000 (BARD)

Total Project Funding: \$40,000

Budget History:

Item	Year 1: 2010	Year2: 2011
Salaries	\$15,500	\$15,500
Benefits	\$ 4,500	\$ 4,500
Total	\$20,000	\$20,000

SUMMARY

- There is a seasonality in attraction of winterform males to the sex attractant pheromone 13-MeC27, both in the field and in laboratory bioassays which seems to coincide with females reaching reproductive maturity and being mated in the field.
- Seasonality in attraction of winterform males to live females was also observed in the laboratory and was delayed in comparison to male attraction to 13-MeC27 in the laboratory.
- Traps baited with 100 ug and 1000 ug of 13-MeC27 consistently caught more males than the 10 ug, 1 ug or 0.1 ug doses or the control.
- The clear screen trap caught significantly more winterform males and females than the clear panel, the yellowish mesh, or the commercial delta trap.
- Identified 3 chemicals that were predominant in summerform female extract compared to male extract (13-MeC27; 2-methylheptacosane, 2-MeC27; and 3-methylheptacosane, 3-MeC27).
- Summerform males are as attracted to 13-MeC27 as to blend of chemicals, both in the laboratory and in the field.
- 13-MeC27 is a female-produced sex attractant pheromone that is attractive to winterform and summerform pear psylla males.

Plans for 2012

- Confirm the trend observed with the seasonality in male attraction to live females and to 13-MeC27 in the laboratory and the field
- Confirm the trend observed with the dose response experiment and the trap design experiment
- Determine the release rate of 13-MeC27 from gray rubber septa in the laboratory