Project Title: Identification of pear tree volatiles attractive to winterform psylla

Report Type: Final Project Report

Primary PI: Jacqueline Serrano
Organization: USDA-ARS
Telephone: 509-454-4461
Email: jacqueline.serrano@usda.gov
Address: 5230 Konnowac Pass Road
Address 2:
City/State/Zip: Wapato, WA 98951

Co-PI: W. Rodney Cooper
Organization: USDA-ARS
Telephone: 509-454-4461
Email: jacqueline.serrano@usda.gov
Address: 5230 Konnowac Pass Road
Address 2:
City/State/Zip: Wapato, WA 98951

Cooperators: David Horton, USDA-ARS in Wapato, WA.

Project Duration: 3 Year **(**+NCE)

Total Project Request for Year 1 Funding: $ 30,000
Total Project Request for Year 2 Funding: $30,000
Total Project Request for Year 3 Funding: $6,000

Other related/associated funding sources: None

WTFRC Collaborative Costs: None

Budget 1
Primary PI: W. Rodney Cooper
Organization Name: USDA-ARS
Contract Administrator: Mara Guttman
Telephone: 509-510-5619
Contract administrator email address: Mara.guttman@usda.gov

Item	2020	2021	2022
Salaries	$8,650.00	$8,866.00	$0.00
Benefits	$2,768.00	$2,837.00	$0.00
Wages	$0.00	$0.00	$0.00
Benefits	$0.00	$0.00	$0.00
RCA Room Rental	$0.00	$0.00	$0.00
Shipping	$0.00	$0.00	$0.00
Supplies	$17,582.00	$16,797.00	$5,000.00
Travel	$0.00	$500.00	$0.00
Plot Fees	$1,000.00	$1,000.00	$1,000.00
Miscellaneous	$0.00	$0.00	$0.00
Total	$30,000.00	$30,000.00	$6,000.00

Footnotes:

Salary and benefits for Biological Science Technician that conducted laboratory bioassays and assisted with field trial.

Supplies for volatile collections and analyses (bags, glassware, pumps, tubing, fittings, solvent, gases, chemicals), field trials (traps, posts, lures, chemicals), bioassay materials (glassware, fittings), and general lab supplies (gloves, pipette tips, GC parts, vials)

OBJECTIVES: Recap, Goals, and Activities:

1) Determine if volatiles emitted by post-dormant (bud-swell) pear trees are attractive to post-diapause winterform pear psylla.

Preliminary results from caged bioassays were promising and suggest that pear tree volatiles may be attractive to winterform psylla. However, the results were not significantly different, likely due to flaws in the bioassay methods. We had difficulty hiring a technician who was meant to conduct bioassays in first two years of project. In Year 3, we collected winterform pear psylla from evergreens at the Moxee farm to use for laboratory bioassays, but due to mechanical failures in the building, the collected insects died before use. We have repeated collections for bioassays to be conducted in Year 4. Both Y-tube and caged bioassays were conducted with pear psylla to determine their attraction to host plants. For all bioassays, we examined variation in responses of winterform and summerform males and females to pear and an overwintering evergreen host. In Year 4, we also conducted a field trial, testing whether volatiles identified from pear were attractive to winterforms.

2) Identify pear tree volatiles that are responsible for attraction of post-diapause winterform pear psylla.

No volatile collections were conducted during Year 1 of funding, due to the timing of the project (February-March) and when research funds were received (late summer 2020). We designed a method to allow us to perform simultaneous collections from multiple trees, which incorporated powerful air and vacuum pumps and manifolds. These materials were purchased and used to build the collection system for implementation in Year 2. The volatile collectors that were used in the collections were purchased as a prefabricated item and were found to be contaminated. Therefore, we had to create our own volatile collectors that have been determined to be free of contaminants. The new collectors were used in Year 3, with the system to sample several trees in the field. However due to undetectable differences in the volatiles of the trees, a different volatile sampling method was in Year 4 so that collections took place in a smaller glass container.

3) Develop a synthetic lure, based on attractive pear tree volatiles, that can be used in a trap to detect, monitor, or manage migrating post-diapause winterform pear psylla.

Due to delays in completing research for other objectives, this objective was eliminated.

SIGNIFICANT FINDINGS

- Preliminary caged bioassays suggested that pear tree volatiles are attractive to winterform psylla.
- Winterform pear psylla were attracted to juniper volatiles and preferred to settle on juniper shoots over pear shoots, but summerforms did not respond to volatiles from juniper.
- Attraction to pear and juniper volatiles varied by season, tree phenology, and psyllid physiology.
- Prefabricated volatile collectors were found to be contaminated with several chemicals, which prevented volatiles emitted by pear trees to be properly analyzed. New, cleaner, and cheaper collectors were made for volatile collections.
- Whole tree volatile collections were too large in volume to detect any volatiles emitted by pear trees before and after budswell.

- Volatile collections with cuttings and pruned stems in 1L jars also revealed minimal results. A more sensitive sampling technique will be needed to sample volatiles from small growing pear leaves. Groups of whole stems and pruned pieces from overwintering trees revealed a small amount of a single major volatile, β-myrcene and (*E*)-4,8-dimethyl-1,3,7-nonatriene, respectively. It is unclear if either play a role in psylla attraction.
- Field trials with two volatiles emitted pear, were not successful in capturing significant amounts of pear psylla, which could be attributed to trap type or lure release rate. Additional studies are needed to optimal combination of trap and lure type.

METHODS

Insect Collection

Psylla were collected from pear trees located at the ARS facility in Wapato and the USDA experimental farm near Moxee. For assays with post-diapause winterform psylla, insects were held overnight in a growth chamber maintained at 41°F with 12:12 (L:D) h photoperiod. Summerform psylla were held overnight at 50°F with a 16:8 (L:D) h photoperiod. Psylla females were dissected, and reproductive development was ranked based on Krysan and Higbee (1990) with ovarian scores ranging between 0 and 7 where 0 indicates no reproductive development and 7 indicates full reproductive maturity.

Plant Collection

Pear shoots were collected from Bartlett pear trees grown in a commercial orchard near Wapato, WA or in an experimental orchard located at the USDA research farm near Moxee, WA. Pear phenology (Larsen 2023) was monitored at both locations to record dates of bud swell (scale separation) and bud burst. Pear seedling whips were removed from cold storage and used for dormant pear in assays with summerform psylla. Juniper shoots were collected from ornamental trees located near Wapato, WA that typically harbor large populations of overwintering pear psylla (Cooper et al. 2019). Each shoot was thoroughly rinsed in water and cut to 10 cm length, and the cut end of each shoot was placed in water.

Laboratory Bioassays

Y-tube olfactometer experiments compared choice of spring winterform, summerform, and autumn winterform psylla to three treatments: 1) pear versus blank, 2) juniper versus blank, or 3) pear versus juniper. The Y-tube olfactometer was setup as described in Horton and Landolt (2007) (Figure 1). Male and female pslla were assayed separately and each insect was observed for 5 min. When an insect entered an arm of the Y-tube, it was considered a choice.

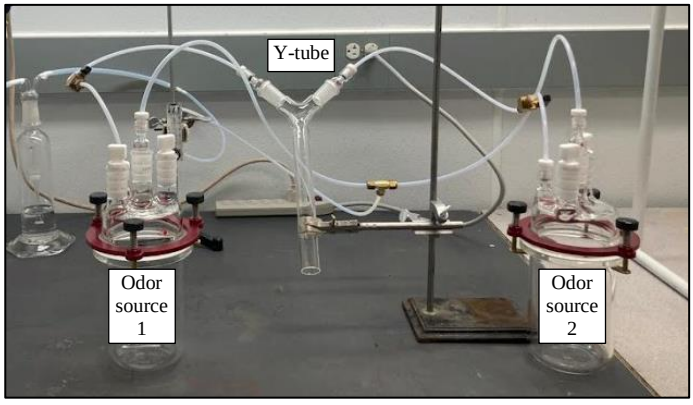

Figure 1. Example of Y-tube olfactometer set up.

Choice preference assays were used to examine attraction of psylla to plant shoots in a greenhouse setting. Treatments included dormant pear, active pear collected directly before the assay, and juniper Three plant shoots (one per treatment) were arranged in each of six cages within a greenhouse. Ten psylla (equal numbers of males and females) were released into the center of each cage. Plant shoots were examined after 24 hours and the number of psylla choosing a shoot was recorded. Assays were then repeated with new plant shoots that were covered with a mesh sleeve cage to prevent psylla from landing on the shoot, and to reduce visual cues.

Collection and Analysis of Pear Volatiles

Volatiles were collected from Bartlett pear trees during the dormant phase through the bud-swell phase when psylla re-entry is known to occur. These collections took place semiweekly as the trees exit dormancy. Phenological growth stage of the tree will also be recorded, following the BBCH identification keys of pome fruit trees.

In 2021 and 2022, volatiles were collected from 5 trees in orchards in Moxee, WA. Briefly, branches were wrapped in polyethylene bags (Figure 2A) that were fitted with an inlet and outlet for filtered air flow to be introduced using vacuum and air pumps. A charcoal filter was attached to the air pump (before the manifold) to introduce clean air into the inlet of the bag (Figure 2B). A volatile collector was connected to the outlet and to the manifold of the vacuum line (Figure 2B). The tubing that is connected to the inlet and outlets of each bag is fitted with a flow meter to ensure constant flow over the trees (Figure 2C). Each collection was conducted over four hours during peak daylight hours (approximately 10:00-14:00). Once the volatile collections were complete, the collectors were extracted with high purity solvent, which were stored in a freezer until analyses.

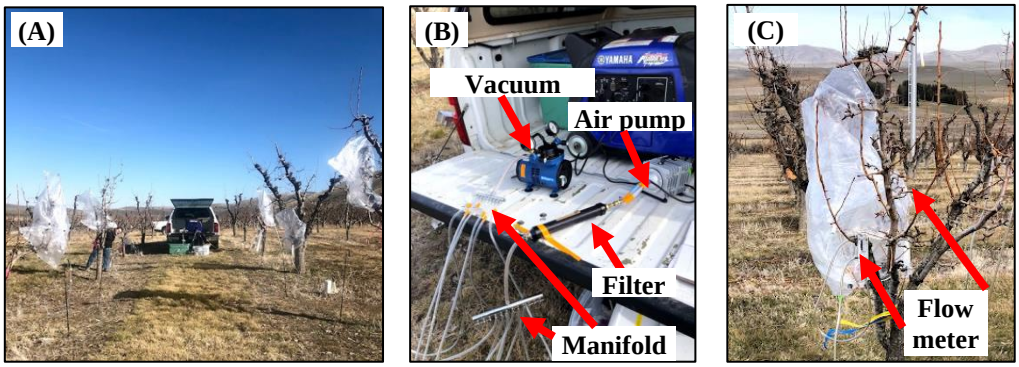

Figure 2. Example of volatile collection set up: (A) Volatiles being collected from 5 Bartlett pear trees at the USDA experimental farm in Moxee; (B) air pump, vacuum pump, and tubing set up; (C) up close image of volatile collection set up on pear tree.

In our second attempt to collect volatiles from trees in the field, we did not detect any differences in volatiles between the tree and the control (bag with no tree) and we also did not see any seasonal differences in volatiles emitted by post dormant trees. We believe because the bags are such a large sampling area in comparison to the small buds or leaf clusters, that it is hard to detect small quantities of volatiles using this collection method. Therefore, we attempted to collect volatiles from cuttings of pear twigs (whole or cut into pieces) by placing them in 1 liter glass containers (Figure 3). The glass containers will also be used to house plants that were also used for Y-tube bioassays.

The extracts were analyzed by coupled gas chromatography-mass spectrometry (GC-MS) to tentatively identify compounds present in the volatile profile of the trees (via mass spectra interpretation). The identification of the compounds was confirmed, when possible, by comparisons or retention times and mass spectra with those of authentic standards. Qualitative and quantitative comparisons were made between extracts of volatiles from pear trees present throughout the duration of the collections. These comparisons were made within and between samples, across difference phenological growth stages.

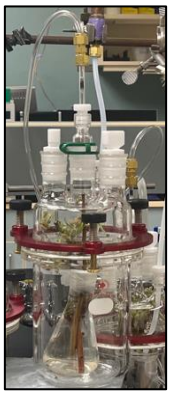

Figure 3. Example of volatile collection set up: 1L glass container with an inlet and outlet port so that volatiles can be collected using laboratory vacuum and onto charcoal collectors.

Field Trial

There were two compounds tested that were identified from pear trees in two recently published papers. The first compound was β-caryophyllene, which was the most abundant compound identified from trees at the BBCH 32-33 stages (Gallinger et al. 2023). The second compound was (*Z*)-3-hexenyl acetate, a compound found to be present on pear in high abundance, with or without the presence of pear psylla (Valle et al. 2023). We tested lures containing each compound individually, and a binary blend of the two. Lures were attached to clear sticky traps (AlphaScents), and each trap was fixed to a wooden stake at least 1 meter from the ground (Figure 4). Four replicates of traps were placed in the periphery of pear orchards, i.e. in Wapato surrounding the USDA-ARS facility and at the USDA-ARS farm in Moxee. Psylla captured on traps were sexed and counted in the laboratory. Lures were replaced biweekly and were made in-house using 4 mL plastic vials.

Figure 4. Example of clear sticky trap used in field assays testing plant volatiles. Lures were suspended from the top of the binder clips, so that they were level with the top of the trap.

RESULTS AND DISCUSSION

Bioassays

Results from preliminary caged bioassays (conducted in 2019) were promising and suggest that pear tree volatiles may be attractive to winterform psylla (Figure 5). However, the results were not significantly different, likely due to flaws in the bioassay methods. In short, a dual choice assay was conducted in a small cage, where 40 psylla were introduced and presented with two traps, one containing an untreated piece of filter paper, and the other containing filter paper treated with volatiles collected from pear trees. Although the results, were not significantly different, they do suggest that the pear psylla may be attracted to pear volatiles.

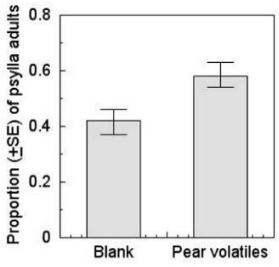

Figure 5. Mean (±SE) number of pear psylla caught in traps baited with a nontreated piece of filter paper ("Blank") and pear volatiles.

We have conducted similar caged bioassays, presenting psylla with juniper, dormant pear, and nondormant pear. In early assays, significantly more spring-collected winterforms settled on exposed juniper shoots compared with dormant or active pear, and it later assays there was a switch, showing significantly more psylla settled on active pear shoots than on other treatments (Figure 6A). Similar results were seen when assays were performed with covered shoots (Figure 6B) indicating that psylla may be using odors to locate preferred plants. Significantly more summerform psylla settled on exposed active pear shoots than on either juniper or dormant pear

shoots (Figure 7) and in assays with covered shoots, about equal numbers of psylla settled on active pear and juniper shoots suggesting that visual or gustatory cues have a significant role in host settling (Figure 7). Results with autumn collected winterforms were dependent on collection date. In both exposed and covered assays, significantly more pear psylla settled on pear shoots than on juniper shoots in assays conducted in November when pear psylla females exhibited ovarian development (Figure 8). In contrast, more psylla settled on juniper shoots than on pear in December when no ovarian development was observed (Figure 8).

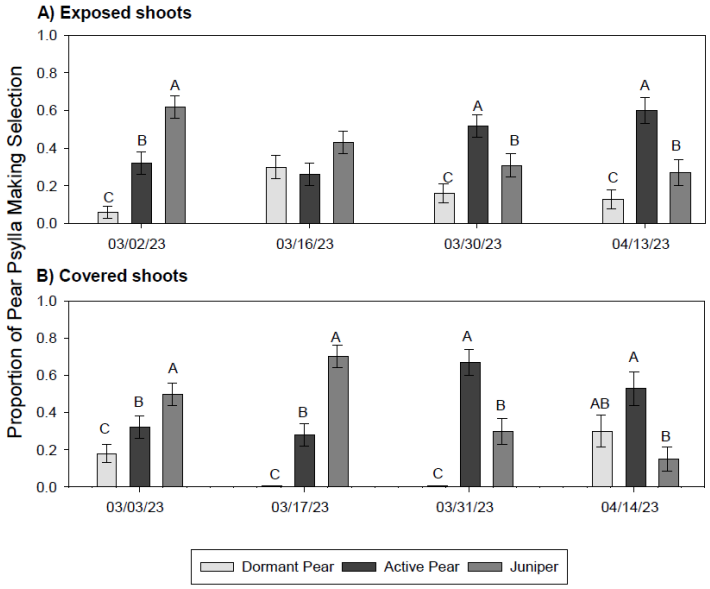

Figure 6. Results of choice preferences studies using spring-collected winterform pear psylla. Shoots were either (A) exposed or (B) covered with a mesh sleeve cage to prevent gustatory cues and to reduce visual cues. Different letters denote significant differences (α=0.05) among means within each date.

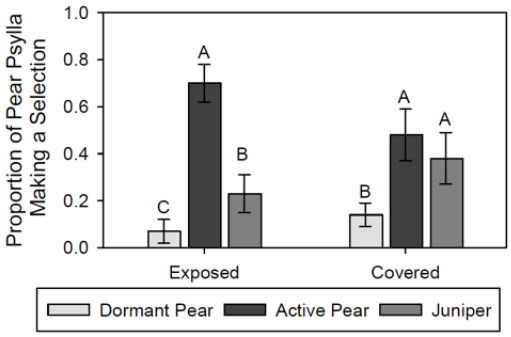

Figure 7. Results of choice preferences studies using summerform pear psylla. Shoots were either exposed or covered with a mesh sleeve cage to prevent gustatory cues and to reduce visual cues. Different letters denote significant differences (α=0.05) among means within each date.

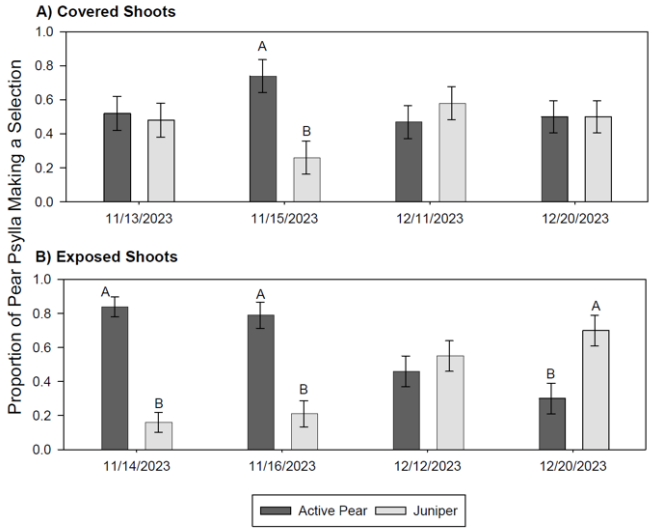

Figure 8. Results of choice preferences studies using autumn-collected winterforms. Shoots were either (A) exposed or (B) covered with a mesh sleeve cage to prevent gustatory cues and to reduce visual cues. Different letters denote significant differences (α=0.05) among means within each date.

Results from Y-tube olfactometer assays are presented in Figure 9. Spring-collected winterforms preferred juniper odors and pear odors over odor blanks in Y-tube preference assays (Figure 9a). When pear psylla were provided a choice between juniper and pear, males and females exhibited different host odor preferences, and results show that females preferred pear odor while males preferred juniper odors (Figure 9A). Assays with summerforms (Figure 9B) indicate that they do not respond to juniper odors, but both sexes chose pear odors significantly more often than odor blanks and juniper odors. Psylla collected in November, showed a strong preference for juniper odors over odor blanks and pear odors over odor blanks (Figure 9C) and males preferred juniper odors over pear odors, but females did not show a preference for either odor source. All psylla collected in November exhibited external characteristics consistent with the winterform morphotype including dark coloration and large body size. However, ovarian development was observed in about 50% of females. By December, we did not observe ovarian development in dissected females. Psylla assayed in mid-December showed a strong preference for juniper odors over both odor blanks and pear odors and chose pear odors and odor blanks equally (Figure 9D).

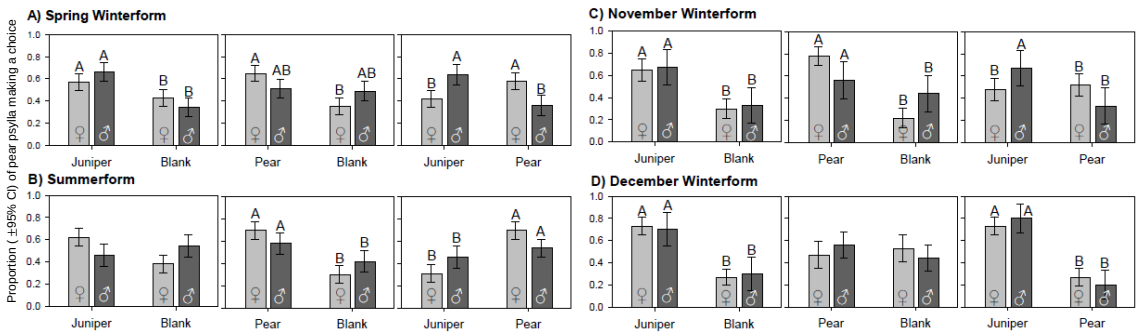

Figure 9. Results of pear psylla Y-tube olfactometer assays with (A) spring-collected winterforms, (B) summerforms, (C) winterforms collected in November, and (D) winterforms collected in December. Different letters denote significant differences among combinations of odors and psylla sexes.

Observation of ovarian development in autumn-collected winterform psylla was highly unexpected and led to interesting bioassay results. Previous studies show that wild pear psylla remain in complete diapause with ovarian development scores rarely exceeding 2 or 3 until February (Krysan and Higbee 1990, Horton et al. 1998, Horton and Landolt 2007). In contrast, 33% of females of the winterform morphotype dissected in early December had ovarian development scores as high as 6 or 7 (mature eggs). Females dissected in mid- to late-December were characterized by a lack of ovarian development consistent with previous studies on pear psylla diapause (Krysan and Higbee 1990, Horton et al. 1998, Horton and Landolt 2007). This shift in physiological status did not correspond when any obvious trends in temperature, however there are several possibilities to explain ovarian development in autumn-collected pear psylla. One possibility is that the strength of diapause varies depending on autumn climates. If this is true, we may expect ovarian development to occur more frequently in autumn winterforms in lower latitudes, and for the frequency of autumn ovarian development to increase in the Pacific Northwest in response to climate change. Regardless, ovarian development was not observed in females by mid-December, and the fate of these females remains unknown. It is possible that reproductively maturing winterforms died by mid-December, dispersed for sampling locations, or reabsorbed eggs. These observations warrant further research on the current state of pear psylla diapause in the Pacific Northwest.

Analyses of volatiles

In March 2019, preliminary volatile collections were conducted with a Bartlett pear tree at the USDA-ARS farm in Moxee, using methods described above. As a control, volatiles were sampled from a collection bag that did not contain a pear tree. Collected volatiles were then extracted and analyzed via GC-MS. Results from this analysis showed that there were differences in volatile profiles between the pear tree and the control, especially during the earlier minutes of the analysis. Additional samples were collected from one tree on a semi-weekly basis during March 2020, and analyzed via GC-MS. Compounds detected in 2019 analyses, were not detected in any of the samples taken in March 2020 (data not shown). During the analyses, there appeared to be issues with old GC-MS instrument used for analyses.

In 2021, a new GC-MS was purchased and installed in the lab and all extracts of volatiles from 2021 were analyzed on the new instrument. It appeared that each of the analyzed extracts contained many peaks/compounds. However, compound identifications revealed that the extracts contained several contaminants, including some related to plastics. Through careful analyses of solvents and collectors, we determined that the collectors were the source of contamination. Because the source of contamination were the volatile collectors, a newer collector needed to be developed and used. The collectors that were used are similarly made to the previous used collectors in that glass tubing was used to house the adsorbent. However, the adsorbent was changed from Porapak Q to thermally desorbed charcoal and there were no plastic components (Figure 10).

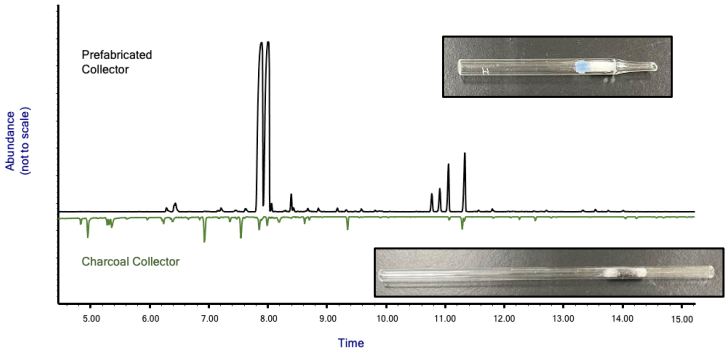

Figure 10. Representative chromatograms of an extract from an unused collector (top trace) and the extract from the new charcoal collectors (inverted trace). The trace representing the extract of the charcoal was scaled up for demonstration purposes.

In 2022, we repeated the volatile collections with the charcoal collectors, and conducted biweekly collections from the end of February through the end of March. It appeared that each of the analyzed extracts contained many peaks/compounds. However, these peaks were present in pear extracts and the controls, which indicates that these compounds are not unique to the trees (Figure 11). In addition, we also saw minimal differences in volatiles emitted by the trees from dormancy through bud burst, approximately BBCH10 (Figure 12). Our results suggest that the area, i.e. size of the bags, is too large to detect the small quantities of volatiles that are emitted by the post dormant trees. We made another attempt to sample volatiles, using custom 1L glass jars, from cuttings taken from pear when it was passed bud break and leaves were beginning to separate (approximately BBCH9-10). This method seemed promising because it was successfully used to sample cherry leaves. However, we found that samples of groups of pear cuttings contained the same compounds as the control flask of water), although sometimes at different abundances (Figure 13). The difficulties in obtaining detectable plant volatiles from early stages of pear growth, indicate that non-destructive sampling methods need to be highly sensitive to detect volatiles from small leaves, such as headspace sampling that can be directly injected into a GC-MS.

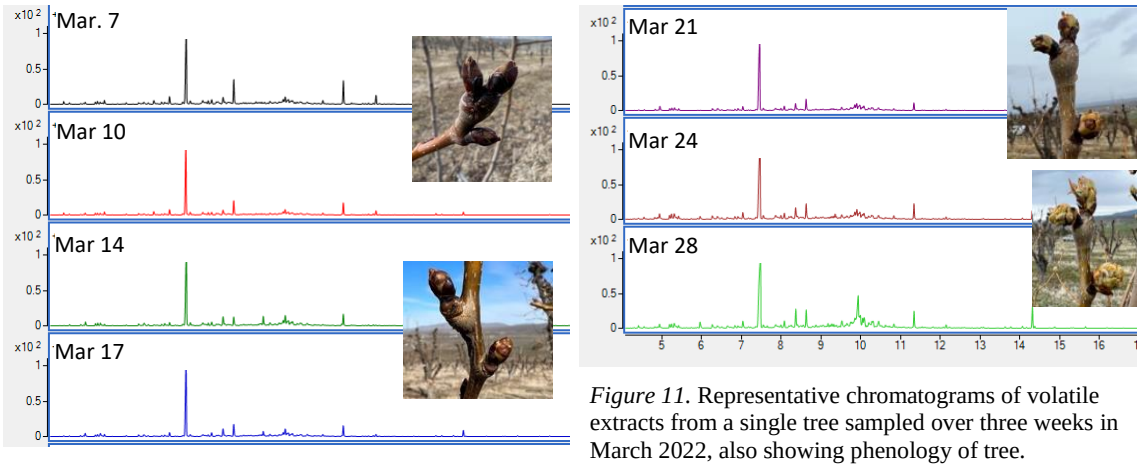

Figure 11. Representative chromatograms of volatile extracts from a single tree sampled over three weeks in March 2022, also showing phenology of tree.

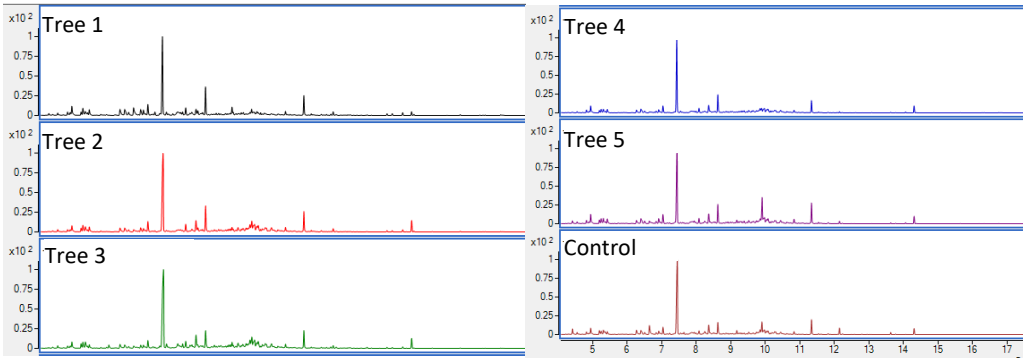

Figure 12. Representative chromatograms of volatile extracts from five different single trees and an empty bag control, sampled on when trees were past bud break, approximately BBCH10.

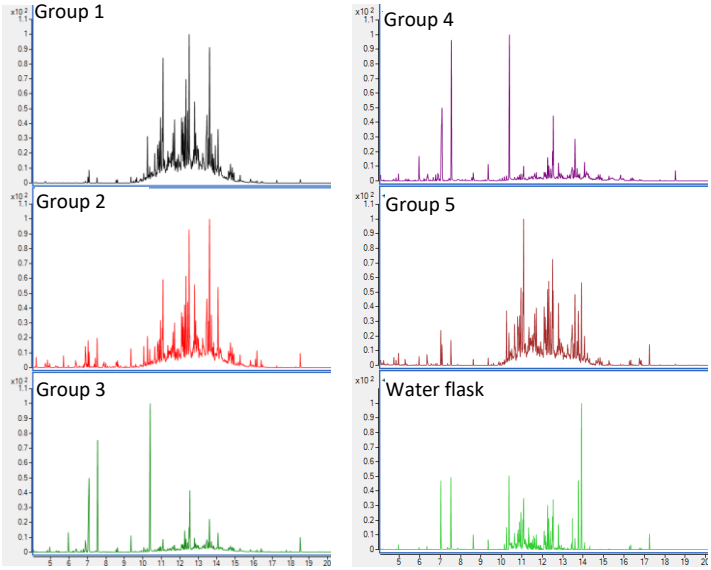

Figure 13. Representative chromatograms of volatile extracts from five different groups of pear cuttings placed in a flask of water, and control (only a flask of water), that were sampled in 1L glass jars (see Figure 3).

Volatiles were also collected from pear that was used for Y-tube bioassays by sampling whole twigs or twigs that were cut into 1-inch pieces (to simulate winter pruning). There was not a significant amount of plant volatiles detected in the sample compared to the background. The major volatile produced from intact pear branches was determined to be β-myrcene, however that compound was not detected in the sample of the cut branches, where the major volatile was another common plant compound, (*E*)-4,8-dimethyl-1,3,7-nonatriene. The importance of these compounds in the attraction of pear psylla is yet to be determined.

Field Trial

Traps baited with volatiles found in two different stages of pear (shoot development or later) did not attract significant numbers of pear psylla at either location. The highest overall capture was recorded on April 4th, for traps baited with β-caryophyllene. There were no obvious trends over time, and most treatments were not different from the control, except for β-caryophyllene on April 4th. An effective psylla trap may need a more complex blend of host plant volatiles, a visual cue like color, or vibration signals used by the psylla for courtship (Jocson et al. 2023), but more field research is needed to determine the best methods to trap pear psylla for pest management purposes.

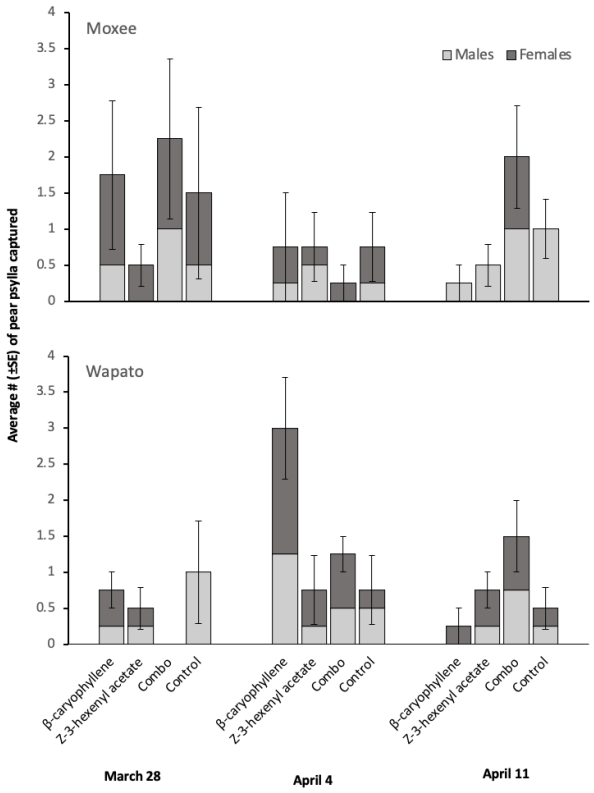

Figure 14. Mean (± 1 SE) numbers of winterform pear psylla caught on clear sticky traps baited with lures containing β-caryophyllene, (*Z*)-3-hexenyl acetate, a combination of the two, or the control. Trap captures are grouped by the dates they were checked, and proportions of males and females are also shown within average counts of the four replicates.

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EXECUTIVE SUMMARY

Project title: Identification of pear tree volatiles attractive to winterform psylla
Key words: pear psylla, host odor, bioassay, behavior, attractant.

Abstract:

Before the start of this project, it was discovered that winterform pear psylla migrate to other hosts, such as evergreen trees, during the fall months where they would overwinter. As temperatures warm, pear psylla break their diapause and disperse from shelter plants and return to pear orchards to begin egg-laying. It is unknown how post-diapause winterform pear locate pear hosts in early spring. Volatile cues seem likely because research from the 1990s demonstrated that psylla do not respond to color in early spring before leaf development on pear. Over the course of the study, we wanted to determine if winterform pear psylla would show behavioral preferences to pear (before and after budbreak) and juniper, a known winter shelter plant. Additionally, we wanted to determine if there were any volatiles emitted by pear as trees emerged from dormancy that could serve as attractive cues to pear psylla. Results of bioassays performed in the lab demonstrate that pear psylla are attracted to plant volatiles emitted by pear and juniper, but behaviors varied by season, tree phenology, and psyllid physiology. We observed a shift in plant settling from juniper to pear in spring months, corresponding with an increase in ovarian development in female psylla and the initiation of pear bud swell, however, it is not clear from our data whether this shift in plant preference is due to changes in pear phenology, insect physiology, or both. Results from volatile collections indicate that more sensitive methods are need to sample budding pear, because passive sampling of cuttings in large glass containers or plastic bags over tree sections did not provide detectable plant volatiles. Field trials with two pear volatiles found in other studies, were not successful in capturing significant amounts of pear psylla, but more research is needed to determine the best trap type and lure device. Our study provides a foundation for further research on chemical ecology and overwinter biology of pear psylla. Additional research is needed to identify specific compounds that elicit behavioral responses by pear psylla and to determine whether they would have practical use for pear psylla management.