FINAL PROJECT REPORT

Project Title: Acoustically based mating disruption of winterform psylla

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Other funding sources

Agency Name: USDA -ARS, Innovation Fund (Awarded Jan 1, 2020 – Dec 31, 2021)
Amount awarded: $21,000
Notes: To purchase equipment (speakers, minishakers, laptop computers) for field tests of disruption

Agency Name: Washington State Commission on Pesticide Registration (Awarded Dec. 2020)
Amount awarded: $24,768
Notes: Funding support for Dowen Jocson to conduct a field trial in 2021.

Total Project Funding: $155,660

Budget History:

Item	6/1/2017 to 5/31/2018	6/1/2018 to 5/31/2019	6/1/2019 to 5/31/2020	6/1/2020 to 5/31/2021
WTFREC Expense				
Salaries[1]	$28,417	$29,554	$30,736	
Benefits[2]	$2,580	$2,683	$2,791	
Wages[3]	$11,040	$11,251	$11,471	
Benefits[4]	$1,124	$1,145	$1,168	
Supplies[5]	$6,000	$3,000	$3,000	
Travel[6]	$3,600	$2,100	$4,000	
Total	$52,761	$49,733	$53,166	$0

[1] Salary for Dowen Jocson for the academic year
[2] Benefits for Dowen Jocson for the academic year; includes health insurance and fringe
[3] Summer wages for Dowen Jocson; summer wages for hourly employee (40 hrs/week; 12 weeks)
[4] Fringe benefits for the PhD student and time-slip employee during the non-academic year
[5] Yr 1 – acoustics equipment, cages, pear whips; Yrs 2 and 3 – acoustics equipment for playback tests and cage trials; pear whips.
[6] Yr 1 – Funds were to be used to visit the USDA facility in Gainesville, FL for training in insect acoustics, but trip was not needed. Yrs 2/3 - Vehicle lease through the state motor pool for use in conducting field research

OBJECTIVES

1. Determine whether pear psylla uses acoustic duetting in mate search activities.
2. Describe the vibrational signals used by psylla in mate location activities.
3. Show (in small cage studies with potted pear plants) that it is possible to slow or disrupt mating by mechanically transmitting these signals to the tree substrate.
4. Show that it is possible to slow or disrupt mating in a field setting by mechanically transmitting signals through the support wires of a trellised pear orchard.
5. Use data obtained in this industry-funded project to leverage funds from other sources

SIGNIFICANT FINDINGS

Years 1-2

- Recruited a Ph.D. candidate (Ms. Dowen Jocson) to complete our research objectives
- Acoustics equipment was purchased and set-up at the Pullman location, and psylla colonies were established. Acoustics assays with summerforms and winterforms were initiated.
- Acoustic signals from male summerform psylla were detected, quantified, and described. This is the first evidence that this species communicates acoustically.

Year 3

- Playback tests were conducted using both live males and the synthesized male signal to confirm that the male signal induces female acoustic response (duetting).
- Males were more likely to sing when they had recently been exposed to female presence.
- Male song syllables increase in pitch (frequency in Hz) as temperatures get warmer.
- ***Leveraged funding***: Applied for Innovation Fund grant (USDA-ARS) that was funded ($21,000)

Year 4

- Conducted a four-week, replicated cage trial with winterforms as a first effort to examine whether psylla mating can be disrupted acoustically
- Both male song playback and white noise (random acoustics) playback reduced total number of offspring per plant at four weeks by ~60% compared to control (no acoustic disruption) plants.
- ***Leveraged funding***: Applied for WSCPR grant to support field-based disruption trials in 2021; the proposal was funded for $24,768.
- ***Leveraged funding*****:** Applied for USDA-NIFA predoctoral fellowship (decision pending).

RESULTS AND DISCUSSION (YEARS 1-4)

Years 1-2. Ms. Dowen Jocson arrived in summer 2018 to begin a Ph.D. program in Entomology. She began her research in autumn 2018. ***Confirm that pear psylla communicates acoustically and describe the signal***. The vibrational signals of psyllids have been described for ~40 species across a range of taxa (Tisechkin 2006, Percy et al. 2006, Rohde et al. 2013, Liao and Yang 2015, Wood et al. 2016), including another pear psyllid (Eben et al. 2014), suggesting this mode of sexual communication is widespread. Our first assays confirmed that pear psylla does communicate acoustically. Assays were done in a soundproof room at the Pullman campus (Fig. 1A). We recorded signals using an accelerometer (Fig. 1B-C), which detects vibrations in the plant surface produced by signaling insects. The signal was sent to a computer (Fig. 1D) where it was translated into a readable form using free software (Raven). We confirmed pear psylla does indeed communicate acoustically. The male signal is a series of 15-25 “pulses” lasting about 10 seconds, followed by a longer phrase of more tightly packed syllables (Fig. 2: upper panel). Duration of an individual call was about 30 seconds, with consecutive calls (3 calls shown in Fig. 2: upper panel) separated by 10 to 15 seconds. The signal is superficially similar to that of a close relative of our pear psylla, the European pear psylla (*Cacopsylla pyri*; Eben et al. 2014). One noticeable difference is that the signal of our pear psylla has substantially higher average frequency (“pitch”; 1320 Hz [Fig. 2: lower panel]) compared to the lower frequency of the *C. pyri* signal at approximately 690 Hz (Eben et al. 2014).

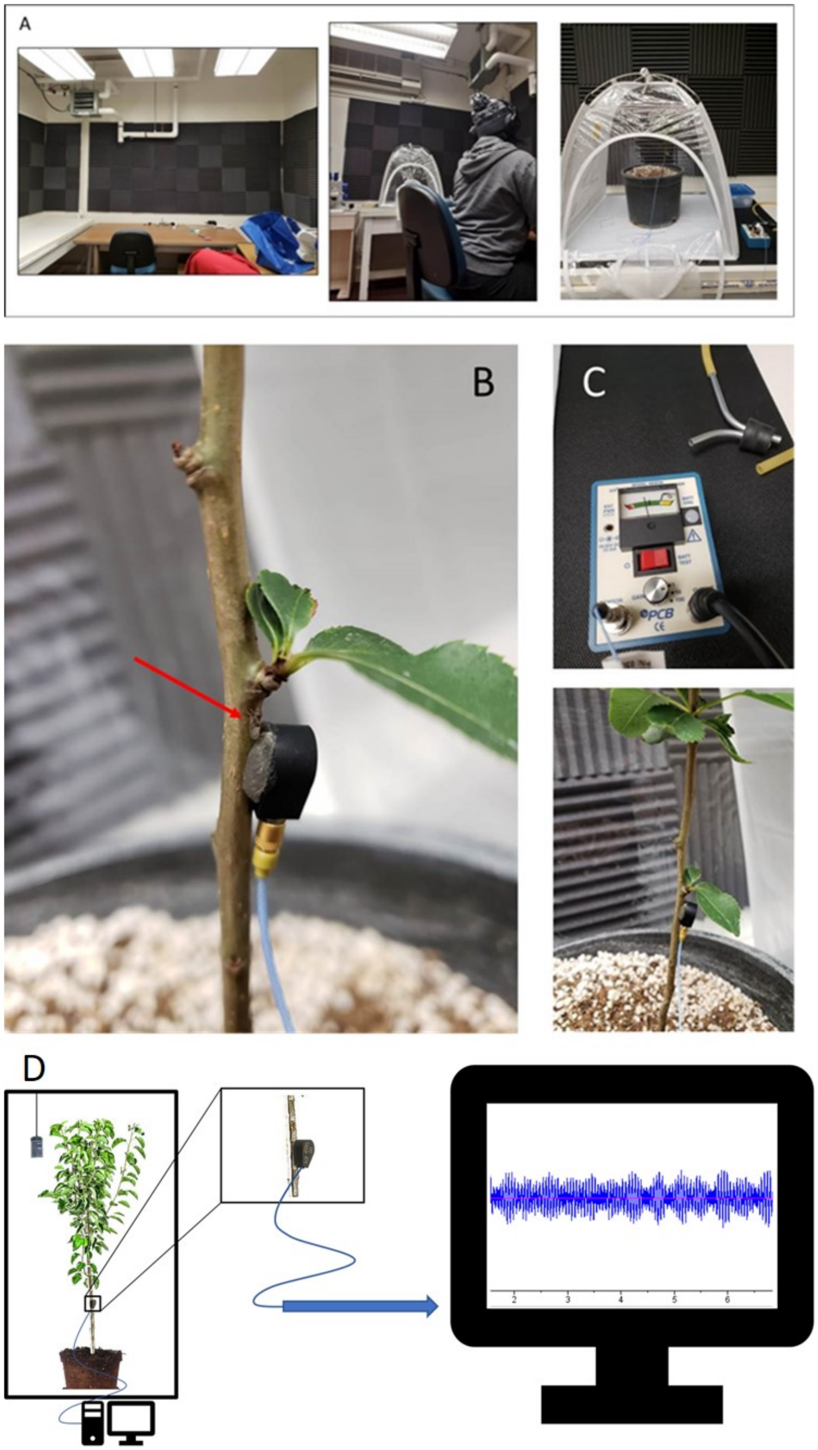

Figure 1. (A) Semi-soundproof room at Pullman location being used in our acoustics assays. (B) Head of accelerometer attached to pear whip (red arrow shows location of a psyllid). (C) Signal conditioner used to power accelerometer head and translate the vibrations. (D) Set-up.

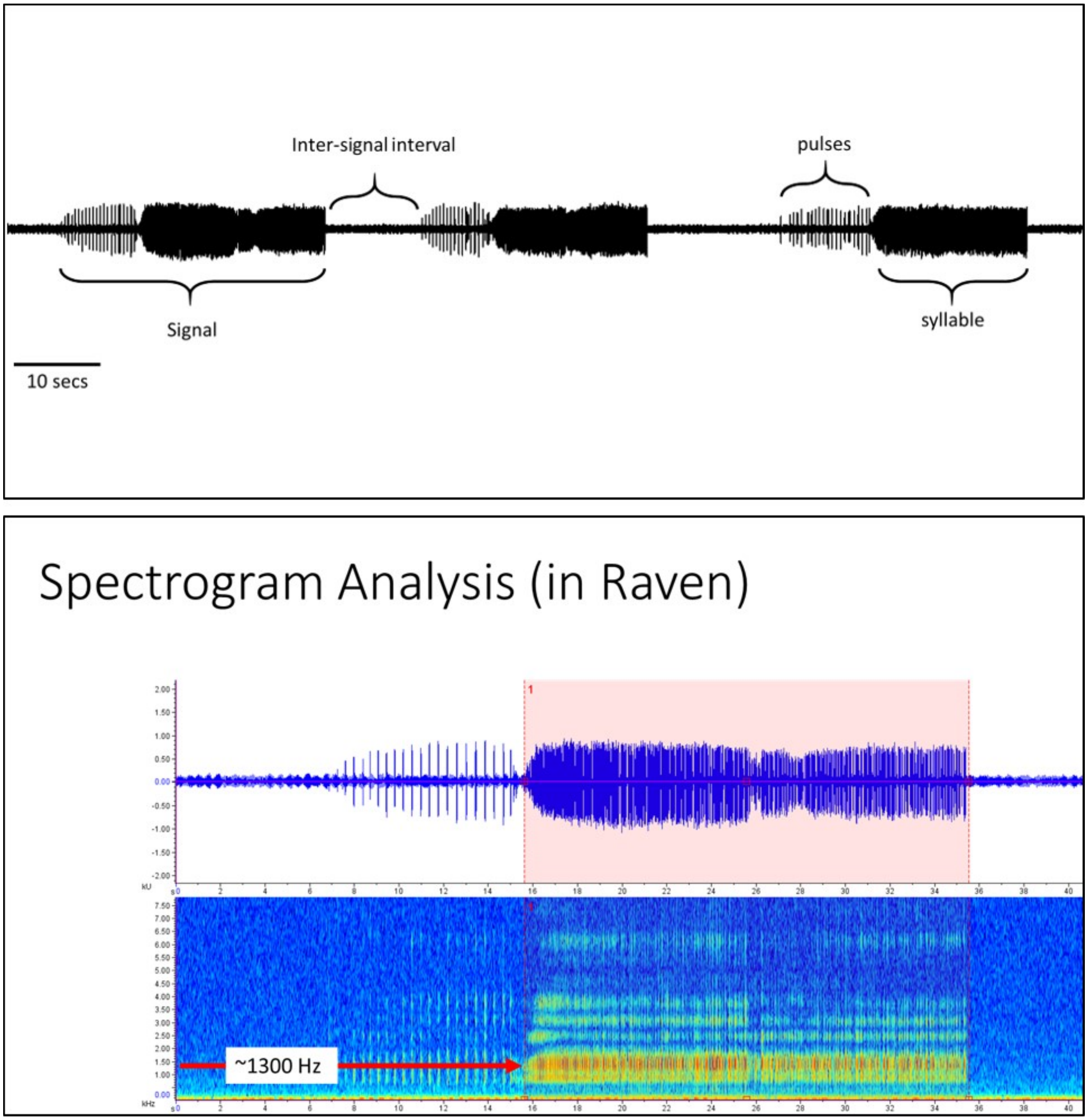

Figure 2. Upper panel shows oscillogram for signaling male summerform pear psylla; lower figure shows sonogram and spectrogram analysis depicting signal frequency concentrated at 1300 Hz.

Year 3. Research in Year 3 examined environmental and biological factors that affect signal traits and willingness to signal. ***Previous exposure of males to females.*** Inconsistent calling by males prompted us to see if exposure of males to females preceding the assay improved calling probabilities. We saw a 4-fold increase in calling probabilities for males previously exposed to females compared to calling by isolated males (Fig. 3). Psyllids appear to use 3 modalities during their mate location activities (Lubanga et al. 2014): acoustic (=vibrational), visual, and olfactory. The exact relationship among these modalities in bringing the sexes together has yet to be fully defined. One prevailing belief is that vibrational duets bring a male and female psyllid to the same general neighborhood on the plant (e.g., perhaps on the same shoot), and that visual and chemical cues are then used to prompt courtship, physical contact, and mating

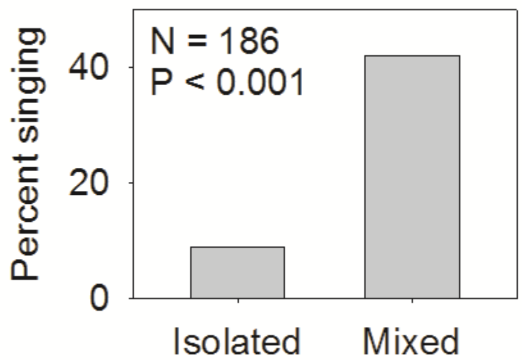

Figure 3. Probability a male summerform will sing as a function of pre-exposure to females. “Isolated”: males from single-sex culture; “Mixed”: males from mixed-sex culture.

(Lubanga et al. 2014). A second idea, yet to be tested, is that the sex pheromone of pear psylla (the hydrocarbon 13-methylheptacosane) discovered by scientists at the ARS laboratory in Wapato, when detected by males, is a “trigger” that prompts males to initiate singing activities.

Temperature. Temperature is likely to affect how rapidly the stridulatory structures of singing psylla vibrate and therefore affect properties of the vibratory signal (see Jocson et al. 2019). Male assays were conducted across a range of temperatures (74 to 90 ºF) to test whether this environmental factor affected signal characteristics. Pitch of the male signal increased with increasing temperature between 74 and 90 ºF (Fig. 4). This result suggests that characteristics of the acoustic signal will shift seasonally under orchard conditions.

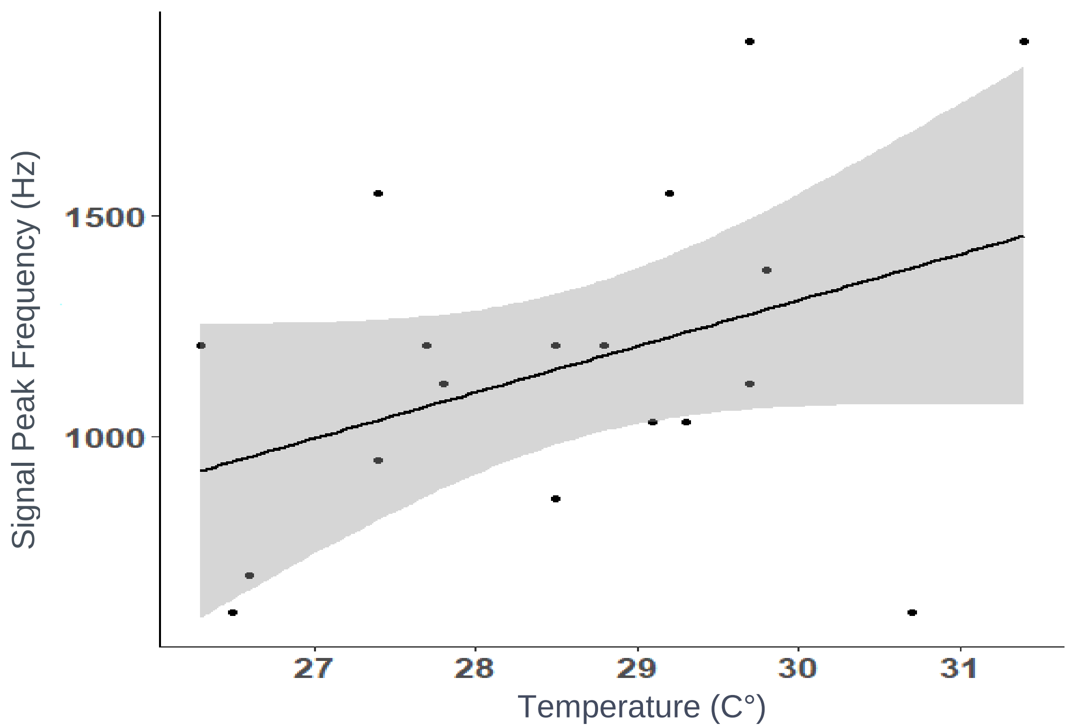

Figure 4: Linear regression that shows that there is a trend of increasing pitch (frequency in Hz) of the male signal as temperature increases (from 74 to 90 ºF). The gray bar shows the 95% CI around the regression line. Assay was conducted with summerforms.

Playback tests of signal and duetting. Our practical aim for this project is to show that a mimic of either the female signal or the male signal, transmitted through pear trees under field conditions, disrupts the mate-seeking behavior of males. While we have detected and described the male signal, we now need a description of the female signal. Eben et al. (2014) showed that females of the European pear psyllid rarely signaled spontaneously but required the male signal to induce her acoustic reply. We assayed recordings from live summerform males as well as the synthetic mimics of the male signal. Signals were sent through pear stems using a Linear Resonance Actuator (the same technology that is used to make your cellphone vibrate) connected to a computer, with the other end attached to a plant hosting one or more female psylla. Our assays showed that female summerforms responded to both the live male signal and to male recordings by sending out vibrational pulses (Fig. 5). Females waited for males to signal and then responded with their own song. The female-song is less complex than the male song and consists only of a series of pulses (highlighted in Figure 5). ***Innovation Fund.*** We applied for and obtained a USDA-Innovation Fund grant ($21,000). Funds are being used to purchase equipment (speakers, minishakers, laptop computer) for cage- and field-based trials of mating disruption through application of acoustic signals.

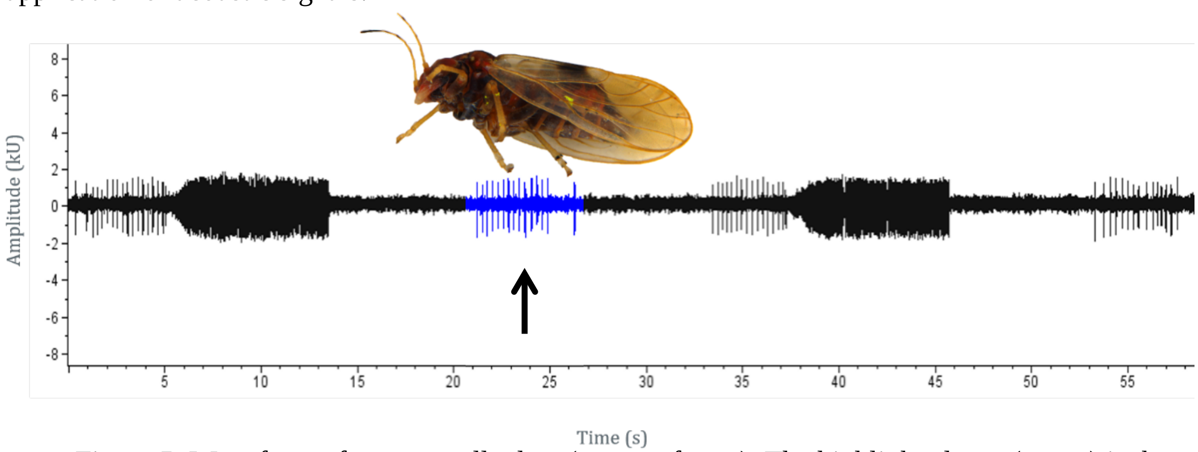

Figure 5: Waveform of a pear psylla duet (summerforms). The highlighted part (arrow) is the female response to the male signal that preceded it.

Year 4. ***Cage trial to examine prospects for disruption.*** Use of acoustic disruption of mating to manage plant-feeding pests has yet to be shown for any insect except as demonstration plots in vineyards, for leafhopper pests (Polajnar et al. 2016). Efforts to disrupt psyllid mating are limited to a cage study with citrus psyllid (Lujo et al. 2016). Our first look at the potential viability of this strategy for psylla control was done in a greenhouse using bug dorms, each containing a potted pear whip (Fig.6C). Twelve playback devices (LRAs; linear resonance actuators) were constructed for the cage study; raspberry pi (small computers) were programmed to do playbacks 24/7 in the greenhouse (Fig. 6A and 6B). Equipment was purchased using Innovation Fund dollars. We had 4 cages per treatment and 3 treatments: male signal playback, white noise playback, and no playback (control). Twenty female winterforms were added to each cage and allowed to settle on the whips. After 48 hours, 10 male winterforms were added to each cage, and the playback devices were activated. After 4 weeks, all adults were removed. Eggs, nymphs, and any new adults were counted from five randomly selected and fully-leaved branchlets per plant, providing estimates of reproduction. Results showed that plants exposed to either the male song playback or the white noise playback had fewer first generation psylla per 5 branchlets than control (no playback) plants (Fig. 7). The male signal and white noise playbacks did not differ significantly, suggesting that even a constant but random background acoustics signal interfered with production of next generation offspring. The results suggest that the playbacks led to delays in mating and thus delays in onset of egg-laying.

Where to next? Funding to support Dowen through 2021 was obtained in December 2020 through a grant submitted to the Washington State Commission on Pesticide Registration (funded for $24,768). Dowen also submitted a pre-doctoral fellowship proposal to the USDA-NIFA (decision pending). We hope to use those dollars to conduct a field test of the disruption concept under an orchard situation.

Ideally, tests will be done in late winter/early spring at a high-density pear orchard under a wire trellis system. Electromagnetic minishakers attached to trellis wires will be used to disseminate the male acoustic signal and a white noise signal to trees. A laptop computer will control the minishakers and signal production (all equipment to be purchased using dollars from the Innovation Fund grant). We will collect winterforms from target trees (those receiving the signal mimics) and control trees located a few rows away. Females will be dissected to determine mating status.

Figure 6. (A) raspberry pi computer for playing back signals. (B) audio interface with a playback device connected to the back. The LRA tip (blue tape) is attacked to the stem of the plant to mimic vibrational communication. (C) Bugdorms set up with playback devices attached to the plants.

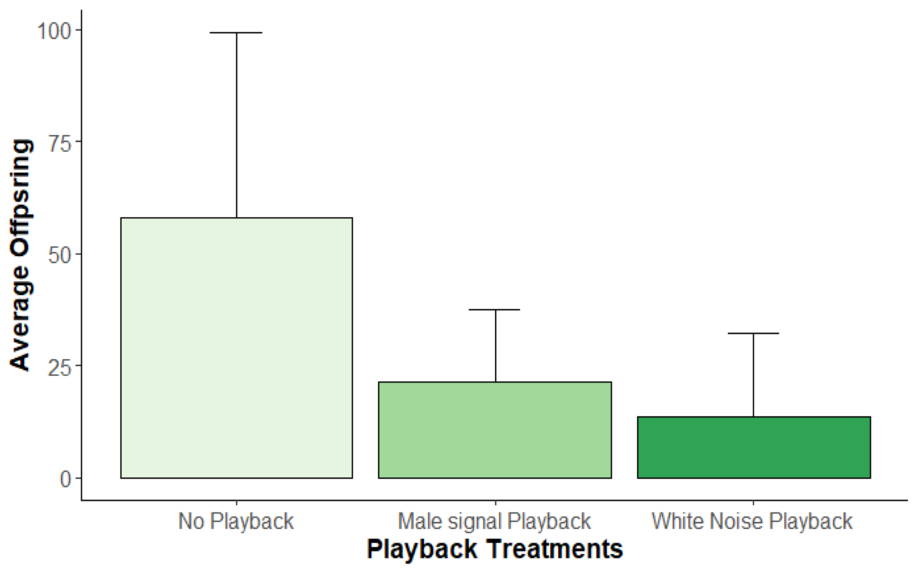

Figure 7: Average number of winterform-produced offspring per 5 branchlets from plants that received no playback, male signal playback, and white noise playback. This shows that the playback treatments were effective in reducing the number of psylla offspring (egg, nymphs, and new adults) compared to plants that did not receive any vibrational playback.

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EXECUTIVE SUMMARY

Project Title: Acoustically based mating disruption of winterform psylla

Key words: pear psylla, pear IPM, mating disruption, psyllid biology

Abstract:
Mate-searching behavior by psyllids includes use of vibratory cues sent through the plant surface as a male-female duet. These signals have been described for over 40 species of psyllids including one species of pear psyllid (*Cacopsylla pyri*), a close relative to the highly damaging species found in North America (*Cacopsylla pyricola*). We confirmed through a series of assays that our pear psylla also engages in a vibrational duet. The male signal is a series of 15-25 "pulses" lasting about 10 seconds, followed by a longer phrase of more tightly packed syllables. Duration of an individual call was about 30 seconds, with consecutive calls separated by 10 to 15 seconds. The signal is superficially similar to that shown by *C. pyri*. Pitch increases with temperature. Males are more likely to initiate signaling if they are pre-exposed to females. Females respond only following detection of the male signal. The female signal is less complex than that of the male, consisting only of a series of pulses.

Practical goals of this work include developing methods to saturate pear orchards with the male or female signal as a way to delay mating of winterform psylla in late winter as they emerge from wintering in an unmated condition. A replicated cage study with virgin winterforms was conducted showing that production of first generation offspring was about 60% lower on potted trees exposed to synthetic versions of the male signal or to random vibration ("white noise") compared to that on control (no signals applied) trees. The results, while highly preliminary, are consistent with the idea that either source of vibration delayed or interfered with successful mating of post-diapause females.

Future objectives include a field trial in a high-density, trellised orchard, in which signals are to be dispersed through trellis wires. These trials are to be supported by funding obtained elsewhere. We used data obtained in this industry-supported project to leverage funding from the USDA Innovation Fund program and from the Washington State Commission on Pesticide Registration.

Presentations (all by Dowen Jocson):

- Field Day August 2019 in Wenatchee, WA (Invited Speaker)
- Pear Day January 2020 in Wenatchee, WA (Invited Speaker)
- Entomological Society of America 2019 10-minute paper presentation, St. Louis, MO
- 3-minute Thesis presentation competition for the College of Agricultural, Human and Natural Resource Sciences (1st place)
- 3-minute Thesis presentation competition for Washington State University (4th place)

Interview with Dowen Jocson:

- Good Fruit Grower: March 1, 2020 issue
    - https://www.goodfruit.com/portfolio-items/march-01-2020/?portfolioCats=983
- ARCS Fellowship Speaker for 2020 Luncheon
    - https://www.youtube.com/watch?v=zCHwh5g-LSU&feature=youtu.be