Project Title: Incorporating *Trechnites* into a psylla biocontrol program

Report Type: Final Project Report

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Cooperators: Steve Castagnoli/Christopher Adams (OSU-MCAREC)

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 39,839
Total Project Request for Year 2 Funding: $ 39,542
Total Project Request for Year 3 Funding: $ 39,769

Other related/associated funding sources: Awarded
Funding Duration: 2021 - 2023
Amount: $245,974
Agency Name: WSDA SCBG
Notes: This grant was submitted using Year 1 data from this project as preliminary data.

Funding Duration: 2022
Amount: $20,596
Agency Name: WSCPR
Notes: This grant was submitted using Year 3 data from this project as preliminary data.

Funding Duration: 2023
Amount: $14,980
Agency Name: WSCPR
Notes: This grant was submitted using Year 3 data from this project as preliminary data.

Funding Duration: 2023-2024
Amount: $224,688
Agency Name: USDA NIFA Postdoctoral Fellowship
Notes: This grant was submitted using data generated from samples collected as part of this project.

Budget 1
Organization Name: USDA-ARS **Contract Administrator:** Mara Guttman
Telephone: 510-559-5619 **Email address:** mara.guttman@usda.gov

Item	2019	2020	2021
Salaries[1]	$17,404[2,3,4]	$17,839[2,3,4]	$18,286[2,3,4]
Benefits	$4,529[2,3,4]	$4,642[2,3,4]	$4,759[2,3,4]
Wages			
Benefits			
Equipment			
Supplies[5]	$8,500	$7,500	$7,000
Travel[6]	$500	$500	$500
Miscellaneous			
Plot Fees			
Total	**$30,933**	$30,481	$30,545

Footnotes:
[1]All salaries include 2.5% COLA increase per year
[2]8 weeks ($23.56/hr) for PCR technician at 32% benefits (Cooper)
[3]~6 weeks for trap collection/psylla dissection technician at 32% benefits (Horton)
[4]Summer technician (GS-3) to work 40 h/wk×12 wk×$12.74/hr assisting all other technicians with the project at 15% benefits rate (Schmidt-Jeffris)
[5]Funds to purchase PCR reagents and other PCR supplies, trapping supplies, pesticide non-target effects bioassay supplies
[6] Travel to commute to orchards and scout for native psyllid host plants

Budget 2
Organization Name: OSU-ARF **Contract Administrator:** Russ Karow
Telephone: (541) 737-4066 **Email address:**
Russell.Karow@oregonstate.edu

Item	2019	2020	2020
Salaries[1]	$2,510[2,3]	$2,572[2,3]	$2,638[2,3]
Benefits	$2,046[2,3]	2,096[2,3]	$2,150[2,3]
Wages			
Benefits			
Equipment[3]			
Supplies			
Travel[4]	$200	$200	$200
Miscellaneous			
Plot Fees			
Total	**$4,756**	$4,868	$4,988

Footnotes:
[1]All salaries include 2.5% COLA increase per yea
[2]Technician at OSU-SOREC ($15.68/hr*80hr) at 81.5% benefits
[3]Technician at OSU-MCAREC ($15.68/hr*80hr) at 81.5% benefits
[4]Travel to commute to orchards and scout for native psyllid host plants

Budget 3

Organization Name: WSU **Contract Administrator:** Katy Roberts/Kim Rains

Telephone: 509-335-2885/509-293-8803 **Email address:** arcgrants@wsu.edu/kim.rains@wsu.edu

Item	2019	2020	2021
Salaries[1]	$1,560[2]	$1,599[2]	$1,639[2]
Benefits[3]	$145	$149	$152
Wages			
Benefits			
Equipment			
Supplies			
Travel[4]	$2,445	$2,445	$2,445
Miscellaneous			
Plot Fees			
Total	**$4,150**	$4,193	$4,236

Footnotes:

[1]Salary includes 2.5% COLA increase per year

[2]Summer technician at $15/hr×8 hr/wk ×13 wks

[3]Benefits: 9.3%

[4]Travel: 50% use of motor pool vehicle for 26 wks ($1,057) and 50 mi/wk with pro-rated total fuel cost=$1,388

OBJECTIVES: Goals, Year 3 activities, and expected results

1. Improve methods for monitoring adult *Trechnites* and for estimating percent parasitism.
In Year 3, we completed assays to compare methods for monitoring *Trechnites* and for estimating parasitism rates. Percent parasitism was estimated using only PCR of pear psylla nymphs, which we have determined to be the most efficient method. A USDA-ARS Post-doc was hired for model development and further testing and will continue for the next ~2 years.

Expected Results. Preliminary results from trap catch, dissections/emergence, and PCR have been summarized. Full model and building of the grower tool will continue in spring & summer 2023. The most efficient method for trapping *Trechnites* and which trap best reflects percent parasitism was completed at conclusion of Year 3 and a peer reviewed manuscript is currently in progress.

2. Define the relationship between counts of adult *Trechnites* and parasitism of psylla nymphs
We will continue processing data to define this relationship. We need to define the relation within time as well to account for rising and possible falling parasitism rates that fluctuate with the life cycle of both *Trechnites* and pear psylla.

Expected results. Using machine learning we have developed a model that can accurately predict parasitism rates within a low margin of error. Results from objectives 1-2 will be combined for two peer-reviewed publications, an extension publication, and an update of the *Trechnites* section in Orchard Pest Management (http://treefruit.wsu.edu/crop-protection/opm/, OPM).

3. Screen additional IPM and organic chemicals for effects on parasite survival and life history.
Experiments to test non-target effects of pesticides on *Trechnites* require a reliable source of *Trechnites* adults and psylla mummies (immature stages of *Trechnites* still in psylla nymphs). Rearing *Trechnites* has proven to be challenging in part because of inconsistencies in the availability of colony-reared early instar pear psylla. An alternative to rearing is collection of mummies directly from the field. We found adequate numbers of mummies could be collected in cardboard bands wrapped around pear tree branches. The cardboard bands are placed in trees in autumn when the parasitized psylla nymphs search for overwintering shelters, and a retrieved in mid-winter. Cardboard bands were placed in trees in winter of 2021 & and we completed pesticide bioassays of *Trechnites spp*. adults in the spring of 2022. Assays on mummies will be conducted in Spring 2023.

Expected results. Summary of pesticide non-target effects will be updated annually, with differences in adult mortality, percent emergence from mummies, percent parasitism, and movement pattern differences between a pesticide and water check as the main results.

4. Examine native psyllids from multiple locations for *Trechnites*.

We concluded examining native psyllid species for parasitism by *Trechnites* through the final year of this project. We have found *Trechnites insidiosus* attacking native, non-pest *Cacopsylla* spp occurring on willows. We have also identified another *Trechnites* species, *T. sadkai*, in the Tieton area near bitterbrush, but it is unclear what hosts these wasps were using. We placed overwintering bands in pear blocks in Tieton, but all emerging wasps were *T. insidiosus*. Fresh and Processed Pear Committee funds were used to leverage additional funds from WSDA to expand this work to include a larger geographical area.

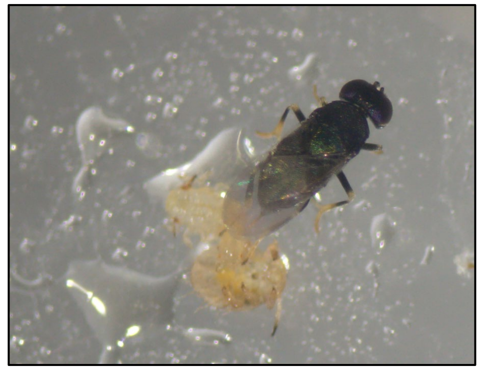
Expected results. Year 1-2 results indicate that *Trechnites insidiosus* does parasitize native psyllids. The new grant funding from the WSDA will allow us to better determine if *Trechnites* regularly parasitizes native psyllids. If so, planting native plants that host these psyllids near pears may improve biological control of pear psylla.

SIGNIFICANT FINDINGS

- 3D-printed tube traps and screened sticky cards continue to be successful at capturing adult *T. insidiosus*

- PCR was determined to be the most effective way of assessing parasitism levels

- Overwintering bands can be effective at obtaining large numbers of *T. insidiosus* for bioassay work and at assessing hyperparasitism levels. We learned in 2022 timing of band placement greatly affects the number of psylla mummies obtained.



Trechnites ovipositing into a pear psylla nymph.

- We produced a model that accurately predicts parasitism rates to within 7.5% for 95% of the observations in the field. The model was trained on a portion of the data collected for Objectives 1 and 2 and tested against the remaining data. Surprisingly, the location of the data was often of least importance to producing accurate results. We believe this model will be generalizable to much of the pear growing region in the PNW.

- 48 *Trechnites sadkai* were found from June to October in beat tray samples from bitterbrush (*Purshia*) located near Tieton, WA. This parasitoid was potentially attacking psyllids that occur on this plant. Tube traps placed near stands of bitterbrush captured both *T. sadkai* and *T. insidiosus*. Several other parasitoid species were collected, including *Tamarixia* spp. from psyllids occurring on bitterbush.

METHODS

1. Improve methods for monitoring adult *Trechnites* and for estimating percent parasitism.

Adult Trechnites. At each of the four locations, five plots were laid out in an orchard. Collection of all data occurred from April-late September at all locations. We discontinued this sampling in the two Oregon research orchards, as *Trechnites* populations remained low. We expanded the use of traps in Oregon but removed the random leaf/targeted nymph samples described below.

Within each plot, we placed one screened sticky card, changed/removed after one week. Work in Year 1 indicated that screened sticky cards were an effective method for monitoring *Trechnites*; these replaced the unscreened sticky cards at all locations. Beat tray samples, which were conducted in Year 1, were discontinued, as they did not adequately reflect *Trechnites* abundance. Leaf samples consisted of up to 20 leaves that are found to contain psylla nymphs, when sufficient quantities were present. An additional sample of 25 leaves was randomly collected from each plot to determine the age distribution of psylla nymphs. We also used 3D-printed cylinder traps to sample for *Trechnites*.

Percent parasitism. PCR was used to detect percent parasitism every year. In Year 1, we also dissected psylla nymphs to assess parasitism. In Year 2, we discontinued dissection and attempted to use emergence cages to monitor percent parasitism instead of dissection. Ten psylla from each plot at a location were placed inside a cage on a detached pear leaf and monitored for emergence of parasitoids. Survival was poor using this method and was discontinued.

2. Define the relationship between counts of adult Trechnites and parasitism of psylla nymphs.

The percent parasitism data allowed us to model how counts of the adult parasitoid in orchards via the three different methods (sticky cards, tray counts, traps) related to actual percent parasitism in the field, improving grower understanding of what level of control to expect when they are scouting for adult *Trechnites*. Counts from each method were compared to percent parasitism to determine if the relationship was consistent between locations and which trap type most closely predicted parasitism levels.

Model development: The postdoctoral researcher has produced a preliminary model that can accurately predict parasitism rates in WA locations. We are currently collecting weather data from our OR cooperators to finish modelling across all PNW locations. At present the model incorporates both sticky card and cylinder trap data to predict parasitism. Optimization of the model will continue in 2023 to reduce potential scouting labor for use in the grower tool.

3. Screen additional IPM and organic chemicals for effects on parasite survival and life history.

We tested 12 products (Actara, Altacor, Assail, Bexar, Centaur, Delegate, Entrust, Fujimite, Lime-Sulfur, Malathion, Neemix and Rimon) in 2022. For each pesticide tested, we examined effects on sprayed adults (% mortality) compared to a water sprayed control. Mummies have been collected to test as well and will be assayed in 2023.

We were unable to test sprayed adults for sublethal effects as removing the adults from the container led to high mortality of *Trechnites*. *Trechnites* adults can only be collected once per year from psylla mummies and 2022 mummy collection was particularly low. We will be able to test the pesticide mortality on the mummies collected in 2022 and may be able to repeat adult exposure bioassays depending on survival numbers.

4. Examine native psyllids from multiple locations for *Trechnites*

Each year, we located *Salix scouleriana*, *Salix prolixa*, and *Ribes* patches in early spring and *Salix exigua*, and *Purshia tridentata* in spring and summer. These plant taxa host native psyllids that are related to pear psylla, and thus could be sources of parasites (including *Trechnites*) that attack pear psylla. Beat tray samples were used to determine if adult psyllids were present. From these samples, psyllid mummies were isolated and the emerging parasites and psyllid host were identified. Collection occured 2-3 times per season, with the timing focused on life cycles of known psyllid species that feed on these plants.

RESULTS AND DISCUSSION

Obj. 1. We completed sampling orchards at four locations. Full analysis and tool building is in process, we discuss preliminary results under Obj 2. Fig. 1 shows comparison of trap types and psylla counts and Fig. 2 shows the comparison between trap types and *Trechnites* catch. Both 3D-printed tube traps and sticky cards collected high numbers of *T. insidiosus*. Cylinder traps and sticky cards both monitor *Trechnites* and psylla much better than beat trays. Screened sticky cards would be effective if the numbers of psylla and *Trechnites* were the only species of interest. For studies also examining larger insects (e.g. lacewings), unscreened sticky card would need to be used. Cylinder traps are better if preservation of the insect for additional research is needed. Parasitism increases with rising adult pear psylla numbers. At peak parasitism we see a decline in adult pear psylla (approx. 2 weeks post adult psylla peak). This led to a population peak of *T. insidiosus* adults captured and continued suppression of pear psylla. We can observe a linked phenology of *Trechnites* and psylla in Fig 3 and 4.

Obj. 2. In 2021, we successfully obtained funding from the WSDA to expand this work and hired a postdoc (Zilnik) with expertise in modelling. Zilnik has prepared a preliminary model to predict parasitism based on trap capture and PCR results from all three years. The model was constructed using machine learning tools. Model training was conducted with 2/3 of the data collected between 2019-2021. The remaining 1/3 of the data were used to test the accuracy of the model. Currently, the model can accurately predict parasitism rate 95% of the time within 10% of the observed value (Fig. 5). The most important variables in the model that contribute to the high accuracy were psylla degree days, psylla nymph counts, and cylinder trap psylla adult counts (Fig 6). *Trechnites* counts from sticky cards and cylinder traps also contributed substantially to model accuracy. At present, the model does not appear to gain more accuracy from location information and it therefore could be generalized to most locations in WA. We will obtain psylla degree day data from Medford, OR and complete the model. During the spring of 2023, additional testing and optimization of the model will be performed. Additional validation with grower orchard data will be conducted in 2023 in Yakima Valley, Wenatchee Valley, and Hood River as part of other ongoing projects.

Obj. 3. We were unable to rear *Trechnites* in sufficient numbers to begin this objective in Year 1. In Year 1 (Oct 2019), we placed cardboard bands in the research orchards in Moxee and Wenatchee. We determined that parasitized psylla nymphs used these bands as overwintering sites and form mummies within the bands. In Feb 2020, we assessed emergence from these bands. At the Wenatchee site, we placed 115 bands in Bartlett trees and 99 bands in Anjou trees. There were 1.1 mummies per band in Bartlett and 0.5 mummies per band in Anjou. From the 186 mummies we collected, 73% had a wasp emerge, most of which were *T. insidiosus*. Other wasps (n=5) were *Dilyta* spp., a hyperparasitoid. Nearly all emergence occurred within 13-14 days of removing the mummies from the cold. We repeated this process in 2020 but returned too few psylla mummies to complete this objective. In 2021, we adjusted our banding procedure and obtained 474 psylla mummies from the 1,200 bands placed (37.8% of bands contained at least 1 mummy). We were able to conduct the pesticide bioassays on 12 compounds in 2022. The results are summarized in Fig 7. As expected, broad spectrum compounds resulted in high mortality rates of *Trechnites* adults. Altacor and Rimon had the highest 24-hour survival rates. Only Rimon showed no difference in survivorship from the control at 48 hours. Compounds recommended to include in IPM programs such as Neemix, Centaur, Lime-Sulfur, and Spinosad had very low survivorship of *Trechnites*. *Trechnites* are very susceptible to many commonly used insecticides and care should be taken to avoid spraying these compounds when *Trechnites* adults are present in the orchard. *Trechnites* is likely more protected in the mummy stage, which will be tested in Spring 2023.

Obj. 4. In 2019, we found *Trechnites* emergence from *Cacopsylla americana* and *C. alba* collected from *Salix rigida/prolixa* and *S. exigua*. *Cacopsylla alba* occurs on catkins of the host or in galls produced by a small midge, and more occasionally on foliage; parasitized psyllids were collected from all structures, but especially from catkins and galls. These are the first records world-wide that *Trechnites* attacks willow-associated psyllids. In both years, *Trechnites* were also collected by tube traps placed near native willows and bitterbrush, demonstrated that the tube traps are also effective in native habitats outside of pear orchards. This work is the first to demonstrate that native, non-pest psyllids in North America might be reservoirs of *Trechnites*, and this opens a new avenue for implementing *Trechnites*-based biological control of pear psylla.

In 2020, we also found *T. sadkai* in beat samples and tube traps in bitterbrush in Tieton, WA, but *T. sadkai* did not emerge from psyllid mummies collected from bitterbush. Old samples from the Tieton area (2002-2003) from both bitterbrush and a neighboring soft pear orchard were consulted. While the bitterbrush samples contained *Trechnites spp.*, the pear orchard samples were only *T. insidiosus*.

In 2021, we found no *Trechnites spp.* in surveys of *Salix rigida/prolixa*. The parasitoid *Prionomitus* was collected frequently in West Yakima and Union Gap. Closer examination of the reproductive morphology of the *T. sadkai* samples revealed that previous findings were likely incorrect and instead we are observing *T. alni*. It remains unclear if *T. alni* would specialize on psyllids and thus be good biological control agent for pear. We were able to collect many psyllid mummies containing *Prionomitus spp*. however further testing is needed to determine if they will attack pear psylla.

In 2022, we collected 20+ parasites from *Cacopsylla alba* mummies spring through late summer, from locations along the Yakima River and Ahtanum Creek, where *Salix exigua* (host of *Cacopsylla alba*) is common. Sex ratio of the *Trechnites* from these mummies was slightly male-biased. This is a multivoltine psyllid, quite different from the typical univoltine life cycle of *Salix Cacopsylla*. Collections of mummies from other *Cacopsylla* (from *Ribes*), univoltine *Cacopsylla* from other *Salix*, and *Cacopsylla* relatives on *Purshia* produced only *Prionomitus*, apparently a poor natural enemy of pear psylla in North America although a better parasite for pear psylla in Europe.

Our multi-year survey indicates that *Salix exigua* is a potential reservoir of *Trechnites*. Studies are planned to confirm that *Trechnites* specimens reared from willow psyllid will attack pear psylla.

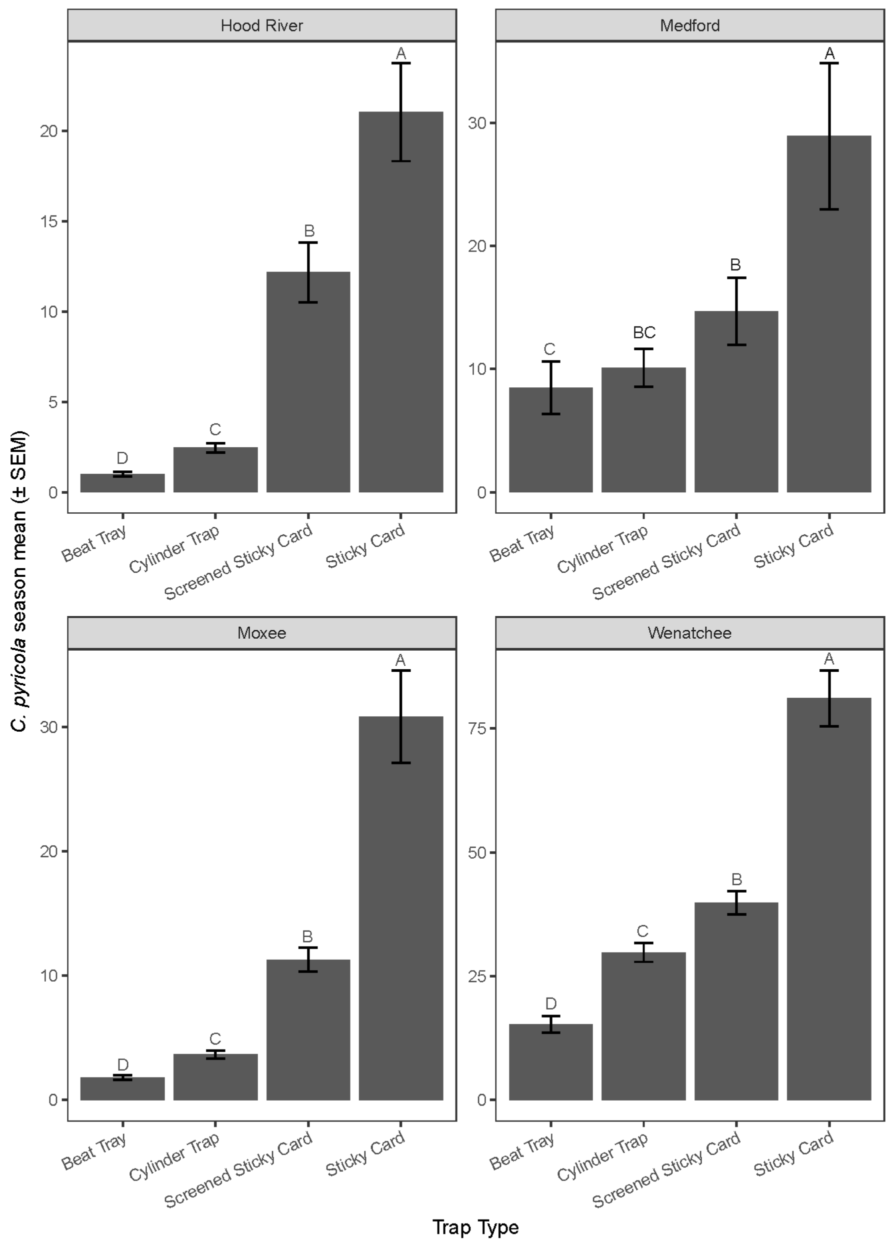


Fig 1. Seasonal mean number of *C. pyricola* by trap type in 2019 (± SEM). Letters indicate means separation between trap type. Note that y-axis varies between locations. Sticky cards (including screened sticky cards) returned higher numbers of *C. pyricola* than all other traps except in Medford, OR. All traps returned higher numbers of *C. pyricola* than the beat tray sampling method.

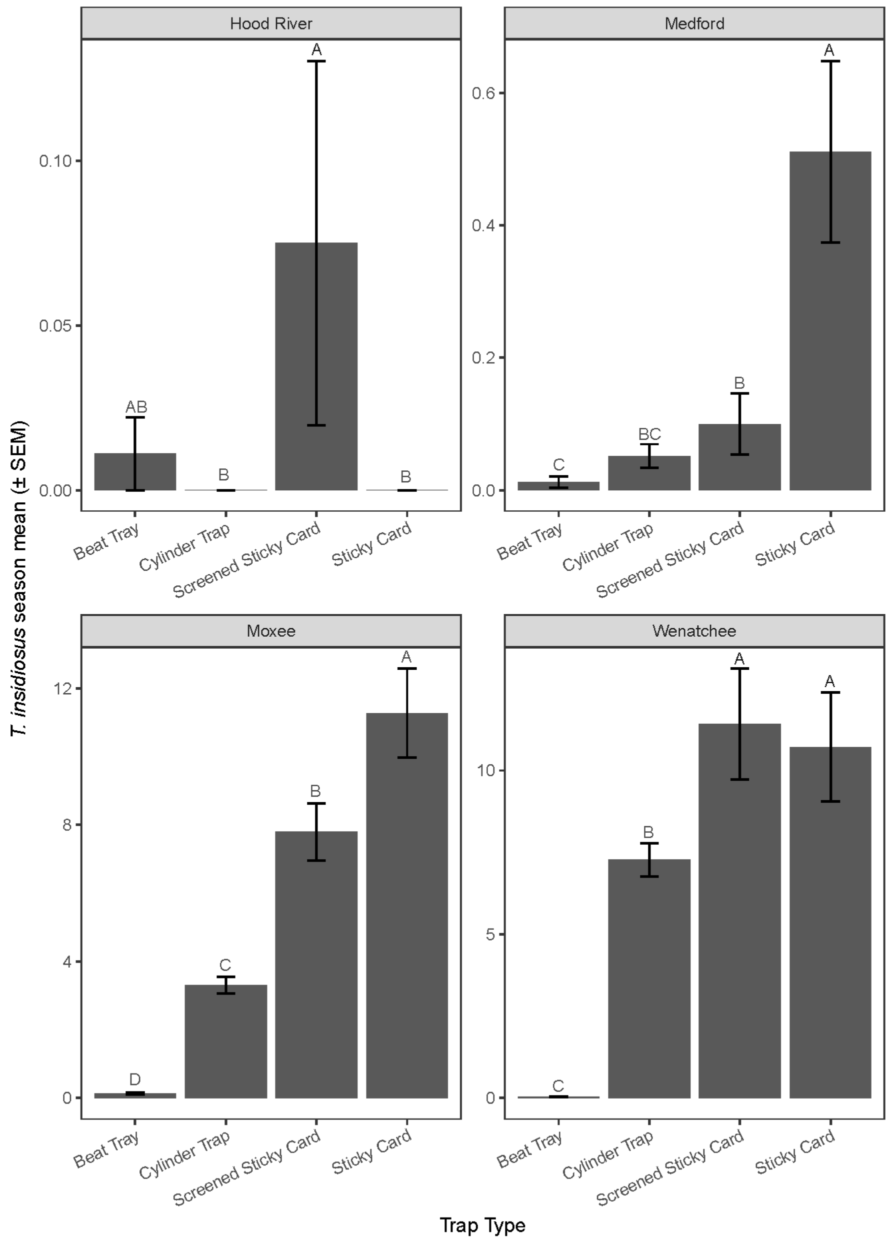


Fig 2. Seasonal mean number of *T. insidiosus* by trap type in 2019 (± SEM). Letters indicate means separation between trap type. Note that y-axis varies between locations. Moxee and Wenatchee, WA returned an order of magnitude larger number of *T. insidiosus* than the Hood River and Medford sites. Hood River, OR was the only location where beat tray sampling recorded more *T. insidiosus* than any trap type except screened sticky cards, likely due to very small numbers of wasps.

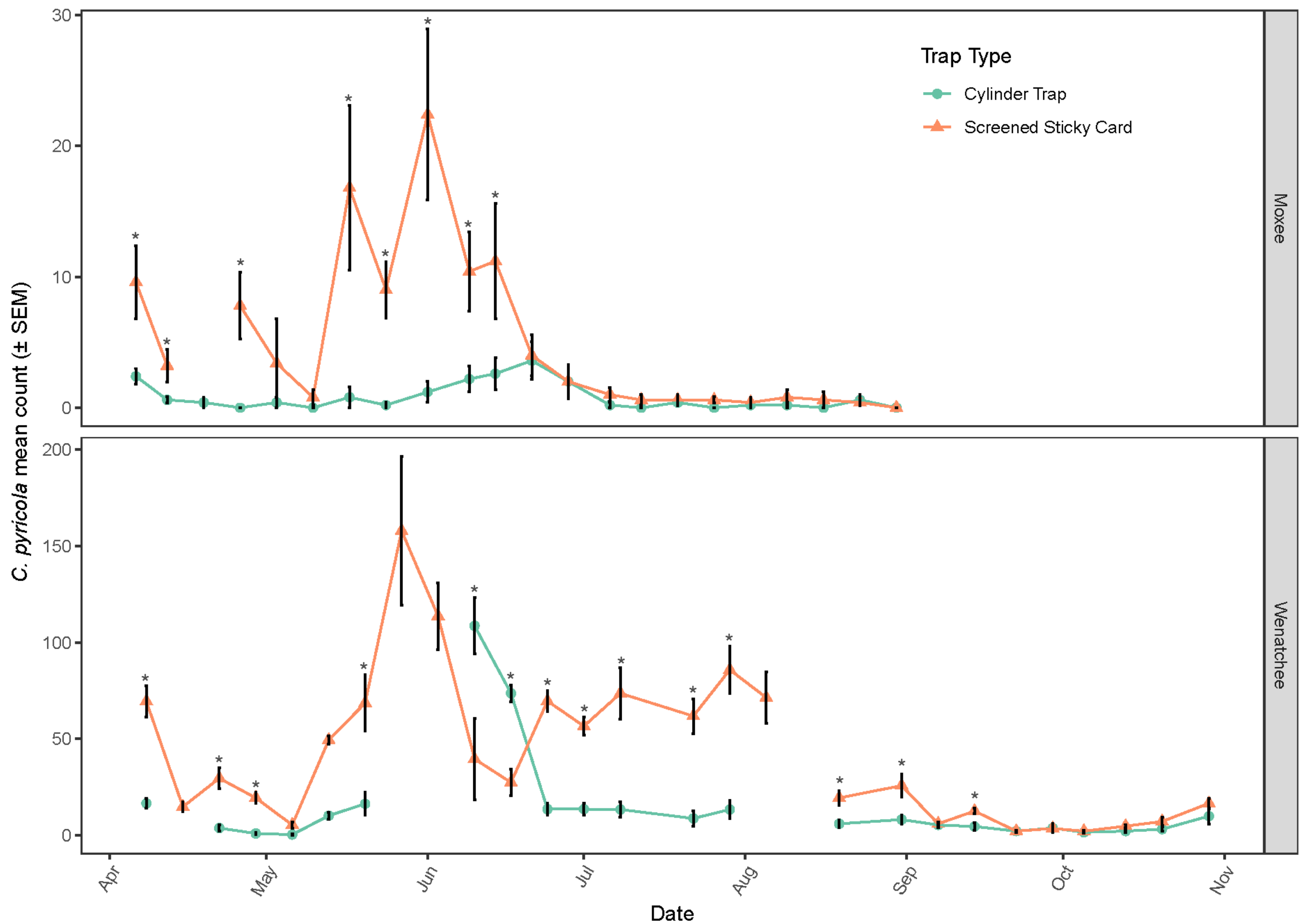


Figure 3. Mean (± SEM) weekly counts of adult *C. pyricola* in 2021. The cylinder trap nearly missed the population peaks in Moxee, though overall trap catch did qualitatively track population growth. Cylinder traps appear to track *C. pyricola* population peaks more accurately at higher population densities

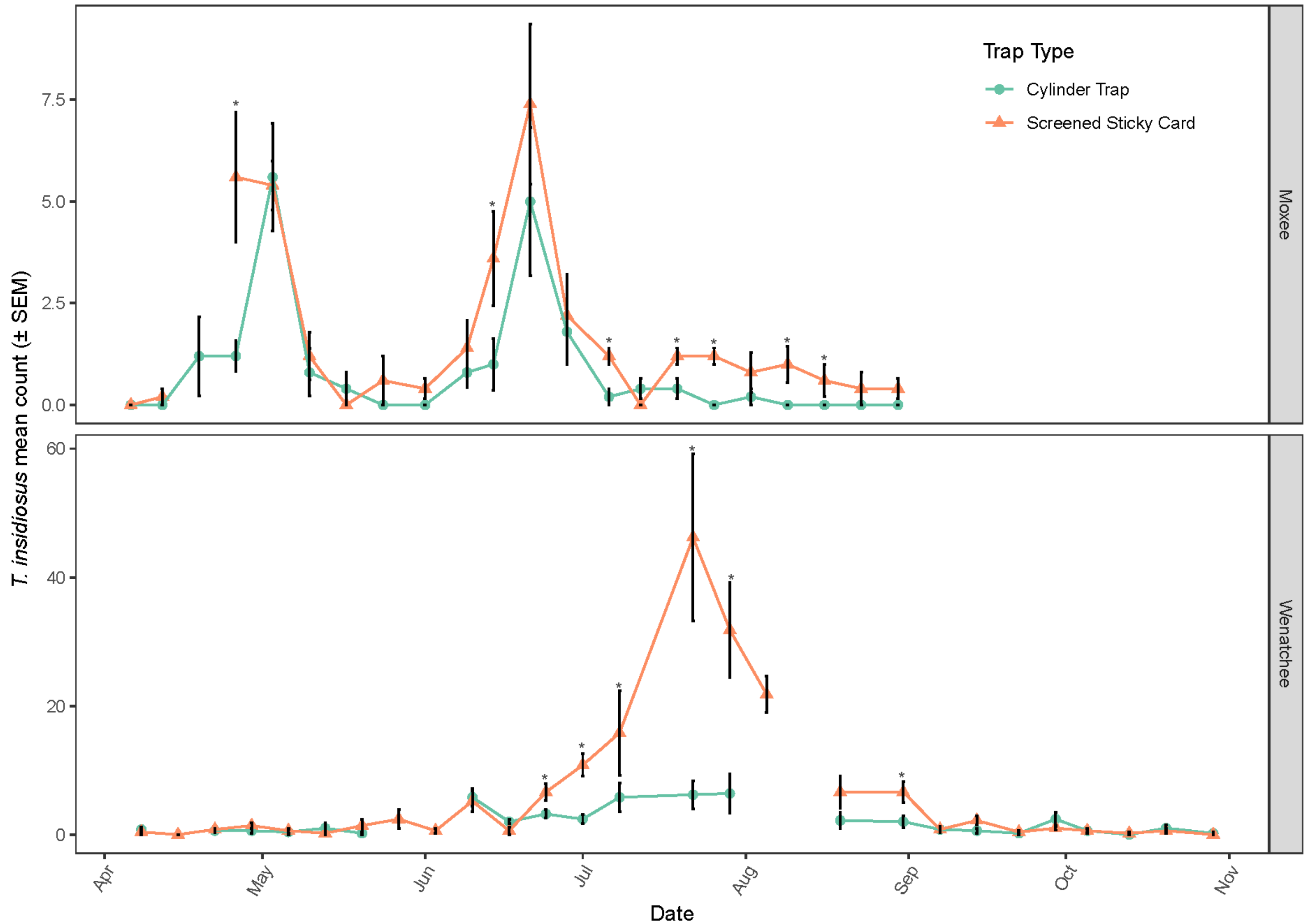


Figure 4. Mean (± SEM) weekly counts of adult *T. insidiosus* in 2021. Wenatchee had a single population peak with more total *T. insidiosus* and Moxee had two population peaks. Notice that cylinder trap and screened sticky card values almost always overlap at lower population densities. Screened sticky cards appear to track high population densities much better than cylinder traps.

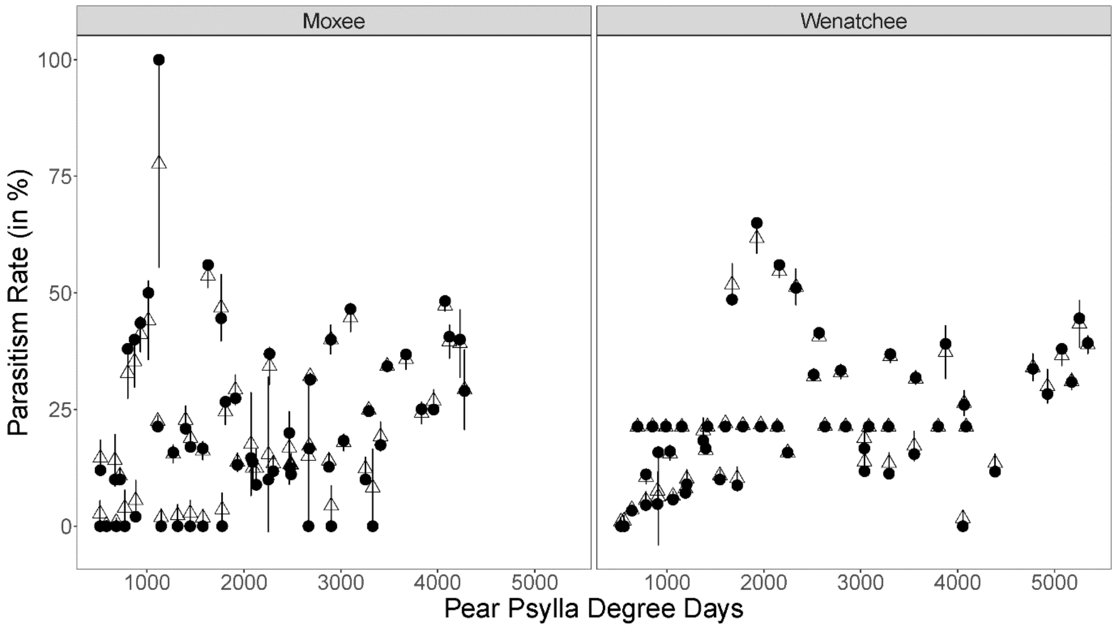


Fig 5. Observed mean parasitism rate (solid circles) vs mean predicted parasitism rate (open triangles) with prediction error. Prediction error generally increased at extreme ends of the parasitism rate (100% and 0%). The model generally performs well (low error) at predicting parasitism rates between 10% and 80%.

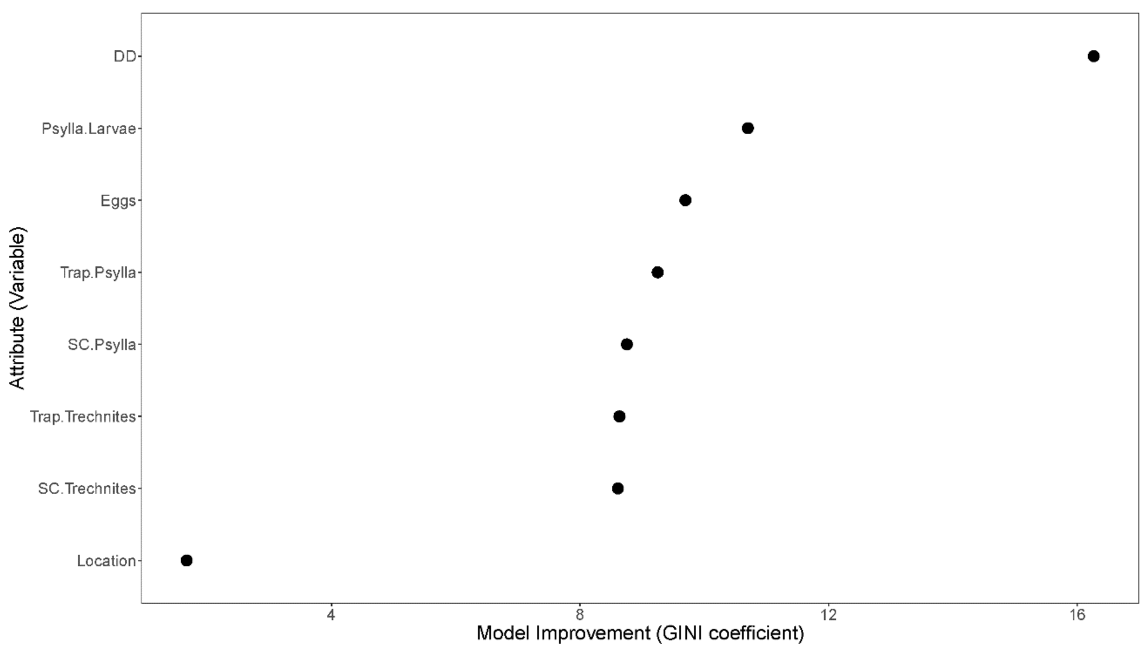


Fig 6. Plot of variable performance in improving predictive power of the parasitism rate model. Psylla degree days (DD) contributed to the most to improving model performance. Further testing will reveal which methods growers should use to monitor psylla and *Trechnites* to get an estimate of their biological control services.

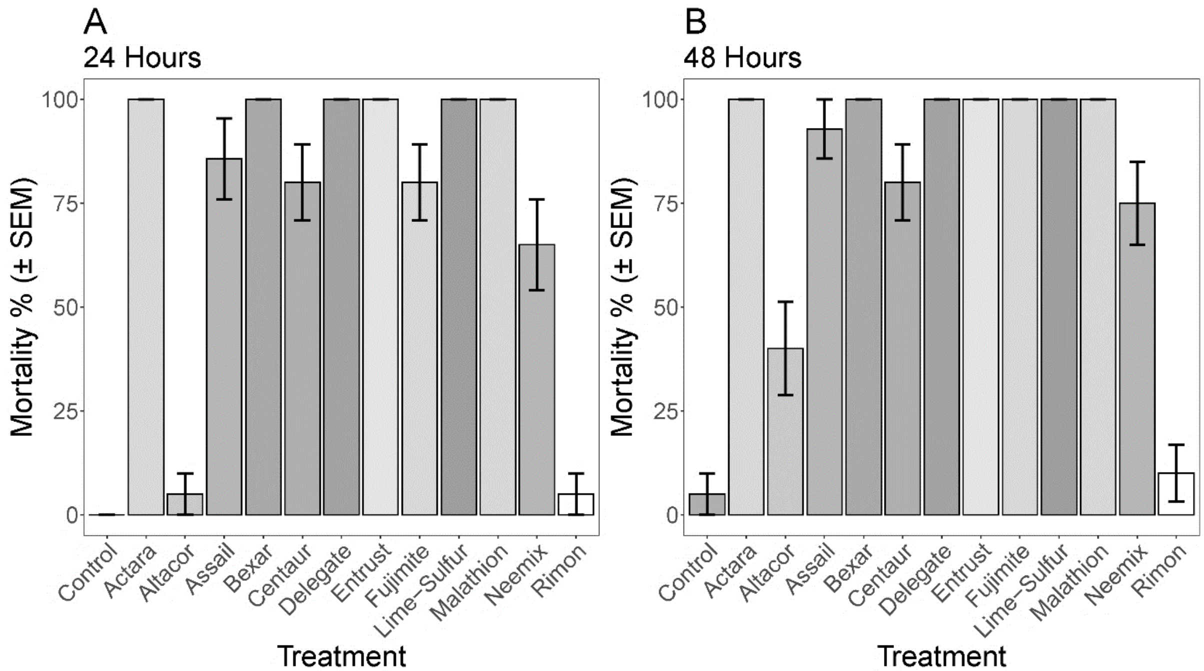


Fig 7. Percent mortality of adult *Trechnites* (± SEM) for the compounds tested at 24 hours (A) and 48 hours (B). All compounds except Altacor and Rimon had increased mortality above the control at 24 hours and only Rimon did not differ from the control mortality at 48 hours. *Trechnites* appears to be extremely susceptible to the majority of compounds used in pear for pest management.