FINAL PROJECT REPORT

Project Title: Ecology of *Trechnites*, an important parasitoid wasp of pear psylla

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Cooperators: Tianna DuPont, Louis Nottingham, Elizabeth Beers, Christopher Strohm (Washington State University); Steve Castagnoli (Oregon State University), Chris Nickelsen (Nickelsen orchards, Hood River, OR).

Budget: $ 30,623 **Year 1 (2018):** $ 30,623

Other funding sources: None

Budget 1
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: (541)7374866, (541)772-5165 **Email address:** l.j.koong@oregonstate.edu

Item	2018
Salaries[1]	$14,440
Benefits[2]	$1,444
Supplies	$4,000
Travel[4]	$1,000
Plot Fees[5]	$775
Total	$21,659

Footnotes:
[1]Salaries: 800hr for a Biological Science Tech. at $18.00/hr (20 hrs per week for 40 weeks, 0.5 FTE).
[2]Benefits: 10% of the wage.
[3]Supplies: Trapping material, containers, insect cages for colonies, insecticides for bioassays, plant enclosures
[4]Travel: Weekly travel to orchards for 40 weeks at $0.535 per mile
[5] Plot fees at MCAREC (0.25 acre) for field enclosure experiments

Budget 2

Organization Name: OSU- SOREC **Contract Administrator:** L.J. Koong

Telephone: **Email address:**

Item	**2018**
Wages	$2,240
Benefits	$224
Plot Fees	
Total	$2,464

Budget 3

Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers

Telephone: 510/559-5769 **Email address:** Chuck.Myers@ars.usda.gov

Item	**2018**
Salaries[1]	$2,500
Supplies[2]	$4,000
Total	$6,500

Footnotes:

[1]Time-slip labor for summer collecting of psyllids and parasitoids

[2]Funds to purchase PCR reagents and other PCR supplies

ORIGINAL OBJECTIVES:

1) To determine the seasonal phenology of *Trechnites insidiosus* in pear orchards and extra-orchard habitats using different monitoring techniques.
2) To determine which floral resources are used by *Trechnites insidiosus* both inside and outside of orchards, and to document use of other psyllid species as alternative hosts.
3) To determine the parasitism capacity of *Trechnites insidiosus* in field enclosures
4) To determine the lethal and sub-lethal effects of common insecticides on larvae and adults of *Trechnites insidiosus*.

SIGNIFICANT FINDINGS

1) *Trechnites* overwinters as larvae in mummified psylla nymphs, and emerge as adults in a single peak during spring (Mid May – Mid June, depending on location). Their population drops during summer, presumably due to low host availability.
2) It was not possible to extract plant DNA fron *Trechnites* to determine floral hosts. Preliminary reports show that *Trechnites* does not parasitize other psyllid species, such as willow psylla.
3) Laboratory experiments showed that, when exposed to *Trechnites,* survival to adulthood of psylla nymphs is reduced by 51%. Furthermore, exposure to *Trechnites* causes psylla nymphs to disperse, potentially contributing to additional mortality without parasitism.
4) Mortality rates in adult *Trechnites* sprayed with Abamectin, Esteem, and Ultor were 82%, 53%, and 26% respectively. Esteem, an insect growth regulator, was surprisingly toxic to adult wasps, while Ultor was the most benign. In larvae (mummified nymphs), mortality with Abamectin, Esteem, and Ultor, was no different than the water control. In all cases the percent unemerged wasps was high, ranging from 87% to 92%.
5) We found a hyperparasitoid complex associated with *Trechnites* and pear psylla, which can potentially affect populations of *Trechnites* in pear orchards. Two different species of parasitoid wasps (and possibly more) also emerged from parasitized nymphs: *Dilyta rathmanae* and *Pachyneuron* sp.

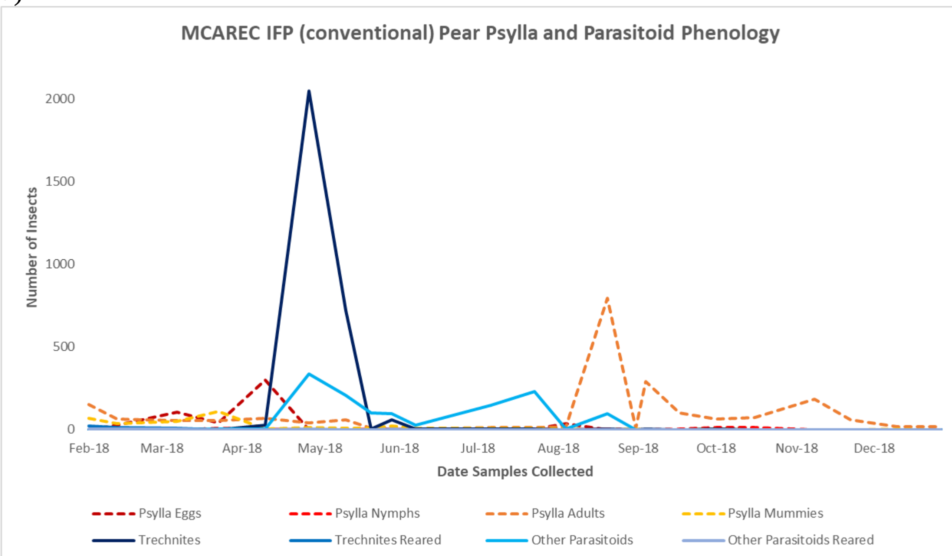
RESULTS AND DISCUSSION

Objective 1: Seasonal phenology of *Trechnites insidiosus*

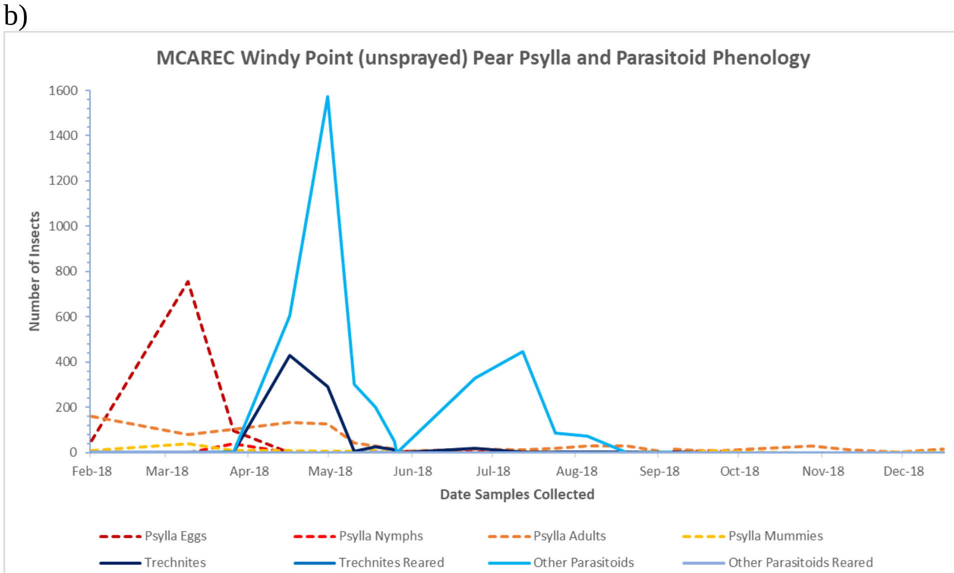
Trechnites overwinters as larvae in psylla mummies in orchards, and adult wasps appear in pear orchards in a single “explosive” peak. In Hood River, most adult *Trechnites* emerged in early May (around 650DD; Fig. 1a, 1b), while in Southern Oregon, *Trechnites* emergence occurred much later (mid-June, around 1700DD; Fig 1c, 1d). In Wapato WA, *Trechnites* emerged at the end of May (around 1500 DD; Fig. 1e). There was very low psylla pressure during the summer, and it is possible that this early abundance of *Trechnites* contributed to keep psylla populations low. The population of *Trechnites* also remained low throughout the season, suggesting that they might be dispersing to other environments, potentially seeking other hosts, or that their population crashed as it might be tightly associated with pear psylla abundance. Number of overwintering mummies collected was low relative to *Trechnites* wasp emergence. This suggests two things, either that overwintering mummies hide on parts of the tree bark where they are difficult to collect, or that additional *Trechnites* adults are migrating into the orchards from other nearby hosts. Overwintering and early spring ecology of *Trechnites* should be further investigated.

Figure 1. Phenology of *Trechnites insidiosus* and *Cacopsylla pyricola* in pear orchards in Hood River OR (a) conventional, (b) unsprayed), Medford OR (c) conventional, d) unsprayed), and Wapato WA (e) unsprayed).

a)



b)



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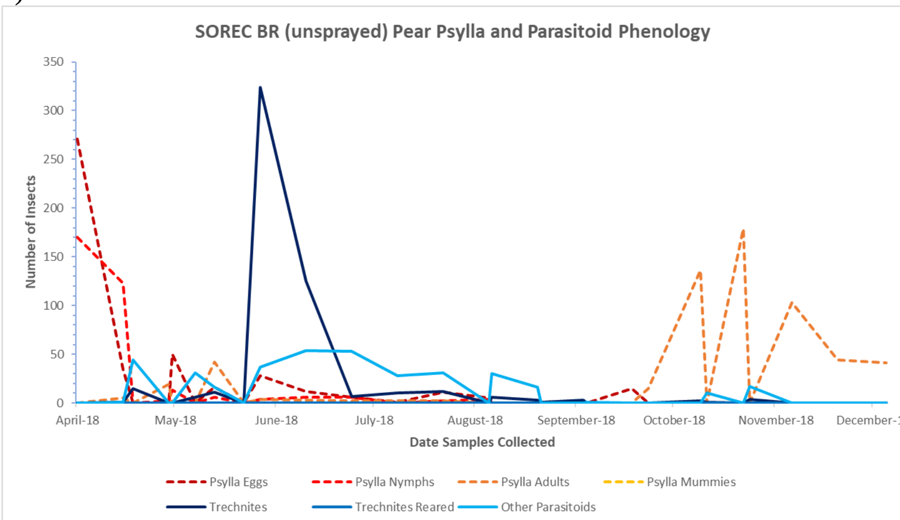
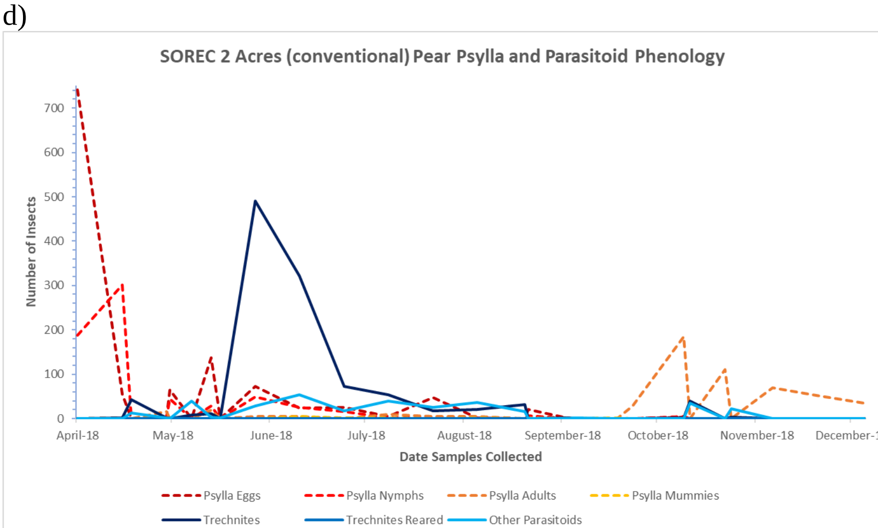


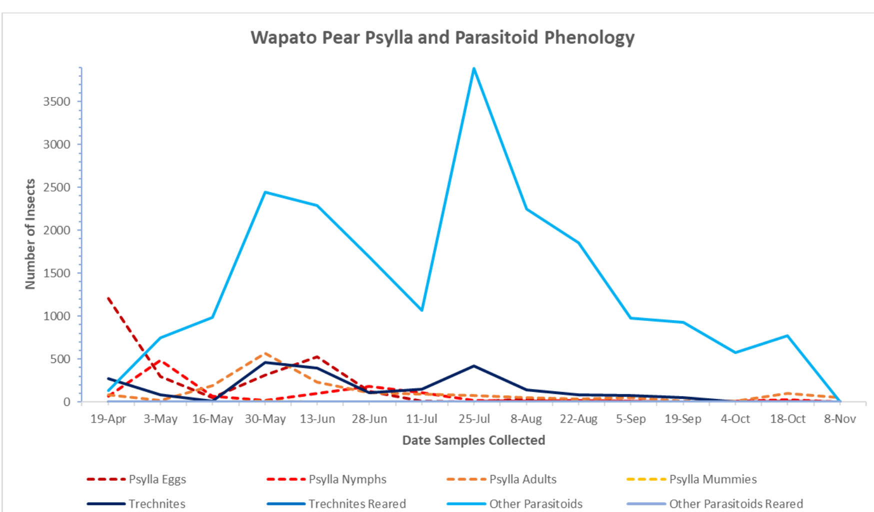
SOREC BR (unsprayed) Pear Psylla and Parasitoid Phenology
Number of Insects
350
300
250
200
150
100
50
0
April-18
May-18
June-18
July-18
August-18
September-18
October-18
November-18
December-1
Date Samples Collected
Psylla Eggs
Psylla Nymphs
Psylla Adults
Psylla Mummies
Trechnites
Trechnites Reared
Other Parasitoids

d)



SOREC 2 Acres (conventional) Pear Psylla and Parasitoid Phenology
Number of Insects
700
600
500
400
300
200
100
0
April-18
May-18
June-18
July-18
August-18
September-18
October-18
November-18
December-1
Date Samples Collected
Psylla Eggs
Psylla Nymphs
Psylla Adults
Psylla Mummies
Trechnites
Trechnites Reared
Other Parasitoids

e)



Wapato Pear Psylla and Parasitoid Phenology
Number of Insects
3500
3000
2500
2000
1500
1000
500
0
19-Apr
3-May
16-May
30-May
13-Jun
28-Jun
11-Jul
25-Jul
8-Aug
22-Aug
5-Sep
19-Sep
4-Oct
18-Oct
8-Nov
Date Samples Collected
Psylla Eggs
Psylla Nymphs
Psylla Adults
Psylla Mummies
Trechnites
Trechnites Reared
Other Parasitoids
Other Parasitoids Reared

Objective 2: Floral resources and psyllid hosts.

We attempted to extract pollen from *Trechnites* wasps to determine which floral resources they use, as it has been done in other insects (i.e., psylla, bees). Due to the diminute size of *Trechnites*, it was not possible to wash pollen from their bodies, therefore we could not amplify plant DNA. Future studies should investigate alternative methods to determine which in-bloom plant species does *Trechnites* visit and feed from.

Other species of psyllid hosts, such as willow psyllid, were examined for *Trechnites* parasitism. Preliminary results show that there is no overlap among the wasp species that parasitize pear psylla and willow psylla, but this work is still in progress.

Objective 3: Parasitism capacity of *Trechnites*

Maintaining *Trechnites* wasps in the laboratory was very challenging, as they are very fragile and experience high mortality when manipulated. We were not able to rear a colony of *Trechnites* or psylla, therefore all our parasitism trials were done with field-collected wasps and psylla nymphs. It was not possible to assess whether field-collected nymphs were already parasitized, so we ran a baseline assay without wasp exposure to find out roughly what proportion of nymphs were already parasitized. To reduce unsuitable conditions, we performed this experiment in the laboratory rather than the field.

Five nymphs were placed in a clear cylindrical container with a fresh pear leaf (Fig. 2), and allowed to develop until adulthood. We tested two treatments, one with no wasp (n = 64), and one where one *Trechnites* wasp was introduced into the container for 72 h (n = 37). Without a wasp, 35% of the nymphs reached adulthood, while only 18% reached adulthood when exposed to one *Trechnites* wasp ($X^2 = 8.12$, df =1, p =< 0.01; Fig. 3). We also observed that nymphs sometimes managed to crawl out of the container through the bottom. In treatments without wasps, 4% of the nymphs were recorded as missing, while 14% of the nymphs went missing in treatments with a *Trechnites* wasp ($X^2 = 4.04$, df =1, p =< 0.04; Fig. 3). This suggests that *Trechnites* exerts non-consumptive effects on psylla nymphs. This phenomenon happens when predators modify the behavior of a prey without consuming it; in this case, psylla nymphs were more likely to disperse when a wasp was present. This shows that the presence of *Trechnites* can affect psylla nymphs even without parasitism, as dispersing nymphs can likely die when moving to different environments. Another option is behavior modification through parasitism, and it is possible that parasitized nymphs seek refuge away from the leaves to protect the developing wasp larva.

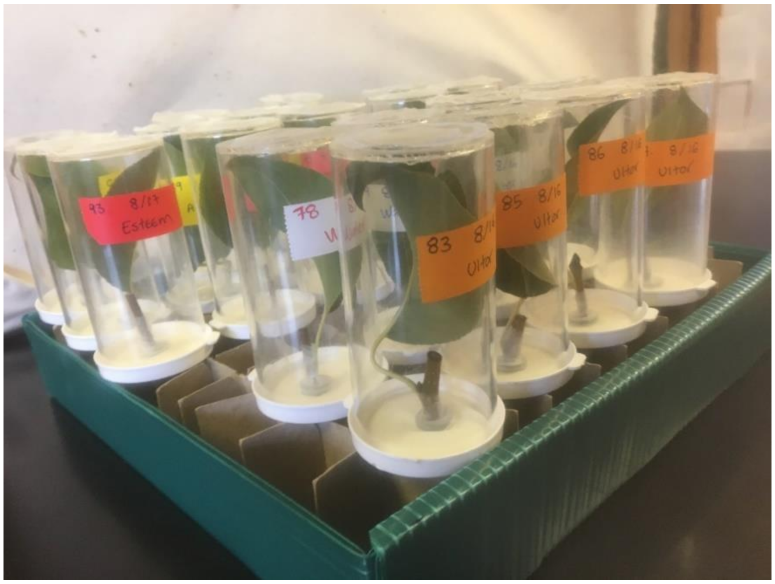


Figure 2. Wasp enclosures to assess parasitism and mortality.

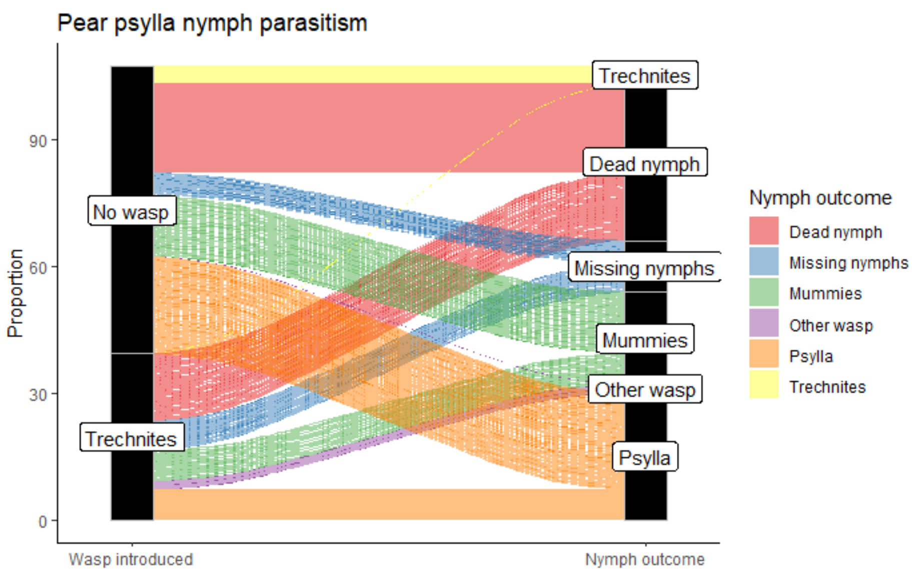


Figure 3. Survival outcome proportions from five psylla nymphs in a leaf container with or without one *Trechnites* wasp.

Objective 4: Insecticide effects on *Trechnites* adults and larvae

Using a potter spray tower at 6 PSI, we sprayed adult *Trechnites* wasps and larvae (developing inside psylla mummies) with 2 mL (0.06 fl oz) of three insecticides at the maximum rate registered for pear psylla: Abamectin 0.15LV (20 fl oz / acre), Esteem 0.86 EC (pyriproxyfen, 16 oz / acre), and Ultor (spirotetramat, 14 fl oz / acre).

A single adult wasp was sprayed in an arena (Fig. 4). After exposure, we kept the wasp for 1 h in the spray arena, and then we transferred the wasp to a leaf container with water and honey. Mortality was recorded after 48h. When sprayed with Abamectin (n = 34), Esteem (n = 32), and Ultor (n = 30), mortality rates in adult wasps were of 82%, 53%, and 26% respectively (Fig. 5). Wasps sprayed with a water control (n = 32) resulted in 15% mortality, with was similar to mortality in Ultor ($X^2 = 0.57$, df =1, p = 0.45), but lower than mortality with Esteem ($X^2 = 8.38$, df =1, p < 0.01), and Abamectin ($X^2 = 15.12$, df =1, p < 0.01). The results with Esteem were surprising, as this is an insect growth regulator targeting juvenile stages and is not supposed to affect adult insects, but it caused greater than expected mortality in adult wasps. Overall, Ultor was the most benign insecticide for adult wasps, and Abamectin was the most detrimental.

We also recorded the number of wasps emerging from a group of ten mummies attached to a filter paper. After being sprayed with Abamectin (n = 28), Esteem (n = 29), and Ultor (n = 28), the percentage of unemerged (dead) wasps from psylla mummies was 87%, 92%, and 88%, respectively (Fig. 6). This mortality was high, but not different from unemerged wasps after being sprayed with a water control (87%, n = 30; $X^2 = 1.65$, df =3, p = 0.64). This could be interpreted as a positive result, indicating that wasp larvae developing inside psylla mummies are protected from detrimental effects of insecticide applications. Unfortunately, wasp emergence from mummies was very low, reflecting either a natural trend in the field or higher mortality due to manipulation in the laboratory.

Tying phenology data with insecticide assay results, it is possible to give some recommendations for spray applications to try to minimize negative impact on *Trechnites.* While Esteem might cause some mortality on *Trechnites* larvae developing in mummies in early spring, this impact is minimal. It is not recommended to apply Abamectin during bloom, but it is essential to time the first application such that it is before peak adult *Trechnites* emergence. Abamectin may not significantly impact *Trechnites* larvae in mummies, but it causes high mortality in adults. If Abamectin is applied during or shortly after adult *Trechnites* emergence, the population will be significantly affected. More research on degree days models is thus necessary to more accurately predict when *Trechnites* wasps emerge. Ultor is usually applied following bloom, after *Trechnites* adult emergence, and this is the most benign product unlikely to significantly affect *Trechnites* populations. Mortality data from other commonly used insecticides is needed.

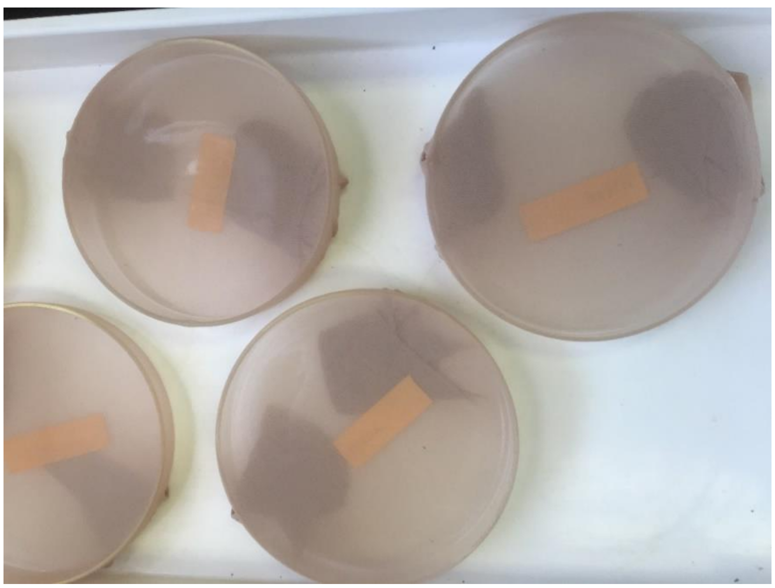


Figure 4. Insecticide assay arenas for spraying adult wasps

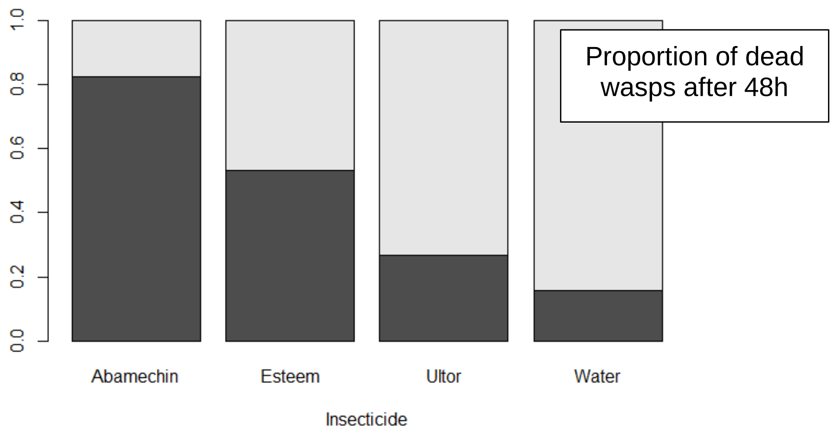


Figure 5. Adult wasp mortality 48 h after insecticide exposure.

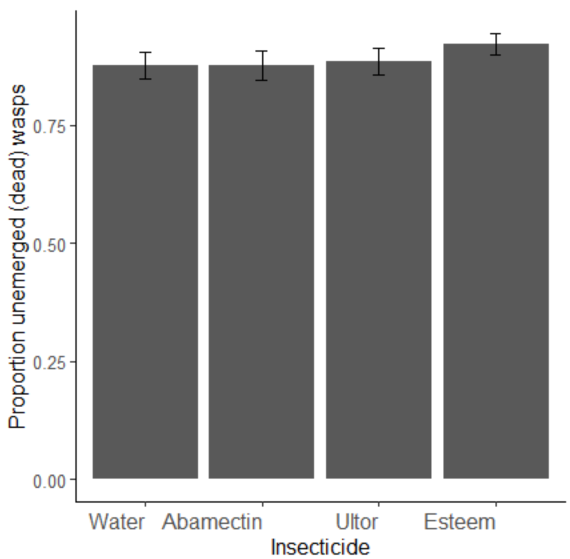


Figure 6. Proportion of unemerged (dead) wasp larvae from mummies after insecticide exposure

Additional results: hyperparasitoid complex

From the insecticide assays, we found that besides *Trechnites* (Fig. 8a), there are at least two more species of wasps that emerge from pear psylla mummies (Fig. 7). These wasps were sent to taxonomists and identified as *Pachyneuron sp.* (Hymenoptera: Pteromalidae; Fig 8b), and *Dilyta rathmanae* (Hymenoptera: Figitidae; Fig. 8c). From previous records (Menke and Evenhuis 1991, Schick 1994), these wasps are reported as being hyperparasitoids; that is, they lay eggs inside *Trechnites* larvae developing inside psylla nymphs. The presence of these hyperparasitoids can affect *Trechnites* populations, and the interactions among parasitoids in pear warrant further research.

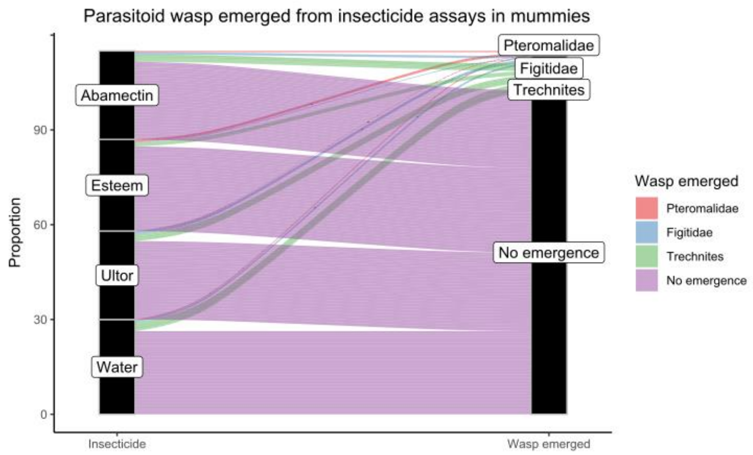


Figure 7. Parasitoid wasp emergence proportions from insecticide assays in psylla mummies.

a)

b)

c)

Figure 8. parasitoids reared from *Cacospsylla pyricola* nymphs. a) *Trechnites insidiosus* (Hymenoptera: Encyrtidae), b) *Pachyneuron* sp. (Hymenoptera: Pteromalidae), c) *Dilyta rathmanae* (Hymenoptera: Figitidae).

Additional results: *Trechnites* DNA detection in psylla mummies

Given that there are at least three species of parasitoid wasps in pear orchards, and that there is a very low rate of wasp emergence from mummies, it is not possible to know which wasp has parasitized a psylla mummy found in the field. We are currently testing DNA identification from mummies. In preliminary assays, we found that using a primer designed for *Trechnites*, it was possible to identify *Trechnites* DNA from parasitized psylla mummies, including empty mummies (the exoskeleton left behind after the wasp emerges; Fig. 9). Future work should focus on designing primers to identify DNA from the other parasitoid wasps found in pear orchards, which will help answer many questions regarding interactions among the parasitoid wasps and pear psylla.

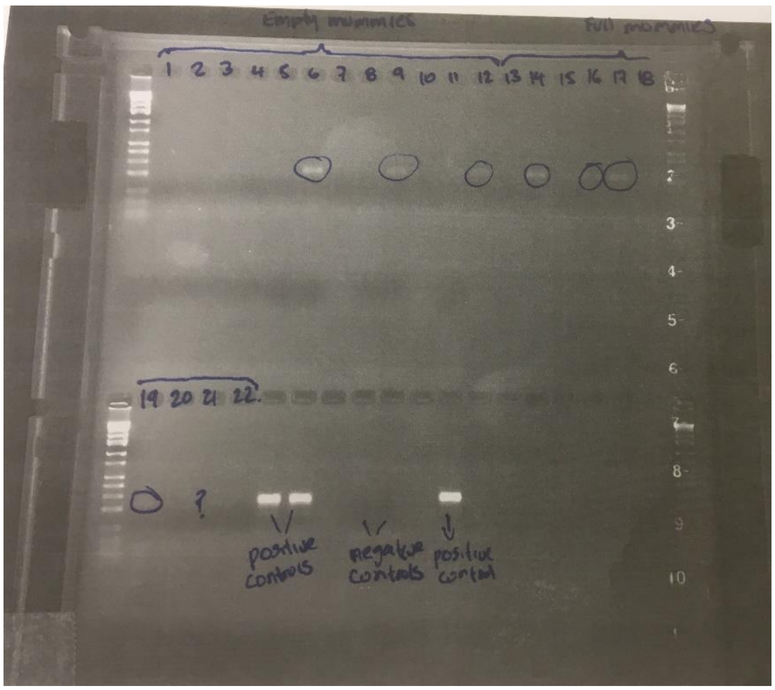


Figure 9. Results from a polymerase chain reaction (PCR) aimed to detect *Trechnites* DNA in parasitized psylla mummies. Circled marks represent samples where *Trechnites* DNA was detected in “empty” mummies (exoskeleton left behind after the wasp emerged), and “full” mummies (with the wasp larva still developing inside).

EXECUTIVE SUMMARY

Trechnites overwintered as larvae in psylla mummies in orchards, and adult wasps appeared in pear orchards in a single "explosive" peak. In Hood River, most adult *Trechnites* emerged in early May (around 650DD), while in Southern Oregon, *Trechnites* emergence occurred much later. In Wapato WA, *Trechnites* emerged at the end of May. There was very low psylla pressure during the summer, and it is possible that this early abundance of *Trechnites* contributed to keep psylla populations low. The population of *Trechnites* also remained low throughout the season, suggesting that they might be dispersing to other environments, potentially seeking other hosts, or that their population crashed as it might be tightly associated with pear psylla abundance. Number of overwintering mummies collected was low relative to *Trechnites* wasp emergence. This suggests that overwintering mummies hide on tree bark where they are difficult to collect, or that additional *Trechnites* adults are migrating into the orchards from other nearby hosts.

When pear psylla nymphs were not exposed to *Trecnhites,* 35% reached adulthood, while only 18% reached adulthood when exposed to one *Trechnites* wasp. We also observed that psylla nymphs were more likely to disperse (presumably to safer environments) when a wasp was present. This shows that the presence of *Trechnites* can affect psylla nymphs even without parasitism, as nymphs can likely die when moving to different environments.

When sprayed with Abamectin, Esteem, and Ultor, mortality rates in adult wasps were of 82%, 53%, and 26% respectively. Wasps sprayed with a water control had 15% mortality, with was similar to mortality in Ultor, but lower than mortality with Esteem, and Abamectin. The results with Esteem were surprising, as this is an insect growth regulator targeting juvenile stages and is not supposed to affect adult insects, but it caused greater than expected mortality in adult wasps. Overall, Ultor was the most benign insecticide for adult wasps, and Abamectin was the most detrimental.

After being sprayed with Abamectin, Esteem, and Ultor, the percentage of unemerged (dead) wasp larvae from psylla mummies was 87%, 92%, and 88%, respectively. This mortality was high, but not different from unemerged wasps after being sprayed with a water control (87%). This could be a positive result, indicating that wasp larvae developing inside psylla mummies are protected from detrimental effects of insecticide applications. However, wasp emergence from mummies was very low, reflecting either a natural trend in the field or higher mortality due to manipulation in the laboratory.

Tying phenology data with insecticide assay results, it is possible to give some recommendations for spray applications to try to minimize negative impact on *Trechnites.* While Esteem might cause some mortality on *Trechnites* larvae developing in mummies in early spring, this impact is minimal. It is not recommended to apply Abamectin during bloom, but it is essential to time the first application such that it is before peak adult *Trechnites* emergence. Abamectin does not significantly impact *Trechnites* larvae in mummies, but it causes high mortality in adults. If Abamectin is applied during or shortly after adult *Trechnites* emergence, the population will be significantly affected. More research on degree days models is thus necessary to more accurately predict when *Trechnites* wasps emerge. Ultor is usually applied after *Trechnites* adult emergence, and this is the most benign product, unlikely to significantly affect *Trechnites* populations. Mortality data from other commonly used insecticides is needed.