CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project: CH-20-103

Project Title: Insecticidal control of leafhoppers in cherries

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Total Project Request: **Year 1:** $81,166 **Year 2:** $84,185

Other funding sources: **None**

WTFRC Budget: ***None***

Budget
Organization Name: Washington State Univ. Contract Administrator: Katy Roberts/Shelli Tompkins
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Item	**2020**	2021
Salaries[1,2]	**52,827**	54,940
Benefits	**18,373**	19,108
Wages[3]	**3,900**	4,056
Benefits	**366**	381
RCA Room Rental		
Shipping		
Supplies[4]	**4,500**	4,500
Travel		
Plot Fees	**1,200**	1,200
Miscellaneous		
Total	**81,166**	84,185

Footnotes: [1] Research assistant professor (Nottingham) at 2% FTE of $7,612.5 per month for 12 months.
[2]Postdoc at 100% FTE of $4,250 per month for 12 months
[3]Summer time slip at 20 hours per week for 13 weeks at $15.00 per hour.
[4]Supplies including potted cherries, greenhouse and colony supplies (cages, soil, pots), bioassay supplies (pipette tips, paper cups, lab sprayer supplies), and PCR diagnostic services.

Objectives:

1. **Perform initial screening on a wide range of insecticides (broad spectrum-conventional, soft-conventional, and organic) against leafhoppers for mortality and feeding suppression.**
   Future goals: Continue screening products, particularly selective-conventional and organic insecticides. Develop methods for colony rearing to allow testing of insecticides on nymphs.
   Deviations: (1) Upon further research into stylet sheath assessment for measuring feeding suppression, we determined that this method may be too time-consuming to justify performing on all insecticides. Many insecticides were very toxic and immediately effective, so such elaborate assays are likely unnecessary. To better gauge the success of selective insecticides, instead, we think examining nymph mortality will provide more useful knowledge to the industry. Because nymphs feed in the ground cover, feeding success is not as important as mortality. (2) We were unable to establish a colony in the lab this year, potentially due to our limited knowledge of necessary plants for development. We suspect that a complex of wild weeds is necessary, such as common mallow, which is difficult to establish in colony given the lack of cultivation for seed. Instead, we relied on field collections for insecticide bioassays. This also proved difficult at first due to high mortality in transport, but we eventually developed a successful method allowing larger collections of leafhoppers from the field.

2. **Determine whether X infected leafhoppers are more susceptible to insecticides than uninfected leafhoppers.**
   Future goals: Continue to store adults that were killed in bioassays, then extract salivary glands for PCR diagnosis of the presence of phytoplasma.
   Deviations: Instead of performing separate bioassays for this hypothesis, we are utilizing individuals from insecticide screening in objective 1 to gain higher samples sizes, test more materials and save time.

3. **Determine residual control timelines for the most effective foliar products.**
   Future goals: Continue to perform residual time-line bioassays for more materials.
   Deviations: Due to lower ability to travel under COVID restrictions, bioassays were performed using potted cherry trees grown outdoors at the TFREC.

4. **Determine the potential for soil applications of systemic insecticides to provide long-term control of leafhoppers and disease transmission.**
   Future goals: Continue to test soil applied materials, potentially larger trees in 2020. Include an additional material, Safari (dinotefuran), which has a label for soil drenches and trunk sprays for cherries grown in nurseries and is known to control other leafhoppers species.
   Deviations: Due to low leafhopper numbers, we decided to eliminate a treatment, Verimark, which had the lowest likelihood for success.

Significant Findings:

- We identified conventional and organic insecticides that caused very high mortality of *C. reductus* (100% mortality across all reps) in direct contact spray bioassays within 24 hours of application.
  - Convention products resulting in 100% mortality were Asana, Malathion, and Actara. Transform WG resulted in ca. 92% mortality between two bioassays.
  - Organic products resulting in 100% mortality were Pyganic and Azera. Cinnerate and TetraCURB Organic resulted in >70% mortality assuming moribund as dead.
- Major improvements were made in collecting, transporting and assaying leafhoppers. Sweep netting with minimal sweeps per collection, storage and transport in mesh cages, and assaying with living plant material and soil all enhanced experimental viability.

- Another potentially important leafhopper species, *Euscelidius variegatus* was discovered in various field sites throughout the season in high abundance, especially in an organic apple block. Very little information on this species exists, but one prior study found it to be a competent vector of X-disease. Adults are larger than *C. reductus* and proved hardier in terms of collection, transport, and lab survival.
- Thiamethoxam and imidacloprid applied as soil drenches resulted in 50-70% mortality of *E. variegatus* leafhoppers 6 days following application.
- Thiamethoxam (Actara) and imidacloprid (Admire Pro) applied as foliar sprays also resulted in ca. 50-70% mortality of *E. variegatus* leafhoppers 6 days following application.

Methods:

Collection and Transport. Sweep netting was performed in commercial cherry and apple blocks near Rock Island, WA. Ten collection trips were made throughout the summer of 2020. Out of these attempts, just three resulted in experiments with usable data. Initially, we attempted aspirate leafhoppers directly out of sweep nets and into vials for transport to the lab. This resulted in very high mortality in transport and in experimental checks (untreated) for those that survived transport and were used in bioassays. To mitigate this issue, we stopped using aspirators and vials, and began dumping all contents from sweep nets directly into 12 x 12" mesh cages (Fig. 1B, in background). This was more efficient than aspirating leafhoppers and their survival in transport increased substantially. However, check mortality in bioassays remained higher than desirable (20-40%) for *C. reductus*. We then adjusted our sweeping technique to involve five sweeps maximum before dumping leafhoppers into mesh cages, to prevent sublethal injury. This was not necessary for *E. variegatus*, a larger and hardier species, but greatly improved the health and longevity of *C. reductus*.

Fig. 1. Leafhopper bioassay arenas and collection cages. A) Closeup of one arena without lid to show cherry leaf in floral tube and soil. B) Multiple arenas with lids in foreground; field collection cages in background.

Contact Spray Bioassays. Arenas were constructed using 8 oz plastic deli cups with slightly moistened soil and excised cherry leaves kept alive by inserting petioles into floral tubes with water (Fig 1A). Leafhoppers were aspirated from collection cages and moved into each arena (5-9 leafhoppers per arena). Each arena was sealed with a plastic lid with a mesh cutout. Once leafhoppers were in all arenas, treatments were applied using hand-pump aluminum spray bottles. Insecticide solutions were sprayed through mesh lids to contact the leafhopper, leaf, and soil, as would occur in the field. Containers with sprayed leafhoppers were then stored for 24 hours in a greenhouse prior to evaluation. To evaluate efficacy of insecticides, leafhoppers were rated as either alive or dead, to be considered dead, leafhoppers needed to be unable to walk. The distinction 'moribund' (impaired but alive) was used in the organic bioassay only (the final bioassay) but will be used in the future for more detail on sublethal effects. Leafhoppers that were clearly alive and standing but could/did not hop when forcibly prodded with forceps were called moribund. Five spray contact bioassays were conducted, however, only two resulted in usable data (the other three had control mortality above 20%). The first conducted tested conventional insecticides (Table 1) and the second tested organic insecticides with the addition of Transform WG as a positive control (Table 2).

Table 1. Conventional Spray Contact Bioassay

Trt.	Per 100 gallons
UTC	-
Asana	14.5 fl oz
Malathion 5EC	44.8 fl oz
Bexar	27 fl oz
Actara	2.75 oz
Transform WG	2.75 oz
TetraCURB conc.	256 fl oz

Table 2. Organic Spray Contact Bioassay

Trt.	Per 100 gallons
UTC	-
Transform WG	2.75 oz
TetraCURB organic	2%
Cinnerate	60 fl oz / 100
Entrust SC	8 oz
Neemix 4.5	16 fl oz
Azera	56 fl oz /acre
Pyganic 1.4 EC	64 fl oz

Systemic Soil Drench and Spray Residue Bioassay. The experiment used Lapins cherry trees (3/4”) on Mazzard rootstock planted in 3.6-gallon injection molded pots. Five cherry trees were assigned to each of five treatments: one untreated control (UTC), two soil applied insecticide treatments, and two foliar applied insecticide treatments (Table 3). Trees were not watered for 72 hours prior to applications. On 15 August, insecticide applications were made between 8:00 and 9:00 am. One-liter insecticide solutions were poured into each pot for soil treatments. Foliar treatments were sprayed using an arm-pump SOLO backpack sprayer, trees were sprayed to just before runoff, about 0.25 liters per tree. Soil drench treatments concentrations were made assuming 400 trees/acre. Soil drench mixes were made to use 1 liter of solution per tree while foliar applications were made to use 0.25 liter/tree (based on amount of spray needed to achieve full coverage). This resulted in soil applications using more A.I. per tree. Trees were lightly watered later that day, but not enough for water to run out from the bottom of the pots to avoid leaching insecticides out of the pots.

Table 3. Systemic Soil Drench Bioassay

Trt.	App.	Max label / acre	Per tree
UTC	-	-	-
Platinum 75 SG	Soil	3.67 oz / ac	0.26 g in 1 liter
Admire Pro	Soil	10.5 fl oz/ ac	0.77 ml in 1 liter
Actara	Foliar	2.75 oz /ac	0.194 g in 0.25 liter
Admire Pro	Foliar	2.8 fl oz / ac	0.21 ml in 0.25 liter

A leafhopper bioassay was conducted 48 hours after treatment applications using *C. reductus* and the same arena methods as the spray contact bioassays, but with the stated modifications to insecticide application method. This first assay resulted in very high check mortality, so the data were not usable. On 19 August, 4 days after treatment, another collection and bioassay attempt was made with *C. reductus* but with similar high check mortality. Luckily, in the same batch of collected insects was another leafhopper species in high numbers which we believe is the lesser known species, *E. variegatus* (Fig. 2C). We used these leafhoppers in a third bioassay beginning on 20 August. This bioassay was successful with almost no check mortality, leading us to further investigate this species. We found just one study in the literature examining *E. variegatus* (Jensen 1969), which determined it to be a competent vector of X phytoplasma, and one other with just a brief mention of its abundance in some orchards (Purcell and Elkinton 1980). We have saved many of these specimens to confirm the species ID and to run PCR analysis for X-phytoplasma.

Results:

Contact Spray Bioassays. Results for contact spray bioassays only include *C. reductus*. The conventional insecticides Asana (esfenvalerate), Malathion 5EC (malathion) and Actara (thiamethoxam) all resulted in 100% mortality of *C. reductus* leafhoppers 24 hours after treatment (Fig. 3). The organic insecticides Pyganic (pyrethrins 1.4%) and Azera (premix of pyrethrins 1.4% and azadirachtin 1.2%) both achieved 100% mortality 24 hours after treatment (Fig. 4). The conventional insecticide Transform WG (sulfoxaflor) resulted in 87.5% mortality in one bioassay (Fig. 3) and 96% mortality in a second when moribund individuals are considered dead (Fig. 4). The conventional materials Bexar (tolfenpyrad) and TetraCURB Concentrate (rosemary oil) provided marginal control at 66.7% and 52% mortality, respectively (Fig. 3). The organic materials Cinnerate (cinnamon oil) and TetraCURB Organic (rosemary oil) provided the next highest level of mortality for organic materials, both at ca. 72%, however many of these individuals were moribund (Fig 4.). The other organic insecticides Neemix (azadirachtin 4.5%) and Entrust SC (spinosad) provided marginal to poor control (Fig. 4).

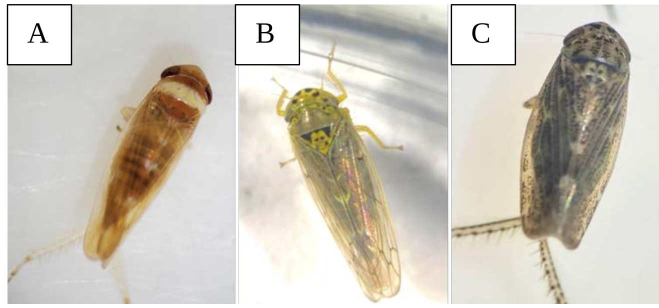

Fig. 2. Washington Leafhoppers: A) *C. reductus*, B) *C. geminatus*, and C) *E. variegatus* (seeking confirmation)

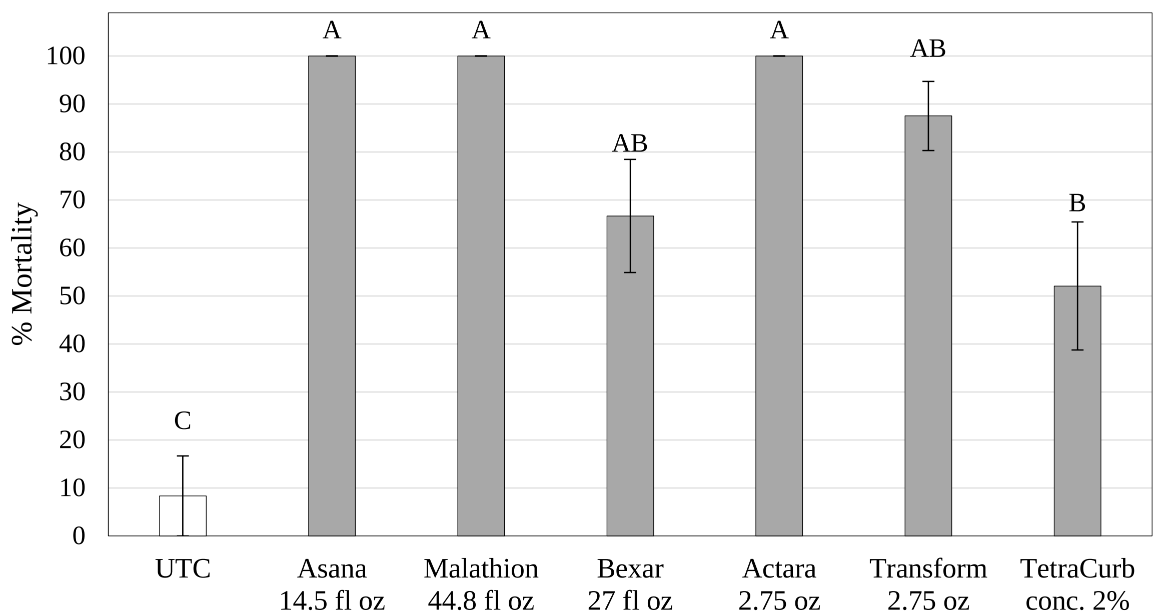

Fig. 3. Conventional Spray Contact Bioassay. Bars show average leafhopper mortality resulting from each insecticide. Bars not sharing a letter are significantly different according to Tukey's HSC (*P* < 0.05)

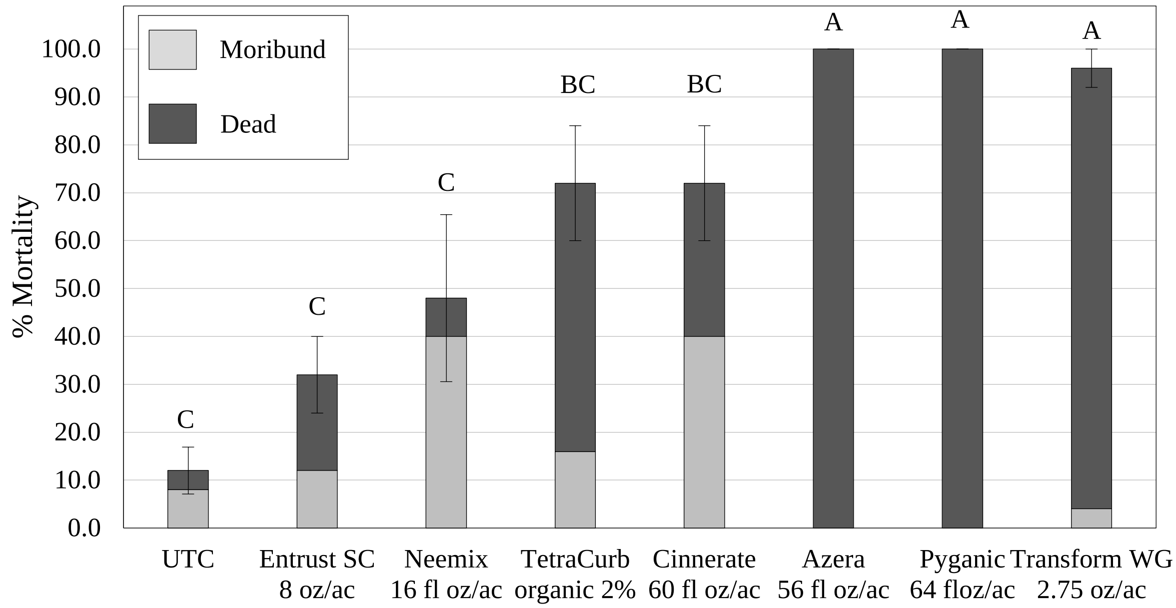

Fig. 4. Organic Spray Contact Bioassay. Bars show average leafhopper mortality resulting from each insecticide. Bars not sharing a letter are significantly different according to Tukey's HSC ($P < 0.05$)

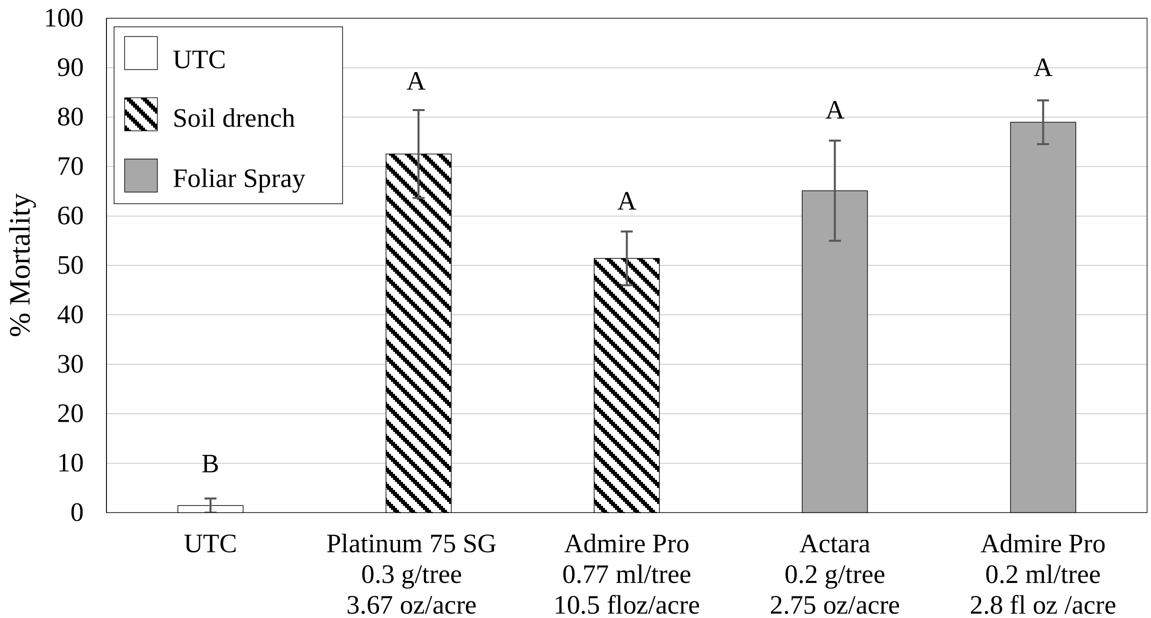

Fig. **5.** Systemic Soil Drench and Spray Residue Bioassay. Bars show average leafhopper mortality resulting from each insecticide product and application method. Bars not sharing a letter are significantly different according to Tukey's HSC ($P < 0.05$)

Systemic Soil Drench and Spray Residue Bioassay. Thiamethoxam and imidacloprid as both soil drenches and foliar sprays exhibited similar control of leafhoppers, which was significantly greater than the check but not overly impressive. However, these data are promising as a preliminary finding considering that this is a first attempt to use a soil drench for control of leafhoppers in cherries to our knowledge, so certain minor adjustments may improve outcomes. In addition, these data demonstrate that residual toxicity of both materials to leafhoppers one week after spray applications was notably lower than direct contact. However, the leafhoppers tested against residues were *E. variegatus* while *C. reductus* was tested against direct sprays, decay information is not conclusive.

Discussion:

Through these 2020 experiments we have identified 5 insecticide materials, 3 conventional and 2 organic, that are highly toxic to leafhoppers upon direct spray contact. The design of contact spray bioassays did not necessarily produce perfect contact with all leafhoppers. Sprays were applied through screen lid cutouts into containers containing soil, large cherry leaves, and leafhoppers, so it is reasonable to suggest that perfect coverage (i.e., coating the leafhoppers with insecticide) did not always occur. We believe that this conservative approach will lend to more accurate predictions of field success.

While these data are preliminary given the few number of experiments, growers and advisors should still find these data useful in making certain spray decisions. Organic growers especially will find these data useful, as very little was known about which materials are toxic to leafhoppers. Pyrethrin containing products such as Pyganic and Azera are highly toxic to leafhoppers and may be used if infestations are high. It should be noted that Azera is a premix product, and the pyrethrin component is likely "pulling the weight". We can assume this because the other component is azadirachtin, the active ingredient in Neemix, which was only moderately toxic to leafhoppers. Pyrethrin products may have some risk for flaring secondary pest through disruption of biological control, however that risk is likely low due to the short residual of these materials. Future experiments should examine lower rates of pyrethrins against leafhoppers, which could lower risks of secondary pest outbreaks. These data also elucidate both effective and ineffective conventional products. Conventional growers will run the risk of flaring secondary pest with any of the products tested, so it is important in the future to develop strategic spray programs based on phenology or trapping to avoid over-spraying and causing secondary pest outbreaks.

Thiamethoxam and imidacloprid products (both soil drenches and foliar sprays) exhibited similar control, 50-70% mortality, of *E. variegatus* leafhoppers, which was significantly greater than the check but not overly impressive. However, these data are promising as preliminary findings considering that this is a first attempt to use a soil drench for control of leafhoppers in cherries, to our knowledge. Additionally, *E. variegatus* seems to be hardier than *C. reductus*, and therefore may also be more tolerant of insecticides. Certain minor adjustments may improve outcomes, such as testing *C. reductus*, increasing rates, or examining mortality 48 and 72 hours after exposure to allow more dying time. Soil drench information may be most important to nursery growers, as both active ingredients are allowable as soil drenches in non-bearing cherry trees (thiamethoxam as product Flagship). Admire Pro can be used as a soil drench in mature, bearing cherries, however this technique will require more testing on larger trees in the field. Additionally, we identified another product, Safari 20SG (dinotefuran), which is supposed to be highly effective on leafhoppers, more mobile in trees than the two products tested, and is legal to use on nursery cherries trees. This will be of interest to explore in 2021 experiments.

A very important finding for future research was determining proper methods for collection and experimenting with these leafhoppers. Now that methods are established and known to be successful, we will be able to conduct more, large scale experiments and provide greater amounts of control information to the industry.

References Cited

Jensen, D. D. 1969. Comparative Transmission of Western X-Disease Virus by Colladonus montanus, C. geminatus, and a New Leafhopper Vector, Euscelidius variegatus1. J. Econ. Entomol. 62: 1147-1150.

Purcell, A. H., and J. S. Elkinton. 1980. A Comparison of Sampling Methods for Leafhopper Vectors of X-disease in California Cherry Orchards1. J. Econ. Entomol. 73: 854-860.