CONTINUING PROJECT REPORT **YEAR**: 1 of 3

Project Title: Identifying sources of X disease in cherry orchards

PI: Tobin Northfield
Organization: WSU-TFREC
Telephone: 509-293-8789
Email: tnorthfield@wsu.edu
Address: 1100 N Western Ave.
City/State/Zip: Wenatchee, WA 98801

Co-PI (2): W. Rodney Cooper
Organization: USDA YARL
Telephone: 509-454-4463
Email: rodney.cooper@ars.usda.gov
Address: 5230 Konnowac Pass Rd.
City/State/Zip: Wapato, WA 98951

Cooperators: Garrett Bishop, Scott Harper, Tianna DuPont

Total Project Request: **Year 1:** $58,400 **Year 2:** $55,849 **Year 3:** $53,707

Other funding sources: **Awarded**
Amount: $249,359
Agency Name: USDA/WSDA Specialty Crop Block Grant
Notes: The PI's on this grant are also on a USDA SCBG grant led by Scott Harper (Northfield and Cooper are co-PIs), that will build on the preliminary ground-truthing of gut content analysis from this grant in part to do fieldwork evaluating alternative host plant use by X disease phytoplasma vectors in the field. The SCBG grant is complementary, but not overlapping with this grant.

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Shelli Tompkins/Katy Roberts
Telephone: 509-665-8271, ext 2/ 509-335-2885 **Email address:** shelli.tompkins@wsu.edu / arcgrants@wsu.edu

Item	2020	2021	2022
Salaries[1]	39,629	41,214	42,863
Benefits[2]	4,478	4,657	4,844
Wages			
Benefits			
Equipment			
Supplies[3]	7,000	4,000	4,000
Travel[4]	2,000	2,000	2,000
Miscellaneous			
Plot Fees			
Total	53,107	51,871	53,707

Footnotes:
[1] new student position
[2] 11.3%
[3] Research consumables (e.g., cages, pots, soil), + molecular tests for disease presence
[4] In state travel

(*Complete the following budget tables if funding is split between organizations,* ***otherwise delete*** *extra tables.*

Budget 2
Organization Name: USDA ARS **Contract Administrator:** Chuck Myers

Telephone: 509-454-4463 **Email address:** Chuck.Myers@ars.usda.gov

Item	2020	2021	2022
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies[1]	5,293	3,978	
Travel			
Plot Fees			
Miscellaneous			
Total	5,293	3,978	

Footnotes:
[1] Molecular supplies for gut content analysis

Objective Recap, Goals, and Anticipated Accomplishments:

Objectives

1. Conduct oviposition tests and life cycle analysis on leafhoppers on five host plants (cherry, clover, dandelion, peach, alfalfa).

Most knowledge we have about life history characteristics of the most common Washington leafhoppers that vector X disease (*Colladonus geminatus* and *C. reductus*) come from a single study on *C. geminatus* conducted in 1952 and 1953 in Dalles, OR (Nielson 1968). The author reared *C. geminatus* leafhoppers on alfalfa plants and peach trees and found that the generation time averaged across the two plant hosts was approximately 60 days. The authors stated that there were no statistically significant differences in the life histories for the two plants, but it would be helpful to get effective estimates for each host type and compare them to other common weeds that may host the X disease phytoplasma. It is also unclear what plants leafhoppers feed on or how other hosts affect their growth and reproduction. Furthermore, *C. reductus* was not included in the study, but is often far more abundant than *C. geminatus* in Washington orchards (*C. reductus* made up 97.5% of the *Colladonus* spp. in our surveys of Wenatchee and Yakima region orchards). Here, we originally set out to build on this research by evaluating the generation time for *C. reductus* and *C. geminatus* on 5 plant species: cherry, white clover (*Trifolium repens*), dandelion (*Taraxacum officinale*), peach and alfalfa. Understanding host plant use will help inform management plans. In our surveys of cherry farms in the Wenatchee and Yakima regions in this project and in the project title, "Field evaluation of leafhopper controls for X disease management" we rarely observed *C. geminatus*, with *C. reductus* being >95% of individuals collected by sweep nets and sticky traps. In response to the abundance of *C. reductus* and lack of knowledge, we focused our trials on this species. Furthermore, when collecting leafhoppers, we noticed they were commonly found on mallow plants, so we included mallow in our trials. In two attempts to start a colony of *C. reductus* with a diverse offering of plants (attempt 1: pea plants, clover, alfalfa; attempt 2: alfalfa, clover, mallow) the leafhoppers died as older nymphs or newly emerged adults, suggesting there was something missing in their diet, and that they may need a diverse diet. We are currently raising *C. reductus* leafhoppers on a combination of peach trees, mallow, alfalfa, dandelion, and clover. Given an apparent need for a diverse diet, we have focused trials on feeding behavior, and used an oviposition test to determine the number of generations per year for *C. reductus*, which is unknown (2 reported for *C. geminatus* in the 1950s), and is unclear from sticky trap data.

2. Evaluate incubation time and acquisition probability for leafhoppers feeding on each, cherry and peach trees and transmission likelihood to cherry, clover, dandelion, peach, and alfalfa.

In our evaluation of acquisition and transmission studies we will follow the methods of previous studies (Jensen 1971, Suslow and Purcell 1982), with the addition of molecular techniques to better evaluate acquisition and transmission success. To evaluate acquisition in year 2 of the project we will identify cherry and peach trees exhibiting X disease symptoms during harvest, and place *C. geminatus* and *C. reductus* leafhoppers in sleeve cages on the diseased trees. After 1 week of feeding (the maximum time needed according to previous research) we will cut the branch off the tree, keeping the sleeve cage intact and place the sleeve cage and branch immediately into a cooler with ice for transport back to the WSU TFREC without allowing leafhopper escape. The leafhoppers collected from cherry trees will then be transferred to greenhouse cages containing one of five potential host plants: cherry, peach, alfalfa, dandelion, or white clover, and replicated 8 times (40 total cages). Each cage will include 3 *C. geminatus* and 3 *C. reductus* leafhoppers, to focus on the potential of the plant to host the disease and allow for either leafhopper species to transfer the disease.

Note: These trials are planned for year two and have not yet been conducted. However, we have preserved the plants from the feeding trials for testing as alternative hosts. To our knowledge, only cherry, peach, and dandelion are known hosts, so testing the herbaceous hosts for phytoplasma after the feeding trials with field-collected leafhoppers is an important step. These samples are currently awaiting molecular sequencing to test for phytoplasma presence.

3. Use molecular analysis on leafhoppers raised on different host plants to evaluate the reliability of gut content analysis to identify previous hosts of leafhoppers collected in orchards.

Research conducted by co-PI Rodney Cooper and colleagues on purple top disease in potatoes (Horton et al. 2018, Cooper et al. 2019), caused by a phytoplasma vectored by beet leafhoppers has included the development of molecular methods to identify previous plant hosts of leafhoppers collected from crops. While the methods have been focused on beet leafhoppers, rather than the *Colladonus* spp. that vector X disease, we expect the methods to be directly applicable to identifying non-cherry plants as sources of leafhoppers. Here, we will use leafhoppers arising from experiments described in objective 1 as a cost-effective evaluation of such methods for cherry-X disease research. These data can then be used as pilot research justifying federal funding identifying alternative leafhopper hosts and their potential importance for disease transmission in cherry orchards. Thus, at the end of the life cycle analysis in year 1 we will send leafhoppers from the field trials to the USDA lab in Wapato for molecular analysis to identify the host plant within the insect's gut. Assuming identification success in year 1, in year 2 we will collect adult leafhoppers from the end of experiments and place them on cherry seedlings, raised separately for each host plant. We will then collect 5 leafhoppers from each seedling at 0, 1, 2, and 3 weeks to identify the timeframe in which the previous host plant can be detected. We have stored leafhoppers from feeding trials and will conduct gut content analysis over the winter months.

Objectives timeline

Objective	Y1	Y2	Y3
1 Life history tests	x	x	
2 Transmission tests		x	x
3 Gut content analysis	x	x	

Significant Findings:

- Of the plants included in the trials (cherry, peach, mallow, alfalfa, white clover, and dandelion), *C. reductus* have a strong affinity for mallow, and to a lesser extent alfalfa. Given how common these plants are in orchard groundcover, these hosts should be considered in management strategies. *C. reductus* may also benefit from a diverse diet, that includes tree feeding.
- Leafhoppers feeding rates on cherry trees ranged from 14% to 51% of the observed feeding, depending the available herbaceous plants.
- Leafhopper feeding rates on peach trees ranged from 22% to 41% of the observed feeding, depending on the available herbaceous plants.
- We observed successful oviposition in August in field conditions, with adults emerging in October, suggesting there are three *C. reductus* generations in the Pacific Northwest. Two of these adult emergence periods typically occur after cherries are harvested.
- Leafhoppers are most active during daylight hours, and we did not observe evidence of leafhoppers moving into trees at night.

Methods:

Feeding trials

We initiated feeding trials in 24in × 24in × 56in (w × w × h) cages with a combination of white clover, alfalfa, dandelion, mallow, Early Red Haven peach trees, and/or Bing cherry trees, with each plant in a separate pot (Figure 1). Each trial lasted 5 days and each cage contained 10-15 leafhoppers, depending on mortality after collection. In the first trial, we conducted observations every two hours from 8am to 11pm. However, leafhoppers rarely moved in the span of the two-hour intervals and did not appear active in observations made at 9pm and 11pm, which were in the dark and made with red headlamps to avoid disturbing insects. Therefore, in subsequent trials, observations were made at 8AM, 1PM, and 6PM, doing 3-minute time searches in each cage. Trials were conducted in environmentally controlled growth rooms set at 75F, with a 16:8 L:D daylength. During each observation, we counted how many leafhoppers were on each plant, what plants they were on and if actively feeding or not by visually observing stylets piercing the plant. We present data only on actively feeding leafhoppers summarized across the insects within a cage.

Figure 1 Feeding trial cages in the growth room.

The trials included the following treatments:

- 2 trials of cherry, alfalfa, clover, dandelion; each with 2 cages
  - Initiated June 11 and August 3, 2020
- 2 trials of peach, alfalfa, clover, dandelion; each with 2 cages
  - Initiated June 11 and August 3, 2020
- 1 trial of cherry, clover, mallow, dandelion; each with 2 cages
  - Initiated September 22, 2020
- 1 trial of peach, clover, mallow, dandelion; each with 2 cages
  - Initiated September 22, 2020
- 1 trial of peach, alfalfa, mallow, dandelion; each with 3 cages
  - Initiated August 22, 2020
- 1 trial of cherry, alfalfa, mallow, dandelion; each with 3 cages
  - Initiated October 6, 2020

Field oviposition test

Based on yellow sticky card data, in the Pacific Northwest *Colladonus* species leafhoppers typically have three periods of abundance: May, late July/early August, and October. However, it is difficult to determine the number of generations per year from yellow sticky card data. This is because the October generation may be the same generation as the August generation, just moving into orchards after loss of alternative host plants. Because leafhoppers overwinter as dormant eggs, we evaluated the potential for eggs laid in field conditions in August to hatch into nymphs. Development of these eggs would then suggest that the August adults represent a distinct generation that gives rise to the adults collected in October. Therefore, during the first week of August 2020 we collected *C. reductus* and placed them in cages 24in × 24in × 24in mesh cages with combinations of herbaceous plants next to the Brunner building at the WSU Tree Fruit Research and Education Center. The cages were monitored periodically to identify the emergence of nymphs and/or adults.

Additional Research: Leafhopper Location and Activity

Studies on the behavior of *Colladonus reductus* within orchards is lacking, leaving unknowns such as when they are most active throughout the day and where they are most abundant within a block. We did not observe activity during daylight hours in the feeding trials, but we were unable to replicate dawn or dusk in the growth rooms (due to non-dimming lights), so we sought to identify whether leafhoppers regularly move vertically from ground cover to canopies in four time periods: morning, mid-day, evening, and overnight. To begin addressing these unknowns we used yellow sticky cards (5 × 7 in) to examine leafhopper abundance at two heights, varying distances from the orchard border, and activity throughout a 24 hr period. In two cherry blocks at 6:00am Aug 5th, we deployed 32 sticky cards, one at each height at four distances from the orchard border (40, 80, 120, and 160 ft), and 16 sticky cards, one at each height. At each location, one trap was tied to a branch at 6 ft and another to a wooden stake at 2 ft from the ground. Traps were collected and replaced at 10:00am, 6:00pm, 10:00pm, and 6:00am the following morning, and *C. reductus* abundance was recorded by height, time, and distance from orchard border.

Figure 2 *C. reductus* feeding on mallow

Results & Discussion:

Feeding trials. We observed active feeding on all plants offered during the feeding trials (Figure 1). In the feeding trials that included cherry trees, the order of *C. reductus* preference appeared to be: mallow, alfalfa, cherry, white clover, and dandelion. Indeed, when offered mallow, alfalfa and a cherry tree we did not observe feeding on dandelion. In the feeding trials that included peach trees, the order of preference appeared to be: mallow, alfalfa, peach, white clover, and dandelion. However, interestingly, when offered mallow, alfalfa and peach together they fed more on peach than alfalfa. The fact that leafhoppers always fed on cherry or peach trees, regardless of what herbaceous plants were there begs the question of whether there is something important about feeding on trees that provide important nutrients to leafhoppers. However, future research is needed to determine whether this is the case.

Field oviposition tests: Adult *C. reductus* leafhoppers collected in the first week of August and introduced to outside cages with mallow and clover readily laid eggs that hatched into nymphs and began reaching the adult stage in October, suggesting that the August generation is a separate generation from the first generation that emerges in May from overwintering eggs and from the October generation that lays eggs that remain dormant for the winter. Given that these two later generations typically occur after cherry harvest, leafhopper control after harvest is likely critically important.

Additional Research: Leafhopper Location and Activity

Leafhoppers were most active mid day and in the evening from 6pm to 10am (Figure 2), although evening catch was much more skewed towards the low trap heights (2’ compared to 6’ height). We conducted this experiment in part to test the theory that leafhoppers move into the trees at night. However, these data do not seem to support this theory, as in the evening hours most trap capture occurred near the ground cover. In addition, we did not observe evidence that leafhoppers were moving in and out of the orchard during different periods of the day. There was consistent capture at the different distances from the edge in our different time periods (data not shown).

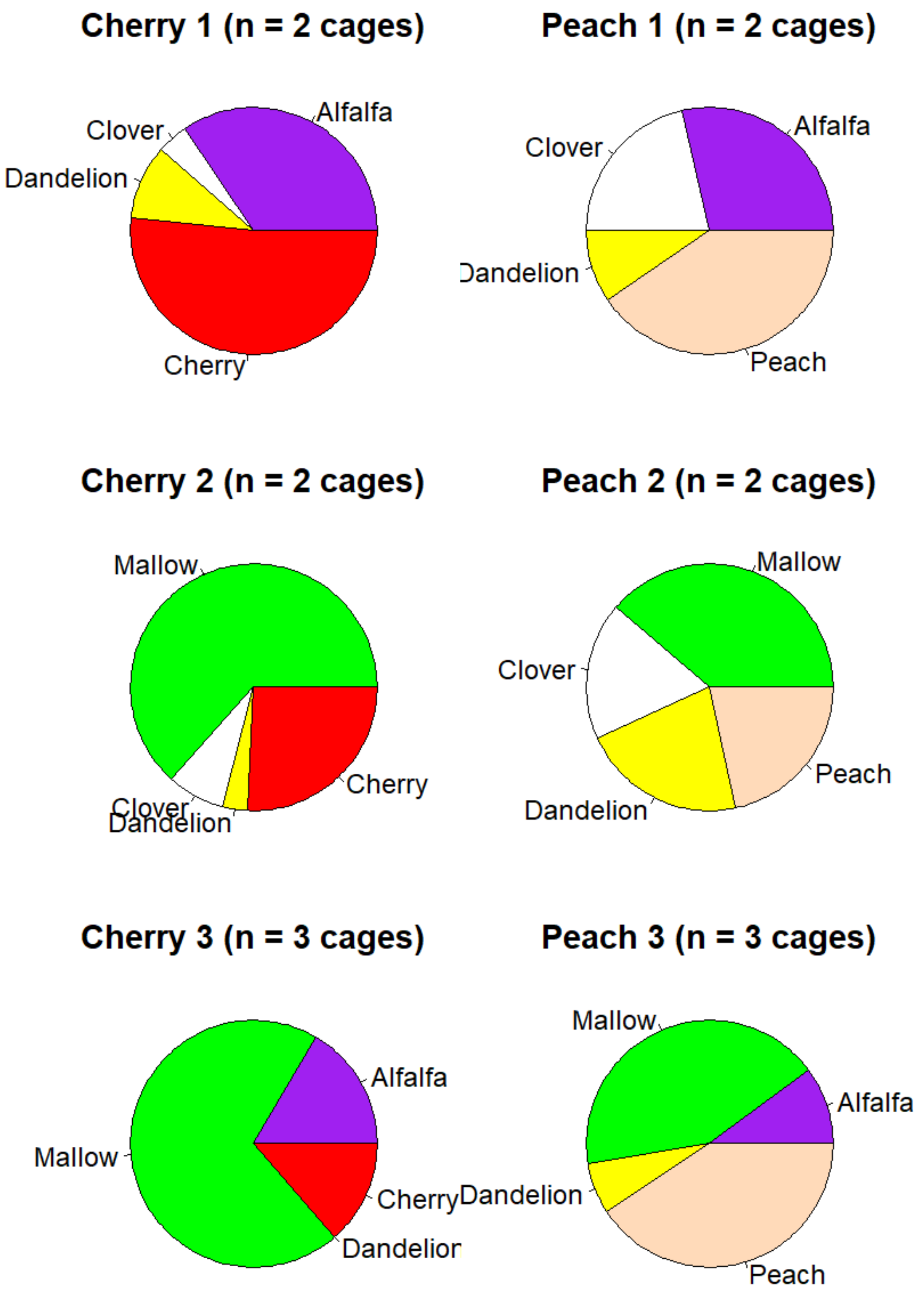

Figure 3 Proportion of feeding observations made on each of the different plant species in cages: cherry (red), white clover (white), dandelion (yellow), alfalfa (purple), peach (peach), or mallow (green) during feeding observations.

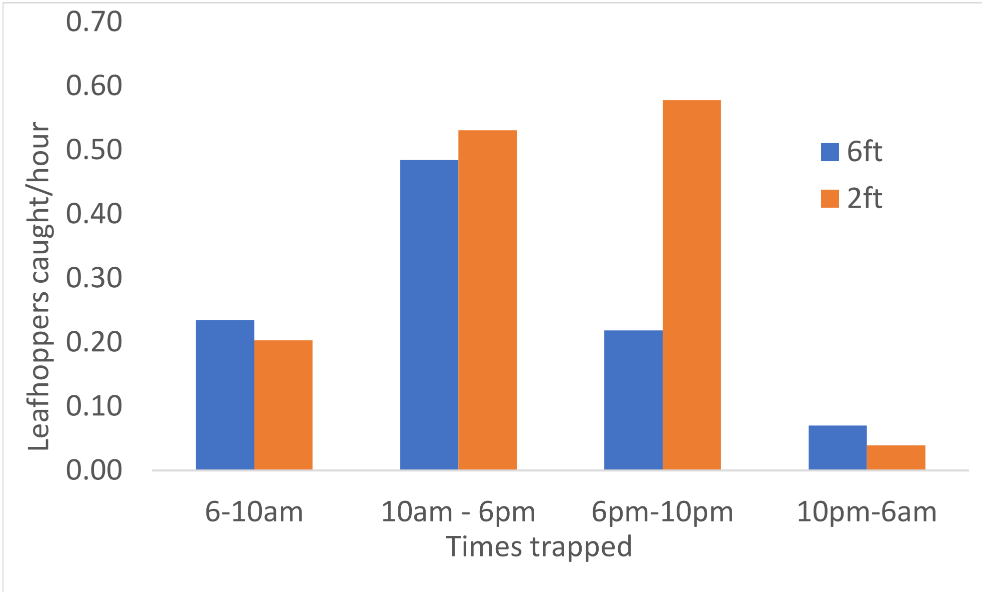

Figure 4 Mean *C. reductus* leafhoppers collected per hour (to account for variable trapping intervals) in a commercial Wenatchee region cherry block over a 24 hour period starting on August 5, 2020. No *C. geminatus* were collected. Traps were hung at 6 feet from a cherry tree or placed below the tree on a 2 foot stake. Data summarize 12 traps at each height.

References

Cooper, W. R., D. R. Horton, M. R. Wildung, A. S. Jensen, J. Thinakaran, D. Rendon, L. B. Nottingham, E. H. Beers, C. H. Wohleb, D. G. Hall, and L. L. Stelinski. 2019. Host and non-host 'whistle stops' for psyllids: molecular gut content analysis by high-throughput sequencing reveals landscape-level movements of Psylloidea (Hemiptera). Environmental Entomology **48**:554-566.

Horton, D. R., W. R. Cooper, K. Swisher Grimm, D. W. Crowder, Z. Fu, T. D. Waters, C. H. Wohleb, K. Frost, A. S. Jensen, and M. Blua. 2018. The beet leafhopper odyssey in North America: a brief overview.

Jensen, D. D. 1971. Herbaceous host plants of western X-disease agent. Phytopathology **61**:1465-1470.

Nielson, M. W. 1968. Biology of the geminate leafopper, *Colladonas geminatus*, in Oregon. Annals of the Entomologial Society of America **61**:598-610.

Suslow, K. G., and A. H. Purcell. 1982. Seasonal transmission of X-disease agent from cherry by leafhopper *Colladonus montanus*. Plant Disease **66**:28-30.