Project/Proposal Title: Field evaluation of leafhopper controls for X-disease management

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Report Type: Final Project Report

Project Duration: 2-Year

Total Project Request for Year 1 Funding: $ 79,864
Total Project Request for Year 2 Funding: $ 82,558

Other related/associated funding sources: Awarded
Funding Duration: 2021 - 2024
Amount: $244,750
Agency Name: USDA/WSDA Specialty Crop Block Grant
Notes: Primarily funds a 3-year evaluation of Extenday ground cover, with comparisons to a selective herbicide-mowing program, and a control. Also, includes roles of groundcover weeds over the season.

Budget 1
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Item	2020	2021
Salaries[1]	50,039	52,040

Benefits[2]	17,325	18,018
Wages		
Benefits		
Equipment		
Supplies[3]	5,000	5,000
Travel[4]	7,500	7,500
Miscellaneous		
Plot Fees		
Total	79,864	82,558

Footnotes:

[1] New postdoctoral researcher position (100% FTE), Louis Nottingham (2%)

[2] 35% (postdoctoral researcher), 25.9% (Nottingham)

[3] Fieldwork consumables and X-disease tests

[4] Domestic travel for research

Objective Recap, Goals, and Anticipated Accomplishments:

1. *Evaluate effects of kaolin clay applied post-harvest on X disease prevalence and density of leafhoppers and predators.*

   As planned, we have finished the two-year trial evaluating the efficacy of kaolin clay to suppress the densities of leafhopper vectors of X-disease (*Colladonus reductus and C. geminatus*) in Yakima and Wenatchee region cherries blocks, as well as Yakima region nectarines.

2. *Evaluate effects of UV-reflective mulch on X disease prevalence and density of leafhoppers and predators.*

   Our shipment of Extenday was delayed by 4 months due to COVID19 preventing us from deploying it in our experimental plots. Thankfully, the growers in Wenatchee region had Extenday in the cherry plots prior to harvest and graciously left it throughout the season for our experimental study. Therefore, in half of our cherry plots (Wenatchee region only) we were able to conduct the first year of evaluating Extenday for suppression and control of the X-disease leafhopper vectors. In 2021 we applied the Extenday to our Yakima region plots as well. We do not present data from two Wenatchee region blocks on Stemilt hill, because we only observed one leafhopper all season in 2020 and did not set up treatments there in 2021. Sampling in 2021 again showed very low leafhopper abundance.

3. *Describe seasonal patterns of leafhopper abundance and map disease incidence in commercial cherry orchards.*
   We tracked the seasonal abundance of leafhoppers over the course of 2020 and 2021. In addition, we mapped disease incidence at harvest in our trial orchards, and we evaluated the spatial distribution of leafhoppers in the different treatments. We found little disease spread over the two funded years of the project. This is partly due to the length of the project covering only 2 years, rather than the proposed 3, such that transmission occurring in 2020 would typically not appear until 2022 or later after a lag in symptoms. Therefore, we instead present differences in the spatial distribution of leafhoppers for the different treatments.

Significant Findings:

- In 2020 Extenday applied postharvest reduced postharvest leafhopper numbers by 81% compared to controls with little to no negative effect on predators in Wenatchee area cherries.
- In 2021 Extenday applied postharvest reduced postharvest leafhopper numbers by 88% and 91% compared to controls in Wenatchee and Yakima region cherries, respectively.
- Extenday provided consistent control despite 50-fold variation in leafhopper abundance in control blocks across Wenatchee and Yakima regions, suggesting it works in both, high-pressure and low-pressure blocks.
- In high-pressure cherry blocks in the Wenatchee and Yakima regions Surround applied postharvest reduced leafhopper numbers by 47-48%, with effects strengthening after the second application.
- Postharvest Surround did not reduce leafhopper numbers in very low-pressure cherry blocks (< 2/trap), or in nectarines, where harvest occurs very late compared to cherries.
- Leafhoppers in Surround-applied blocks appear to forage more in the groundcover than trees compared to control blocks, based on trap capture at 2ft, 4ft and 6ft.

- In control replicates, leafhopper numbers were highest in the middle of the replicate, and in Surround-treated replicates, the numbers were highest at the edge of the replicate adjacent to the untreated buffer trees. Extenday numbers were low throughout the block.
- The project served as preliminary data for a funded WSDA/USDA Specialty Crop Block Grant to compare Extenday to weed management for leafhopper control.

Methods:

We evaluated two control methods (kaolin clay and Extenday groundcover) as additions to the spray rotation currently used on commercial cherry plots in the Wenatchee region (4 plots in one location, and 2 in another) and the Yakima region (2 plots in Wapato) and evaluated kaolin clay in 2 Yakima region nectarine blocks (in Wapato). In 2021 we dropped two of the Wenatchee blocks due to very low leafhopper numbers in 2020 (only one collected all year). We targeted blocks with 1-10% disease prevalence to ensure that the block has disease to control, but that the disease prevalence is not high enough to risk block removal prior to the end of the experiment. However, at the start of the experiment in 2020 we realized that there was very high disease prevalence in the Yakima region cherry blocks, which were removed after the 2021 trial.

Each replicate includes 12 rows with 200 feet of row, with three treatment locations randomized in a split-plot design (Figure 1). Thus, each plot included 36 rows, split in thirds for the three treatments. We evaluated leafhopper abundances and disease prevalence in the middle four rows and used the other rows as buffer rows to reduce spillover effects of the other treatments.

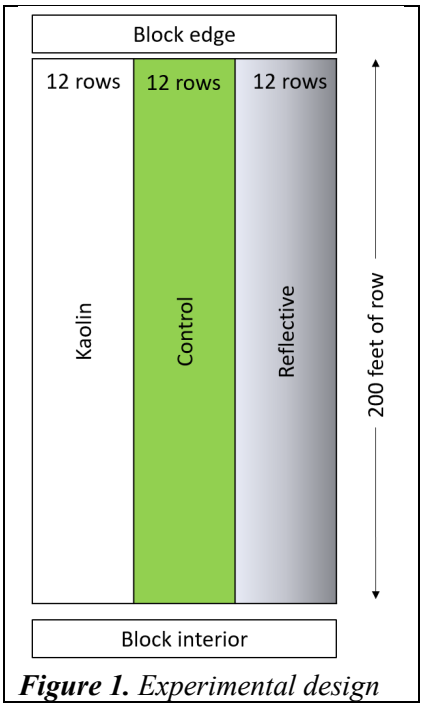

Figure 1. *Experimental design*

Prior to harvest, disease incidence and location within the block was surveyed and recorded for the Yakima region plots. After harvest completion, treatments were applied to assigned plots. In cherry blocks kaolin plots received four kaolin (Surround WP) sprays, one in July, August, September, and October (Table 1) on top of the grower's baseline insecticide program. Kaolin was sprayed at 50 lb/acre and 200 gal/acre. In nectarines, the first application occurred in August, given the later harvest relative to cherries. The postharvest Surround treatment aligns with a typical spray to reduce doubling, and doubling will be recorded in each plot next year. Our order of Extenday was delayed 4 months due to COVID19. Thankfully, our cooperator in the Wenatchee region had Extenday which was deployed in our trial plots from May 27 – October 30. This gave us four replicates of Extenday for the 2020 season. In 2021, we expanded the Extenday treatments to include the 2 Yakima region cherry blocks as well. The nectarine blocks were too small to include Extenday treatments. We did not return to the Wenatchee region blocks that only collected 1 leafhopper in 2020, although collection as part of another project demonstrated there are still extremely low leafhopper numbers there. In our 2021 Wenatchee region cherry blocks we also included a mowing treatment, where the treated area was mowed 5 times instead of 3 for the others. However, this had little effect on the ground cover composition or leafhopper numbers, so for the sake of space we do not present those results.

Figure 2 *5x7 Yellow sticky cards suspended at 2, 4, and 6 ft from the ground.*

After initial treatment application, leafhopper abundance in each treatment (Kaolin clay, Extenday, Control) replicate was monitored using 10 yellow sticky cards (5 × 7 inch) (Fig. 1) in the middle four rows. A yellow sticky card was tied to a cherry tree branch 4 ft from the ground and 25 ft in from each corner of the plot, and two sets of three yellow sticky cards were hung in the middle rows at 2, 4, and 6 ft from the ground using a bamboo pole and braided fishing line (Fig. 2-3). Sticky cards were deployed July 23rd in the Wenatchee region plots and July 31st in the Yakima region plots in 2020 and in 14 and 23 July 2021 in Wenatchee region and Yakima region, respectively. Cards were collected and replaced every two weeks through October, and collected cards were returned to the lab to record leafhopper abundance by species (*Colladonus montanus reductus* and *C. geminatus*). More than 99% of leafhoppers were *C. m. reductus*, so we do not present *C. geminatus* data, apart from 2021 Yakima region cherries that had very few leafhoppers and we then present all known vectors, where we present total vector numbers. Periodical beat sheet sampling was conducted to observe population densities within the tree canopy, but the very low numbers relative to sticky cards suggested it was not an effective method of sampling. During winter months, sticky cards at the Wenatchee region site with all treatments were examined for natural enemy abundance to evaluate non-target effects. To analyze the data we used a generalized linear mixed model, where we assumed a negative binomial error distribution (typical for count data), random effects of replicate block, date, and trap location, a treatment effect, a trap height effect, and a trap by treatment interaction. The random effect for date models variation in leafhoppers over time and accounts for the fact that data collected on the same date will be more similar than those collected on different dates. The random effect for replicate block accounts for the spatial design, that multiple traps occur in the same block and therefore counts within the same block will be correlated, and similarly the random effect for trap location accounts for the fact that the same trap location was sampled multiple times throughout the season. We assumed the control was the baseline

treatment, such that treatment effects model differences between the control and each treatment (Extenday and Surround), the height effect evaluates differences between the heights traps were placed at, and the interactions between trap and height evaluate whether the distribution of leafhoppers across the three heights was different for Surround or Extenday, than in the control blocks.

Table 1. Kaolin clay application timing for cherries and rate by county in 2020. Similar dates and rates were used in 2021.

	KC 1st app	KC 2nd app	KC 3rd app	KC 4th app	Rate
Wenatchee region	Jul 21, 2020	Aug 6, 2020	Sep 4, 2020	Oct 7, 2020	50 lbs/acre 200 gal/acre
Yakima region	Jul 29, 2020	Aug 10, 2020	Sep 9, 2020	Oct 15, 2020	50 lbs/acre 200 gal/acre

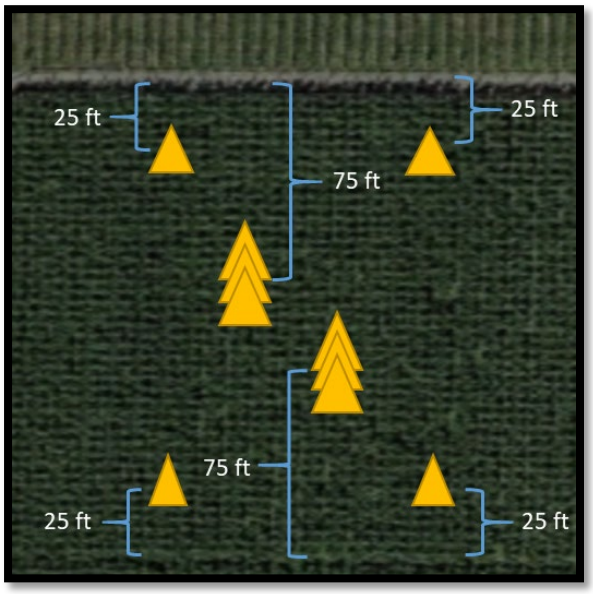

Figure 3 *Trap deployment layout for each Surround, Extenday, and Control experimental plot.*

No-Choice Surround Feeding Study

Kaolin clay (i.e. Surround) covered trees have been shown to reduce feeding and survivorship of other leafhoppers. However, while collecting traps in the Surround sprayed cherry plots, we observed leafhopper presence on leaves frequently. To empirically test if *C. m. reductus* leafhoppers will locate and feed on Surround covered cherry trees we conducted a no-choice feeding study. On

Sep 29 2020, we placed four field collected adult *C. m. reductus* in each of five cages with only Surround covered cherry tree leaves (collected from a sprayed experimental plot (Fig. 7)) and five cages with only non-sprayed cherry trees. We then observed leafhopper feeding behavior at 24, 28, and 46 hrs after initial set-up, recording the number alive, dead, on-plant, off-plant, and actively feeding. Due to space limitations we do not present these data graphically, but the leafhoppers readily feed on both treated and untreated plants.

Results & Discussion:

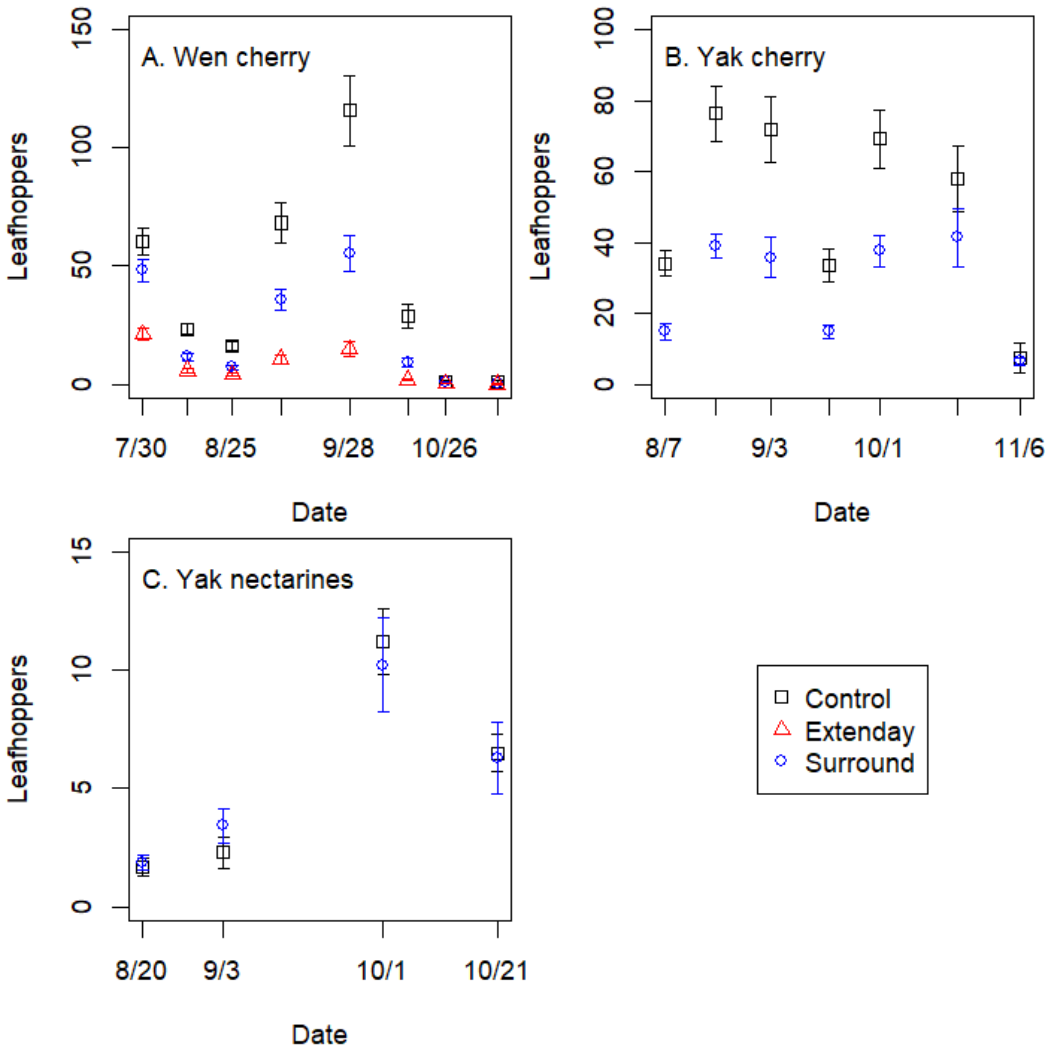

Figure 4. 2020 Leafhopper counts *Mean* C. m. reductus *leafhoppers per trap in 2020 averaged across four plots in control (black squares), Extenday (red circles), or Surround (blue squares) subplots in Wenatchee region cherry blocks (A), Yakima region cherries (B), or Yakima region (C). Dates represent midpoints of 2-week sample periods, except for the September 10th date, which was a 3-week period.*

Objective 1. Surround-kaolin clay.

In 2020, kaolin clay reduced *C. m. reductus* by 47% averaged across the season in each our Wenatchee region (lsmeans comparison: $t = 6.827$, $P < 0.0001$) cherry blocks and our Yakima region

(lsmeans comparison: $t = 8.820$, $P < 0.0001$) cherry blocks, with effects strengthening after the first sample date when only one application had been applied (Figure 4). In addition, leafhoppers were collected at lower heights in treated cherry blocks in Wenatchee (height by Surround interaction: z = -6.080, P < 0.0001), and Yakima (height by Surround interaction: z = -6.502, P < 0.0001) regions.

2021 leafhoppers. As in 2020, in 2021 Surround reduced leafhopper numbers by 48% in Wenatchee region cherries (lsmeans comparions: $t = 23.805$, $P < 0.0001$). This difference is strongest in higher traps (height by Surround interaction: z = -3.879, P = 0.0001; Figure 6), suggesting that the Surround is working to reduce leafhopper movement in and around the tree canopy. In the Yakima region block we observed a 99% drop in leafhopper numbers in control blocks from 2020 to 2021, and in 2021, when leafhopper numbers (all vectors, due to low numbers of *C. m. reductus*) averaged 0.55 per trap we found no significant additional effect of kaolin clay (lsmeans comparion: $t = 2.175$, $P = 0.0777$). However, as in the Wenatchee region it did reduce the height at which leafhoppers were typically caught (height by Surround interaction: $z = -2.179$, $P = 0.0293$). In the Yakima region nectarines, where the harvest date was much later we found no effect on leafhopper numbers within the block in 2020 when leafhopper means were approximately 5, and had only a slight reduction in 2021 when the means were much higher (Figures 4, 5). The effects of Surround reducing the height at which they were captured was not quite significant in 2020 (height by Surround interaction: $z = -1.739$, $P = 0.0820$), but was significant in 2021 (height by Surround interaction: $z = -2.135$, $P = 0.0328$).

Surround effects on predators. In our evaluation of Wenatchee region blocks, the kaolin clay treatment had very little effects on natural enemies, except for lacewings, which had lower densities in the first two weeks of the trial in kaolin-treated blocks (Figure 7).

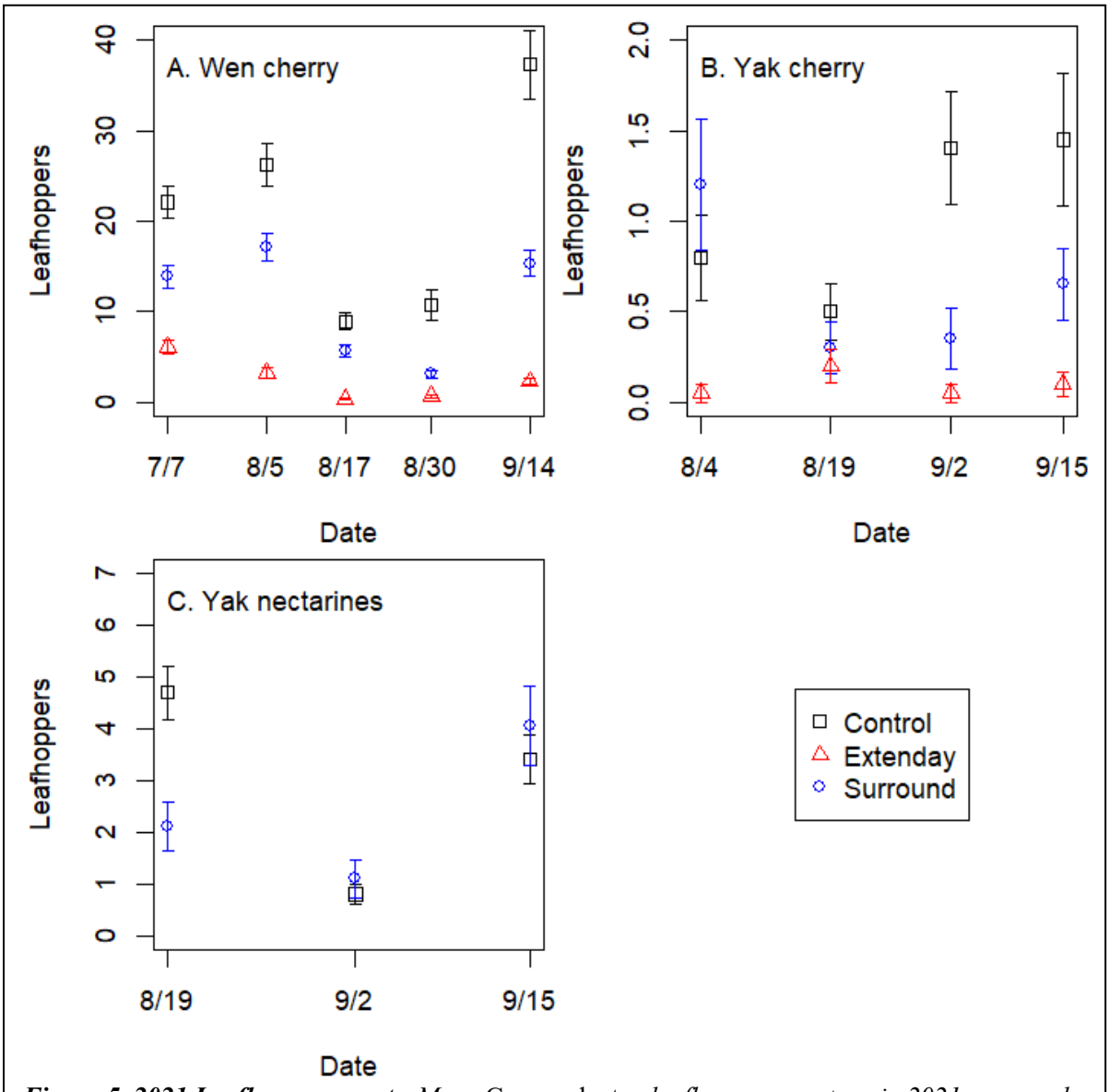

Figure 5. 2021 Leafhopper counts. *Mean* C. m. reductus *leafhoppers per trap in 2021 averaged across four plots in control (black squares), Extenday (red triangles), or Surround (blue circles) subplots in Wenatchee region cherry blocks (A), Yakima region cherries (B), or Yakima region (C). Dates represent midpoints of 2-week sample periods. The Yakima cherry block includes all known vectors combined (*C. m. reductus*,* C. geminatus*,* S. acutus*, and* E. variegatus*), due to a relatively high abundance of all species.*

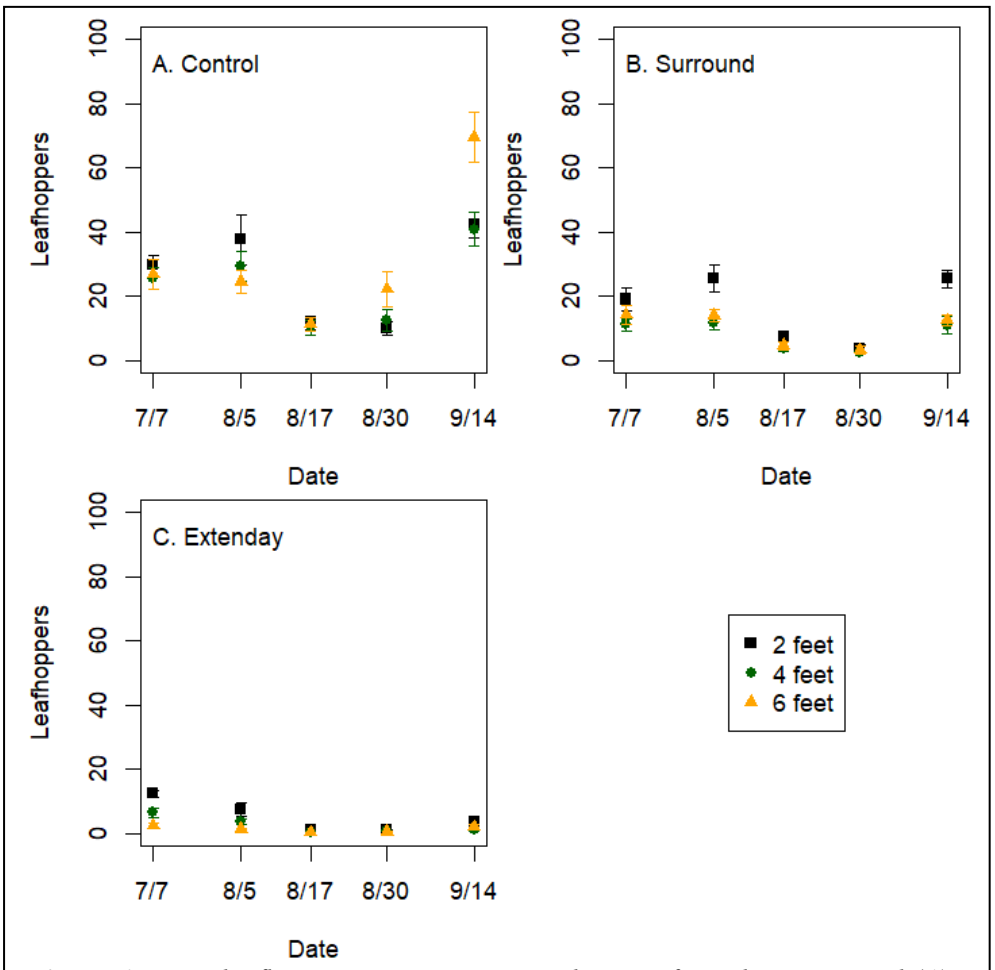

Figure 6. *Mean leafhoppers per trap averaged across four plots in control (A), Surround (B), or Extenday (C) subplots in Wenatchee region cherry blocks, on traps placed at 2 feet (black squares), 4 feet (green circles) or 6 feet (orange triangles). Dates represent midpoints of 2-week sample periods. Data presented are for 2021, and 2020 had similar trends, as do data for Yakima region cherries in 2020 and 2021.*

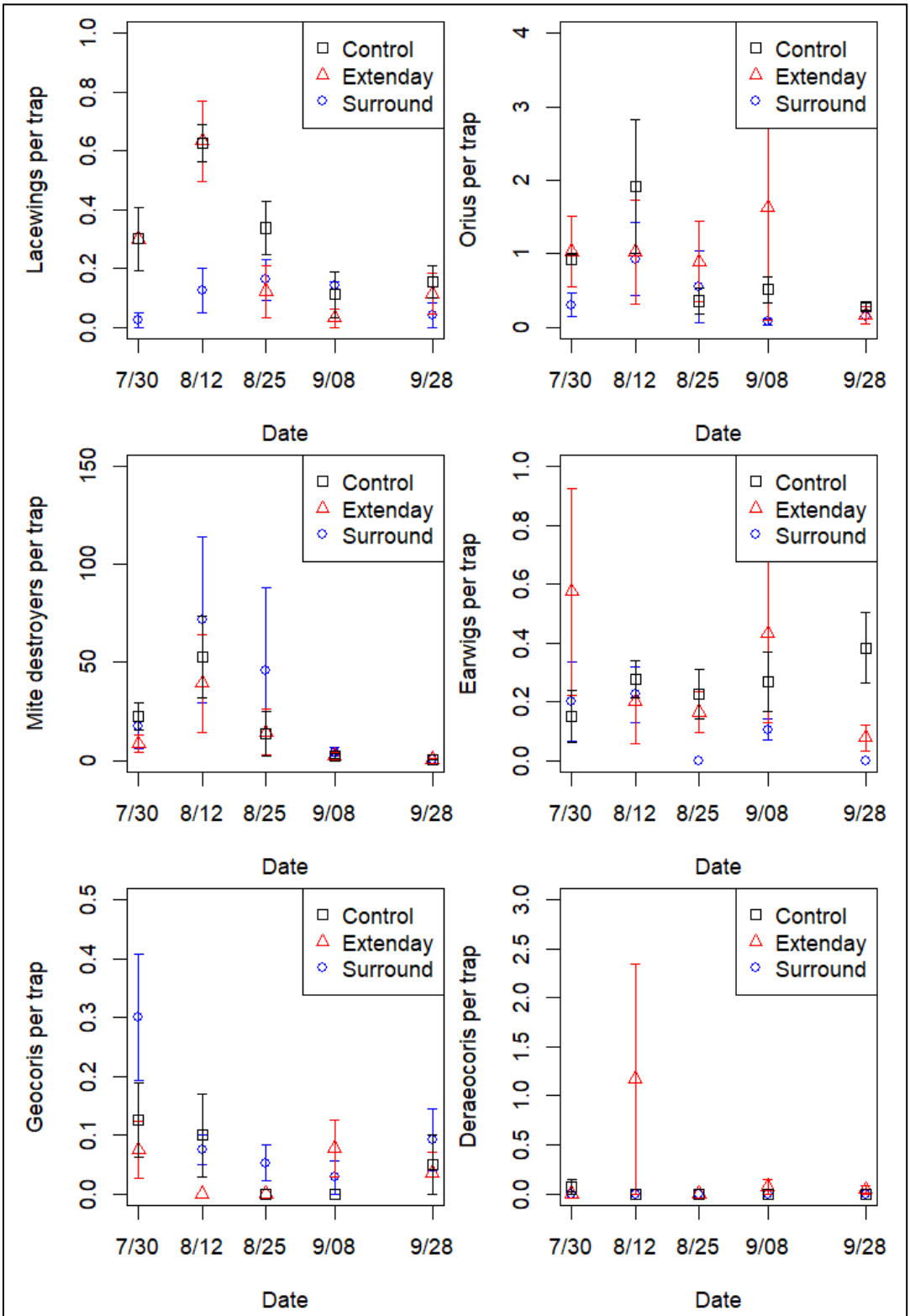

Figure 7. 2020 Predator counts. *Mean predators per trap averaged across four plots in control (black squares), Extenday (red triangles), or Surround (blue circles) subplots in Wenatchee region cherry blocks. Dates represent midpoints of 2-week sample periods, except for the September 10th date, which was a 3-week period.*

Objective 2. Extenday ground cover.

In 2020, Extenday reduced leafhopper numbers by 81% (lsmeans comparison: $t = 13.907$, $P < 0.0001$) and reduced the height at which they were captured (height by Extenday interaction: $z = -2.402$, $P = 0.0163$) in the 4 Wenatchee region cherry plots, providing the best control (Figure 4). Similarly, in 2021, Extenday provided 88% and 91% reductions in Wenatchee (lsmeans comparison: $t = 23.805$, $P < 0.0001$) and Yakima (lsmeans comparison: $t = 5.472$, $P < 0.0001$) region cherries, respectively (Figures 4, 5). The consistency in this control is impressive, given the roughly 50-fold difference in peak leafhopper abundance in the control blocks in the two regions (Figure 5). Extenday also reduced the height at which leafhoppers were caught in Wenatchee region blocks (height by Extenday interaction: $z = -4.907$, $P < 0.0001$), but not in Yakima region blocks (height by Extenday interaction: $z = -0.355$, $P = 0.7229$). These findings suggest that using a ground cover such as Extenday can work to decimate leafhopper populations for high-pressure blocks (e.g., peaks of more than 100 leafhoppers/ trap in Wenatchee 2020 cherry controls) or low-pressure blocks (e.g., peaks of less than 2 leafhoppers per trap in Yakima cherries in 2021). Furthermore, Extenday did not impact the predator community (Figure 5), apart from ground-foraging *Geocoris* bugs, which were rarely collected on sticky traps in any of the treatments.

Given that leafhoppers were collected most in groundcover, the Extenday appears to provide control by simply covering up the weedy hosts that leafhoppers commonly feed on (see continuing report on "Identifying sources of X disease in cherry orchards"). Interestingly, in 2021 detailed analysis of the Wenatchee blocks using Extenday to improve cherry color showed that when Extenday was applied over developing nymphs, the adults appeared shortly after, showing that nymphs can persist under the Extenday. However, once the adults emerged, the populations never recovered, suggesting that the adults did not stay to oviposit under the Extenday cover. Thus, ideal control is likely a pre-harvest nymph treatment paired with Extenday applied after harvest. Furthermore, weed management within tree rows may help with control, as particular traps with higher leafhopper counts were associated with weeds growing over the Extenday from the weed strip. It is likely that it is the physical barrier, rather than the reflective nature of the Extenday that provides the control, but this has yet to be tested.

Objective 3. Phenology and spatial distribution

Phenology. In general, we found three periods of peak abundance of *C. m. reductus* (the most abundant species), in June, August, and September/October (Figure 8). In 2020 Wenatchee region blocks the initial peak was very small, potentially due to control measures in the block and vertical netting surrounding the block. During the first generation we found much higher adult numbers outside the block, indicating low abundance was due to control, rather than phenology. The generation time of *C. m. reductus* is approximately 56 days (Severin & Klostermeyer 1950), so it was feasible that the three peaks of abundance were different generations. To be sure, we collected adult leafhoppers in August from Wenatchee cherries in 2020 and reared them in cages outside the lab. Those adult leafhoppers laid eggs that emerged as adults in October, suggesting that the August and October peaks represent separate generations. Sampling efforts in these plots are ongoing in 2021, as we anticipate leafhopper capture through the end of October.

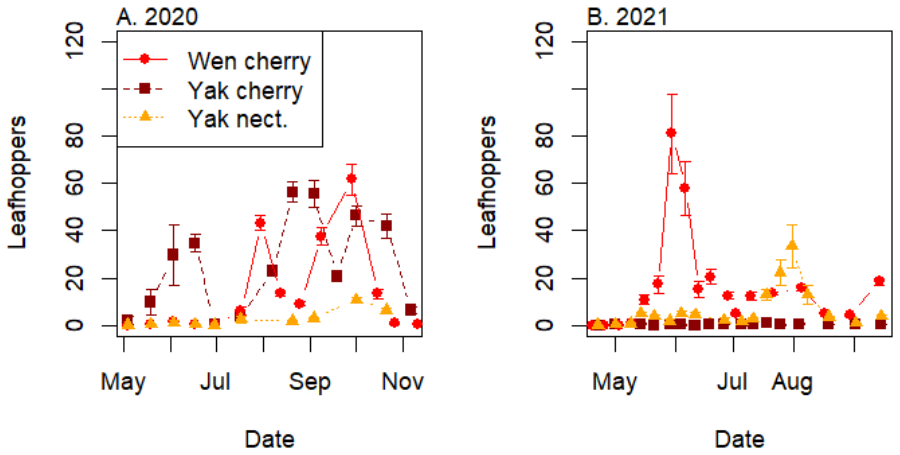

Figure 8. Phenology. *Patterns of abundance in untreated blocks in 2020 (A), and 2021, in Wenatchee region cherry blocks (red circles), Yakima region cherry blocks (dark red squares), or Yakima region nectarine blocks (orange triangles).*

Spatial distribution. We evaluated the spatial distribution of the different treatments to measure the likelihood of edge effects in controlled blocks. Consistently, the mean number of leafhoppers per trap were highest in the center of control blocks, and highest along the edge of Surround-treated blocks, where the edge of the block is adjacent to untreated border rows (Figure 9). This suggests spillover effects along the row where leafhoppers were moving into the Surround-treated blocks. In Extenday-treated blocks the leafhopper numbers were low throughout the block (Figure 9). Given the shortened timeframe of the funded project relative to the lag between transmission and symptoms as well as the initial degree of infection in the blocks (high disease incidence in Yakima region cherries and very low in Wenatchee region blocks), we were unable to evaluate the distribution of disease prevalence.

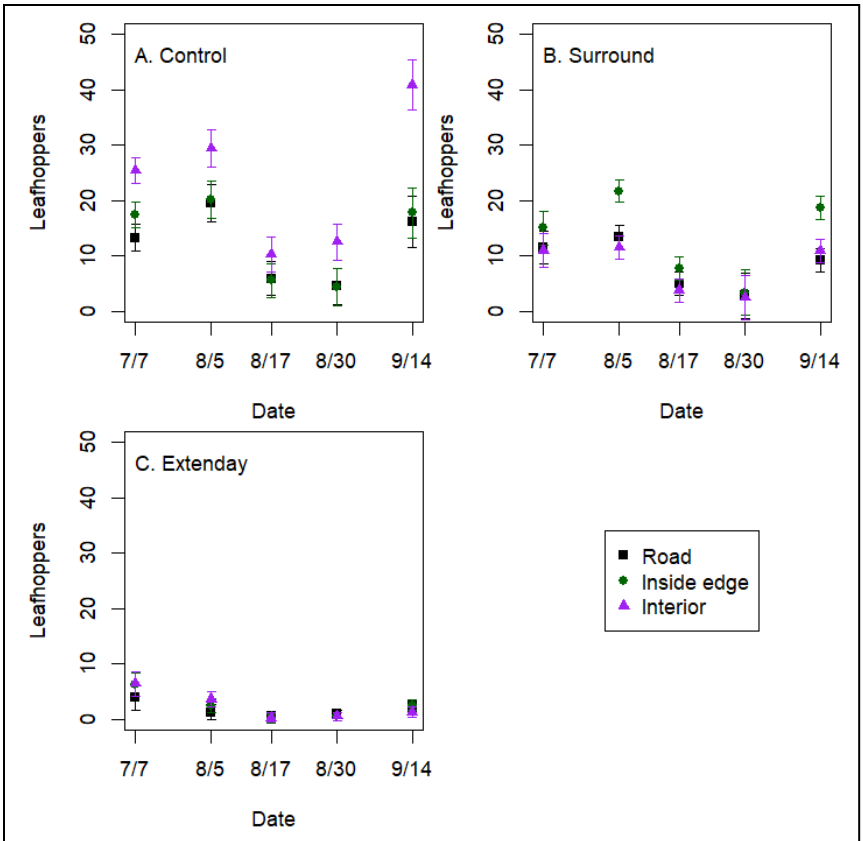

Figure 9. Spatial distribution of leafhoppers. *Abundance of* C. m. reductus *leafhoppers in Wenatchee region cherry blocks in untreated control blocks (A), blocks treated with Surround (B), or treated with Extenday (C). Counts include only traps placed at 4 feet high, either 25 feet from the road (black squares), 25 feet from the edge adjacent untreated buffer trees (green circles), or in the center of the block (purple triangles).While data represent Wenatchee region cherries in 2021, we found similar results in 2020, and Yakima cherries in 2020, and 2021.*

Future plans: We are still collecting leafhoppers from traps in the 2021 season, which extends through October. Given the successful control provided by Extenday, we will use funds provided by a WSDA/USDA Specialty Crop Block Grant to continue Extenday trials and add in a more conventional weed management program to see if similar control can be attained by reducing broadleaf abundance.

References

Severin, H. P., & Klostermeyer, E. C. (1950). *Colladonus geminatus* and *C. Montanus*: Life Histories on Virus-Infected and on Healthy Plants. *Hilgardia*, *19*(18), 553–560.

Executive summary

Project Title: Field evaluation of leafhopper controls for X-disease management

Key words: X-disease, Little cherry disease, *Colladonus* leafhoppers

Abstract:

X-disease is currently at epidemic levels in Pacific Northwest stone fruit orchards. A recent survey of 26% of the PNW cherry industry acreage revealed losses of $120 million due to a combination of X-disease and Little cherry virus 2. While the symptoms of these pathogens are indistinguishable, recent test results suggest the vast majority (93%) of diseased trees are infected with X-disease phytoplasma rather than Little cherry virus 2. The leafhoppers that vector X-disease are abundant after harvest, when phytoplasma titers are high, suggesting this is a key time for phytoplasma transmission. Here, we evaluated two postharvest cultural controls, Surround-kaolin clay and Extenday ground cover, that deter leafhoppers from feeding on trees at the block (Extenday) or plant (Surround) level and potentially prevent transmission. Extenday was effective, reducing leafhoppers by 81% in Wenatchee region cherries in 2020 and 88-91% in Wenatchee and Yakima region cherries in 2021, compared to controls. In year two there was a 50-fold difference in peak densities across the control blocks in Wenatchee and Yakima regions, but these effects were consistent. In 2020 and 2021 high-pressure cherry blocks in the Wenatchee and Yakima region, Surround reduced season-wide leafhopper numbers by 47-48%. Furthermore, the Surround reduced the mean height at which leafhoppers were caught compared to the control plots, suggesting they may not be moving into the trees as much. In Yakima region cherries the clay did not significantly reduce leafhopper numbers, but mean counts were very low, peaking at less than 2 leafhoppers per trap (2-week trapping period). Surround also did not reduce leafhopper numbers in nectarine blocks, potentially due to the late harvest date. In summary, we have identified two cultural controls, with one (Extenday) effective at reducing leafhopper numbers in either high pressure or low pressure blocks, and another (Surround) that reduced leafhopper numbers in high pressure blocks, although not to the same effect as Extenday and also appeared to reduce the movement up into trees from the groundcover.