Project Title: Tactics to improve natural enemy releases in tree fruit

Report Type: Final Project Report

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Cooperators: Steve Arthurs (BioBee); Chuck Weaver (G.S. Long & Parabug); Brent Milne (McDougall Fruit); Dave Keller, Sean Gilbert, Rob McGraw, & Tony Mena (Gilbert Fruit), John Haas & Matt Klaus (G.S. Long), Mike Brown (Gebbers Farms), Teah Smith (Zirkle Fruit), Greg Newman (NWFM) [note: pear grower cooperators will be specified in pear report]

Project Duration: 2-Year, 3 Year

Total Project Request for Year 1 Funding: $102,558*
Total Project Request for Year 2 Funding: $106,033*
*50% by WTFRC Apple Crop Protection, 50% by FPC/PPC Pear

Other related/associated funding sources:
<u>**Awarded**</u>

Funding Duration:	2020-2023
Amount:	$36,614
Agency Name:	BioBee
Notes:	In-kind match of commercial insectary insects, Artemia (brine shrimp cysts on tape), and shipping costs for beneficials to be used in this project. Itemized estimate provided by BioBee.

Funding Duration:	2020-2023
Amount:	$720
Agency Name:	Parabug, Chuck Weaver private contractor
Notes:	In-kind match of drone pilot labor for releasing insects as part of Obj. 2. ~$18/acre × 10 drone-treated acres per trial × 2 trials (apple & pear) × 2 years.

Funding Duration:	2021-2022
Amount:	$29,968
Agency Name:	Western IPM Center, project initiation grant
Notes:	This project expanded the efforts in this grant by providing support

to conduct grower input sessions and a needs assessment survey. The WIPMC grant was also used to start a grant team and stakeholder advisory group that submitted the WSARE grant (below).

Funding Duration: 2020-2023
Amount: $348,733
Agency Name: Western SARE
Notes: This was a complementary (non-overlapping) project, specifically focusing on earwig releases in apple and pear, on the ground and by drone.

<u>Requested</u>
Funding Duration: June 2024 – May 2027
Amount: $350,000
Agency Name: Western Sustainable Agriculture Research and Education (WSARE)
Notes: This project proposal used the data gathered from "Tactics to improve natural enemy releases in tree fruit" to develop targeted questions that will allow for the creation of best management practices for lacewing releases in tree fruit.

Funding Duration: June 2024 – May 2027
Amount: $81,139
Agency Name: Washington Tree Fruit Research Commission (ACP)
Notes: The WSARE proposal above includes funding for one lead technician's salary and extension activities. Due to budget limitations, we were unable to request salary for additional research support. Therefore, this funding request is for an assistant for the lead technician so that the research can be completed. We will be informed of the funding decision in March.

Funding Duration: 2024-2026
Amount: >$15,000
Agency Name: BioBee
Notes: In-kind match for the above WSARE project; commercial insectary lacewings (Awarded: will receive if the above is funded)

Funding Duration: 2024-2026
Amount: ~$7,500
Agency Name: Zirkle Fruit
Notes: In-kind match for the above WSARE project; commercial insectary lacewings and drone pilot labor/fees (Awarded: will receive if the above is funded)

Funding Duration: June 2024 – May 2027
Amount: $109,581
Agency Name: Washington Tree Fruit Research Commission (ACP) & Fresh and Processed Pear Committee Research
Notes: New funding request to pursue research on whirligig mite releases and conservation. In addition to unrelated work in potatoes, this proposal was brought about by results from Obj. 1 of this project and other projects in pears.

WTFRC Collaborative Costs: None

Budget 1*

Organization Name: USDA-ARS **Contract Administrator:** Mara Guttman
Telephone: 510-559-5619 **Email address:** mara.guttman@usda.gov
Station Manager/Supervisor: Rodney Cooper **Email Address:** rodney.cooper@usda.gov

Item	2021	2022
Salaries[1,4]	$17,458	$17,894
Benefits[1,4]	$5,587	$5,726
Wages	$0	$0
Benefits	$0	$0
Equipment	$0	$0
Supplies[2]	$6,500	$6,500
Travel[3]	$0	$0
Miscellaneous	$0	$0
Plot Fees	$0	$0
Total	**$29,545**	**$30,120**

Footnotes:
[1]GS-5 technician for 6 months per year, 100% FTE at 32% benefits, Year 2 includes 2.5% COLA increase. Technician would assist WSU postdoc (see below) with sampling in all locations. This technician will also assist the postdoc with surface sterilization and PCR for gut content analysis.
[2]Funds to purchase PCR reagents and other PCR supplies for gut content analysis, trapping supplies, and some commercial nutritional supplement products (others provided as in-kind match).
[3]Fuel to field sites will be provided by USDA base funds and is not requested.
*50% by WTFRC Apple Crop Protection, 50% by FPC/PPC Pear
[4]This funding (both years) has been deobligated by USDA-ARS and WTFRC has made it available for WSU, to partially support a graduate student who is assisting with this project

Budget 2*
Organization Name: WSU
Contract Administrator: Stacy Mondy
Contract administrator email address: anastasia.mondy@wsu.edu
Station Manager/Supervisor: Chad Kruger **Email Address:** cekruger@wsu.edu

Item	2021	2022
Salaries[1]	$52,827	$54,940
Benefits[2]	$18,373	$19,108
Wages[3]	$1,200	$1,248
Benefits[3]	$113	$117
Equipment	$0	$0
Supplies	$500	$500
Travel	$0	$0
Miscellaneous	$0	$0
Plot Fees	$0	$0
Total	**$73,013**	**$75,913**

Footnotes:
[1]Nottingham salary ($7,612.50/mo × 12 mo × 2% FTE = $1,827 Year 1, Year 2 reflects 4% COLA increase) + Postdoc salary ($4,250/mo × 12 mo × 100% FTE = $51,000 Year 1, Year 2 reflects 4% COLA increase). Nottingham to supervise data collection efforts in pear in the Wenatchee area and advise on project methods and data summary. WSU Postdoc will be based at the USDA-ARS facility in Wapato, WA and supervised by Schmidt-Jeffris. The postdoc will be responsible for leading data collection and summarizing project results. Due to difficulties in finding a qualified postdoc candidate, we have expanded our search to also include an associate in research, which would have a similar salary, but be hired at the M.S. level. The associate in research (Daniel Hausler) was hired in early 2022.
[2] Benefits rate for Nottingham is 29.9% ($547 Yr 1, $569 Yr 2). Benefits rate for postdoc is 35% ($17,826 Yr1, $18,539 Yr2).
[3]Summer technician at $15/hr×8 hr/wk ×10 wks, 9.4% benefits rate, salary includes 4% COLA increase in Year 2
*50% by WTFRC Apple Crop Protection, 50% by FPC/PPC Pear

Note: This report contains apple-related content only. Pear results will be presented in the pear report. Findings from preliminary work (2020-2021) are also included to provide context for the chosen treatments and results of the 2022-2023 research.

OBJECTIVES

Obj. 1. Improve retention of released natural enemies. We tested whether commercially available food supplements (Artemia cysts on tape, *Ephestia* eggs on cards) and lures (methyl salicylate) increased retention of released natural enemies and also examined whether they recruited resident natural enemies and decreased pest populations. Only Artemia cysts were used in 2023 (*Ephestia* eggs were dropped). All fieldwork and pest/natural enemy counts are completed and analyzed for this project, but the molecular work is not yet complete. Several factors caused significant delays, including a move to a new lab space (which needed repairs before use) and the need to change our gut content protocols; we determined that neither pear psylla nor orchard aphid pests amplify well with COI universal primers. To overcome this, a colleague (B. Ohler) designed a pear psylla primer and we adapted aphid primers from another lab – these must be run as a separate PCR from the COI primers, increasing the number of samples we are running. Finally, the need to identify lacewings using molecular techniques (see below) added many additional samples to our workflow. The molecular work will be completed before the project term date (June 2024).

Obj. 2. Determine cost-effectiveness and efficacy of natural enemy release by drone. In 2022, this objective was modified to include comparison of additional treatments, including mealybug destroyer larvae, lacewing cards, multiple species of lacewings, and releasing lacewings as larvae versus eggs. We determined that the 0.25-acre plot trials were not an adequate method for testing drone releases and instead focused entirely on various ground-based methods for releasing lacewings in 2023. An objective specifically testing lacewing releases by drone at a large scale was included in the proposed WSARE project (see other/related funding sources).

SIGNIFICANT FINDINGS

Mealybug destroyer releases are likely not a viable tactic for controlling mealybugs in orchards. They were marginally successful in the 2020 trial, but in the three following trials (2021-2023), they did not lower mealybug counts and rapidly dispersed from the orchard. Because this insect costs $680-950/acre to release, the low chance of success means that fruit growers should avoid using this insect and other options should be explored for mealybug control.

Convergent ladybeetles tested as part of the 2023 mealybug destroyer study (as a comparison treatment) also rapidly dispersed from the orchard and did not lower mealybug counts. This provides preliminary evidence that adult ladybeetles are not suited for orchard releases, but should be investigated further.

Lacewing identification became a critical component of this project. We determined that the "*Chrysoperla carnea*" we purchased for trials in 2021 were actually *C. externa* (purchased as larvae) and *C. plorabunda* (purchased as eggs). *Chrysoperla externa* can be separated from other lacewings visually under magnification, but to distinguish between "resident" lacewings and the released *C. plorabunda*, we had to develop molecular methods. We determined that the COI gene, which we are using in our gut content analysis, can also be used to separate resident from released lacewings. It is important to note that the lacewing species present in orchards that is often referred to as "*C. plorabunda*" is likely *C. johnsoni* and therefore a different species that what is commercially available. However, *C. plorabunda* is native to Washington (found outside of orchards) and therefore likely to be a better climate match that *C. rufilabris*.

Lacewing releases varied in efficacy. In 2021, releases of *C. plorabunda* eggs or *C. rufilabris* larvae reduced aphid abundance by 57% and 43%, respectively. In the following two years, none of the lacewing release treatments reduced aphid abundance. We attributed this to very high initial aphid counts in 2022 and poor survival of released lacewings in 2023, potentially due to the use of organic pesticides. In general, low numbers of released lacewings were recovered, but recovery was very poor in 2023 compared to 2021-2022. Therefore, when determining efficacy of beneficial releases, scouts should focus on pest numbers, not necessarily natural enemy recovery; however, presence or absence of the released natural enemy can give some sense of survival. We determined that releases need to be conduced earlier in the season than anticipated (mid-April in southern Washington) to get an adequate head-start on aphids.

Tactics for retaining and recruiting natural enemies had highly variable results between sites and years. In general, methyl salicylate lures showed some promise for recruiting lacewings and *Stethorus*. Food supplements may have increased *O. insidiosus* retention.

***Orius insidiosus* releases** were performed as part of the retention experiments, but data from these trials also allowed us to access the efficacy of this predator for pest control. One release of *O. insidious* (2,000/acre) reduced adult thrips on sticky cards by 50% in both apple trials. Evaluations of thrips damage did not occur as part of this work, but should be included in future studies. More frequent releases (at lower rates) may be more effective and economical.

Whirligig mite was found in abundance on beat trays in some of our study locations. The role of this predator in North American orchards has received little attention, but research from Ireland and preliminary work from other projects suggest that it may be an important orchard natural enemy. It recently became available for purchase in the U.S. (Oregon only).

Grower survey and discussion, 2021-2022. Leveraged funding from the Western IPM Center allowed us to conduct a grower survey and a series of listening sessions (in collaboration with Tianna DuPont and Ashley Thompson). 132 growers and consultants responded, representing 43,868 apple and pear acres. 37 respondents (28%) are using biocontrol releases occasionally or annually on 7,842 acres costing them $153 per acre on average. The main natural enemies they are releasing are lacewings (29%), lady beetles (28%), and predatory mites (25%). The main barrier to adoption of releases was lack of knowledge/recommendations on how to release successfully (52%). Five stakeholder input sessions were conducted in 2021-2022 in Omak, Wenatchee, Yakima, Hood River, and Medford with a total of 60 participants. The input sessions identified the following as critical research areas: (1) information to make natural enemy releases more effective/useful, (2) evidence of efficacy, (3) what species to release, (4) where to purchase, (5) release timings, (6) release rates, (7) a list of common release mistakes and how to avoid them, (8) on farm success stories, (9) consistent supply, (10) proper placement in the tree/orchard, and (11) pesticide toxicity to natural enemies. Information from the survey and sessions was used to support the pending WSARE grant application to expand the work on lacewings.

RESULTS AND DISCUSSION

Obj. 1. Improve retention of released natural enemies

The study was conducted two commercial organic apple orchards (Wapato, WA in 2022 and Benton City, WA in 2022). The Wapato orchard had a very high ant population, so we changed locations in 2023 in case the ants were affecting our release treatments. The Wapato orchard primarily had rosy apple aphid (RAA) with some green apple aphid (GAA), whereas the Benton City orchard had primarily RAA with some woolly apple aphid (WAA). The Benton City orchard was chosen in part because it typically has serious WAA infestations, but overall aphid abundance was uncharacteristically low in 2023.

There were five treatments consisting of combinations of lure use (Predalure, methyl salicylate), food supplements (Artemia, brine shrimp cysts on tape Fig. 1 + *Ephestia* eggs on cards), and releases (100,000 "*C. carnea*" lacewing eggs + 2,000 *Orius insidiosus* per acre): (1) Predalure + Foods + Release, (2) Predalure + Release, (3) Food + Release, (4) Release only, and (5) No-release control. In

Fig. 1. Ladybeetle feeding on Artemia tape

2022, the "Food" treatment only used Artemia tape (the *Ephestia* eggs were dropped). Rates for the food treatments and lures were: 1 lure/plot, 50 m Artemia tape/plot, and 35,000 *Ephestia* eggs/plot (1 card/30 tags). Each combination was replicated in the orchard 5 times in 0.25-acre plots. Aphids and lacewings were counted prior to release and then once weekly after release. Aphids were counted in the field by counting the number of infested leaves (GAA, RAA) or number of colonies (WAA) per 3 shoots each on 9 trees in the center of the plot. Beat tray samples were collected from the 9 center trees of each plot and all natural enemies from the tap counts were collected and stored in ethanol for identification and use in molecular gut content analysis. Two sticky cards were also hung in each plot to monitor adult natural enemies. The "*C. carnea*" have been tentatively identified as *C. plorabunda* (see lacewing results in Obj. 2).

We conducted an additional study using a similar design in the USDA research orchard in Moxee, WA. Because of the size of the orchard, we removed the no-release treatment. In both years, we only tested the Artemia tape (no *Ephestia* eggs). In 2022, we only released *O. insidiosus*, while in 2023, we also released *C. carnea* eggs.

In the six trials (2 commercial apple, 2 research apple, 2 pear), only 8 *O. insidious* were recovered. However, the consistent decrease in thrips counts in plots where *O. insidiosus* were released indicates that this predator remained in plots long enough to reduce pest populations. Although it was most commonly found 1-2 weeks post-release, in the 2022 commercial apple and 2023 research apple orchards, *O. insidiosus* were found over a month after release. This species is not native to Washington and has never been found in an area where it was not recently released, therefore all recovered *O. insidiosus* are from that year's releases. Of the few *O. insidiosus* found, 75% of them were recovered from plots with supplementary foods. The two individuals recovered from plots without foods were found one month post-release, when the foods were likely completely consumed/decayed. Therefore, there is some evidence that the Artemia tape increased retention of *O. insidiosus* in the field. In future studies examining efficacy of *O. insidiosus* for thrips control in apples, the use of releases in combination with Artemia tape should be explored.

Molecular identification of the *carnea*-group lacewings recovered from the retention trials is ongoing (see lacewing release results in Obj. 2 for more information on lacewing identification). All samples have been processed and sequenced. Sequences have been aligned and we are currently constructing computationally-intensive phylogenetic trees to determine which collected individuals "match" the controls directly removed from insectary bottles. This analysis is anticipated to be completed in February 2024. Based on preliminary analysis, no treatment increased

Commercial apple

	Lures		Foods	
	2022	2023	2022	2023
Aphids	increase	not abundant	no effect	not abundant
Thrips	increase	no effect	no effect	slight decrease
Campylomma	no effect	increase	no effect	no effect
Lacewings	slight increase	no effect	no effect	no effect
Ladybeetles	no effect	no effect	no effect	no effect
Stethorus	increase	no effect	no effect	decrease
Syrphids	no effect	not abundant	no effect	not abundant
Whirligigs	not abundant	slight decrease	not abundant	no effect
Spiders	no effect	no effect	no effect	no effect

Research apple

	Lures		Foods	
	2022	2023	2022	2023
Aphids	not abundant	decrease	not abundant	no effect
Thrips	decrease	no effect	no effect	no effect
Brown mites	decrease	no effect	no effect	no effect
Apple rust mite	no effect	no effect	no effect	no effect
Campylomma	not abundant	not abundant	not abundant	not abundant
Lacewings	increase	no effect	no effect	no effect
Ladybeetles	no effect	no effect	no effect	no effect
Stethorus	increase	no effect	no effect	decrease
Syrphids	not abundant	increase	not abundant	no effect
Whirligigs	not abundant	no effect	not abundant	increase
Spiders	increase	no effect	no effect	no effect

Results summary from retention trials.

retention of released lacewings. However, applications of methyl salicylate lures timed for approximately when released lacewings become adults (as opposed to during the release) may increase the likelihood that the adults remain in the orchard.

In the commercial apple orchard in 2022, releases of *O. insidiosus* and *C. plorabunda* decreased green apple aphids by ~50% compared to the no-release control (Fig. 2). Rosy apple aphids were also present but appeared to be unaffected by our treatments. Seasonal thrips counts were reduced by 30% and counts immediately after release were reduced by 50% (Fig. 3). Surprisingly, all combinations of the lure and food treatments increased green apple aphid abundance relative to the release-only treatment – bringing aphid levels back to nearly that of the no-release control (Fig. 2). It is possible that these treatments caused changes in the natural enemy community within the plots, potentially resulting in competition or increased intraguild (between natural enemies) predation that may have reduced aphid biological control. Lures also increased abundance of *Stethorus* by 62%. Pest mite populations were very low in this orchard, so we could not determine if the increased *Stethorus* populations in Predalure plots resulted in improved biological control.

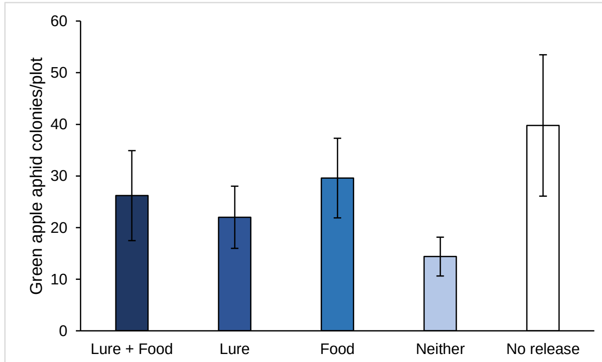

Fig. 2. Releases decreased GAA, but foods and lures increased GAA. 2022 seasonal sums.

In the 2023 commercial apple orchard, aphid populations were too low to discern differences between treatments. Pest thrips seemed to increase in the “release only” treatment, but this effect was not seen in the treatment with releases in combination with food (Fig. 4). Lure treatments had slightly more *Campylomma* and fewer whirligig mites. In this trial, *Stethorus* did not increase in the lure treatments, but instead decreased in the food treatments.

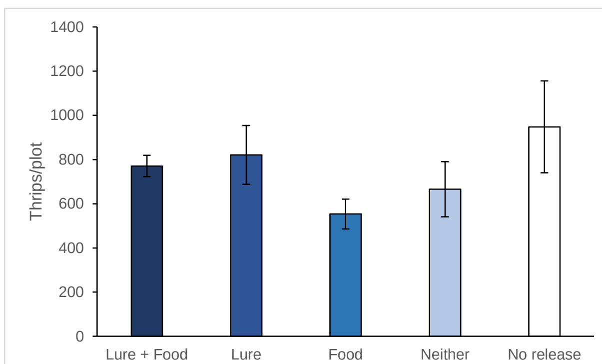

Fig. 3. Releases decreased thrips, especially in the food treatment. 2022 seasonal sums.

In the 2022 retention trial in the Moxee research orchard, lure treatments had fewer brown mites (Fig. 5). Thrips were 48% less abundant in plots with lures. Because this trial was conducted in July, aphids were scarce and the effects of treatments on aphids could not be assessed. Lures increased abundance of lacewings by 100% and spiders by 50%. Surprisingly, *Stethorus* populations were 45% lower in lure plots – they appeared to just “follow” where brown mites were higher.

In 2023, the Moxee trial was conducted earlier in the season. Aphid counts were halved in plots with lures (Fig. 6). Lacewing and *Stethorus* abundance did not differ between treatments, but syrphid counts were higher in the lure treatments; syrphids may have been responsible for the decrease in aphid abundance in the lure treatments. Whirligig mites were more abundant in the food treatments.

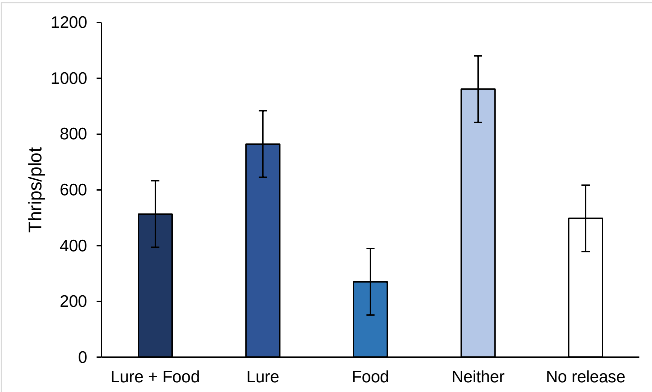

Fig. 4. Releases decreased thrips, but only in the food treatment. 2023 seasonal sums.

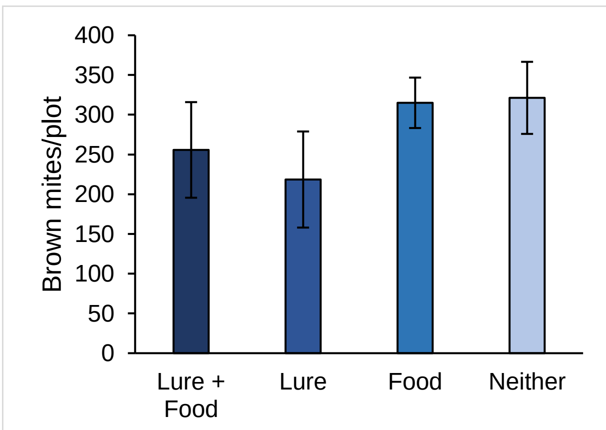

Fig. 5. Lures decreased brown mites. 2023 seasonal sums.

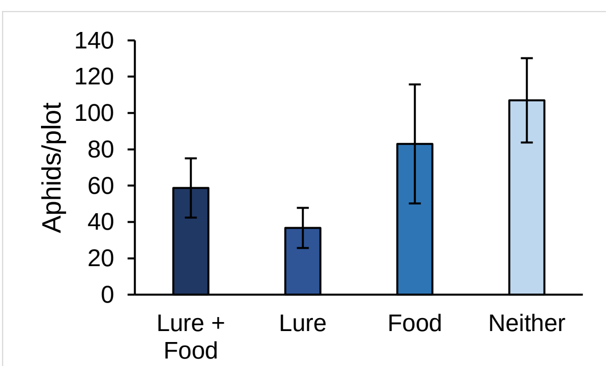

Fig. 6. Lures decreased aphids. 2023 seasonal sums.

Between sites and across years, there was very little consistency in the effects of the treatments. Taken in combination with the pear data, *Stethorus* generally increased in plots with lures and may exert control on mites while rapidly moving between plots. Lacewings also showed a similar, although weak, trend. Because natural enemies interact with each other and pests over time, it is difficult to discern if changes in natural enemy abundance due to treatments are due to predation amongst themselves or changes in pest densities. The gut content work, which should be completed by June 2024, may provide additional information about these relationships.

Obj. 2. Determine cost-effectiveness and efficacy of natural enemy release by drone

Year	Mealybug Destroyer Treatments
2020	5,000 adults/acre by ground May 14
	5,000 adults/acre by ground June 17
	2,000 adults/acre by ground June 17
	5,000 adults/acre by drone June 17
	No release control
2021	1,000 adults/acre by ground on May 27
	1,000 adults/acre by drone on May 27
	No release control
2022	2,000 adults/acre by ground May 20
	2,000 larvae/acre by ground May 18
	2,000 adults/acre by ground May 20
	No release control
2023	2,000 adults/acre by ground on June 22
	"Grower standard" = 20,000 convergent ladybeetle adults/acre by ground on June 23
	No release control

Mealybug destroyers, 2020-2023. All trials were conducted in organic commercial apple orchards. In 2020, mealybug destroyer adults released by hand in either May or June caused a numerical ~3× decrease in mealybug populations compared to the no-release control, but this effect was highly variable between plots. The drone release did not cause a decrease. Recovery of the mealybug destroyers was moderate 1 and 2 weeks after the early season release: ~3 per plot, then ~1 per plot, respectively (27 then 9 per acre). After this period, only 1 mealybug destroyer was found across all plots each week for the rest of the sampling period (0.5 mealybug destroyers/acre). The plots in this trial were small (0.11 acres), so we sought to conduct the work at a larger scale the following year.

In 2021, we examined mealybug destroyer releases in one-acre plots (five replicates/treatment), comparing drone versus ground releases of 1,000 mealybug destroyers per acre to a no-release control. We found very few mealybug destroyers 1 day after release and no mealybug destroyers 8 days after release; they likely dispersed due to low pest density in this orchard. The 1-day recovery was lower in the drone (0.4/acre) compared to the hand-release treatment (3.3/acre). There were no differences between treatments in mealybug counts.

In 2022, mealybug destroyers released as larvae or adults were never recovered after release, although there were enough mealybugs in plots for them to feed on. There were no differences in mealybug counts between treatments. A series of organic fire blight and mildew sprays made during the releases may have negatively affected the mealybug destroyers: Serenade+Previsto on May 19, ProBlad Verde+Merivon+FireLine+FireWall on May 20, and Serenade+Previsto on May 23. The effects of these pesticides on natural enemies are not well described. In a preliminary lab trial, we found that Serenade at field rate did not cause any mortality in adult mealybug destroyers after 24 h, but follow-up studies are needed.

In 2023, we sought to compare mealybug destroyers to a “grower standard”: releases by the grower of convergent ladybeetles (20,000/acre). No mealybug destroyers were recovered post-release. One convergent ladybeetle was found pre-release (indicating that they were already present in the orchard) and three were found throughout the entire four-week post-sampling period. These may have been released or “resident” ladybeetles. The treatments did not differ in mealybug counts. Mealybug destroyers do not appear to be a reliable control method for mealybugs in apples and cannot currently be recommended due to their high cost (~$0.30/insect).

Year	Lacewing Treatments	Release Date
2021	100,000 "*C. carnea*" eggs/acre sprinkled	May 5th
	20,000 "*C. carnea*" larvae/acre sprinkled	
	100,000 *C. rufilabris* eggs/acre sprinkled	
	20,000 *C. rufilabris* larvae/acre sprinkled	
	No release control	
2022	100,000 "*C. carnea*" eggs/acre sprinkled	May 12th
	100,000 *C. rufilabris* eggs/acre sprinkled	May 4th
	100,000 *C. rufilabris* eggs/acre cards	May 4th
	100,000 *C. rufilabris* eggs/acre drone	May 5th
	20,000 *C. rufilabris* larvae/acre sprinkled	May 4th
	No release control	
2023	100,000 "*C. carnea*" eggs/acre sprinkled	May 11th
	100,000 "*C. carnea*" eggs/acre cards	
	100,000 *C. rufilabris* eggs/acre sprinkled	
	100,000 *C. rufilabris* eggs/acre cards	
	20,000 *C. rufilabris* larvae/acre sprinkled	
	No release control	

Lacewing release efficacy, 2021-2023. All trials were conducted in organic commercial apple orchards. In 2021, we purchased *Chrysoperla rufilabris* (Fig. 7) and “*Chrysoperla carnea*”. We found that the *C. carnea* larvae (which came from a different insectary than the “*C. carnea*” egg order) were actually *C. externa*. Both *C. externa* and *C. rufilabris* can be visually distinguished from other lacewings under magnification. Species of *Chrysoperla* lacewings in the “*carnea*-group” cannot be separated using visual characteristics. This includes the insectary “*C. carnea*” sold as eggs and our native *Chrysoperla* lacewings (*C. johnsoni* is the most common). The most reliable method of identification is

Fig. 7. A released *C. rufilabris* seen in the orchard in post-release sampling

mating song analysis, which requires multiple, live specimens of both sexes. We determined that the COI gene (although it cannot be used to definitively identify a lacewing to species) can be used to “match” DNA sequences of field-collected lacewings to those taken directly from the shipping container; this method can therefore distinguish “released” and “resident” lacewings. A collaborating lacewing biologist (K. Taylor, University of Maryland) will conduct song analysis on specimens from this insectary in 2024 to provide a definitive identification. Preliminary comparison of genetic sequences from other insectaries indicates this is likely *C. plorabunda*.

In 2021, the *C. plorabunda* egg and *C. rufilabris* larvae releases reduced aphid counts compared to the control (Fig. 8), but the other treatments did not. *Chrysoperla externa* (“*C. carnea*” larvae) is likely a poor climate match for our area because it is native to the southeastern U.S. Low numbers of larvae of the released lacewing species were found throughout the trial (1-5 per treatment, across 8 weeks of sampling) (Fig. 9). Therefore, when determining efficacy of beneficial releases, scouts should focus on pest numbers and the presence/absence of the released natural enemy, not necessarily the abundance of the released natural enemy. We also found several species of native, non-released *Chrysopa* lacewings. *Chrysopa* larvae were not found until three weeks after our releases and then in lower numbers than our released lacewings (Fig. 9). This indicates that our treatments gave this orchard a head start in aphid management compared to the no-release control. All adult lacewings that were found during the trial were *Chrysopa*, therefore we did not find evidence that the released lacewing larvae fully developed and reproduced.

In 2022, none of the treatments caused a reduction in aphids (Fig. 8). Pesticide applications were made much more frequently in this orchard compared to the orchard used in 2021. Initial aphid counts were 13-times higher in this trial than in 2021 (Fig. 8). However, recovery of lacewing larvae was higher than in 2021 (Fig. 9). None were recovered from the drone treatment or the control (Fig. 9). There were also resident populations of *Chrysoperla* lacewings, which also would have contributed to biological control. This provides some initial evidence that drones may be a poor delivery mechanism for lacewing eggs, but this should be further evaluated in larger plots, potentially with larvae. Two adult *C. rufilabris* were found during the trial, indicating that this species can complete development in Washington orchards. Additionally, the *C. rufilabris* larvae found 8-weeks post-release (Fig. 9) is likely a second generation, indicating that the adults reproduced in the orchard. Genetic analysis of the *carnea*-group adults is still being conducted to determine if any of the released insectary *C. plorabunda* reproduced in the orchard. These results indicate that early season organic spray programs should be evaluated for effects on lacewings.

In 2023, applications of organic pesticides (including spinosad) were made more frequently than the prior two trials. Genetic analysis of the *carnea*-group adults is still being conducted to determine if any of the released insectary *C. plorabunda* reproduced in the orchard. None of the treatments differed from the control in aphid counts (Fig. 8) and recovery of released lacewings was

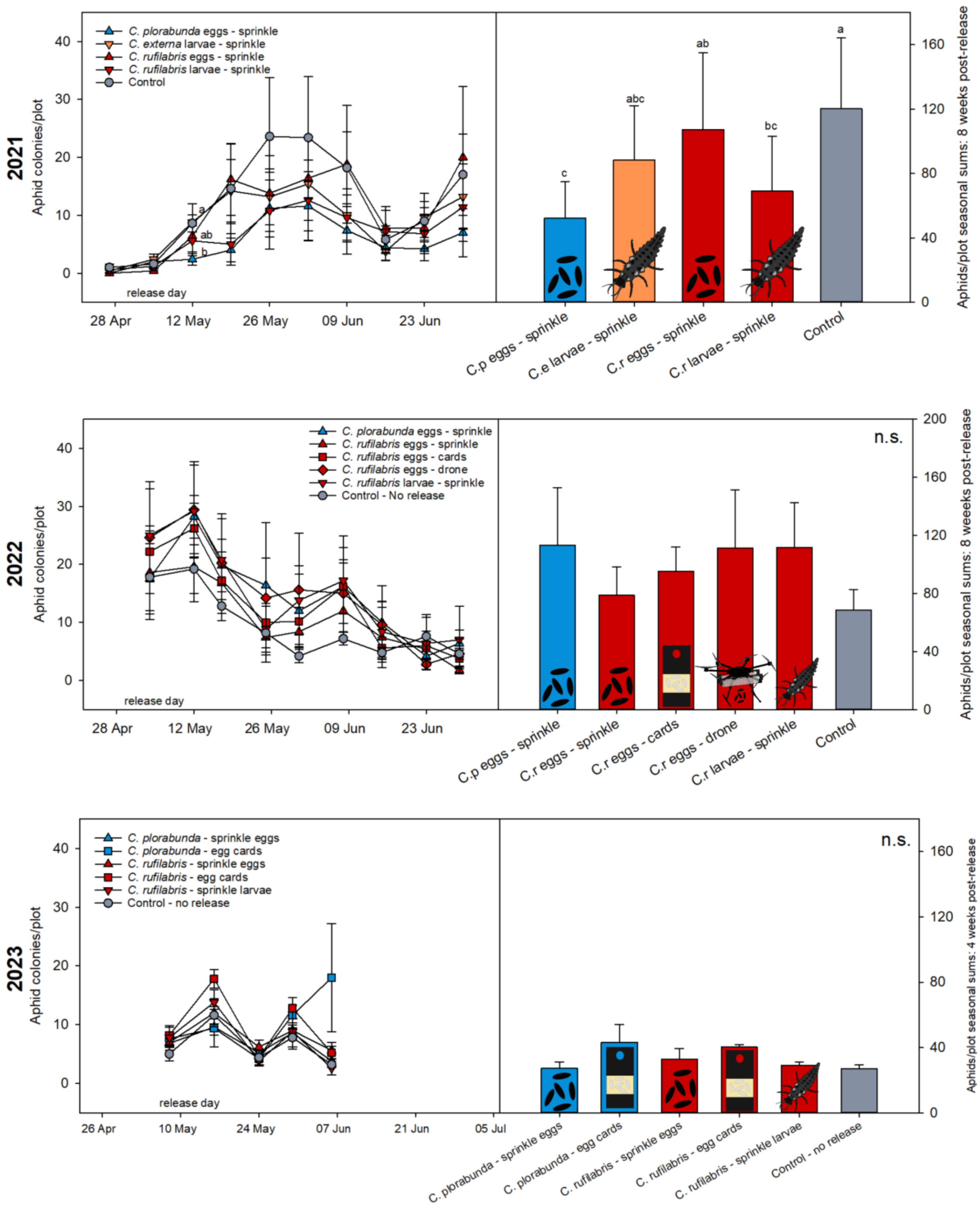

Fig. 8. Aphid counts (all species) by date and seasonal sums following single releases of lacewings released at a rate of 100,000 eggs/acre or 20,000 larvae/acre.

very low compared to the two previous years (Fig. 9). Collectively, the 2022-2023 results highlight the importance of using a selective spray program when conducting releases and releasing as early as

Year	Lacewing spp.	Released stage	Release method	Lacewing larvae/plot Pre-release	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Total
2021	*C. plorabunda*	eggs	sprinkled	0	0.2	0	0	0.2	0	0	0	0	0.4
	C. externa	larvae	sprinkled	0	0.6	0	0.4	0	0	0	0	0	1
	C. rufilabris	eggs	sprinkled	0	0	0.4	0	0	0	0	0	0	0.4
	C. rufilabris	larvae	sprinkled	0	0.8	0	0.4	0.2	0	0	0	0	1.4
	Resident *Chrysoperla*	-	-	0	0	0.04	0	0	0	0	0	0	0.04
	Resident *Chrysopa*	-	-	0	0	0	0.04	0.04	0.04	0	0	0	0.12
2022	*C. plorabunda*	eggs	sprinkled	0	0	0.2?	0	0	0	0	0	0	0.2?
	C. rufilabris	eggs	sprinkled	0	0	0	0.2	0	0	0	0	0	0.2
	C. rufilabris	eggs	card	0	0.4	1.6	0.2	0	0	0	0	0	2.2
	C. rufilabris	eggs	drone	0	0	0	0	0	0	0	0	0	0.0
	C. rufilabris	larvae	sprinkled	0	0.2	0.6	0.2	0	0	0	0	0.2	1.2
	Resident *Chrysoperla*	-	-	0.04	0.04	0.20	0.08	0.04	0.04	0	0	0	0.4
	Resident *Chrysopa*	-	-	0	0	0.10	0.03	0.07	0.27	1.20	0.53	0.17	2.4
2023	*C. plorabunda*	eggs	sprinkled	0	0.8?	0	0.2?	0					1?
	C. plorabunda	eggs	card	0	0.2?	0	0	0					0.2?
	C. rufilabris	eggs	sprinkled	0	0	0	0	0					0.0
	C. rufilabris	eggs	card	0	0.2	0	0	0					0.2
	C. rufilabris	larvae	sprinkled	0	0.2	0.2	0	0					0.4
	Resident *Chrysoperla*	-	-	0	0.30	0.15	0.05	0.10					0.6
	Resident *Chrysopa*	-	-	0	0	0	0.30	0.33					0.6

Fig. 9. Captures of released lacewings post-release and comparison to resident populations of *Chrysoperla* and *Chrysopa* (using control plots). The date of first capture is indicated in red (lighter shading in gray scale). "?" indicate a *carnea*-group lacewing was recovered, but identification as a resident or released individual has not yet been complete. The *C. rufilabris* larvae found on Week 8 in 2022 is likely a second generation.

possible. Early releases provide both a "head start" on resident natural enemies and make it more likely that aphid populations are at levels that lacewings can suppress. All molecular work for this project is anticipated to be completed by June 2024, including molecular identification of captured adult lacewings (2022-2023 samples) and gut content analysis of captured larvae (2021-2023 samples).

Of the natural enemies we have tested, lacewings appear to be the most promising and cost-effective. This project has allowed us to identify areas where more information is needed to provide best practice recommendations for releasing lacewings in orchards. Most importantly, these lacewing efficacy trials were conducted in one orchard each year, leaving results susceptible to factors unique to that particular location (spray programs, pest pressure, regional differences). Our pending proposal with Western SARE will address the remaining questions needed to develop best practice recommendations for releasing lacewings in orchards. This will include testing different release rates, organic pesticide compatibility, multi-site trials, and large-scale drone release assessments.

EXECUTIVE SUMMARY

Project title: Tactics to improve natural enemy releases in tree fruit

Key words: lacewing, mealybug destroyer, *Orius insidiosus*, lures, supplementary foods

Abstract:

Growers have experimented with releases of natural enemies to control pests in organic apples, but there are currently no best practice recommendations for releases in orchards. The purpose of this project was to determine which natural enemies and release methods showed the most promise for controlling orchard pests. We also examined the potential of lures and supplementary food products for recruiting resident natural enemies and retaining released natural enemies. Releases of mealybug destroyers (2,000 or 5,000/acre) showed promised in a preliminary trial (2020), but in the three following trials (2021-2023), they did not lower mealybug counts and rapidly dispersed from the orchard. Because this insect costs $680-950/acre to release, the low chance of success means that fruit growers should avoid using this insect and other options should be explored for mealybug control. Lacewing releases varied in efficacy. In 2021, releases of *C. plorabunda* eggs (100,000/acre) or *C. rufilabris* larvae (20,000/acre) reduced aphid abundance by 57% and 43%, respectively. In the following two years, none of the lacewing release treatments reduced aphid abundance. We attributed this to very high initial aphid counts in 2022 and poor survival of released lacewings in 2023, potentially due to the use of organic pesticides. Across all years, releases of lacewing larvae resulted in higher recapture than releases of eggs. Low numbers of released lacewings were recovered in all trials, even in treatments where aphid abundance decreased. Therefore, when determining efficacy of beneficial releases, scouts should focus on pest numbers, not necessarily natural enemy recovery; however, presence or absence of the released natural enemy can give some sense of survival. Releases should be conducted earlier in the season than anticipated (mid-April in southern Washington) to get an adequate head-start on aphids. Multi-site studies are needed to fine tune recommendations, but early season (mid- to late April) releases appear to be critical for success. Tactics for retaining and recruiting natural enemies had highly variable results between sites and years. In general, methyl salicylate lures showed some promise for recruiting lacewings and *Stethorus*. Food supplements may have increased retention of released *O. insidiosus* and subsequently reduced thrips abundance. The use of lures after a lacewing release should be investigated to determine if they encourage released lacewings to remain in the orchard after they develop into adults.