USING DRONES AND AN ATTRACTANT TO IMPROVE BIOLOGICAL CONTROL OF MEALYBUGS AND SPIDER MITES IN WASHINGTON WINE GRAPES

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Final Report 2022-24 Washington State Wine Commission Research Grant Program

1. SUMMARY

Field evaluation of drone-released beneficial insects and mites in central Washington wine grapes for improved biological control of mealybugs and spider mites, demonstrated good potential for this strategy for enhancement of biological control. There was a trend towards reduced mealybug populations in blocks with drone releases of *Cryptolaemus* beetles and substantial suppression of

spider mite populations was achieved in all treatment blocks. The study also provided a powerful demonstration of the ability of methyl salicylate to attract substantial numbers of a diversity of beneficial insects and mites in wine grapes and improve biological control of mealybugs, spider mites and likely other grape pests as well.

Spider mite populations were substantially reduced in all treatment blocks compared to the untreated control. Lowest numbers of spider mites were recorded in the methyl salicylate (MeSA) blocks (with or without drone application of beneficials), but the numbers in the drone application blocks were also substantially below the number recorded in the control blocks. Predatory mite abundance was greatest in MeSA treatments followed by the drone + MeSA treatments. The majority of predatory mites we found during sampling were native predatory mites. Fewest mealybugs on leaves were recorded in the drone treatments. Data on season-long percentage infestation of grapevine trunks indicated that fewest mealybugs occurred in drone-treated blocks. In general, the two treatments incorporating methyl salicylate dispensers produced greater numbers of beneficial insects and mites that included lacewings, ladybeetles, predatory bugs and predatory flies.

While the overall results indicate substantial potential for the use of drones in delivering insectary-reared biological control agents for enhanced mealybug and spider mite control, there are opportunities for improved efficacy of the treatments and clarification of outcomes. The following are suggested changes to experimental protocol that we believe will better define the potential of using drones and methyl salicylate for improved IPM in wine grape vineyards in future research.

1. Separation between treatment blocks of at least 100 meters
2. Replacement of methyl salicylate dispensers after 6 weeks
3. Use of native Washington predatory mites for drone-release
4. Time drone releases to occur late in the day when temperatures are falling.

2. Final Report

3. Project Title: Using Drones and an Attractant to Improve Biological Control of Mealybugs and Spider Mites in Washington Wine Grapes

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5**. Objectives and Experiments Conducted to Meet Objectives**

1. Assess quality of insectary beneficials and impact of drone release on viability

2. Evaluate the efficacy of drone-released natural enemies (ladybeetles/predatory mites) in suppressing populations of mealybugs and spider mites in WA wine grape vineyards.

3. Evaluate the efficacy of slow-release dispensers of methyl salicylate to attract natural enemies of mealybugs and spider mites and improve biological control with or without drone-released ladybeetles and predatory mites.

6. Summary of Major Research Accomplishments and Results by Objective:

1. Assess quality of insectary beneficials and impact of drone release on viability (2022)

Beneficial insects (*Cryptolaemus* [mealybug destroyer beetles]) and predatory mites (*Neoseiulus californicus*) were obtained from a commercial insectary for our vineyard releases in mid-June and mid-August. Upon arrival (via Fedex), samples of ~100 beetles and ~100 mites were immediately taken from the shipment and held in the laboratory (20-25 °C) at WSU-Prosser and assessed for mortality after 1 and 24 hours (Table 1). Beetles were held in small cups (20/cup) and mites were distributed among 10 leaf discs laid on wet cotton wool. Mortality was assessed by the ability of beetles and mites to make coordinated movement when prodded with a needle.

June 14

Species	No. Held	No. Dead (1 hr)	No. Dead (24 hr)	% Survival (24 hr)
N. californicus	**106**	**0**	**13**	**82**
Cryptolaemus	**100**	**0**	**4**	**96**

August 17

Species	No. Held	No. Dead (1 hr)	No. Dead (24 hr)	% Survival (24 hr)
N. californicus	**114**	**0**	**27**	**64**
Cryptolaemus	**100**	**1**	**3**	**97**

Table 1. Survival of mealybug destroyers and predatory mites in the laboratory in mid-June and mid-August for up to 24 hrs after arrival from a commercial insectary.

Shipment-induced mortality of *Cryptolaemus* beetles was minimal (3-4%). The survival of *N. californicus* predatory mites was not as good (64-82%). However, our release rate of 23,000 mites per treatment block should have offset this.

We also assessed the survival of beetles and mites after they were dropped from the drone in the field on the day of release. To do this, we placed trays in the vine rows to collect the dropped beetles and mites and held them in the laboratory for 24 hours at 20-25˚C. Since numbers retrieved were small, we combined the data from the two releases.

***Cryptolaemus* beetles: 100% survival (n = 7)**

Predatory Mites: 94% survival (n = 36)

Drone release did not appear to be a significant mortality factor for either *Cryptolaemus* beetles or predatory mites.

We did not conduct post-arrival and release survival experiments in 2023, but it should be noted that one shipment of insects arrived in very poor condition (very high mortality) and had to be replaced. The possibility of exposure to excessive heat enroute or other conditions that might cause excessive mortality to the shipped insects is always present.

2. ***Evaluate the efficacy of drone-released natural enemies (ladybeetles/predatory mites) in suppressing populations of mealybugs and spider mites in WA wine grape vineyards (2022).***
3. ***Evaluate the efficacy of slow-release dispensers of methyl salicylate to attract natural enemies of mealybugs and spider mites and improve biological control with or without drone-released ladybeetles and predatory mites (2022).***

<u>Spider Mites</u>

When analyzed over the entire period of monitoring, spider mite populations were substantially reduced in all treatment blocks compared to the untreated control (Figure 1).

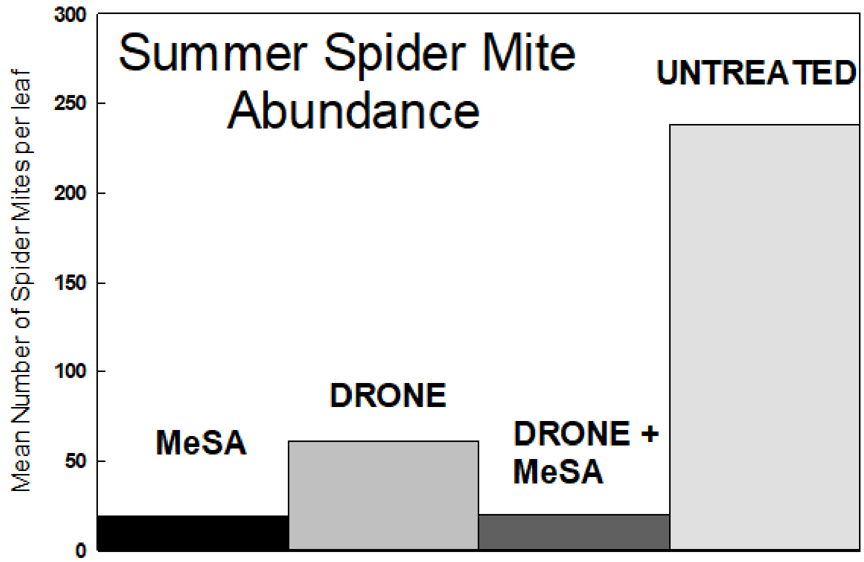

Figure 1. Spider mite abundance during June-September 2022 in Drone, MeSA, Drone + MeSA and untreated wine grape blocks.

Lowest numbers of spider mites were recorded in the methyl salicylate (MeSA) blocks (with or without drone application of beneficials), but the numbers in the drone application blocks (mean 60 mites/leaf) were also substantially below the number recorded in the control blocks (mean 240 mites/leaf).

Predatory mites (Phytoseiidae) are one of the major predators of spider mites, which is why we drone-released an insectary-reared California species of predatory mite, *Neoseiulus californicus*. Although viability of these mites before and immediately after release appeared to be reasonable (see 6.1), they did not seem to persist well in the vineyard. The majority of predatory mites we found during post-drone release sampling were native predatory mites, *Galendromus occidentalis* (Western Predatory Mite), suggesting that *N. californicus* might have provided effective control after the release by drone but showed poor establishment.

Predatory mite abundance was greatest in the MeSA and drone + MeSA treatments. Fewer predatory mites were found in the drone or untreated blocks (Fig. 2). It is known that MeSA is attractive to predatory mites in the laboratory, but this has rarely been shown in the field.

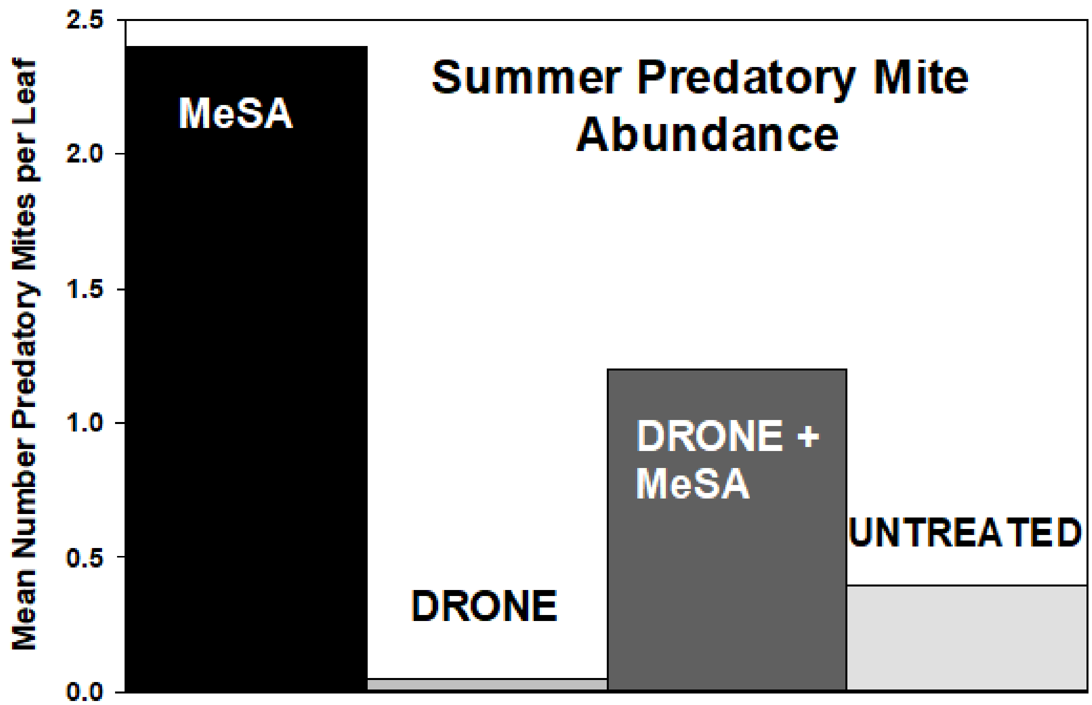

Figure 2. Predatory mite abundance during June-September 2022 in Drone, MeSA, Drone + MeSA and untreated wine grape blocks.

These data suggest that methyl salicylate dispensers may play a substantial role in improving biological control of spider mites by increasing populations of predatory mites. However, spider mites are consumed by a large network of predators and the methyl salicylate dispensers in our study attracted predators other than predatory mites (see following sections). Good suppression of spider mites was also seen in the drone treatments, despite predatory mites being least common in these treatments. The *Cryptolaemus* ladybeetles we applied by drone for mealybugs also feed on other prey and may have also consumed spider mites. Alternatively, there may have been some 'leakage' of other spider mite predators from the MeSA treatments into the drone-treated blocks.

Mealybugs

When analyzed over the entire period of monitoring, the number of mealybugs on sampled leaves was least in the drone treatment blocks (means 22-25/leaf). The greatest number of leaf-dwelling mealybugs were found in the MeSA treatment and untreated blocks (mean 30-34/leaf) (Fig. 3).

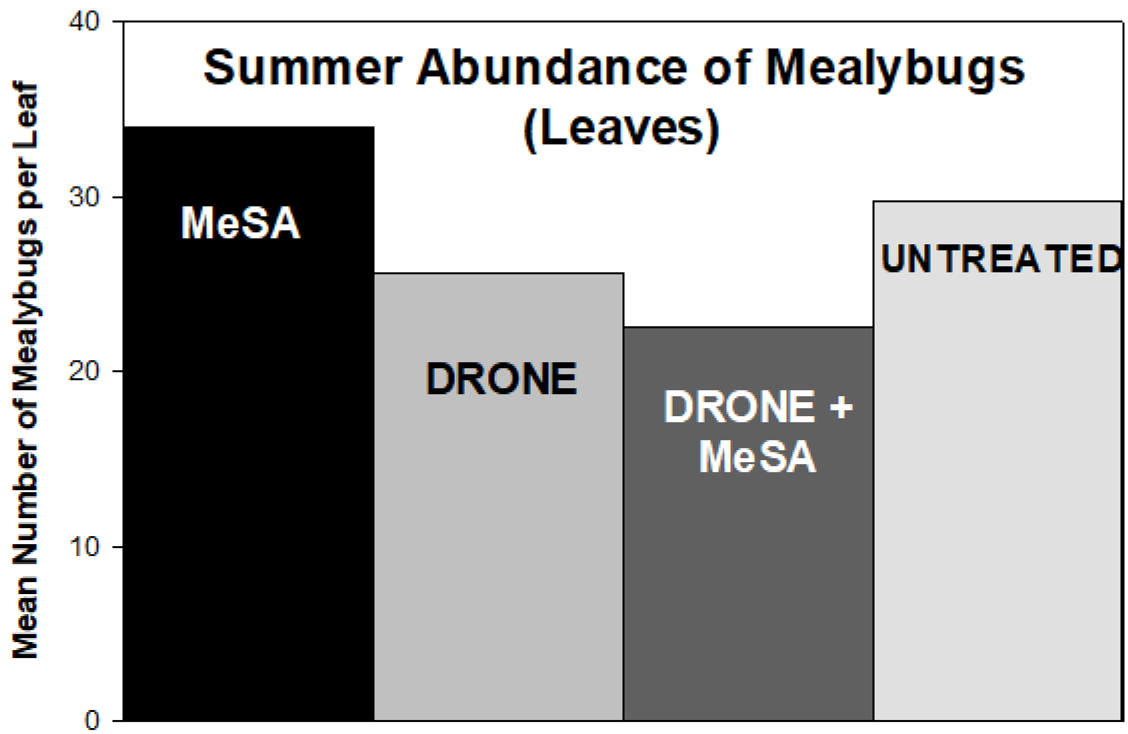

Figure 3. Leaf-dwelling mealybug abundance during June-September 2022 in Drone, MeSA, Drone + MeSA and untreated wine grape blocks.

Trunk Counts: Mealybug populations were also assessed by fortnightly examination of grapevine trunks. On each examination date, 10 grapevine trunks were randomly selected within each treatment block and examined for the presence or absence of mealybugs. Some data were also collected on numbers of mature mealybugs, but it was difficult to make accurate counts of immature mealybugs. Data on season-long percentage infestation of grapevine trunks within each experimental treatment indicates that fewest mealybugs were found on trunks within the drone-treated blocks, but like the data on mealybug infestation of leaves, this suppression was moderate compared to untreated blocks (Fig. 4).

These data suggest that the drone releases of *Cryptolaemus* ladybeetles had a moderate impact on mealybug populations. The cooperating grower was satisfied with mealybug population levels in the entire experiment block, considering them lower than previous years. The higher number of mealybugs in the MeSA treatment is surprising, because MeSA is known to attract many other kinds of mealybug predators including lacewings. We suspect that the relatively close proximity of treatments to each other may have obscured, confused or minimized real effects, in this instance.

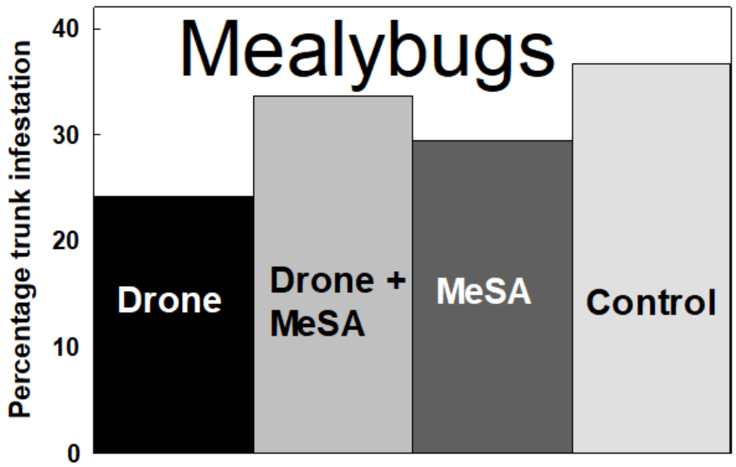

Figure 4. Mean percentage infestation of grapevine trunks by mealybugs in different treatments during June-September 2022

Results for these Objectives in 2023

<u>Spider Mites</u>

Spider mite populations in Drone release and Drone plus MeSA treatments were lower than populations in the control treatment by about 50% (Fig. 5). This was not as substantial a decrease as seen in 2022 (~80-90%) but still substantial. Numbers of predatory mites (*G. occidentalis*) were good in all treatments, suggesting a significant natural population already present in the vineyard (Fig. 6). There was no evidence that our drone releases of predatory mites enhanced this population. There was some evidence that MeSA dispensers enhanced predatory mite populations which is in accordance with previous research by our laboratory and others.

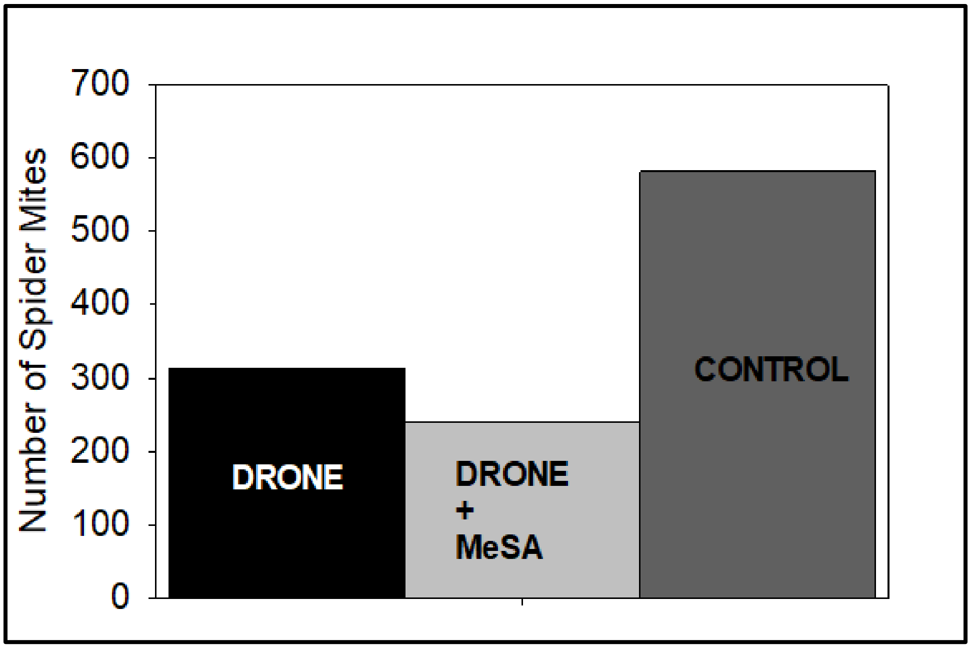

Figure 5. Total numbers of spider mites recorded in Drone, Drone plus MeSA and Control treatments during June-September 2023.

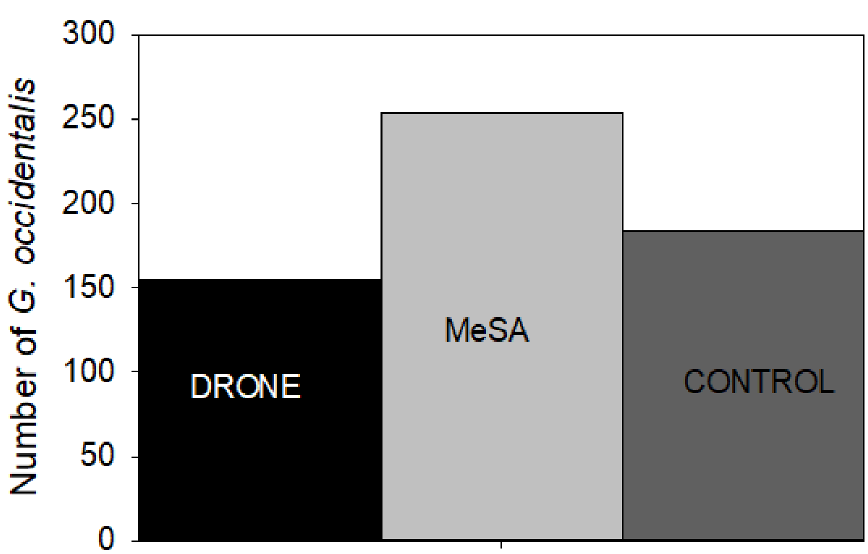

Figure 6. Total numbers of predatory mites (Galendromus occidentalis) recorded in Drone, Drone plus MeSA and Control treatments during June-September 2023.

Mealybugs

Trunk counts of mealybugs showed about 33% fewer mealybugs in the Drone treatment compared to the control. Suppression was less in the MeSA and Drone plus MeSA treatments (Fig. 7).

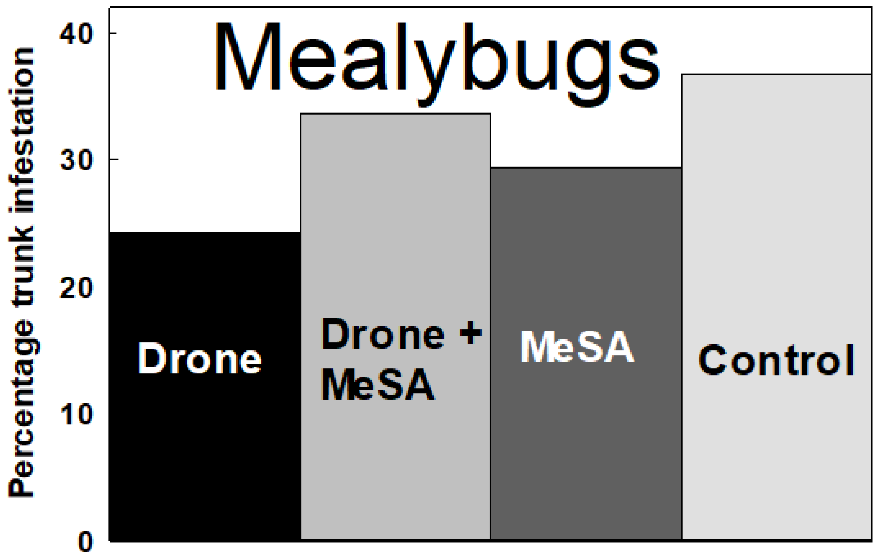

Figure 7. Percentage infestation of grapevine trunks by mealybugs during June-September 2023

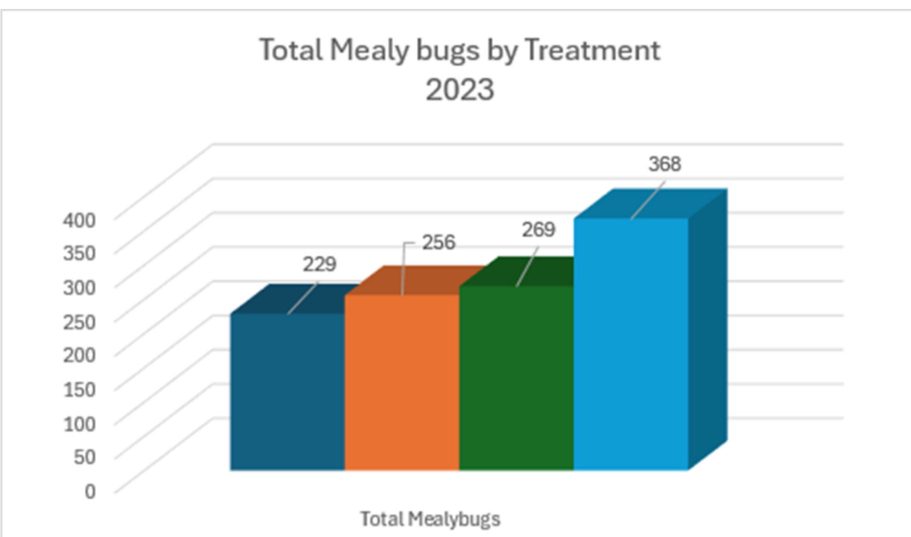

Figure 8. Total number of female and nymphal mealybugs on sticky traps during June-September 2023 (Dk Blue = Drone, Orange = MeSA, Green = Drone + MeSA, Blue = Control

There was also a substantial treatment impact on leafhopper populations with all treatments suppressing leafhopper populations (Fig. 9). Most suppression occurred in the MeSA blocks (T2). Curiously, suppression was also substantial in the drone blocks (T1).

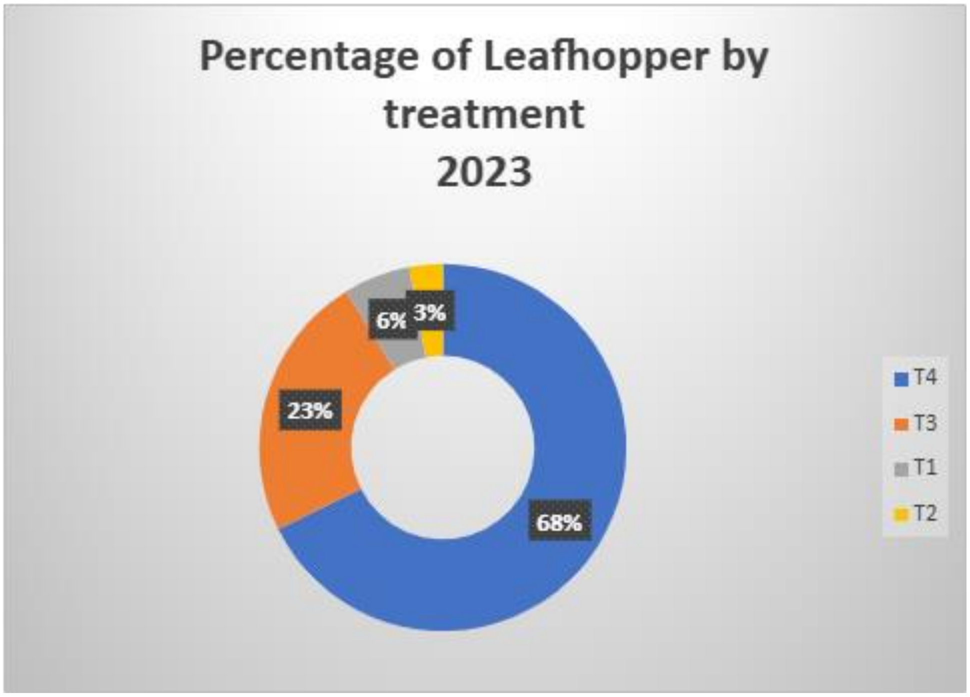

Figure 9. Leafhopper populations sampled in the trial (June-September 2023) on sticky traps expressed as a percentage found in each treatment.

4. ***Evaluate the efficacy of slow-release dispensers of methyl salicylate to attract natural enemies of mealybugs and spider mites and improve biological control with or without drone-released ladybeetles and predatory mites (2022 & 2023).***

Slow-release dispensers of methyl salicylate were included in this research to see if 1) they could improve the residence time and sustainability of drone-released beneficials and 2) improve biological control further by attracting a diversity of naturally occurring beneficial insects and mites.

2022: In general, the two treatments incorporating methyl salicylate dispensers (MeSA only and MeSA + Drone releases) recorded greater numbers of beneficial insects and mites overall. Some beneficial insects known to be attracted to MeSA responded in very good numbers. Green Lacewings have been shown by our lab and others to be strongly attracted to MeSA and this was seen in the current study (Figs. 10 and 11).

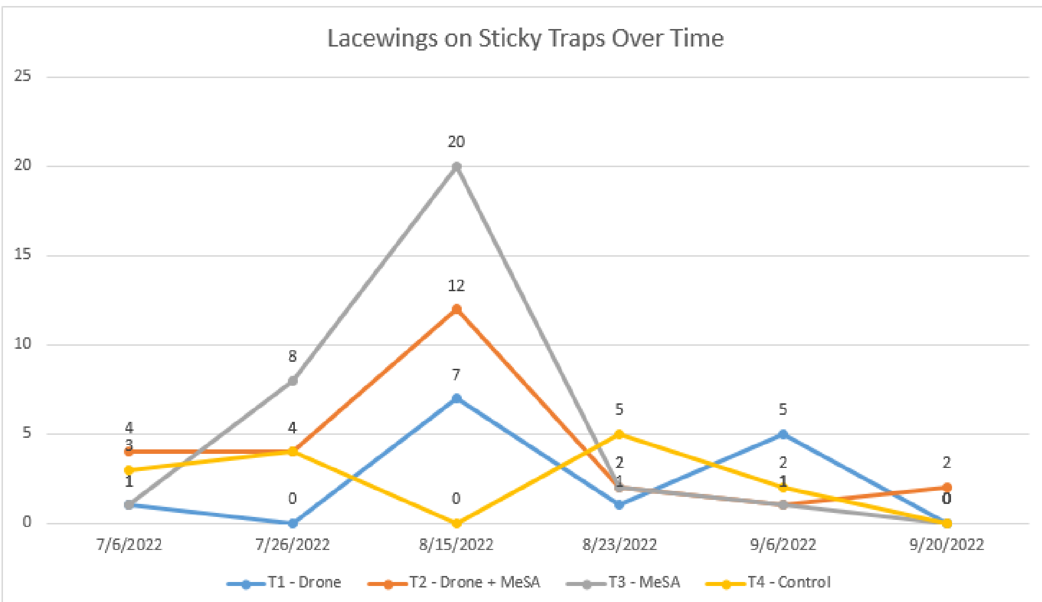

Figure 10. Numbers of Green Lacewings (Chrysoperla spp.) attracted to sticky traps in different treated wine grape blocks during June-September 2022.

The fall in numbers of lacewings observed in MeSA treatments from late August onwards may represent depletion of the dispensers. Release rate from the dispensers increases in hot weather and the above average temperatures in July 2022 may have caused premature depletion.

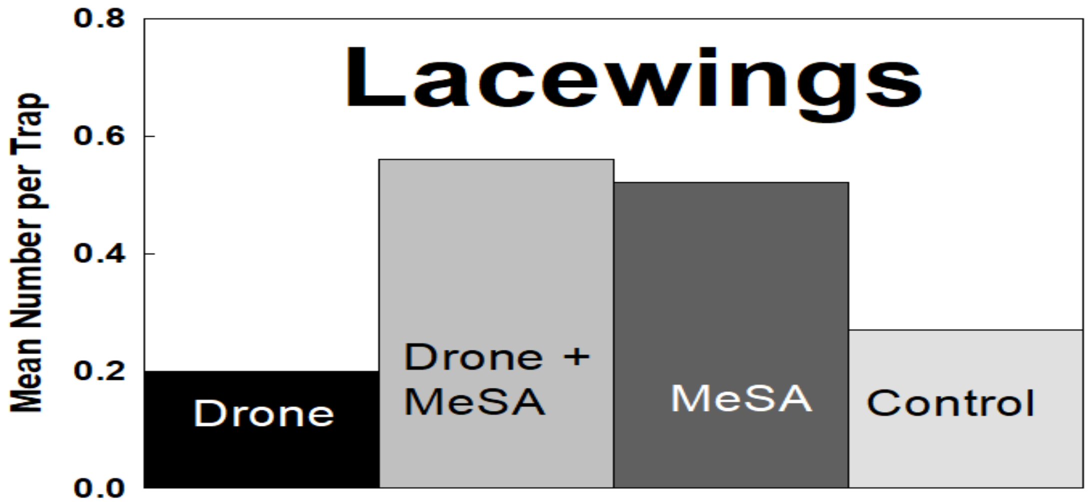

Figure 11. Season-long mean sticky trap catches of Green Lacewings (Chrysoperla spp.) in Drone, Drone + MeSA, MeSA and control (untreated) wine grape blocks during June-September 2022.

Green Lacewings are important predators of mealybugs and spider mites. Augmentative biological control of mealybugs with lacewings has been attempted in California and may also be an option for Washington wine grapes.

Ladybeetles (3 native species combined) responded similarly to Green Lacewings with more than three times as many found in sticky traps season-long in the two MeSA treatments as in the control or drone treatments (Fig. 127).

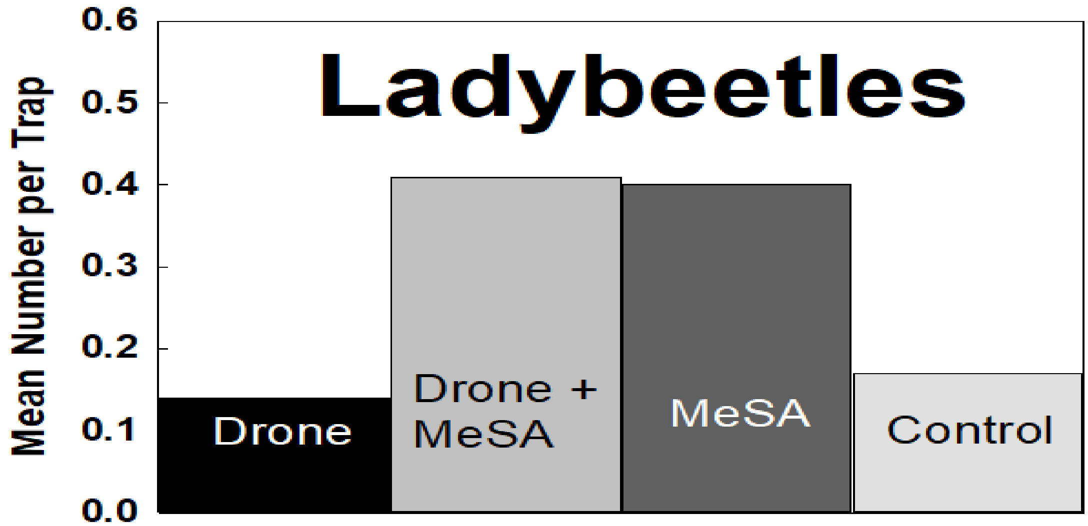

Figure 12. Season-long mean sticky trap catches of Ladybeetles in Drone, Drone + MeSA, MeSA and control (untreated) wine grape blocks during June-September 2022.

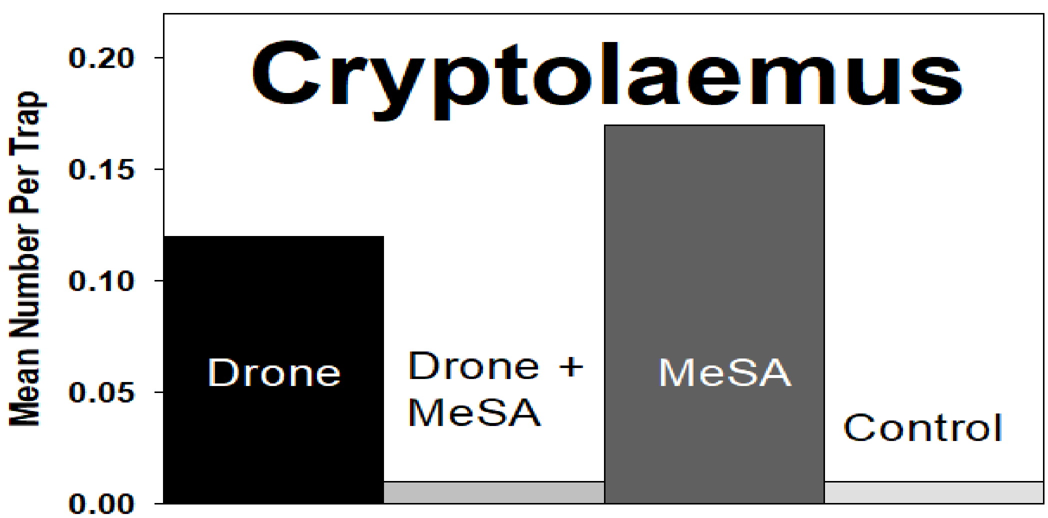

Figure 13. Season-long mean sticky trap catches of Cryptolaemus ladybeetles in Drone, Drone + MeSA, MeSA and control (untreated) wine grape blocks during June-September 2022.

The ladybeetle species we released by drone, the Mealybug Destroyer (*Cryptolaemus*), was mostly trapped in the blocks it was released in, and also the MeSA blocks. Very few were found in untreated and curiously, in the Drone + MeSA blocks (Fig. 13). Numbers trapped overall, were small and it may be that yellow sticky traps are not optimal for assessment of *Cryptolaemus* populations.

Predatory bugs (e.g. Big-eyed bugs, Minute pirate bugs) are important predators of mealybugs and spider mites and most were found in greater numbers in the MeSA treatments. For example, nearly three times as many Big-eyed bugs (*Geocoris* spp.) were found in the two MeSA treatments compared to the control (Fig. 14).

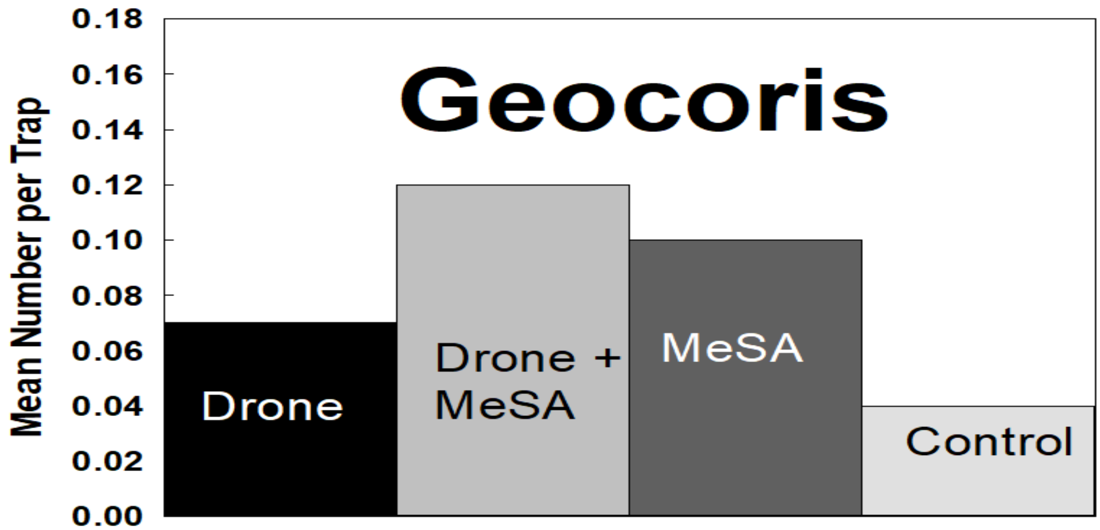

Figure 14. Season-long mean sticky trap catches of Big-Eyed Bugs (Geocoris spp.) in Drone, Drone + MeSA, MeSA and control (untreated) wine grape blocks during June-September 2022.

Predatory fly species (e.g. Long-legged flies (Dolichopodidae)) also responded significantly to the two MeSA treatments with 3-4 times as many trapped, compared to the control (Fig. 15).

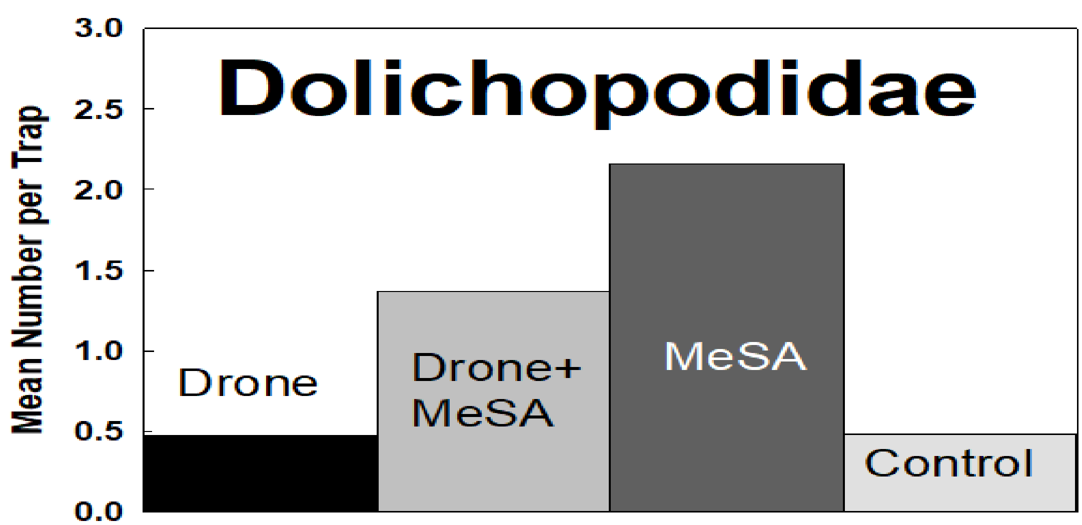

Figure 15. Season-long mean sticky trap catches of predatory Long-Legged Flies (Dolichopodidae) in Drone, Drone + MeSA, MeSA and control (untreated) wine grape blocks during June-September 2022.

2023: All treated blocks attracted and harbored greater numbers of beneficial insects than the control blocks. Most beneficial insects were found in the Drone + MeSA blocks. Populations in these blocks were 43-48% greater than in the controls (Fig. 16).

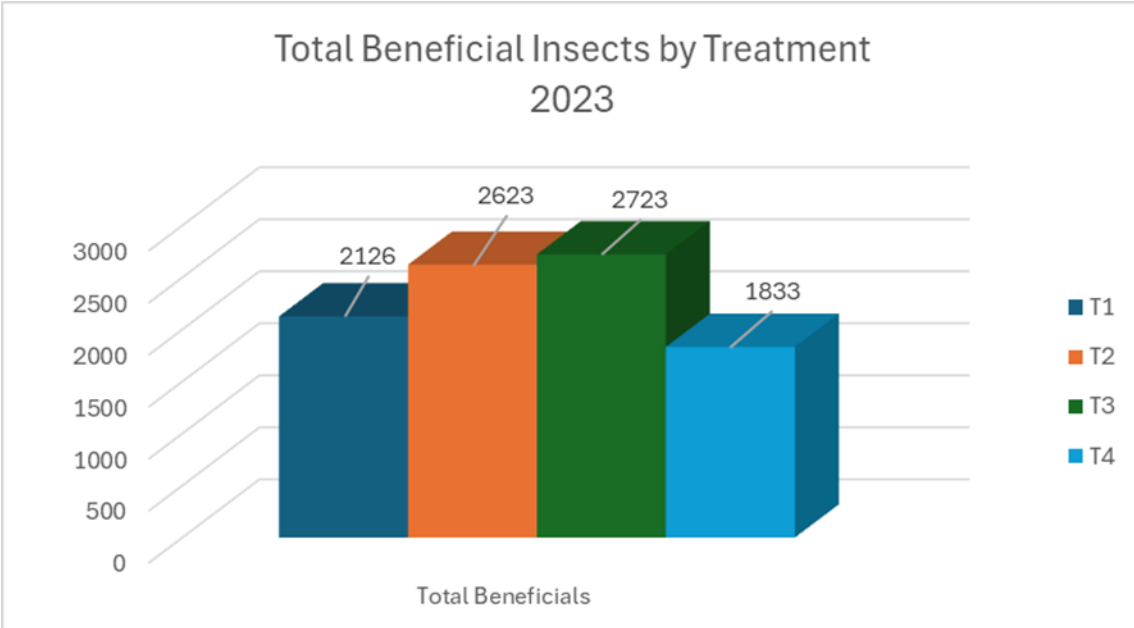

Figure 16. Season-long totals of all beneficial insects in treatment and control blocks in 2023 (T1: Drone, T2: MeSA, T3: Drone + MeSA, T4: Control).

The Spider Mite Destroyer (*Stethorus* spp.), a specialized predator of spider mites, occurred in the MeSA blocks (T2, T3) at densities that were about three times greater than in the control blocks (T4) (Fig. 17).

Figure 17. Season-long totals of Spider Mite Destroyers (Stethorus spp.) in treated and untreated blocks in 2023 (T1: Drone, T2: MeSA, T3: Drone + MeSA, T4: Control)

At the end of the first year of study, the research team decided to add the new objective of comparing the cost of manual release to drone release since that data was available. Efficacy of drone versus manual release was not tested here, but we are able to provide a preliminary evaluation of the time and approximate cost comparison.

Some beneficial insects and mites ordered from the commercial insectary were released manually by the grower hosting the study, in other vineyards.

Cryptolaemus were manually released on 5 acres of Gewurztraminer. The grower mixed the predatory beetles with sawdust and used a spoon to sprinkle them on approximately every 10th plant. This involved stopping for each dose. This process took about 5 hours.

The *N. californicus* predatory mites were manually released on about 5 acres of Sémillon and Merlot using an ATV riding every other row. The grower used an empty bulk spice container and sprinkled the predatory mites on the vines while moving down the row. The grid was every other row and about every 5th plant. This process also took about 5 hours. This approach treats 10% of the vines within the treatment block.

The manual release took about 1 hour per acre and the grower estimated his cost at $20 per hour.

The cost of manual release was therefore similar to the commercial rate charged by UAV-IQ and other drone companies performing drone-based biocontrol release (approximately $20 per acre). However, it is worth noting that to achieve a similar cost of release, the manual release wasn't as homogeneous as the drone release as it was performed only in a few rows and plants and covered only 10% of each acre. Furthermore, the drone can treat an acre in less than 1 minute, making it at least 60 times faster than manual release before accounting for the reduced coverage of the manual release. The feasibility of dedicating 1 man-hour per acre for manual release is also questionable for medium and large vineyards.

Summary of Results and Opportunities for Improvement

Our evaluation of drone-release of beneficial arthropods (mites and beetles) in wine grapes for improved biological control of mealybugs and spider mites demonstrated great potential for this novel IPM augmentation strategy. There was a trend towards reduced mealybug populations in blocks with drone releases of *Cryptolaemus* beetles and substantial suppression of spider mite populations was achieved in all treatment blocks. The study also provided a powerful demonstration of the ability of methyl salicylate to attract substantial numbers of a diversity of beneficial insects and mites in wine grapes.

While the overall results indicate substantial potential for the use of drones in delivering insectary-reared biological control agents for enhanced mealybug and spider mite control, and warrants further research, there are opportunities for improved efficacy of the experimental treatments and clarification of outcomes. The following are suggested changes to the research protocol that we believe will better define the potential of using drones and methyl salicylate for improved IPM in wine grape vineyards.

5. The one-acre blocks used for the replicates of each treatment need to be better isolated from other treatments, to guard against 'spillover' between treatments. In 2022, we used narrow one-acre blocks separated by 50 meters. We believe this is insufficient to prevent beneficial insects traversing between blocks/treatments as the season progresses. Future research should aim to use square one-acre blocks separated by at least 100 meters.
6. Some of the results show a diminishing in impact of methyl salicylate in attracting/retaining beneficials from mid-August onwards. This may have been because dispensers became depleted after hot summer conditions. Future research should incorporate replacement of methyl salicylate dispensers after 6 weeks.
7. Washington native predatory mite species should be used for drone application instead of the California predatory mite (*Neoseulus californicus*). The latter was chosen because of its lower cost (one third of the cost of WA natives). Most of the predatory mites found in our study were the Washington native Western Predatory Mite (*Galendromus occidentalis*), suggesting perhaps, poor survival of *N. californicus.* The California species is unlikely to survive Washington winters, whereas augmented populations of *G. occidentalis* would survive and be sustainable. Future research should consider using the Washington native predatory mites, *G. occidentalis* and/or *Neoseiulus fallacis,* despite their greater cost.
8. In 2022, the second drone release of beneficials occurred on a day when temperatures reached very high levels (100 ˚F +) soon after release. These conditions were likely harmful to the released beetles and predatory mites. Future research should consider drone releasing beneficials later in the day, when temperatures are falling. This would allow the

beetles and mites time to find optimal habitat niches overnight, before the heat of the following day.

Note that we used most of these protocol improvements in 2023

1. Outreach and Education Efforts - Presentations of Research:

A successful field day was held on August 19th, 2022 at the field trial site near Benton City, Washington. Despite relatively short notice and limited advertising, we attracted a good audience of about 30 interested growers, grape industry and pest management personnel. A reporter from the 'Good Fruit' magazine was in attendance and a write-up on the field day was published in March 2023.

David James presented a talk 'Enhancing Biological Control of Mites and Mealybugs using a Drone and an Attractant' at the Grape Society Annual Meeting held in Grandview, Washington on November 18th, 2022. He presented another talk in January 2024 entitled "Using drones and an attractant to enhance biocontrol of GMB and TSSM" at the G S Long Annual Conference in Yakima in January 2024. Our research on drones and biocontrol also featured in a March 2024 WAVEX webinar presented by David James.

An article entitled "The biological war on Mealybugs and Mites: battle report from Red Mountain" was published in Spring 2023 in the WSU Viticulture and Enology Extension News.

During the study, a drone pilot from a local community college that has a precision agriculture program was trained on the use of drones and the specialized equipment used to release beneficial insects and mites. He was also educated about the basic principles of biocontrol and the handling of biocontrol products to maintain viability. This will benefit local students as these lessons will be incorporated into the local curriculum.. It likely will lead to employment for local pilots serving in Washington state.

2. Research Success Statements

This study has demonstrated great potential for using drones as a vehicle for distributing insectary-reared beneficial insects and mites in wine grape vineyards. It also highlighted the potential of using methyl salicylate as a beneficial insect attractant enhancing and retaining a wide diversity of endemic beneficial insects and mites in vineyards. The prospect of synergy between this attractant and drone releases of beneficials seems very likely. Future research will determine more precisely the level of efficacy and cost-effectiveness of each strategy as well as the benefits of simultaneous use.

3. **Funds Status:** 100% expended. Spending of funds proceeded in accordance with the strategies outlined in the funding proposal.