

Annual Report – Crop Year 2016
Department of Pesticide Regulation – Pest Management Alliance Program

Project Title: “Area-wide Integrated Pest Management Program for Virginia Creeper Leafhoppers (*Erythroneura ziczac*) in North Coast Vineyards”

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Grant Agreement: PML-15-G001

Results to Date

(1) Regional Monitoring and Newsletter

(1a) Monitoring Sites, Sampling Frequency, and Data Summary

The regional leafhopper monitoring effort in 2016 included a total of 12 vineyard sites, 7 in Mendocino County and 5 in Lake County. Sites were selected to provide a representative sample of vineyards throughout the major wine grape growing regions within each county where Virginia creeper leafhopper (VCLH) is present. In Mendocino, sites where VCLH is not present (i.e. Redwood and Potter Valley) were included in order to catch any potential expansion of VCLH populations into new areas.

Overwintering leafhopper adults were sampled from the vineyard floor on March 2. Weekly sampling was then initiated on March 21 and May 26 in Mendocino and Lake County, respectively, as the overwintering adults began to move from the vineyard floor into the vine canopy. Monitoring in Lake County was initially delayed because it was not originally scheduled for the 2016 program, but after some discussion with growers and project team members it was decided that monitoring would be possible and beneficial to the area-wide program.

In Mendocino County, weekly regional monitoring included counts of leafhopper adult, egg, and nymph densities as well as parasitism rates. This work was carried out by Lucia Varela and Ryan Keiffer (Ag. Technician, UCCE Mendocino). In Lake County, monitoring included weekly counts of leafhopper adults and nymphs, which was carried out by collaborating PCAs (Broc Zoller, Bill Oldham) and vineyard managers (Randy Krag). Additionally, counts of leafhopper eggs and parasitism rates at the Lake County sites took place every 6 weeks.

Monitoring data was summarized and interpreted in a weekly “Leafhopper Newsletter” that was sent via email to growers in both counties. As of September 2016 there are 73 total subscribers to this newsletter, which includes 58 growers and PCAs who oversee more than 10,000 vineyard acres in Mendocino and Lake County. The remaining subscribers include county, state and UC ANR personnel (9) as well as the project team members (6). In total, 26 newsletters with summaries of the regional monitoring were sent out during the 2016 season (April 11 to October 5). Additional newsletters will be sent out during the winter with relevant information and updates about this area-wide VCLH IPM program, such as summaries of seasonal parasitism data and overwintering leafhopper densities. An archive of these newsletters can be found at: http://ucanr.edu/sites/vclh/VCLH_newsletter/

(1b) Regional Population Trends in 2016

Prior to bud break, overwintering densities of VCLH adults (Fig. 1) were highest in the McDowell Valley, Hopland, Talmage, and Ukiah areas, likely reflecting the high populations that were observed in this region in 2015. No overwintering leafhoppers were sampled in Lake County. Over the course of the 2016 growing season, densities of VCLH were higher in Mendocino County than in Lake County (Figs. 2-3). Within Mendocino County, the highest densities of VCLH nymphs and adults were observed in the McDowell Valley, Hopland, Talmage, and Ukiah areas, both in the early season (May/June, Figs. 2A & 3A) and late season (July/Aug., Figs. 2B & 3B) periods. Densities of WGLH were also higher in Mendocino County, although differences between the two counties were less pronounced. WGLH populations were especially high in the Redwood and Potter Valley areas, where VCLH has not been reported.

VCLH & WGLH Overwintering Adults - March 2016

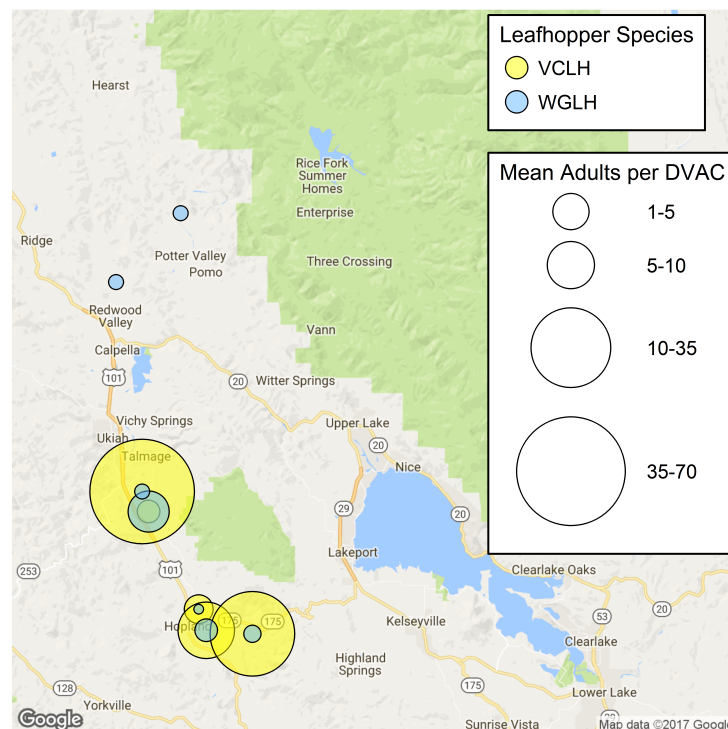
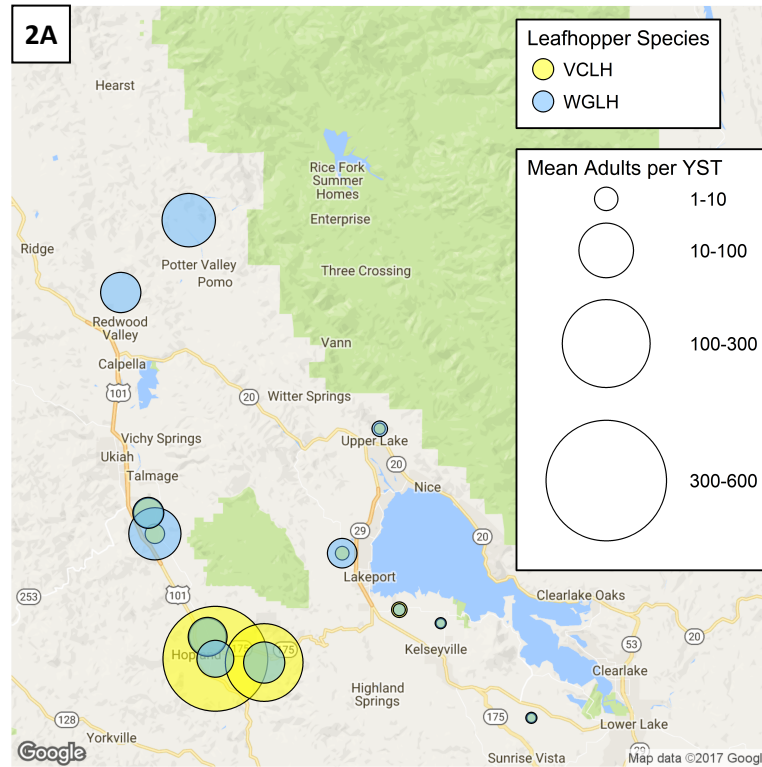


Fig. 1. Overwintering leafhopper adults were sampled on March 2, 2016. Adults were sampled from the vineyard floor using a D-VAC type insect vacuum. Densities represent mean number of adults per vacuum sample.

VCLH & WGLH Adults - Early Season - 2016



VCLH & WGLH Adults - Late Season - 2016

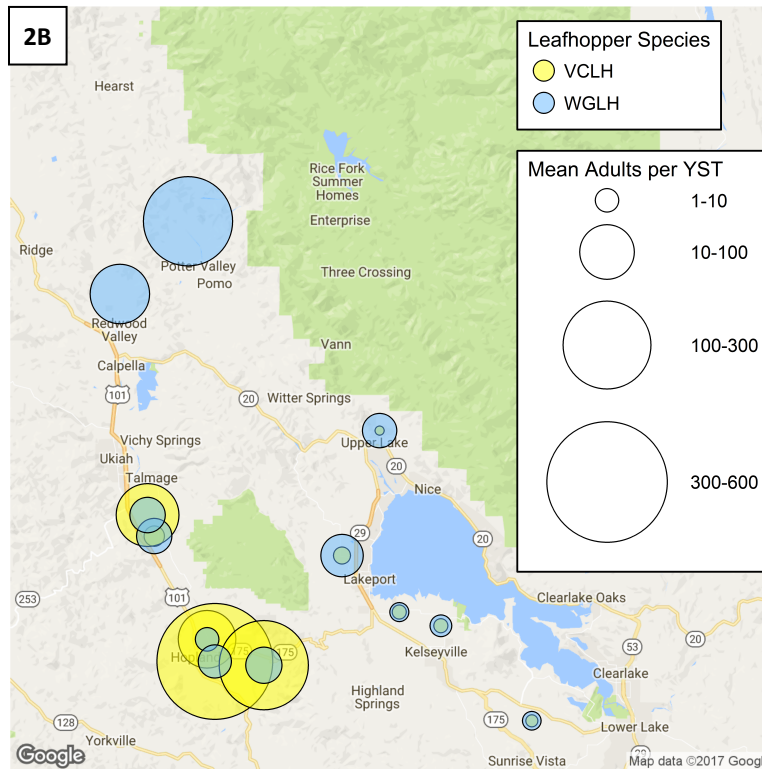
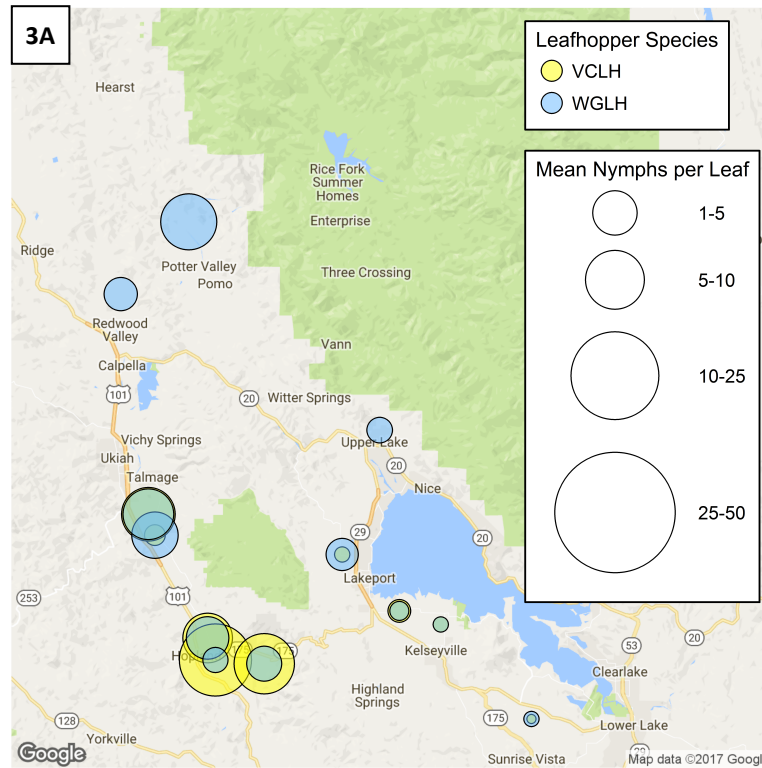


Fig. 2. Leafhopper adults in the early season (May/June, 2A) and late season (July/Aug., 2B) periods. Densities represent mean number leafhopper adults per yellow sticky-trap (YST) during peak flight in each period.

VCLH & WGLH Nymphs - Early Season - 2016



VCLH & WGLH Nymphs - Late Season - 2016

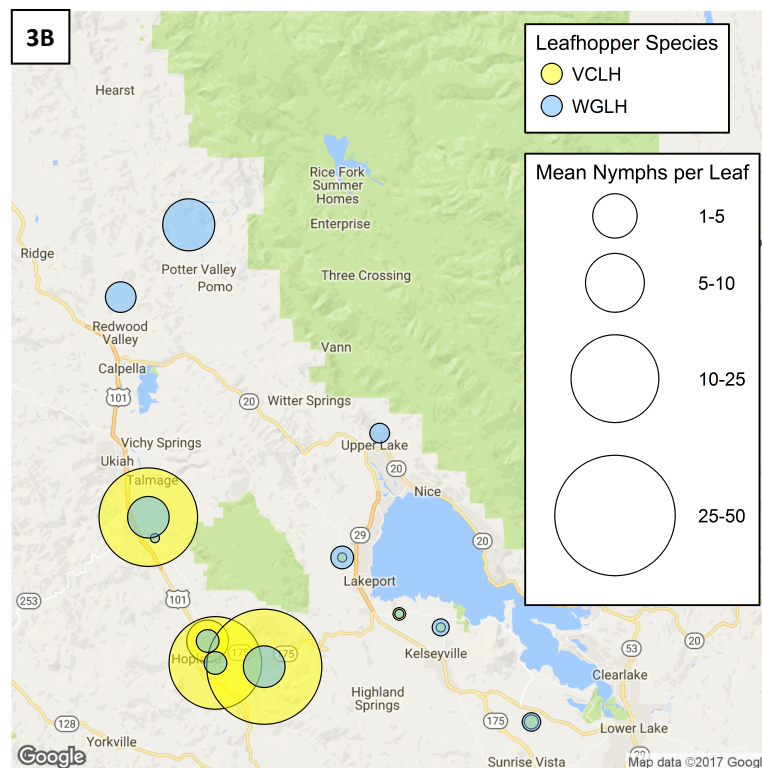


Fig. 3. Leafhopper nymph densities in the early season (June, 3A) and late season (Aug., 3B) periods. Densities represent the peak mean number of nymphs per leaf during each period.

(1c) Timing of Leafhopper Activity in the Vine Canopy

While adults of both leafhopper species appeared to move into the vine canopy at approximately the same time (Fig. 4A), VCLH egg deposition began earlier than WGLH (Fig. 4B). Earlier VCLH egg deposition can lead to the earlier appearance of VCLH nymphs (Fig. 4C), which has implications for the timing of monitoring and sprays for this pest.

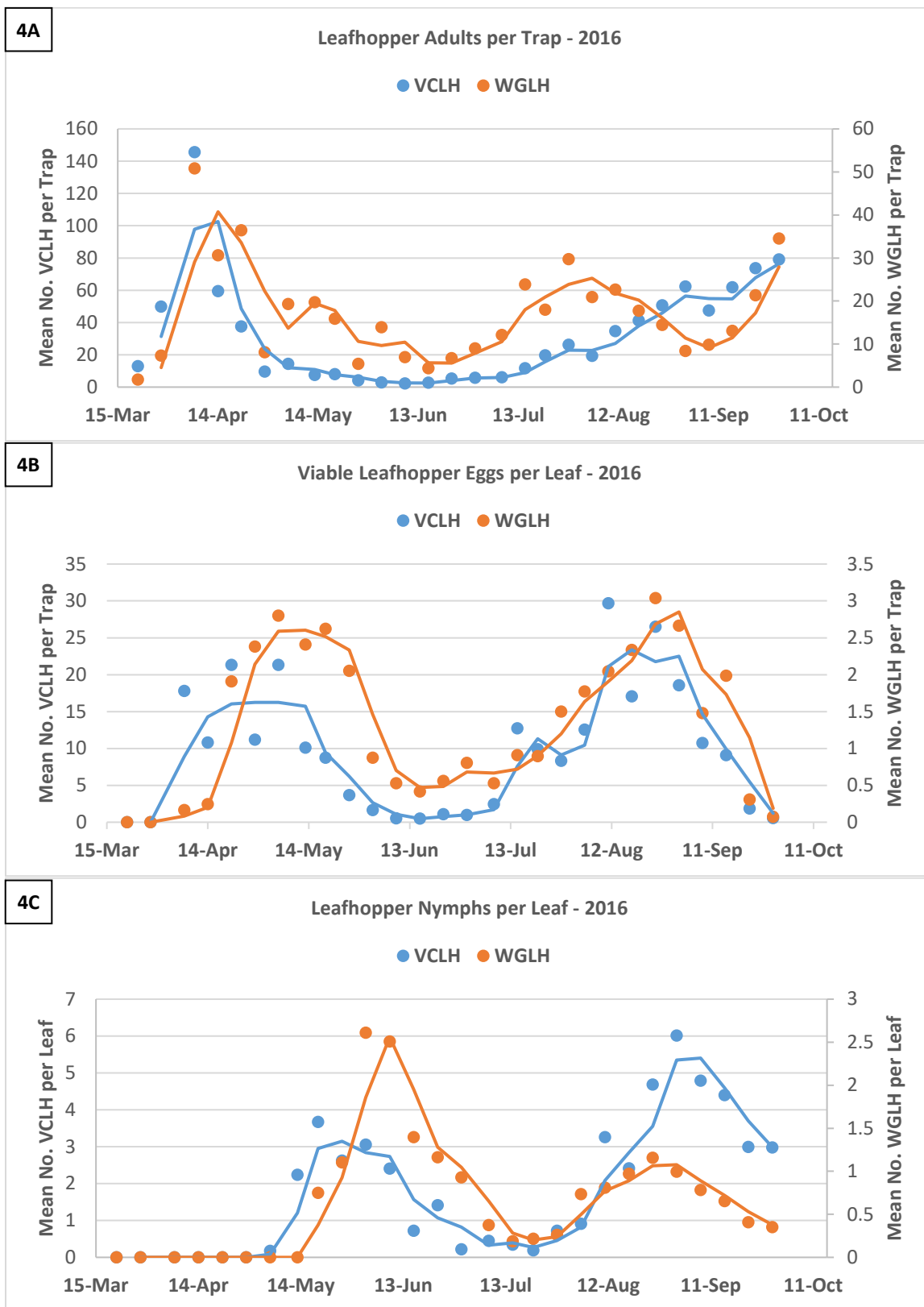


Fig. 4. Mean weekly number of leafhopper adults per trap (4A), and viable eggs (4B) and nymphs per leaf (4C). Earlier egg deposition by VCLH leads to the earlier appearance of nymphs, which has implications for the timing of monitoring and sprays.

(1d) Leafhopper Life-Stage Development

Early in the season, both VCLH and WGLH have relatively distinct life-stages. For example, in Fig. 5 a series of separate, successive peaks can be seen for adults, eggs and nymphs early in the season. Later in the season these life-stages begin to overlap, which has implications for the efficacy of sprays that target a particular life stage.

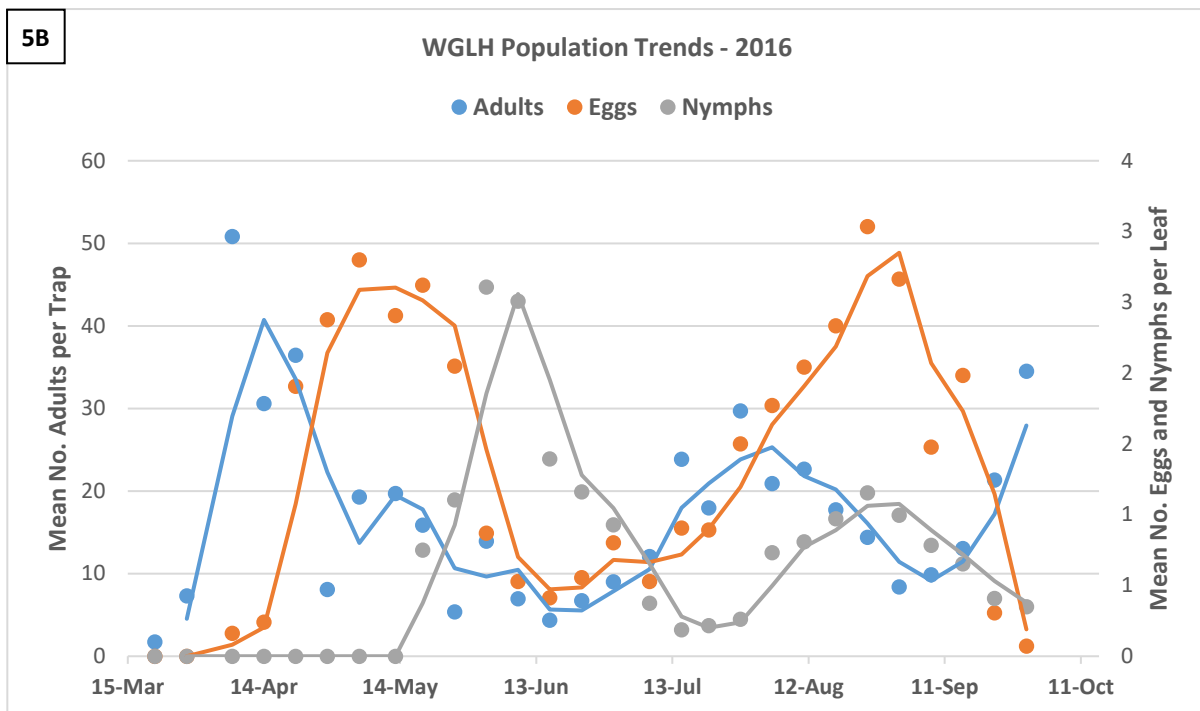
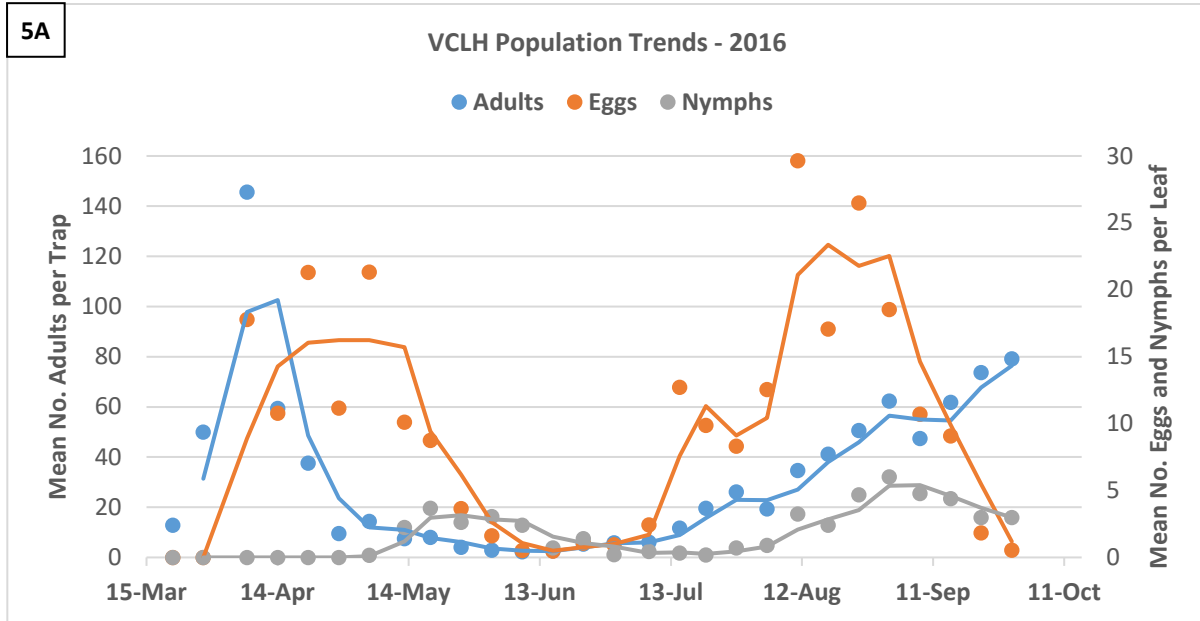


Fig. 5. Weekly densities of VCLH (5A) and WGLH (5B) adults, eggs and nymphs averaged across all sites. Notice how life-stages are relatively distinct early in the season, but then begin to overlap later in the season.

(1e) Predator and Parasitoid Populations

Densities of insect predators that are known to feed on leafhopper eggs, nymphs and/or adults were generally higher earlier in the season, although some species such as *Orius* sp. were in greater abundance later in the season (Fig. 6). The most abundant early season predators included Cantharidae, *Chrysoperla* sp. and Syrphidae. Predaceous beetles in the family Coccinellidae were present in low abundance throughout a majority of the growing season. Certain predators were found in very low abundance (*Geocoris* sp., *Hemerobius* sp., *Agulla* sp.) or not at all (*Nabis* sp.). No specific evaluation of leafhopper predation was conducted, so it is unclear what effect these predators may have had on VCLH or WGLH populations.

Early season populations of *Anagrus* spp. parasitoids were low, which is common while they are initially colonizing vineyards. Once established, they can then build up a large population by the end of the season (Fig. 7), which is what was generally observed across all vineyards. Most of these *Anagrus* spp. are likely *A. daanei* or *A. erythroneurae*, although no specific counts were recorded due to the use of yellow sticky-traps, which prevents identification beyond genus.

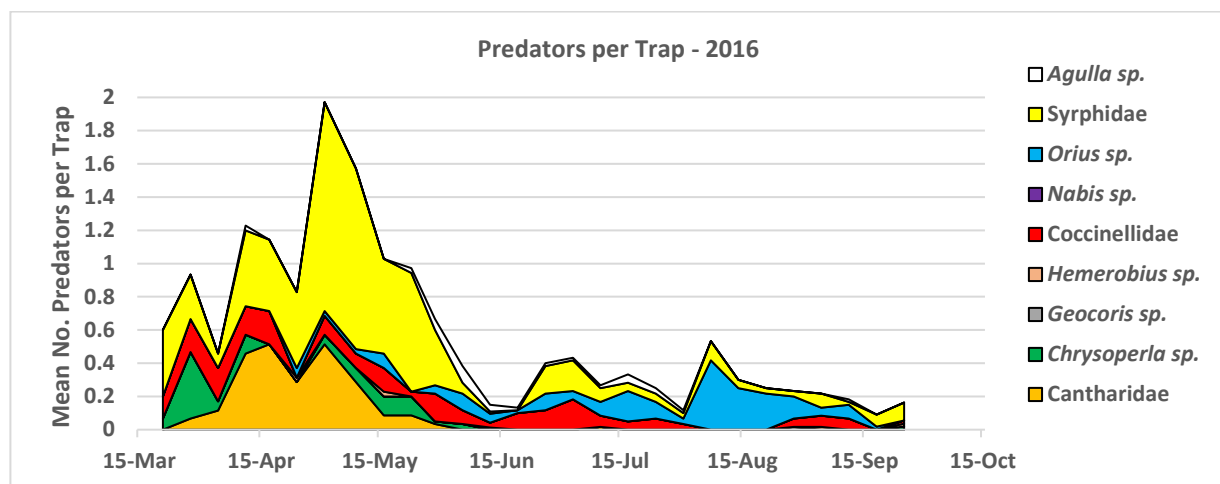


Fig. 6. Densities of predators in the vine canopy. Cantharidae, *Chrysoperla* sp. and Syrphidae were most abundant in the early season, while *Orius* sp. was more abundant in the late season.

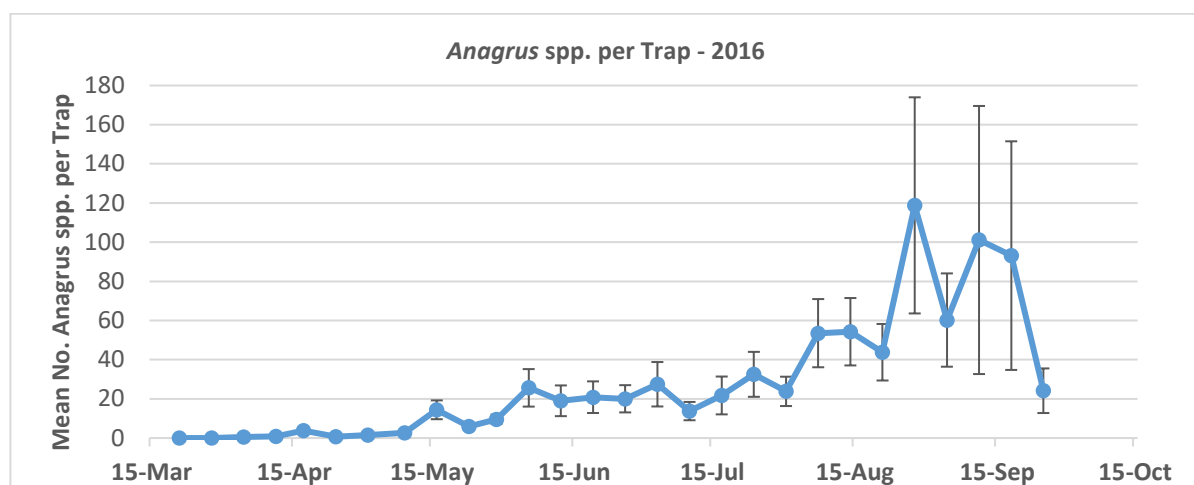


Fig. 7. Weekly densities of *Anagrus* spp. were initially low, but then steadily increased over the growing season. Note that these counts do not differentiate between *A. daanei* (the key VCLH parasitoid) and *A. erythroneurae*.

(2) *Anagrus daanei* Rear and Release Program

(2a) Greenhouse Rearing Program

In July 2015, as part of an earlier research program funded by the American Vineyard Foundation, *Anagrus* parasitoids for control of VCLH were released in Mendocino County. The parasitoids released were a mix of *Anagrus daanei*, *A. erythroneuræ* and *A. tretiakovæ* reared from grape leaves collected in the Sacramento Valley that contained mixed leafhopper populations. While these releases did lead to significant increase in VCLH parasitism (87-91% of newly laid eggs following release), the approach was less than ideal since only 45% of the 5,018 total *Anagrus* parasitoids released were actually *A. daanei*, the key parasitoid that attacks VCLH.

The *A. daanei* rear and release program was greatly improved in 2016. Greenhouse colonies were established in October/November 2015 with a pure strain of *A. daanei* from the Sacramento Valley. Starting in early spring 2016, these colonies were augmented by adding additional grape vines and leafhopper adults and then expanded over the summer by building more cages and acquiring additional greenhouse space. At present, a total of 4 greenhouse colonies have been established. Each colony contains 48 grape vines. Between April and September, 12 vines are removed every week from each colony and replaced with fresh vines. The vines that are removed are each placed into an individual emergence chamber, which is then checked 3 times a day (8am, 12pm, 5pm) for emerging *A. daanei*. The *A. daanei* collected from each emergence chamber are aggregated into a single large vial that contains a strip of filter paper soaked in 50% honey solution, which provides a food source for the parasitoids. The large vial is then held in an incubator at 12°C until the *A. daanei* are brought to a vineyard release site.

Emergence of *A. daanei* peaks 2-5 days after grape material has been placed into the emergence chambers (Fig. 8). As such, grape material was removed from the colonies and placed into emergence chambers on Saturday, then the *A. daanei* were collected Monday – Thursday and finally released into a vineyard on Friday morning. All remaining *A. daanei* that were collected on Friday were released back into the greenhouse colonies at the end of the day. Yield of *A. daanei* was also maximized by making general improvements to the design of the emergence chambers (reducing moisture in the containers) and large vial for parasitoid aggregation (daily provision of honey solution). By doing so, mortality of *A. daanei* following emergence was reduced from approximately 25% to 12%.

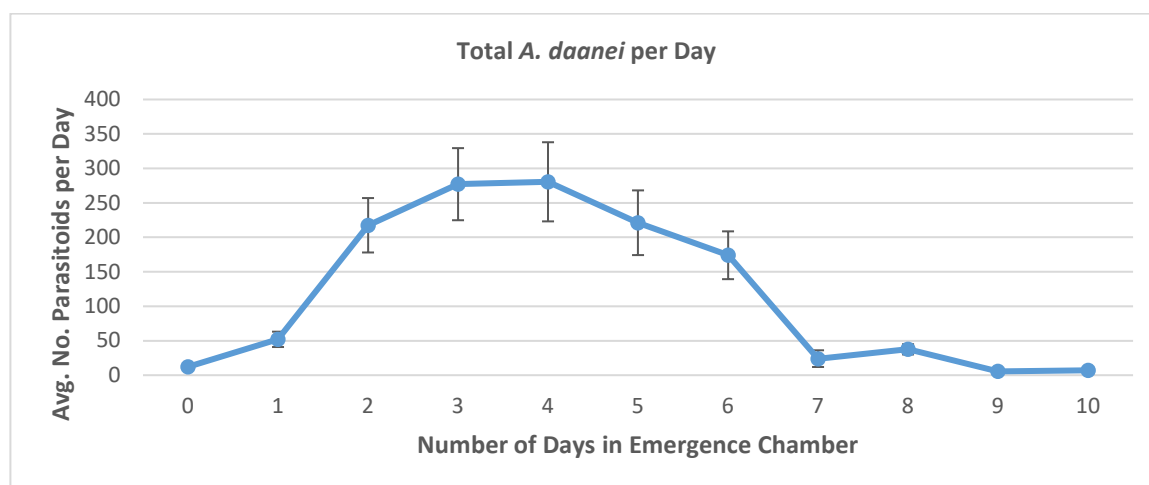


Fig. 8. Total *A. daanei* produced per day. Peak emergence occurs between days 2-5.

(2b) “Field Colony” Rearing Program

One of the main limitations to the greenhouse colonies of *A. daanei* is the availability of grape vine material, a large quantity of which is required to provide suitable oviposition sites for VCLH and, subsequently, *A. daanei*. Each colony contains 48 potted grape vines, 12 of which are replaced each week for rearing out the *A. daanei*. Between March – September approximately 1,300 healthy grape vines are required to maintain healthy colonies (6 months x 4 weeks/month = 24 weeks x 12 vines/colony/week = 288 vines/colony x 4 colonies = 1,152 vines + 48 initial vines/colony = 1,344 vines).

To overcome this limitation of the greenhouse colonies, another approach used in 2016 involved an attempt to create “Field Colonies”, in which untreated vineyard acreage with high densities of VCLH would be inoculated with *A. daanei* early in the season and then subsequently sampled later in the year to rear out parasitoids for release at additional sites. Sites for the Field Colonies were located near Hopland (1 site) and Davis (2 sites). Additional vineyard sites were scouted near Lodi (4 sites), but they did not contain any significant population of VCLH.

Initial surveys of the Field Colony sites found very low densities of VCLH and low parasitism rates (Fig. 9). The Hopland site was inoculated with *A. daanei* on April 23 and May 26. The Davis sites were never inoculated, since in previous years high densities of *A. daanei* naturally occurred at these sites. Grape material collected from the Davis sites on June 24 yielded low densities of *Anagrus* spp. and likely even lower densities of *A. daanei*, since previous surveys have indicated that only about 45-50% of the *Anagrus* spp. reared from these sites is *A. daanei*. Given the low yield and quality of parasitoids from the Field Colony sites, the *A. daanei* reared from the greenhouse colonies were prioritized for release in 2016.

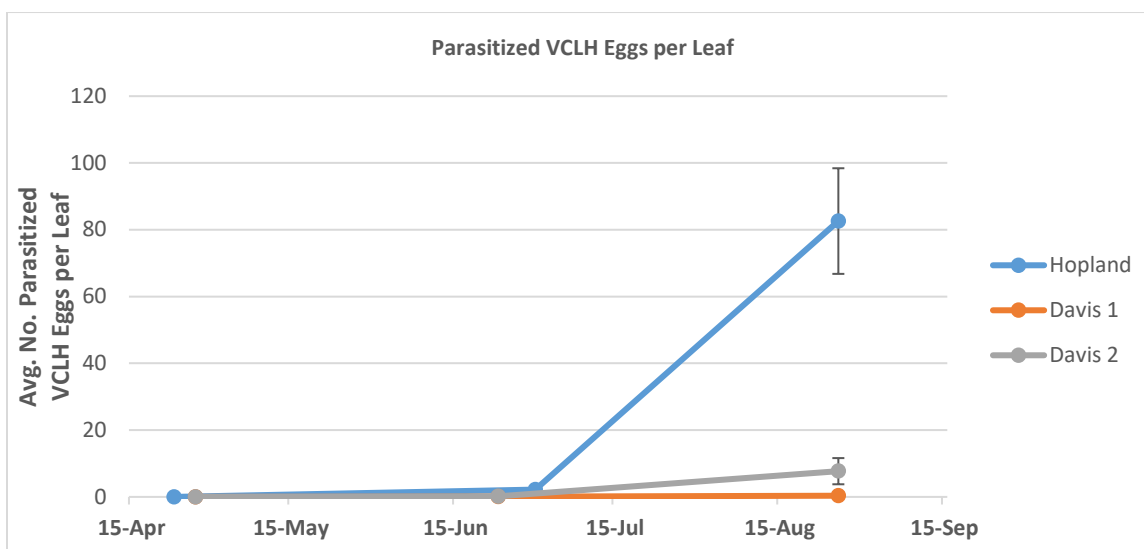


Fig. 9. Low densities of VCLH eggs and parasitism rates led to low yield of *A. daanei* from the “Field Colonies” effort.

(2c) *A. daanei* Release Program

Using material from the greenhouse colonies, multiple batches of *A. daanei* were released once per week between April 23 and September 2, 2016 (Fig. 10). During this period 27 release events took place and a total of 15,342 *A. daanei* were released across 9 sites, 7 in Mendocino and 2 in Lake County (Fig. 11). Parasitoids were typically released at 1 site each week, although in some weeks populations were high enough so that releases could be made at 2 sites. On average 568 ± 71 *A. daanei* were released each week and a total of $1,704 \pm 340$ were released per site. For each release, 10 voucher specimens were collected to confirm parasitoid identity.

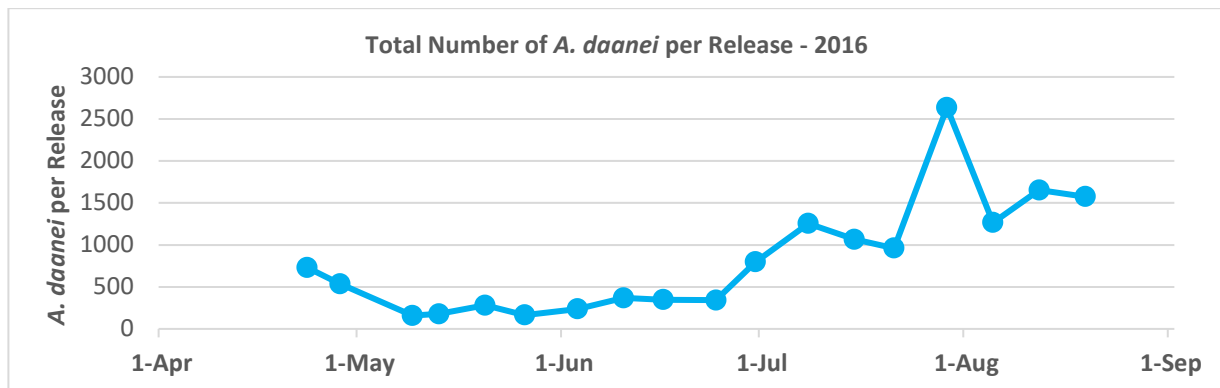


Fig 10. Total number of *A. daanei* per release. Parasitoids were released in greater abundance later in the season as greenhouse production and aggregation methods were improved.

Total *A. daanei* Released - 2016

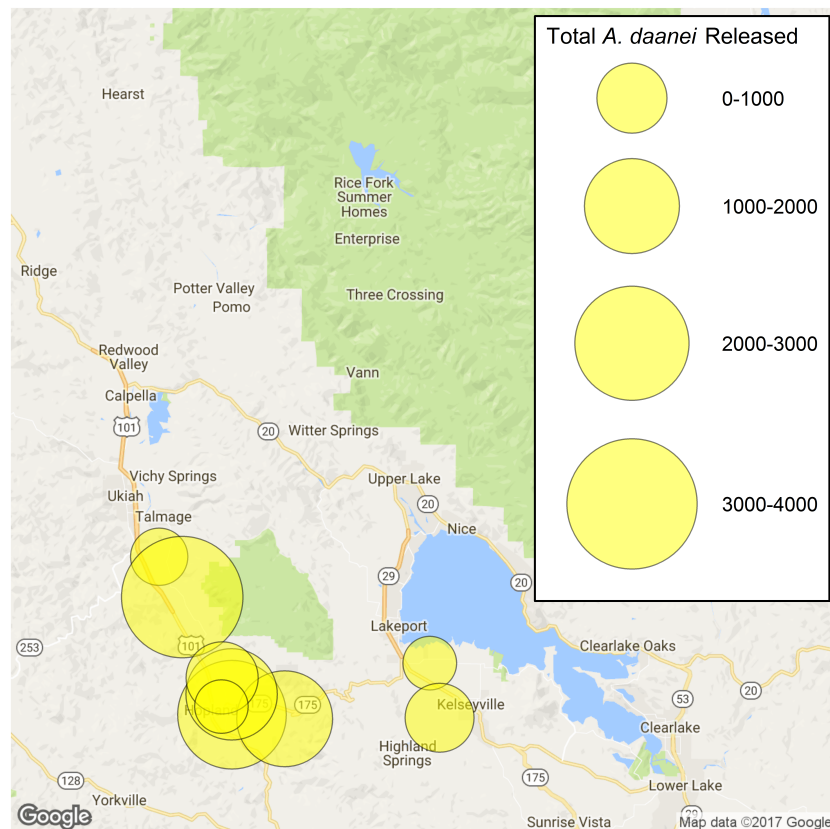


Fig. 11. Total *A. daanei* released in 2016

At each release site, parasitism of VCLH and WGLH was evaluated prior to release and then approximately once per month after that. Parasitism was evaluated at the release point (Release Point), 100 m away from the release point (Release Point + 100 m) and at a no-release control site (Control) located > 1000 m away from the release point (Fig. 12). During follow-up monitoring at each site, a subset of parasitized VCLH eggs were isolated and the *Anagrus* spp. was reared out and identified. Differences in parasitism rate between the sites was evaluated with logistic regression. When a significant difference was detected, Tukey contrasts were used to separate plots.



Fig. 12. Example layout of a paired *A. daanei* release point and control site. Parasitism rates were evaluated before and after each release at (1) the release point, (2) 100 m away from the release point, and (3) at a control site >1000 m away from the release point.

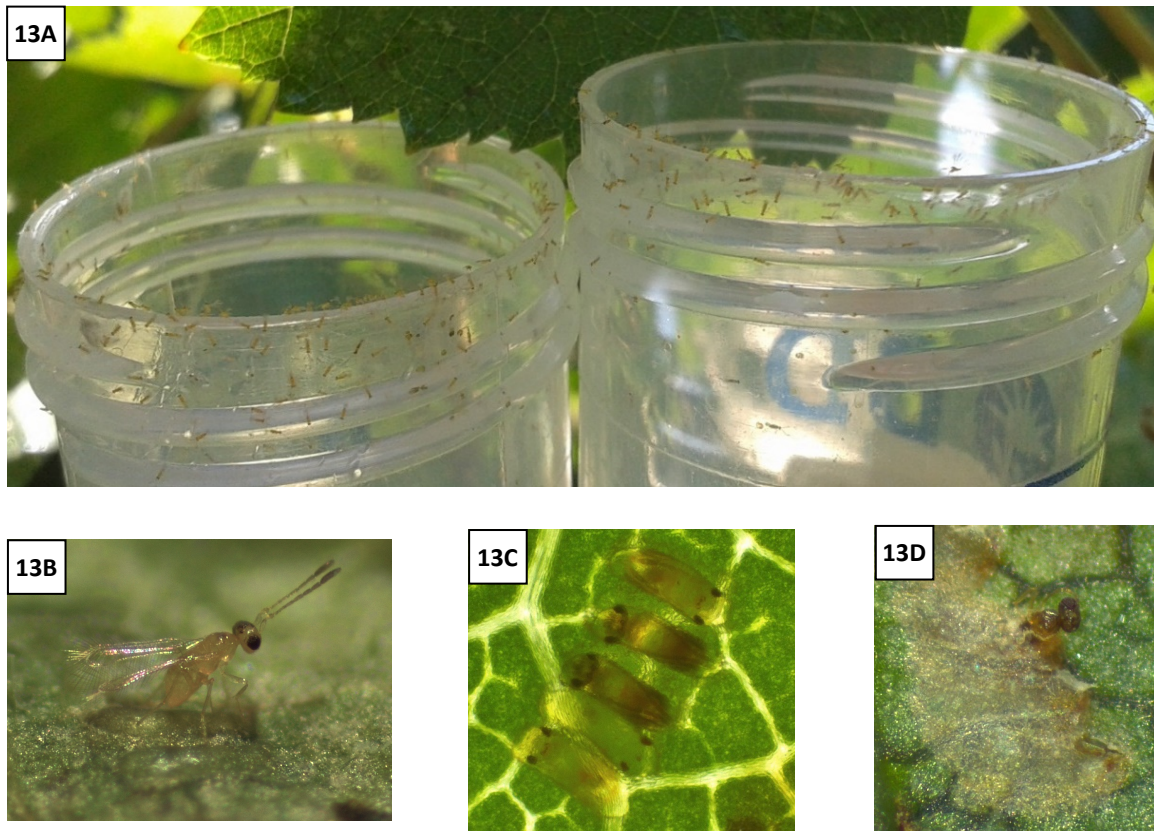
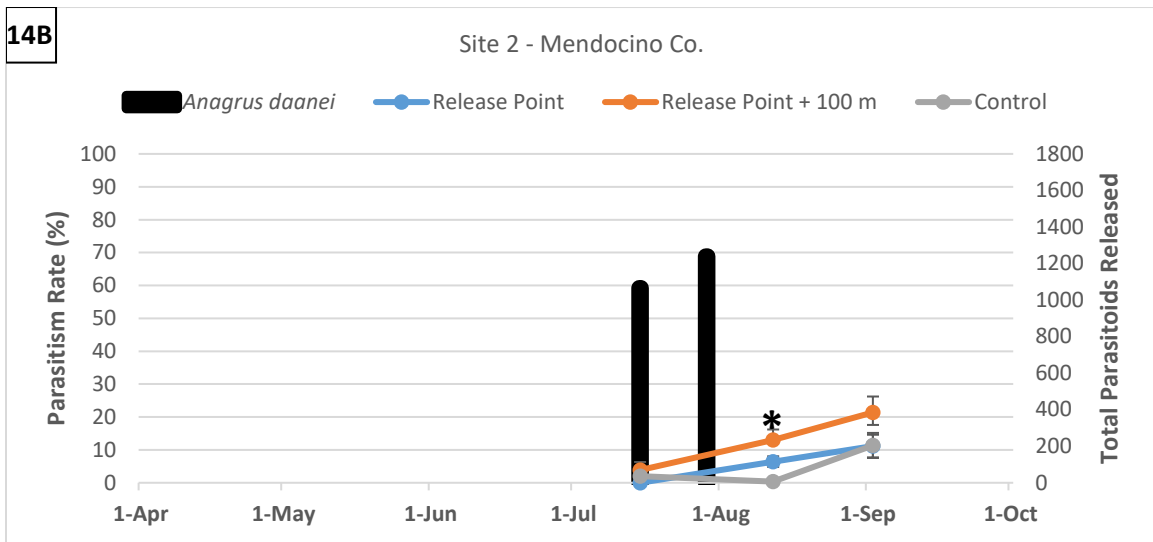
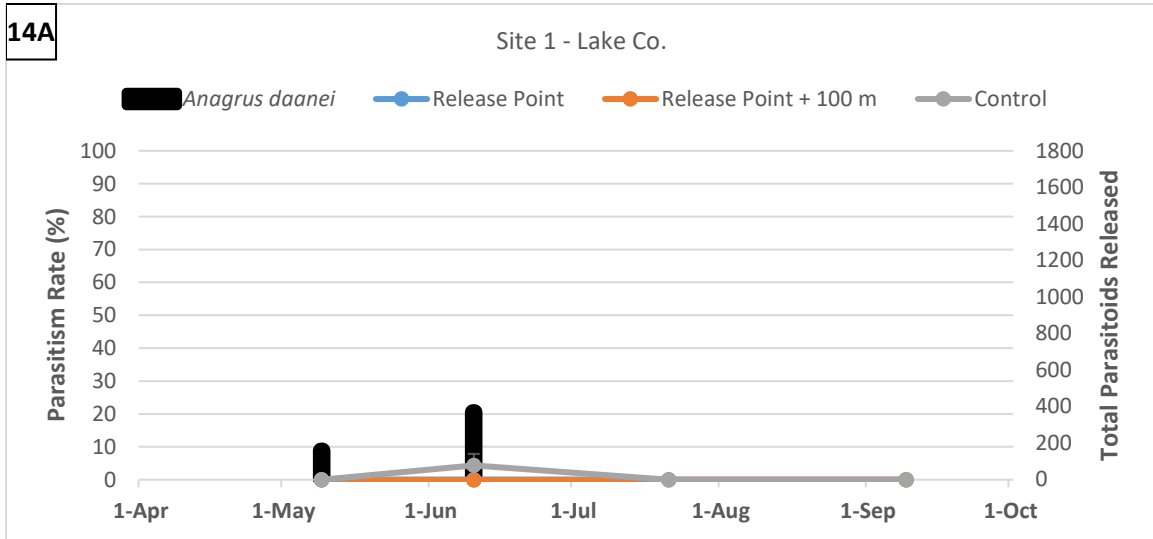
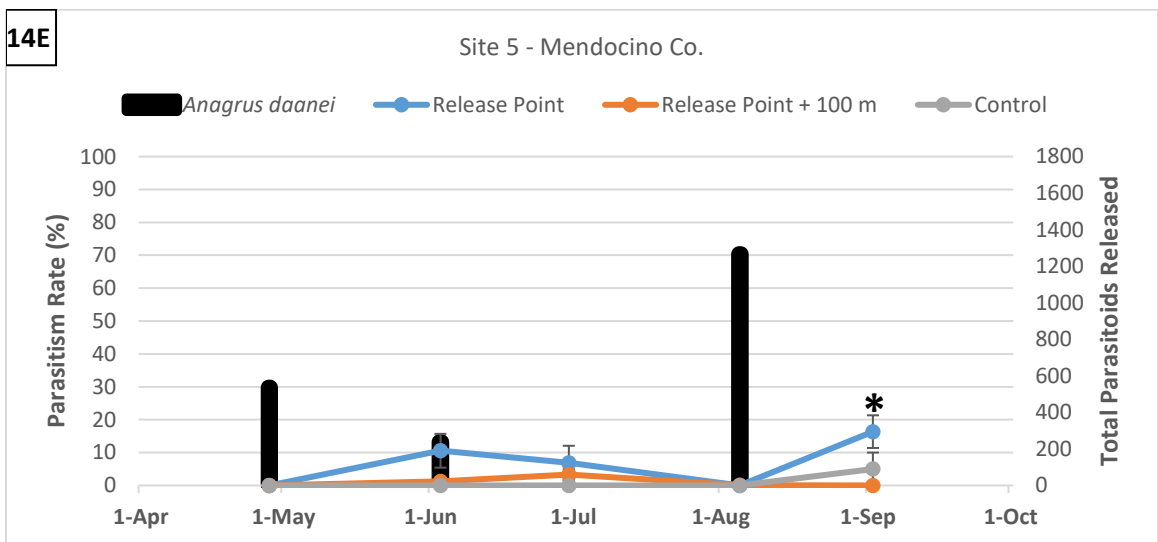
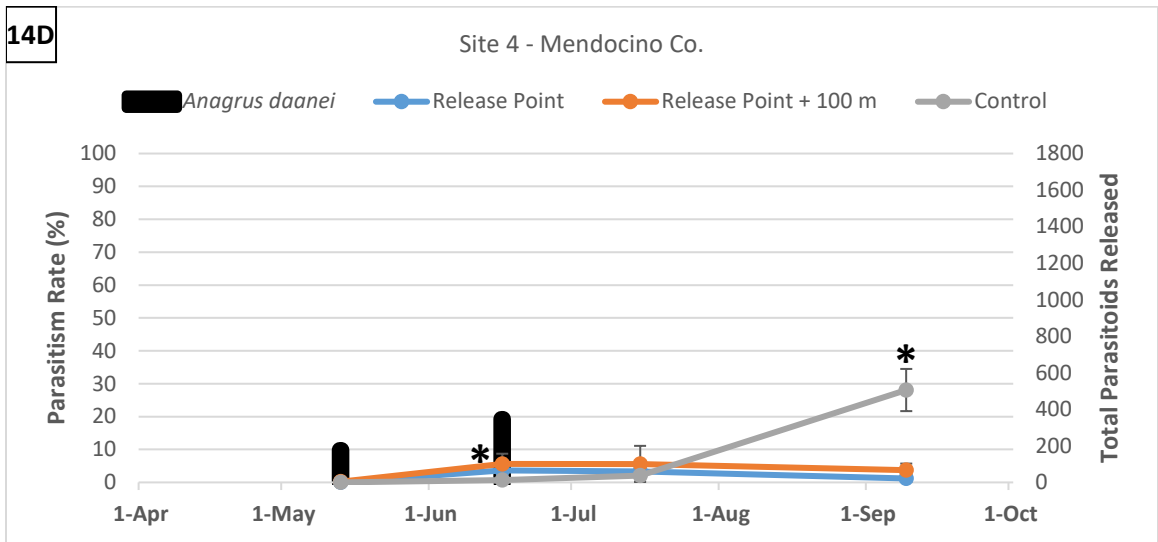
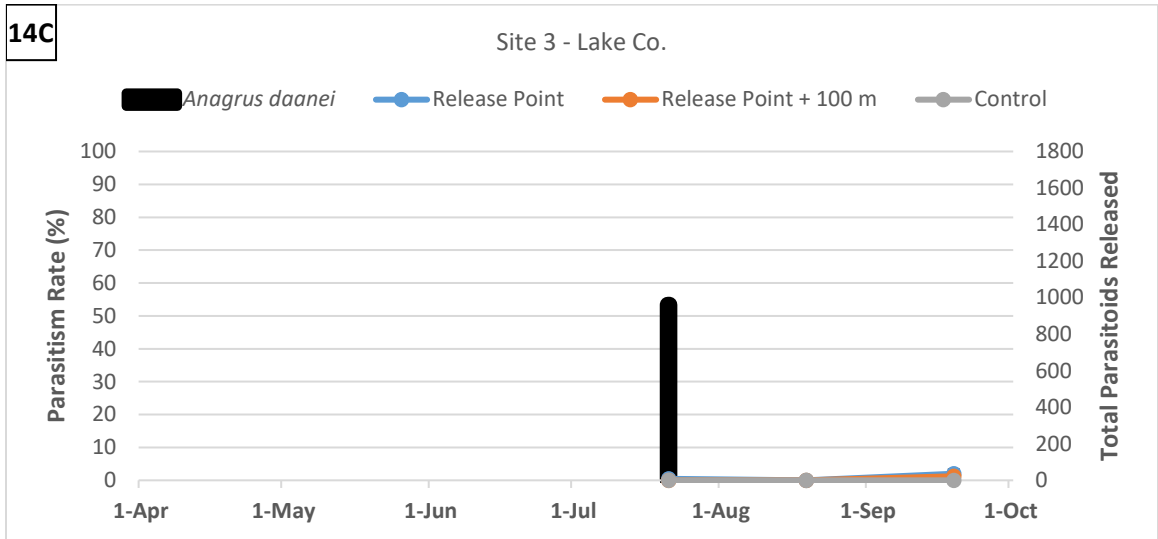
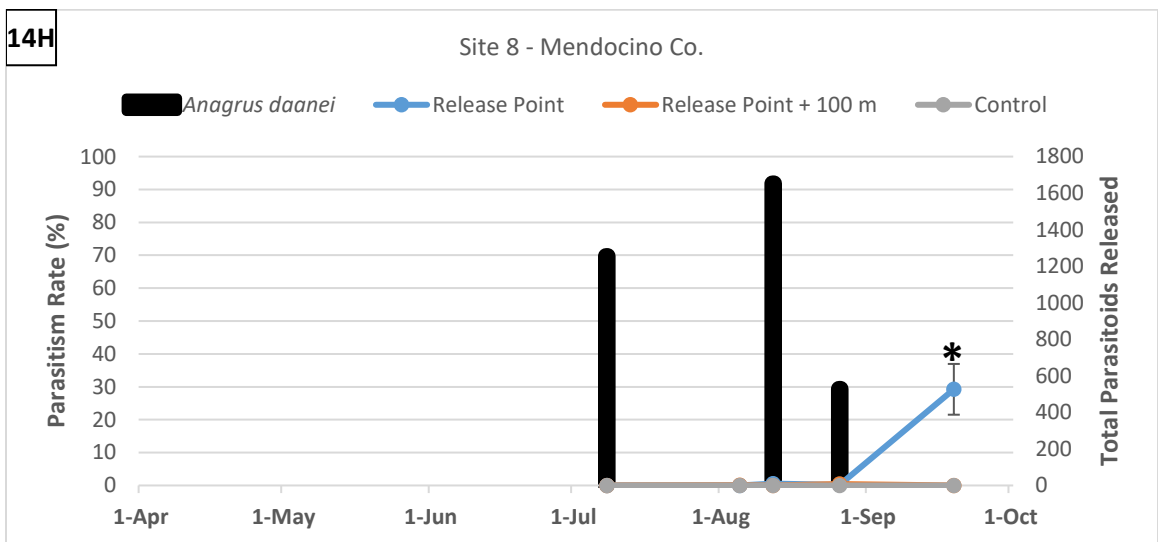
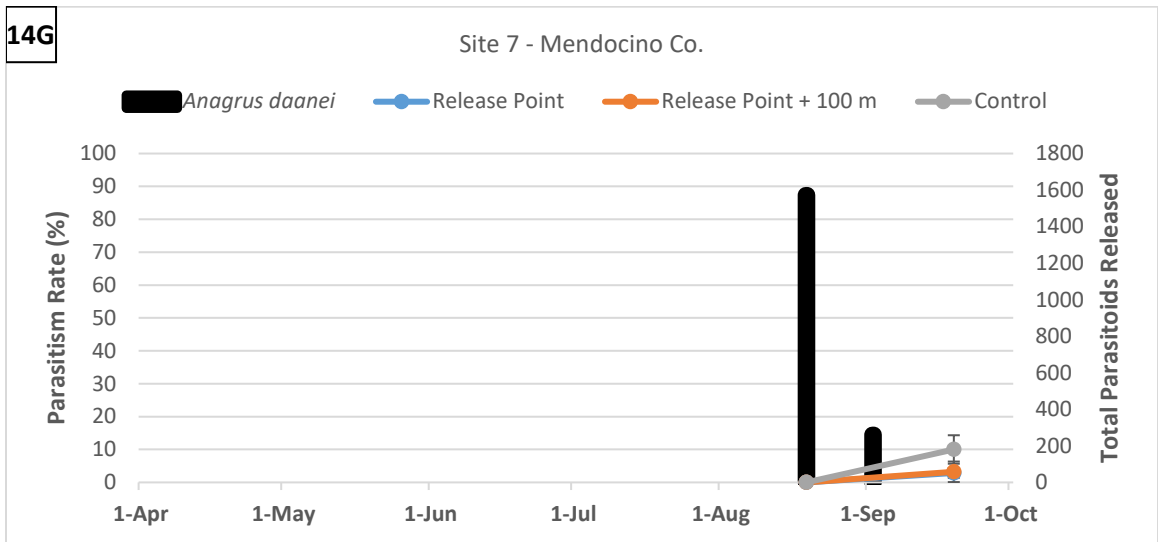
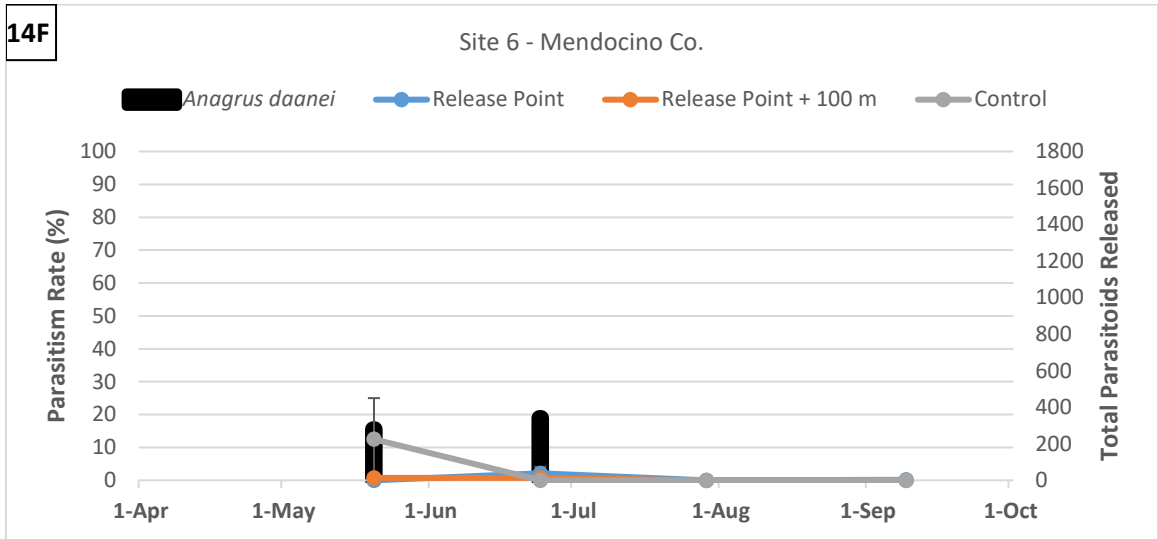


Fig. 13. (13A) *Anagrus daanei* release in a vineyard, note the parasitoids aggregated around the edge of the vials; (13B) *A. daanei* in the process of parasitizing a VCLH egg; (13C) parasitized VCLH eggs with well-developed *A. daanei* visible, the two dark spots at the tip of each egg are the eyes of the adult parasitoid; (13D) *A. daanei* emerging from VCLH eggs, the parasitoid chews a hole in the top of the egg and then pushes itself out over about 30-45 minutes.

Impacts of the *A. daanei* releases varied across sites. Significant increases in parasitism at the Release Point relative to Control plots was observed at 4 of the 9 sites (Fig. 15). In the following graphs, black bars represent quantity of *A. daanei* released while the colored lines indicate VCLH parasitism rates. Asterisks indicate significant differences ($P < 0.05$) between the Release Point and Control site.







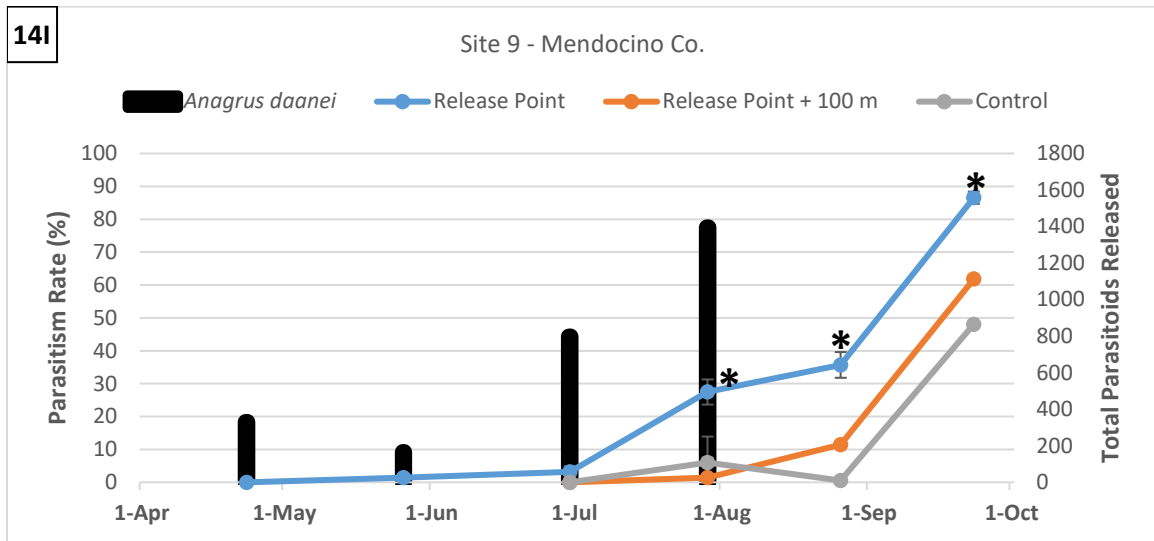


Fig. 14. Quantity of *A. daanei* released at each site (black bars, right Y-axis) relative to pre- and post-release VCLH parasitism rates (colored lines, left Y-axis). Graphs have been scaled equally in order to compare differences in number of parasitoids released and relative changes in VCLH parasitism rates. Asterisks indicate significant difference ($P < 0.05$) between “Release Point” and “Control” plots.

The extent to which *A. daanei* had an impact on parasitism rates at the Release Point is likely related to densities of healthy VCLH eggs at the time of release (Fig. 16A) and/or total quantity of *A. daanei* released (Fig. 16B). For example, at some sites a large quantity of *A. daanei* was released, but without adequate densities of healthy VCLH eggs there was no opportunity for the parasitoids to establish and therefore no observable effect. There are other factors that could have influenced the efficacy of the *A. daanei* releases as well, such as application of chemical controls around the time of release and/or making a release out of sync with VCLH biology (i.e. not releasing parasitoids during peak VCLH oviposition period), although this is difficult to discern from the current data.

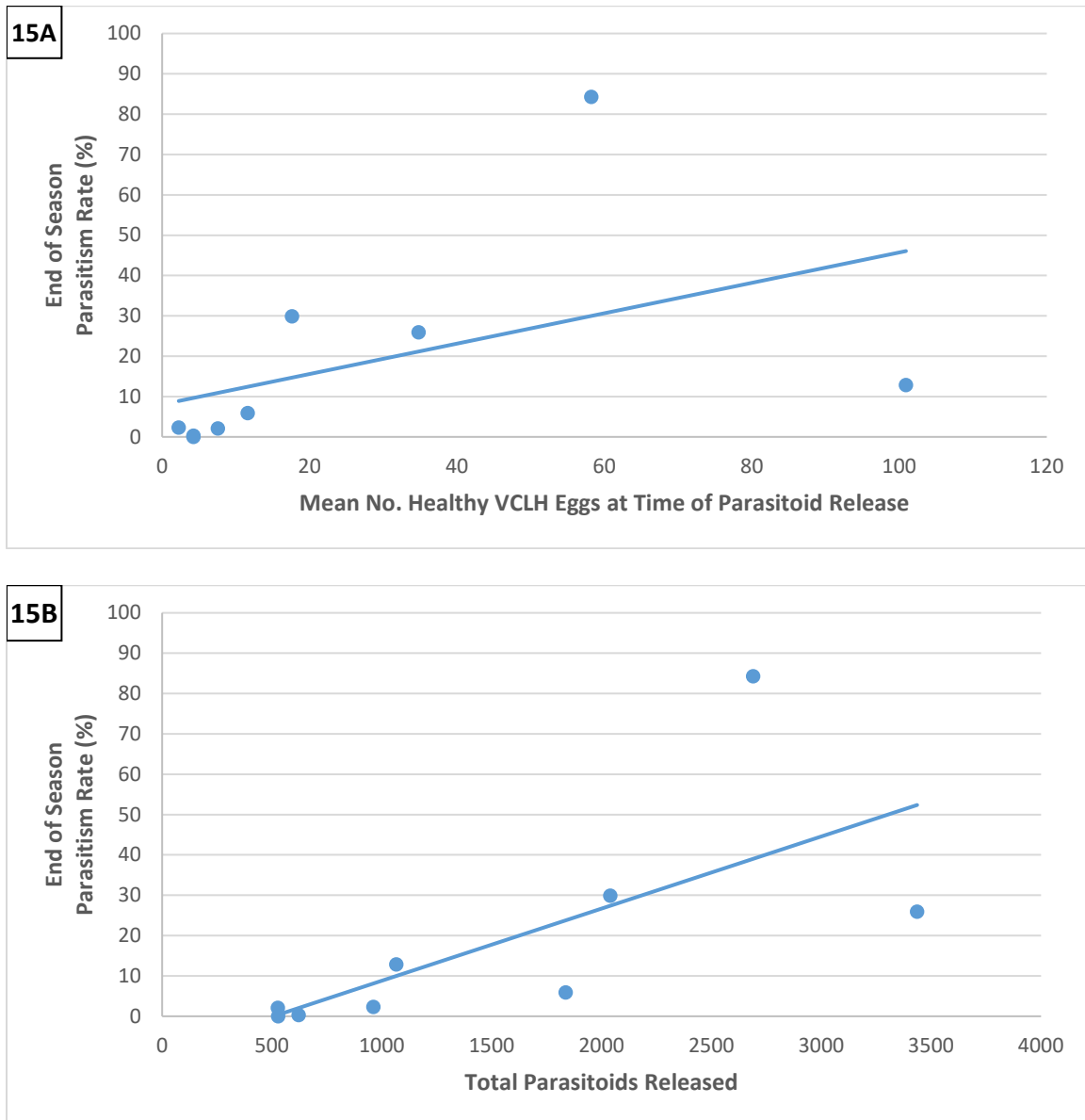


Fig. 15. Release of *A. daanei* tended to have greater impact at sites with higher densities of healthy VCLH eggs (15A) and/or at sites where greater numbers of *A. daanei* were released (15B).

(2c) Genetic Evaluation of A. daanei Populations

Outbreaks of VCLH in the North Coast are due, in part, to the fact that resident *A. daanei* in this region appear to only attack WGLH, although the parasitoid is known to attack both WGLH and VCLH. The identification of an *A. daanei* population in the Sacramento Valley that readily attacks VCLH immediately raised questions as to whether or not the two populations might actually be different species. This is especially relevant given that *A. daanei* was formerly referred to as *A. epos*, which Triapitsyn (1998) revealed to be a complex of multiple *Anagrus* species, each with a different preference for the multiple *Erythroneura* leafhoppers that attack commercial grapes in California.

To date, morphological comparisons of the Sacramento and Mendocino *A. daanei* populations has not revealed any differences to indicate that these may be two different species. This year specimens of *A. daanei* from both populations were submitted to genetic evaluation with a focus on the CO1 and ITS2 regions of the genome. Preliminary results have indicated a small amount of variation unique to the Sacramento Valley population, although further analysis is needed to fully elucidate these differences. As such, next year *A. daanei* specimens from the two populations will undergo more detailed evaluation focusing on a broader range of the genome using RADseq and anchored hybrid enrichment.

(3) Grower Outreach and Education

(3a) Leafhopper Newsletter

As previously mentioned, data from the regional monitoring effort was summarized in a “Leafhopper Newsletter” which was sent via email to growers each week. The newsletter also included information on identification of WGLH and VCLH nymphs and an update on the most recent *A. daanei* releases. At present there are a total of 73 subscribers, which includes 58 growers and PCAs who oversee more than 10,000 vineyard acres in Mendocino and Lake County. The remaining subscribers include 9 county, state and UC ANR personnel and the 6 project team members.

(3b) Grower Meetings

A “Tailgate Talk” was held on July 7, 2016 at a vineyard near Hopland. There were 22 attendees representing 9 vineyard operations, including the 3 largest growers in the area, along with multiple regional PCAs and state and county officials. The area-wide IPM team provided an update on regional leafhopper population trends and parasitism rates, the *A. daanei* release program, and an overview of the project website. This was followed by an open discussion with growers about their experiences managing VCLH and the value of various cultural and chemical controls.

The “Mendocino-Lake IPM Seminar” took place on Nov. 18 at the Hopland Research and Extension Center. A summary of findings from the 2016 area-wide IPM program was presented, followed by more open discussion with growers about VCLH management. More than 60 growers and PCAs were in attendance.

(3c) Project Website (<http://ucanr.edu/sites/vclh>)

The project website was established in December 2015. In addition to general information on project background, VCLH biology and leafhopper identification, the website now includes research summaries from crop years 2013-2016 as well as an archive of the Leafhopper Newsletter. So far this year the website has recorded a total of 568 unique users and/or sessions. Of those, 341 came from the United States (mostly California) and most visits were directed towards the leafhopper newsletter. Peak traffic regularly occurred following the release of the weekly Leafhopper Newsletter (Fig. 16).

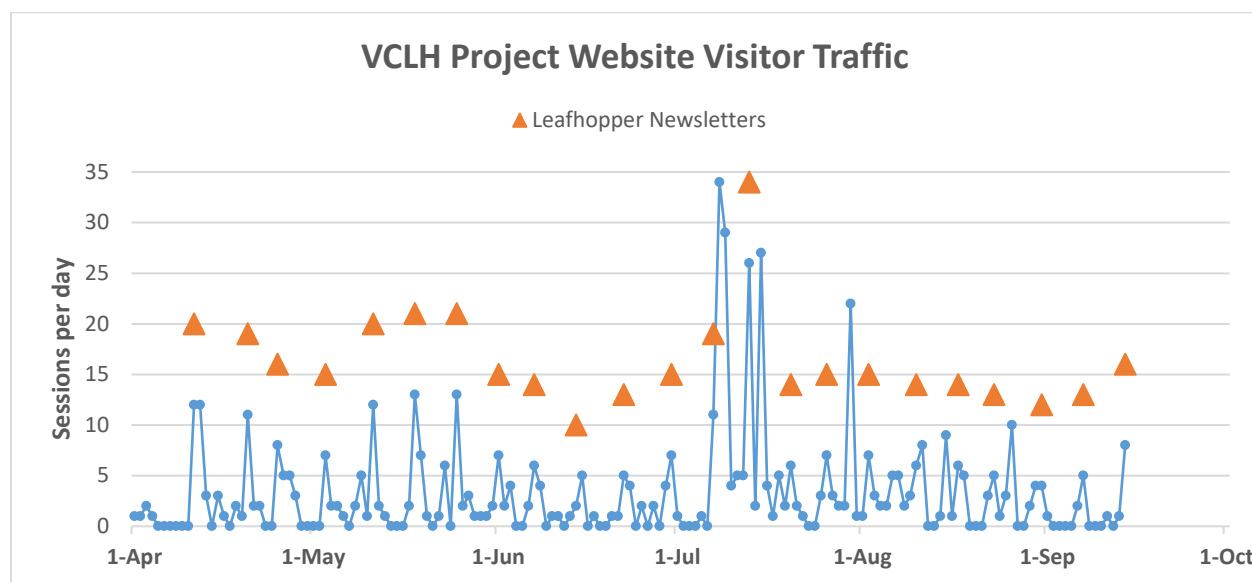


Fig. 16. Visits to the project website tended to peak following the release of the weekly Leafhopper Newsletter as well as after the “Tailgate Talk” on July 7.

Problems Encountered

(1) Regional Monitoring

One goal of this project was to generate “hot spot” maps using data collected from the regional monitoring effort. These maps are generated by taking data from the regional monitoring sites and interpolating it across the entire county, thereby providing growers with a better idea of regional trends in VCLH densities, especially in areas that are not in immediate proximity to the monitoring sites. While data from the monitoring effort was regularly presented to growers as line graphs in the Leafhopper Newsletter, hot spot maps would have provided growers with an alternate, additional way to interpret the data.

Working with the GIS and Ecological Mapping Unit at the Hopland Research and Extension Center, preliminary hot spot maps were successfully generated in June 2016. Unfortunately, map resolution was very limited due to the small number of sites (7) included in the regional monitoring effort. While this was initially thought to be an adequate sample size for such maps, it is now clear that data from at least 10 sites must be provided in order to generate a useful map.

(2) *A. daanei* Rear and Release Program

While rearing, aggregation and release of *A. daanei* has been greatly improved over the past 2 years, the ability to release large numbers of parasitoids early in the season still remains a challenge. This is primarily due to the difficulty of supporting robust VCLH and *A. daanei* colonies over the winter and building up their populations earlier in the spring than would typically occur in the field. Timing releases to coincide with key stages of the VCLH life-cycle is also important (i.e. during early season egg deposition). Small numbers of *A. daanei* available from the colonies early in the season limited the number of release sites during this period. Furthermore, release sites with high densities of VCLH must be targeted and prioritized, especially for early season releases. This would improve the likelihood of *A. daanei* establishment. Finally, the use of “Field Colonies” proved unnecessary, as they yield a significantly lower density of *A. daanei* relative to the greenhouse colonies.

(3) Grower Outreach and Education

No major problems were encountered with the grower outreach and education component of this project.

Milestones Achieved

(1) Regional Monitoring

Far more sites were included in the 2016 regional monitoring effort than was originally planned for. Seven sites, rather than five, were monitored in Mendocino County. Additionally, five sites were also monitored in Lake County, which was not scheduled to participate in the regional monitoring effort until 2017. The Lake County monitoring was especially unique because it was carried out entirely by collaborating growers and PCAs. Their participation in the 2016 monitoring effort provides valuable experience for this type of program and will greatly improve the 2017 monitoring effort in that county.

Regional VCLH monitoring requires the coordinated activity of multiple growers, PCAs, and UCCE personnel spread throughout the North Coast and Bay Area regions – all of whom are dealing with a variety of competing research and production demands beyond the scope of the VCLH area-wide program. In light of this, the project team successfully developed methods and routines to coordinate regular data collection from 12 vineyard sites across 2 counties and the ability to rapidly process and summarize it to produce the weekly Leafhopper Newsletter.

(2) *A. daanei* Rear and Release Program

Major improvements were made in 2016 to the *A. daanei* rearing, aggregation and release program. Greenhouse colonies were expanded which led to increased production of *A. daanei*. Parasitoid yield was also improved by reducing mortality during the *A. daanei* aggregation process. The increased production of *A. daanei* allowed them to be released in larger numbers, earlier in the year, and at more sites and counties than in any previous year (Table 1). Finally, *A. daanei* releases did lead to increased parasitism at 4 of the 9 sites.

Table 1. Comparison of *A. daanei* rear and release program in 2015 and 2016

Event	2015	2016
No. of Colonies	1	4
First <i>A. daanei</i> Release	July 29	April 23
Release Events	8	27
Total <i>A. daanei</i> Released	2,258	15,342
Release Sites	1	9

(3) Grower Outreach and Education

The regular circulation of the Leafhopper Newsletter was an outstanding success. Many growers have indicated that this was a useful and reliable resource that improved their ability to manage VCLH populations in their vineyard. In particular, weekly announcements of VCLH nymph emergence and development likely improved spray timing, especially during the first generation, which is important for season-long control of VCLH.

Another avenue for grower outreach and education has been the project website (<http://ucanr.edu/sites/vclh>), which now contains a variety of content related to VCLH ecology and management as well as annual summaries of research, recorded talks and news items related to VCLH in California vineyards. The website does appear to have regular traffic, which seems to be mostly drawn in by the Leafhopper Newsletter.

Finally, grower meetings over the past year (Nov. 2015 and July 2016) have all been well-attended by a variety of growers, PCAs, and state/county personnel. While these events do include formal presentations on various aspects of VCLH ecology and management, they also provide a forum for growers and researchers to discuss VCLH management and research findings to date.

Plans for the Following Year

All of the activities from 2016 will be continued and in many cases expanded in 2017.

(1) Regional Monitoring

The regional monitoring effort in 2017 will closely resemble the effort that took place in 2016, although monitoring in Lake County will start earlier in the season, include a higher level of detail and possibly more sites.

In order to provide more data points for both the hot spot maps as well as the ecological modeling component of this project, efforts will be made to bring in more collaborating growers/PCAs to collect information on pest densities from a broader range of sites.

(2) *A. daanei* Rear and Release Program

Greenhouse colonies of *A. daanei* will be expanded in spring 2017. Greenhouse space has already been acquired for additional new colonies. Grape material will also be added to the colonies earlier in the year (i.e February) in order to build up greater populations even earlier in the year than previously. In combination, this will hopefully allow for the release of higher quantities of *A. daanei* (i.e. >500/release) earlier in the season (i.e. April 1) and at more sites. In 2017, parasitoids will be released at no less than 5 sites in Lake County and 5 sites in Mendocino County.

(3) Grower Outreach and Education

Printed material with guides on leafhopper identification will be developed to complement the guides currently available online (project website, UC IPM, and Leafhopper Newsletter).

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