1. Summary

Project Title: Egg parasitism of the Virginia creeper (*Erythroneura ziczac*), a newly invasive leafhopper pest in California (Year 3)

Principal Investigators: Kent Daane, Glenn McGourty, Serguei Triapitsyn, Lucia Varela

Summary:

Organic grape growers in Mendocino and Lake County have been experiencing severe outbreaks of the Virginia creeper leafhopper (*Erythroneura ziczac*) since 2011. Feeding by *E. ziczac* causes leaf stippling and reduced photosynthesis which can impact crop yield and quality. High populations of *E. ziczac* adults in the fall can also be a nuisance, flying into the eyes, nose, and mouth of workers manually harvesting grapes. The primary natural enemies of *E. ziczac* are the small egg parasitoids *Anagrus daanei* and *Anagrus tretiakovae* (Hymenoptera: Mymaridae). A related pest, the Western grape leafhopper (*Erythroneura elegantula*) is also parasitized by *A. daanei* as well as *Anagrus erythroneurae*. *Erythroneura ziczac* and *E. elegantula* are commonly found together in North Coast vineyards. *Anagrus daanei* is the parasitoid species of most importance for *E. ziczac* control, whereas *A. tretiakovae* is rarely found in California.

Our approach to improving *E. ziczac* control involves a combination of short- and long-term strategies. Short-term work focuses on grower outreach/education to improve pest identification and timing of chemical controls while long-term strategies are focused on the identification, evaluation and introduction of *Anagrus* parasitoids to improve biological control.

In 2015 we held multiple outreach events (March 6 and November 20) and a field day (July 29) to review *E. ziczac* identification, management and provide updates on research progress and findings to date. There were also presentations made about the importance of leafhoppers in general in the transmission of grape "red blotch". We also initiated a regional monitoring program to keep growers informed about the seasonal development of leafhopper populations in Mendocino County. Each week, at multiple vineyard sites, data were collected on adult leafhopper flights, egg deposition, nymph densities and parasitism rates. A summary of this data was then circulated to growers via a weekly email newsletter (http://ucanr.edu/sites/vclh/VCLH_Newsletter/). Finally, we established a project website (http://ucanr.edu/sites/vclh/) to serve as a repository of information on management of *E. ziczac*.

Our previous research has shown that although *A. daanei* is present in the North Coast, it does not attack *E. ziczac*. Surveys across northern California have identified another population or "strain" of *A. daanei* in the north San Joaquin Valley that will readily parasitize *E. ziczac*. As such, we proposed to collect, augment and release this novel strain of *A. daanei* into Mendocino and Lake County vineyards in order to establish biological control of *E. ziczac*. In 2015, more than 2,000 *A. daanei* were collected from Yolo County and released into a Mendocino County vineyard. Following these releases, parasitism of *E. ziczac* at the release site increased from 0% to 87-91%.

Also of importance is to note that in June 2015 our research group received a large grant from the California Department of Pesticide Regulation "Pest Management Alliance Program" that will provide funding for this research program through March 2018. These funds will allow for the continued importation and release of *A. daanei* into Mendocino/Lake County vineyards, as well as support the on-going outreach and education efforts with growers, including the regional monitoring of leafhopper populations.

2. Annual or Final Report

This is a final report (year 3)

3. Project Title and UGMVE proposal number

"Egg parasitism of the Virginia creeper (*Erythroneura ziczac*), a newly invasive leafhopper pest in California." (Proposal #2016-1493)

4. Principal Investigator/Cooperator(s)

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Report authors (for citation): Wilson, S. H., McGourty, G., Varela, L. G., Triapitsyn, S. V., and Daane, K. M. 2016. Egg parasitism of the Virginia creeper (*Erythroneura ziczac*), a newly invasive leafhopper pest in California. American Vineyard Annual Reports, Crop Year 2015.

5. Objective(s) and Experiments Conducted to Meet Stated Objective(s):

(1) Improve biological control of *E. ziczac* in the North Coast by introducing *A. daanei* from the North San Joaquin Valley.

Potted Vines Approach

Parasitoids were initially collected by inoculating sets of potted grape vines (var. CS 06) with *E. ziczac* eggs (197 ± 53.8 eggs/vine) and then exposing the vines at vineyard sites in Yolo County with known populations of *A. daanei*. After one week of exposure, the potted vines were brought to the greenhouse and placed into parasitoid emergence chambers, which consisted of opaque cylindrical containers (12" diameter x 36" height) with a clear vial attached to the top. The *Anagrus* parasitoids exhibit both gravi- and photo-taxis, which upon emergence from *E. zizac* eggs drives them to move to the top of the container and into the vial. Vials were checked 3 times daily for *Anagrus* emergence. All parasitoids were aggregated into a larger container that contained a strip of filter paper soaked in 50% honey solution. The parasitoids were then held at 10°C in an incubator without light until release. A previous pilot study had shown that *Anagrus* parasitoids could be successfully held under these conditions for an average of 29 ± 5.7 days.

The first set of potted grape vines was exposed on June 11 (16 vines) and subsequent cohorts were exposed on June 27 (10 vines) and July 16 (8 vines). After retrieving the potted vines, they were held in emergence chambers in the greenhouse (24°C, 40% RH, photoperiod 16:8 L:D) for a three week period. Recovery of *Anagrus* was surprisingly low, with an average of only 115 ± 24 total *Anagrus* collected over each 3-week emergence period (Figure 1). Additionally, during the field exposure period both Western grape leafhopper (*Erythroneura elegantula*) and Variegated leafhopper (*E. variabilis*) had laid eggs on the potted vines which were subsequently parasitized by *Anagrus* spp. Thus the already small quantity of *Anagrus* recovered from the potted vines was found to be a mix of *A. daanei* (55%) and *A. erythroneurae* (45%), the latter of which likely coming from the eggs of *E. elegantula*.

Established Colony of A. daanei *at UC Berkeley greenhouse*

Rather than release the small quantity of *Anagrus* spp. collected from the potted vines, we decided to introduce them into a greenhouse colony of *E. ziczac* in order to augment their densities for a future release date. While both *A. daanei* and *A. erythroneurae* were introduced into the *E. ziczac* colony, it is assumed that the absence of any suitable hosts for *A. erythroneurae* kept them from establishing. Subsequent identification of parasitoids from this colony have so far indicated that only *A. daanei* is present.

"Field Rearing" Approach

Given the small number of *Anagrus* recovered using the potted vines, a new approach was developed in July 2015 that involved the direct collection of parasitoids from vineyards in the Yolo County area with very high leafhopper populations. Starting July 23, grape leaves were collected each week and placed into emergence chambers, which were then monitored for parasitoid emergence over a 3-week period. Initially 360 leaves were collected each week, but on August 7 this was doubled to 720 leaves per week. Weekly collections took place through August and the last collection was on September 11, for a total of 9 collection/emergence periods between July 23 and September 18. For each round of collection, batches of 30 leaves were placed into an individual emergence chamber. This new method produced much higher rates of recovery, with an average of 716 ± 154 total *Anagrus* collected in each emergence period (Figure 1). As with the potted vines, these leaves from field sites also contained a mixture of *E. ziczac*, *E. elegantula* and *E. variabilis* eggs and thus a mixed population of *A. daanei* (45%), *A. erythroneurae* (52%) and *A. tretiakovae* (3%) were recovered. Regardless of this mix, we were still collecting a much greater number of *A. daanei* relative to the previous approach.

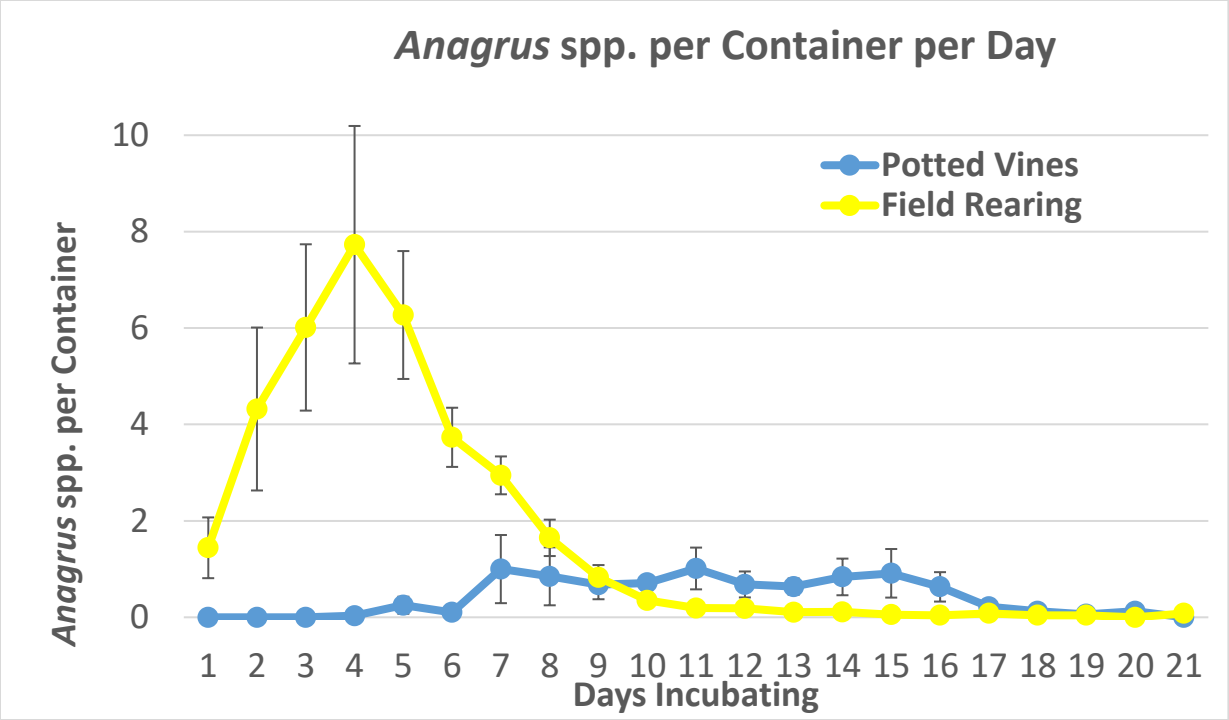

Figure 1. *Anagrus* spp. emerged earlier and in higher abundance using the "field rearing" approach.

(2) Implement an area-wide IPM program to promote grower awareness and adoption of best management practices for control of *E. ziczac* in the North Coast.

Grower Meetings

Grower meetings were held on March 6, July 29 and November 20 to review *E. ziczac* identification, management and provide updates on research progress and findings to date.

Regional Monitoring and "Leafhopper Newsletter"

Additionally, UCCE personnel carried out a regional leafhopper monitoring program between April – October 2015. Each week, adult leafhopper flights, egg deposition, nymph densities and parasitism rates were evaluated at seven vineyard sites spread throughout Mendocino County (McDowell Valley, Hopland, and Ukiah/Talmage area). The idea was to provide growers with information on leafhopper population development in order to raise awareness of leafhopper "hot spots" and help them improve spray timing to better coincide with the most vulnerable leafhopper life-stages. We have also been working with the Hopland Research and Extension Center (HREC) GIS and Ecological Mapping Unit to develop a "data-store" for this regional monitoring effort. Georeferenced pest counts will be used to generate regional maps of leafhopper densities and parasitism rates. Database and map development is still in progress.

Project Website

A project website was developed in October 2015 (http://ucanr.edu/sites/vclh) which will serve as a clearing-house for all information related to this Virginia creeper leafhopper research program.

6. Summary of Major Research Accomplishments and Results by Objective

(1) Improve biological control of *E. ziczac* in the North Coast by introducing *A. daanei* from the North San Joaquin Valley.

Release of A. daanei *Leads to Increased Parasitism of* E. ziczac

The first *Anagrus* release took place on July 29, in a vineyard near Hopland, CA. Subsequent releases were made at this site every seven days until the last release on September 18 (Figure 2). Pre- and post-release evaluations of *E. ziczac* parasitism were conducted at the release point, 100 m. away from the release point, and at a control site two km. away where no parasitoids were ever introduced (Figure 3).

Some parasitism of *E. ziczac* was recorded prior to the release, with 6% parasitism in the area 100 m. away from the release point (Figure 3). This is thought to be the result of natural re-adaptation of the endemic *A. daanei* in the Mendocino/Lake County area to *E. ziczac*. Re-acceptance of *E. ziczac* by the local *A. daanei* population has been expected throughout the duration of the project, although the rate at which this would take place was unclear. At an earlier stage of this project, an experiment in which *A. daanei* from Mendocino County was isolated with large quantities of *E. ziczac* eggs indicated that even in a no-choice situation the parasitoid would not attack *E. ziczac* (see report for crop year 2014). Apparently after multiple years of exposure to *E. ziczac* under field conditions, some proportion of the regional *A. daanei* population is now beginning to attack *E. ziczac* in Mendocino County.

Regardless, the subsequent *Anagrus* releases did appear to significantly increase *E. ziczac* parasitism rates both at the release point as well as 100 m away (Figure 3). Later in the season some parasitism of *E. ziczac* at the no release control site was observed as well. Similar to the pre-release parasitism that was observed, this is likely due to the re-adaptation of the regional *A. daanei* population to *E. ziczac*. Alternately, since this trend was observed after the releases had begun, movement of *Anagrus* from the release point to the control site cannot necessarily be ruled out, as these minute parasitoids may be able to move great distances as "aerial plankton", although there is little information on *Anagrus* dispersal. Following the release, parasitized *E. ziczac* eggs were isolated and the parasitoid reared out for identification. In all instances, the parasitoids were identified as *A. daanei*.

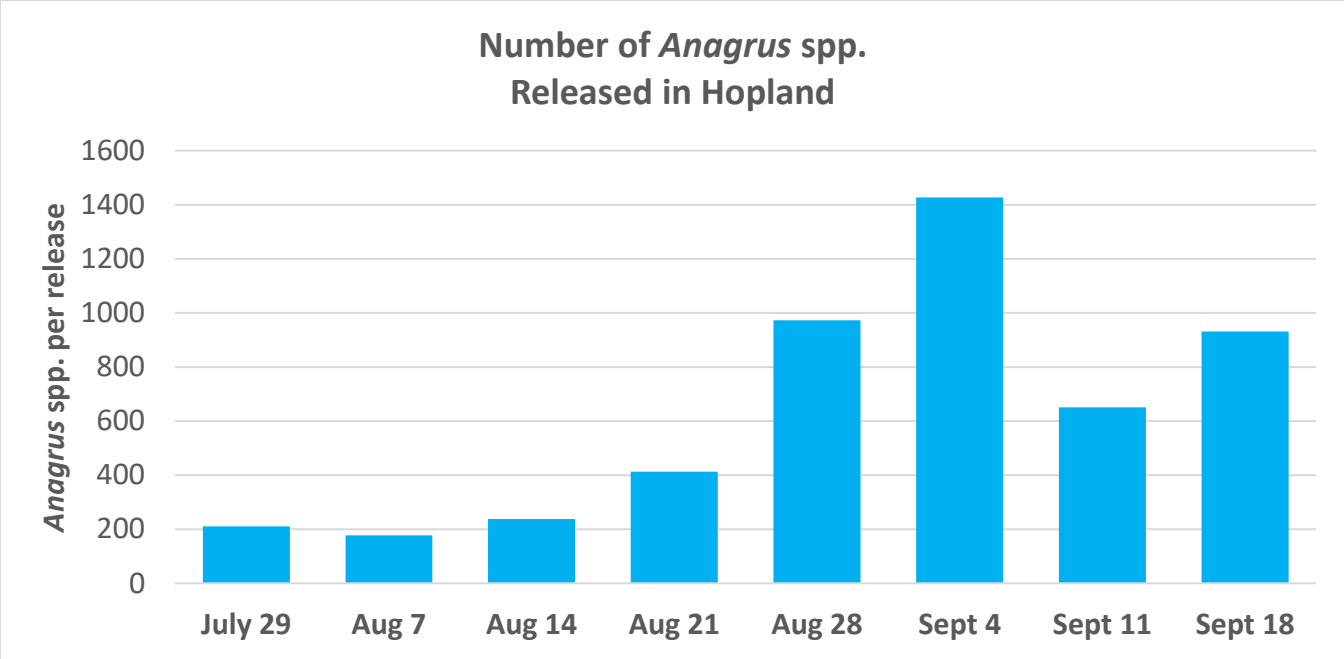

Figure 2. Total *Anagrus* spp. per release.

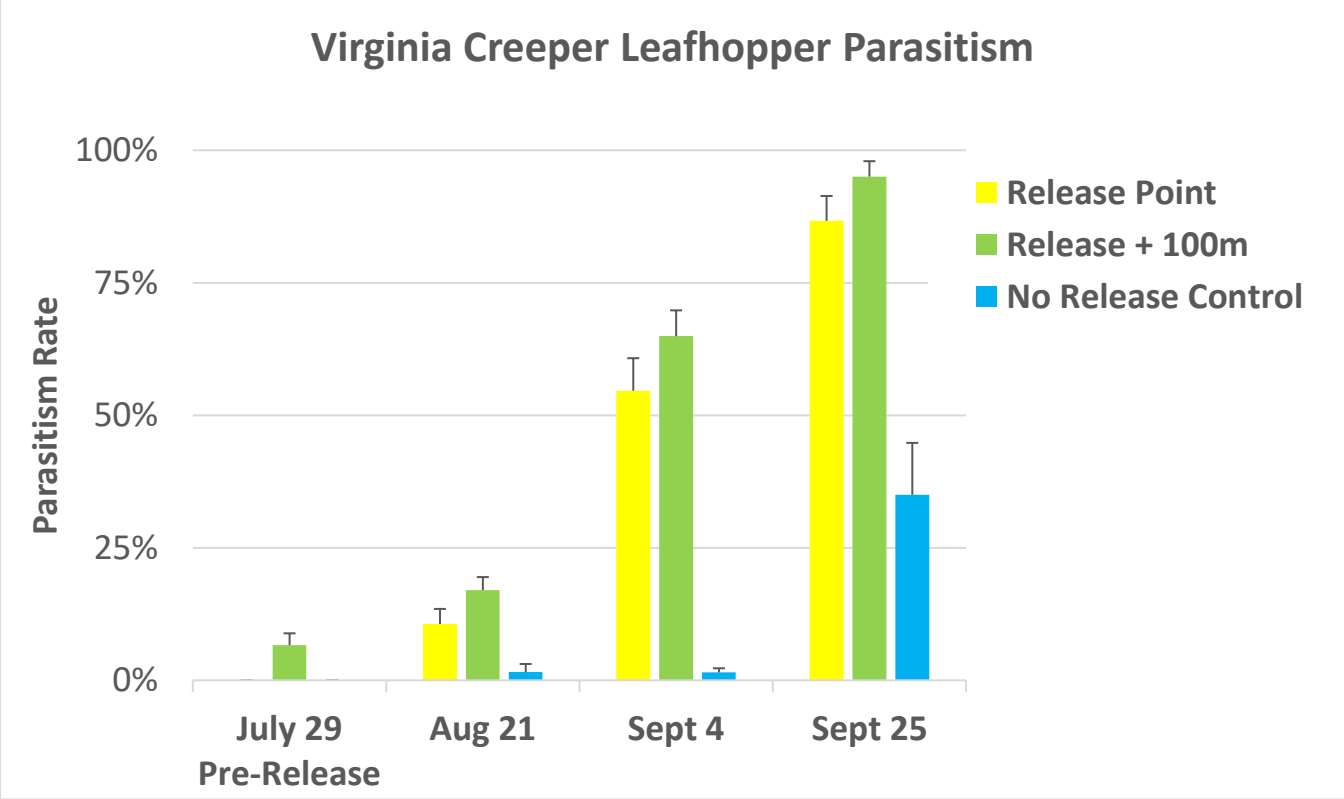

Figure 3. Pre- and post-release evaluations of *E. ziczac* parasitism.

Improved Collection and Rearing Methods for A. daanei

The methods initially proposed to collect and rear *A. daanei* proved inadequate. Exposing potted vines inoculated with *E. ziczac* eggs in Yolo County did not produce enough *A. daanei* to merit a field release in Mendocino County. Furthermore, this approach required a 33-day cycle from vine inoculation to parasitoid release (Table 1). As such, a new method that relied on grape leaves from heavily infested vineyards in Yolo County proved much more suitable and produced *Anagrus* in greater abundance. The initial 3-week emergence period was shortened to 1 week after the first few cycles demonstrated that a majority of the *Anagrus* would emerge within this short initial period. Not only did this approach produce much higher numbers of *Anagrus*, but it only required a 7-day cycle from leaf collection to parasitoid release (Table 1). Finally, and most important, subsequent releases of *A. daanei* into a Mendocino County vineyard led to significantly higher parasitism of *E. ziczac* (Figure 3).

The colony of *A. daanei* that was established at UC Berkeley in July 2015 has been expanded into a new greenhouse facility that will accommodate much higher populations of the parasitoid. As such we are currently in the process of collecting *A. daanei* from this colony for early season releases in Mendocino County. This compliments the "field rearing" approach, which is not as effective in the early season when grape vine canopies are still just beginning to develop.

Table 1. Comparison of the two approaches to collect and rear *Anagrus* spp. for release

Approach	Task	Days
Potted Vines	Collect leafhoppers	1
	Inoculate vines	3
	Expose vines	7
	Anagrus emergence period	21
	Release *Anagrus*	1
	Total days/release	33
	Total *Anagrus*/release	115 ±24
Field Rearing	Collect leaves	1
	Anagrus emergence period	7
	Release *Anagrus*	1
	Total days/release	9
	Total *Anagrus*/release	716 ±154

(2) Implement an area-wide IPM program to promote grower awareness and adoption of best management practices for control of *E. ziczac* in the North Coast.

Grower Meetings

In 2015 we held multiple outreach events (March 6 and November 20) and a field day (July 29) to review *E. ziczac* identification, management and provide updates on research progress and findings to date. These meetings were well-attended (>20 growers / vineyards represented at each meeting) and, more importantly, represented a large portion of the organic vineyard population and/or vineyards with concerns about *E. ziczac*. There were also

presentations made about the importance of leafhoppers in general in the transmission of grape "red blotch", but these are not included in this review.

Regional Monitoring and "Leafhopper Newsletter"

Growers expressed great interest in the regional monitoring of leafhopper populations, although it is unclear to what extent they relied on this data to time their own practices. There were 44 total subscribers to the "Leafhopper Newsletter" in 2015, which included growers, PCAs, and UCCE and state personnel (i.e. CDFA). Subscription has now grown to 66 individuals as of April 2016.

Project Website

Since its launch in October 2015, the "Virginia Creeper Leafhopper" website has been averaging 105 sessions per month. We expect this to increase as the website is further promoted through our outreach and education events.

7. Outside Presentations of Research:

Presentations

"Biology and Control of Virginia creeper leafhopper in Mendocino and Lake Counties" Virginia Creeper Leafhopper Seminar - UCCE Mendocino County. Hopland, CA. 30 people in attendance. Mar. 6, 2015. ***Presented by Dr. Lucia Varela***

"Update on invasive species: management and control" North Coast California Association of Pest Control Advisers Spring Continuing Education Seminar. 55 people in attendance. May 21, 2015. ***Presented by Dr. Lucia Varela***

"Updates on vineyard pests: mites, leafhoppers, moths, other arthropods or exotic pests" Sonoma County Winegrape Commission Pest Control Adviser Breakfast Meetings. Windsor, CA. 100 people in attendance. Mar. 4 – July 7, 2015 (5 repetitions). ***Presented by Dr. Lucia Varela***

"Monitoring and control measures updates of pests of pears and grapevines" Mendocino Pest Control Advisers Breakfast Meeting. Ukiah, CA. 36 people in attendance. Mar. 12 – Sept. 30, 2015 (7 repetitions) ***Presented by Dr. Lucia Varela***

"Virginia creeper leafhopper research updates" UCCE Virginia creeper leafhopper Field Day. Hopland, CA. 35 people in attendance. 35 people in attendance. July 29, 2015. ***Presented by Dr. Lucia Varela***

"Hands-on insect identification demonstration: sharpshooters and leafhoppers" Sonoma Winegrape Commission Sustainable Grape Day. Forestville, CA. 90 people in attendance. July 31, 2015. ***Presented by Dr. Lucia Varela***

"Biology of Virginia Creeper Leaf Hopper in 2015 Season and Organic Pesticide Trial" Lake and Mendocino County IPM Seminar (UCCE Lake/Mendocino). Nov. 20, 2015. ***Presented by Dr. Lucia Varela***

"Improving Biological Control of the Virginia Creeper Leafhopper" Current Wine and Wine Grape Research. Davis, CA. Feb. 9, 2015. ***Presented by Dr. Houston Wilson***

"Area-wide IPM Program for Virginia Creeper Leafhopper" Grower Meeting – UCCE Mendocino. Hopland, CA. Mar. 6, 2015. ***Presented by Dr. Houston Wilson***

"Biological Control of the Virginia Creeper Leafhopper (*Erythroneura* ziczac): A New Invasive Pest of Northern California Vineyards" Entomology Society of America – Pacific Branch Annual Meeting. Coeur d'Alene, ID. Apr. 13, 2015. ***Presented by Dr. Houston Wilson***

"Area-wide IPM Program for Virginia Creeper Leafhopper – Update" Grower Meeting – UCCE Mendocino. Ukiah, CA. July 29, 2015. ***Presented by Dr. Houston Wilson***

"Biological Controls of Leafhoppers" 2015 Continuing Education Series "Pests – What's Bugging You?" Napa County Farm Bureau, UCCE, Agricultural Commissioner's Office. Yountville, CA. Nov. 3, 2015. ***Presented by Dr. Houston Wilson***

"Update on Virginia Creeper Biological Control" Lake and Mendocino County IPM Seminar (UCCE Lake/Mendocino). Nov. 20, 2015. ***Presented by Dr. Houston Wilson***

"When do natural enemies work and why do they fail?" San Francisco IPM Technical Advisory Meeting. San Francisco, CA. Feb. 2015. ***Presented by Dr. Kent Daane***

Publications
No new publications in 2015.

Strategy for Communicating Results to End-Users:

We will continue to present relevant findings and updates on *E. ziczac* management to a variety of grower and industry stakeholders. Venues would likely include grower and professional society meetings (i.e. Allied Grape Growers, County Farm Bureau, Association of Applied Insect Ecologists etc.). We have already made plans for a grower-researcher meeting to be held in Mendocino and Lake County in June 2016. Research results will also be published in both scientific and industry journals (i.e. Environmental Entomology, Practical Vineyard and Winery, UC ANR publications etc.). Information will also be made available through county extension websites. We have also developed a "Leafhopper Newsletter" (currently with 66 subscribers) to provide weekly updates on regional leafhopper population development. Additionally, we have created a project website (http://ucanr.edu/sites/vclh/) to provide further information to growers about Virginia creeper leafhopper and this associated research program.

8. Research Success Statements

This research program has directly benefitted wine grape growers by increasing awareness and knowledge of *E. ziczac* management, this includes pest identification, habitat management to support overwintering parasitoids, timely information on *E. ziczac* life stage development and product selection and timing of use.

Wine grape growers also benefit from this research program via reduced demand for chemical controls as a result of increased biological control of *E. ziczac* following the introduction of *A. daanei* from the northern San Joaquin Valley population.

Since there is adequate overwintering habitat for *A. daanei* throughout the North Coast (primarily *Rubus* spp.), it is possible that the *A. daanei* released through this research program will establish in the region, leading to persistent biological control benefit to wine grape growers that will extend well beyond the time frame of this AVF project.

9. Funds Status

All funds are being appropriately spent and we foresee that funds will be completely used by the end of the granting period. Salary positions include partial funding for a post-doctoral researcher (Dr. Houston Wilson). Houston was responsible for carrying out the parasitoid collection-release program and developing the project website. Serguei Triapitsyn identified the *Anagrus* specimens to species and coordinated the molecular work at UC Riverside. Lucia Varela carried out the regional leafhopper monitoring effort, including all pest counts and weekly announcements. Glenn McGourty and the Mendocino County extension office assisted the grant by providing their lab tech Ryan Keiffer to help Lucia and Houston with field work. Kent Daane provided guidance for experimental design and laboratory space at the UC Berkeley Oxford Tract greenhouse. Travel costs include trips to field sites in Mendocino, Lake, and Yolo County. Supplies and expenses costs were primarily used for mounting, identification and molecular work with *Anagrus* specimens and field and laboratory supplies for the *Anagrus* collection and release work.

Acknowledgements

We extend our thanks to the numerous growers, vineyard managers and pest control advisors throughout Mendocino and Lake County who have collaborated with us on this project. We would not be able to conduct this work without your support and input.

Funding for this project in crop year 2015 has been provided by grants from the American Vineyard Foundation and the California Department of Pesticide Regulation Pest Management Alliance Program.