Conservation Biological Control of the Western Grape Leafhopper (Hemiptera: Cicadellidae): Plant Host Preference of the Parasitoid *Anagrus erythroneurae* (Hymenoptera: Mymaridae) in Napa and Sonoma, California

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ABSTRACT

Sustainable pest management is a major concern in large agricultural productions. In Northern California viticulture, the Western Grape Leafhopper often threatens plant health and crop quality. As an alternative to noxious chemicals, the surrounding habitat can be manipulated to favor beneficial insects that naturally control pest populations. These parasitoids, *Anagrus erythroneurae*, require an alternate host while the target pest is overwintering as an adult in the fallen leaf litter. After collecting monthly samples from plant genera common throughout the vineyards and surrounding landscapes, I reared parasitoids and examined their abundance to determine their relative plant preference. While previous studies have revealed blackberry (*Rubus* spp.) and rose (*Rosa* spp.) as suitable refuges, I found that mint (*Mentha* spp.), catnip (*Nepeta* spp.), and *Ceanothus* spp. also sustain significant parasitoid populations. Altering the surrounding plant community and increasing refuge availability within the vineyards supports a stable population that can more efficiently provide favorable ecosystem services. Future studies should explore which leafhopper species are prevalent on these host plants and how the pest populations are affected by increasing the abundance of these plants in the surrounding habitat.

KEYWORDS

Erythroneura elegantula, viticulture, overwintering habitat, alternate host, pest management

INTRODUCTION

Conservation control is a sustainable method of pest management, involving habitat manipulation in favor of native natural predators. More broadly known as biological control, this method typically utilizes a specialized parasitoid that attacks only the target pest (Mackie 1931). This specialized predator has minimal non-target impact, while controlling the pest population (Murphy *et al.* 1998).

In wine vineyards, the parasitoid wasp *Anagrus erythroneurae* provides an effective control for the Western Grape Leafhopper, *Erythroneura elegantula* (WGLH). These leafhoppers reduce plant photosynthetic capacity, thus decreasing plant health (Miles *et al.* 2012). Although not a major pest, leafhoppers can experience outbreaks, through their short 20- to 30- day life cycle, significantly reducing crop yield for the season (Jackson 2008). The impact of these outbreaks can be reduced by their specialized parasitoid, *Anagrus erythroneurae*, which requires an egg host to complete its 15- to 21- day life cycle (Doutt and Nakata 1965). Mated *Anagrus* spp. females oviposit their eggs directly into the leafhopper host egg, which provides nutrients for the immature wasp, and ultimately leads to pest mortality (Prischmann *et al.* 2007). This obligate association is interrupted by the adult overwintering stage of the leafhoppers, which leave the parasitoid without an egg host (Mackie 1931).

When the grape leaves fall to the ground in the cold season, WGLH overwinters in leaf litter, forcing the parasitoid to leave the vineyard in search of an alternate host (Doutt and Nakata 1965). Instead of locating their minute hosts directly, *Anagrus* spp. take advantage of the specialized association that WGLHs have with specific plants (Murphy *et al*. 1998). Thus, the parasitoids locate their host by searching for plants that the pest typically nests on.

Just as the parasitoid uses the plant to locate its host, the plant can also be used to locate the parasitoid. For example, vineyards with surrounding habitat abundant in blackberry are found to support a large *Anagrus* spp. population (Doutt and Nakata 1965). After this discovery, blackberries were planted artificially, but the parasitoids were not nearly as successful, due to a lack of appropriate riparian habitat characteristics for blackberry, such as soil moisture and canopy coverage (Flaherty *et al*. 1985). French prune trees were later suspected as an ideal refuge (Kido *et al.* 1984), although a study using rubidium markers revealed their main benefit to be windbreak, which could be also accomplished using any tall, rigid structure (Corbett and Rosenheim 1996). Other potential refuges include wild rose and apple trees, while hairy-leaved varieties were found to be more resistant to leafhopper attack (McKenzie and Beirne 1972, Triapitsyn 1998).

In the process of determining the preferred host plant for this parasitoid, many *Anagrus* spp. had been identified as *Anagrus epos* since its discovery in 1965. More recent studies revealed multiple species within the group formerly known as *A. epos* (Trjapitzin 1995). Of these species, *Anagrus erythroneurae* makes up 95% of the parasitoids emerging from WGLH (Trjapitzin and Chiappini 1994, Triapitsyn 1998, Lowery *et al.* 2007). In addition, their success can be attributed to a higher degree of host specificity and relative abundance when compared to other *Anagrus* spp. (Williams and Martinson 2000).

Once the species complex was defined, more studies focused on the plant host preferences of *A. erythroneurae* in various regions of North America. In New York, wild grape varieties (*Vitis* spp.) were found to harbor significant parasitoid populations in the winter period (Williams and Martinson 2000). In Washington and Oregon, the parasitoid was found on blackberry (*Rubus* spp.), willow (*Salix* spp.), and rose (*Rosa* spp.), also during the winter months (Prischmann *et al.* 2007, Wright and James 2007). In Canada's British Columbia, *A. erythroneurae* was also found on rose (*Rosa* spp.), willow (*Salix* spp.), and grape (*Vitis* spp.), in addition to blackberry (*Rubus* spp.), apple (*Malus* spp.), and mint (*Mentha* spp.) (Lowery *et al.* 2007). These studies reveal a regional variation in pest-parasitoid relationships that require more investigation.

Most studies track relative abundance of *Anagrus* spp. only during the winter period, though it is important to understand their movement throughout the year to support a sustainable population. Additionally, no studies have been conducted in California to determine their species interactions specific to this region.

In my study, I explored the preferred alternate host plants of *Anagrus erythroneurae* on a seasonal basis in the counties of Napa and Sonoma in Northern California. To do so, I collected monthly samples of common plant genera in the surrounding habitat for 12 months. These samples were used to rear *Anagrus* spp. to the adult stage, when they were collected for later species identification. Based on previous studies in other regions, I expected to find a high abundance of *A. erythroneurae* on wild grape, blackberry, rose, and apple during the winter months, which would reflect their preference for these refuges when WGLH is not accessible. Their relative abundance on each plant species was then compared over one-year sampling period to show which species are the most important during certain seasons.

METHODS

Sampling sites and plants collected

Plants were collected from six discrete vineyard sites throughout Napa and Sonoma counties, located in Northern California. These sites varied from entirely landscaped to natural riparian, representing the continuous variations of habitats that surround vineyards. Within these various sampling locations, there was an equally diverse array of plant species that were either naturally occurring in the area or planted by the grower for a variety of reasons, with intentions ranging from aesthetics to potential means of biological control.

In all of the twelve monthly collections, there were 76 plants that were uniquely identified to the genus or species level. Plant genera sampled were representative of those commonly found in the planted and natural areas surrounding vineyards. Sampling procedures targeted leafy growth when possible; leafhoppers preferentially feed on the underside of leaves, where they feed on the phloem from the vein (McKenzie and Bierne 1972).

Sampled plants were widespread within and between sites, allowing for replicates within a season. Throughout the year, some plants were not available for sampling, due to reduced abundance. Some plants were only identified to genus, due to material limitations.

For each unique plant ID number, clipped samples from individual plants were placed into 4-gallon plastic bags. Once the sample was taken, each plastic bag was stored inside of a paper grocery bag to protect and isolate the samples. Sample sizes were standardized by volume to account for varying densities across plant species.

Rearing parasitoids

To determine which *Anagrus* spp. are found on each plant genera, wasps were reared out in the lab. At the Oxford Tract Insectary, I weighed collected plant samples in grams then placed them into individual emergence containers, with the intention that any parasitized leafhopper eggs containing *Anagrus* spp. will emerge during the observation period. To encourage rapid emergence, room conditions simulated summertime in light and temperature: 15-hour photo period

(06:00-21:00) at 22°C. These containers consisted of a large paper tube, 30cm in diameter and height, with a 15cm diameter hole cut in the top, which was topped with a 4 oz funnel, closed with a micro centrifuge tube. The funnel was covered with tin foil to exclude light from the entire container with the exception of the top tube. Since insects are drawn to light, they gathered in the top vial where they were easily collected, following their emergence, on a daily period.

Specimen collection

Since *Anagrus* spp. are known to complete egg development in approximately 15- 20 days, I checked the samples on a daily basis for 3-4 weeks per sampling period to allow buffer time to ensure that all collected specimens had emerged (McKenzie and Beirne 1972). Monthly sampling began in January 2013 and ended in December of the same year, composing a 12-month dataset. Collected wasps were kept individually in 95% ethanol at 17°C for long-term storage. For species identification, samples were sent to Sergei Triapitsyn, the current authority on *Anagrus* spp.

Statistics and visualization

For data analysis, I used a Shapiro-Wilk test for normality, with a standard alpha level of 0.05 (R Commander 2013). If non-normality was detected, a post-hoc Tukey test was used to determine significant differences between sampled plants. All data was visualized in a 100% stacked bar graphs in Microsoft Excel to normalize for uneven sampling across seasons.

RESULTS

Seasonal variation

Across plant genera, I found the greatest *Anagrus erythroneurae* abundance during the spring and summer periods. Relative abundance across seasons was not normally distributed ($W = 0.3474$, $p \ll 0.05$). Catnip (*Nepeta* spp.) was the only plant to carry significant difference across all sampled plant genera (TukeyHSD, $p < 0.05$). Normalized relative abundance still showed some trends by visualization (Figure 1).

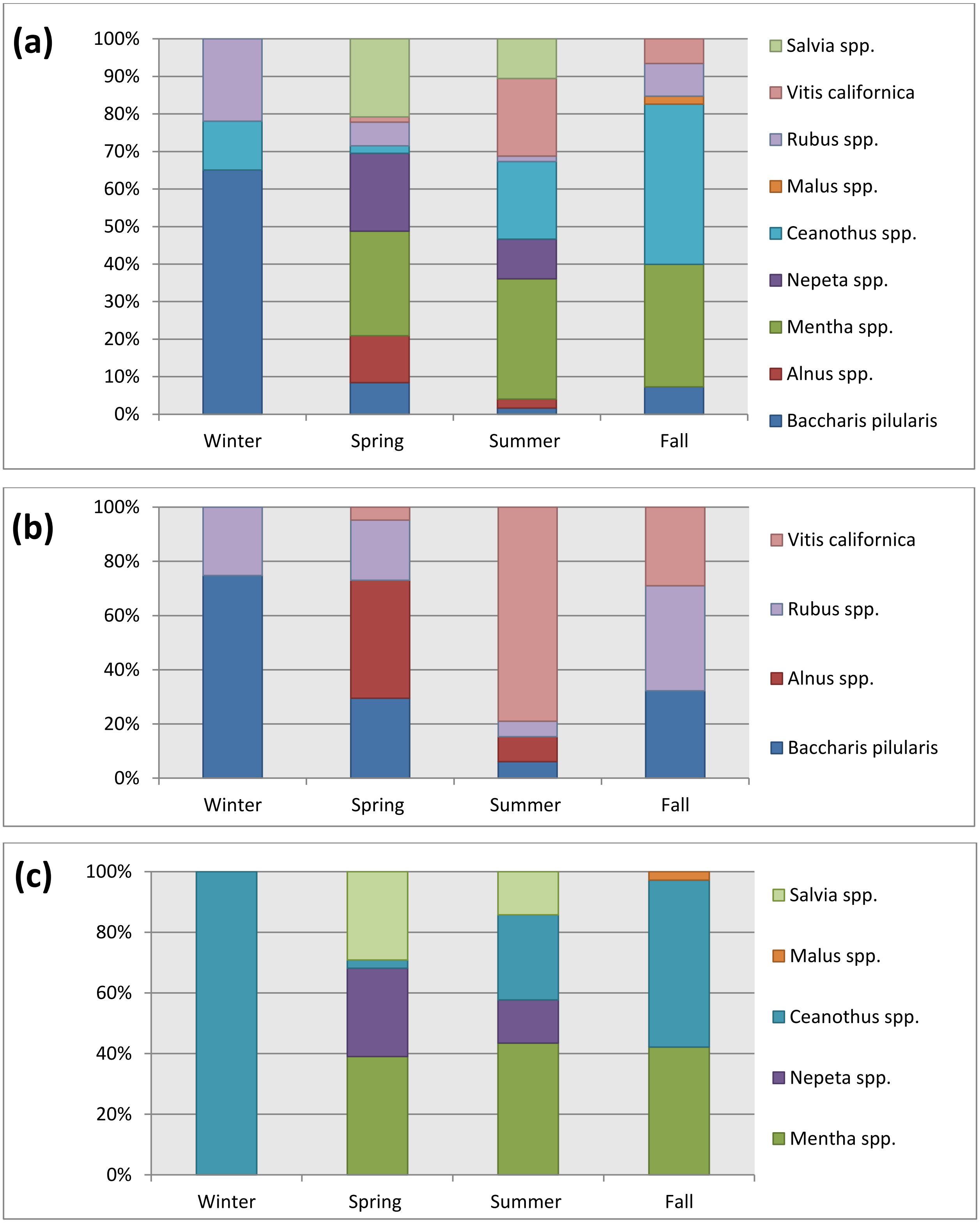

Figure 1. Relative seasonal abundance of *A. erythroneurae* by plant genera. 100% stacked columns show the relative abundance of the parasitoid by season on: (a) all sampled plant genera, (b) naturally-occurring plant genera, and (c) landscape plant genera.

Over the 12-month sampling period, I consistently found *A. erythroneurae* on the following plants in every each season: catnip (*Nepeta* spp.), California lilac (*Ceanothus* spp.), blackberry (*Rubus* spp.), and coyote brush (*Baccharis pilularis).* Overall, the plant with the highest abundance of parasitoids was catnip, as suggested by the TukeyHSD. The majority of parasitoids reared from this plant were collected in the winter and spring seasons (Table 1). Of the native plant genera, coyote brush produced a relatively large amount of parasitoids throughout the year, particularly in the winter season (Figure 1a, Table 1).

Table 2. Summary of plant genera hosting *Anagrus erythroneurae*. Totals represent the number of individuals collected from each genera for sampling from January to December 2013. L or N indicates plant origin as landscaped or naturally-occurring, respectively.

Plant Family	Common Name	L/N	Mean Parasitoids Collected/ Sample			
			Winter	**Spring**	**Summer**	**Fall**
Lamiaceae	Mint (*Mentha* spp.)	L	0	6.71	20.00	7.50
	Catnip (*Nepeta* spp.)	L	42.5	60.25	3.67	7.00
	Sage (*Salvia* spp.)	L	0	5.00	6.56	0
Rhamnaceae	California lilac (*Ceanothus* spp.)	L	1.19	0.47	12.89	9.80
Rosaceae	Apple (*Malus* spp.)	L	0	0	0	0.50
	Blackberry (*Rubus* spp.)	N	2.00	1.53	0.94	2.00
Vitaceae	Wild grape (*Vitis californica*)	N	0	0.33	12.89	1.50
Asteraceae	Coyote brush (*Baccharis pilularis)*	N	5.94	2.03	1.00	1.67
Betulaceae	Alder (*Alnus* spp.)	N	0	3.00	1.50	0

Plant genera without parasitoids

Some sampled plant genera, such as madrone (*Arbutus* spp.), manzanita (*Arctostaphylos* spp.), pea (*Pickeringia* spp.), spruce (*Picea* spp.), and pear (*Pyrus* spp.), did not yield any parasitoids in the scope of my study.

DISCUSSION

Outside of previous literature, the study revealed an abundance of *Anagrus erythroneurae* on several alternate host plants that had not yet been found to host the parasitoid. While previous studies found *A. erythroneurae* abundant on rose (*Rosa* spp.) and blackberry (*Rubus* spp.), the overwhelming majority of our collected parasitoids emerged from catnip (*Nepeta* spp.), California lilac (*Ceanothus* spp.), and mint (*Mentha* spp.) in Napa and Sonoma vineyards. Although rose and blackberry hosted fewer parasitoids than expected, the number of individuals collected from these samples confirmed their viability as alternate host plants. There are many factors affecting these host-parasitoid interactions, the majority of which vary across vineyard ecosystems. Therefore, it's important to consider the reason for the discrepancy in our findings in relation to the literature.

Abundance parameters

Because an efficient and consistent parameter to measure parasitoid abundance was not available, I compromised by looking at the number of *Anagrus erythroneurae* emerged per sample. All of the sampled plants have varying physical characteristics, making it difficult to compare them by weight. Buckeye, for instance, has very large dense seeds that were randomly collected in our sampling. On average, these buckeye samples were extremely heavy in relation to other plant samples of a comparable size. Furthermore, the sampling method provided a relatively consistent measure of volume across sampling periods and plant genera. By focusing on standardization of sample size, predictable variation was minimized to focus on that caused by biological factors, such as plant genera or seasonality.

Seasonal variation

Throughout an annual cycle, the majority of *Anagrus erythroneurae* were collected during the development transition periods because multiple generations are reproducing. In addition to expected seasonal variation, the rainy season often resulted in samples with significantly higher moisture levels in the sample containers. Since humidity was not regulated, this expedited the molding process that would eventually occur in all samples, potentially reducing the period of optimal parasitoid emergence conditions. Most samples developed a considerable amount of mold by the end of the three-week emergence period, though these wetter samples often molded before two weeks had passed.

Looking at catnip (*Nepeta* spp.) in particular, there was a large spike in parasitoid abundance during the winter and spring periods. This is important for biological control because this period coincides with the pest's return to the vineyard canopy. If a large population of the parasitoid is present in the surrounding habitat, followed by a drop in the summer season, it is highly possible that these individuals migrated to the vineyard canopy, thus parasitizing the pest at the beginning of the season. If parasitoid populations can be supported during this vital transition period, they can more effectively control the returning pest population.

Plant genera without parasitoids

Although many of the sampled plants were found to host *Anagrus erythroneurae*, there were others that the parasitoid was not reared from. Considering the multiplicity of variables that contribute to successful emergence of a parasitoid in the lab, it is certainly possible that any of these plant genera have the potential or do currently host *Anagrus erythroneurae*, though it was not captured in our sampling. Some potential barriers to emergence include poor plant health, trauma from collection and transport, predation within the emergence container, inability to arrive in collection vial, among many others. In the case of blackberry (*Rubus* spp.), planting in non-riparian habitats produces unhealthy individuals, as revealed by previous studies, and thus the benefits from individuals naturally occurring along stream banks cannot be expected from them (Williams 1984, Wilson et al. 1989). Similarly, some other plants that may be favorable refuges in certain conditions may have been unfavorable during the 2013 sampling period.

Limitations and future directions

Many factors contributed to this study, some of which may have influenced the trends in the results. Due to visibility of physical characteristics, only collected females could be identified to the species level (Prischmann et al. 2007). Fortunately, the majority of individuals collected were female, though it would be interesting to look at sex ratios to better understand their reproductive capacity.

Because this research was situated in a profitable industry, I was limited in sampling discrete vineyards. Each vineyard presents a unique ecosystem, created by both natural and human-influenced traits. There are no such continuous habitats larger than the vineyard scale, thus each ecosystem has a different variety of plants and insects, both native and non-native, planted and invaded, that experience novel interactions. Though this may seem overwhelming, it sheds light on the countless interactions that remain to be discovered. Future studies could explore the impact of plant proximity to the vineyard edge to examine parasitoid dispersal behavior and find the optimal spacing to establish alternate host plant refugia.

Since favorable plant hosts were identified, future studies should also explore the impact of altering the habitat to support *A. erythroneurae* populations throughout the year. Additionally, it would be beneficial to investigate the leafhopper species present on these potentially beneficial host plants so that other potential refuges could be predicted, based on their leafhopper populations.

Broader implications

Overall, use of conservation control is a much more biologically and economically sustainable means of pest management. Although there are countless factors that contribute to the health of an ecosystem, parasitoid abundance inversely impacts the health of the pest population, with less adverse effects than other control methods such as pesticides and mechanical removal. By integrating biological control methods with other pest management practices, ecosystem health can be maximized, with minimal impacts from limited pesticide applications and strategic habitat manipulation.

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Appendix A: Sampling Logistics

January 16, 2013
February 9, 2013
March 2, 2013
April 2, 2013
May 21, 2013
June 18, 2013
July 23, 2013
August 4, 2013
September 11, 2013
October 6, 2013
November 7, 2013
December 11, 2013

Table A1. Collection dates for plant samples from Napa and Sonoma. On these dates, I went to each of the vineyard sites to collect plant samples from selected plant genera in attempt to rear *Anagrus erythroneurae* to determine individual plant viability as overwintering refuge.