Evaluation of Overwintering Habitat of the Western Grape Leafhopper (*Erythroneura elegantula*) in and around North Coast Vineyards

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ABSTRACT

The Western Grape Leafhopper (*E. elegantula*) can be a very destructive vineyard pest when in high enough concentrations. Though there is a lot known about how to manage them in vineyards during the growing season, not much is known about where they go during the overwintering phase of their life. I found out where these pests go during this period by sampling them with a D-Vac over a four month period to get rough estimates of what plants they are found on and in what numbers. *E. elegantula* was found on a number of plants such as California Buckeye and *Ceanothus*, they were found in very high densities on mint, which was the only statistically significant species after using a Two way Tukey HSD.

KEYWORDS

overwintering, leafhoppers, *Erythroneura elegantula*, vineyard, grapes

INTRODUCTION

The dominance of "conventional" agriculture, (e.g. the large scale monoculture farming with the use of many synthetic inputs) has led to numerous environmental and human health problems. To increase yield, farmers use high impact inputs like fertilizers and pesticides. These inputs combined with other practices such as repeated tillage of soil have depleted soil nutrients, exposed humans and the ecosystem to toxic chemicals (Koureas et al. 2012; Reganold et al. 1987; Carpenter 2011), and disrupted natural systems meant to cycle nutrients within the biosphere. This switch has made people more reliant on external inputs leaving them vulnerable when problems arise, leaving with no natural systems to act as a buffer (Altieri and Nicholls 2012). For example, if farmers use pesticides on a pest, yet it kills the pest's predator while the pest gains resistance, either a larger application or a different pesticide must be used or risk higher losses than before the pesticide was used (this cycle is termed pesticide treadmill). Agroecology provides a safe and viable alternative as it mimics natural systems to cycle nutrients and promote natural systems without the use of external inputs or toxic chemicals (Altieri and Nicholls 2012). Benefits of Agroecological practice include the purification of water, control of pests, and increased resilience to disaster (droughts, storms, etc.). One facet of pest control is the use of natural pest management.

Nature has used food webs to keep all organism populations in balance, and agroecology, by mimicking these processes, would be able to help the agricultural industry naturally control pests. Normally in nature food webs keep themselves in check with multiple species in all trophic levels interacting and preventing any one species from taking over the ecosystem. Many times when farmers use pesticides, herbicides, and fungicides, it kills nearly all life, those that are beneficial and detrimental to crops, and disrupts natural cycles (Altieri and Nicholls 2012). In many cases the species we deem pests are the quickest to both recover and gain resistance to these toxins, while their predators lag behind, leading to lower yields than before any inputs were used, falling victim to the pesticide treadmill (Altieri and Nicholls 2012). Agroecology can mimic natural ecology to control pest by providing suitable conditions for predators of the pests, whereas chemicals can eliminate these predators and exacerbate the problem, causing unregulated growth of a pest (Ponti et al. 2005) . The wine grape industries in Sonoma and Napa

Valley are two prime examples of where a pest population has multiplied and is causing damage due to lack of natural regulation in winery monoculture.

In Sonoma and Napa Valley the Western Grape Leafhopper (*Erythroneura elegantula*) causes serious damage to the grape vines, but studying and promoting its predators and other natural defenses can help to keep this pest under control (Booij et al. 1992; Bentley et. al 2008). *Anagrus spp.* is a small parasitic wasp that is a specialist parasitoid of the *E. elegantula*, which uses its ovipositor to lay its own eggs in the *E. elegantula* eggs, which feed on the developing leafhopper. Because the leafhopper overwinters as an adult and doesn't lay eggs during this period, Anagrus cannot control the pest population over winter, instead using another host (Corbett and Rosenhall 1996). Where the *E. elegantula* actually goes in these California vineyards is thought to be on the vineyard floor (Bentley 2008). Studies on similar species and climates have been conducted (Cerutti et al. 1991; Decante et al. 2006; Mazzoni et al. 2008) , these studies were not conducted in the North Coast and data is almost always exclusive for a specific geographic area. These exact data cannot be followed in another area of the world with another species because flora differs across geographic regions, and therefore responses would vary depending on the area. Despite all the information on *Anagrus spp.* and other natural enemies of the leafhopper during the growing season, there is limited research on its habitat and habits during the winter months, which could serve as valuable source of information in controlling this pest. Because of the fact that *E. elegantula* cannot reproduce over the winter, this means that this would be the time where decreasing the population could make the biggest difference. Because of the huge difference than can be made in managing these pests in such a short amount of time, it shows why knowing where *E. elegantula* overwinters is so indispensable to their management..

This study is an evaluation of *E. elegantula* overwintering habitat use in vineyards and surrounding habitat to try and follow the lifecycle and preferred surroundings of the *E. elegantula* over the winter as an adult. I will collect a variety of specimens or different species of plants, in attempts to catalog trends or increased leafhopper populations seen on specific plants. This will be used to ID which plants and areas *E. elegantula* prefer to stay when they overwinter to be used mainly by wine growers as a pest control tool. Based off of data from similar studies (Wang et al. 2010; Zimmerman et al. 1996), my hypothesis is that the *E. elegantula* overwinter on specific vegetation outside of the vineyard in order to survive.

METHODS

Site Selection

In order to determine which vineyards in Sonoma and Napa to test, I first looked at our lab's data from previous months to determine which vineyards had any number of *E. elegantula* in the previous season. The second factor was whether or not there was substantial area of vegetation within or outside of the vineyard that could provide habitat. The designated size of area with vegetation had to be ≥ 400 m^2. Any site that had both of these were selected for the study, and sites were randomly spread throughout the North Coast.

Collection Method

I collected all of the *E. elegantula* through the use of a large D-Vac system (a vacuum designed to collect insects). The D-Vac we used was a modified leaf vacuum with an attachment 2.5 meters long and a bucket at the end of the attachment. The area of the bucket opening was 0.062 m^2 and 1.5m deep. Inside the bucket I placed a small mesh bag with elastic straps to hold in in place inside the bucket. Each single species sample came from vacuuming five thrusts of the bucket into a single plant species, and then closing and tying the bag closed to keep all the insects gathered (Zimmerman 1996). Though collecting using sticky traps and beating sheets were considered, the habits of the *E. elegantula* combined with how collection was planned would not have gathered accurate data. Because gathering would be throughout the day, the temperature would affect results from the beating sheets depending on whether or not the *E. elegantula* would be flying around (midday), or resting on the leaves (early morning) (Booij 1992;Zimmerman 1996). Using sticky traps isn't completely accurate either, having the possibility of by-catch from individuals merely passing by the plant , not only the ones living on it. In addition, many of these traps would have been left out for a month at a time, and winter rains would skew data results as glue is washed off of the traps. By using the D-Vac I avoid both of these problems because the vacuum is too strong for *E. elegantula* to fly away even while they are active, and the samples are gathered and bagged instantly.

Plants to Sample

To pick which of the plants to sample, I chose the dominant species that were in the surrounding vegetation of the sites at each vineyard. Each plant species needed to be present in at least 2 of the vineyards sites, with at least 1-4 samples per species per site. Because of the plants being more or less abundant depending on the site or area, the exact number of samples varied per species of plant. With the exception of vineyard floor, all of the plants sampled were not directly in the vineyard.

Sampling

For each sample, I ran the D-Vac on a species of plant and if there was a high enough abundance of the plant, I would take additional samples. This consisted of starting the leafblower, thrusting it into an area of the plant with the vacuum on, and agitating the plant for a few seconds, repeating four more times for each sample. Once all of the samples were collected and taken back to the lab, they were frozen for at least 4 days in a -18^{o}C freezer to make sure everything in the sample was dead. I sampled all of these plants once per month starting on December 15th and ending on April 15th.

Data Analysis

I analyzed the data by going through a number of steps. In order to first get the numbers for the data, I counted the *E. elegantula* from the D-Vac samples by hand, separating out and identifying the insects from each sample with a microscope to verify the exact number of *E. elegantula* (Booij 1992). I made a chart containing number of leafhoppers, date collected, and plant species from each of the samples and used this for data to analyze. I first ran these numbers through R with a Shapiro-Wilk test to see whether the distribution of the leafhoppers on the plants was normal or not. After finding that it was not (with a p-value of 2.2e-16) I then log transformed the data so I would able to run an ANOVA test on the different categories. The ANOVA showed that the only significant category was the plant species in determining the leafhopper density, with the date not having a significant enough effect on the data (the species degrees of freedom was 14 and had an f-value of 7.088). I then ran the leafhopper number against plant species using a Two-way Tukey HSD test to see which plants were statistically significant in determining *E. elegantula* presence. The only plant that had a p-value less than 0.05 was the mint.

RESULTS

After I sampled the different species of plants for leafhopper densities, it became very evident that the *E. elegantula* did not randomly scatter when vines went dormant, but instead had a definite choice in vegetation. Mint overwhelmingly had the highest densities of leafhopper per plant at an average value of 10.3, while Toyon, Vineyard floor, and riparian floor all had less than 1 (See **Figure 2**). Even though 14 other species of plants were sampled, many within one hundred feet of the plants that were found to have *E. elegantula*, I didn't find of them on any of these other species.

Plants found with *E. elegantula*	**Plants without *E. elegantula***
CA Buckeye (Aesculus californica) California Lilac (*Ceanothus*) Mint (*Mentha*) Olive (*Olea europaea*) Rosemary (*Rosmarinus officinalis*) Toyon (*Heteromeles arbutifolia)* Vineyard Edge Vineyard Floor	Blackberry (*Rubus)* CA Bay (*Umbellularia californica*) Citrus (*Citrus*) Coast Live Oak (Quercus agrifolia) Coyotebush (*Baccharis pilularis*) Elm (*Ulmus)* Rose (Rosa)

Figure 1. Presence/Absence of *E. elegantula* per plant species. Of all the sampled plants, the plants on the left were the only species that yielded any *Erythroneura elegantula.*

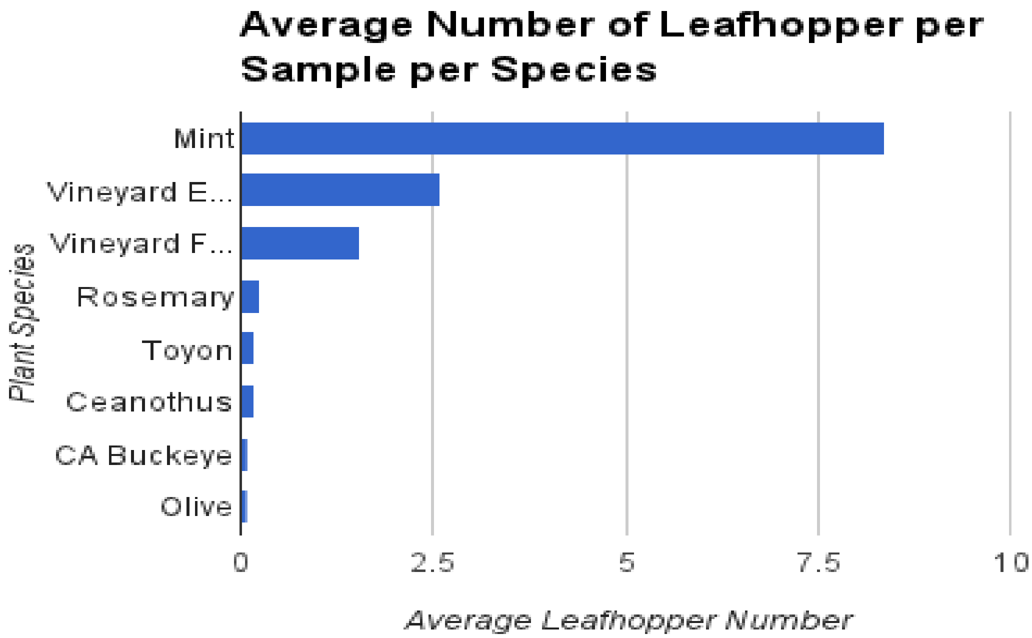

Figure 2. **Leafhoppers per sample.** Blue lines indicate the average number of *E. elegantula* per sample for each species of plant.

DISCUSSION

Introduction

After completing my study, I discovered that *E. elegantula* overwhelmingly prefer mint in addition to a handful of other plants to live on over the course of the overwintering phase of its life as an adult. Though mint was the only statistically significant plant species, count data shows that vineyard edge and vineyard floor also have high numbers of *E. elegantula* as well as Rosemary,Toyon, Ceanothus, California Buckeye, and Olive. These results are an important step in following the life history of *E. elegantula* and may provide a useful tool in their management.

Plant Species Preference

After running all the counts of the *E. elegantula* and the average numbers of specimens per plant, it shows that the *E. elegantula* prefers to overwinter on plants in the *Mentha* genus. I don't know why this is from the way the sampling was conducted, but is is clear they are found in very high densities on these plants. Whether it is reacting to a compound that the mint has or for some other reason is unknown, but whatever the reason is, the prefer *Mentha* over all other nearby plants.

The second highest densities of leafhopper were on the vineyard floor and vineyard edge. Though other papers did say that *E. elegantula* liked to overwinter in grasses and on the vineyard floor (Cate 1975), my results showed that they only seem to be in high numbers in grasses, while plain vineyard floor without grasses or other annuals had no leafhoppers. Vineyard edge and vineyard floor had similar plant combinations, usually with a mixture of grasses and annual weeds and flowering plants, though the exact combination changed from site to site.

I have a hypothesized a few reasons for why the leafhoppers choose these plants over others to overwinter on. The first is that the density of the leaf matter on both of these categories of plants (mint and vineyard floor/edge) is very high, and act as good insulators, allowing the leafhoppers to stay warm enough during the winter (Cate 1975). Leafhoppers are able to survive temperatures down to -12°C, it isn't for extended periods of time, and need some way to keep their body temperature high enough (Cate 1975). The second is that these plants (excluding mint) grow more quickly as annuals than a perennial like a toyon or coyotebush, and therefore have a higher percentage of younger leaves. Because plants C:N ratio increases with age, annuals will consistently have higher percentages of nitrogen for insects to ingest and therefore higher nutrient density in the leaves for the *E. elegantula* to eat. Stippling was evident on some of these plants, but whether *E. elegantula* is the species actually ingesting these plants or whether they just gather on these plants is unknown.

The last plants that had numbers of *E. elegantula* on them didn't have much in common with each other except for the fact that they were perennial. Again these plants had stippling but it is unknown whether it is from *E. elegantula* or other insects. Though Rosemary,Toyon, Ceanothus, California Buckeye, and Olive had *E. elegantula* whereas others like Rose and Elm didn't, does not mean that the leafhoppers are never found on the other plants. This could have resulted from samples being taken during the day, samples only on days that weren't raining or a variety of other factors. More sampling needs to be done to confirm definite presence or absence on all of these plants.

Limitations/Future Directions

One of the biggest limitations that this project encountered is the limited number of data. Though I cannot sample every plant and must instead do smaller samplings, there still needs to be more data overall. Most of the data was from mostly biodynamic or organic farms, and only about one that uses pesticides which may skew the data having to do with its effect on leafhopper densities. In addition the study applies only to vineyards in this area and can't be used for sites outside of the area or for a different leafhopper species. To more thoroughly test this, either a test would have to be run in the lab with all the tested species of plants and see which plants the leafhoppers choose, or plant each of the species at all of the vineyards, and measure total relative density on each of the plants. As for the problem of whether *E. elegantula* is eating the plants or simply living on them would have to be done by devising an experiment to keep them enclosed with the plants while preventing other insects from getting in to see if it is indeed *E. elegantula*.

Broader Implications/Conclusion

In conclusion, though other factors have some change in leafhopper numbers, the *E. elegantula* does have definite overwintering plant preference for mint and other plants. Despite knowing this, there is no proof that these plants are used for food but could instead be used to live on for protection (which would fit in with my theory on warmth from leaf density). Because of this however it raises the question of whether it would be better to completely remove the mint, or to leave it and use it as a trap crop to keep high densities of *E. elegantula.* Because of its slow lateral growth, using mint as a trap crop would be an easy addition to vineyards without having to worry about them taking over (depending on the species). By having the pests cluster on these plants in a very small area, farmers could use pesticides, burning, vacuuming, or other techniques to keep *E. elegantula* numbers low during the overwintering period since they are unable to reproduce then. If however the mint was the main food source, or for some reason is the plant that they require to survive overwintering, then the solution would be simpler and just to remove all of the mint from the vineyards to reduce *E. elegantula* numbers in the overwintering period.

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