Growing season and summer habitat preference of Western Grape Leafhopper, *Erythroneura elegantula,* in California North Coast vineyards

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ABSTRACT

The increased demand for holistic, integrated pest management techniques has intensified the need to better understand key pest ecology. This experiment sought to fill gaps in Western Grape Leafhopper, *Erythroneura elegantula,* life history and ecology. The focus of this study was to uncover potential overwintering host plants that aid WGLH during their winter diapause period. A custom built D-Vac machine was used to sample various plant species surrounding Northern California wine vineyards. Sampling was conducted for an entire year, thus allowing for a better understanding of the key transition periods and filling of gaps in leafhopper ecology outside of the vineyard. Experiment results showed that leafhoppers occupy the vineyard floor and weedy vegetation when not occupying the vine canopy, but also showed a significant preference for various species of mint plants during non-growing season periods. These results were obtained by comparing seasonal average leafhopper densities amongst several plant species using non-parametric analysis of variance tests. The results of this study and its implications on comprehensively understanding leafhopper ecology could potentially lead to better farm management recommendations for farmers that want to steer away from conventional, industrial agriculture methods.

KEYWORDS

Habitat assessment, biological control, integrated pest management, farm landscape management, diapause

INTRODUCTION

Modern agriculture is often characterized by industrialized methods of production, which includes monoculture systems that require many inputs derived from chemicals, fossil fuels, and manipulation of the land (Gliessman 2007). These conventions have negative externalities that affect climate, ecosystems and human health in both short-term and long-term levels because they are generalized into a "one-size fits all" approach in very different regions (Gliessman 2007).

In order to move away from these industrialized methods, ecologically sound pest management should consider the surrounding environment to utilize natural ecosystem services. Ecologically based pest management concepts attempt to move away from the antiquated notion of total pest management (TPM; removal of all pests entirely), and towards integrated pest management (IPM; various methods used to control the majority of pest population) (Cumming and Spiesman 2006). Heterogeneous farming systems and biological control are promising resources in the field of ecologically based pest management. Various studies have demonstrated that landscape biodiversity can influence ecosystem services, such as biological control (Russel 1989, Andow 1991). However, the ecological factors responsible for this relationship are not fully understood (Tscharntke et al. 2007). It is crucial to explore specific characteristics within these surrounding landscapes that are influencing the on-site ecosystem services throughout the year.

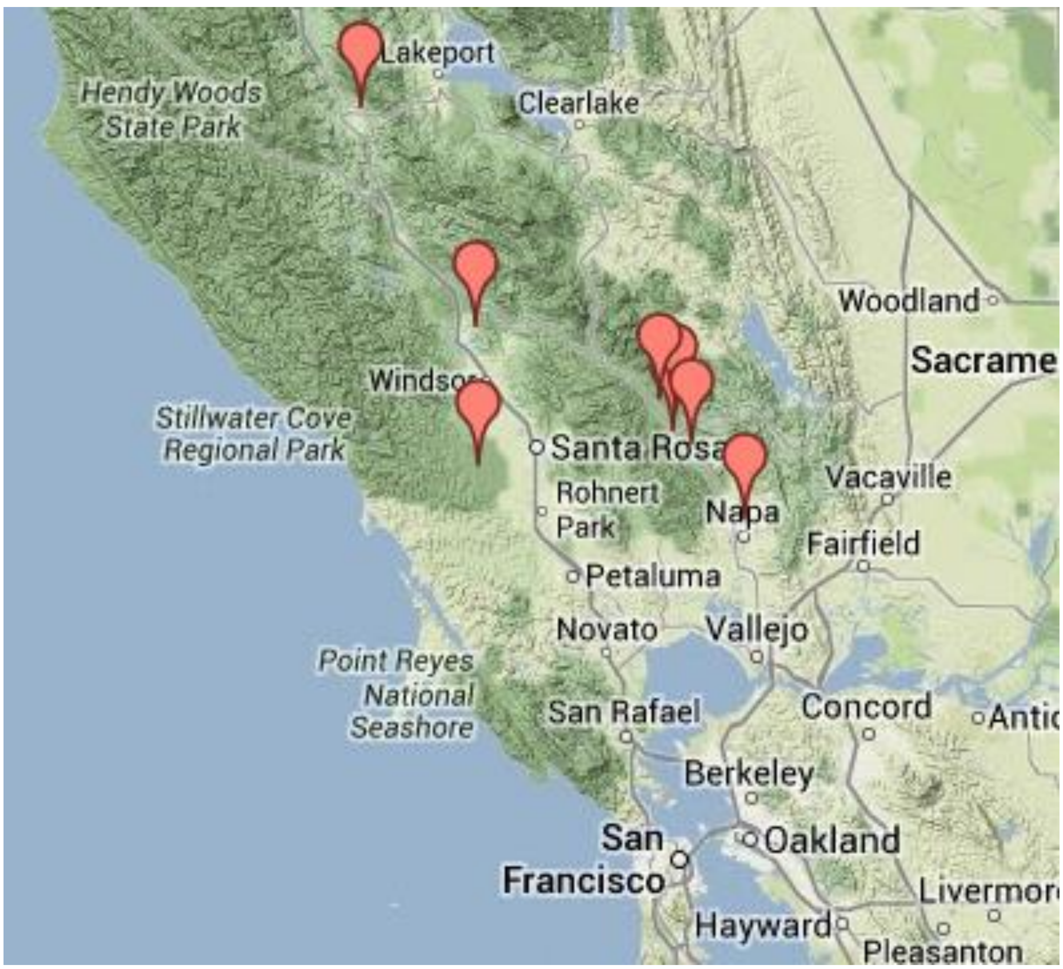
In regards to pest management, California's wine industry is no exception to modern agriculture. Regionally intensive expansions of wine vineyards in Napa/Sonoma Counties utilize large monoculture plots as the main method of production. This is known as the resource concentration hypothesis, which means monoculture plots are susceptible to pest outbreaks due to the large expanse of resources for target pests (Root 1973). The key pest in North Coast vineyards is *Erythroneura elegantula*, Western Grape Leafhopper (Daane and Costello 2000). The leafhoppers feed on grape leaves with piercing/sucking mouthparts in order to reach sexual maturity, which reduces the grape vine's photosynthetic capabilities (Daane and Costello 2000, UC IPM 2008). This can potentially lead to huge economic losses (Daane and Costello 2000). The implementation of ecosystem services such as biological control into pest management becomes an added source of pest control. Biological control, in this system, revolves around parasitoid wasps, *Anagrus spp.*, parasitizing leafhopper eggs to prevent leafhopper nymph emergence, thus controlling pest population densities (Root 1973, Letourneau 1987, Andow 1991, Landis et al. 2000). However, there are several factors in landscape diversity influencing biological control.

The focus of this study is to further understand the Western Grape Leafhopper life cycle in order to use this knowledge more effectively when creating biological control focused farm management recommendations. Specifically, I am interested in potential alternative plant species that may be beneficial to western grape leafhopper development throughout the year. Not much is known of off-site western grape leafhopper ecology; except, western grape leafhoppers undergo a winter diapause period, a time in which the insect delays development until more suitable conditions become available (Cate 1975). However, there are those rare winter days that surpass the leafhopper diapause threshold of 50°F; at which point, the leafhopper's metabolic rate increases to the point that it begins respiration once more and must feed on secondary hosts plants to meet their energy demands (Cate 1975). After this dormant period, they rely on secondary plant species to develop until the primary grape vines become available for them to reach sexual maturity (Cate 1975). Most literature cites their diapause habitat as leaf litter and surrounding shrubs, without any specificity regarding plant species. So it is unclear which plants are responsible for WGLH being able to survive outside the vineyard. Because of this, I believe there will be no specific plant that WGLH prefers outside of the vineyard. By sampling various plant species in the landscapes found surrounding grape vineyard sites on a monthly basis, I intend to compile a series of observations to determine any trends or transition phases that may reveal WGLH host plants throughout the year. Learning more about the off-site ecology of western grape leafhoppers and uncovering specific beneficial plants may lead to the development of more rigorous vineyard landscape management techniques. This knowledge, coupled with that of *Anagrus* habitat preferences, would lead to more effective land management recommendations that maximize ecosystem services.

METHODS

Study Sites

I conducted my research at several participating wine grape vineyard sites across California's North Coast region, which includes Napa, Sonoma, and Mendocino Counties (Map 1). These sites include Grgich Hills American Canyon and Rutherford sites; Constellation Tokalon, Landslide, and Hoffman sites; Napa Valley Reserve; Joseph Phelps Vineyards; and Campovida Vineyards. Mediterranean climate dominates these areas, which lead to warm, dry summers and mild, wet winters. All of the vineyard research sites were located adjacent to patches of natural and semi-natural habitat. Common natural habitat types found in this region include: chaparral, riparian, and oak woodland, among others.



Map 1. Participating vineyards in overwintering study.

Data Collection

My sampling targeted the various dominant plant species that comprise the natural habitats found in and around the research vineyard sites. I took samples once a month from January to December 2013, which allowed me to quantify changes in leafhopper densities on various plant species throughout the year. There is a gap for September 2013 due to lack of transportation and other resources. I took samples using a custom built D-VAC. The D-VAC was constructed from an inverted leaf blower with a 5-gallon bucker attached at the end to amplify the suction area to $0.67m^2$ (Figure 1). A paint strainer bag would then be placed over the bucket portion to catch insects as they are vacuumed off of the vegetation. Each sample consisted of 5 thrusts (~5 seconds/thrust) with the D-VAC into different areas of a given plant. I then repeated this three times on three different individuals for each plant species (3 samples/plant species evaluated). Specific plant species were selected based on previous habitat studies and plant availability in the vineyards. Plant species used for analysis, however, were limited to years for which a sample was taken for each month throughout the year. This was done to meet statistical analysis requirements. Each sample had a unique sample identification number for the month and plant species sampled. Samples were then taken back to the laboratory where they were processed to sort out the WGLH from other insects and plant debris. I was trained to identify western grape leafhoppers by Houston Wilson in the Miguel Altieri lab. This year round study allowed me to make inferences about leafhopper habitat preferences during key transition periods between winter/spring overwintering and summer wine grape growing season.

Figure 1- Custom built D-VAC apparatus.

Data Analysis

I used the statistical program R to analyze my data. I utilized the latest version of R, 3.0.2 “Frisbee Sailing”, to carry out my statistical analysis. Leafhopper average densities were recorded for each month on each plant to track changes during key transition phases. I divided my data into subsets by sample period to run a Kruskal-Wallis Non-Parametric ANOVA test on each season because my data was not normal after running a Shapiro Test. This test assesses any significant difference in leafhopper densities on various plant species outside of the vineyard. Default settings on R determine whether or not there is a difference between any of the plant species ($p<0.05$), but not which plants specifically. In order to determine which specific groups are different from one another, I ran a Mann-Whitney Post Hoc test by rank on the ANOVA objects to determine specific plants. Additionally, I ran the same significant test protocol on a different subset of data divided by plant species throughout the year to determine whether leafhoppers are found consistently on these alternate plants throughout the year. I assumed that my D-VAC method effectively captures insects on the plants and that these samples are representative of actual leafhopper densities found on each plant species in order to infer habitat preference.

RESULTS

Plant Assessment

I assessed the plants in the surrounding habitat at the study sites to determine what condition the plants were in, whether leaves were present or not and if there existed signs of stippling on the leaves. Leaf presence and condition affected how I sampled the various plant species, as I often selected to go for the plants with leaves and stippling present since this is a good indicator of leafhopper presence. I stored these samples in a freezer to conserve them until I could process them at a later time.

Leafhopper Densities

I sampled three replicates of the seven plant species in different sites every month for a year to get a density distribution over the year on a given plant species (Fig 1). On average, the amount of leafhoppers found on the vineyard floor decreased from making up 88% of the total WGLH sampled in the summer to only making up 11% of the total sampled in the winter.

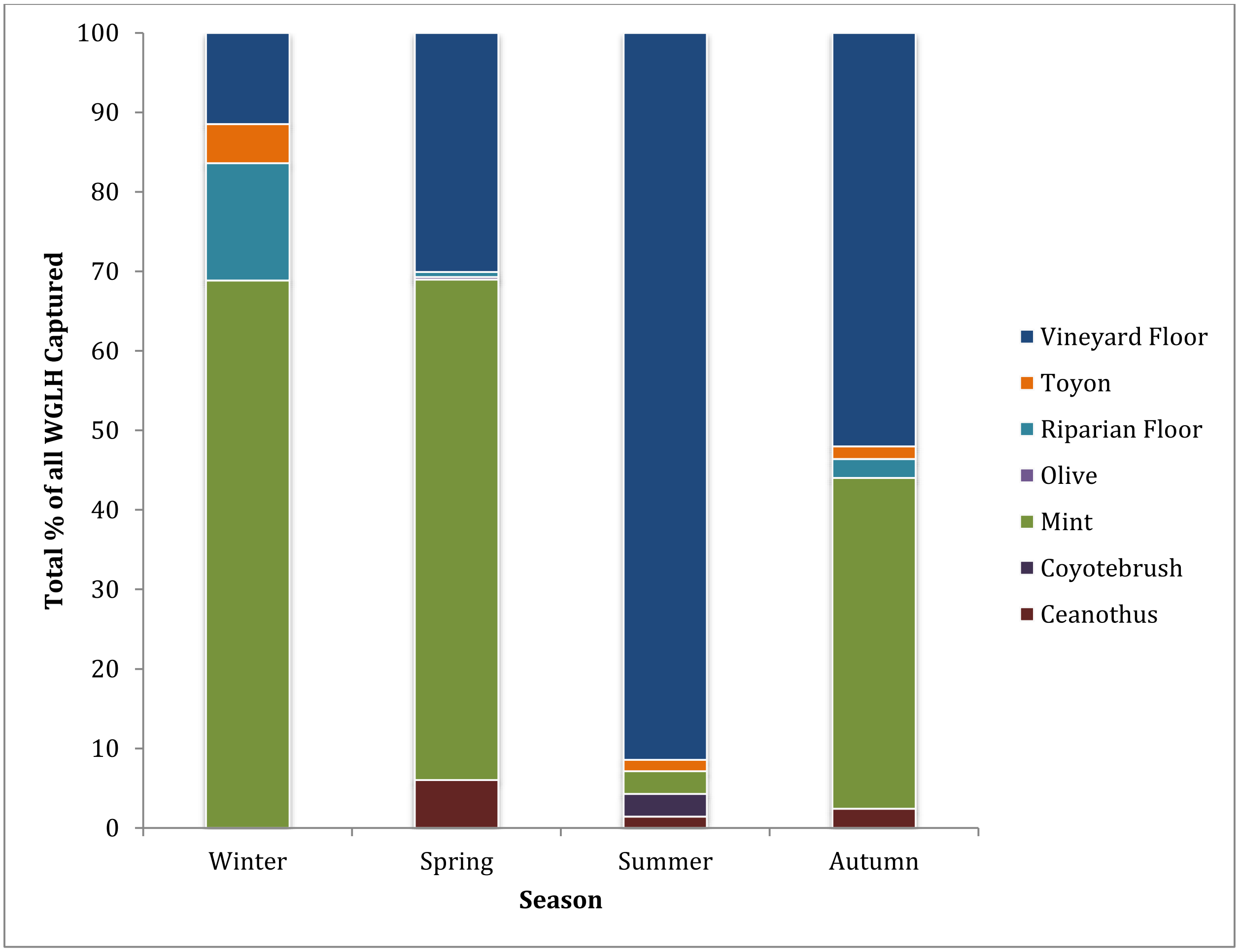


Fig. 1 Year-long breakdown of total percentage of all western grape leafhopper densities sampled by season.

When not in the vineyard floor, leafhoppers show a preference for aromatic plant species such as ceanothus and mints. Most plant species had little to no leafhopper activity throughout the year. The alternate plants as a group showed greater average densities in the non-growing season periods (2.25 ± 5.4) than the low numbers during the growing season (1.30 ± 0.1). The vineyard floor on the other hand had its smallest values during the winter with an average density of 0.7 ± .15. The breakdown of seasonal leafhopper counts can be found in Figure 2 and shows mint having relatively high averages during the winter (22.1 ± 8.9) and lowest averages during summer(0.22±.22).

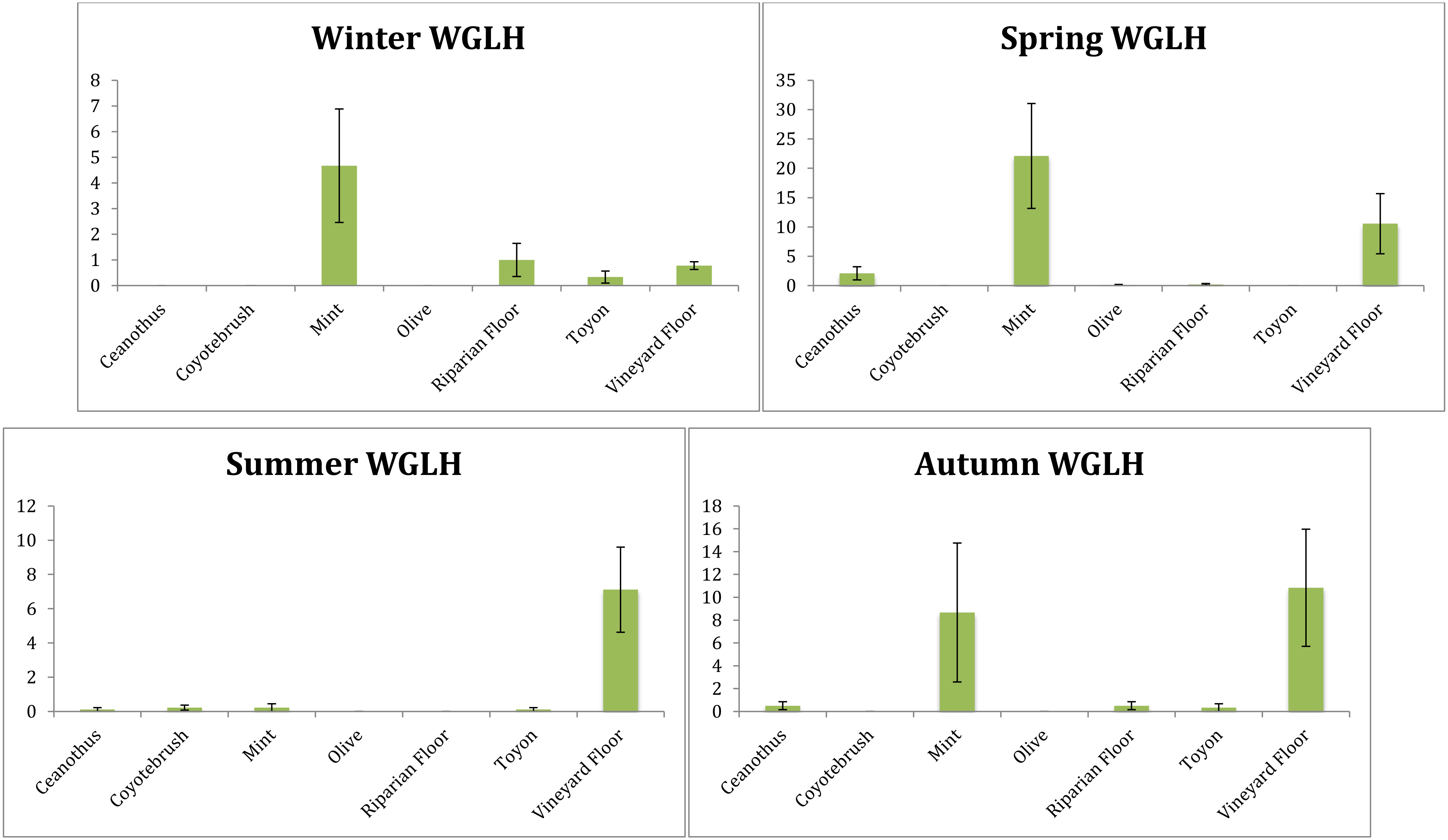


Fig 2. Leafhopper average density breakdown for each plant by seasons. Mint has high average densities outside of the year.

Data Analysis Results

A Shapiro-Wilk normality test revealed that the data was not normally distributed (W=0.3276, p-value < 2.2e-16). This led to the utilization of a Kruskal-Wallis non-parametric ANOVA test to assess any significant differences between the observed averages. A Kruskal Wallis test revealed a significant difference in the plants for every season with most seasons having a p-value well below 0.05 (Table 1). The significant results from the Kruskal tests require a post hoc test. I chose to run a Mann-Whitney test for rank to specifically reveal which plants were statistically significant from one another. The post-hoc tests using Mann-Whitney tests with no p-value correction shows significant differences between mint and ceanothus (p-value <0.00), coyotebrush (p-value <0.00), olive (p-value<0.00), and toyon (p-value<0.00); and significant differences between the vineyard floor and ceanothus (p-value=0.02) and coyotebrush (p-value<0.00) during the winter [Table 2]. It also shows significant differences between mint and ceanothus (p-value=0.03), coyotebrush (p-value<0.00), olive (p-value<0.00), riparian floor (p-value<0.00), and toyon (p-value<0.00); and differences between the vineyard floor and coyotebrush (p-value<0.00), olive (p-value=0.01), toyon (p-value<0.00), and riparian floor (p-value=0.01) during the spring. During the summer, only the vineyard floor was statistically different than all the other plants with p-values all under 0.05. The autumn had mint statistically different than ceanothus (p-value=0.01), coyotebrush (p-value<0.00), olive (p-value<0.00), riparian floor (p-value=0.01), and toyon (p-value<0.00); and the vineyard floor different than coyotebrush and olive with p-values of > 0.02.

Table 1. Kruskal-Wallis test results showing significance for every season. A post hoc Mann-Whitney required for each season.

Season	Kruskal-Wallis X^2	Degrees of Freedom	p-value
Winter	26.758	6	0.0001607
Spring	25.349	6	0.0002942
Summer	16.811	6	0.01
Autumn	22.934	6	0.0008188

Table 2. Mann-Whitney Post-Hoc test results for each season showing differences between plants.

Winter	Ceanothus	Coyotebrush	Mint	Olive	Rip.floor	Toyon
Coyotebrush	1	-	-	-	-	-
Mint	0.0007	0.0007	-	-	-	-
Olive	1	1	0.0007	-	-	-
Rip.floor	0.597	0.5973	0.117	0.597	-	-
Toyon	1	1	0.0089	1	1	-
Vin.floor	0.022	0.0022	0.2454	0.351	1	1
Spring	**Ceanothus**	**Coyotebrush**	**Mint**	**Olive**	**Rip.floor**	**Toyon**
Coyotebrush	0.6608	-	-	-	-	-
Mint	0.0339	0.0002	-	-	-	-
Olive	0.7664	1	0.0003	-	-	-
Rip.floor	0.8555	1	0.0006	1	-	-
Toyon	0.6608	1	0.0002	1	1	-
Vin.floor	0.3325	0.0063	0.9407	0.0106	0.0175	0.0052
Summer	**Ceanothus**	**Coyotebrush**	**Mint**	**Olive**	**Rip.floor**	**Toyon**
Coyotebrush	0.585	-	-	-	-	-
Mint	1	0.683	-	-	-	-
Olive	0.374	0.169	0.374	-	-	-
Rip.floor	0.374	0.169	0.374	1	-	-
Toyon	1	0.585	1	0.374	0.374	-
Vin.floor	0.035	0.07	0.035	0.014	0.014	0.035
Autumn	**Ceanothus**	**Coyotebrush**	**Mint**	**Olive**	**Rip.floor**	**Toyon**
Coyotebrush	0.1757	-	-	-	-	-
Mint	0.0111	0.0027	-	-	-	-
Olive	0.1757	1	0.0027	-	-	-
Rip.floor	1	0.1757	0.0111	0.1757	-	-
Toyon	0.6733	0.4047	0.0077	0.4047	0.6733	-
Vin.floor	0.1044	0.0284	1	0.0284	0.1044	0.0608

The same protocol was used to determine if leafhoppers were consistently found on plants throughout the year. A Kruskal Wallis test was performed on subsets of data divided by plant species by season and resulted that only mint has significantly different leafhopper densities on it throughout the year p-value<0.00) [Table 3]. I then conducted another Mann-Whitney post-hoc test on mint throughout the year and resulted that summer leafhopper densities are statistically different than spring leafhopper densities (p-value=0.01) and autumn leafhopper densities (p-value< 0.00) [Table 4] [Figure 3].

Table 3. Kruskal-Wallis test results for plant species densities throughout the year.

Plant Species	Kruskal-Wallis X^2	Degrees of Freedom	p-value
Ceanothus	7.025	3	0.0711
Coyotebrush	5.5054	3	0.1383
Mint	11.3484	3	0.009984
Olive	2.6667	3	0.4459
Riparian Floor	5.1313	3	0.1624
Toyon	2.2222	3	0.5276
Vineyard Floor	2.488	3	0.4774

Table 4. Mann-Whitney post hoc test results for mint plant leafhopper densities throughout the year.

Season	Autumn	Spring	Summer
Spring	0.6355	-	-
Summer	0.0014	0.0120	-
Winter	0.2845	0.2244	0.236

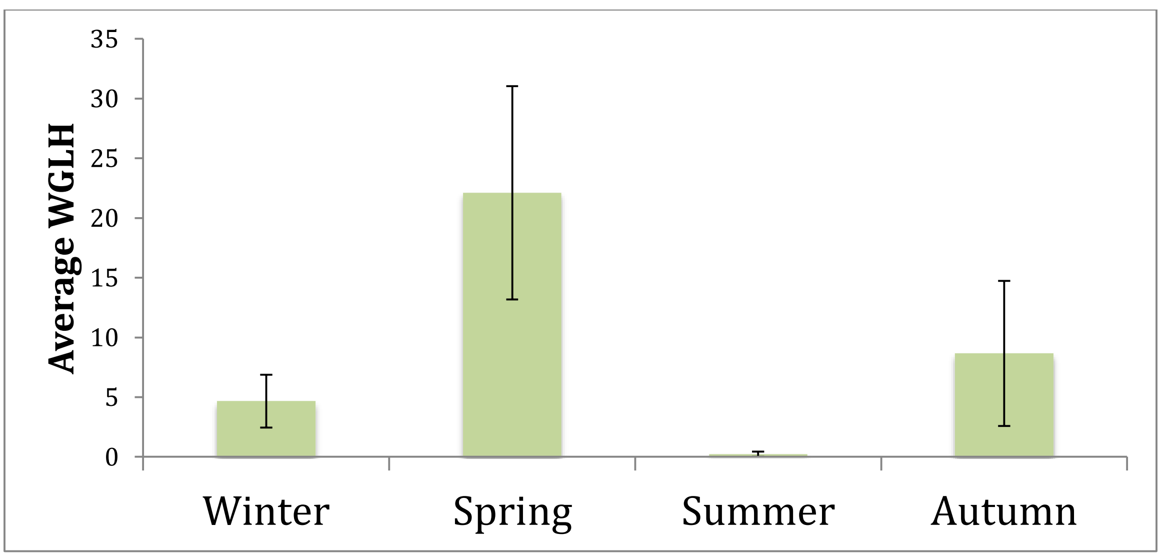


Fig. 3- Average seasonal western grape leafhopper densities on mint.

DISCUSSION

Western grape leafhopper habitat preference in California North Coast wine vineyards varies significantly throughout the year. Data was analyzed at seasonal basis to assess whether there is a significant difference in habitat preference. During the growing season, leafhoppers showed a significant preference for the vine floor, which makes sense since leafhoppers primarily rely on cultivated wine grapevines. However, leafhoppers seem to prefer various species of mint surrounding vineyards during other times of the year. Particularly, leafhoppers tended to prefer mint species as a habitat choice for the winter diapause season, which may be contributing to its successful reemergence in the spring. Additionally, leafhoppers did not equally show this preference throughout the year, which may mean that leafhopper habitat changes throughout the seasons. This may be a significant pattern, but may also be due to sampling error from inconsistencies in the data collection and processing. Despite this, this new data could lay the foundation for future habitat preference assessment projects moving forward to make actual recommendations to growers in the future and minimize leafhopper success rates of reemergence in following years.

Western Grape Leafhopper: Habitat Preference Outside of Growing Season

Leafhopper habitat preference seemed to change during the year. The seasonal densities seemed to reveal a pattern of change when starting and ending the growing season. The two main host sites in my study appeared as mint and the vineyard floor (Figure 4). The sudden drop at the beginning of summer could be the leafhopper movement back to the vine canopy (UC IPM 2008). Throughout the growing season (July-October) there seems to be an increasing amount of leafhoppers in the vineyard floor, perhaps a sign of the leafhoppers preparing for migration out of the vine canopy for winter diapause (UC IPM 2008). With this apparent migration, there also exists a delayed increase in leafhopper densities on mint plants surrounding the vineyard.

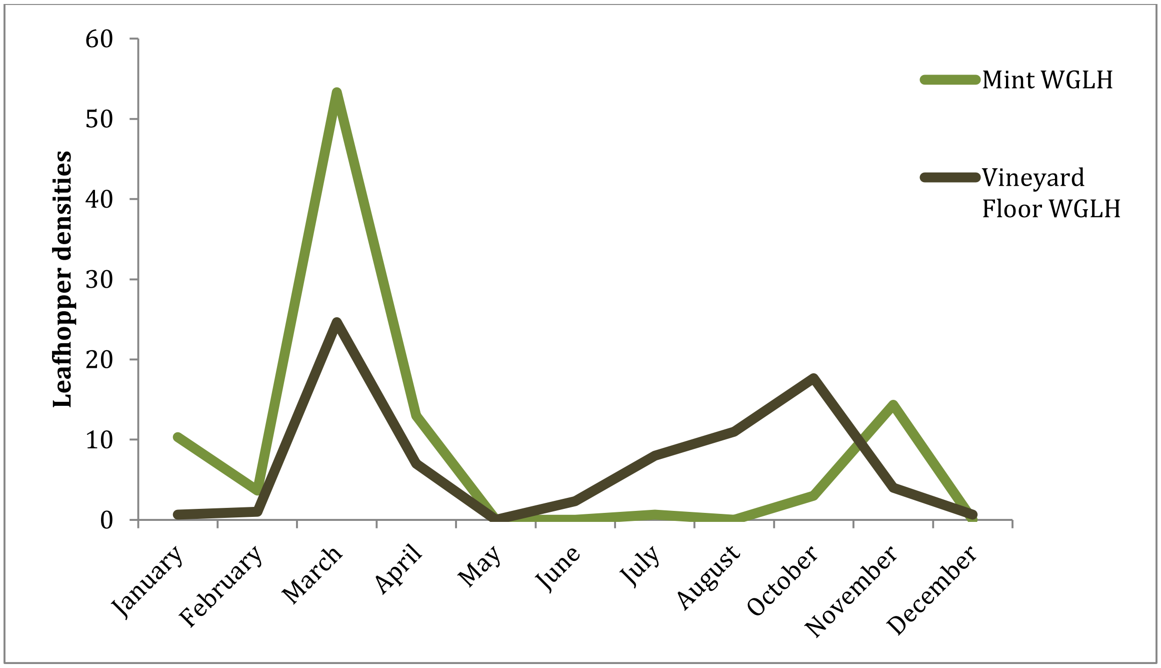


Fig. 4- Time series plot of leafhopper densities throughout the year by month.

Potential for Habitat Preference

The statistically significant findings on mint plants throughout year could mean that leafhoppers do not just randomly disperse into the surrounding weedy floor/debris but instead also seek out certain plant species that could aid their spring reemergence. This would change the way leafhopper life cycle is understood (Figure 5). Leafhoppers need to feed on a host plant on the rare winter days that are warmer than 50°F which requires them to fulfill their metabolic needs due to respiration (Cate 1975). The winter showed the significantly different densities on mint plant that lead to this assumption that mint is a potentially beneficial plant habitat for WGLH to diapause on over winter. This notion is supported by the fact that mint can survive light frosts over winter seasons, which would make it an even more plausible resource for leafhoppers to feed on warm winter days. These mint plants were all clearly outside of the vineyard so there's some uncertainty as to why leafhoppers showed this affinity to mint plants. These results, thus, run counter to previously believed ideas that leafhoppers simply drop and diapause in the vineyard floor leaf litter (Cate, 1975).

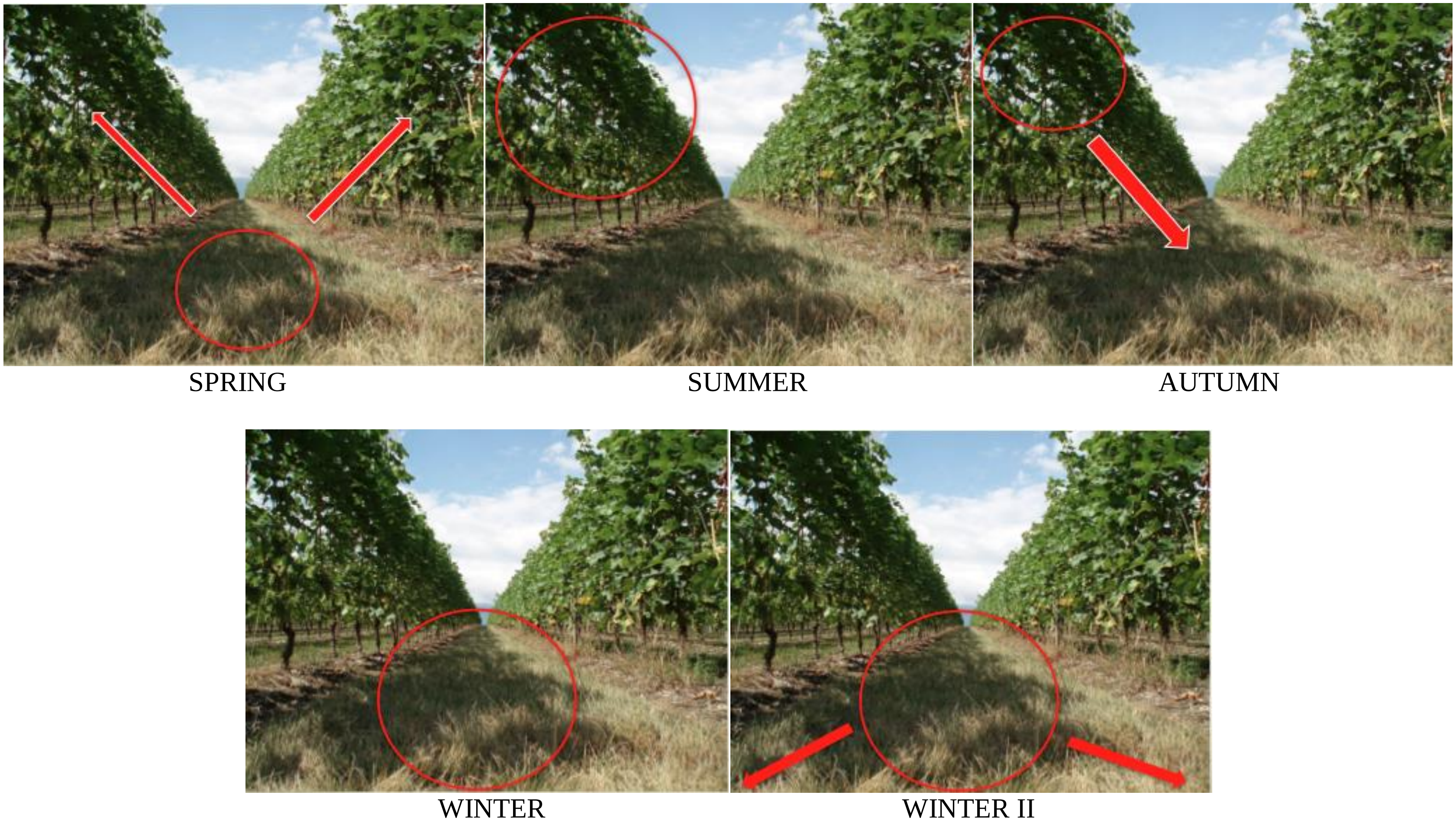


Fig. 5- Leafhopper life cycle as known now and how it can potentially be occurring. During Spring, leafhoppers begin reemerging from the previous winter diapause and move back to the vine canopy. During Summer, leafhoppers feed and reproduce in the vine canopy and have up to 2-3 generations a season. In Autumn, leafhoppers begin to move to the vineyard floor as the leaves begin to drop from the grapevine. Previously, it was thought leafhoppers entered diapause and just stay in the leaf litter and debris. But now with new data, it could be that leafhoppers leave the vineyard floor and also diapause on other plants outside the vineyard aside from the floor.

Habitat Preference Consistency of Western Grape Leafhoppers

Additionally, the data also refuted my idea that there would be consistent density counts of leafhoppers on the plants they were found on throughout the year. The average densities for plant species with leafhopper presence varied greatly throughout the year and showed no consistent pattern whatsoever. This could either be due to faulty data collection/processing or leafhopper habitat preference changing throughout the year. For example, mint, which appeared to be a plausible alternate host plant, had fluctuating average density counts throughout the year. Mint had generally high counts in the winter months, a surge in spring, and a lack of leafhopper presence in the summer months, before their density shifts over to the vine canopy in late summer. This could hint at plausible signs that leafhopper habitat preference changes throughout the year to accommodate metabolic needs and a movement around the vineyard site throughout the year. This would signify a more dynamic ecology to leafhopper life cycle than previously thought.

Limitations and Improvements

There were various obstacles that could have affected the outcome of these results. As noted before, there were many inconsistencies in the sampling and processing procedures due to participating in an ongoing project. The most glaring flaw is the lack of data for the month of September, which was due to lack of resources-transportation costs- to sample for that month. Potential links to leafhopper habitat preference will not be known during this crucial transition month from summer growing period to harvest. There was a general lack of data for many plants, some of which were a result of leafhopper ecology, but some may be due to processing errors in the lab. Human error is always an issue, so this could be the source for some inconsistent observations. Additionally, there were some flaws in the sampling protocol that may have affected the observed density counts. I did not collect all of my samples under the same conditions, which may influence how active leafhoppers were at a certain time due to their metabolic needs (Cate 1975). Some sites were sampled early in the cooler mornings while others were samples in the warmer afternoons, simply due to time lost travelling to all the sites. I also did not count leafhoppers in earlier stages of development due to the lack of ability to identify the nymph stages, so it could be that there were leafhoppers present on plant but not accounted for due to being in earlier instar stages of life.

Broader Implications and Future Directions

A better understanding of leafhopper habitat presence means a more comprehensive understanding to leafhopper ecology that can be applied in integrated pest management programs. The lack of support for leafhopper habitat preference from this study does not mean that recommendations cannot be made for farm landscape management. There were certain patterns in leafhopper density counts on various plants that could suggest the removal of these plants from the landscape to avoid that secondary resource. By removing these plants that leafhoppers tend to benefit from, growers can remove a potential source of leafhopper re-emergence in their vineyards. These results, coupled with results found for parasitoid wasp habitat preference, can lead to an improved management plan that maximizes wasp populations and minimizes leafhopper populations via biological control (Daane 2000). Additionally, the vineyard floor is a source of continued leafhopper presence, so managing it becomes inherently important to reduce the number of leafhoppers in diapause that survive to breed in following years.

Moreover, there are several directions to go moving forward from this study. It would be beneficial to get more data from more vineyards for a longer period of time and for multiple times a month. Additionally, sampling more plant species throughout the year can lead to a more robust data set. This would improve the data set by increasing the depth of data to analyze and increase the level of inference that can be drawn from such a study. Several more research questions arise from the outcome of this study as well. For example, why do leafhoppers prefer certain plants to others? What, potentially, about these plants is beneficial to leafhoppers? And so on.

REFERENCES

Andow, D. A. 1991. Yield loss to arthropods in vegetationally diverse agroecosystems. Environmental Entomology 20: 1228-1235.

Cate, J.R. 1975. Ecology of *Erythroneura elegantula* in grape agroecosystems in California. Thesis, University of California, Berkeley, CA, USA.

Cumming, G. S., and B. J. Spiesman. 2006. Regional problems need integrated solutions: pest management and conservation biology in agroecosystems. Biological Conservation 131:533-543

Daane, K. and M. Costello. 2000. Variegated and Western Grape Leafhopper *Raisin production manual.* Oakland: University of California, Agriculture and Natural Resources, 2000. 173-180.

Gliessman S.R. 2007. Agroecology: The ecology of sustainable food systems. CRC Press, 2007. 3-20

Landis, D. A., S. D. Wratten, and G. M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual Review of Entomology 45: 175-201.

Letourneau, D. K. 1987. The Enemies Hypothesis: tritrophic interactions and vegetational diversity in tropical agroecosystems. Ecology 68:1616–1622.

Murphy, B. C., Rosenheim, J. A., & Granett, J. 1996. Habitat diversification for improving biological control: Abundance of *Anagrus epos* (hymenoptera: Mymaridae) in grape vineyards. Environmental Entomology 25(2): 495-504

Root, R.B. 1973. Organization of plant-arthropod association in simple and diverse habitats: the fauna of collards (Brassica oleracea). Ecological Monographs 43(1): 95-124.

Russell, E. P. 1989. Enemies Hypothesis: a review of the effect of vegetational diversity on predatory insects and parasitoids. Environmental Entomology 18(4): 590-599.

Tscharntke, T., R. Bommarco, Y. Clough, T. O. Crist, D. Kleijn, T. A. Rand, J. M. Tylianakis, S. van Nouhuys, and S. Vidal. 2007. "Conservation biological control and enemy diversity on a landscape scale." Biological Control 43(3): 294-309.

UC IPM website. Oct 2008. Grape Leafhoppers. April 2013. http://www.ipm.ucdavis.edu/PMG/r302300111.html