Assessing Overwintering Habitat Preferences of *Anagrus* spp. in and around North Coast Vineyards

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ABSTRACT

The counties of Napa and Sonoma are home to many expansive vineyards, and thus also prime victims for the attacks of the pest *Erythroneura elegantula*, Western grape leafhoppers (WGLH). This leafhopper negatively impacts the vineyard by consumption of leaf cell contents, and fecal spotting on the fruits. Their leaf consumption directly affects the photosynthetic yield, as their damage causes a reduction in the chlorophyll surface area of the leaf. However, with the identification of *Anagrus* spp., a microscopic parasitoid wasp, as a natural enemy of the WGLH, growers need to know what plants will attract these natural enemy populations. I conducted a fine-tuned study, in order to more definitely confirm the overwintering preferences of *Anagrus* spp., using emergence chambers. Anagrus emergence was measured daily from the day after sampling up to 21 days. From sampling the same plants consistently from various vineyards over five separate sampling dates from November 2012-March 2013, I found that plants from genera *Nepeta*, *Baccharis*, *Ceanothus*, and *Rubus* significantly yielded more *Anagrus* spp.

KEYWORDS

Anagrus, alternate host, landscape ecology, leafhopper, conservation biological control

INTRODUCTION

In agriculture it is critical to consider the surrounding environment of any cultivated area and its functional biodiversity, especially in relation to the agricultural products of the agroecosystem— particularly in the case of vineyards, grapes. (Paoletti et al. 1992, Banks 2004, Steinbauer et al. 2006). Functional biodiversity is the biological variety in the context of the specific ecosystem services provided by each organism that make up the entire ecosystem (Moonen and Bàrberi 2008); this view is embodied in the agroecology approach to agriculture (Francis et al. 2003). According to agroecology, the knowledge of the ecological makeup of an area, such as a vineyard, is crucial to the success of the agricultural product. This determination of success hinges heavily on a healthy, ecologically-friendly treatment of both the cultivated and non-cultivated environment, thus enabling the conservation of such biodiversity (Nicholls et al. 2001, Altieri et al. 2005). Although the mechanisms behind the positive role of enhancing biodiversity in increasing agricultural yields remain unknown, a positive correlation between the two is widely acknowledged (Marquard et al. 2009, Chase 2010). Therefore, to achieve understanding and subsequently holistic management of the vineyard ecosystem, researchers must understand the biology of surrounding organisms and their interactions that impact the vineyard.

The counties of Napa and Sonoma are home to many expansive monoculture vineyards by in the United States, and, due to the lack of biodiversity, they are prime for the attacks of the pest *Erythroneura elegantula* (Hemiptera: Cicadellidae) Osborn 1928, its common name: Western grape leafhopper (WGLH) (Thies et al. 2011). The WGLH negatively impacts the vineyard by consuming leaf cell contents and leaving fecal spots on the leaves (stippling). There is also a sociological aspect to their negative effects: they become a nuisance in, and sometimes even a barrier to the pickers during the harvest season (Altieri et al. 2005). The WGLH leaf consumption directly affects the photosynthesis of the grape plants, as the damage causes a reduction in the chlorophyll surface area of the leaf, and thus reduces yield (Prischmann et al. 2007). In investigating the biology of WGLH and its interactions with other organisms, researchers found that only two members of the *Anagrus* genus – *A. erythroneurae* Triapitsyn and *A. daanei* Triapitsyn – are specialists targeting the eggs of the WGLH (Prischmann et al.

2007, Williams and Martinson 2000, Zimmerman et al. 1996). However, due to the microscopic nature of the size of these egg parasitoids, this thesis will not assess the parasitoids at the species level, but at the genus level, as "*Anagrus* spp." Promoting *Anagrus* spp. populations in the vineyards would be beneficial to control for WGLH pest damage (Landis et al. 2000, Tscharntke et al. 2005, Bianchi et al. 2006). Instead of the alternative of employing pesticides which easily affect non-target organisms (Daane et al. 2008), growers may choose methods of integrated pest management (IPM), which often take the form of biological control of pests through use of natural enemies, such as the *Anagrus* wasp.

With the identification of a natural enemy of the WGLH, scientists opened up the possibility of conservation biological control of the leafhopper by the *Anagrus* wasp. In 1996, researchers collected data which supported the importance of the biology of surrounding environments, especially as possible overwintering refuges for *Anagrus* wasp (Corbett and Rosenheim 1996, Thies et al. 1997, Bianchi and Van Der Werf 2003, Miliczky and Horton 2005, Debras et al. 2007), due to their season-dependent compatibility with the WGLH to use as host. *Anagrus* spp. cannot overwinter on grapevines, because the WGLH takes the form of an adult during winter, a stage *Anagrus* wasp cannot parasitize. (Prischmann et al. 2007, Zanolli and Pavan 2011). Therefore, during the winter, *Anagrus* spp. is forced to leave the grapevines and must rely on an alternate host to successfully overwinter (Doutt and Nakata 1965, Williams 1984, Murphy et al. 1996, Williams and Martinson 2000, Zanolli and Pavan 2011).

Researchers have consistently shown a direct association between the abundance of a natural enemy and non-crop habitat (Altieri 1993, Tracker 2002, Boller et al. 2004, Pfannenstiel et al. 2012). There have been several studies proposing possible non-crop habitats for its overwintering, such as the *Rosa* spp. (wild rose) (Mckenzie and Beirne 1972) and *Rubus* spp. (blackberry) (Williams 1984, Hesami 2008) plants. However, in 1998, Triapitsyn conducted a taxonomic review, effectively invalidating many previous studies conducted, which had incorrectly identified *Anagrus* spp. to species, all as *Anagrus epos*. These misidentifications are readily attributed to the microscopic nature of *Anagrus* spp. specimens. Therefore, to further this work it is important to revisit these identified preferences of "*A. epos*." Furthermore, researchers must continue to identify other potential host plants so growers can incorporate this information into their decision-making to enhance beneficial insect activity. However, none of these previous studies investigated the overwintering *Anagrus* spp. populations in the vineyards of Northern California, the focus of my study.

This thesis will provide more information to Northern California grape-growers with the overwintering preferences of *Anagrus* spp., in order that they may know which plants are efficacious in mitigating WGLH pest damage. Thus, I will investigate the overwintering biology of *Anagrus* wasp as a parasitoid of the WGLH through assessing *Anagrus* spp. emergence from hedgerow and other non-vegetative crop habitats surrounding vineyards in Napa and Sonoma County. This information can then be incorporated into the biological control system for enhancement and further promotion of the *Anagrus* wasp as a natural enemy of the WGLH. I will investigate nine genera of plant species (*Aesculus*, *Baccharis*, *Ceanothus*, *Heteromeles*, *Nepeta*, *Olea*, *Quercus*, *Rubus*, and *Ulnus*) for evidence of overwintering *Anagrus* spp. in Northern California vineyards. I predict *Anagrus* spp. to be found on these bushes, as according to results from previous studies focusing on other locations within and outside the United States (Mckenzie and Beirne 1972, Williams 1984, Hesami 2008). Due to the thus far inconclusive nature of the vegetation-specific studies, I expect an equal proportion of parasitism, as indicated by individual *Anagrus* wasp emergence counts, on the nine plant genera, because of a lack of definitive analysis on actual overwintering data for *Anagrus* spp. in and around North Coast vineyards.

METHODS

Study site selection

I chose study collection sites consisting of at least twelve separate patches (> 400 m^2) of natural habitat found adjacent to identified vineyards in Napa and Sonoma County. These significant patches of natural habitat increase the variety of plants from which I choose to collect material, to increase the breadth of my sampling scheme. Furthermore, they comprise of mostly Oak woodland and riparian vegetation, plants most prevalent in, and thus representative of, the natural habitats commonly found on the North Coast.

Sampling scheme

Within each study site, I identified candidate plants with a higher prevalence in dominant habitats of the North Coast and a higher probability of hosting overwintering Anagrus for sampling: a total of 122 plants. However, not all 122 plants were sampled every time. These 122 plants comprise of multiple plants of the same genus, spread out across multiple vineyards throughout Napa and Sonoma County. From November 2012 – March 2013, I collected vegetation samples a total of five times, once each month. Using a pair of garden clippers, I sampled the first six-to-twelve inches of first-year growth (Lowery et al. 2007, Zanolli and Pavan 2011), altogether at least 100 grams of vegetation from each candidate plant. These samples were collected into double-layered bags comprised of plastic trash bags, each lined in a grocery brown bag.

Checking *Anagrus* spp. emergence

Emergence chamber set-up

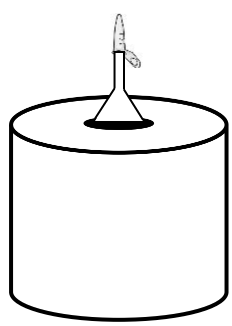

Figure 2.1 - The emergence chamber

Once I completed the collection of plant material, I placed each double-layered bag of collected plant material into separate emergence chambers constructed of rounded cardboard buckets (30cm x 30cm) with blacked-out lids in the laboratory at 26°C, 14:10 hours [light:dark] cycle, and 50% relative humidity (RH) to simulate spring/summer conditions (Lowery et al. 2007) for four weeks to encourage *Anagrus* wasp emergence. The lids had a hole (2" diameter) around which I glued a funnel, wide mouth down covering the hole. At the other, thin end of each funnel I attached a microcentrifuge tube, into which the *Anagrus* flew after emergence (Figure 2.1).

Checking emergence

For each of the five sampling dates, I checked for *Anagrus* spp. emergence on a daily basis, starting from the day after collection. To determine emergence, I placed a piece of white paper behind the microcentrifuge tube, in order to preliminarily detect by eye any possible candidates. If any candidates were spotted, I removed the tube and replaced its cap; then I confirmed its identity using a microscope. Any false candidates were promptly cleaned out of the tube and replaced onto the original funnel. If the candidate were actually *Anagrus*, I streamed carbon dioxide gas into the tube for temporary unconsciousness. At times when the carbon dioxide gas was not available, I alternatively froze (at 32 °C) the *Anagrus* wasp for 20-30 minutes, or as long as necessary to induce unconsciousness. After confirming unconsciousness using a microscope, I looked for the presence or absence of antennae clubs to determine the *Anagrus* spp. specimen to female or male, respectively. Using a size 3/0 paint brush, I then transferred the *Anagrus* wasp from the tube to a glass vial. Afterwards, I filled the glass vial with 95% ethanol to preserve the specimen for future identification of each *Anagrus* wasp to species. Then I recorded the plant identity (preliminarily numerically labeled), sex, emergence date (day of detection), and sampling date. The preserved specimens will later be identified to the species level by experts in UC Riverside.

Data analysis

Plant genus

Using appropriate statistical methods, I was able to meaningfully assess the results of the study, given the collected data: total count of *Anagrus* spp., emergence date, sex, and plant type from which they were reared. Throughout the entire sampling scheme, 122 separate plants were sampled, some from the same genus multiple times, but this was taken into account through using the parameter “average emergence” for all analyses. I only considered a small portion of these plants in my data processing, in order to analyze a representative sample of the greater selection of plants present in and around North Coast vineyards (Table 2.1). The nine chosen plant genera most completely represent the non-crop vegetation in and around North Coast vineyards, as they were found in Oak woodland or riparian habitats (Doutt and Nakata 1973, Williams 1984).

Plant genus	**Common name**
Aesculus	CA Buckeye
Baccharis	Coyotebrush
Heteromeles	Toyon
Nepeta	Catnip
Olea	Olive
Quercus	Oak
Rubus	Blackberry
Ulnus	Elm

Table 2.1 These are the nine selected plant genera for data analysis, due to their representativeness for plant genera of the area in and surrounding North Coast vineyards.

Upon evaluating the recorded emergence data for these nine plant genera using numerical summaries on R Commander (package for R statistical programming), I found that it was not normal. Therefore, after log-transforming average emergence (according to the number of times sampled each sampling date), I conducted a one-way analysis of variance (ANOVA) on emergence for plant genus, applying the null hypothesis of an equal frequency distribution of *Anagrus* spp. emergence consistent amongst all collected plant genera, given their independence.

RESULTS

Testing for Normality across Sampling Dates

As the study progressed, I accordingly adapted our sampling scheme to gather clippings from the same genus from different plants across various vineyards, in order to ensure a more accurate representation of the North Coast vineyards, instead of, for example, obtaining clippings from the same three plants of one particular vineyard, for one plant genus. A normality test using 95% confidence intervals from one-way ANOVA (df=4, f=1.06) showed no difference in emergence count (log-transformed) amongst all five collection dates (11/4/2012, 12/8/2012, 1/16/2013, 2/9/2013, 3/2/2013). This confirmed the validity in comparing *Anagrus* spp. emergence from the same plant across all sampling dates: the established basis needed for the subsequent analyses.

Plant genera and *Anagrus* emergence

The primary angle from which to analyze the *Anagrus* spp. emergence is based in the plant genera from which the *Anagrus* emerged in the temperature-controlled laboratory room. For this analysis, I informally determined by observation that within the first two weeks since collection, I can readily identify plants with a high probability (>50%) of rearing *Anagrus* spp. The nine genera chosen are ones most representative of the overwintering habitat in and around vineyards. They were also ones that were collected from a larger variety of site types, thereby eliminating other uncontrolled variables. I made note of these genera for increased collection for the next sampling date. After log-transforming the data for one-way ANOVA, I found that *Nepeta*, the catnip, significantly resulted in more *Anagrus* spp. emergence, compared to the other eight genera. The next level of significance involved three genera: *Baccharis* (coyotebrush), *Ceanothus* (CA lilac), and *Rubus* (blackberry). The rest of the chosen nine genera did not result in significantly differing emergence data, compared with each of the remaining genera (Figure 3.1).

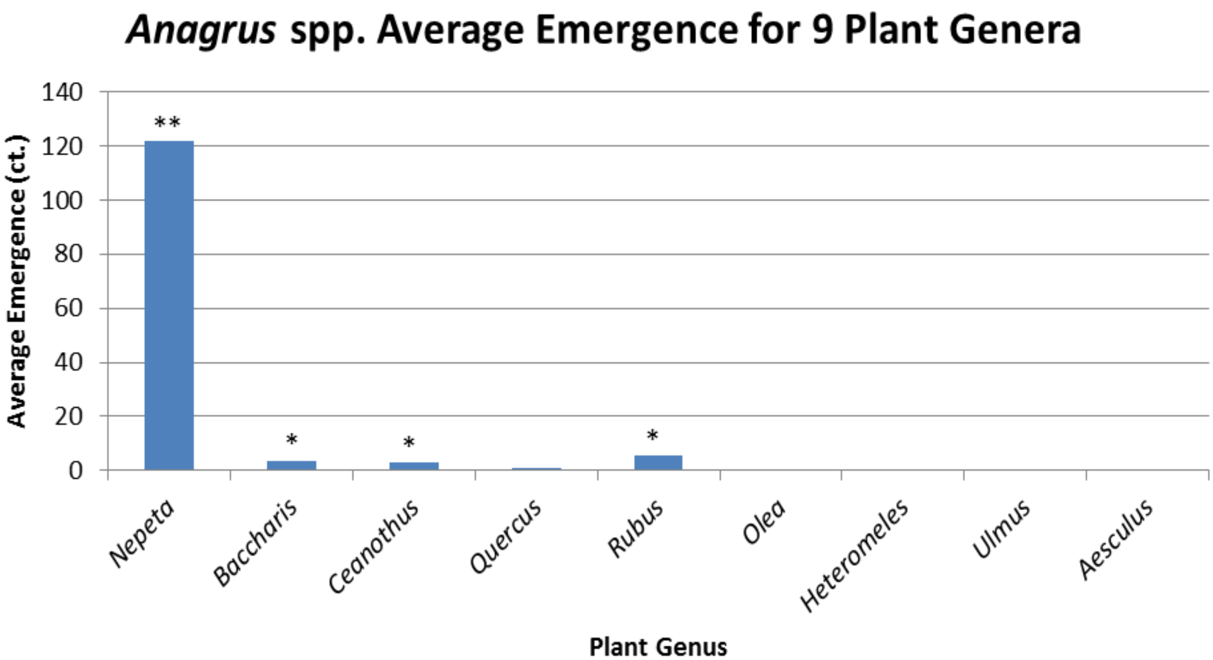

Figure 3.1 The average emergence for the 9 plant genera resulted in having *Nepeta* the highest, and the next level of significance included *Baccharis*, *Ceanothus*, and *Rubus*.

DISCUSSION

The accumulated results of *Anagrus* spp. emergence data seem to confirm that *Anagrus* spp. exhibits discrimination in the plant's role as overwintering habitat based on plant genera. The differences that exist among emergence dates amongst the plants show that the parasitoid displays a variety of preferences in emergence, depending on the other (largely unknown) factors.

Although effective in identifying overwintering habitat for *Anagrus* spp., this sampling regime has its limitations in definitively determining the effect of incorporating "x" amount of specific plants into a vineyard for pest control, due to its time constraints and lack of comprehensiveness, in terms of assessing all possible plant genera within a defined vicinity of the vineyards. Simultaneously this study sits well within the context of current research conducted on overwintering biology of *Anagrus* spp. that can parasitize the Western grape leafhopper, by confirming many of the preferred plant families in a more reliable, more direct manner (emergence chamber method) instead of sticky traps, as is often the method of choice

(Williams and Martinson 2000, Prischmann et al. 2007). Therefore, my investigations contribute well into the growing pool of knowledge, regarding conservation biological control and how to evaluate its efficacy before implementation.

Emergence date and Anagrus emergence

Emergence date is an important factor to consider for this study. The observed range of emergence dates for this study suggests that *Anagrus* emergence occurs for most plants (other than 154) within the first three weeks, with emergence peaking at the end of week two, regardless of sampling collection date. This is supported in the data provided by previous studies, placing *Anagrus* spp. lifecycle development time around 15-20 days (Hesami 2008, Usmani 2012), or generally two to three generations for every leafhopper generation (Cate 1975, Williams 1984, Williams and Martinson 2000). As a result of the direct measurement methods provided by emergence chambers, my collected data offers in-depth analysis on the emergence behavior of *Anagrus* spp. The results have implications for biological control mechanisms and *Anagrus* spp. overwintering behavior, because different plants would host different *Anagrus* species, thus affecting the emergence times for the *Anagrus* spp., and thus their movement into the vineyard.

Limitations

The nature of this research requires an intensive, cohesive stream of consistent sampling in the same manner over a large period of time. My chosen methodology needs a specified amount of labor per sample, of which it is ideal to collect around fifty samples, each with its own emergence chamber in the temperature-controlled laboratory, due to the physical space limitations of the room. Each of the five times I sampled the plants, the entire process from collection to transfer into the buckets, took 10-12 hours, transportation time included. Furthermore, intensive labor is required post-sampling, in order to collect data daily for emergence and the emergence factors. Therefore, this study cannot possibly be replicated past a certain number of times for each plant genus, no matter the amount of resources obtained, unlike other studies. This is due to the extensive, thorough manual checking required daily. . The same concern exists for the understanding of the *Anagrus* spp. biology, and subsequently using the knowledge in conjunction with the collected data to understand the results in their biological context. Furthermore, for the purpose of this thesis with its time constraints, I was not able to obtain the species identification of *Anagrus* spp. in time to confirm whether the collected specimens actually consist of species that actually parasitize the Western grape leafhopper eggs.

Future directions

In the future, researchers may proceed in sampling to determine *Anagrus* spp. emergence during the summer. Future steps also include looking into the actual mechanisms of dispersal for *Anagrus* spp. all-season behavior. Understanding further biology of *Anagrus* spp. development lifecycle and overwintering host preferences would also be key next steps, including, most importantly, complete alternate host identifications. Future research would function as alternate hosts' prevalence in relation to the Western grape leafhopper pest presence in Northern California vineyards.

Broader Implications

This study sets the stage for increasing the literature bank for implementation of conservation biological control, and therefore provides appropriate basis for understanding the underlying overwintering biology for both the natural enemy and the pest. Throughout the study, interesting observations about early emergence from overwintering habitat (early March) are an important addition to the knowledge, helping growers to decide when and what to plant near their vineyards. Specific recommendations include catnip (*Nepeta* spp.) and coyotebrush (*Baccharis* spp.), which are both dominant plants preexisting in the region. However, before any incorporation is attempted, it is critical to understand how it might impact the Western grape leafhopper populations in and around the vineyards.

It is essential to formulate methodology for subsequent research regarding *Anagrus* spp. emergence behavior around spring/summer conditions and analyze actual dispersal mechanisms directly, whether it is through marking or microscope views. My study determined that out of the nine plant genera, the highest frequencies of Anagrus spp. were reared from *Nepeta*, *Baccharis*, *Ceanothus*, and *Rubus* spp. Therefore, further research in identifying the specific leafhopper populations existent on these plants would provide more conclusive evidence on the alternate hosts used by *Anagrus* spp. during the winter. Once these leafhoppers are identified, researchers can then pinpoint other plants favored by the leafhoppers, and consequently find *Anagrus* spp. overwintering preferences. These alternate hosts are all leafhoppers, lending information about their natural preferences for hosts—that is, that because a leafhopper naturally disperses amongst the same handful of families for feeding, so *Anagrus* spp. must likewise do so, in order to accommodate for location of leafhopper for parasitism (Williams and Martinson 2000).

This research contributes vital information for *Anagrus* spp. overwintering biology, required for evaluating potential implementation of conservation biological control, and its alternative manifestation – namely, intercropping alternate host preferences into the vineyards of Napa and Sonoma County.

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