Pre-release Efficacy Test of the Proposed Biological Control Agent *Arrytinis hakani* on the Invasive Weed *Genista monspessulana*

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ABSTRACT

In weed biological control, conducting a pre-release efficacy test can help ascertain if potential biological control agents will be capable of controlling the target plant. Currently, the United States Department of Agriculture (USDA) is investigating the phloem-feeding psyllid *Arrytinis hakani* as a potential agent for the exotic invasive weed, *Genista monspessulana*. In this experiment, I tested if *A. hakani* was capable of killing the target plants by putting insect densities, ranging from 4 to 16 A. hakani nymphs per test plant, on the plants for six weeks. At the end of this period, I removed the insects from the test plants and assessed the damage by comparing final and initial plant parameters for test plants with different insect densities. I determined that although increasing *A. hakani* densities had a significant negative impact on number of leaflets grown and percent change in height, the psyllid did not cause leaf senescence or destroy any of the plants even at its highest density. It is possible that this damage did not occur because insect mortality during the course of the experiment was high enough that densities of 12 and 16 insects were not maintained, even though insects were replaced weekly. Because *A. hakani* did not have enough impact to destroy the test plant, it cannot be endorsed as a strong candidate for biological control without further research.

KEYWORDS

Herbivory, agent efficiency, French Broom, *Candidatous liberibacter europarus*, leaf senescence

INTRODUCTION

Classical biological control is used ubiquitously to mitigate damaging invasive species, effectively controlling invasives so that native ecosystems can be maintained. In classical biological control, an exotic invasive biocontrol organism is released to combat a specific target invasive organism. This practice has been commonly used in the United States since the 1900's, and has a strong history of being successful and safe. As of 2005, 350 organisms had been released to control 130 weed species worldwide; of these, only eight had impacts on non-target plants, and forty-one of the weeds were successfully controlled (McFadyen 2000, Julien and Griffiths 2008). However, while biological control is a well-tested method of mitigation, the effectiveness of this practice is less redoubtable. One review of biological control projects examined thirty-eight successful weed biocontrol mitigations, and found that of the 132 agents released in these projects, only fifty-four actually had an impact on controlling the invasive (McFayden 2003). Thus, less than half of the biological control agents released were actually effective at controlling their target plant. Likewise, 54% of successful weed biocontrol projects in which multiple agents were released had only one agent which was responsible for the damage inflicted on the target invasive (Denoth et al. 2002). Introducing more invasives to an area than are necessary is common in classical biological control, and it has the potential for negative impacts on ecosystem diversity. Ineffective agents, unlike effective agents, will not depend on a depleting food source (the target plant) and will thus be able to maintain higher population densities, which can increase populations of predators and parasitiods, causing indirect competition effects (Thomas and Reid 2007). The international biological control community has taken steps towards addressing the problem of ineffective agents in the "The Code of Best Practices for Classical Biological Control of Weeds." (1999) Points (3) and (8) of the twelve listed mandates state that it is best practice to "Select agents with the potential to control the target" and to "Stop releases of ineffective agents" (Balciunas 2000).

One way to help reach these goals and ensure that released agents will be effective at controlling their target weeds is to run a pre-release efficacy test. This type of test assesses the impact that the proposed biological control agent has on the invasive weed. This pre-release evaluation is a tool that has the potential to minimize the chances of releasing ineffective agents (e.g. Colpetzer et al. 2004, Goolsby et al. 2004, Williams 2005, Ding et al. 2006, cited in Y.

Zhang et al. 2012).Choosing only those agents that have a strong impact on the target plant reduces the number of species used, and thus reduces the risk direct and indirect risk to non-target plants and the ecosystem.

One scenario where the use of a prerelease efficacy test could be used is in the mitigation of French Broom, the weedy legume, *Genista monspessulana (G. monspessulana)* (L.) Johnson (Fabaceae), found along the western coast of the United States.G. monspessulana, is a native of the Mediterranean area of Europe and western North Africa which was introduced into the U.S. in the mid-1800s (Sheppard 2003). Since then, the weed has spread along the western coast of the United States. It has proliferated to the point where it is now listed among the most invasive wild-land plants in the state of California (Bossard 2000) since it outcompetes natives for space and resources. *Genista monspessulana (G. monspessulana)* is also an extensive invasive in Australia, and to mitigate it, a phloem-feeding psyllid, *Arytinnis hakani* Loginova (Hemiptera:Psyllidae) also native to countries surrounding the Mediterranean region, was released in 2008 after having been found already present in the region (Henry 2008). However, it has not been approved for release in the United States. While the SDA has conducted extensive research on the specificity of *A. hakani*, its impact on the target weed has not yet been assessed. Until the impact on plant caused by this agent is ascertained, it is uncertain whether or not it will actually be effective at damaging the target plant, *G. Monspessulana*.

In this study, I examined the impacts of *A. hakani* on the invasive weed *G. monspessulana* to assess if this psyllid will be an effective biological control agent. To determine the amount of impact inflicted on the *G. monspessulana,* I conducted a quarantine experiment to compare how the number of *A. hakani* placed on individual plants affected the amount of damage inflicted. I expected that by increasing the number of *A. hakani* present on each plant I would observe an increase in the damage inflicted on the plant. I hypothesized that the relationship between the number of *A. hakani* and damage to the plant would be linear, and would reach a maximum when the number of insects equals the number of leaflets on the plant (*L. smith, unpub data*). This estimate is consistent with previous laboratory observations which suggest that one insect per leaflet may be the maximum number of psyllid nymphs that can be reared to the adult stage on small potted plants *(L. Smith, unpublished data).*

METHODS

Study site

I conducted my experiment at the United States Department of Agriculture (USDA) Exotic and Invasive Weeds (EIW) department located in Albany, CA. I performed the experiment in quarantine because *A. hakani* has not been approved for release in the United States.

Data collection and experimental methods

Material Preparation

I performed the methods for the experiment in four steps: pre set-up, set-up, monitoring, and take-down. In the pre set-up, I maintained colonies of insects and plants so that there were adequate numbers of both species at approximately the same stage and size throughout the experiment. The original colony of *A. hakani* was maintained on potted French Broom plants in a quarantine room under fluorescent lighting (12hr photoperiod) at room temperature (ca. 19°C) and humidity (22% RH). Two weeks before each repetition of the experiment, I put ten pairs of adult *A. hakani* (one male, one female) on ten different *G. monpessulana* clippings to oviposit. The time it takes for eggs to develop into second instar nymphs (L_2) is approximately two weeks (*L. Smith, unpublished data*), and the average fecundity of a female is about 6-10 eggs per day at 18°C. Thus, by setting up ten pairs of *A. hakani* two weeks before I setup the experiment, I ensured that I would have at least 120 useable L_2 nymphs — enough for three repetitions of the five treatments: 0, 4, 8, 12, and 16 *A. hakani*. I used L_2 nymphs because this was the youngest stage that could be easily handled. Thus I had approximately five weeks before the nymphs reached adulthood (*L. Smith, unpublished data*) at which point they could start reproducing—at six weeks the eggs they laid could hatch and I would no longer have control over the number of insects on each plant.

To ensure that I had test plants of the same original height throughout the experiment, I germinated two cohorts of fifty plants apiece, one on 9/4/12 and one on 9/20/12, so that I would have enough variation in plant height to choose plants of approximately the same size during the

four weeks of setup for the experiment. These plants were grown in a greenhouse at the USDA at room temperature and humidity. Finally, before I set up a repetition of the experiment, I thoroughly cleaned each plant, examining them with a microscope to find and remove insects from the greenhouse.

Set-up

I set up ten repetitions of the five levels of treatment (0, 4, 8, 12, and 16 *A. hakani* per plant) over the course of four weeks. The first week, I set up reps 1 and 2 of the experiment. Prior to adding the *A. hakani,* I measured the height of each plant, from the cotyledons to the shoot apical meristem, using digital calipers. Next, I counted the number of leaflets of each of the ten plants. I used a pin on the end of a dowel with the aid of a microscope to transfer the *A. hakani* nymphs to these test plants. After this, I put a plastic cup, with the bottom cut-out, over the plant. I put a screen over the top of the cup, and put a cut-out lid over the top; this ensured that each plant was isolated, but still ventilated enough to keep the plants from molding. Finally, the treated plants were put into a quarantined incubator at 18°C (the optimal temperature for L_2 *A. hakani* development) and 25% humidity with a 12hr photoperiod (*L. Smith, unpublished data*)

I then repeated this set-up process for repetitions 3-10 over the course of four weeks (setting up two to three repetitions per week). The set-up process was performed on one-week incremental dates due to time restraints.

Maintenance

For the third stage of the experiment, I monitored the plants on a weekly basis. Monitoring included watering the plants and checking for the presence of arthropods other than *A. hakani*. Also, I counted the number of *A. hakani* on each test plant with the aid of a microscope. I replaced missing or dead insects with ones at the same nymphal stage. After six weeks of maintaining the test plants and *A. hakani* populations, the insects had matured to adults and laid eggs—all insects were removed one week after they reached adulthood, before the eggs hatched.

Take down

I recorded final measurements for each repetition after the test plants had been infected for six weeks. For data take-down, I removed each plant from the incubator and captured all *A. hakani* remaining on the plant using an aspirator for adults and a pin on the end of a dowel for the eggs. Next, I measured the final plant height, the stem diameter (using digital calipers) and recorded the number of leaflets on each plant. I cut off the above-ground plant, and weighed it on a digital scale to obtain the wet biomass weight. Additionally, I washed the roots of each plant, patted them dry with a towel, and took a wet biomass weight measurement in milligrams.

Data Analysis

To analyze my data, I used the software program R for all statistical tests. Before I began analyzing the data to answer my study questions, I used a Shapiro-Wilk test of normality to make sure that the different levels of treatment (0,4,8,12, and 16 *hakani*/plant) for the ten repetitions had normal distributions. I discovered that the different levels of treatment had normal distributions for change in number of leaflets, but not for other growth change parameters.

Next, I assessed the rate of mortality for *A. hakani* nymphs by graphing a linear regression of the mortality rates at different stages and insect densities. Next, I analyzed the data using a one-way ANOVA, paired t-test to compare the different mortality rate for every nymphal stage within a treatment level, and Tukey HSD test to compare the different rates of mortality for different stages and densities of *A. hakani*. Additionally, I graphed the average number of *A. hakani* (determined by averaging the original number of insects with the number of insects still living on each plant at the end of one week) vs. the original number of insects to determine the shape of the curve of the actual numbers of insects per plant.

I then used a one-way ANOVA to compare the effect of treatment on the response variables: percent change in plant height, percent change in number of leaflets, and above-ground and root biomass, to determine if the number of *A. hakani* actually did have an impact on plant growth and health. If my ANOVA gave a p-value $< .05$, I considered it statistically significant. In conjunction with my ANOVA tests, I used a Tukey HSD test to see which level of treatments had means different from each other. Finally, I also used a Linear Regression or

GLM model (depending on whether the data was normal of not) to determine the change in growth parameters vs. an average number of *A. hakani*, estimated by averaging the number of *A. hakani* found present on the plants each week with the initial number put on the plant.

Because the plants did not experience any drying of leaves due to the presence of *A. hakani*, I could not test my original hypothesis that when the number of *A. hakani* nymphs equals the number of leaflets on the French Broom, the plant will experience significant damage (significant damage is defined as 50% or more of the above-ground plant being dry and dead).

RESULTS

Set-up: initial plant parameters

The response variables measured at the beginning of the experiment, plant height ($p= 0.552$, Shapiro-Wilks = .98) and number of leaflets ($p> .05$, Shapiro-Wilks = 595) were non-normal, as expected from biological data with only 10 replications of the test plants.

Maintenance: mortality rate of *A. hakani*

At both higher insect densities and at older nymphal stages, *A. hakani* mortality increased (Fig. 1). Mortality rate was considerably lower for densities of 4 *A. hakani* per test plant than for all other densities ($p < .005$). Also, L_2 mortality was significantly lower than all other nymphal stages at the same density ($p < .05$). Mortality rates at L_3 and L_4 nymphal stages were not significantly different from each-other and neither were L_4 and L_5; all other nymphal stages were significantly distinct from each-other ($t_{(50)}= 1.56$: $p < .05$)

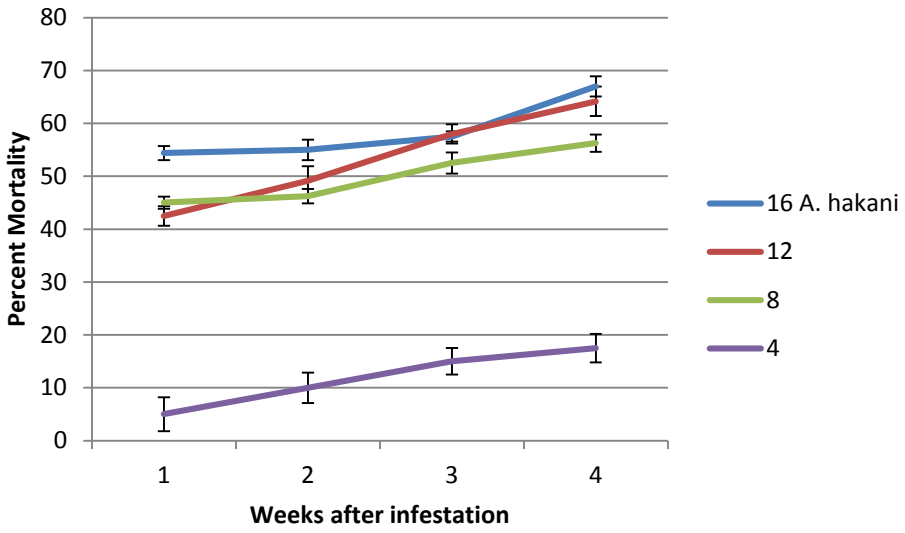

Fig. 1 Percent Mortality Rate for Densities of *A. hakani* (ARHA) (mean±Sd). Increasing mortality of *A. hakani* at both higher densities and older instar stages—mortality is significantly lowest as densities of four *A. hakani* per test plant

The estimated average numbers of insects present on each test plant

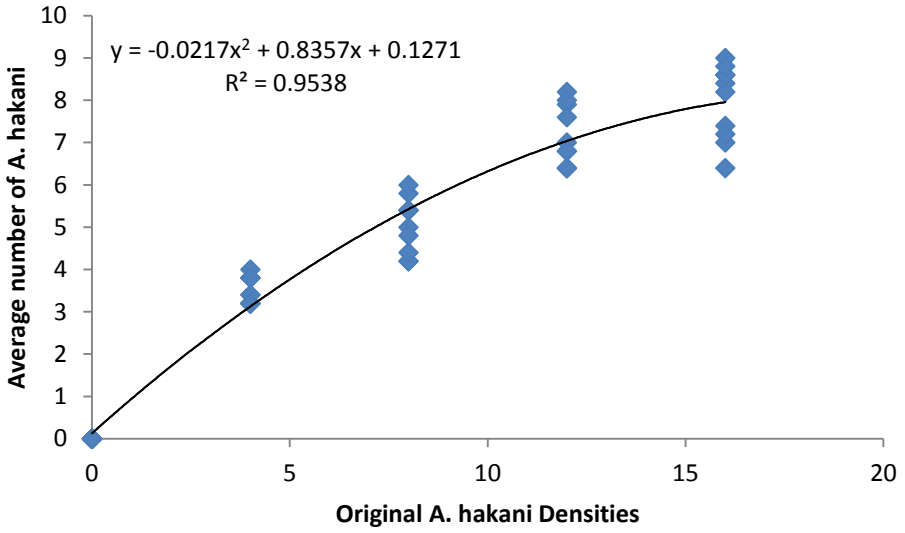

Fig. 2 Average number of insects vs. Original number of insects. Insect density follows a curve declining in rate at approximately seven insects per plant.

Final data: impact of *A. hakani* on the test plants

I determined whether or not the change in number of leaflets, the percent change in height, the ratio of aerial/root biomass, and the final stem diameter were normal using a Shapiro-Wilks test (Table 2) as well as histogram and quantile-quantile plots. I concluded that delta leaflets was normal, whereas the percent change in height, aerial biomass, root biomass, ratio of aerial to root biomass and the stem diameter were not normal.

Table 2. Normalcy of final plant parameters, according to the Shapiro-Wilks test ($p<.05$)

	Delta Leaflets	% Change in Height	Aerial Biomass	Root Biomass	Aerial/Root Biomass	Stem diameter
P-value	0.21	0.011	.000067	.0089	0.00034	4.93e-07
Shapiro-Wilks	0.97	0.93	.96	.97	.89	0.79

I found that there was no significant relationship between psyllid density and the ratio of aerial to root biomass. Fig 3, $\chi(.45,, N=50)= 0.5$, $p=0.96$. By excluding some outliers, both change in number of leaflets(Fig 4, $F_4=6.69$ $p=0.00031$) and percent increase in plant height (Fig 5, $F_4=4.16$ $p=0.0062$,) were significant.

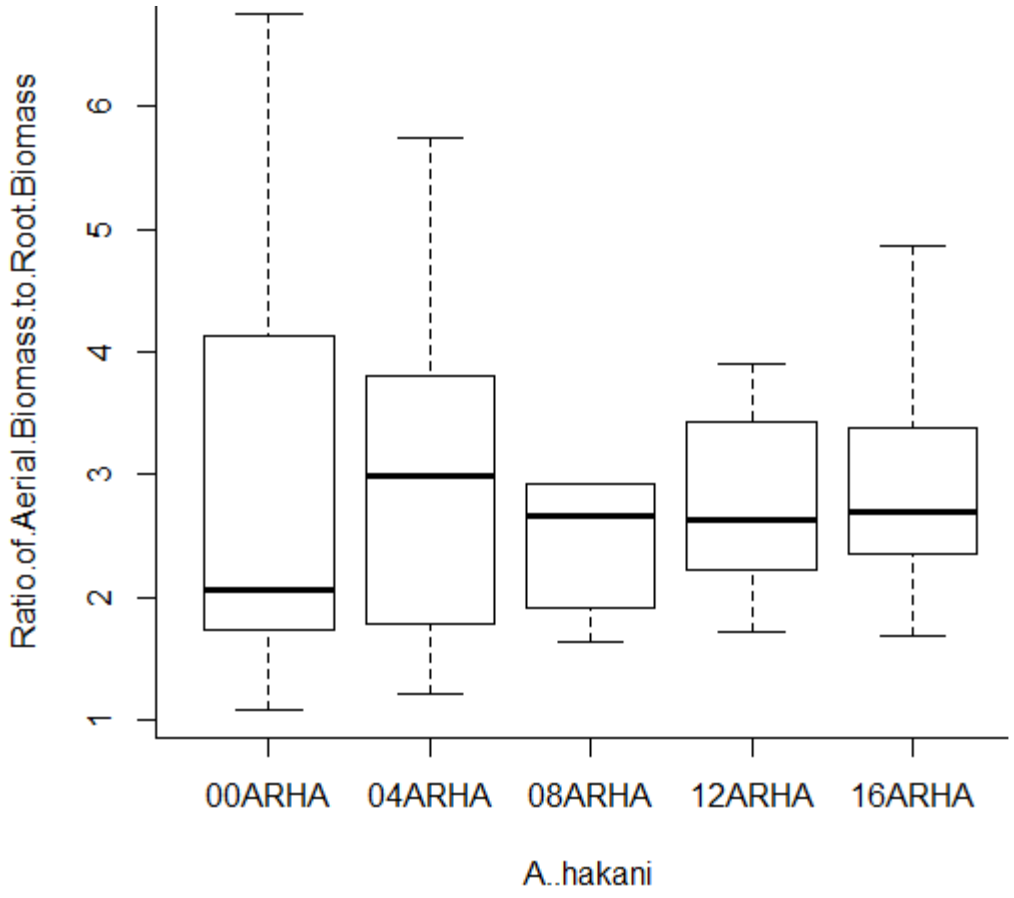

Fig 3. Aerial/root Biomass Per *A. hakani* Densities. Kruskal-Wallis (mean±Sd). χ (.77, N=4) = 0.58, p = 0.96) The means look very similar.

For change in number of leaflets, I ignored two outliers because these test plants had very low densities of insects (0 and 4 treatment levels) and yet experienced more severe damage than any of the other plants(they dropped all of their leafs), so it was likely that they suffered from a disease, such as root rot. Without outliers, (2 outliers ignored out of 50 pieces of data) the data was significant (Fig. 4, ANOVA, $F_{(4,42)}$ =4.34, p=0.0049). There were significant differences between the groups of 4 and16 A. hakani ($t_{(44)}$=-2.84, p=0.046), and 0 and 16 A. hakani ($t_{(44)}$ = -3.6 p=0.0070). Additionally, a linear regression also shows that the data was significant, following a line with a negative linear slope (y = -0.5239x + 16.895, R^2 = 0.2459. The data was

more significant than found using the ANOVA (Linear Regression: $F_{(1,45)} = 14.68$, p=0.0003928)

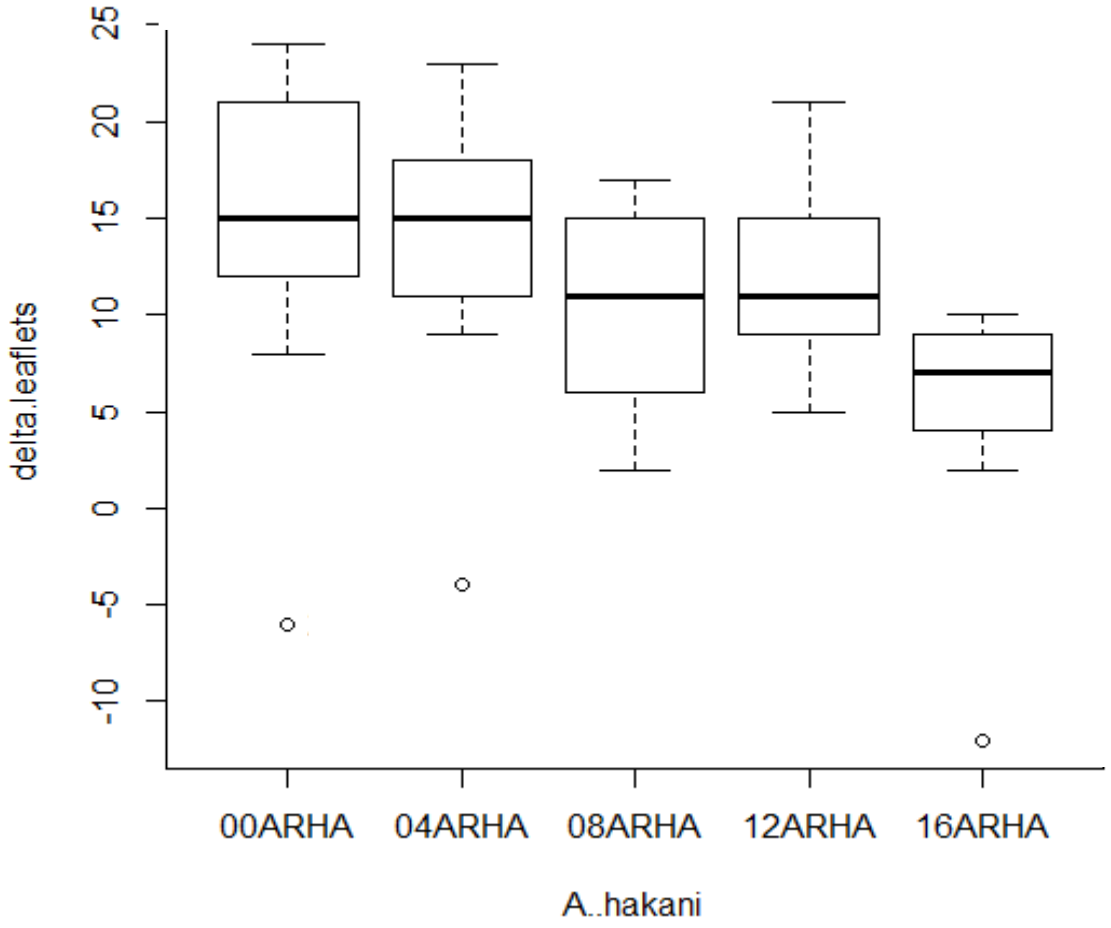

Fig. 4. One-way ANOVA (mean±Sd) change in number of leaflets. The relationship is significant (ANOVA, $F_{(4,42)} = 4.34$, p=0.0049).Tukey Test: there is a difference between the means of change in numbers of leaflets for 4 and 16, and 0 and 16 and groups of A. hakani densities

I took into consideration insect mortality by plotting the average number of *A. hakani* present (a weighted average of survivorship for the different nymphal stages on each plant) vs. response variables. The linear regression of delta leaflets (final number of leaflets – initial number of leaflets) vs. number of *A. hakani* was significant ($F=14.47_{(1,45)}$ p= 0.00043), though not as close of a fit as determined using a linear regression for the original number of *A. hakani*. The data followed a negatively linear slope: $y = -1.0786x + 17.782$ $R^2 = 0.2684$ (Fig 4.)

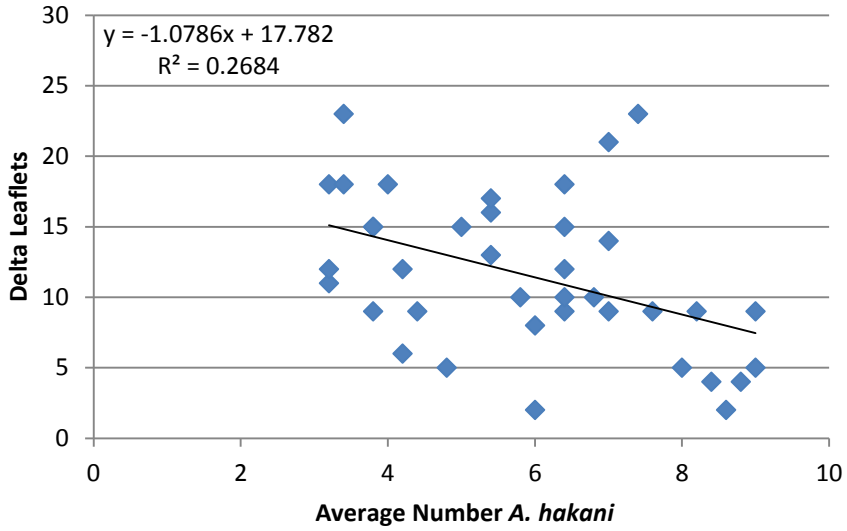

Fig. 5. Average Number of *A. hakani* vs. Delta Leaflets final number of leaflets – initial number of leaflets): Linear regression. Y = -1.0786x + 17.782 R² = 0.2684 From this model, we can see that there is a trend towards decreasing number of leaflets vs. average number of A. hakani, but the trend is not very strongly linear.

For percent change in plant height, one outlier out of 50 data points was removed because it was unusually tall. Because this test plant had an earlier planting date (8/24/2012) than the other plants used in its repetition (9/11/2012) it is possible that this test plant grew significantly larger than the others because it was older. Ignoring this data point, the relation between insect densities and percent increase in plant height was significant $\chi(5, N=48) = 7.6$, $p = 0.04829$. There were significant differences between 12 and 0 *A. hakani* ($t_{(36)}=-3.34$, $p=0.014$) and 12 and 4 *A. hakani* ($t_{(36)}=-2.95$, $p=.039$). A linear regression for this data, which was significant ($F=8.5_{(1,47)}$, $p=0.0054$) shows a negative linear slope: ($y = -1.6432x + 56.289$, $R^2 = 0.1155$) with a significance greater than that given by the Kruskal-Wallis test.

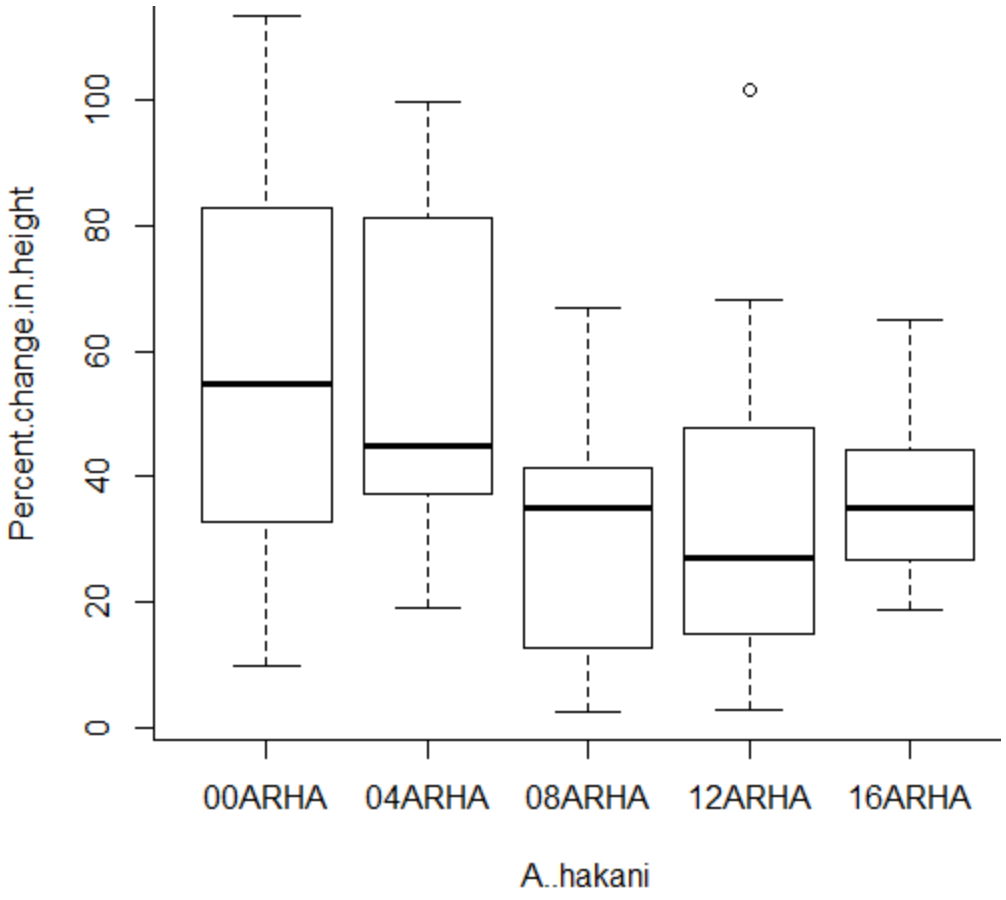

Fig. 6. One-way ANOVA: (mean±Sd). Percent increase in plant height. The data is significant (ANOVA: $F_{(4,44)}$=3.42 p=0.0161). Tukey Test: there is a difference between the means of ARHA densities of 12 and 0 and 12 and 4.

I accounted for *A. hakani* mortality and the impact on increase in plant height. As with the change in number of leaflets, I calculated an average number of *A. hakani* present on each plant throughout the course of the experiment, and graphed this against percent increase in height. This relationship was significant, ($F=8.62_{(1,47)}$ p= 0.0051) and negatively linear (y = -3.2313x + 58.042, R^2=.102). The fit of the linear regression line was stronger after weighted average of insect mortality was accounted for (p= 0.0051), rather than when the initial levels of

treatment of *A. hakani* were assumed (p=0.0054). Also, the slope of the line was more steep (m_0=-1.6432x, m_1= -3.2313)

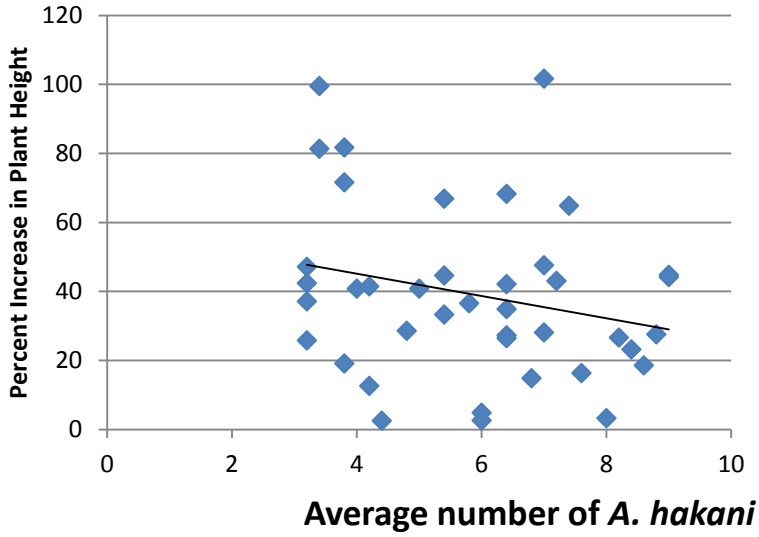

Fig. 7. Average *A. hakani* vs. Percent Change in Plant Height. Linear Regression (y = -3.2313x + 58.042, R^2=.102), ($F=8.621_{(1,47)}$ p= 0.005131). From this model, we can see that there is a significant trend towards decreasing number of leaflets vs. average number of *A. hakani*, even if the data des not conform to a very clearly linear trend.

DISCUSSION

Introduction

This study contributes critically to our understanding of the suitability of the proposed biological control agent *A. hakani* by quantifying its impact on the invasive weed *G. monspessulana.* The main purpose of this test was to determine what density of *A. hakani* was capable of destroying *G. monspessulana* plants. In this experiment, I determined that although *A. hakani* had an impact on *G. monspessulana*, the damage was not significant at insect densities predicted from previous incubator studies.Without further research, *A. hakani* is not a viable biological control agent for *G. monspessulana.*

Plant mortality

Primarily, the results of the study showed that although *A. hakani* had some impact on plant development, it is not likely to be a strong biological control agent for *G. monspessulana*. Specifically, when insect densities in my experiment were the same as those found in an incubator study at the USDA, there was no significant damage to the test plant. The initial incubator study suggested that suggest that *A. hakani* should decimate plants when there is a ratio of 1 insect to 1 leaflet (*L.Smith, unpubl data*) however, in this experiment, 2 insects per leaflet did not cause enough plant damage to test the original hypothesis that the plant would experience > 50% damage at densities of 12 and 16 *A. hakani* per test plant.

In addition to the lack of visible plant damage, there was also no premature leaf abscission on any of the test plants throughout the experiment suggesting that A. hakani may not be a viable control agent.Leaf abscission caused by Psyllids has been observed in many studies on Psyllids (Hemiptera:Psyllidae): *Boreioglycaspis melaleucae* on *Melaleuca quinquenervia* (Cav.) (Center et al. 2007, Morath et al. 2006), *Aphalara itadori* on Knotweed (Grevstad 2013), and *Diclidophlebia lucens* on *Miconia calvescens* (Burckhardt 2005). Leaf abscission resulting from leaf senescence is believed to be caused by a phytotoxic salivary exudate the Psyllid releases into the plant (Hodkinson 1974) as it feeds on the phloem along the main delivery channels from growing leaves (White 1993). Lack of this abscission indicates that *A. hakani* may not be as strong of biological control as other agents that were effectively released.

Plant growth

Increasing the number of psyllids made an impact on plant growth parameters, although it had no impact on biomass or biomass allocation. Lack of impact on the aerial to root ratio with increasing numbers of Psyllids was also discovered to be true for the Psyllid *Boreioglycaspis melaleucae* on the invasive tree *Melaleuca quinquenervia* (Franks et al 2006), although *Boreioglycaspis melaluecae* had a significant impact on plant biomass, and in some cases even destroyed the plant. These results indicate the possibility that biomass allocation is not a good indicator of plant damage resulting from psyllid herbivory, although overall biomass is.

Although *A. hakani* did not destroy the plant as hypothesized, the psyllid did have an impact on plant growth. There was a marked linear decrease in number of leaflets correlated with density of *A. hakani* (Fig 3.) This correlation supports the hypothesis that more psyllids relates to more damage, also observed in studies of post-release efficacy assessments of a specialist psyllid on *Melaleuca quinquenervia* (Franks et al. 2006) There was a key difference between this study and my own, however; in the study on *Melaleuca quincuenrvia*, leaf senescence was observed on all infested test plants so delta leaflets was due to loss of leafs, rather than from growth retardation as in my experiment.

In addition to its impact on change in number of leaflets, *A. hakani* also affected vertical plant growth; increasing numbers of insects related to decrease in percent change of height. Unlike the linear trend found in change in number of leaflets, percent increase in height was lowest at densities of 12 *A. hakani*, rather than 16 *A. hakani* per test plant. It is possible that this trend indicates that the damage caused by *A. hakani* levels off at the density of 12 insects per plant, or that the increasing insect mortality at increasing insect densities made the actual value of insects for 12 and 16 treatments to be too similar to distinguish between the two (on average, treatments with 12 *A. hakani* actually had only 6.8 insects present, and treatments with 16 A. hakani had a close 7.6 insects at on average). The hypothesis that the damage did not decline linearly due to the non-linear increase in insect density is also supported by the rate of change of the slope for number of *A. hakani* present per test plant, which decreases at 12 insects per leaflet (Fig. 2). Additionally other studies on psyllid impact on plants observed that psyllids had an impact on plant growth, but the trend relating plant growth to psyllid density was only observed after four months (Franks et al 2006). In my experiment, insects were removed from the test plant after six weeks, after only one generation of insects, and the final plant parameters were immediately taken. However, it is also possible that there was simply too much variation in the data to determine a clear trend.

Causes of plant damage

There are several possible explanations as to why *A. hakani* was capable of reducing plant growth, but not inflicting damage to the plant such as leaf abscission or desiccation. One possible explanation is that there were not enough insects present to decimate the plant. This is

supported by the data showing that decrease in plant height "levels off" as the rate of actual insect densities begins to level off. However, in a study correlating number of the Psyllid *Boreioglycaspis* sp. on the invasive tree *Melaleuca quinquenervia*, *Boreioglycaspis* impacted plant height, biomass, and leaf senescence, it was found that the Psyllid caused damage at lower densities than found in my study. In the study of Boreioglycaspis melalucae, insects were maintained at densities of 15 to 50 insects per seedling (seedlings size was approximately 26.1±0.13 cm tall); at the beginning of my experiment, *G. monspessulana* seedlings were 5.04-6.87cm tall, with a maximum of 16 insects per plant, so the density was clearly

Another speculative possibility is that *A. hakani*, which has coevolved with *G. monspessulana*, is capable of destroying the plant single-handedly; it is possible G. monspessulana has evolved a tolerance for *A. hakani*. *G. monspessulana* has developed quantitative defenses, such as tannins or isoflavanoids, as resistance to specialist herbivores (Müller-Schärer et al. 2004) such as *A. hakani*. *A. hakani* may be negatively affected by these quantitative defenses, and cannot on its own create severe damage to the plant. However, results from a study examining *A. hakani* feeding on French Broom suggest that *G. monspessulana* quantitative defenses may not affect *A. hakani* (Herrerra et al. 2011)

Finally, non-fatal damage may support the hypothesis that *A. hakani* acts as vector for a pathogen that damages the plant. This mechanism would explain why there was not a decrease in biomass/root ratio with increasing insect density, expected if the insects were feeding enough to damage the plants. Additionally, it would explain why there was not a more clear relationship between insect densities and plant damage—particularly percent change in height, which was lowest at densities of 12 insects per plant rather than 16 insects per plant; it would only take one insect to transmit the pathogen, so increasing the number of insects only increases the probability that the plant will contract the pathogen, not the actual damage experienced by the plant. Currently, *A. hakani* is being investigated for the presence of the pathogen *Candidatous liberibacter europarus*, found only in psyllids, such as the Citrus Psyllid *Diaphorina citri and the* potato/tomato psyllid *Bactericera cockerelli* (Gottwald 2010*).* In Citrus, *C. liberibacter europarus* is transmitted via the citrus Psyllid and causes some initial damage in growth parameters to the plant, but does not kill saplings for approximately 5-7 years. (Cen et al. 2012). If *A. hakani* had transmitted the pathogen to the French Broom, we would observe low level

damage as we did in the experiment, but the plants would not be controlled until the pathogen had remained in the plant for more time.

Study limitations

A. hakani should not be ruled out as a possible biological control agent without noting several limitations which may have impacted the study. Primarily, the results we obtained from this quarantine experiment may not match field results simply because of the differences in field versus lab conditions. In the experiment each infected test plant was placed in a cup and insect mortality was so high because the insects felt crowded and stressed in this environment. This would seem to be supported by the increasing mortality rate as the insects aged into adults, becoming larger and more mobile and requiring more space. Additionally, one study found that insect mortality of *A. hakani* in quarantine where the insects were free to move between plants had a survival rate to adulthood of 25%. (Henry et al. 2008). This percent mortality is noticeably higher than the survival rate to adulthood of 11% that we found, indicating that in our study, insects mortality was abnormally high, which was likely caused by the use of cups in the experiment. Finally, we may not have observed the damage predicted from the incubator study because we had an inflated estimate of the number of *A. hakani* present since we did not account for mortality affecting the actual number of insects present. If we were to increase the number of *A. hakani*, we might yet see the damage we anticipated.

Future directions

Because *A. hakani* did cause some damage to plant growth, it cannot be ruled out as a biological control agent; however, further study on *A. hakani's* impact on *G. monspessulana,* particularly focusing on the mechanism of this impact, is required before it can be considered a viable biological control agent. One option for further research would simply be to repeat the experiment with higher densities of *A. hakani*. By increasing the numbers of insects present, we could determine at what densities, if any, the insect would be able to destroy the plant. A follow-up for this study would be to address whether or not *A. hakani* can be naturally maintained at these densities in the field. Another area of potential further study is to examine if the insect's

phloem-feeding is what has actually caused damage to the plant, or whether the damage could be caused by a possible pathogen like *Candidatus Liberibacter europaeus.*

Broader implications

Pre-release efficacy tests offer valuable insights into the suitability of potential biological control agents. In this experiment, I determined that *A. hakani* did not mitigate the *G. monspessulana* to the degree that would make it a valuable biological control agent. While the lack of impact observed offers an interesting field for future study, the main results of the study shows that *A. hakani* is likely to be an ineffective agent, and thus according to the Biological Code of Best Practices for Invasive Weeds, *A. hakani* is not a strong biological control candidate. In such a case where not only the principals of proper biological control, but also temporal and monetary efficiency are at stake, it is only logical to suggest that pre-release efficacy tests be used more ubiquitously across biological control programs.

ACKNOWLEDGMENTS

Special thanks to USDA ARS Research Entomologist Lincoln Smith, for his and continued advice and support in all stages of the experiment, from inception to final draft. Also, thanks to my excellent coworkers at the USDA, Irene Wibawa and Caroline Nunn who made sure that I knew lab procedures and offered advice and support as I conducted my experiment. Thanks to Chris Pavia, for continually peer-reviewing my work, and finally thanks to my peer-review group, including Sage Farrell, Natalie Solares, and Michael Scott, for their feedback on all drafts of this paper.

REFERENCES

Balciunas, J. K. 2000. A proposed Code of Best Practices for classical biological control of weeds. N. R. Spencer (ed.), Proceedings of the X International Symposium for the Biological Control of Weeds. 1999, Bozeman, MT. 435.

Burckhardt, D. Hanson, P.; Madrigal, L. 2005. *Diclidophlebia lucens*, n. sp. (Hemiptera: Psyllidae) from Costa Rica, a potential control agent of *Miconia calvescens (Melastomataceae)* in Hawaii. Proceedings of the Entomological Society of Washington 107(4), 741-749.

Cen, Y., Zhang L. , Yulu, X., Guo J., Deng X., Zhou W., Sequeira R., Gao J., Wang Z., Yue J., and Gao Y. 2012. Detection of '*Candidatus Liberibacter Asiaticus'* in *Cacopsylla* (*Psylla*) *citrisuga* (Hemiptera: Psyllidae). Florida Entomological Society. 95(1), 304-311.

Center, T.D., Pratt, P.D., Tipping, P.W., Rayamajhi, M.B., Van, T.K., Wineriter, S.A., Dray Jr F. Allen, Purcell, M.F., 2006. Field colonization, population growth, and dispersal of Boreioglycaspis melaleucae Moore, a biological control agent of the invasive tree Melaleuca quinquenervia (Cav.) Blake. Biological Control. 39(2), 363–374.

Conrad, K. A., K.,Dhileepan. 2007. Pre-release evaluation of the efficacy of the leaf-sucking bug *carvalhotingis visenda* (heteroptera: Tingidae) as a biological control agent for cat's claw creeper *macfadyena unguis*-cati (bignoniaceae). Biocontrol Science and Technology, 17(3), 303-311.

Franks, S.J.; Kral, A.M.; Pratt, P.D. 2006. Herbivory by introduced insects reduces growth and survival of *Melaleuca quinquenervia* seedlings. Environmental entomology 35 (2), 366-372.

Goolsby, J.A., R. Zonngveid and A. Bourne. 2004. Prerelease Assessment of Impact on Biomass Production of an Invasive Weed, *Lygodium microphyllum* (Lygodiaceae: Pteridophyta), by a Potential Biological Control Agent, *Floracarus perrepae* (Acariformes: Eriophyidae). Environmental Entomology 33 (4), 997-1002

Gottwald, Tim R. 2010. Current Epidemiological Understanding of Citrus Huanglongbing Annual Review of Phytopathology 48(6)

Grevstad F, R. Shaw, R. Bourchier, P. Sanguankeo, G. Cortat, R. C. Reardon. 2013. Efficacy and host specificity compared between two populations of the psyllid *Aphalara itadori*, candidates for biological control of invasive knotweeds in North America. Biological Control. 65, 53-65

Henry K., S. Ivory.,A. L Lush, and J. Kent. 2008. Application for the approval of the release of the Cape Broom Psyllid *Artyinnis hakan*i, a potential biological control agent for Cape Broom *Genista monspessulana*. Final Report Land and Water Australia Project No.

SARDI1/SARD002240

Herrera A.M., R. I. Carruthers, N. J. Mills. 2011. No evidence for increased performance of a specialist psyllid on invasive French broom. Acta Oecologica. 37 79-86

Hodkinson, I. D. 1974. The biology of the Psylloidea (Homoptera): a review. Bull. Entomology. Review. 64(2), 325-339.

Julien, M.H. and M.W. Griffiths1998. Biological control of weeds. A world catalogue of agents and their target weeds. CABI Publishing, Wallingford. 39, 516-524.

Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution, 17(4), 164-170.

Louda, S. M., R. W. Pemberton, M. T. Johnson, and P. A. Follett. 2003. Nontarget effects: the Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. Annual Review of Entomology 48, 365 -396.

McClay, A. S., and J. K. Balciunas. 2005. The role of pre-release efficacy assessment in selecting classical biological control agents for weeds—applying the Anna Karenina principle. Biological Control 35(3), 197-207.

Morath, S.U., P.D., Pratt, C.S., Silvers, , T.D Center. 2006. Herbivory by *Boreioglycaspis melaleucae* (Hemiptera: Psyllidae) accelerates foliar senescence and abscission in the invasive tree *Melaleuca quinquenervia*. Environmental Entomology 35, 1372–1378.

Müller-Schärer, H., U. Schaffner, T. Steinger. 2004. Evolution in invasive plants: implications for biological control. Trends Ecological. Evolution. 19, 417–422

Percy, Diana M., Roderic D.M. Page and Quentin C.B. Cronk. 2004. Plant–Insect Interactions: Double-Dating Associated Insect and Plant Lineages Reveals Asynchronous Radiations. Systems Biology 53 (1), 120-127.

Sheppard A., and T., Thomann Proceedings of the XI International Symposium on Biological Control of Weeds. 2005. Quantitative field surveys for the selection of biological control agents for *Genista monspessulana*, based on host range and efficacy assessment. 60, (7) 133- 137

Shivankar V. J. and C. N. Rao. 2010. Psyllids and their management. Pest Management in Horticultural Ecosystems. 2 (16), 1-4

Vitousek, P.M., D. A.Cala, L., L.,Loope, M., Rejmanek and R., Westbrooks. 2007. Introduced species: a significant component of human-caused global change. New Zealand J. Ecol. 21 1–16

Wink M., The role of quinolizidine alkaloids in plant-insect interactions 1992. E.A. Bernays (Ed.), Insect–Plant Interactions 12(8), 131–166