Chemical defense of *Artemisia frigida* and *Hypericum perforatum* against grasshopper herbivory

BIOS 35503: Practicum in Environmental Field Biology

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Summer 2007

ABSTRACT

Nearly all plants have some form of defense against herbivory and pathogens (Baldwin 1998).

Induced chemical defense response to insect herbivory is very common in plants, occurring in

approximately 110 species in 30 families (Zong and Wang 2006, Baldwin 1998). *Artemisia*

frigida and *Hypericum perforatum* are two species abundant on the National Bison Range in

Western Montana that are known to have inducible chemical defenses against herbivory. The

goal of this study was to answer the following questions: Can the chemical defenses of *H.*

perforatum and *A. frigida* be induced to defend against grasshopper herbivory? Which of the

two species has stronger chemical defenses against grasshopper herbivory? and Does plant-plant

signaling occur between individuals of *H. perforatum* or *A. frigida*? It appears from the results

of laboratory and field feeding trials that inducible responses can be seen in both plant species.

A. frigida appears to have stronger chemical defenses than *H. perforatum*. Also, plant-plant

signaling may occur in *A. frigida* individuals, but not *H. perforatum*. Further studies need to be

conducted on the plant-plant signaling that occurs between *A. frigida* and *H. perforatum*.

KEY WORDS: chemical defense, induced response, plant-plant signaling, grasshopper
herbivory, *Artemisia frigida*, *Hypericum perforatum*, *Melanoplus sanguinipes,* National Bison
Range

INTRODUCTION

Nearly all plants have some form of defense against herbivory and pathogens (Baldwin

1998). Most plants utilize more than one form of defense integrating chemical, physical, and

other defensive strategies which can vary in time, space, and intensity (Agrawal and Rutter

1998). The chemical defenses of plants can be induced or constitutive (constant) (Baldwin 1998,

Sirvent and Gibson 2002, Agrawal 1998). Induced responses are changes in a plant following

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herbivory or infection that lower the preference, performance, or pathogenicity of the attacker on the plant (Agrawal 1999). Induced response to insect herbivory is very common in plants, occurring in approximately 110 species in 30 families (Zong and Wang 2006, Baldwin 1998).

It is a very common belief that induced response to herbivory is a cost-saving measure for plants (Fagerstrom et al. 1987, Clark and Harvell 1992). When the defenses are not utilized, the plant will save energy and nutrient resources, however, empirical evidence for this view is weak (Agrawal 1998). Like any other type of chemical defense, induced chemical responses have costs as well (Baldwin et al. 1994, Baldwin 1998). Chemical defense requires resources to be allocated from other uses such as growth or reproduction (Herms and Mattson 1992). For example, 6% of the nitrogen content of an induced *Nicotiana attenuata* plant is involved in induced toxin production (Baldwin et al. 1994). Also, there may be a time lag between attack and activation of the defense which can leave the plant vulnerable for some time (Baldwin 1998). While the answers provided by cost-benefit analyses of induced defense may appear incomplete, many studies have focused their attention on how plant fitness is directly affected by induced responses to herbivory (Agrawal 1998, Karban et al. 1999). Karban et al. (1997) claim that the variability in induced chemical responses increases the effectiveness of the defense and decreases herbivore performance.

In addition to the local effects of induced responses on one particular leaf or plant, inter-plant and intra-plant signaling of induced responses also occurs (Karban et al. 2000, Preston et al. 2001, Pickett et al. 2003, Frost et al. 2007). When attacked by herbivores, many plants release volatile chemicals into the air which act as signals for neighboring undamaged plants which increase their defenses based on the cue (Picket et al. 2003). These signals may also be important for individual plants to overcome vascular constraints since a volatile signal may get to

far away branches faster than chemicals sent through the vascular system (Frost et al. 2007). Inter-plant signaling has been shown to occur between species such as *Artemisia tridentata* and *Nicotiana attenuata* (wild tobacco) (Karban et al. 2000, Kessler et al. 2006).

Understanding chemical defenses has implications for range management and agriculture. This study chose to examine two plant species with known chemical defenses: *Artemisia frigida* and *Hypericum perforatum.* Both species are very abundant in Western Montana and on the National Bison Range where this study took place. They are also among the few species in the area that had not yet senesced at the time of the study. *A. frigida* is native to the region, while *H. perforatum* is invasive.

Artemisia frigida commonly known as Fringed Sage or Prairie Sagewort is a perennial subshrub in the Asteraceae Family. It is native from Alaska to Mexico and its chemical defenses are studied frequently (Taylor and Lacey 1994). *A. frigida* varies in its palatability to livestock, but it is sometimes eaten by sheep and wildlife and is an indicator of overgrazing (Taylor and Lacey 1994). A member of the same genus, *A. tridentata*, or sagebrush, constitutively emits a mixture of at least 23 different volatile compounds including monoterpenes, sesquiterpene lactones, coumarins and flavonoids (Muller et al. 1964, Personius et al. 1987). Among these compounds is methyl jasmonate, a volatile compound which can induce a number of plant responses (Preston et al. 2001). Damage to the plant increases the absolute quantity of methyl jasmonate released (Preston et al. 2001). The plant-plant communication of *A. tridentata* extends to plants of other species, causing increased herbivore resistance for plants within 10-15 cm of an induced sagebrush plant (Karban et al. 2000). Despite these defenses, there are historical examples of major herbivory occurring on *A. tridentata.* In 1935 a grasshopper plague wiped out

approximately 50 percent of the *A. tridentate* near the Little Powder River in Wyoming and Montana (Allred 1941).

Hypericum perforatum commonly known as St. Johnswort or Klamath weed is a perennial forb native to Europe. It was first discovered in the United States in 1793. It grows well on disturbed sites especially in the West and is considered by many ranchers to be a weed species. In Montana it is listed under the highest threat level - Category 1 – of noxious weed according to the state Noxious Weed List (Pokorny and Sheley 2003). It is an undesirable species for grazing livestock and causes phytochemical reactions in light skinned animals (Pokorny and Sheley 2003). Biological control for *H. perforatum* dates back to the introduction of *Chrysolina* species in 1944 and is among the oldest programs of biological control in North America (Zangerl and Berenbaum 2005). Today, *H. perforatum* is still considered by many to be a problematic species although beetle introductions have been somewhat successful in controlling the problem (Zangerl and Berenbaum 2005).

Despite being a weed, *H. perforatum* can be economically important. Wildcrafters collect the plant for the herbal industry because of its links to treatment for mild depression (Sirvent et al. 2002). The same group of chemicals that makes *H. perforatum* a problem for ranchers also gives it its pharmaceutical benefits. These compounds are known as hypericins and include five compounds: hypericin, pseudohypericin, protohypericin, protopseudohypericin, and cyclopseudohypericin (Sirvent et al. 2002). Like most phototoxins, hypericins, are believed to be involved in the chemical defenses of *H. perforatum.* It has been shown that hypericin levels can be induced by abiotic and biotic elicitors (Sirvent and Gibson 2002).

To test the chemical defenses of these two plants against herbivory on the National Bison Range, I decided to use grasshoppers as the herbivores in this study. Grasshoppers are the major

invertebrate herbivore consuming in total more plant biomass than mammals with the species *Melanoplus sanguinipes* making up 50-70% of all grasshoppers (Belovsky and Slade 2000). *M. sanguinipes* feeds on a mixed diet of grasses and forbs. Introduced weeds tend to act as a steady supply of their food in some regions (Pfadt 1994). These characteristics make *M. sanguinipes* a potential herbivore of *H. perforatum* and *A. frigida* and a good representative herbivore for this study.

The goal of this experiment was to examine the chemical defense of *Artemisia frigida* and *Hypericum perforatum* against grasshopper herbivory. This study focused on the induciblity of these defenses in response to herbivory as well as the communication between neighboring plants and the induction that occurs. This study hopes to answer the questions: Can the chemical defenses of *H. perforatum* and *A. frigida* be induced to defend against grasshopper herbivory? Which species has stronger chemical defenses against grasshopper herbivory? and Does plant-plant signaling occur between individuals of *H. perforatum* or *A. frigida*?

Hypotheses

1. *H. perforatum* and *A. frigida* will both exhibit inducible responses against grasshopper herbivory in field and laboratory studies.

2. Direct induction will be a stronger defense against grasshopper herbivory than plant-plant induction from neighboring damaged plants.

3. *A. frigida* will exhibit within-species plant-plant signaling for chemical defense while *H. perforatum* will not exhibit within-species plant-plant signaling.

4. Damaged *A. frigida* plants will be able to induce chemical defenses in neighboring *H. perforatum* plants through plant-plant signaling.

5. The chemical defenses of *A. frigida* will be stronger at repelling grasshopper herbivory than *H. perforatum*.

METHODS

Defense Induction Treatments

For both field and laboratory tests four defense induction treatments were applied. This could be achieved by 3 different plant setups in the mesocosms (Figure 1). One treatment was "Non-Induced" which included a plant individual that was not clipped and next to an unclipped individual. The next treatment was "Directly-Inducted Only". This was a plant that was clipped with a neighboring plant that was unclipped. The next treatment was "Neighbor-Induced Only". This was an unclipped plant that was next to a clipped plant. The assumption here is that the clipped neighbor may send airborne induction signals to the unclipped plant. The final treatment was "Directly-Induced & Neighbor-Induced". This was a clipped plant that had a clipped neighbor. For this treatment induction effects would not only come directly from the plant itself, but also could be sent as airborne signal.

Field Feeding Trial

In order to examine the chemical defenses of *Artemisia frigida* and *Hypericum perforatum* against grasshopper herbivory, I established and monitored mesocosms on the National Bison Range in July of 2007. During the week of July 1-7, I established thirty-six wire mesh exclusion cages (0.33 m^2 base and approximately 0.50 m high with a triangular peaked top) at the Triangle grasshopper study site on the National Bison Range. I removed all plants from each 0.33 m^2 plot by clipping above ground biomass 5 to 10 cm above the ground. I dug two

holes side by side, 15 cm apart in the middle of the plot in order to add potted plants at a later time. I then installed the exclusion cages over the plot, burying the bottom 10 cm. I drove a 60 cm stake into the ground at each of the four corners of the mesocosm and tied the cage to the stake with wire to stabilize and secure the mesocosm. In order to remove insects not involved in this study, I added a strip of insect tape to the mesocosm until the addition of plants and grasshoppers.

On July 22, I harvested *A. frigida* and *H. perforatum* plants from the National Bison Range adjacent to the Triangle study site. In order to not excessively damage the roots, I kept the roots and attached soil intact and then transferred the plants to pots for transportation. To make biomass measurements of the plants, I brought the plant specimens back to the lab. For both species, I carefully removed soil from the roots and then weighed the whole plant to determine an initial biomass. After these measurements, I repotted the plants with additional soil. On July 24, I transported the plant specimens back to the Triangle study site at the National Bison Range where I added them to the already established mesocosms. In 18 of the mesocosms I added two *H. perforatum* specimens in the pre-dug holes. In the other 18 mesocosms I added two *A. frigida* specimens in the pre-dug holes. The following day I clipped the leaves on specimens of both *A. frigida* and *H. perforatum* in order to induce anti-herbivory responses. When a plant was clipped, I clipped half off of all leaves on 15% of the total number of branches on the plant. For each species, I clipped both plants in six cages, providing eight "Directly-Induced & Neighbor Induced" plants for the experiment and 4 controls for the treatment. I clipped one of the two plants in six cages, providing four "Directly-Induced Only" plants for the experiment and four "Neighbor-Induced Only" plants for the experiment and two controls for each treatment. I clipped neither plant in six cages providing eight "Non-Induced"

plants for the treatment and 4 controls for the treatment. When clipping, I collected all the plant biomass that fell from the plant and brought it back to the lab to weigh. This weight was accounted for in calculating the change in biomass used for analyses.

On July 26, I added 7 *M. sanguinipes* individuals to the treatment cages and added no grasshoppers to the control cages. For the next 4 days (July 27 to July 30), I returned to the Triangle study site each morning (from 800 to 1200 MST) to check the status of the grasshoppers. I recorded the location of each grasshopper (on cage, ground, plant or under pot) and whether it was dead or alive. If a grasshopper was dead I removed it and then replaced it with a new grasshopper. If the location of a grasshopper was unknown then a new grasshopper was added. On July 30, I checked the status and location of the grasshoppers and then ended the feeding period. I removed the potted plants from the cages and transported them back to the lab for biomass measurements. I measured biomass in the same way as before by removing as much soil from the roots as possible without damaging the roots and then weighing the plant.

Laboratory Feeding Trial

In order to examine the chemical defenses of *Artemisia frigida* and *Hypericum perforatum* against grasshopper herbivory in a more controlled setting than the field trials, I conducted laboratory feeding trials from August 1-3. On August 1, I conducted a trial with *H. perforatum.* I clipped both plants in seven feeding containers, providing eight "Directly-Induced & Neighbor Induced" plants for the experiment and six controls for the treatment. I clipped one of the two plants in seven feeding containers, providing four "Directly-Induced Only" plants for the experiment and four "Neighbor-Induced Only" plants for the experiment and three controls for each treatment. I clipped neither plant in seven feeding containers providing eight "Non-

Induced" plants for the treatment and six controls for the treatment. On August 2, I conducted a trial with *A. frigida* with the same treatment setup as *H. perforatum*. On August 3, I conducted a trial with both *A. frigida* and *H. perforatum* mixed. Each feeding container had one specimen of each species. In seven cages I clipped both *H. perforatum* and *A. frigida*. In seven cages I left both *H. perforatum* and *A. frigida* unclipped. In seven feeding containers I clipped *H. perforatum* and left *A. frigida* unclipped. In seven cages left *H. perforatum* unclipped and clipped *A. frigida*. Of each seven manipulations, four served as treatments and three served as controls. I conducted all the feeding trials in 5.7 liter clear plastic rectangular containers with mesh covers. I added 5 *M. sanguinipes* individuals to the treatment containers and no grasshoppers to the control containers.

The day before each lab trial, I harvested whole plants in and near the Triangle study site on the National Bison Range. I potted the whole plants in pots for transport back to the lab. That day I also collected grasshoppers in and near the Triangle study site and starved them over night. One hour before the start of each trial, I randomly selected branches of some plants and clipped half the leaves on them and then marked these branches with flagging. On other plants I randomly selected and marked branches but did not clip them. Then, I clipped all the branches at their base, weighed them for initial biomass, and added them to the feeding trial containers. After both plant samples were in each trial container, I added the grasshoppers. I let the feeding trials run for 12 hours from approximately 900 to 2100 MST. At the end of the trial I removed the grasshoppers and then removed the plant samples and weighed them to determine the final plant biomass.

Herbivory Calculations

For both the in-field and laboratory feeding trials, I measured changes in total plant biomass in grams. In order to standardize for change in biomass due to handling, desiccation, and other factors, I calculated the total plant biomass change minus the average mass loss that was observed for the control of each treatment. I calculated the mean biomass change ratio (control biomass$_{after}$/control biomass$_{before}$) for the control for each of the four treatments. The equation for the herbivory is:

$$(\text{Experimental biomass}_{before} * \text{Mean control biomass change ratio}) - \text{Experimental biomass}_{after}$$

This way the herbivory only included the effects on the plant biomass due to grasshoppers and removed the changes in biomass due to handling, desiccation and other factors. The units for this value would still be grams. In order to make the grasshopper herbivory in lab and field setups comparable, I transformed the data to have the units: grams of plant biomass per individual grasshopper per 12 hour period.

Statistical Analysis

I performed statistical analyses using SYSTAT 9 (Systat Software Inc., Point Richmond, California, USA). In order to see if induction of chemical defenses had an effect on plant biomass loss in the field and laboratory, I performed one-way ANOVAs with grasshopper herbivory as the response variable and induction treatment as the factor for each plant species in the field and in the lab. I also performed a two-way ANOVA with grasshopper herbivory as the response variable and induction treatment and species as variables to see if grasshopper herbivory varied between plant species and if the different plant species responded differently to

the different treatments. In order to be able to compare both plant species for their responses to given treatments I transformed the data to take into account the fact that the biomass loss between each species was significantly different. To do this, I determined the average biomass loss per grasshopper per 12 hour period of the Non-Induced treatment (unclipped next to an unclipped plant) for lab and field trials and species. Then, I divided the average biomass loss per grasshopper per 12 hour period for all four treatments by the average biomass loss per grasshopper per 12 hour period of the Non-Induced treatment. This way, the different species could be compared to how they reacted to treatments even though the plant species lost significantly different amounts of biomass.

RESULTS

Field Feeding Trial

Plant biomass loss was significantly greater for *Hypericum perforatum* than *Artemisia frigida* (two-way ANOVA, $F = 11.329$, $df = 1$, $p = 0.002$) during the four day field trials. Using the transformed data standardized for species, *H. perforatum* and *A. frigida* had significantly different plant biomass losses in response to different defense induction treatments (two-way ANOVA, $F = 18.555$, $df = 3$, $p < 0.0001$) (Figures 1 and 2). For *H. perforatum*, the Neighbor-Induced Only treatment had the greatest loss in plant biomass with an average of 0.020 ± 0.0031 g/grasshopper/12 hours and was significantly greater than all of the other defense induction treatments (one-way ANOVA, Bonferroni Post-hoc test, $p < 0.0001$). The three other treatments had plant biomass losses that were less than zero or equivalent to zero when considering standard error (Figure 2). While not significantly different, plant biomass loss in the Non-Induced treatment (0.0013 ± 0.0018 g/grasshopper/12 hours) was biologically greater than the plant

biomass loss in the Directly-Induced & Neighbor-Induced treatment (-0.0059 ± 0.0017

g/grasshopper/12 hours) (one-way ANOVA, Bonferroni Post-hoc test, p = 0.076). For *A. frigida*,

plant biomass loss was not significantly different for any of the defense induction treatments

(one-way ANOVA, F = 1.530, df = 3, p = 0.238). In fact, none of the treatments had positive

plant biomass losses when considering standard error (Figure 3).

Grasshoppers in mesocosms with *A. frigida* had higher daily mortality than those with *H.*

perforatum with mortality reaching over 25% between day 2 and day 3. Grasshoppers in *H.*

perforatum mesocosms had mortality rates of about 19% during the same period from day 2 to

day 3 (Figure 4).

Laboratory Feeding Trial

Plant biomass loss was significantly greater for *H. perforatum* than *A. frigida* (two-way

ANOVA, F = 8.782, df = 1, p = 0.005) during the 12 hour laboratory trials. Using the

transformed data standardized for different species, *H. perforatum* and *A. frigida* had

significantly different plant biomass losses in response to different defense induction treatments

(two-way ANOVA, F = 2.995, df = 3, p = 0.042). For *H. perforatum*, plant biomass loss was not

significantly different for any of the defense induction treatments (one-way ANOVA, F = 0.631,

df = 3, p = 0.603). However, three of the treatments (Directly-Induced & Neighbor-Induced,

Non-Induced, and Neighbor-Induced) had positive values for plant biomass loss (Figure 5). For

A. frigida, there may be a biologically significant trend for greater plant biomass loss in some

defense induction treatments than others (one-way ANOVA, F = 2.667, df = 3, p = 0.075). The

Directly-Induced Only treatment had more plant biomass loss (0.0020 ± 0.0017 g/grasshopper/12

hr.) than the Directly-Induced & Neighbor-Induced treatment (-0.0018 ± 0.00070

g/grasshopper/12 hr.) (Figure 6).

For the mixed lab trial, plant biomass loss was not statistically different between either

species or any of the defense induction treatments.

DISCUSSION

It appears from this study that the chemical defenses against grasshopper herbivory are

stronger for *A. frigida* than *H. perforatum.* In both laboratory and field trials, grasshopper

herbivory was greater on *Hypericum perforatum* than *Artemisia frigida.* Also, in the field trials,

grasshopper mortality rates were greater in mesocosms with *A. frigida* than *H. perforatum.* No

previous scientific studies have compared the chemical defenses of these two species, so further

studies to confirm these results should be conducted. Still, this finding may have larger

ecosystem impacts in the fields of range management, agriculture and invasive species

management.

From this study, I may be able to conclude that *H. perforatum* exhibits induced response.

This is supported by the fact that grasshoppers avoided directly-induced plants in the field trial.

For *H. perforatum* in field trials*,* grasshopper herbivory was greater on the Neighbor-Induced

treatment than any other treatment. It might seem logical that the Non-Induced treatment would

experience greater herbivory than the Neighbor-Induced treatment, however, this was not

observed. For the Neighbor-Induced treatment, unclipped *H. perforatum* specimens were placed

near clipped *H. perforatum* specimens in the same container. If the clipped *H. perforatum*

specimen did not send chemical signals to the unclipped specimen, then the grasshoppers would

be faced with a feeding choice between the un-induced unclipped specimen and the induced

clipped specimen. It would seem logical that the grasshoppers would feed much more on the

unclipped specimen since its defenses would not be induced. On the other hand in the Non-

Induced treatment, two unclipped *H. perforatum* specimens were placed nearby in the same

container. Since the chemical defenses would not be induced in either specimen than

grasshoppers should have an equal preference for feeding on each specimen and the grasshopper

herbivory pressure would be divided in half between the two. Also in field trials with *H.

perforatum,* there appeared to be a preference for grasshoppers to feed on the Non-Induced

treatment over the Directly-Induced & Neighbor Induced treatment. This result supports other

studies that show that chemical defenses against herbivory are inducible in *H. perforatum*

(Sirvent and Gibson 2002, Kirakosyan et al. 2004). However, induced response in *H.

perforatum* was not observed in the laboratory feeding trials since grasshopper preference for the

Neighbor-Induced treatment was not seen.

For both laboratory and field trials, grasshopper herbivory on *A. frigida* appears to have

been very low. In the field trials, no herbivory was detectable in any defense induction

treatment. For the laboratory trials, only the Directly-Induced treatment had a positive value for

grasshopper herbivory and this value was close to zero. From this I can conclude that

grasshopper feeding on *A. frigida* is very low, but since none of the values are statistically

different from each other, no conclusions can be made about the induciblity of chemical defenses

in *A. frigida* or its plant-plant signaling. If future studies were to increase the grasshopper

herbivory levels than differences between defense-induction treatments may be observed.

It would have been useful to include a species that is known to have no chemical defenses

against herbivory for comparison to *H. perforatum* and *A. frigida*. An undefended species would

provide a baseline for overall grasshopper herbivory. This would help determine if grasshoppers

were choosing to feed or not feed depending on the chemical defenses of the plants and not some other variable in the experimental design. Also, grasshopper mortality in the field trials could then be attributed to starvation or flaws in the setup of the mesocosms if grasshopper mortality occurred despite being fed a desirable plant.

The one mixed lab feeding trial that examined *A. frigida* and *H. perforatum* defense induction on each other could be expanded in future studies. A field version of this experiment could test inter-species inter-plant signaling occurs between *A. frigida* and *H. perforatum*. Also, the lab feeding trial could be repeated with more replication since this trial only had a sample size of 4 for each treatment. There is evidence in the literature that *A. frigida* may be able to signal to *H. perforatum* to prime its defenses against herbivore attack. Sirevent and Gibson (2002) showed that methyl jasmonate can elicit increase hypericin production in *H. perforatum*.

Understanding how induced response and plant-plant signaling work in *A. frigida* and *H. perforatum* is important to gaining a better understanding of plant defenses against herbivory in general. A better knowledge of chemical defenses will help in agriculture to set a threshold under which pest levels must be kept in order to avoid losses to economically important plants (Agrawal 1999). Manipulating induced response may be a technique used by farmers to increase yields of their crops (Agrawal 1999). Understanding chemical defenses also helps in better management of invasive species (Zangerl and Berenbaum 2005). For invasive species with chemical defenses such as *H. perforatum* a well-designed biological control program must take into account the plant's resistance to herbivory.

ACKNOWLEDGEMENTS

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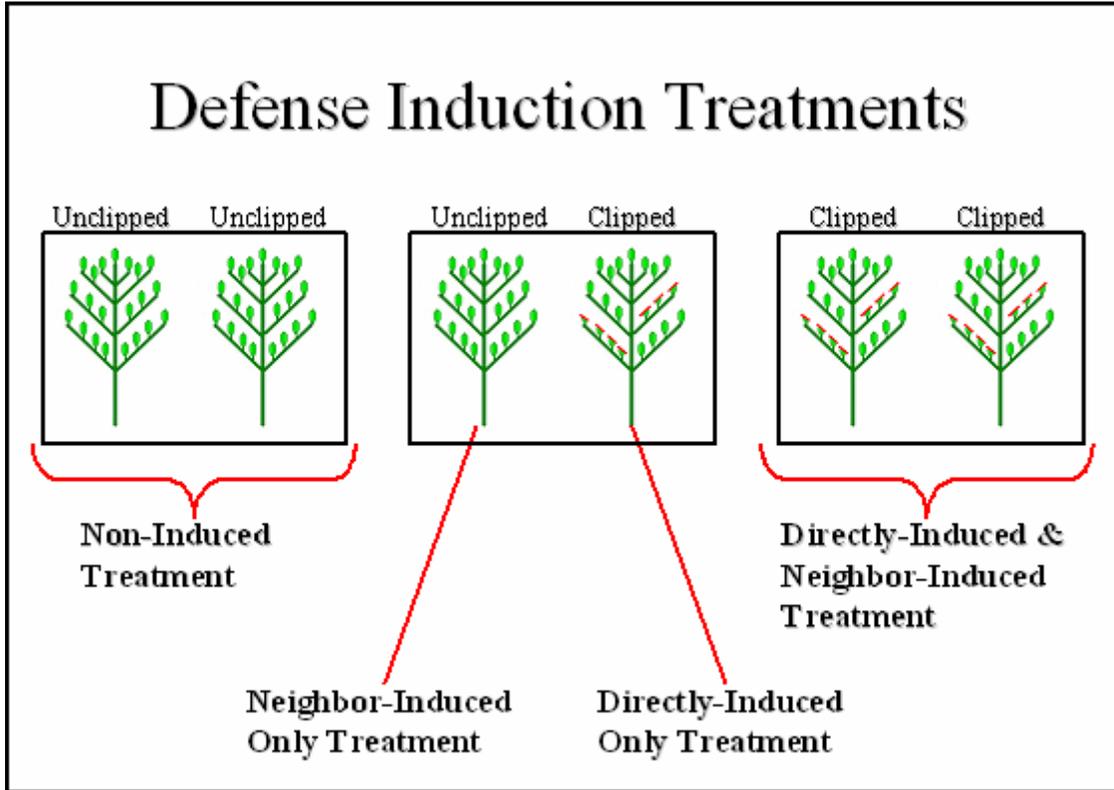
Figure 1. Diagram of the three mesocosm setups and the four induction treatments that result.
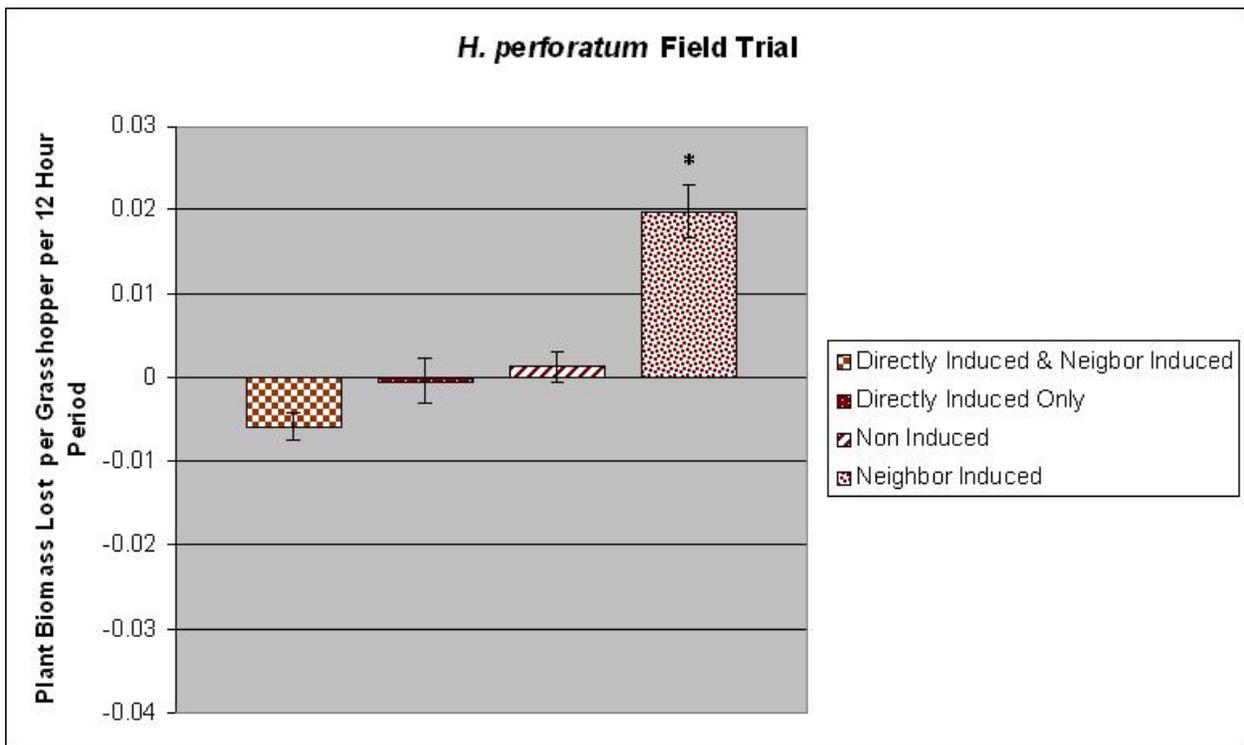
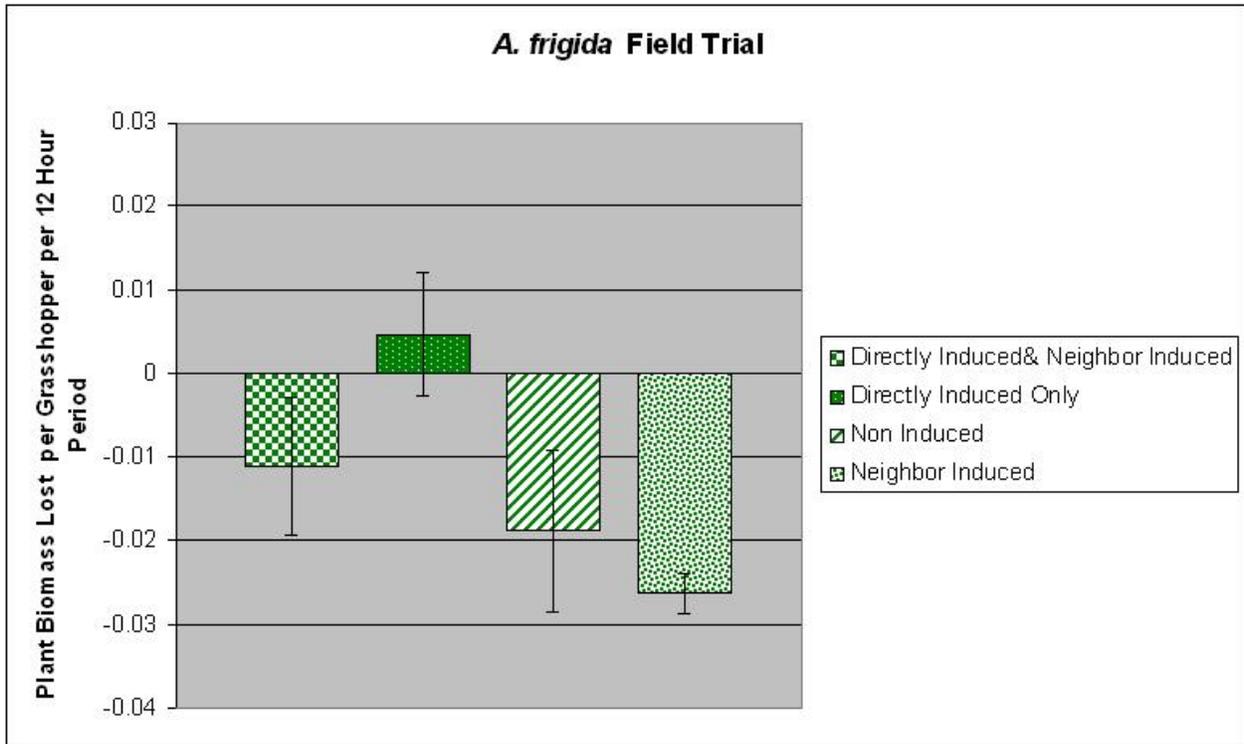**Figure 2.** Grasshopper herbivory in 4 day *H. perforatum* field trials (F = 21.862, df = 3, p < 0.0001)

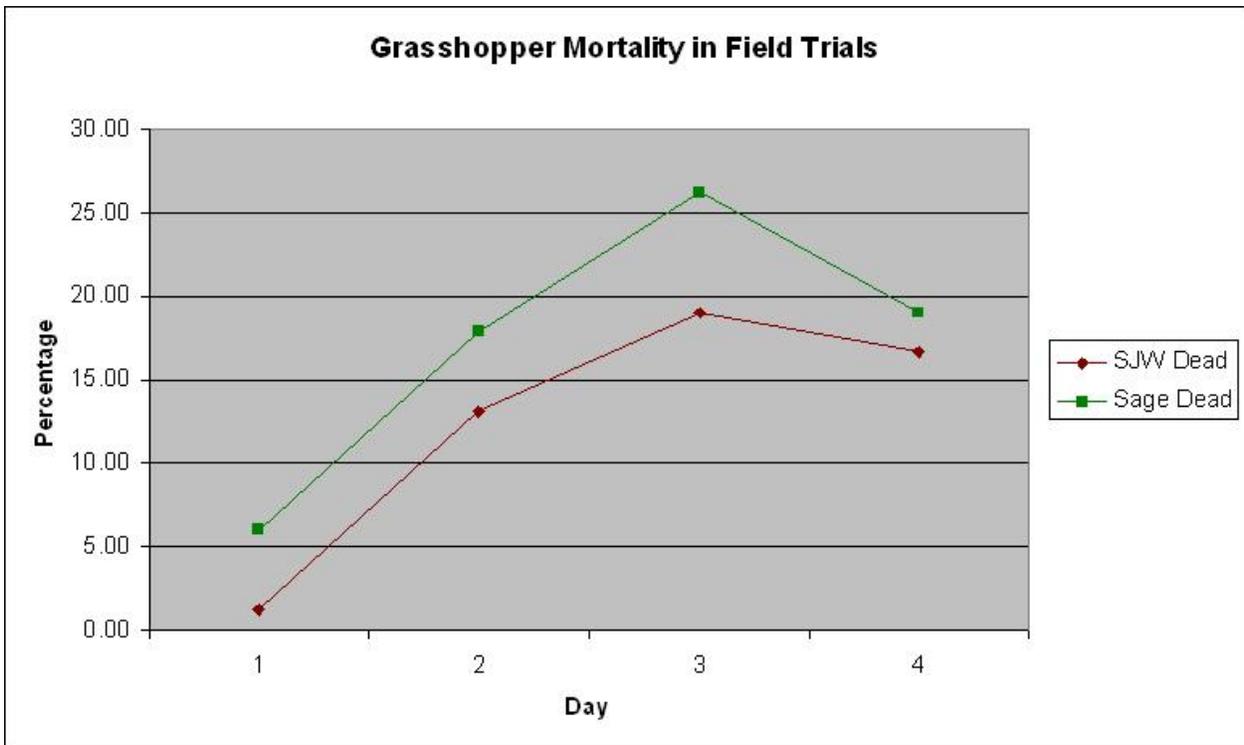**Figure 3.** Grasshopper herbivory in 4 day *A. frigida* field trials (F = 1.530, df = 3, p = 0.238).

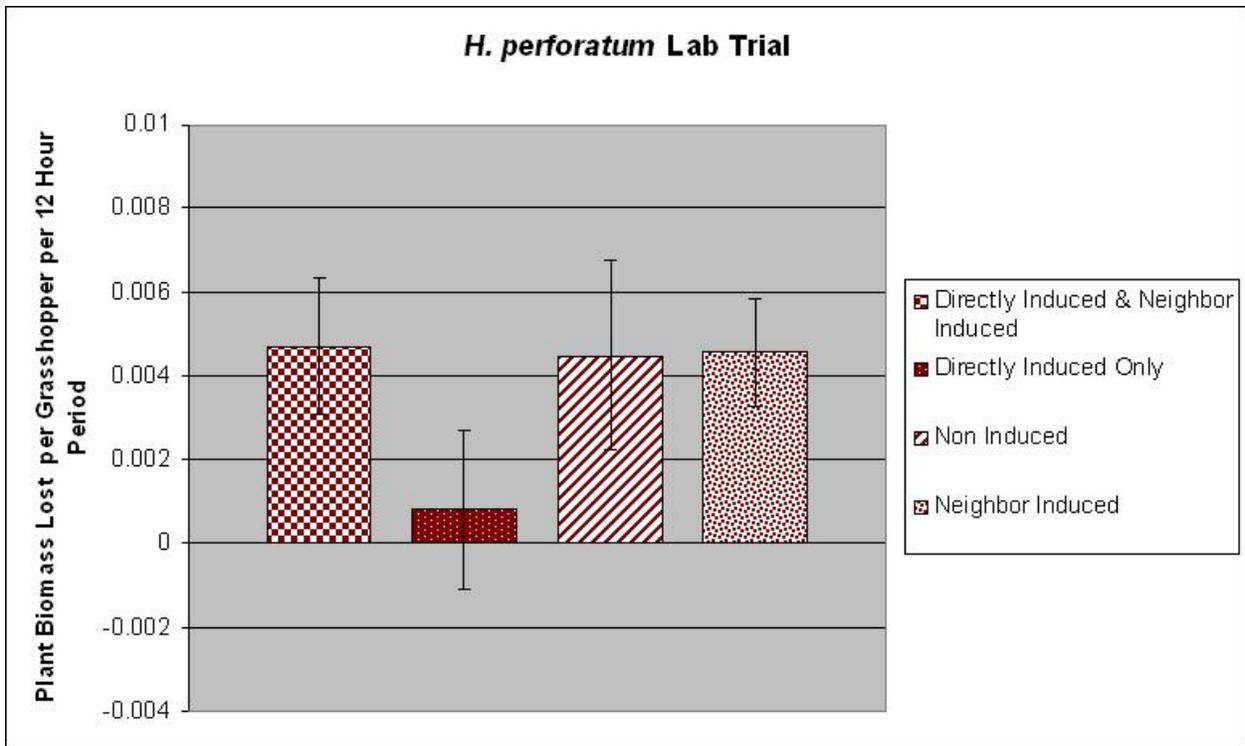**Figure 4.** Grasshopper mortality in 4 day field trials for all treatments.

Figure 5. Grasshopper herbivory in 12 hour *H. perforatum* laboratory trials (F = 0.631, df = 3. p = 0.603).
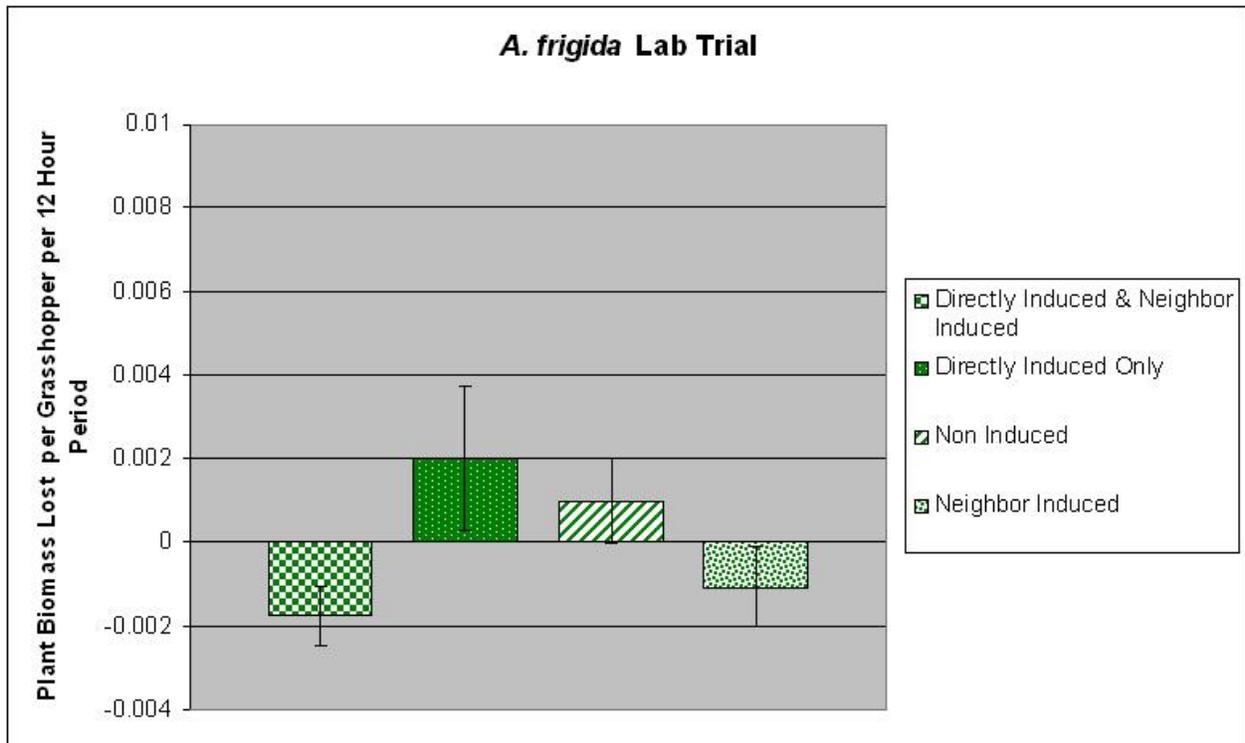

Figure 6. Grasshopper herbivory in 12 hour *A. frigida* laboratory trials (F = 2.677, df = 3, p = 0.075).