

Clonal integration of induced resistance to herbivory damage in *Chamerion angustifolium*

Hannah Carey
Structured Undergraduate Research Program
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hcarey@stedwards.edu

Amanda Buchanan
Mentor
Amanda.buchanan@bio.fsu.edu

ABSTRACT

A plants ability to resist high levels of herbivory may increase performance and fitness, then be favored by natural selection (Agrawal et al 1998, Karban 1993). The objectives of this study were to find if *Chamerion angustifolium*, Fireweed, develops an induced resistance to herbivory damage and if itclonally integrates this defense. *Adoxus obscurus*, a specialist beetle to *C. angustifolium*, was the selected herbivore to damage leaf tissue in this experiment. *A. obscurus* presented unpredictable and irregular levels of herbivory damage. In this experiment 60 different genets were selected at three different temporal blocks. The results showed no significant difference between the controlled and damaged genets in terms of leaf surface area and beetle weight loss. *C. angustifolium* may potentially have the ability to tolerate the higher levels of herbivory present in this experiment.

INTRODUCTION

The loss of tissue from herbivory damage places biotic pressures and stress on plants. Increasing resistance to herbivores allows plants to improve fitness and increase performance (Agrawal et al 1998, Karban 1993). However, plant resistance to herbivory can also elevate negative costs because defense can be energy intensive (Agrawal 1998). Plants that develop induced resistance to herbivory damage may be favored by natural selection in environments with large populations of herbivores, but the benefits of this defensive mechanism may be outweighed by costs in areas at low risk for herbivory (Agrawal et al 1998).

Plant defense theory says that certain conditions must initiate a plant to defend itself in the form of induced resistance. The attack is unpredictable and not fatal to the plant. This experiment places an irregular level of herbivory attack upon a single ramet to test if the entire genet reacts. Also, defense is costly to the plant in terms of performance and fitness. If *Chamerion angustifolium* does not show any clonal integration of inducible defense, it could be the possibility that the defense is too costly to the entire genet. When herbivores are abundant amongst the plant, those plants that have induced defense are more likely to have increased growth and reproduction over the plants lacking the resistance (Karban 1992). This could show a possible evolutionary trend amongst the clonal plant, *C. angustifolium*. If the benefits of induced defense significantly outweigh costs, natural selection will favor plants with the ability to defend (Agrawal et al 1998).

Plants may respond to biotic stresses by adaptation in either the form of resistance or tolerance (Agrawal et al 2004). Resistance reduces stress by creating a defense. Tolerance allows for there to be minimal negative implications to plant fitness while herbivory damage is occurring (Agrawal et al 2004). This experiments tests whether or not *C. angustifolium* induces herbivory resistance. However, the plant could ultimately tolerate the higher levels of herbivory present in this experiment.

As might be expected, theory on plant induced resistance show that the defenses fluctuate temporarily and spatially (Agrawal et al 1998). The costs of resistance may explain the reasons for fluctuating induced defenses. The presence and absence of herbivores influences the defense expression (Rhoades 1979). Such costs may consist of the energy input to gain resistance or that the defenses to a specific herbivore may bring its predators and parasites (Agrawal et al 1998). The cost of defense could ultimately lead to inhibiting another factor necessary for plant fitness.

The physical connection of ramets within a clonal plant allows for the potential to communicate and share herbivory defenses. If undamaged ramets of clonal plants do not induce resistance when another ramet is damaged, it may be explained by either the cost of resistance to herbivory or a lack physiological connection between ramets of the same genet. If one ramet is damaged, the warning signal may be sent out into the entire genet preventing further herbivory damage (Stuefer 2005). If the clonal plant is able to transmit the induced resistance to the entire genet, the plant will be able to see maximum benefits.

If the costs of induction is low to the plant and if clonal integration is high, then ramets in addition to the damaged ramet are more likely to induce resistance. However, if the costs of defense are high and if clonal integration is low, only the damaged ramet or near ramets are likely to induce resistance to herbivory. I examine the spatial patterns of induced resistance in a clonally

integrated plant to ask if plants induce resistance to herbivory damage and if the distance from the damaged ramet influences levels of resistance. If *Chamerion angustifolium* induces resistance and the resistance is beneficial to the genet, then each ramet will show the resistance.

In this experiment, I tested for induced resistance in *Chamerion angustifolium* to examine patterns of herbivory defense in clonally integrated plants. I ask the two questions about herbivory resistance in *C. angustifolium*, (1) does *C. angustifolium*, induce resistance to specialist beetle, *Adoxus obscurus*, herbivory damage, and (2) does damage confer resistance to undamaged ramets to the damaged genets.

METHODS

Natural History of *Chamerion angustifolium*

C. angustifolium, commonly known as fireweed, is a clonal herbaceous perennial plant, which frequently colonizes damaged areas such as those occurring after forest fire or avalanche (Myerscough 1980). A clonal plant's ramets grow from a single rhizome. *Chamerion angustifolium* often grows densely in identifiable clumps to determine the rhizome of origin for the ramets. *Adoxus obscurus* is a specialist beetle that feeds on fireweed leaving long striations of leaves. The beetle is often observed on top leaves of ramets and is present throughout *C. angustifolium* growing season.

Experimental Design

To test whether induced herbivory resistance occurs, 60 *C. angustifolium* plants with ten or more ramets were selected haphazardly. Damage treatments were imposed in three temporal blocks of 20 plants occurred over a 4-week period. Fifteen plants were selected for manipulation of herbivory damage and five were selected as control for each of the three blocks.

A. obscurus individuals were caught and maintained for about 6 hours with minimal food available for a period of starvation. Three to four individuals were netted to the top 10 centimeters of a single ramet of each experimental plant. These individuals were left on the plant an average of 5 days until about forty-percent of the 10 cm of ramet was damaged. To ensure this damage occurred, more *A. obscurus* individuals are added into the nets or they remained netted for a longer duration of time.

After sufficient damage occurred to the single ramet, three leaves from the genet were selected to conduct a bioassay. One leaf from the damaged ramet and from ramets five and fifteen away from the damaged ramet were collected. New *A. obscurus* individuals were collected for the bioassay, and one individual was placed with each leaf for 36 hours. The initial and final weight of the *A. obscurus* and the initial surface area of the leaves were measured.

Data were analyzed with an ANOVA (JMP program) to test the differences in bioassay beetle weight among four predictor classes: damaged, 5cm, 15cm, and control, and a separate ANOVA to test the differences in bioassay leaf area.

RESULTS

Figure 1

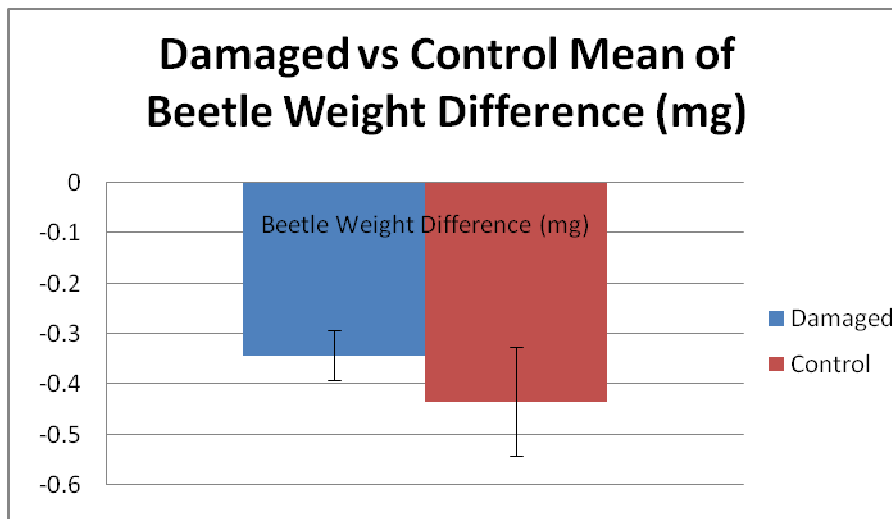


Figure 2

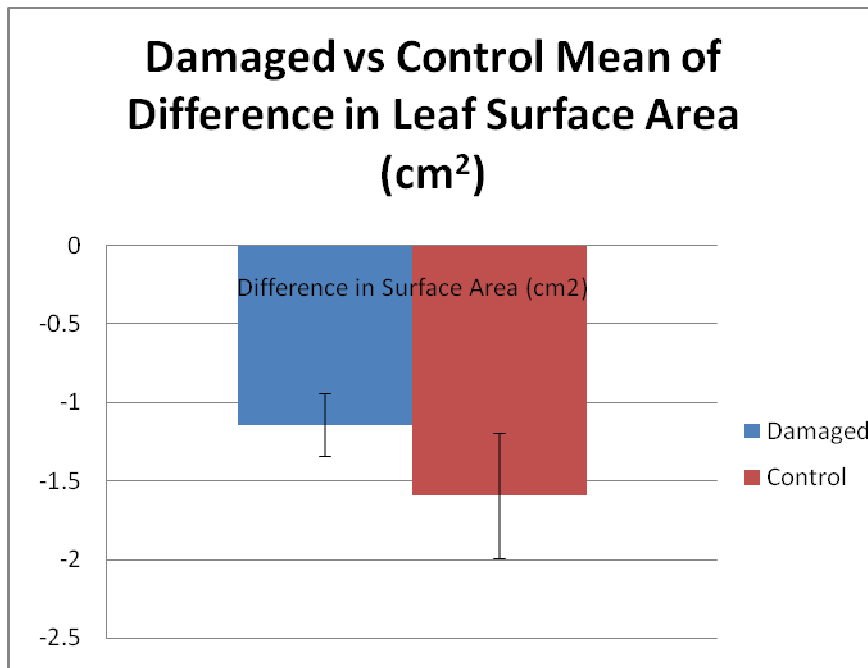


Figure 3

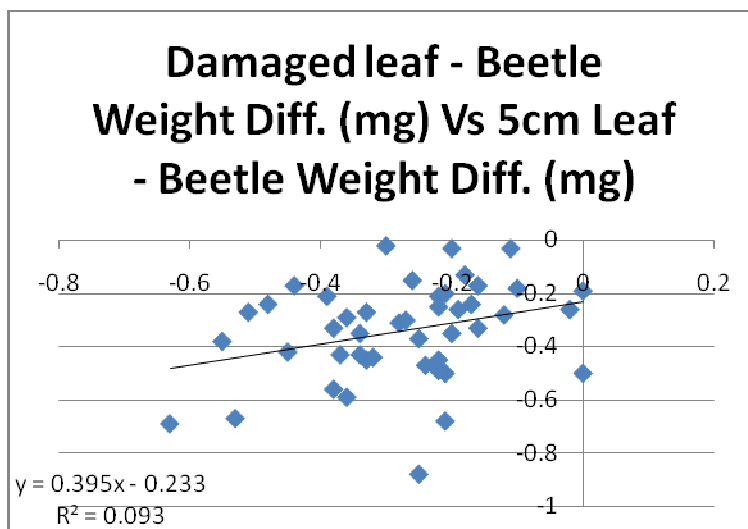


Figure 4

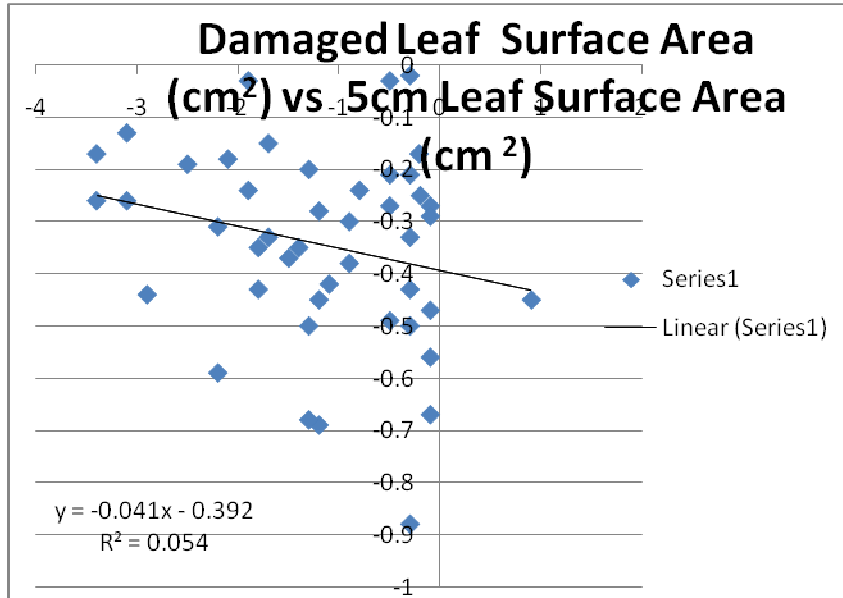


Figure 5

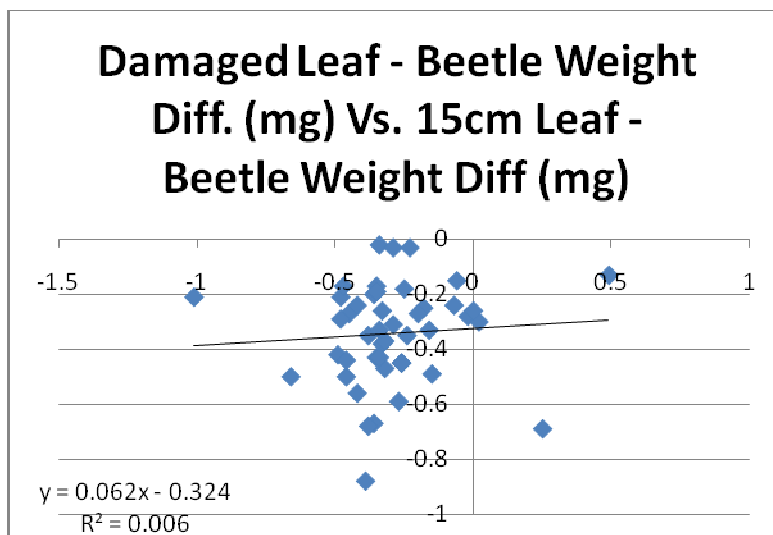
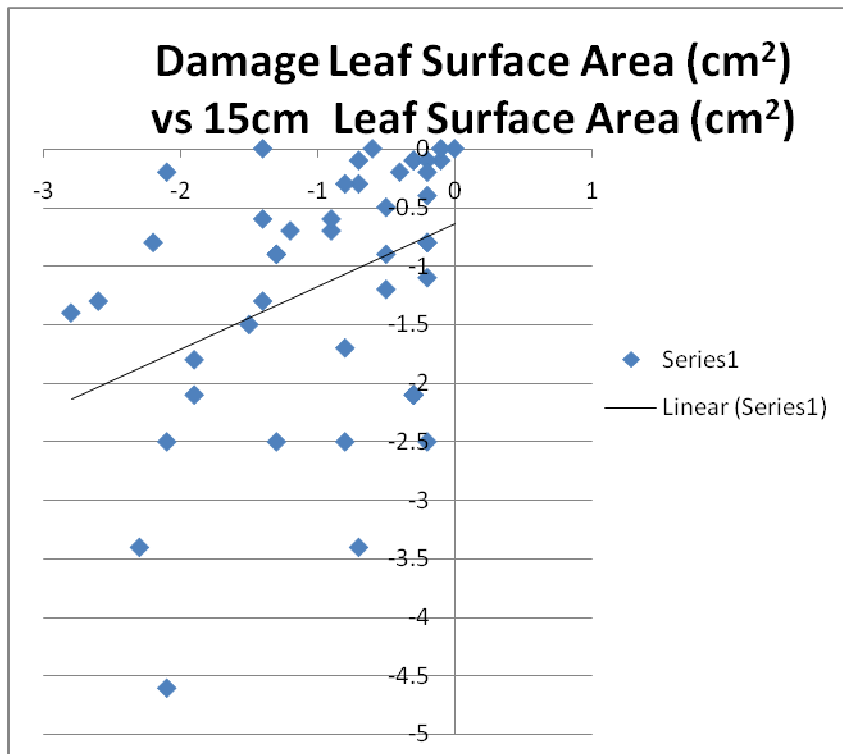


Figure 6



To compared the damaged and control treatments, a t-test was used.

Figure 1 is a graph of *A. obscurus* weight difference from before and after the bioassay. The standard error was 0.1079. The confidence level used was 0.95.

Figure 2 shows a graph of control versus damage in terms of difference in leaf surface area. The standard error was 0.3971. The confidence level used was 0.95.

Figure 3 shows a relationship of the damaged ramets to the ramets 5cm away in terms of beetle weight difference. An ANOVA was used to calculate this relationship. The p-value was 0.0417 and a RSquare value of 0.092. Figure 4 also shows the relationship of the damaged ramets to the 5cm away ramets in

terms of difference in surface area. The ANOVA test showed a p-value of $<.0001$ and a RSquare value of 0.342.

Figure 5 shows the relationship of the damaged ramets to ramets 15cm away in terms of difference in beetle weight. An ANOVA test calculated an RSquare value of 0.0059 and a p-value of 0.6144. Figure 6 shows the relationship of the damaged ramets to the ramets 15cm away from the damaged in terms of leaf surface area. An ANOVA test calculated a p-value of 0.0092 and an RSquare value of 0.1476.

DISCUSSION

The results show no significant difference between the damaged and controlled *C. angustifolium* to support evidence of induced resistance to herbivory damage. However, significant results were derived from testing the relationships between different ramets of same plants. Damaged ramets to ramets 5cm away from the damaged plant in terms of beetle weight showed slight significance with a p-value of 0.041. Also, damaged ramets to ramets 15cm away from the damaged ramet showed significance in terms of surface area with a p-value 0.0092. These results ultimately do not confer clonal integration of an induced resistance.

The lack of a difference between the damaged and control treatments could possibly be explained by *C. angustifolium* tolerance to *A. obscurus* damage to leaf tissue. *A. obscurus* specialist relationship to *C. angustifolium* could explain an evolutionary trend for genets to tolerate the seasonal damage caused

by the beetle. Introducing an unfamiliar herbivore that presents new forms of damage to *C. angustifolium* could potentially force the plant to react differently.

The genets used in the experiment face regular levels of herbivory damage while also having one ramet subject to dramatically increased herbivory damage. Completely eliminating all types of herbivory to the genets could pose a different reaction from the plants. However, this experimental design would not depict a real-life occurrence for *C. angustifolium*.

However, understanding the relationship of plant induced defenses and herbivory helps identify the dynamic between plants and the herbivores feeding from the plant. Herbivores play a critical role in plant cycles. The process of plant defenses can eventually create diversification of a plant species and the herbivores attacking the plant if there is a “counteradaptation” (Stamp 2003). There are also conservation implications, if a plant is unable to become resistant to herbivory damage and other factors, and, biodiversity can be harmed. If clonal plants can use integration to transmit herbivory defense, then the fitness of the clonal plant will increase.

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