Resource allocation shifts in response to herbivory and the consequences for tolerance in *Solanum lycopersicum*

A Senior Honors Thesis in the Department of Biology, Tufts University

Timothy Korpita, Class of 2012

Dr. Colin Orians

Table of Contents

Abstract

Herbivory is a major stress to which plants have adapted a number of strategies designed to mitigate its negative consequences. There is increasing evidence that one of these strategies, tolerance, or the ability to recover from the herbivory event, can be affected by a shift in resource allocation patterns induced by herbivory. Based on the evidence for this induced resource shift, a model was proposed that attempts to explain the mechanisms behind it and its adaptive significance. The model predicts that in response to herbivory, resources will be remobilized from attacked tissues to storage in herbivore inaccessible tissues, and these stored resources will be utilized later in regrowth of the lost and damaged tissues, ultimately increasing tolerance. The goal of this thesis was to characterize and quantify this resource allocation shift, confirm experimentally the predicted increase in tolerance and mechanisms behind it, and provide directions for future study. The data presented in chapter 2 demonstrate a resource shift and a subsequent increase in tolerance. The data presented in chapter 3, show that there is not a net removal of resources from herbivore accessible tissues as predicted by the induced sequestration model. Based on these results, and evidence from the literature, we refine and expand the induced sequestration model in chapter 4, as well as suggest avenues for further investigation.

Acknowledgments

There are a great number of people who deserve thanks for their part in helping me complete this thesis: first and foremost, my thesis committee, Dr. Colin Orians, Dr. George Ellmore, and Dr. Sara Gomez, for perpetual support and guidance throughout the process. I would also like to thank Dr. Stephen Fuchs for help and supplies in my attempt to find the hypothesized vegetative storage protein, and Dr. Mitch McVey and Dr. Juliet Fuhrman for their molecular biology advice and expertise given to the same end. I thank Michael Grossi for his continual logistical support for many years over my Tufts career. I may not have been able to collect the quantity of data that I did were it not for the assistance of Michael Rogove. I should also thank the many members of the Orians lab that contributed to this project at some point: Colleen Butler, Minda Berbeco, Alexandra Thorn, Adam Steinbrenner, Sarabeth Buckley, Nicole Soltis, Selena Ahmed, and Tegan Morton, who along with Anne Madden and Nooria Al-Wathiqui, deserves additional thanks for keeping Barnum always entertaining. I should also thank the Trimmer Laboratory, in particular Dr. William Woods, for the continual supply of *Manduca sexta* larvae from which I collected way more caterpillar regurgitant than any person should in a lifetime. Finally, I would like to thanks those friends and family outside of the Biology department who came in to help with experimental harvests during "crunch time": Daniel Korpita, Rajesh Reddy, and Andrew Parker. Thank You all.

Chapter 1: Introduction

Induced Sequestration: Current Knowledge and Model Predictions

Plants are not entirely passive organisms that sit idly by and take whatever their environment throws at them. They interact with their environment, and the stresses and heterogeneity found within it, and modify their form and features so that interaction is as productive as possible in terms of reproductive output (Sultan, 2000). This phenotypic plasticity, as it is called, is an essential feature of many forms of life, but is of particular importance for sessile organisms like plants who cannot respond to their environment behaviorally.

Examples of this phenotypic plasticity, when they are the result of environmental stresses, are generally characterized as "responses". The plant detects the stress, and then responds by modifying its phenotype to mitigate the negative effects of the stress. One of the significant stresses a plant faces is herbivory, and plants respond to this stress in a number of ways, such as the production of defense compounds (Karban and Baldwin, 1997). By responding with the production of defense compounds only when an herbivore is detected, rather than producing them constitutively, the plant saves valuable resources and can apply them to other functions (Agarwal and Karban 1999). Another response to herbivory, for which there is increasing evidence, is an inducted shift in within plant resource allocation pattern (Orians et al., 2011).

Schwachtje et al. 2006 demonstrated that simulated herbivory increases carbon allocation to the roots in *Nicotina attenuata.* Other studies that also used radiolabeling showed similar effects in different systems. Gomez et al., 2010 showed an increased export of recently assimilated carbon and nitrogen from tomato leaves that were treated with methyl jasmonate, a damage cue in plants often used to simulate herbivory. Babst et al., 2005 and Babst et al., 2008

showed that herbivory and simulated herbivory also increased carbon partitioning to the stem and roots in *Populus tremuloides,* as well as decreasing the starch concentration of the leaves. It has also been demonstrated in tomato that *Manduca sexta* (a specialist herbivore) herbivory increases the concentration of amino acids associated with carbon and nitrogen transport (Steinbrenner et al., 2011).

These results led to the development of a model that describes this phenomenon and explains its adaptive significance to plants (Orians et al., 2011). The hypothesis is that shift in resource allocation will allow the plant to better tolerate the herbivory. Tolerance, or the ability to recover from a stress, is thought to be somewhat dependent on the resources a plant has stored that can be mobilized for recovery (Tiffin, 2000). The idea behind induced sequestration is that the plant responds to herbivory by upregulating storage in organs that are not accessible to the herbivore attacking it. Attack by a leaf chewing insect (or a treatment that simulates such an attack), should, according to the model, result in an increase in storage in the stem and roots. It follows that there should be a decrease in the allocation of resources to those tissues, such as the apex and leaves, that are vulnerable in order to support this increase in storage. According to the model, these storage reserves should be mobilized to support the regrowth of leaf area once the herbivore has moved on. The assumption for regrowth is a simple one: more stored reserves will result in increased regrowth capacity means increased fitness. The resources relevant to the model will vary depending on the specific situation, though both carbon based and nitrogenous resource allocation patterns have been documented to be affected by herbivory (Babst et al., 2008, Gomez et al., 2010). Our preliminary results suggest that nitrogen may be more relevant in our system, with significant changes in protein concentrations in multiple tissues where there

were no significant changes in starch or soluble sugar in response to simulated herbivory (Korpita, unpublished data).

Many of these predictions remain unsupported or poorly supported by experimental evidence, specifically the hypothesized remobilization of stored resources and improved tolerance. The best documented component of the model is the movement of resources out of the accessible tissues during an herbivory event. The accumulation of resources in storage organs is not well verified, and the resources may just as easily be converted into some other form (defense compounds, for example) and be sent back into the herbivore accessible tissues. There is also limited evidence for the notion that these resource allocation shifts increase tolerance, although data from the Schwachtje et al., 2006 paper were interpreted to support it indirectly.

The goal of this thesis was to examine some of these predictions of the induced sequestration model. We designed experiments that allowed us to A) determine if herbivory results in a net export of resources from vulnerable tissues, B) determine if resources accumulated in herbivore inaccessible tissues, and C) if the resources allocation shift induced by herbivory results in increased tolerance of the herbivory. Based on the results of these experiments, we refine the induced sequestration model, and provide extensive suggestions for further inquiry in the conclusion.

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Chapter 2: Data Chapter 1

Simulated Herbivory Results In Increased Tolerance of a Defoliation Event

Introduction:

Plants experience a variety of stresses, biotic and abiotic, and have evolved adaptations that allow them to both avoid these stresses and to recover from them once they have occurred. One of the major stresses plants face is that of herbivory. Plants can respond to herbivory in a number of ways. For example, they can produce defense compounds that have a direct negative effect on the herbivore (Karban and Baldwin, 1997). Plants are also capable of tolerating herbivory, recovering after the herbivore has moved on (Tiffin, 2000). Tolerance may be a particularly important strategy in situation where an herbivore has evolved the ability to detoxify the plant's chemical defenses (Strauss and Agrawal, 1999), such as is the case with *Manduca sexta* and *Nicotiana attenuata* (Wink and Theile, 2002).

Mechanistically, a plant's capacity to tolerate damage is thought to dependent in part on the stored resources of the plant. Plants with more storage are more tolerant (Van der Meijden, 1988). Interestingly, there is increasing evidence that the levels of stored resources in the plant, like the levels of defense compounds, can be induced by herbivory. Holland et al. (1996) found that herbivory resulted in increased accumulation of carbon isotopes in the roots of grasses. Babst et al. (2008) showed that Gypsy Moth herbivory on *Populus* resulted in the increased export of carbon from leaves. Schwatchje et al., 2006 showed an increase in carbon allocated to the roots, as well as a decrease in soluble sugar concentration in sink leaves after simulated herbivory treatments in tobacco. Babst et al., (2005) showed a decrease in starch concentrations in methyl jasmonate treated leaves of *Populus*. Henkes et al (2008) showed that jasmonic acid treatment of roots also modified the way they partition carbon. These studies all show a shift in resource allocation in response to the real or simulated herbivory event, in a way that moves

them away from tissues the plant recognizes as in danger. It is hypothesized that this shift in resources allows the increased levels of storage necessary to tolerate the herbivory event (Orians et al. 2011). The idea is that the resources that would have been consumed by the herbivore had they not been removed from vulnerable tissues will instead be stored temporarily, and then used to regrow the lost tissues. Most studies to date have focused on documenting the shift in carbon allocation, but considering the often limiting nature of nitrogen in the environment, it is not surprising that shifts in nitrogenous resources have also been documented (Gomez et al. 2010; Newingham et al., 2007). This shift in resource allocation could potentially have negative effects on the growth and development of the plant, if it were taking resources away from them for storage. Alternatively, in a carbon limiting situation, the plant could increase photosynthesis to compensate for the carbon being shifted to storage. This change in photosynthesis rates in response to damage has been documented in a number of systems (reviewed in Welter, 1989).

Although the case for an herbivore induced shift in resources is relatively strong, it is less clear if those shifts in resources are just transiently stored and then used to regenerate lost tissues. It is possible that those resources are converted for some defense purpose, are used to increase the photosynthetic rate of a less photosynthetically active tissue such as the stem, are stored more permanently, or some combination of the above. Schwatje et al., 2006 addressed this question experimentally, and they demonstrated that *N. attenuata* plants manipulated to silence transcription of the protein kinase GAL83 (the silencing of which they show to be driving the herbivore induced shift of carbon resources to the roots in *N. attenuata*) results in prolonged flowering after herbivore attack compared to the non-silenced controls. The authors interpret these results to show that the resources mobilized to less vulnerable tissues by the herbivory induced silencing of GAL83 are being used to help the plant tolerate the herbivory.

It has been shown that simulated herbivory induces multiple changes in metabolism in tomato as well. Gomez et al., showed that partitioning of radiolabeled nitrogen shifts towards the roots after methyl jasmonate application in tomato, and that this shift was relatively rapid (4hours) (Gomez et al., 2010). Changes in the levels of a whole suite of metabolites was also documented in tomato plants 24 hours after herbivory by both the specialist herbivore *Manduca setxa*, as well as the generalist *Helicoverpa zea* (Steinbrenner et al., 2011). The specialist induced changes were mainly in metabolites associated with carbon and nitrogen transport. In *H. zea,* the generalist herbivore, shifts were mainly seen in defense molecule precursors. This led to the model for induced sequestration presented in Orians et al., that hypothesizes that the benefits of induced sequestration will outweigh the costs in particular sets of circumstances, such as nutrient limiting conditions, and may not in others (Orians et al., 2011). It is when these factors align that one would most expect to see an induced sequestration response from the plants. Earlier work in the lab also demonstrated that the stems are the tissues most relevant for storage in tomato, and that after a simulated herbivory event, protein concentrations increase in the stems and decrease in the apex relative to controls (Korpita, unpublished data).

In this study we investigated the effects of this documented resource shift, both during a simulated herbivory event, and after a defoliation event requiring regrowth, on plant physiological indicators. Using "induced sequestration" as a framework we predicted that simulated herbivory would pull resources away from growth toward storage, and thus retard growth compared to uninduced controls. We also expected that, after severe defoliation, the herbivory-induced plants should have more resources available in storage tissues for regrowth and may ultimately produce more flowers.

Methods:

Plants

Tomato (*Solanum lycopersicum*, First Lady II Cultivar) plants were grown in potting mix in 10 cm x 10 cm x 20 cm square pots. The plants were grown in a greenhouse under natural light (experiment took place in June/July). Plants were fertilized three times a week with 50 ml of ½ strength Hoagland solution for the duration of the experiment.

Damage Period

We began treatments approximately 5 weeks after germination, when the plants were 24.7 cm tall on average (7 leaf stage). There were 3 treatments: 2 damage treatments (mechanical and herbivore cue; see below) and an undamaged control group. In each damage treatment, three leaves (leaves 2, 3 and 4, counting down from the top) were damaged. Leaf one was defined as the newest leaf that was over 50% expanded.

For the two damage treatments a fabric wheel was used to make puncture wounds along the edge of every leaflet of each leaf. After damage, 80 µl of *Manduca sexta* regurgitant or deionized water were applied with a paintbrush. Caterpillar regurgitant was collected from 5th instar *Manduca sexta* larvae fed on tomato plants for at least 48 hours and stored at -80 ˚C until further use. The chemical cues present in *Manduca* saliva can elicit a specific response compared to mechanical damage (Halitschke et al., 2003; Kessler and Baldwin, 2002). The water treatment was designed to test for the effects of mechanical damage alone. Plants in the control group were not damaged. There were 15 replicates per treatment.

We treated the plants for five consecutive days, during which data was collected (see below). On day 6, five plants/treatment were harvested for chemical analysis to determine

whether damage resulted in differential resource partitioning. The remaining plants were subjected to the defoliation treatment. We removed all leaflets (including the leaflets of the undamaged and previously damaged leaves 2, 3, and 4) and the apex of the plant with a sterile razor blade. All tissues removed were massed and plants were monitored for regrowth.

Starting on day 0 (the day before damage treatments began), chlorophyll measurements were made on the terminal leaflet of leaves 5, 2, and 0. Three measurements were made and averaged together with a handheld chlorophyll meter (Opti-Sciences, CCM 200) for each leaf. These measurements were collected through day 6. Over the same period, height from cotyledon to the base of the newest visibly separated leaf in the apex, stem width at marked points between leaves 3 and 4 and 2 and 3, the length of leaf 0 and the terminal leaflet of leaf 0, and number of leaves were measured.

Regrowth

We monitored the defoliated plants for regrowth over the following 23 days. At 9 and 13 days post defoliation, chlorophyll was measured in the new apex. In addition, we recorded the number of new leaves and the length of the longest new leaf on days 1, 4, 7, 10, 13, 16, 19, and 23. On day 23 the number of flower buds, open or closed, was recorded. Those flowers recorded at the harvest were the only ones to appear during the regrowth phase.

At harvest (day 23 post-defoliation), plants were divided into 4 tissues, massed, and put into 50 mL Falcon tubes and flash frozen in liquid nitrogen. The 4 tissues were roots, stem, regrown leaves, and regrown apex. The regrown apex was the new shoot that grew out of the nodes of the two highest defoliated leaves, and was where the majority of the growth after defoliation took place. The regrown leaves were all other regrowth.

Statistics

Statistical Tests were performed using SPSS Student Version 18.0 (IBM). One Way ANOVAs were performed to test for significant differences between treatment types. Tukey's Honest Significant Difference Tests were performed to investigate differences between individual pairs of means.

Results

During Damage Period

All of the plants, regardless of treatment, showed a decline in chlorophyll content. The steepest decline in the treated groups took place between the readings taken on day 1 and day 3. In the expanding leaf (leaf 0), the decline in chlorophyll (mean±S.E.) was 15.58 (±1.39), 12.69 (±1.45), and 9.12 (±1.89) units, for the Manduca, Water, and No Damage treatments, respectively (figure 1a). The difference in the decline between the Manduca and No Damage treatments was significant ($F= 4.119$; $p = 0.023$, Tukey HSD, $p = 0.017$), while the Water treatment did not differ from the other two treatments (Tukey HSD, both $p > 0.10$). In the youngest fully expanded leaf (leaf 2), the same pattern was observed [10.14 (±1.81) units, 6.54 (±1.59) units, and 4.25 (±1.99), for Manduca, Water, and No Damage treatments, respectively](figure 1b). These differences were marginally significant, however ($F= 2.704$; $p = 0.079$). The changes in leaf 5 were minor and did not vary by treatment ($F= 1.529$, $p= 0.228$).

The mean height change between days 0 and 6 was 5.67(±0.37) cm, 6.45 (±0.28) cm, and 7.29 (±0.32) cm, for Manduca, Water, and No Damage treatments, respectively. The difference in growth between Manduca and No Damage treatments was statistically significant ($F= 6.167$; p

= 0.004, Tukey HSD, p = 0.003), but again the Water treatment did not differ from the other treatments (Tukey HSD, both p > 0.10) (figure 2).

Regrowth

The majority of the regrowth took place off of a new shoot that grew from the most apical node. By day seven post-defoliation, the mean length of this new growth was 5.17 (±0.23) cm, 4.97 (±0.48) cm, and 3.78 (±0.40) cm, for the Manduca, Water, and No Damage treatment groups, respectively. The difference in regrowth between Manduca and No Damage treatments was statistically significant (F= 3.818; p = 0.035, Tukey HSD, p = 0.042), but the other two pairwise combinations were not significant (Tukey HSD, p = 0.091 and p = 0.928) (figure 3).

The Manduca treatment had an average of 15.4 new leaves by day 7 post defoliation. This was significantly higher (F = 4.668, p = 0.018, Tukey HSD, p = 0.018) than the average number of new leaves in the No Damage treatment, 12.2. Again the number of leaves in the Water treatment did not differ significantly from either the Manduca or No Damage treatments (Tukey HSD, p = 0.746 and p = 0.089) (Figure 4).

The Manduca treatment had 3.60 flowers on average at the time of harvest (23 days post defoliation). This was significantly higher (F= 3.927, p = 0.032, Tukey HSD, p = 0.024), than the average number of flowers on No Damage treatment plants, 2.00. Water treatment plants had an average of 2.40 flowers, which was not significantly different from either other treatment (Tukey HSD, both p > 0.10) (Figure 5).

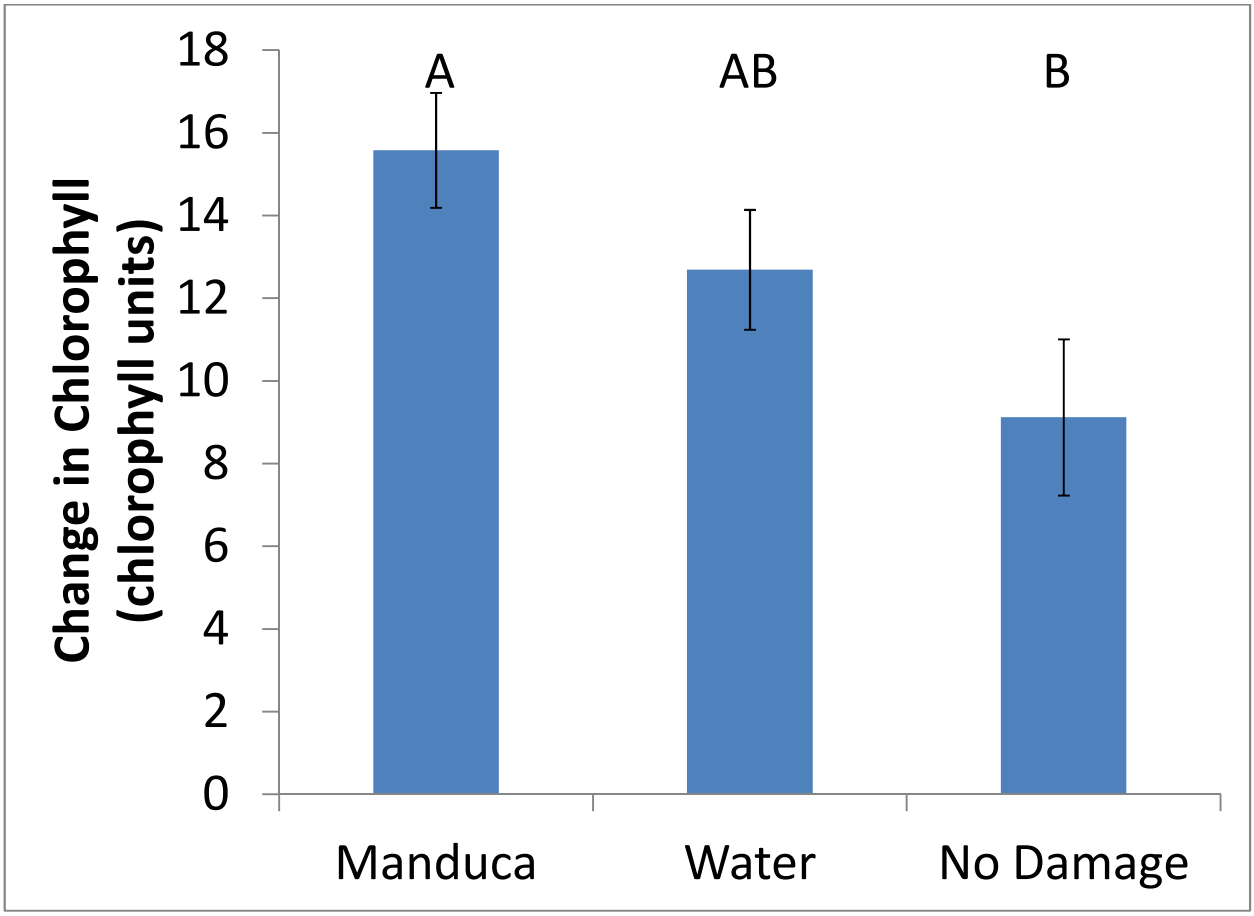

Figure 1a. Mean Decrease in Chlorophyll content between Day 1 and Day 3 of damage treatments on Leaf 0. Chlorophyll measurements were taken on the terminal leaflet of leaf 0, in triplicate, and averaged together. Bars represent means ± standard error. Means were compared using a One Way ANOVA and Tukey's HSD test. The means annotated by like letters were not significantly different ($p > 0.05$).

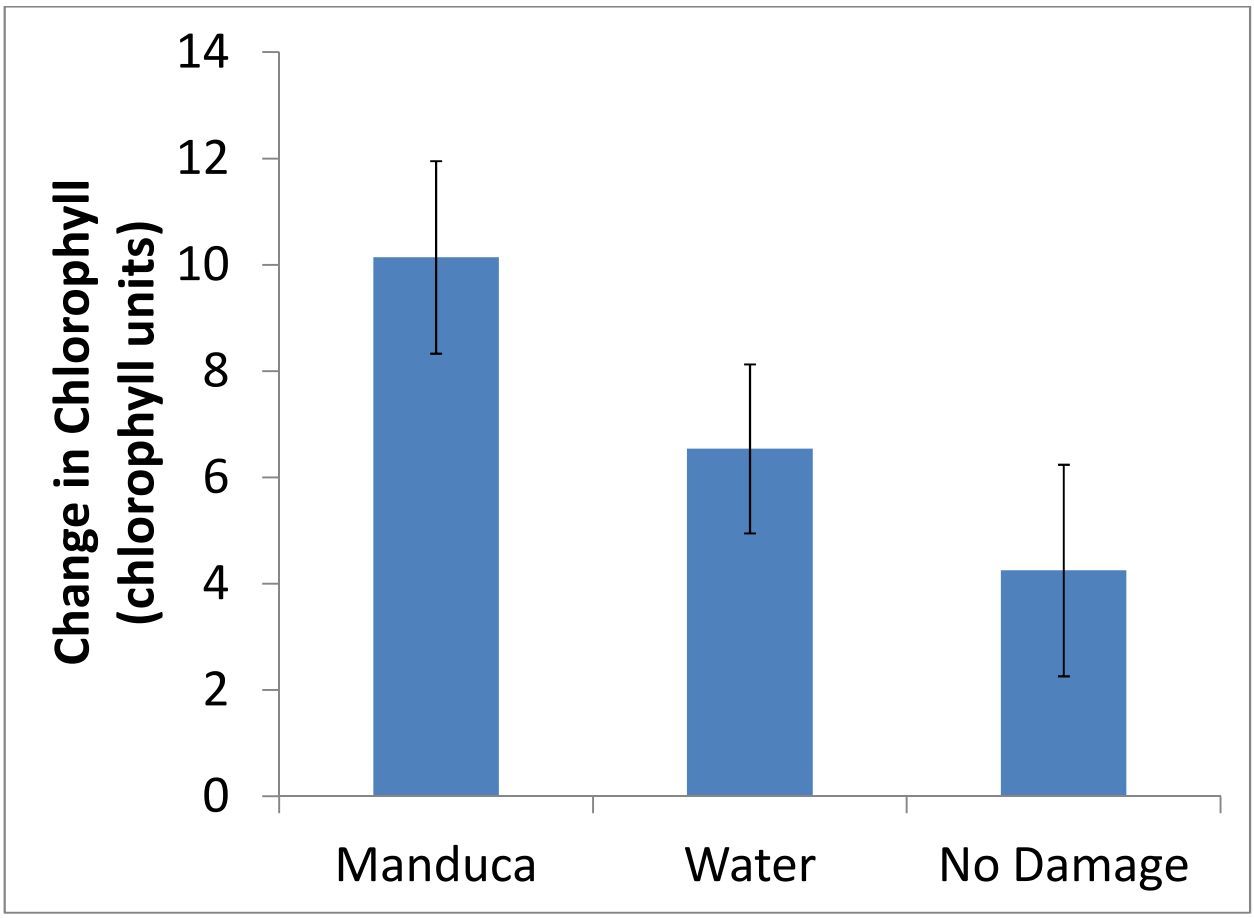

Figure 1b. Mean Decrease in Chlorophyll content between Day 1 and Day 3 of damage treatments on Leaf 2. Chlorophyll measurements were taken on the terminal leaflet of leaf 2, in triplicate, and averaged together. Bars represent means ± standard error. Means were compared using a One Way ANOVA and Tukey's HSD test. The means presented here were at most marginally significantly different ($p = 0.079$).

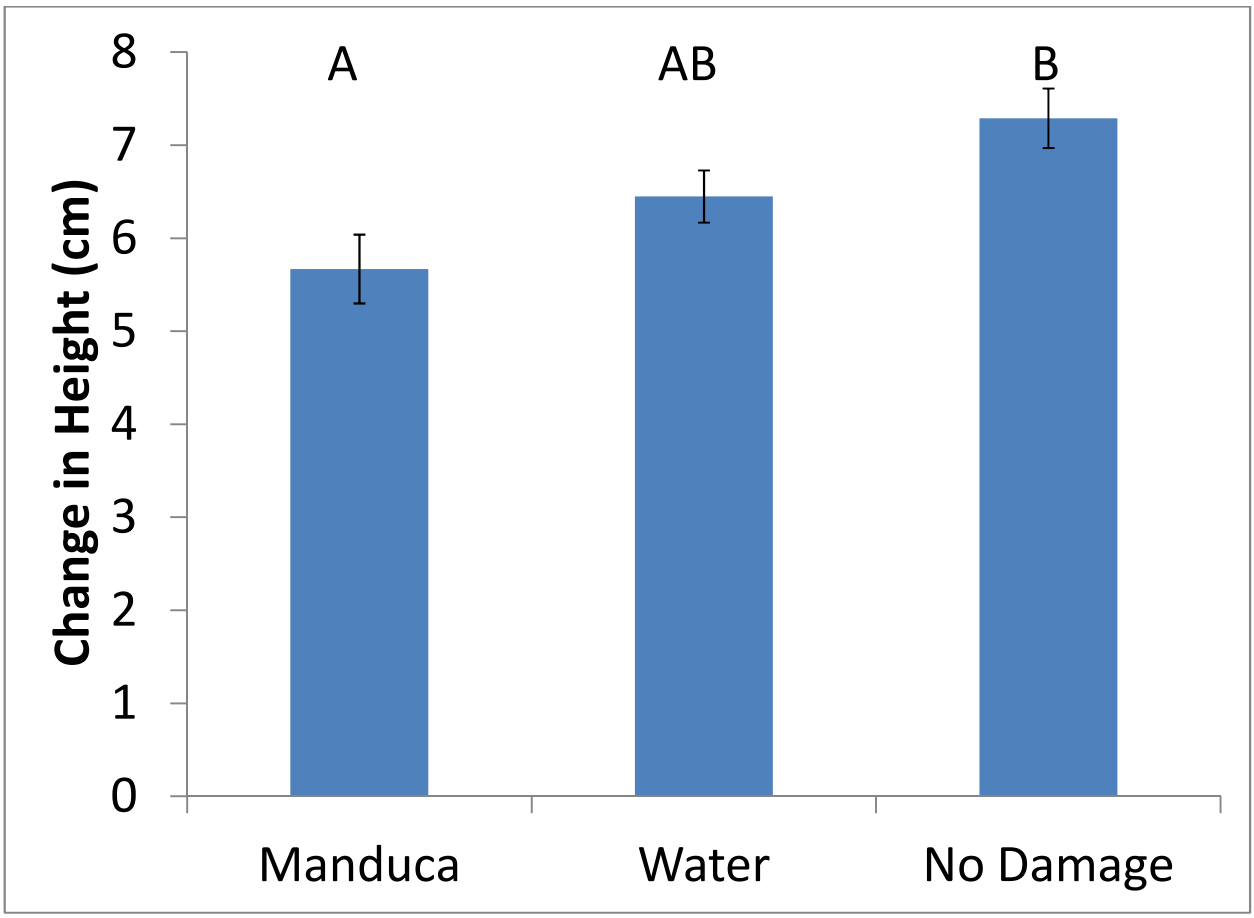

Figure 2. Mean Height Increase During Damage Treatment. Bars represent means ± standard error. Means were compared using a One Way ANOVA and Tukey's HSD test. Like letters represent means that were not significantly different ($p > 0.05$).

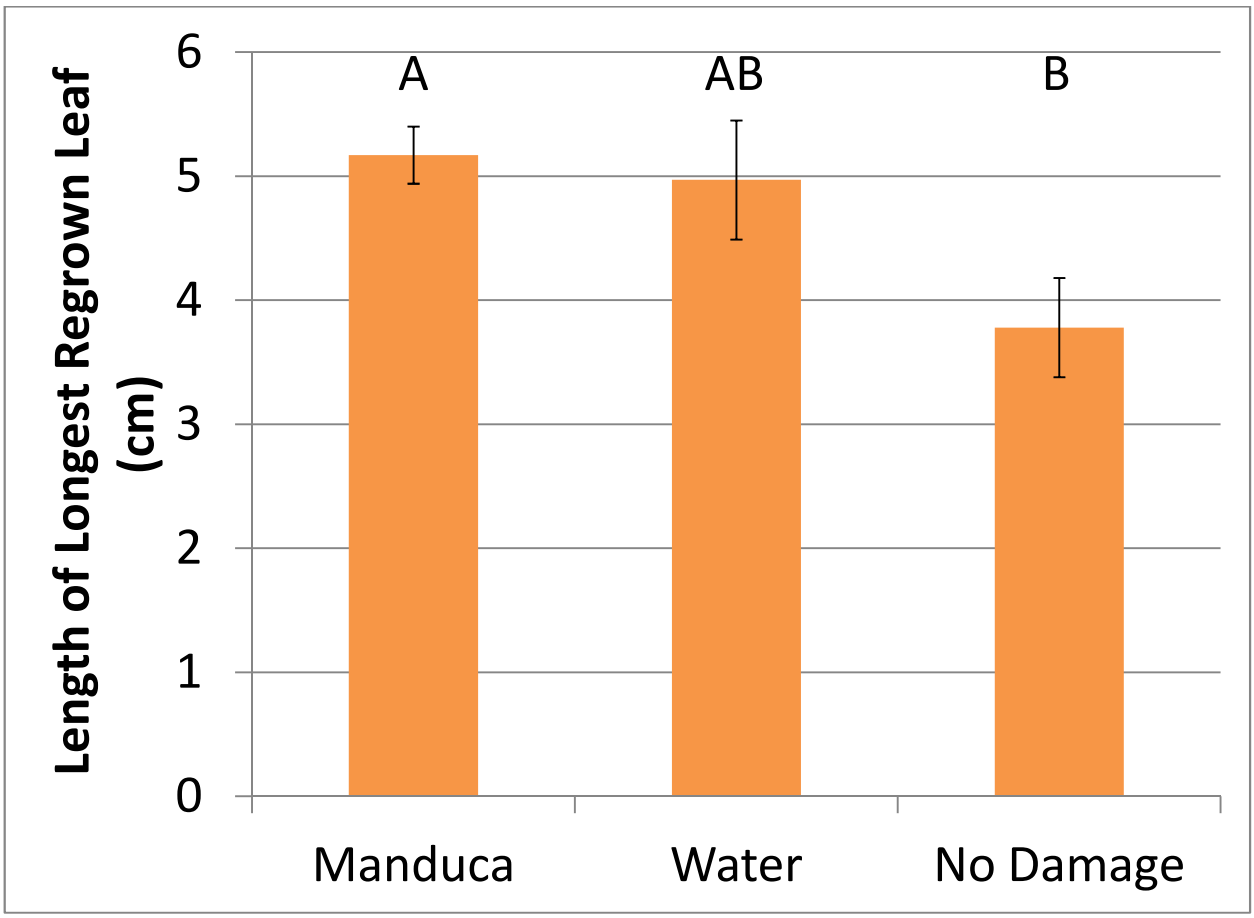

Figure 3. Mean Length of Longest Regrown Leaf on Day 7 Post-Defoliation. Bars represent means ± standard error. Means were compared using a One Way ANOVA and Tukey's HSD test. Like letters represent means that were not significantly different ($p > 0.05$).

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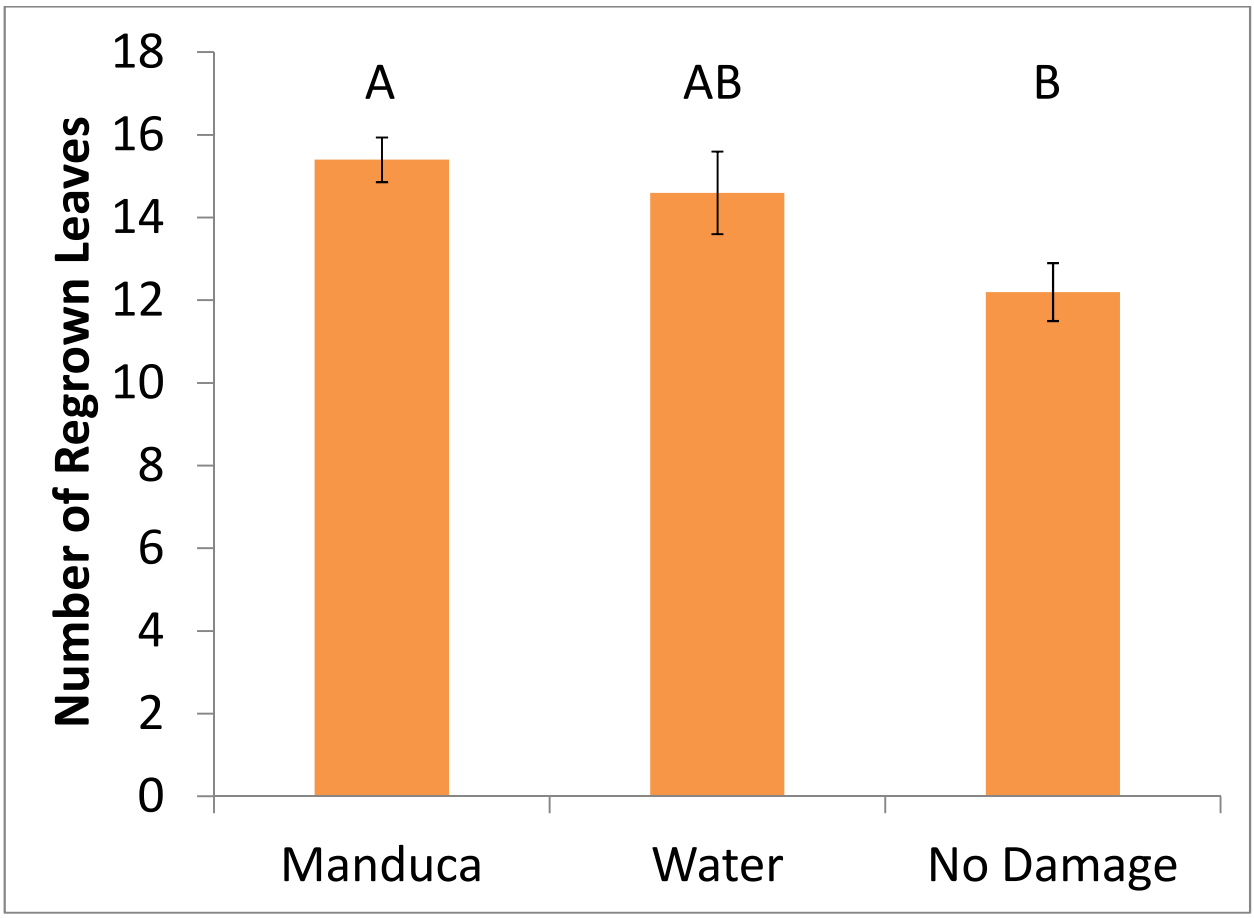

Figure 4. Mean Number of Regrown Leaves at Day 7 Post-Defoliation. Bars represent means ± standard error. Means were compared using a One Way ANOVA and Tukey's HSD test. Like letters represent means that were not significantly different ($p > 0.05$).

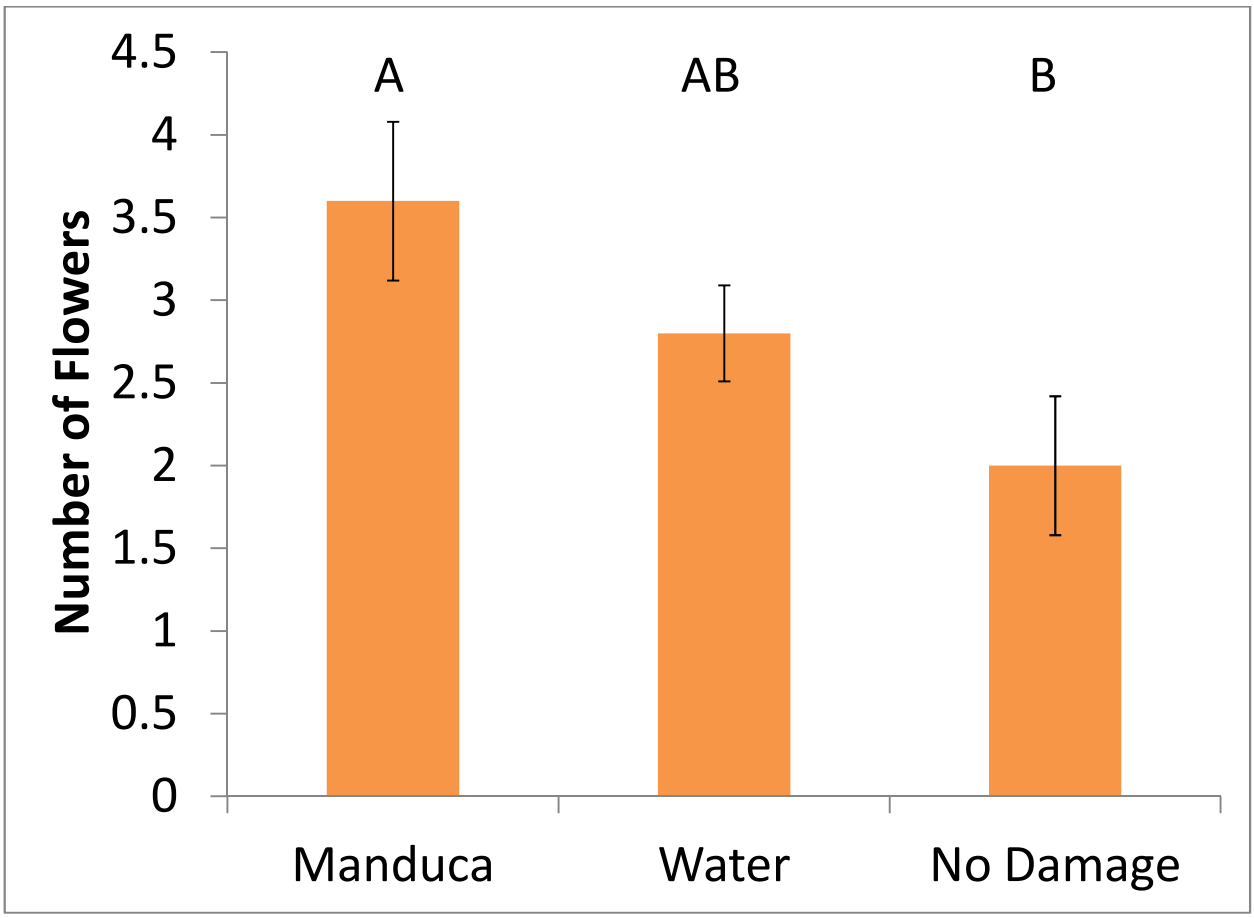

Figure 5. Mean Number of Flowers at Harvest. Bars represent means ± standard error. Means were compared using a One Way ANOVA and Tukey's HSD test. Like letters represent means that were not significantly different ($p > 0.05$).

Discussion

These results show that the damage treatment has a negative impact on growth. This is significant because the pinwheel treatment does not remove photosynthetic area. The observed decrease in growth is therefore likely the result of a shift in primary metabolism elicited by the damage. It appears from these data that during an herbivory event, the tomato plants are devoting less of their resources toward new growth. It would not be unreasonable to suggest that these resources are being used to support some function other than storage, such as the production of defense compounds. Some of the resources that would otherwise go toward growth are undoubtedly going toward these other sinks (Herms and Matson, 1992), but there is also

evidence that has shown that some of the resources in vulnerable tissues (leaves) are exported to less vulnerable tissues (roots and stems) and stored in response to damage cues (Gomez et al. 2010) (Korpita, unpublished data). Our results with the chlorophyll measurements suggest that this was happening in our plants as well. It has been hypothesized that these resources are temporarily stored so that they may be used in regrowth after the herbivore has moved on (Schwatje et al 2006; Orians et al., 2011).

Our results from the regrowth phase of the experiment directly support this hypothesis. Plants that had been induced to sequester resources by the damage treatment were clearly able to tolerate the loss of all their leaf material better than the undamaged control. They recovered leaf area at a faster rate, and were able to produce on average more flowers. The resources they stored during the regrowth were remobilized into new tissues so that the plants could recover faster.

This experiment on its own cannot very well determine what resource is most relevant to regrowth in this particular system, but our previous work on this system suggest that nitrogenous resources may be more relevant than carbohydrates. Most of the induced sequestration work to date has focused on carbon (Holland et al. 1996; Schwachtje et al. 2006; Babst et al., 2005; Babst et al. 2008; Kaplan et al. 2008), but in well-lit areas with species with lots of non-leaf photosynthetic area, nitrogen is likely what is limiting recovery from defoliation, and therefore could be most relevant to induced sequestration in this system. The data presented here hint at Nitrogen being relevant, since chlorophyll concentration decreased faster in the *Manduca* group than the untreated group. Since chlorophyll content has been shown to correlate with nitrogen (Evans, 1989), this suggests less nitrogen remained in the leaves of treated plants. We will address this issue of nitrogen with data presented in the following chapters.

These results confirm the post attack advantage of the induced sequestration response to plants. This response is likely to be more advantageous in certain scenarios than others (Orians et al , 2011). In some situations, a tolerance strategy may be the most effective option for a plant, and the utilization of stored resources if often an important component of plant tolerance of stresses (Tiffin, 2000). It follows logically that the more resources available for remobilization, the better the plant will tolerate the stress, and there is experimental evidence to support this (Van der Meijen et al, 1988). Since stored resources cannot be used for growth, defense, or reproduction, the inducibility of the response allows plants to maximize both storage and the use of resources for other functions. At least in the circumstances provided by this experiment, that induction was enough to increase the tolerance of the plant to defoliation. These results do not, on their own, show that this increased tolerance was because of a shift in resources out of vulnerable tissues and into storage however, but that will be addressed in the next chapter.

Future investigations of herbivory induced tolerance of damage should focus on verifying what we are interpreting as increased tolerance due to greater storage is in fact due to storage, and not other factors known to increase tolerance, such as the activation of dormant meristems (Tiffin, 2000). If different levels of damage result in different levels of resource shifts, they should show different levels of tolerance to the loss of leaf material. Confirming this correlation experimentally would provide further support for the induced sequestration model.

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Chapter 3: Data Chapter 2

Simulated Herbivory Results in a Within Plant Shift in the Allocation of Carbon and Nitrogen

Introduction

Plants are constantly responding to stresses from their environment with shifts in metabolism, both locally and systemically (Metlen et al., 2009; de Kroon et al., 2005). One of the major stresses they face is herbivory, and they can respond to herbivory in diverse ways (Karban and Baldwin, 1997). The two main categories of strategies are resistance, the production of defense traits that deter herbivores, and tolerance, where plants recover from the herbivory event after it has occurred (Karban and Baldwin, 1997). Any strategy to mitigate the negative effects of herbivory, whether it falls under resistance or tolerance, should naturally have some cost to the plant (Strauss et al., 2002; Tiffin, 2000; Hermes, and Matson, 1992). The resources that they are investing in these strategies are in general not available to be put toward growth and reproduction. To minimize the fitness cost of these strategies, plants often do not express them constitutively but will instead induce them only when the herbivore attacks. This way those resources that would otherwise have been wasted in herbivore mitigation can be used for growth and reproduction when herbivores are absent (Argawal and Karban, 1999).

The induction of resistance mechanism is well documented, but there is increasing evidence that tolerance strategies may also be inducible (Orians et al., 2011). For example, Babst et al. (2005) showed a decrease in starch concentrations in methyl jasmonate treated leaves of *Populus*. Holland et al. (1996) found that herbivory resulted in increased accumulation of carbon isotopes in the roots of grasses. Babst et al. (2008) showed that Gypsy Moth herbivory on *Populus* resulted in the increased export of carbon from leaves. Schwatchje et al., (2006) showed an increase in carbon allocated to the roots, as well as a decrease in soluble sugar concentration

in sink leaves after simulated herbivory treatments in tobacco. Gomez et al., (2010) showed an increase of nitrogen export toward the roots in tomato. These results are significant for the induced sequestration model because they in general show the shift of resources from herbivore accessible tissues toward herbivore inaccessible tissues. The idea is that these exported resources will be stored, and then used for regrowth. It is believed that tolerance is somewhat dependent on the stored resources a plant has to remobilize (Tiffin, 2000). By exporting and sequestering resources that would otherwise be lost to the herbivore, the plant should be better able to recover when the herbivore moves on (Orians et al., 2011). Our results presented in Chapter 2 show that this is in fact the case in tomato.

Most of the studies to date used to support the induced sequestration model actually only demonstrate an increase in export of recently assimilated resources out of herbivore-vulnerable tissues. It is implicit in the induced sequestration model that resources removed from vulnerable tissues be stored until the herbivore moves on, when they will be remobilized for regrowth. The experimental evidence for this part of the model is extremely limited. There is some evidence that simulated herbivory results in increased concentrations of protein in the stem tissue of tomato plants (Korpita, unpublished data).

The goal of this experiment was to document this hypothesized removal, sequestration, and remobilization of resources in plants that experienced simulated herbivory before an artificial complete defoliation. Because of the previous results (Gomez et al., 2010, Steinbrenner et al., 2011, Korpita 2012 chapter 1, Korpita 2012 unpublished) suggesting that the nitrogenous resources may be the most relevant in the tomato system, nitrogen is the focus of the analysis.

Methods:

Plants

Tomato (*Solanum lycopersicum*, First Lady II Cultivar) plants were grown in potting mix in 10 cm x 10 cm x 20 cm square pots. The plants were grown in a greenhouse under natural light (experiment took place September through October) supplemented with metal halide lamps for a 16 hour photoperiod. Plants were fertilized three times a week with 50 ml of ½ strength Hoagland solution for the duration of the experiment.

Damage

We damaged plants approximately 5 weeks after germination, when the plants had 7 leaves that were more than 50% expanded. There were 3 treatments: 2 damage treatments (mechanical and herbivore cue; see below) and an undamaged control group. In each damage treatment, three leaves (leaves 2, 3 and 4, counting down from the top) were damaged. We defined leaf 1 as the newest leaf that was over 50% expanded (see appendix 1 for detailed illustration).

For the two damage treatments we used a fabric wheel to make puncture wounds along the edge of every leaflet of each leaf. After damage, 80 µl of *Manduca sexta* regurgitant or deionized water were applied with a paintbrush. Caterpillar regurgitant was collected from 5th instar *Manduca sexta* larvae fed on tomato plants for at least 48 hours and stored at -80 ˚C until further use. The chemical cues present in *Manduca* saliva can elicit a specific response compared to mechanical damage (Halitschke et al., 2003; Kessler and Baldwin, 2002). The water treatment was designed to test for the effects of mechanical damage alone. We did not damage control plants at all. Each harvest consisted of 6 replicates per treatment.

Harvests

We harvested the first set of plants on day 0, the day before damage treatments began (see Figure 1 below for complete description of experimental timeline). We damaged the plants for five consecutive days. The second harvest took place on day 2, approximately 24 hours after the first damage treatment. This harvest was done because previous work indicated that many metabolic changes take place in in this time period in tomato (Steinbrenner et al., 2011). The third harvest took place on day 6, as well as the defoliation (see below). The first post-defoliation harvest took place on day 11, 5 days after defoliation, and the final harvest took place on day 20, 14 days after defoliation.

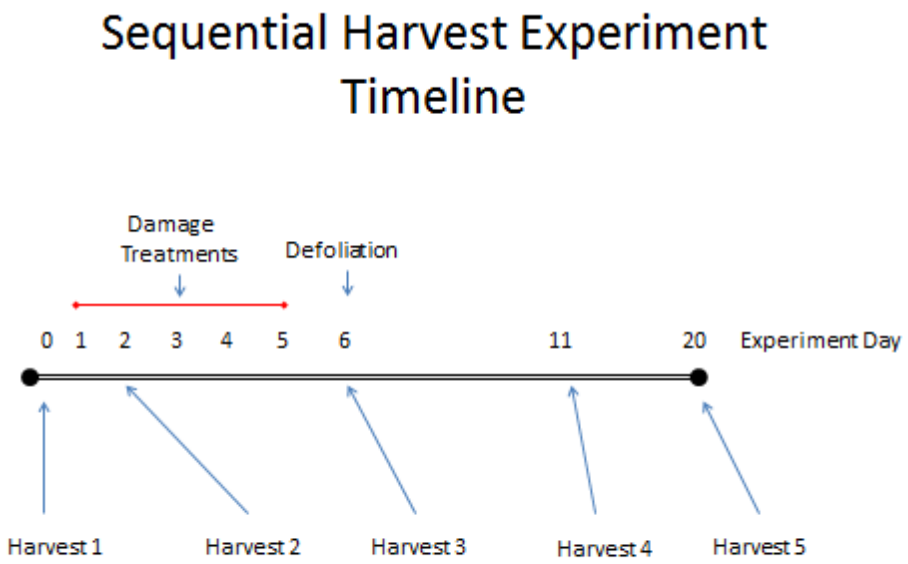

Figure 1. Timeline of Experimental Treatments and Harvests.

Defoliation

The plants remaining after the first three harvests were defoliated on day 6. We removed all leaves (including the undamaged and previously damaged leaves 2, 3, and 4) and the apex of

the plant with a sterile razor blade. All tissues removed were massed and plants were left to regrow.

Analysis

During harvests, we divided plants by tissue, massed, put in 50 mL Falcon tubes, and flash frozen in liquid nitrogen. They were stored in a -80^{o}C until analysis. The plant was divided into six tissues for harvests that took place before the defoliation: Roots, stem, old leaves, damaged leaves, young leaves, and apex (see supplemental material for diagram). The old leaves were defined as those below the damage leaves (below the leaf marked as #4 at the start of the experiment). The young leaves were defined as those leaves above the damaged leaves that were below the apex. The apex was all the tissue above the fork of top two leaves that were less than 25% expanded. During the harvests after defoliation, plants were divided into stems, roots, and regrown leaf tissue.

The frozen plant tissues were then lyophilized, massed again, and then pulverized to a fine powder in a mechanical pulverizer. This material was analyzed for percent carbon and percent nitrogen in a CHNS analyzer in CN mode (vario MICRO cube, Elementar).

Calculations

Data presented as concentration of carbon or nitrogen in a tissue are simply the result of the calculations made by the CHNS analyzer's software. To obtain the carbon or nitrogen content of a tissue, we multiplied the corresponding concentration by the dry mass of the tissue. The data presented as proportions are the carbon or nitrogen content of that tissue divided by the sum of all the plants' individual tissues' content.

Statistics

Statistical Tests were performed using SPSS Student Version 18.0 (IBM). One Way ANOVAs were performed to test for significant differences between treatment types. Tukey's Honest Significant Difference Tests were performed to investigate differences between individual pairs of means.

Results

For complete results, see Tables 1 and 2.

HARVEST 3 (Day 6)

Vulnerable Tissues

Apex

At harvest three, after 5 days of simulated damage, the proportion of the plant's total dry mass made up by the apex did not differ significantly across treatments ($F = 2.23$, $p = 0.14$) (Figure 4), nor did the concentration of nitrogen in the apex ($F = 1.87$, $p = 0.19$). Differences in the proportion of the total nitrogen in the plant found in the apex were marginally significant across treatments ($F= 3.12$, $p = 0.07$). The Manduca treatment, Water treatment, and No Damage treatment apices contained 7.3% (± 0.8), 8.8% (±1.0), and 10.2% (± 0.5) (all data show are means ± S.E.), of the total plant nitrogen in the apex on average, respectively (Figure 2). The difference was only marginally significant between the Manduca and No Damage treatment (Tukey HSD, $p = 0.06$), the other pairwise combinations were not significant ($p > 0.10$).

The concentration of carbon in the apex differed significantly across treatments ($F=5.92$, $p = 0.01$). The carbon concentrations were 436.98 mg/g (±3.80), 429.48 mg/g (±3.12), and 421.15 mg/g (±2.76), for the apices of the Manduca, Water, and No Damage treatment plants, respectively (Figure 3). Only the difference between the Manduca and No Damage treatment was

significant (Tukey HSD, p = 0.01), the other pairwise combinations were not significant (p > 0.10). The differences in the proportion of the total plant carbon located in the apex did not differ significantly across treatments, however (F=1.89, p = 0.19). Likewise, the carbon to nitrogen ratio of the apex tissue did not differ significantly across treatments (F=1.24, p = 0.32).

Young Leaves

There was a statistically significant difference across treatments in the proportion of the total plant dry mass that was found in the young leaves (F=4.40, p = 0.03). 11.2% (±1.0), 7.5% (±1.6), and 6.5% (±0.9) of the total plants dry mass was found on average in the young leaves of Manduca, Water, and No Damage treatment plants, respectively. Only the difference between the Manduca and No Damage treatments were significant (Tukey HSD, p = 0.03). The other pairwise combinations were not significant (p > 0.10). The concentration of nitrogen in the young leaves did not differ significantly across treatments (F=0.64, p=0.54). There were significant differences, however, between the treatments, in terms of proportion of the total plant nitrogen found in the young leaves (F=4.739, p = 0.025). 16.9% (±1.4), 11.5% (±2.2), and 9.9% (±1.3) of the total nitrogen in the Manduca, Water, and No Damage treatment plants, respectively, was found in the young leaves. Only the difference between the Manduca and No Damage treatment was significant (Tukey HSD, p = 0.03), the other pairwise combinations were not significant (p > 0.08).

The concentration of carbon did not differ significantly across treatments (F=1.89, p=0.19), but there were significant differences between treatments in terms of proportion of total plant carbon found in the young leaves (F=4.95, p=0.02) (Figure 3). 11.6% (±1.0), 7.7% (±1.6%), and 6.6% (±0.9) of the total carbon in the Manduca, Water, and No Damage treatment plants, respectively, was found in the young leaves. Only the difference between the Manduca

and No Damage treatment was significant (Tukey HSD, p = 0.02), the other pairwise combinations were not significant (p = 0.08). The carbon to nitrogen ratio of the apex tissue did not differ significantly across treatments (F=0.34, p = 0.72).

Damaged Leaves

There were no significant differences between (all p > 0.10) for the data from the damaged leaves, except for the concentration of carbon (F=5.66, p=0.02). Concentrations of carbon were 398.38 mg/g (±2.89), 391.83 mg/g (±2.25), and 384.63 mg/g (±3.41) in the damaged leaves of the Manduca, Water, and No Damage treatment plants, respectively. Only the difference between the Manduca and No Damage treatment was significant (Tukey HSD, p = 0.01), the other pairwise combinations were not significant (p > 0.10).

Old Leaves

There were no statistically significant differences (all p > 0.10) across treatments in the old leaves at harvest 3.

Storage Tissues

Stem

There was a statistically significant difference across treatments in the proportion of the total plant dry mass that was found in the stems (F=5.318, p = 0.018). 33.0% (±1.4), 35.6% (±0.4), and 37.6% (±1.0) of the total plant dry mass was found, on average, in the stems of Manduca, Water, and No Damage treatment plants, respectively. Only the difference between the Manduca and No Damage treatments were significant (Tukey HSD, p = 0.01). The other pairwise combinations were not significant (p > 0.10). There were no significant differences between the concentrations of nitrogen in the stem tissue (F=0.90, p = 0.43), but there were

significant differences in terms of the proportion of the total nitrogen in the plant found with the stems (F=5.03, p=0.02). 19.7% (±1.1), 21.8 (±0.4), and 24.6% (±1.5) of the total nitrogen found in the plant was located in the stems of the Manduca, Water, and No Damage treatment plants, respectively. Only the difference between the Manduca and No Damage treatments were significant (Tukey HSD, p = 0.02). The other pairwise combinations were not significant (p > 0.10).). There were no significant differences between the concentrations of carbon in the stem tissue (F=1.22, p = 0.32), but there were significant differences in terms of the proportion of the total carbon in the plant found with the stems (F=7.10, p=0.01). 33.1% (±1.4), 36.3 (±0.4), and 38.4% (±0.9) of the total carbon found in the plant was located in the stems of the Manduca, Water, and No Damage treatment plants, respectively. Only the difference between the Manduca and No Damage treatments were significant (Tukey HSD, p = 0.01). The other pairwise combinations were not significant (p > 0.08). The carbon to nitrogen ratio of the stem tissue did not differ significantly across treatments (F=0.97, p = 0.40).

Roots

There were no statistically significant differences (all p > 0.10) across treatments in the data collected from the roots at harvest 3.

HARVEST 5 (Day 20, 14 Days after defoliation)

<u>Regrown Leaf Material</u>

There were no significant differences in the proportion of the plants dry mass at harvest five that was made up of regrown leaf material (F=1.19, p =0.33). Nor were there significant

differences in the proportion of the total plant nitrogen or carbon found in the regrown leaves (F=1.66, p=0.22) (F=0.76, p=0.48). There were, however, significant differences across treatments in the concentration of nitrogen (F=10.96, p=0.001) (Figure 5) and carbon (F=7.76, p=0.01) (Figure 6). The new leaf material nitrogen concentrations were 31.87 mg/g (±1.06), 42.08 mg/g (±1.26), 38.70 mg/g (±2.17), for the Manduca, Water, and No Damage treatment plants, respectively. The Manduca treatment was significantly different from both the Water (Tukey HSD, p=0.001) and the No Damage treatment (p=0.02). The new leaf material carbon concentrations were 391.70 mg/g (±4.50), 422.05 mg/g (±6.86), 412.50 mg/g (±5.15), for the Manduca, Water, and No Damage treatment plants, respectively. The Manduca treatment was significantly different from both the Water (Tukey HSD, p=0.004) and the No Damage treatment (p=0.05). The carbon to nitrogen ratio also differed significantly across treatment (F=9.23, p=0.002). The carbon to nitrogen ratios were 12.353 (±0.417), 10.067 (±0.290), and 10.779 (±0.432) for the Manduca, Water, and No Damage treatment plants, respectively. The Manduca treatment was significantly different from both the Water (Tukey HSD, p=0.002) and the No Damage treatment (p=0.03).

There were no significant differences (all $p > 0.08$) in the dry mass, concentration or proportion of carbon or nitrogen, or the carbon to nitrogen ratio in the stems or roots of the plants from harvest five.

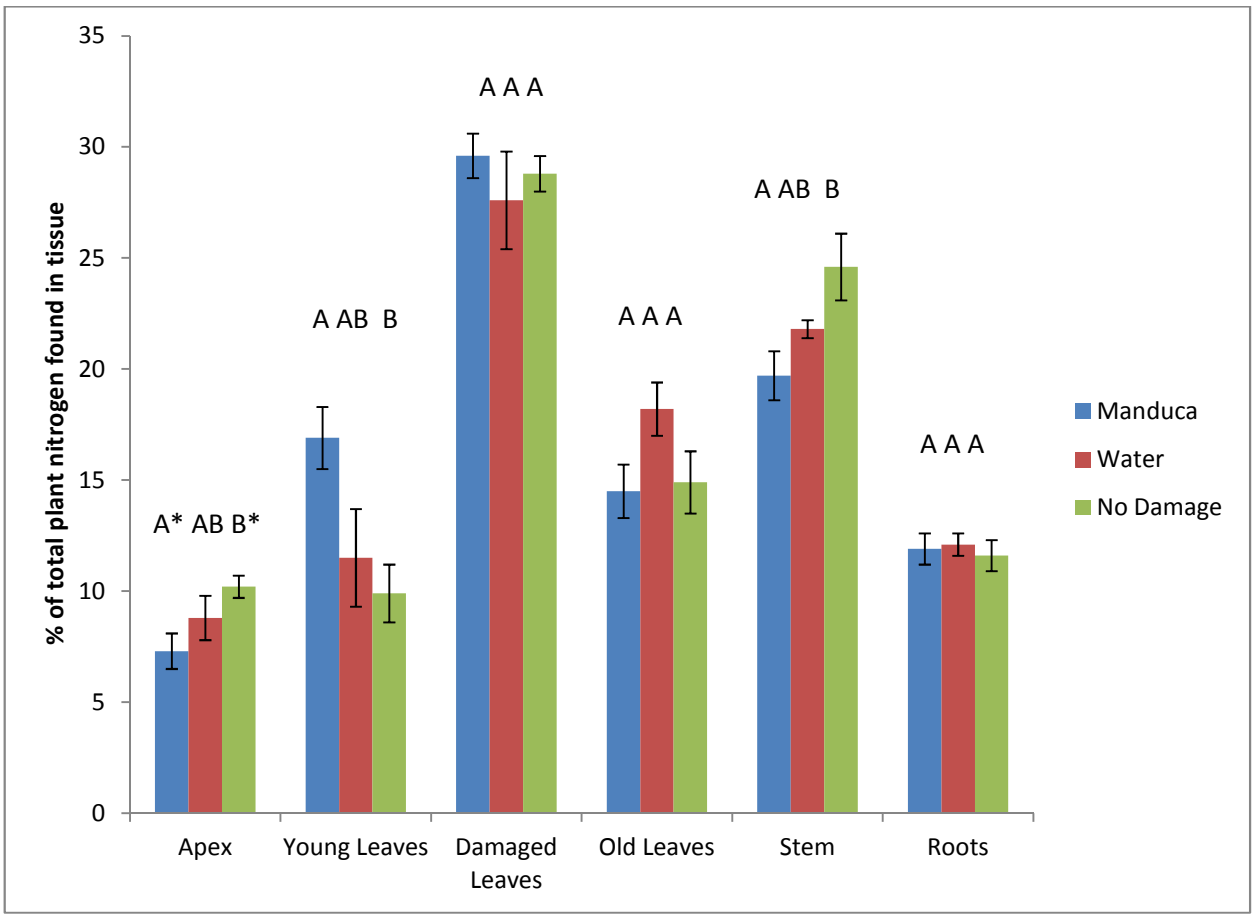

Figure 2. Mean proportion of total plant nitrogen found in tissue in plants collected at harvest 3. Bars represent means ± standard error. Means were compared using a One Way ANOVA and Tukey's HSD test. The means annotated by like letters were not significantly different ($p > 0.08$). Unlike letters accompanied by an asterisk (*) represent differences that are only marginally significant ($0.08 > p > 0.05$).

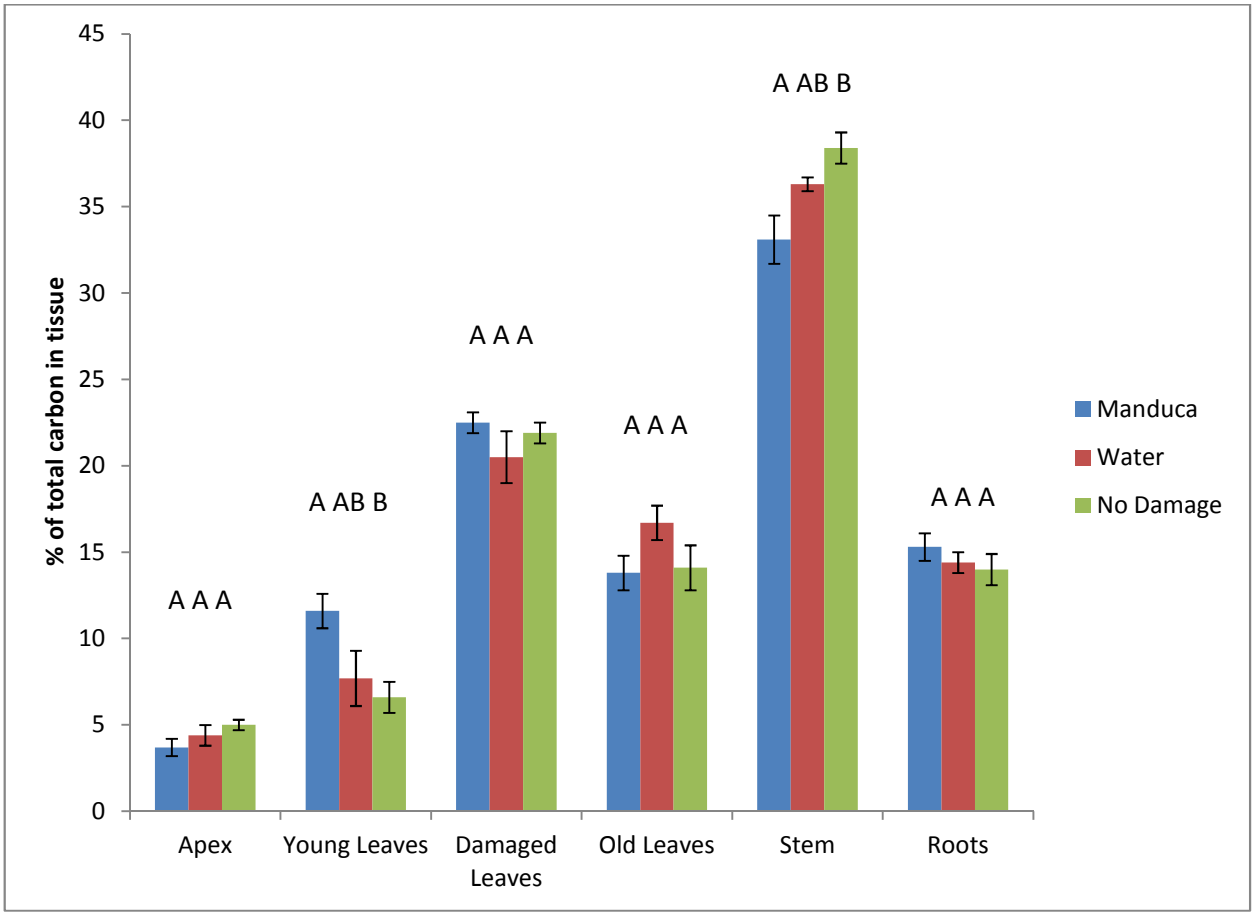

Figure 3. Mean proportion of total plant carbon found in tissue in plants collected at harvest 3. Bars represent means ± standard error. Means were compared using a One Way ANOVA and Tukey's HSD test. The means annotated by like letters were not significantly different ($p > 0.08$). Unlike letters accompanied by an asterisk (*) represent differences that are only marginally significant ($0.08 > p > 0.05$).

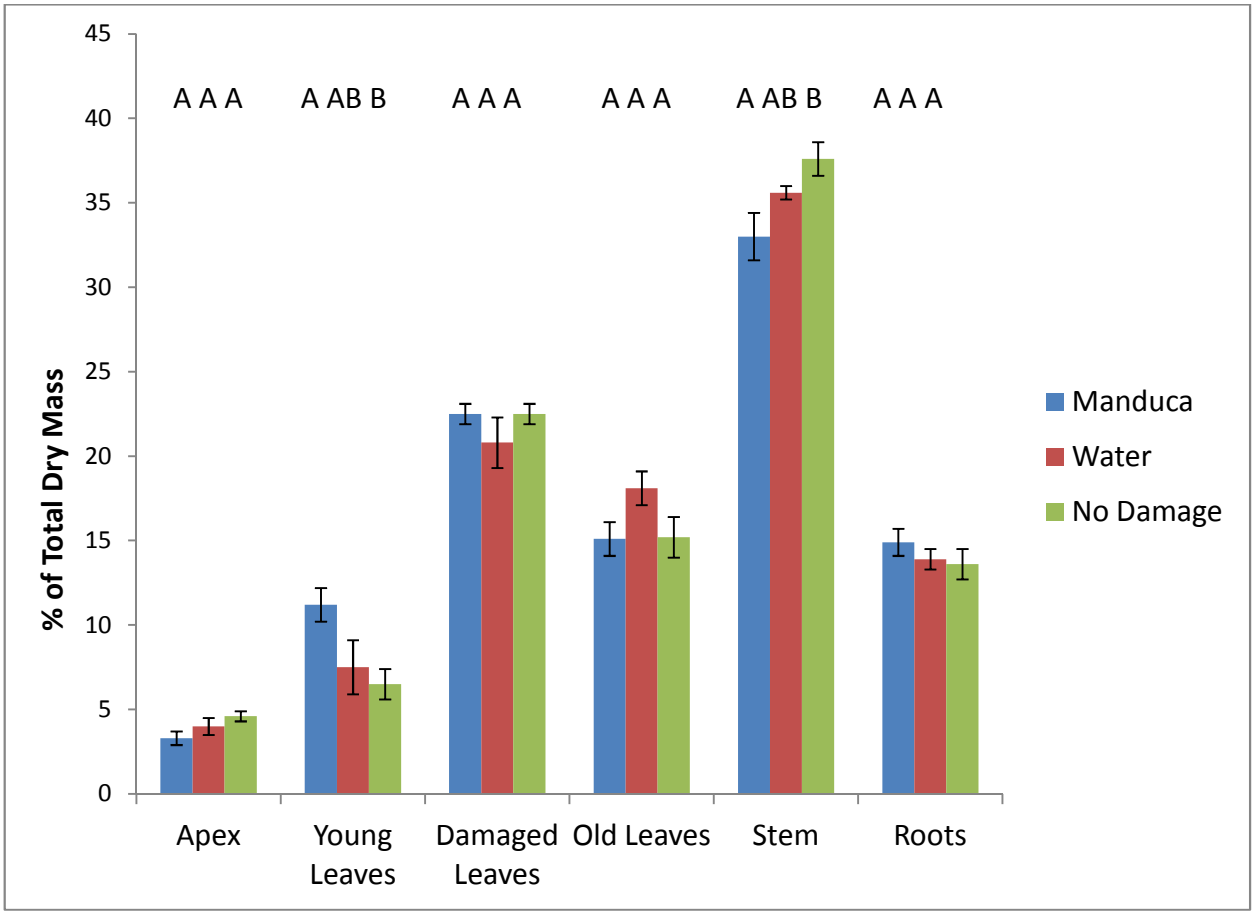

Figure 4. Mean proportion of total plant dry mass found in tissue in plants collected at harvest 3. Bars represent means ± standard error. Means were compared using a One Way ANOVA and Tukey's HSD test. The means annotated by like letters were not significantly different ($p > 0.08$). Unlike letters accompanied by an asterisk (*) represent differences that are only marginally significant ($0.08 > p > 0.05$).

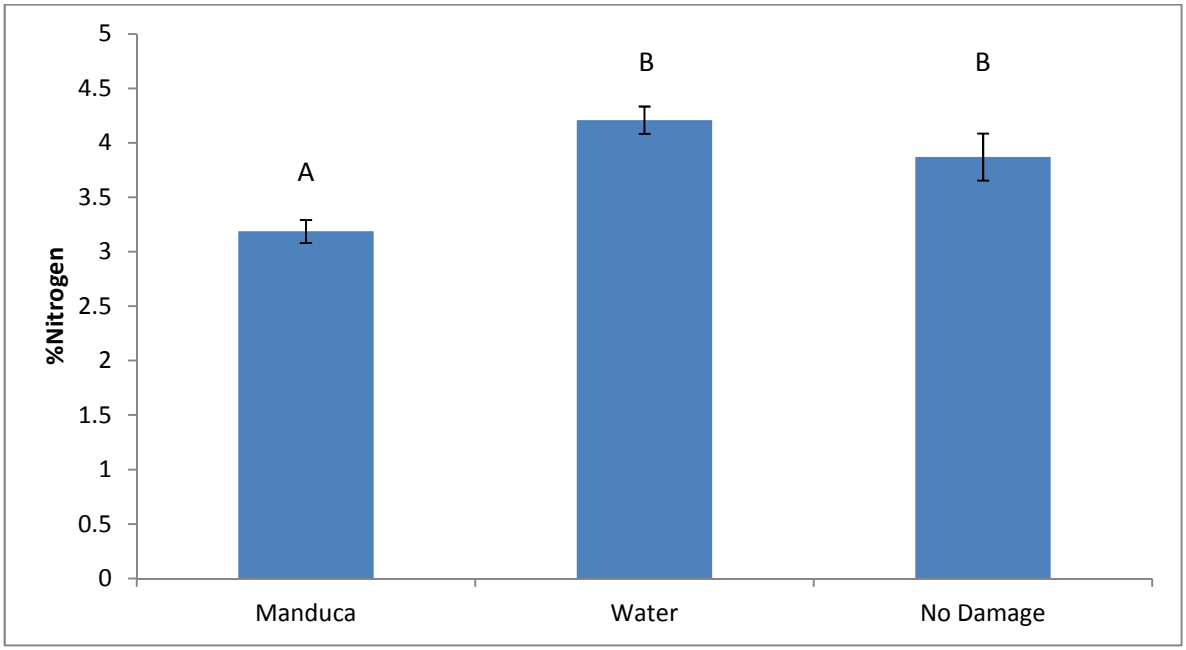

Figure 5. Mean concentration of nitrogen in regrown leaf material collected at harvest 5. Bars represent means ± standard error. Means were compared using a One Way ANOVA and Tukey's HSD test. The means annotated by like letters were not significantly different ($p > 0.08$). Unlike letters accompanied by an asterisk (*) represent differences that are only marginally significant ($0.08 > p > 0.05$).

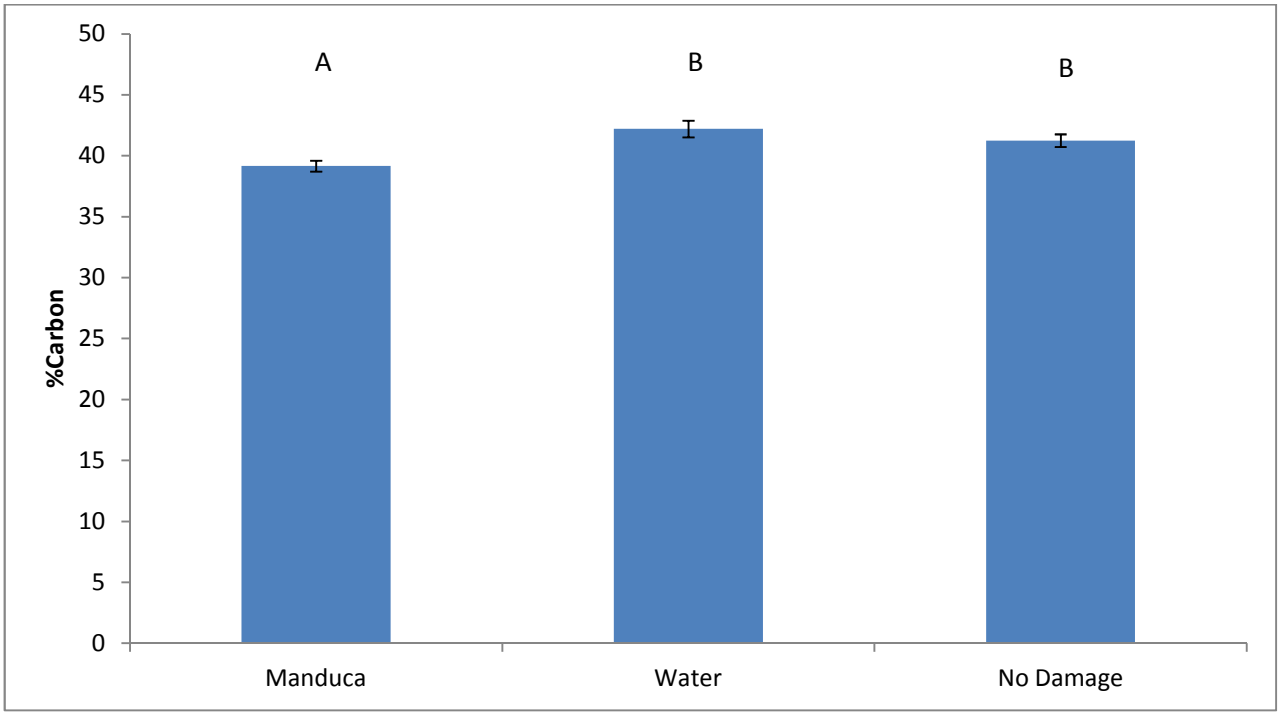

Figure 6. Mean concentration of carbon in regrown leaf material collected at harvest 5. Bars represent means ± standard error. Means were compared using a One Way ANOVA and Tukey's HSD test. The means annotated by like letters were not significantly different ($p > 0.08$). Unlike letters accompanied by an asterisk (*) represent differences that are only marginally significant ($0.08 > p > 0.05$).

Nitrogen

Tissue	Treatment	Measurement (units)	Harvest 1 (Day 0)	Harvest 2 (Day 2)	Harvest 3 (Day 6)	Harvest 4 (Day 11)	Harvest 5 (Day 20)
Apex	Manduca	Conc. (mg/g)	NA	33.98±1.81	33.38±0.78	NA	NA
	Water	Conc. (mg/g)	NA	33.78±0.61	30.80±1.19	NA	NA
	No Damage	Conc. (mg/g)	44.70±1.81	34.07±0.39	30.80±1.24	NA	NA
	Manduca	Proportion (% of total)	NA	**8.1±1.2 a**	7.3±0.8	NA	NA
	Water	Proportion (% of total)	NA	**7.5±0.5 ab**	8.8±1.0	NA	NA
	No Damage	Proportion (% of total)	7.0±0.6	**4.7±0.5 b**	10.2±0.5	NA	NA
Young Leaves	Manduca	Conc. (mg/g)	NA	25.75±1.91	23.00±1.17	NA	NA
	Water	Conc. (mg/g)	NA	24.60±0.75	21.58±0.88	NA	NA
	No Damage	Conc. (mg/g)	36.48±1.85	25.02±1.08	21.40±1.22	NA	NA
	Manduca	Proportion (% of total)	NA	14.7±2.0	**16.9±1.4 a**	NA	NA
	Water	Proportion (% of total)	NA	10.1±1.7	**11.5±2.2 ab**	NA	NA
	No Damage	Proportion (% of total)	8.8±0.7	13.6±2.7	**9.9±1.3 b**	NA	NA
Damaged Leaves	Manduca	Conc. (mg/g)	NA	21.87±1.56	20.15±1.02	NA	NA
	Water	Conc. (mg/g)	NA	21.07±0.41	18.27±0.72	NA	NA
	No Damage	Conc. (mg/g)	29.05±2.10	20.70±1.15	17.95±0.78	NA	NA
	Manduca	Proportion (% of total)	NA	30.4±1.2	29.6±1.0	NA	NA
	Water	Proportion (% of total)	NA	31.5±1.1	27.6±2.2	NA	NA
	No Damage	Proportion (% of total)	33.6±1.1	29.0±2.2	28.8±0.8	NA	NA
Old Leaves	Manduca	Conc. (mg/g)	NA	16.63±1.43	14.78±1.18	NA	NA
	Water	Conc. (mg/g)	NA	16.05±0.46	13.80±0.60	NA	NA
	No Damage	Conc. (mg/g)	21.43±1.20	14.88±0.89	13.60±0.33	NA	NA
	Manduca	Proportion (% of total)	NA	14.6±1.2	14.5±1.2	NA	NA
	Water	Proportion (% of total)	NA	17.5±1.3	18.2±1.2	NA	NA
	No Damage	Proportion (% of total)	19.5±1.5	19.4±1.3	14.9±1.4	NA	NA
Stem	Manduca	Conc. (mg/g)	NA	10.75±0.68	9.07±0.31	9.45±0.38	8.90±0.34
	Water	Conc. (mg/g)	NA	9.70±0.19	8.40±0.16	8.80±0.27	9.60±0.36
	No Damage	Conc. (mg/g)	13.00±1.06	9.53±0.35	9.13±0.65	8.50±0.11	8.62±0.26
	Manduca	Proportion (% of total)	NA	19.9±1.0	**19.7±1.1 a**	62.0±1.1	53.4±2.4
	Water	Proportion (% of total)	NA	18.9±0.6	**21.8±0.4 ab**	59.4±0.8	56.9±1.2
	No Damage	Proportion (% of total)	18.1±0.7	18.7±1.3	**24.6±1.5 b**	59.7±2.7	56.8±2.8
Roots	Manduca	Conc. (mg/g)	NA	13.72±0.38	12.20±0.43	11.47±0.51	11.15±0.20
	Water	Conc. (mg/g)	NA	13.15±0.21	11.92±0.22	11.63±0.22	11.72±0.28
	No Damage	Conc. (mg/g)	15.80±0.97	12.75±0.24	11.88±0.20	11.38±0.13	11.15±0.22
	Manduca	Proportion (% of total)	NA	12.2±0.6	11.9±0.7	35.2±1.7	27.6±1.9
	Water	Proportion (% of total)	NA	14.4±0.5	12.1±0.5	37.3±0.8	30.9±1.0
	No Damage	Proportion (% of	13.0±1.1	14.5±0.9	11.6±0.7	36.6±2.1	28.5±1.2

		total)					
Regrown Leaves	Manduca	Conc. (mg/g)	NA	NA	NA	24.80±1.64	**31.87±1.06 a**
	Water	Conc. (mg/g)	NA	NA	NA	25.03±1.80	**42.08±1.26 b**
	No Damage	Conc. (mg/g)	NA	NA	NA	26.38±1.84	**38.70±2.17 b**
	Manduca	Proportion (% of total)	NA	NA	NA	2.8±1.1	19.1±4.0
	Water	Proportion (% of total)	NA	NA	NA	3.4±1.1	12.2±1.1
	No Damage	Proportion (% of total)	NA	NA	NA	3.7±1.2	14.7±2.1

Carbon

Tissue	Treatment	Measurement (units)	Harvest 1 (Day 0)	Harvest 2 (Day 2)	Harvest 3 (Day 6)	Harvest 4 (Day 11)	Harvest 5 (Day 20)
Apex	Manduca	Conc. (mg/g)	NA	418.68±3.74	**436.98±3.80 a**	NA	NA
	Water	Conc. (mg/g)	NA	421.03±3.11	**429.48±3.12 ab**	NA	NA
	No Damage	Conc. (mg/g)	429.03±1.16	423.30±1.65	**421.15±2.76 b**	NA	NA
	Manduca	Proportion (% of total)	NA	**4.5±0.8 a**	3.7±0.5	NA	NA
	Water	Proportion (% of total)	NA	**3.9±0.3 ab**	4.4±0.6	NA	NA
	No Damage	Proportion (% of total)	3.8±0.4	**2.3±0.2 b**	5.0±0.3	NA	NA
Young Leaves	Manduca	Conc. (mg/g)	NA	409.10±4.14	412.37±5.73	NA	NA
	Water	Conc. (mg/g)	NA	405.78±4.43	407.55±4.00	NA	NA
	No Damage	Conc. (mg/g)	424.17±3.38	411.30±4.33	399.18±4.02	NA	NA
	Manduca	Proportion (% of total)	NA	10.4±1.3	**11.6±1.0 a**	NA	NA
	Water	Proportion (% of total)	NA	6.9±1.2	**7.7±1.6 ab**	NA	NA
	No Damage	Proportion (% of total)	5.6±0.6	9.2±2.0	**6.6±0.9 b**	NA	NA
Damaged Leaves	Manduca	Conc. (mg/g)	NA	387.43±4.57	**398.38±2.89 a**	NA	NA
	Water	Conc. (mg/g)	NA	394.17±4.96	**391.83±2.25 ab**	NA	NA
	No Damage	Conc. (mg/g)	391.83±6.79	395.53±5.56	**384.63±3.41 b**	NA	NA
	Manduca	Proportion (% of total)	NA	24.0±0.9	22.5±0.6	NA	NA
	Water	Proportion (% of total)	NA	24.1±0.9	20.5±1.5	NA	NA
	No Damage	Proportion (% of total)	25.1±1.1	22.1±1.3	21.9±0.6	NA	NA
Old Leaves	Manduca	Conc. (mg/g)	NA	360.82±3.95	364.57±2.99	NA	NA
	Water	Conc. (mg/g)	NA	372.75±10.58	365.20±5.37	NA	NA
	No Damage	Conc. (mg/g)	361.63±4.16	354.88±10.64	364.32±4.51	NA	NA
	Manduca	Proportion (% of total)	NA	**14.0±0.8 a**	13.8±1.0	NA	NA
	Water	Proportion (% of total)	NA	**16.5±1.2 ab**	16.7±1.0	NA	NA
	No Damage	Proportion (% of total)	18.1±1.3	**18.3±1.1 b**	14.1±1.3	NA	NA
Stem	Manduca	Conc. (mg/g)	NA	391.23±4.93	399.48±1.00	397.63±4.84	413.18±4.10
	Water	Conc. (mg/g)	NA	396.02±2.42	403.22±1.42	390.03±3.20	415.25±3.51
	No Damage	Conc. (mg/g)	384.07±4.42	393.07±1.96	402.15±2.86	401.13±2.82	418.60±1.06
	Manduca	Proportion (% of total)	NA	32.1±1.3	**33.1±1.4 a**	67.5±1.3	67.7±1.0
	Water	Proportion (% of total)	NA	31.5±0.8	**36.3±0.4 ab**	66.8±0.4	67.6±1.2
	No Damage	Proportion (% of total)	29.6±0.8	30.5±1.7	**38.4±0.9 b**	68.1±2.1	69.9±1.8

Roots	Manduca	Conc. (mg/g)	NA	385.27±3.85	408.68±3.39	393.88±6.25	406.67±6.75
	Water	Conc. (mg/g)	NA	386.20±5.90	410.28±2.22	391.62±3.40	396.98±6.32
	No Damage	Conc. (mg/g)	395.27±6.02	391.33±5.79	407.05±3.02	391.48±4.28	399.38±5.53
	Manduca	Proportion (% of total)	NA	15.1±0.8	15.3±0.8	31.3±1.6	27.4±1.6
	Water	Proportion (% of total)	NA	17.2±0.5	14.4±0.6	31.8±0.4	28.7±1.1
	No Damage	Proportion (% of total)	17.9±1.4	17.6±1.1	14.0±0.9	30.6±2.0	26.0±1.3
Regrown Leaves	Manduca	Conc. (mg/g)	NA	NA	NA	1.16±0.10	**391.57±4.50 a**
	Water	Conc. (mg/g)	NA	NA	NA	0.77±0.16	**422.05±6.86 b**
	No Damage	Conc. (mg/g)	NA	NA	NA	0.66±0.09	**412.50±5.15 b**
	Manduca	Proportion (% of total)	NA	NA	NA	1.3±0.06	4.9±1.1
	Water	Proportion (% of total)	NA	NA	NA	1.5±0.05	3.6±0.3
	No Damage	Proportion (% of total)	NA	NA	NA	1.3±0.04	4.1±0.7

Dry Mass

Tissue	Treatment	Measurement (units)	Harvest 1 (Day 0)	Harvest 2 (Day 2)	Harvest 3 (Day 6)	Harvest 4 (Day 11)	Harvest 5 (Day 20)
Apex	Manduca	Mass (g)	NA	**0.228±0.037 a**	0.203±0.025	NA	NA
	Water	Mass (g)	NA	**0.217±0.016 ab**	0.292±0.045	NA	NA
	No Damage	Mass (g)	0.150±0.008	**0.127±0.013 b**	0.333±0.033	NA	NA
	Manduca	Proportion (% of total)	NA	**4.2±0.7 a**	3.3±0.4	NA	NA
	Water	Proportion (% of total)	NA	**3.6±0.3 ab**	4.0±0.5	NA	NA
	No Damage	Proportion (% of total)	3.4±0.4	**2.1±0.2 b**	4.6±0.3	NA	NA
Young Leaves	Manduca	Mass (g)	NA	0.565±0.089	0.687±0.063	NA	NA
	Water	Mass (g)	NA	0.403±0.080	0.550±0.131	NA	NA
	No Damage	Mass (g)	0.230±0.013	0.520±0.109	0.463±0.062	NA	NA
	Manduca	Proportion (% of total)	NA	9.9±1.3	**11.2±1.0 a**	NA	NA
	Water	Proportion (% of total)	NA	6.7±1.3	**7.5±1.6 ab**	NA	NA
	No Damage	Proportion (% of total)	5.2±0.5	8.7±1.9	**6.5±0.9 b**	NA	NA
Damaged Leaves	Manduca	Mass (g)	NA	1.335±0.026	1.368±0.045	NA	NA
	Water	Mass (g)	NA	1.447±0.058	1.473±0.085	NA	NA
	No Damage	Mass (g)	1.127±0.049	1.302±0.098	1.612±0.122	NA	NA
	Manduca	Proportion (% of total)	NA	23.9±0.8	22.5±0.6	NA	NA
	Water	Proportion (% of total)	NA	23.9±0.9	20.8±1.5	NA	NA
	No Damage	Proportion (% of total)	24.8±1.1	21.5±1.3	22.5±0.6	NA	NA
Old Leaves	Manduca	Mass (g)	NA	**0.838±0.051 a**	**0.920±0.073 a**	NA	NA
	Water	Mass (g)	NA	**1.067±0.124 ab**	**1.300±0.090 ab**	NA	NA
	No Damage	Mass (g)	0.905±0.112	**1.208±0.077 b**	**1.095±0.124 b**	NA	NA
	Manduca	Proportion (% of total)	NA	**15.0±0.9 a**	15.1±1.0	NA	NA
	Water	Proportion (% of total)	NA	**17.4±1.3 ab**	18.1±1.0	NA	NA
	No Damage	Proportion (% of total)	19.3±1.2	**20.1±1.4 b**	15.2±1.2	NA	NA
Stem	Manduca	Mass (g)	NA	1.790±0.122	**2.032±0.157 a**	2.330±0.089	2.518±0.117
	Water	Mass (g)	NA	1.895±0.122	**2.557±0.105 ab**	2.293±0.102	2.328±0.193
	No Damage	Mass (g)	1.383±0.126	1.827±0.149	**2.668±0.096 b**	2.560±0.163	2.507±0.092
	Manduca	Proportion (% of total)	NA	31.8±1.3	**33.0±1.4 a**	67.2±1.7	67.2±0.8
	Water	Proportion (% of total)	NA	31.1±0.6	**35.6±0.4 ab**	66.9±0.4	66.7±1.5
	No Damage	Proportion (% of total)	29.8±0.9	30.1±1.9	**37.6±1.0 b**	67.5±2.3	69.0±1.9

Roots	Manduca	Mass (g)	NA	**0.850±0.046 a**	0.907±0.050	1.100±0.095	1.032±0.062
	Water	Mass (g)	NA	**1.058±0.047 ab**	0.997±0.044	1.090±0.064	1.017±0.043
	No Damage	Mass (g)	0.822±0.122	**1.048±0.061 b**	0.968±0.082	1.190±0.122	0.982±0.71
	Manduca	Proportion (% of total)	NA	15.2±0.7	14.9±0.8	31.5±2.0	27.7±1.7
	Water	Proportion (% of total)	NA	17.5±0.6	13.9±0.6	31.7±0.5	29.7±1.4
	No Damage	Proportion (% of total)	17.5±1.3	17.4±1.1	13.6±0.9	31.3±2.2	26.9±1.5
Regrown Leaves	Manduca	Mass (g)	NA	NA	NA	0.043±0.018	0.195±0.048
	Water	Mass (g)	NA	NA	NA	0.049±0.018	0.120±0.007
	No Damage	Mass (g)	NA	NA	NA	0.054±0.016	0.148±0.026
	Manduca	Proportion (% of total)	NA	NA	NA	1.3±0.6	5.1±1.1
	Water	Proportion (% of total)	NA	NA	NA	1.4±0.5	3.5±0.3
	No Damage	Proportion (% of total)	NA	NA	NA	1.3±0.4	4.1±0.6

Carbon:Nitrogen

Tissue	Treatment	Measurement	Harvest 1 (Day 0)	Harvest 2 (Day 2)	Harvest 3 (Day 6)	Harvest 4 (Day 11)	Harvest 5 (Day 20)
Apex	Manduca	Ratio	NA	12.482± 0.623	13.121±0.293	NA	NA
	Water	Ratio	NA	12.476± 0.153	14.036±0.479	NA	NA
	No Damage	Ratio	9.670±0.354	12.435± 0.168	13.768±0.471	NA	NA
Young Leaves	Manduca	Ratio	NA	16.278± 1.059	18.143±0.885	NA	NA
	Water	Ratio	NA	16.547± 0.351	19.010±0.628	NA	NA
	No Damage	Ratio	11.768±0.565	16.597± 0.730	18.905±0.901	NA	NA
Damaged Leaves	Manduca	Ratio	NA	18.150± 1.222	20.055±1.129	NA	NA
	Water	Ratio	NA	18.752± 0.483	21.601±0.776	NA	NA
	No Damage	Ratio	13.788±0.860	19.364± 0.956	21.600±0.807	NA	NA
Old Leaves	Manduca	Ratio	NA	22.397± 1.662	25.338±1.710	NA	NA
	Water	Ratio	NA	23.304± 0.834	26.677±1.056	NA	NA
	No Damage	Ratio	17.151±0.998	24.227± 1.491	26.853±0.621	NA	NA
Stem	Manduca	Ratio	NA	37.044± 2.129	44.319±1.510	42.373±1.579	46.753±1.750
	Water	Ratio	NA	40.897± 0.780	48.086±0.909	44.554±1.545	43.520±1.448
	No Damage	Ratio	30.395±2.129	41.499± 1.472	45.179±2.987	47.234±0.709	48.812±1.537
Roots	Manduca	Ratio	NA	**28.180± 0.719 a**	33.719±1.276	34.584±1.081	36.493±0.479
	Water	Ratio	NA	**29.383± 0.392 ab**	34.500±0.769	33.716±0.624	33.980±0.959
	No Damage	Ratio	25.396±1.278	**30.738± 0.656 b**	34.318±0.788	34.412±0.521	35.897±0.939
Regrown Leaves	Manduca	Ratio	NA	NA	NA	16.362±1.158	**12.353±0.417 a**
	Water	Ratio	NA	NA	NA	16.076±0.782	**10.067±0.290 b**
	No Damage	Ratio	NA	NA	NA	15.538±0.946	**10.779±0.432 b**

Table 1. Compiled Results for Sequential Harvest Experiment. Within each harvest, mean dry mass and mean proportion of total dry mass, mean concentration of nitrogen and carbon and mean proportion of total plant nitrogen and carbon, as well as the mean carbon to nitrogen ratio of all tissues that were present at that harvest are shown. Harvest/tissue combinations in bold had statistically significant differences between treatments ($p < 0.05$). All $n = 6$. Like letters represent differences in means that were not statistically significant.

Defoliated Material

Tissue	Treatment	Nitrogen conc. (mg/g)	Carbon conc. (mg/g)	Dry Mass (g)	Carbon:Nitrogen Ratio
Apex	Manduca	33.24±0.73	446.66±8.47	**0.209±0.017 a**	13.475±0.190
	Water	32.72±0.79	441.29±4.99	**0.207±0.023 a**	13.589±0.284
	No Damage	30.72±0.77	433.96±5.52	**0.277±0.020 b**	14.224±0.283
Young Leaves	Manduca	21.87±0.60	**410.22±2.33 a**	**0.802±0.043 a**	18.980±0.488
	Water	21.94±0.57	**409.94±2.04 a**	**0.584±0.053 b**	18.844±0.378
	No Damage	20.28±0.51	**402.59±1.87 b**	**0.596±0.053 b**	20.035±0.436
Damaged Leaves	Manduca	18.57±0.56	390.94±2.50	1.457±0.035	21.361±0.601
	Water	18.52±0.48	392.21±1.55	1.496±0.037	21.385±0.483
	No Damage	17.41±0.39	390.22±1.79	1.573±0.052	22.595±0.494
Old Leaves	Manduca	13.50±0.57	362.27±1.84	**0.832±0.047 a**	27.514±0.961
	Water	14.18±0.47	366.75±2.49	**1.093±0.061 b**	26.246±0.710
	No Damage	13.57±0.33	366.37±2.20	**1.080±0.068 b**	27.240±0.630

Table 2. Compiled Results for Defoliated Material from Harvest Three of Sequential Harvest Experiment. Within each harvest, mean dry mass, mean concentration of nitrogen and carbon, as well as the mean carbon to nitrogen ratio of defoliated tissues are shown. Harvest/tissue combinations in bold had statistically significant differences between treatments ($p < 0.05$). All n = 18. Like letters represent differences in means that were not statistically significant.

Discussion

Our results demonstrate that herbivory does modify the allocation of resources within a plant, and give a whole plant description of the carbon and nitrogen budgets of the plant for the duration of an herbivory event, from the start of damage through regrowth. Many of the observed modifications relative to the control plants, however, were not in the direction predicted by the induced sequestration model. When in interpreting these results, is important to remember that the data presented are all dynamic and interacting with each other in complex ways.

In terms of the relative dry mass of tissues, not surprisingly, the apices of the Manduca treated group harvested after 5 days of damage were smaller than the controls (Table 1). This makes sense in the context of induced sequestration, as the apex is the most actively growing part of the plant. This growth should continue as normal in the control plants, but continuing to invest resources in the expansion of a tissue that could be lost to an herbivore (as in the treated groups) would be risky. Additionally, if the wounding induces storage, there should be fewer resources available for growth. There is evidence that the application of methyl jasmonate, an important plant signaling molecule involved in the response to damage, limits leaf expansion (Moore et al., 2003). Steinbrenner et al., 2011, found a decrease in a number of soluble sugars in the apex in response to herbivory. It is not clear if these resources were being actively sequestered or just pulled into a different resource pool. Alternatively, growth at the apex may be limited in the damage groups by the induction of the production of defense compounds, since that production requires the investment of resources (Cipolini et al., 2003). The concentration of nitrogen in the apex was not significantly different from the controls, but the proportion of the total nitrogen in the plant was significantly lower in the apex. If the resources diverted from growth were going largely to defense investment in the apex, we would not expect to see this decrease, because those resources (carbon and nitrogen) would still be found in the tissue, just in

a different form. Just because the defense investment is not taking place in the apex does not mean that those resources are going toward storage.

The young leaves did not respond similarly to the apex. The young leaves made up a greater proportion of the dry mass as well as the total plant nitrogen in the Manduca treatment than in the No Damage treatment. There was not a net export of nitrogen from the young leaves, as we might expect under the induced sequestration model since they are herbivore accessible tissues. This effect is surprising, but optimal defense theory tells us that the relative value of a plant tissue will determine the amount of investment in their defense (Stamp, 2003). The young leaves are very valuable given their high photosynthetic capacity relative to older leaves, so they are expected to be well defended. There is evidence that much of the nitrogen going into the young leaves during the damage period was defense related, because in chapter 2 we showed chlorophyll levels decreased more rapidly in the treated groups during damage treatments, and chlorophyll is usually a good indication of the nitrogen status of a plant or tissue (Evans, 1989), but nitrogen content actually increased relative to controls based on the CN analysis results from this experiment (Table 1). This suggests that much of the nitrogen going into the young leaves is not being used to support photosynthesis, but rather secondary functions, such as defense. This still does not explain why we are seeing an increase in size in the young leaves in the damaged plants when the rest of the plant is actually growing less. It is possible that if expansion in the apical meristem is limited by some signaling from the jasmonic acid pathway, and investment in the damaged leaves is limited to defense, there are more resources available for growth in the young leaves. This hypothesis requires, however, that the mechanisms by which damage in the focal leaves modifies leaf expansion systemically, but on a tissue specific level.

The damage leaves showed an interesting consistency across treatments. There were no significant difference in any of the variables measured, though there did appear to be a slight increase in the concentration of nitrogen and slight decrease in the dry mass. Once again, as in the young leaves, there is clearly not a net export of nitrogen from these herbivore accessible leaves. Our analysis is unable to differentiate between different kinds of nitrogen. There are massive changes going on in the damaged leaves during an herbivory event (Steinbrenner et al., 2011), but apparently these changes maintain a balance in the overall nitrogen and carbon budget of the damaged tissues. This is surprising given the results of Gomez et al., 2010, which showed that the export of amino acids increased in plants treated with methyl jasmonate. Our results suggest that either this export is limited temporally (measurements in Gomez et al. were made 4 hours after treatment), or it is matched by an increase import of nitrogen from other parts of the plant. Less surprisingly, we also did not document any changes in the old leaves. These leaves are probably less relevant to the induced sequestration response, since they have relatively fewer resources in them to begin with (Korpita, unpublished data).

The results from the storage tissues also unfolded in unexpected ways. The induced sequestration model predicts that there should be an increase in concentration of resources in the herbivore inaccessible tissues in damaged plants. When protein, and not simply nitrogen, was measured in a previous experiment, there was in fact a significant increase in the stems in plant that were treated with simulated herbivory (Korpita, unpublished data). Unexpectedly, the pattern in nitrogen from the harvest three data did not display the same result. When the decreased size of the stem in the treated group is factored in, there is actually a significantly smaller proportion of the total plant carbon and nitrogen located in the stem. This does not mean, however, that storage of nitrogen was not taking place in the stems of these treated plants, only

that if it was, there was at least a proportional increase in the allocation of other components to the stem. Percent carbon and nitrogen measurements are naturally relative and the results of one will depend on the results of the other, as well as the concentration of other unmeasured elements. Still, the lower proportion of nitrogen in the herbivore inaccessible tissue of the stem does not exactly fit with the induced sequestration model. Much of the stem's growth occurs at the apex, and growth at the apex is retarded by the damage treatment. So these results may just an artifact of the decrease in apex growth rather than an "intentional" decrease in resource allocation to the stem tissue.

The material from the final harvest also show mixed results for our expectations based on the induced sequestration model. We had expected to see an increase in nitrogen in the stems at harvest 3 in the treated groups relative to the control, and then this excess nitrogen would be used to support greater regrowth after the defoliation event. The nitrogen levels in the treated groups' stems would return to the similar levels as the control groups as the excess nitrogen was used up in the faster regrowth. The regrown leaf material would be roughly equivalent in concentrations, but the difference would be in the biomass of the tissue the treated groups were able to regrow. We know from the results presented in the previous chapter that the greater regrowth does occur. The results from harvest three do not show an excess of nitrogen in the stem after the damage treatments, so although the concentration of nitrogen in the stems were not significantly different at harvest five, that cannot really be considered a "return" to baseline, as we had hypothesized. The damage treated plants did produce more regrown leaf material on average, though not significantly so due to high variation. Surprisingly, the concentrations of carbon and nitrogen were significantly lower in the Manduca treated group, though this could be due simply to their slightly greater size. The regrown leaves also represented a greater proportion, though not

significantly so, of the total plant nitrogen and carbon, which is what would be predicted by the induced sequestration model.

Where previous studies (Babst et.al 2008; Schwatchje et al., 2006; Gomez et al., 2010) have examined the induction of the *movement* of resources after herbivory or herbivory simulation, we specifically examined how herbivory influences the *accumulation* of these mobilized resources. Having previously demonstrated (chapter 2) that the induced sequestration model is correct in predicting that the induced shift in resource allocation will allow plants to better tolerate herbivory, we also attempted to verify that this was in fact related to the changes in resource allocation patterns. On the first note, we did show that simulated herbivory drastically changes the resources allocation patterns between tissues within plants, and this is largely driven by changes in the relative sizes of the tissues, though the direction of change was not always what the model we presented in the introduction would suggest. Similarly, where changes in concentration did occur, as often as not they were in the opposite direction as was predicted. The results from the regrowth harvest were also ambiguous in terms of the predictions made in this thesis' introduction. Due to high variation, most of the results were not significant. Those that were, the concentration of carbon and nitrogen in the regrown leaf material, is what we least expected to change, and may be different for reasons not directly related to their treatment.

It is important to remember when looking at these results, that they are measurements of total carbon and nitrogen in the plant tissues. These are just corollaries for the measurements that are actually relevant for the plants regulation through the induced sequestration response, though in practice impossible. These are measurements of the labile resource pool, those resources which are not tied up in another function, like a cell wall or DNA molecule. These are the only

resources that the plant can use and "decide" what to do with them (invest in storage, growth, or defense). Our ultimate goal is to understand what "choices" the plant is making during an herbivory event and what influences the "decision". To a certain degree, our measurements reflect historical "choices" made by the plant. Unfortunately, it is an imperfect reflection, because the "decisions" made by the plant rarely have clear cut, independent implications for the allocation of elemental carbon and nitrogen, but we can still get a very good idea of what is the functional result for the plant.

Moving forward, it will be important to incorporate more direct measurements of the three main possible "choices" (storage, defense, and growth/reproduction) that a plant can use its labile resource pool for in experiments that examine the induced sequestration model. We have a pretty good understanding of how herbivory induces defense production (Karban and Baldwin, 1997). Growth is not terribly difficult to measure, though looking into the underlying mechanisms such as leaf expansion would be useful. Understanding the mechanisms that result in the herbivore induced storage of resources will be important to our understanding herbivore induced resource allocation shift (discussed further in the conclusion). Once we have an accurate measurement of the mechanisms that result in all the possible "choices" for the labile resource pool, and can investigate them simultaneously, we will be able to more fully understand how plants adaptively modify their phenotype to deal with herbivory and other stresses.

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Chapter 4: Conclusion

Induced Sequestration: Model Refinement and Future Directions

The experiments of this thesis returned interesting results, and furthered our understanding of the induced sequestration response. We demonstrated with the results presented in chapter 2 that plants treated with simulated herbivory show a decreased investment in growth during the treatment and are better able to recover from the loss of leaf area relative to untreated plants. The results from the second experiment, presented in chapter 3, showed that there are a number of changes in relative resources allocation that occur during herbivory, and that this influences performance during regrowth.

While the results of the regrowth experiment played out almost exactly as expected based on our predictions from the induced sequestration model, the sequential harvest experiment showed that the mechanisms that result in this increased tolerance are more complex than expected. First of all, there was a net movement of nitrogen into herbivore accessible tissues. This is surprising because we anticipated that the plant would modify its overall allocation to minimize potential loses to the herbivore, and given previous results that show an increased export of nitrogen from the roots in response to simulated herbivory (Gomez et al., 2010). The total nitrogen (and carbon as well) budget of the plant, which we measured, includes a vast array of molecules with many different functions, and this net influx was likely driven by upregulation of defense compounds outweighing the removal of other labile nitrogen sources.

The lack of evidence from the second experiment of an increase in resource allocation to the storage tissues was also surprising. The most likely explanation, based on our previous results (Korpita unpublished data), is that in the stem of tomato, our analysis was incapable of identifying the changes in the labile resource pool amidst the consistency of the relatively much

larger non-labile pool across treatments. Compared to other tissues, a very high proportion of the carbon and nitrogen of the tissue is bound in non-labile, structural elements, (cell walls, etc.). These resources, while they would be picked up by our analysis, we would not expect them to participate in the induced sequestration response. So with these results, I still do believe that storage is taking place in the stems in tomatoes in response to herbivory, just this analysis did not pick it up. This lack of detected storage made it difficult to verify that stored resources were preferentially remobilized to support regrowth, another prediction of the model.

When we incorporated the distribution of biomass in treated plants into our results, we found some patterns that we expected based on our current predictions for the induced sequestration model, and others that were quite surprising. Expected was the observed decrease investment in apex tissue in treated groups, but unexpected was the apparent increase in proportional mass in the young undamaged leaves and decrease in proportional mass in the stems relative to control groups. The relative dry mass of tissues might change regardless of if there were sequestration of resources induced by herbivory, but its inclusion in our analysis is relevant. Ultimately, there are two potential, non-mutually exclusive scenarios in which the predictions outlined in the induced sequestration model could play out and still be correct. The resource allocation shift could happen in such a way so that the plant has a smaller proportion of its resources in herbivore accessible tissues. Possibly independent of this, or as a cause or result of it, the plant could decrease storage in accessible tissues and upregulate them in inaccessible tissues. The theoretical "goal" of the plant would be the same in either scenario, to have more resources available for the tolerance of the herbivory event. Our results go solidly against the first scenario. A greater proportion of resources are located in tissues accessible to the herbivore. We have to keep in mind, however, that the induced sequestration response of the plant is

constrained by other factors. There are a number of limits to plant phenotypic plasticity (DeWitt et al., 1998). The plant has other requirements that have to be met, such as a certain leaf area to fix carbon, that limit the degree to which its resource allocation patterns can be specifically tailored to respond to the herbivore.

Since the first scenario is not supported, yet the documented resource shift did increase tolerance, we have to assume that the second scenario is taking place while simultaneously the opposite of the first scenario is occurring. We have to assume the plant is sequestering resources, and will mobilize them after the herbivore passes on. It did not show up in our analysis with this experiment, but did in previous studies (Korpita, unpublished data). Our analysis, of purely carbon and nitrogen is likely insensitive in tissues, such as stems, where the labile resource pool in far outweighed by the non-labile pool.

Moving forward with the induced sequestration model, it will be important to investigate the mechanisms underlying the induced sequestration response. What signaling pathways are driving the change in resource allocation patterns? Where exactly are the shifted resources coming from, where are they going, how do they get there, and what form do they take when they are there? This will allow us to better understand what shifts in resource allocation are actually the result of active regulation by the plant, and what effects may just be a side effect of other manipulations.

For nitrogenous resources at least, some of the answers may lie in vegetative storage proteins (VSPs). VSPs are a diverse category of proteins, defined by their function, which is to transiently store nitrogen and be preferentially broken down when the nitrogen is needed, and perform no other major function during their lifetime (Staswick, 1994). This could be during leaf

flushing in deciduous trees after a dormant winter, or when a strong nitrogen sink such as a seed pod begins to outstrip the formerly excessive nitrogen uptake of a leguminous plant (Staswick, 1994). If the herbivory damage treatment induces the expression of a VSP in the stem of tomato that could both be the storage in an herbivore inaccessible tissue predicted by the induced sequestration model, and a major sink for nitrogen driving the export from the vulnerable tissues that Gomez et al. (2010) and Steinbrenner et al. (2011) provide evidence for. There is substantial evidence to suggest that a VSP could be playing this role. First of all, methyl jasmonate, a well-known defense response elicitor (Thaler et al., 1996, McConn et al., 1997) upregulates the production of VSPs in soybean (Staswick 1994), a related VSP in *Arapidopsis thaliana* (Berger et al., 1995 and *Brassica napus* (Rossato et al., 2002). An unrelated VSP is also upregulated in *Medicago sativa* by jasmonate application, which also modifies the N partitioning of the entire plant (Meuriot et al., 2004). Wounding also upregulated the production of a soybean VSP related protein in poplar (Veljanovski et al., 2010). There is also evidence that soybean VSP and its interspecific relatives are derived evolutionarily from an acid phosphatase (Staswick, 1994). Acid phosphatases are known to have protective properties (Williamson and Colwell, 1991). The acid phosphatase VSP in *Arabidopsis* was shown to have anti-herbivore properties (Liu et al, 2005). If a similar protein were expressed as part of the induced sequestration response, it could be both storing nitrogen temporarily, as well as serving a defense function, particularly useful during an herbivore attack. The discovery of a wound induced VSP, particularly one that is preferentially degraded during regrowth, would be a true smoking gun confirming the existence of induced sequestration. An attempt was made during this thesis to find this protein, but tomato stem proved a difficult tissue to perform protein analysis on, given its high structural carbohydrate and low protein concentration.

If, as we are proposing, there is a storage protein massively upregulated in the tomato stem, it begs the question, where is the nitrogen used in the VSP coming from? The answer is likely from a diverse array of sources, both recently assimilated and removed and exported from the leaves, but given its incredible abundance in plant photosynthetic tissue, Ribulose 1,5 biphosphate carboxylase (Rubisco) degradation could be the source of a huge quantity of the nitrogen going into a VSP. Rubisco has been previously shown to be an important source of mobilizable nitrogen (Schiltz et al., 2004). Multiple stresses have been shown to accelerate the degradation of Rubisco (Feller et al., 2007), and this could theoretically be controlled by the same pathways controlling VSP production in the stem (jasmonate pathway).

If these predictions prove true, it will allow better characterization of the induced sequestration response. Instead of measuring total nitrogen or total protein, we could measure the shifts in abundance of these specific proteins, assuming that their shifts are what is driving the observed effect of increased tolerance. This should allow us to better separate constitutive storage reserves or those that are merely the consequence of another response from those specifically upregulated by the damage.

The goal of future studies should be to attempt to measure the "choices" an herbivore attacked plant is making with regards to the allocation of its resource pool, how that varies across tissue types, and the effect of these choices on tolerance. The three main options are putting resources into defense, growth, or storage. If representative mechanisms driving each of these three options are quantified simultaneously, across tissues, we should be able to get a great picture of the "decision" made with the labile resource pool. Defensive compounds can be directly quantified, and growth measurements are somewhat straight forward. If the mechanisms underlying the induction of storage becomes better understood, we could measure that response

and determine an approximate proportion of the resources available for a response to herbivory, used in each possible "choice". With an experiment like that we will be able to determine the relative significance of an induced storage response to the overall strategy of the plant to mitigate the negative effect of herbivores.

To conclude, I would like to summarize the details of the induced sequestration model that we have refined based on our analysis of the results from the experiments presented in this thesis, and well as the literature. Many of these predictions remain unsupported by data, but should be viewed as a working model and used to direct further investigation. When a plant is attacked by a leaf chewing herbivore, its metabolism will reconfigure in a number of ways. In the tissues accessible to the herbivore, some of the Rubisco is broken down into its amino acid constituents, and chlorophyll is broken down. Some of this nitrogen is exported, and much of it is converted to defensive compounds. The growth pattern of the vulnerable tissues are modified on a tissue specific level, minimizing new investments in growth in tissues with local damage, as well as yet unexpanded leaf material. The nitrogen mobilized from the degradation of Rubisco in the leaves is sent to the storage tissues, where some of it is metabolized to defense compounds, which are sent back into the leaf tissue, and the remainder is used in the synthesis of storage protein in the stems. Carbon allocation shifts are not as significant, and are limited to the tissues whose growth rates are modified. After the damage has ended, the stem storage protein will be preferentially broken down to supply amino acids to regrowth and repair of the damaged tissues. Ultimately, this allows the plant to produce more flowers and seeds than it would be able to if the damage did not induce these resource allocation shifts.

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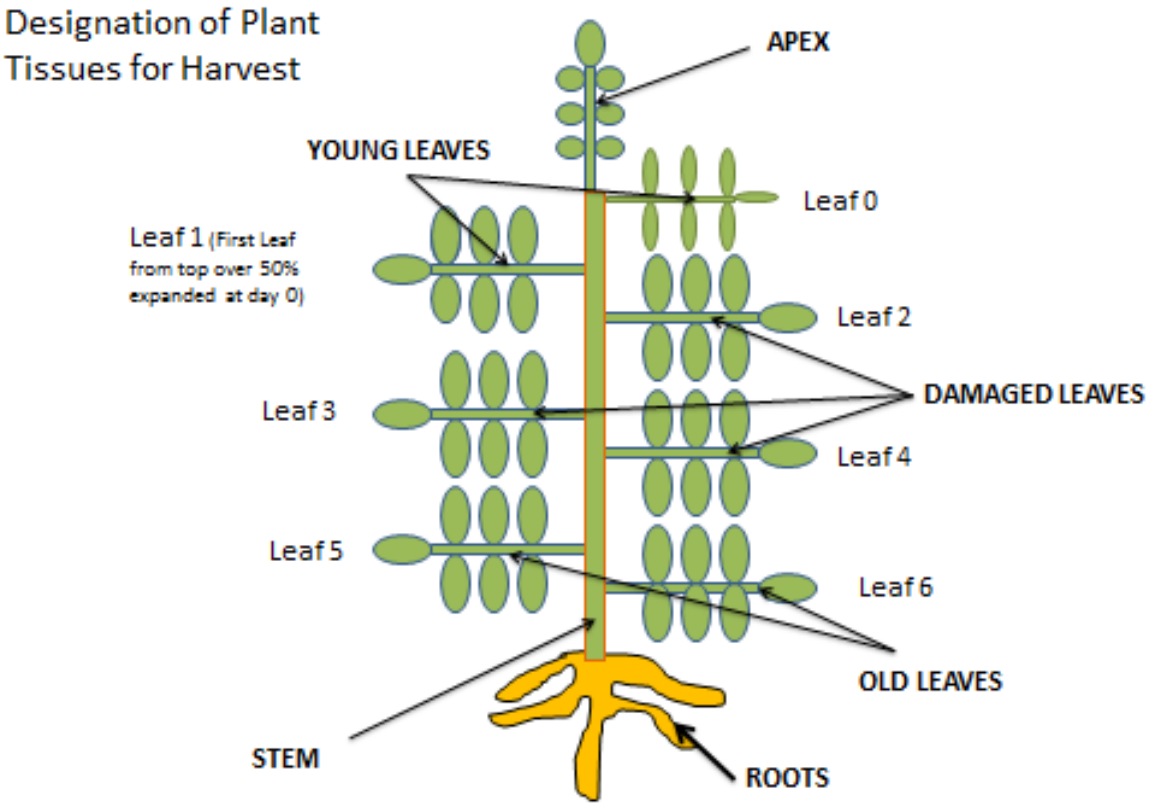
Appendix 1


Designation of Plant Tissues for Harvest
APEX
YOUNG LEAVES
Leaf 0
Leaf 1 (First Leaf from top over 50% expanded at day 0)
Leaf 2
DAMAGED LEAVES
Leaf 3
Leaf 4
Leaf 5
Leaf 6
OLD LEAVES
STEM
ROOTS