

**IMPACT OF ATTACK BY GALLMAKERS ON THEIR ANCESTRAL AND
NOVEL PLANT HOSTS IN A GOLDENROD-INSECT SYSTEM**

by

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ABSTRACT

Through coevolution reduced virulence and increased tolerance may evolve. Novel plant hosts may experience greater impact from herbivores compared to ancestral hosts. I tested this using *Solidago altissima* and *Solidago gigantea* as host plants and *Eurosta solidaginis* and *Rhopalomyia solidaginis* as gallmakers. In 4 out of 8 data sets, both gallmakers significantly reduced the aboveground biomass (by about 30%) in the novel host. (Overall, 7 out of 8 data sets showed a trend in the expected direction.). Coupled with previous data sets from Heard and Kitts 2012 on *Gnorimoschema gallaesolidaginis*, 6 out of 10 data show significant support for this coevolutionary hypothesis (9 out of 10 in the expected direction; sign test $P = 0.011$). In addition to herbivory, plants may also experience abiotic stressors. By inducing water stress and clipping on *S. altissima* and *S. gigantea* I found that *S. altissima* experiences synergistically greater damage when two stressors are present.

Keywords: *S. altissima*; *S. gigantea*; gallmakers; coevolution; virulence; tolerance; drought; insect-plant interactions; host shifts; synergy

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"Never give up, Never surrender"

-Galaxy Quest

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CHAPTER 1- GENERAL INTRODUCTION

What is coevolution and its importance

Earth's biodiversity and richness are shaped by many evolutionary forces and mechanisms. Species often interact with one another, giving rise to potential coevolution. Janzen (1980) was among the first to coin the term "coevolution" on a macroevolutionary level as "an evolutionary change in a trait of the individuals in one population in response to a trait of the individual of a second population, followed by an evolutionary response by the second population to the change in the first". This process has played an important role in shaping our current biodiversity as well as catalyzing some key events in earth's history (Thompson 1994; Janzen 1980). Coevolved symbiosis lead to the formation of mitochondria and chloroplasts, which gave rise to eukaryotic cells and plants. Some of the more recent applications of coevolution can be found in the medical field. Many treatments against pathogens lose effectiveness over time as a result of coevolution in resistance and/or tolerance. Through detailed understanding of how our immune system coevolves with pathogens, we can develop more appropriate treatments for infections such as malaria or hepatitis C (Rehermann 2009; Hill et al. 1997). In recent years there has been an increase of research done on this evolutionary force, highlighting its importance and economic value to our society.

Coevolution in the context of host-enemy interactions

The examples mentioned previously highlight coevolution in the context of host-enemy interactions. Certain traits in hosts might be favored through coevolution in response to being attacked by enemies. A host's resistance and tolerance might be affected, and the enemies' virulence also might change as they coevolve. It is in the best

interest of the host to avoid the attack in the first place. This avoidance is known as resistance, and can become more common as the host will evolve appropriate traits. However, once the natural enemy overcomes this resistance, the impact of damage can be mitigated by the host's tolerance. Through growth, reproduction, and other mechanisms, the host can maintain its fitness despite parasite attack (Fornoni 2011). As hosts and their enemies coevolve, higher tolerance and resistance might be favored for the host. The enemy may also evolve higher or lower virulence depending on what maximizes its reproductive output (Alizon et al. 2009). If the enemy is an obligatory parasite that benefits from its host dying, it might be beneficial to evolve higher virulence because a host's death favors parasite's transmission to new hosts (Berenos et al. 2009). For instance, some nematodes need to kill their host in order to be able to spread (Poinar 1975). However, if the parasite depends on the host's wellbeing to exploit important resources, then it is likely to evolve lower or intermediate virulence (Alizon et al. 2009).

Although many studies have investigated the relationship of resistance and virulence for host-enemy interactions, tolerance has been poorly studied. It is difficult to separate tolerance and virulence in such close associations, and in many cases tolerance goes unstudied, or is confounded, in comparisons with virulence. For example, Heard and Kitts 2012 were able to measure herbivore impact on ancestral and novel plant hosts from insect-induced galls; however, they were unable to separate if the impact difference was due to reduced virulence in herbivores or increased tolerance in plants. Medical researchers have started to investigate the genetic makeup of tolerance and virulence of specific parasites and hosts. This might not be the clearest or most feasible way to tease virulence apart from tolerance; however, it is a start.

Coevolution in context of plant-herbivore interactions

Plant-insect communities form a large portion of the earth's biodiversity, playing an important role in crop production, ecosystems, and the global economy. Understanding how the underlying evolutionary mechanisms, such as coevolution, function with respect to such associations is helpful in understanding speciation and abundance of species, as well as the impacts of insects on host plants. In most cases insect herbivores influence primary productivity of plants by damaging or reducing their photosynthetic area and fitness (Barbour et al. 1987). In most cases, however, attack by insect herbivores does not directly cause mortality of adult plants; and this is particularly true for gallmakers, which fit comfortably into the host-parasite category as described above (Abrahamson and Weis 1997).

Herbivorous insects can damage plants in a wide variety of ways. Defoliating and chewing insects form the majority of herbivores, and use their mandibles to feed on external plant tissue (Carlos and Pellmyr 2002). Insects can also damage plants by extracting plant fluids through specialized tubular mouthparts (Carlos and Pellmyr 2002). Gallmakers, on the other hand, are a more specialized class of herbivore that can manipulate their plant host's tissue to form a gall, or tumour-like structure. They spend part of their life cycle in the protective casing of the gall, feeding on highly nutritious plant tissues of their hosts (Abrahamson and Weis 1997). Such specialization can form a strong coevolutionary bond which may also lead to formation of host-specific races and, later, speciation (Stireman et al. 2005). Host shifts are a common occurrence among insect herbivores (Thompson and Pellmyr 1991). Most commonly the herbivore expands its diet to include a host closely related to its original host, and in doing so, it forms a new

association without any previous evolutionary history. This might occur for exotic hosts as well, which expand their range and invade a new community. Colonization of new hosts and its ecological consequences can lead to divergence in the host's traits and lead to speciation (Thomas et al. 1987; Fordyce 2010). Conservation challenges may also arise if insects start attacking exotic hosts, and vice versa (Shapiro 2002).

In most plant-insect associations, plant defense mechanisms such as tolerance should increase with coevolution, as selection will favor more tolerant plants. At the same time, at least some herbivores might decrease their virulence toward the plant host because this will increase the availability of resource for exploitation. However, when a novel plant-herbivore association forms, as with a recent host shift, increased tolerance and decreased virulence will take time to evolve. Hence, the novel host might experience higher herbivore impact initially, as compared to the ancestral host (Heard and Kitts 2012). Such response might not be true for all plant-insect associations, however there is a strong adaptive indication for increased tolerance from herbivore damage (Stowe et al. 2000; Nunez-Farfan, Fornoni and Valverde 2007).

Coevolution and introduced species

The idea of host shifts might be extended and applied to introduced and invasive species. When an introduced species expands its geographic range it is faced with multiple potential new hosts (when it's an insect), or herbivores (if it's a plant) (Keane and Crawley 2002; Maron and Vila 2001; Colautti 2004). A lot of research has been conducted on the enemy release hypothesis, which states that an introduced species will escape natural enemies in the new range and therefore have more resources to enhance its fitness (Keane and Crawley 2002;). For example, when plants invade a new habitat, they

may face weaker selection from herbivores (enemy escape), and therefore reallocate the resources previously used for defense towards other plant tissues (Zangerl and Berenbaum 2005). While this expectation seems intuitive, it might also be possible for an introduced plant species to experience greater enemy pressure, because it is impacted more by novel herbivores than by native herbivores in its original range. Coevolutionary theory predicts that a novel host might experience higher herbivore impact than a coevolved native host (Hallett 2006). Application of this theory, which has only recently started to get more attention, might help us further understand why some species become invasive while others do not, and give an alternative to the enemy release hypothesis.

Invasive species and biocontrols

Plant-herbivore interactions play an important role in agriculture and our economy, particularly when it comes to invasive species and conservation approaches towards them. Using biocontrol in managing invasive species requires care and planning. Biological control is the introduction of some sort of natural enemy in order to reduce the adverse effect of the pest. Effectiveness of a biocontrol program is measured based on a reduction of the pest abundance below a target level, usually related to an economic threshold (Holt and Hochberg 1997). A common mistake in controlling a target species is to assume that it is evolutionarily independent from other species. Many successful biocontrol programs have involved the application of parasitoids and microbial agents (Channer and Gowen 1992; Waage and Greathead 1988). Poor understanding of how species can coevolve with the biocontrol agents may lead to release of the pest from control. As an example, a baculovirus pathogen was applied to control the rhinoceros beetle (*Oryctes rhinoceros*), which is a serious pest on coconut and oil palms in South

Asian islands (Young 1986). Beetle densities were suppressed for a while, but after a few years outbreaks started to recur, suggesting a possible evolved resistance and/or tolerance towards the control agent. Based on experimental data, it was found that the pathogen's virulence had decreased in beetle populations from agricultural habitats (Zelazny et al. 1989). Therefore the effectiveness of biocontrol can depend on the potential or historic coevolutionary relationship between the agent and its target host. Poor understanding of coevolution in biocontrols might lead to biocontrol release or in some instances unpredictable impacts on non-target species (Louda et al. 2003). Given coevolution it might be difficult to have long term appropriate biocontrols, however, in-depth understanding of coevolution can benefit in understanding and predicting such interactions so that biocontrols are applied appropriately.

Host shifts in sympatric populations

When species expand their dietary range coupled with geographic range, these new habitats might have strong effects on the species and mask any coevolutionary patterns. This problem might be mitigated by studying coevolution in sympatric host shifts between closely related species and specialist herbivores. Although such shifts may be less common, they have similar implications as geographic host shifts. Ancestral and newly established coevolved associations may be compared, giving a clearer understanding of how species coevolve with time and how their traits change in response. This understanding can then be applied to biocontrols and invasive species conservation.

Environmental effects on coevolved associations

Even when we study sympatric populations and host shifts, environmental fluctuations might impact coevolutionary patterns and complicate our inference. Changes

in the environment, such as climate change, can affect plant-herbivore interactions in many ways, influencing (for example) their defense mechanisms or life histories (Bale et al. 2002). This in turn may disturb the strength of plant-herbivore interactions and may alter the surrounding ecosystem composition. Abiotic stress such as drought can lead to drastic changes in plant metabolism and defense mechanisms, potentially leaving the plants vulnerable to higher impact from herbivores (Gutbrodt et al. 2011; Haugen et al. 2008). With increasing global temperatures we might expect such stressors to interfere with plant-insect associations more often, and more severely. For example, Bansal et al. 2013, have found synergistic impacts on plants under simultaneous drought stress and herbivore attack . If drought stress in combination with herbivory inflicts greater damage than herbivory by itself, then abiotic conditions will modify the herbivore impact that would otherwise be expected from the coevolved relationship. Therefore it is not only important to study how coevolution influences invasive species, biocontrols, and host shifts, but also how environmental changes might impact the coevolutionary force, since this interaction may overpower and mask the strength of herbivore impact that would have occurred otherwise.

Outline of thesis research

This thesis addresses two major questions regarding coevolution in plant-insect associations. First, I propose that ancestral plant-herbivore interactions, in gallmaker-goldenrod system, experience higher tolerance and lower virulence compared to novel plant-herbivore interactions. This would be because ancestral associations have had time for coevolution to shape these traits, whereas novel associations have not. Differences in these traits between novel and ancestral interactions are assessed by measuring herbivore

impact (measured as reduction in aboveground biomass), because as discussed earlier, it is difficult to separate tolerance from virulence. Second, I explored whether environmental conditions such as drought have an important effect on already coevolved relationships, and if in the short term they can overpower effects of coevolution, masking such patterns of reduced virulence and increased tolerance.

Gallmakers and goldenrods can be used as a model system to study the evolution of herbivores' impact on their hosts. On several occasions gallmakers attacking goldenrods have undergone host shifts between goldenrod species (Abrahamson and Weis 1997; Stireman et al. 2005). *Rhopalomyia solidaginis/capitata* (Diptera: Cecidomyiidae), *Gnorimoschema gallaesolidaginis* (Lepidoptera: Gelechiidae), and *Eurosta solidaginis* (Diptera: Tephritidae) each possess an ancestral and novel host-pair association with *Solidago altissima* and *S. gigantea*, respectively (Stireman et al. 2005). The hypothesis that novel host associations should experience higher herbivore impact initially (time varying based on species and other environmental factors) compared to ancestral associations can therefore be explored using these gallmakers and their appropriate goldenrod hosts. The interaction of *Gnorimoschema* with its ancestral and novel hosts has already been found to follow these predictions (Heard and Kitts 2012). Heard and Kitts 2012 found that aboveground biomass of the novel host compared to the ancestral host was significantly reduced from *Gnorimoschema* attack, hence indicating that a higher impact was inflicted on the novel host. Patterns of impact for *Eurosta* and *Rhopalomyia* in the study system are unknown . A further benefit of using the goldenrod gallmaker system is that each of these host shifts occurred in sympatric populations. This helps to

reduce any environmental confounding that might impede inference when host shifts happen in association with geographic range shifts.

I collected galled and ungalled goldenrods in two sympatric populations, in order to compare herbivore impact between novel and ancestral herbivore-host association. I found support for the coevolutionary hypothesis of novel plant hosts experiencing higher herbivore impact than ancestral plant hosts. This supports the hypothesis that herbivore-host coevolution can lead to increased tolerance and/or decreased virulence.

One set of collections (Iowa 2012), however, contrasted with our other results: there was more impact on the ancestral host than the novel one. I speculated that abiotic stress, such as drought, could have caused this. For my second objective I applied drought and mechanical damage to *S. altissima* and *S. gigantea* to test whether drought conditions can affect plant defense mechanisms and thus mask coevolutionary effects on strength of herbivore impact. I found that drought and mechanical damage have synergistic effects on growth in *S. altissima*. This supported my speculation that drought can lead plants to experience greater impact from herbivores. Failing to make this realization would have led me to incorrect conclusions about the coevolutionary pattern herbivore impact on the *Solidago* system.

My results can further strengthen the field of coevolutionary theory in plant-insect interactions, particularly in gallmaker-goldenrod associations, and be applied towards invasive species management and biocontrols, bringing insight into plant-insect communities and economic benefits.

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**CHAPTER 2- IMPACT OF ATTACK BY *EUROSTA* AND *RHOPALOMYIA* ON
THEIR ANCESRAL AND NOVEL *SOLIDAGO* HOSTS**

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Abstract:

Galling insects and their plant hosts may have close relationships leading to potential coevolution. New host pairs form through host shifts, and may lead to increased insect virulence and decreased plant tolerance on a novel plant host compared to the ancestral host. *Rhopalomyia solidaginis/capitata* (Diptera: Cecidomyiidae), and *Eurosta solidaginis* (Diptera: Tephritidae) are specialist gallmakers that possess an ancestral and a novel association with *Solidago altissima* and *S. gigantea*, respectively. We measured the aboveground biomass of both plant species to assess herbivore impact. With both gallmakers we found that the novel host (*S. gigantea*), in 4 out of 8 data sets from collections, experienced significantly greater impact in reduced aboveground biomass (by about 30%) compared to the ancestral host (*S. altissima*). In 7 out of 8 datasets, the trend was in the expected direction (sign test, $P = 0.011$).

Introduction:

It is rare for selection to act on one species independently (Leimu et al. 2008). Quite often selection pressures affect a framework of interacting species, where adaptation by one species may impose selective forces on other interdependent species (Berenos et al. 2009; Thompson 1994). These close relationships may lead to coevolution, especially in host-enemy relationships (Berenos et al. 2009; Day and Burns 2003). This has been most thoroughly explored for hosts and parasites, in which the parasite induces damage, with more virulent parasites inflicting greater damage, and reduces the host's fitness (Strauss and Agrawal 1999; Read 1994). In response, some hosts may develop tolerance as a defense mechanism. A tolerant host reduces the negative

impact from the enemy through variety of mechanisms that do not cause fitness loss (Raberg et al. 2009; Boots 2008; Barker 1993; Clarke 1986).

As hosts and their parasites coevolve, selection pressures may favor host adaptations that give higher tolerance, reducing the impact of parasites on fitness (Raberg et al. 2009; Boots 2008; Raberg et al. 2007; Carr et al. 2006; Kover and Schaal 2002; Simms and Triplett 1994). In addition, selection on most specialist enemies should favor reduced virulence towards the hosts, unless the enemy depends on host's damage for transmission, in which case selection will favor increased virulence (Alizon et al. 2009; Ewald 1994). Virulence involves a trade-off between the exploitation of host resources and damage inflicted on the host. If there is too much damage to the host, in many cases this will lead to less resources being available to the parasite (Alizon et al. 2009; Frank 1996; Anderson and May 1979). If hosts are expected to evolve higher tolerance, and enemies lower virulence, as in our case as explained below, then the overall effect should be a decrease in the impact on host fitness over evolutionary time.

It is difficult to separate changes from virulence from changes in tolerance, because they are in a sense two sides of the same coin. When damage is inflicted through virulence, the damage is counteracted with the host's tolerance, and the joint outcome of the two factors is the parasitic impact (Restif and Koella 2004). Recent molecular research has made progress in analyzing different strains of viruses allowing us to quantify virulence strength (Peever et al. 2000; Upton et al. 1991). Once the strength of virulence is known, tolerance can be found as the byproduct of the impact from the enemy. A classic example of this is the myxoma virus in Australia's rabbit population. When the virus epidemic killed most of the rabbits, through coevolution they developed

higher tolerance to the virus, and in response the virus also developed lower virulence (Fenner and Woodroffe 1953). In the experimental setting, Best and Kerr (2000), could-cross infect tolerant and control rabbits with different strains of virus to measure tolerance. Peever et al. (2000) could also measure variation in tolerance and virulence of chestnut blight fungus by using genetic nucleotide sequencing to identify virus isolates. With the use of genetic tools and sequencing of virus strains, future studies might be able to independently measure virulence and tolerance and their interactions with coevolved associations.

While these expectations for host-enemy coevolution have largely been developed for animal and microbial systems, many plants and their insect herbivores may also fall into such framework (Sternberg et al. 2012; Heard and Kitts 2012). This is especially true for some specialist herbivores, such as gallmakers, which can have very close relationships with, and severe impacts on, their plant hosts. Since severe damage to the host should only decrease gallmaker transmission rates (that is, gallmaker survival and reproduction), we expect to see a reduced instead of increased virulence over evolutionary time. Gallmaker performance and benefits from damaging the plant will depend on resources available for exploitation, hence higher virulence might restrict those resources and therefore high virulence will not be beneficial. Tolerance as an herbivore defense strategy has been widely documented in plants (Raberg et al. 2009; Raberg et al. 2007; Carr et al. 2006; Kover and Schaal 2002; Simms and Triplett 1994). However, few studies have tested the prediction that, with coevolution, gallmaker impact will decline over time as plant hosts evolve higher tolerance towards herbivore damage, and gallmakers evolve lower virulence (Heard and Kitts 2012).

While many herbivores display host specificity, through evolutionary time it is common for them to switch plant hosts and thus extend their host range (Stireman et al. 2005). Novel plant-insect relationships can differ greatly from ancestral ones in many ways, but one important difference is that following a host shift, the herbivore has a long coevolutionary history with the ancestral host but a short one with the novel host. If tolerance and virulence evolve through time, we therefore predict that the novel host will experience higher impact from the herbivore, hindering plant growth and development.

It is important to study host shifts and how coevolution changes plant-herbivore interactions after the establishment of novel herbivore-host interactions because these factors can affect plant and insect abundance, community structure, as well as biodiversity (Fornoni 2011; Bush and Smith 1998). Studying how tolerance and virulence evolves can give us insight into speciation and how novel host-parasite races occur.

Gallmakers and goldenrods can be used as a model system to study the evolution of herbivores' impact on their hosts. On several occasions gallmakers attacking goldenrods have undergone host shifts between goldenrod species (Abrahamson and Weis 1997; Stireman et al. 2005). *Rhopalomyia solidaginis/capitata* (Diptera: Cecidomyiidae), *Gnorimoschema gallaesolidaginis* (Lepidoptera: Gelechiidae), and *Eurosta solidaginis* (Diptera: Tephritidae) each possess an ancestral and novel host-pair association with *Solidago altissima* and *S. gigantea* respectively (Stireman et al. 2005). The hypothesis that novel host associations should experience higher herbivore impact initially, compared to ancestral associations, can therefore be explored using these gallmakers and their appropriate goldenrod hosts. Supporting such predictions, Heard and Kitts (2012) have found greater impact on the novel host *S. gigantea* compared to *S. altissima* from

Gnorimoschema's attacks, but patterns of impact for *Eurosta* and *Rhopalomyia* are unknown.

We compared the impact of herbivory on novel and ancestral hosts for *Eurosta* and *Rhopalomyia*, using field collections. However, since the host shift in all instances occurred from *S. altissima* to *S. gigantea*, it would be hard to differentiate if *S. gigantea* experiences more herbivore impact due to the novelty of its coevolutionary association, or simply because it is susceptible to higher damage in general. In order to correct for this confounding effect, we examined the impact of mechanical damage to stems and leaves (clipping and defoliation), hypothesizing that because such damage isn't more novel to either species, they should respond similarly to it. Together, our analyses revealed a pattern of greater impact by herbivores attacking novel hosts, consistent across gallmaker species and across (most) sites and years.

Methods:

The following analysis involved several steps, with results from early steps used in subsequent steps. For clarity we describe these intermediate results in this Methods section.

1: Study System

We studied gallmaker impacts on the goldenrod species *Solidago altissima* L. (Asteraceae), and its close relative *Solidago gigantea* Ait. Their distribution ranges across most of North America, where they are commonly found growing in prairies, old fields, and disturbed habitats (Abrahamson and Weis 1997). These species are broadly sympatric, and at many sites coexist without interference (Abrahamson and Weis 1997). *S. altissima* and *S. gigantea* are perennials that can reproduce sexually through insect pollination and asexually via rhizomes, which overwinter and produce new shoots the

following spring. Plants usually flower through late summer to fall, when they start to senesce. Both species are hosts to many herbivores, of which *Eurosta solidaginis* (Fitch) (Diptera:Tephritidae) and *Rhopalomyia solidaginis* (Loew) (Diptera: Cecidomyiidae), are gallmaking specialists (Abrahamson and Weis 1997). Each of these gallmakers has undergone a host shift from *S. altissima* to *S. gigantea*, thus making those species ancestral and novel hosts, respectively (Stireman et al. 2005). The host shift from *Rhopalomyia* is the oldest, dating at least 2 million year ago, with little to no gene flow between the two host races. *Eurosta*, in contrast, shows considerable gene flow and its host shift is much more recent, dating no more than 200,000 years ago, and *Gnorimoschema gallaesolidaginis* (Riley) had a host shift between the times of the other two gallmakers (Stireman et al.2005).

A *Eurosta* female oviposits a single egg into the stem of a plant, with the larva later producing a large ball gall approximately 3-5 cm in size, by producing chemicals and hormones that mimic plant hormones. The overwintered larva emerges as an adult in late spring, where it mates and oviposits more eggs. Because the larva feeds on plant tissue within the gall, it hinders plant's fitness and reduces sexual reproduction (Abrahamson and Weis 1997).

A female *Rhopalomyia solidaginis* can oviposit multiple eggs into a plant's terminal buds. This in turn produces a bunch gall, where many leaflets are clustered together providing multiple chambers for larvae. *Rhopalomyia* may produce two generations per year. The first one is where overwintered larvae emerge from the ground and feed upon plant tissue while later in spring forming a single chamber gall in the plants' apical meristem (Wise et al. 2006; McEvoy 1988). A few weeks later adults

emerge, mate, and females oviposit in goldenrod's apical meristems (Wise et al. 2006; McEvoy 1988). In our study we focus on this second generation that produces the clustered bunch gall, since it probably produces more damage to the plant allowing us to detect clearer patterns.

2: Mechanical damage : greenhouse and field experiment

We first examined mechanical damage on *S. altissima* and *S. gigantea* to test if the two species respond to generalized damage in a similar way, vs. the alternative that one species is intrinsically more fragile to tissue damage or removal. If the two species respond to mechanical damage in a similar way, then we can compare the herbivore impact between ancestral host *S. altissima* and novel host *S. gigantea*, ascribing any differences in impact to differences in the co-evolutionary ages of the two associations.

We applied mechanical damage through lopping and defoliation on *S. altissima* and *S. gigantea*. Lopping consisted of removing the terminal one quarter of the stem, and defoliation was applied by clipping every other leaf on the plant's main shoot. Clipping represented a less severe damage upon plants that might be reflective of more generalized herbivores, such as leaf chewers. We consider lopping to be similar to, but more severe than, an attack by *Eurosta* or *Rhopalomyia*, because gallmaker attack often damages apical meristems, while lopping removes them completely. The greenhouse experiment, in which only lopping was applied, provided us with tight experimental control and therefore a precise estimate of plant response to damage. A complementary field experiment, in which both lopping and defoliation were applied, allowed us to observe how plants respond to mechanical damage in a more competitive natural environment.

In October 2012 we dug up 45 *S. altissima* and 49 *S. gigantea* rhizomes from a field in Fredericton NB. Collected rhizomes were planted in winter of 2012 at the UNB greenhouse in 13x13x15 cm pots, using standard "Pro-mix BX" substrate (Premier Tech, Riviere-du-Loup, QC). We randomized the pots by bench position and added 5 milliliters of 14-14-14 slow releasing fertilizer (Nutricote) per pot. After each plant reached 50 cm in height, it was assigned either to the lopping treatment, or to a control group. Treatment assignments altered such that lopped and control plants were paired. By day 105 most plants were beginning to senesce and we collected them by clipping the stems at the soil level. Collected ramets were dried for 48 hours at 70 °C in a drying oven, and later separated into different components: leaves, stem, and flowers (including phyllaries, peduncles, and associated tissues). Each component was then weighed in grams to the nearest second decimal.

We repeated this experiment with 43 individuals of *S. altissima* and 35 individuals of *S. gigantea* in summer of 2013 by following the same procedure as above.

We also performed a mechanical damage field experiment where 53 triplets of *S. altissima* and 53 of *S. gigantea* were located and tagged in Fredericton, NB in early summer of 2013. A triplet consisted of three neighbouring, similar-sized ramets. After plants reached 50 cm in height, lopping or defoliation was applied to one randomly chosen plant within a triplet, with the third plant undamaged for a control. At day 105 after treatment application, plants were clipped at ground level and processed in a similar manner as the plants from the "greenhouse experiment".

Within each pair or triplet the difference in total biomass was taken between lopped and control plants, and defoliated and control plants. This provided us with an

estimate of the mechanical damage impact. We ran a one-way ANOVA to compare the impact of lopping between plant species, and then the impact of defoliation between plant species.

3 :Gallmaker impact collections:

The simplest analysis of *Rhopalomyia* and *Eurosta* impact would consist of directly comparing biomass between galled and ungalled plants. This analysis, being similar to that of the "mechanical damage field experiment", would provide us with an estimate of impact from damage. However, results from such an analysis might be misleading because it assumes that the gallmakers' choice of ramets to attack is random with respect to plant size. This assumption will fail for gallmakers that prefer larger plants, as is the case with *Eurosta* and *Gnorimoschema* (Heard and Cox 2009; Hartnett and Abrahamson 1979) (it is not known if such preferences exist for *Rhopalomyia*). If gallmakers prefer larger plants, then the galled-ungalled plant size difference will underestimate true herbivore impact, since galled ramets would be larger than the ungalled ones (at least at time of galling, and possibly even after the impact of galling reduces their growth). Therefore, we estimated herbivore impact with a more complicated procedure using stem width to estimate a hypothetical unattacked size for each galled ramet ("Correcting for gallmaker preference", section 4; also see Figure 2.1). This correction yields a better estimate of gallmaker impact in each triplet, which can then be compared between ancestral and novel hosts.

Collections took place at two sites in Iowa USA, since these sites contained both goldenrods infected by both gallmakers while growing sympatrically. At the first site, Kent Park, 23 km north-west of Iowa City, IA (41.73 N, 91.729 W), *S. altissima*

dominated a drier hillside prairie, while *S. gigantea* dominated an adjacent moist bottomland. The second site was a mesic old field at Iowa Lakeside Laboratory, located about 100 km north-east from Sioux City, IA (43.38 N, 95.18 W), where the goldenrod species were well interspersed. We collected at Kent Park in late August 2012, and at both sites in late September 2013 and early September 2014. For each species and each gallmaker we collected 45 triplets (similar sample sizes had sufficient power to detect a difference in Heard and Kitts 2012), where each triplet consisted of a galled ramet and the two closest ungalled ramets of the same species. Such triplet analysis removes local environmental and some genetic variation since the plants of a triplet share a common habitat (and if they belong to a single genet, also the same genotype).

Selected plants were chosen by walking through a site and inspecting the area for galled ramets. Once a galled ramet of interest was detected, the next nearest galled plant was collected (along with its ungalled neighbours); this procedure avoided bias for larger galls or galls on larger plants, which are more evident to the searcher. Only plants with a single gall were collected. Processing and weighing of the ramets followed the same procedure as in the "mechanical damage experiment:2".

4: Correcting for gallmaker preference

The risk of underestimating gallmaker impact as a result of insect preference for larger plants can be mitigated by finding a proxy that can be used to estimate an attacked ramet's hypothetical size in the absence of attack – that is, the size to which it would have grown, had it not been galled. We suspected that basal stem width could provide such a proxy (Clought and Scott 1989). If ramet biomass is correlated with stem width, and if post-attack growth in stem width can be estimated, we can use it to find the hypothetical

unattacked ramet size. We used additional data from the mechanical damage experiments to develop a procedure for using stem width to correct estimates of herbivore impact in galled ramets. We began by asking whether stem width is correlated with plant biomass, and if growth in stem width changes in a predictable way due to inflicted damage.

In each mechanical damage experiment we measured stem width of each plant at day 0 (day of treatment application) and day 105 (day of collections). Stem width was measured using digital calipers to the nearest second decimal in mm, approximately 5 cm above soil. After the plant was processed and dried we once again measured the stem width at 5 cm from the bottom. Since stem widths for the Iowa field collections were determined post drying, we needed to convert growth measures based on fresh stem widths to a correction applicable to dry stem widths.

In all three experiments (two in the greenhouse and one in the field), fresh stem width predicted dry stem width extremely well (all $r^2 > 0.82$, $p < 0.001$, Table 1). Pooling data from all three experiments yielded a regression equation of $y = 0.79 X + 0.09$, $r^2 = 0.90$ (Figure 2.1), $P < 0.0001$. We use this equation to convert fresh stem width to dry stem width in all subsequent calculations.

Table 2.1: Linear regressions of dry stem width and fresh stem width from mechanical field and greenhouse experiment.

Mechanical damage experiments	r^2	p-value	Slope	Intercept
Greenhouse (2012)	0.88	< 0.0001	1.08	0.52
Greenhouse (2013)	0.83	< 0.0001	1.06	0.67
Field (2013)	0.86	< 0.0001	0.17	0.20
Combined	0.90	< 0.0001	1.14	0.29

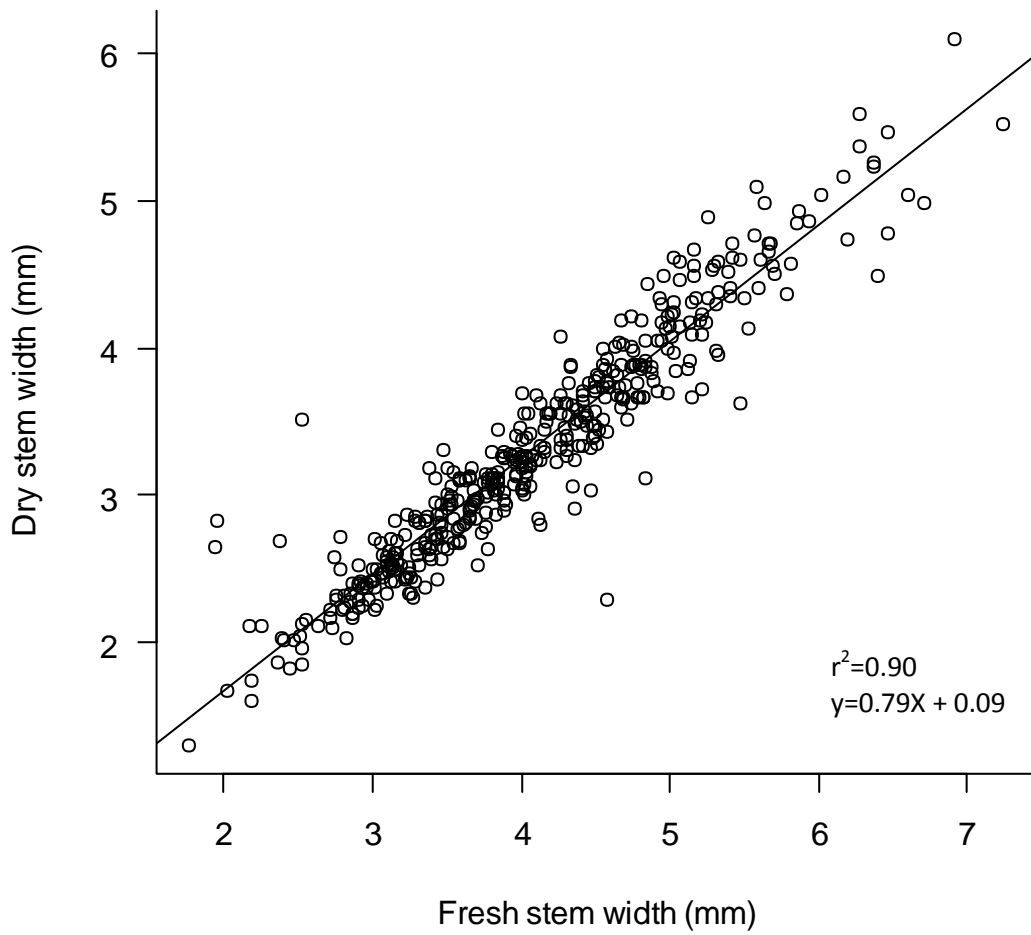


Figure 2.1: Linear regression between fresh and dry stem widths from mechanical and greenhouse experiments .

We calculated the fresh stem width difference between plants at day 0 and plants at day 105 for all three mechanical damage experiments, calling this value stem growth. We then compared stem width growth between control and lopped plants using a two way ANOVA (Figure 2.2; Table 2.2).

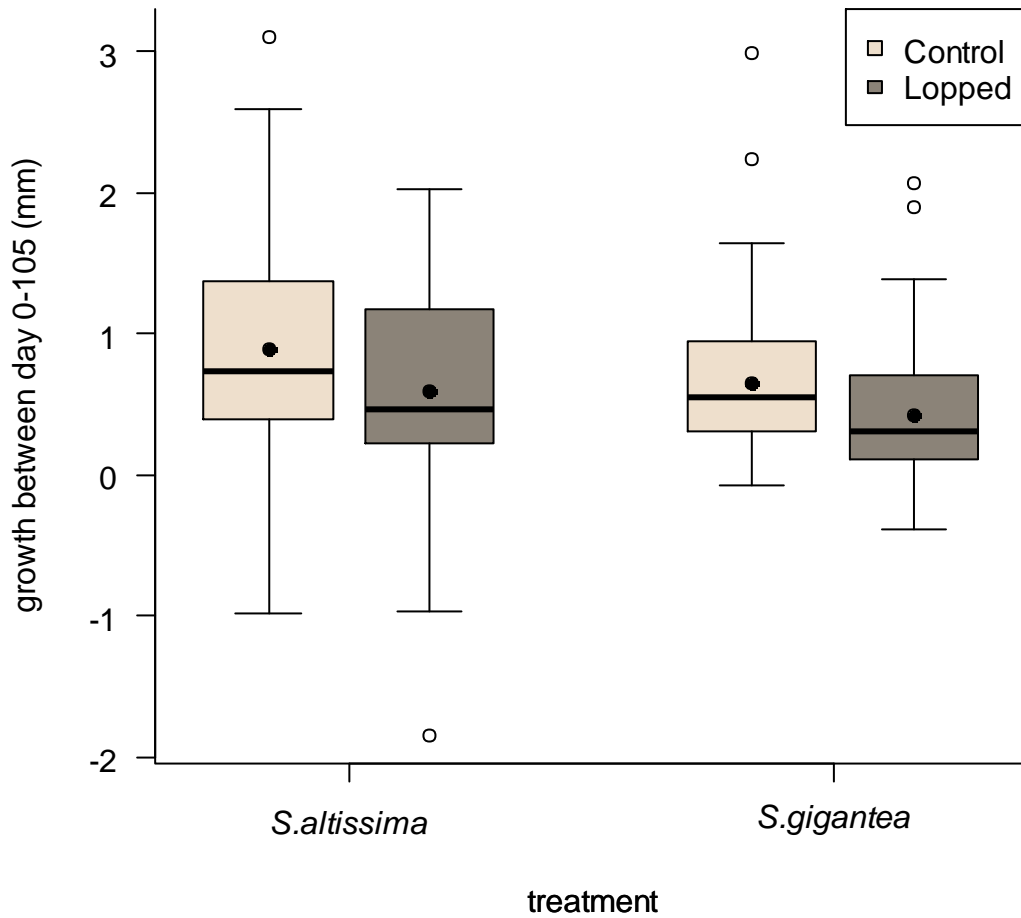


Figure 2.2: Comparing the means of growth in stem width between day 0 to day 105, across all three mechanical damaged experiments, for *S. altissima* and *S. gigantea* in lopped and control plants. Box plot summarizing the (25% and 75%) lower and upper quartiles, with whiskers representing the 5th and 95th percentile. Horizontal line representing the median and the point the mean.

Table 2.2: Two-way ANOVA comparing the means of stem width growth between lopped and control plants in *S. altissima* and *S. gigantea* across all three mechanical damage experiments.

	df	MS	F	P
Treatment	1	6.29	18.22	<0.0001
Species	1	3.61	10.46	<0.0001
Treatment*Species	1	0.09	0.26	0.61
Residuals	342	0.35		

Growth of stem width significantly decreased between lopped and control plants ($P < 0.0001$). In each mechanical experiment for each species we compared means of stem width growth between lopped and control plant (results not shown), and found that growth decreased by 28-62%. Pooling data sets from all the mechanical experiments resulted in growth decrease of 34% in *S. altissima* and 36% in *S. gigantea*. Since correcting stem width by 28% or 62% did not make any significant difference in the interpretation of our final results, for ease of computation we used 30% to correct the stem width in galled ramets.

We assume that gallmakers and lopping impose similar damage to plants, since they both damage meristematic tissue. By dividing the galled ramets stem width by 0.7 we found the hypothetical stem width in the absence of damage.

Stem width was a good predictor of aboveground biomass (Figure 2.3; Table 2.3). Using ungalled plants from our Iowa collections, we derived 10 transformation equations, to derive the hypothetical unattacked aboveground biomass, for each site, per plant species (results not shown). By plugging in the appropriate slope and intercept from exponential regressions into: $\ln y = \ln a + bx$ (where 'ln(a)' is the intercept and 'b' is the slope), we calculated our hypothetical unattacked aboveground biomass (y) from our corrected stem width (x) (see Table 2.3).

Table 2.3: Exponential regressions using a linear model of ungalled aboveground biomass as a function of ungalled stem width from Iowa collections. Testing if the slope is different from 0.

	Species	Slope	Intercept	r²	p-value
Kent Park 2012	<i>S. altissima</i>	0.52	-0.26	0.63	P<0.0001
	<i>S. gigantea</i>	0.46	0.18	0.75	P<0.0001
Kent Park 2013	<i>S. altissima</i>	0.71	-0.52	0.84	P<0.0001
Lakeside 2013	<i>S. altissima</i>	0.88	-0.90	0.89	P<0.0001
	<i>S. gigantea</i>	0.88	-1.19	0.85	P<0.0001
Kent Park 2014	<i>S. altissima</i>	0.72	-0.64	0.78	P<0.0001
	<i>S. gigantea</i>	0.76	-0.75	0.81	P<0.0001
Lakeside 2014	<i>S. altissima</i>	0.95	-1.13	0.85	P<0.0001
	<i>S. gigantea</i>	0.97	-1.36	0.89	P<0.0001

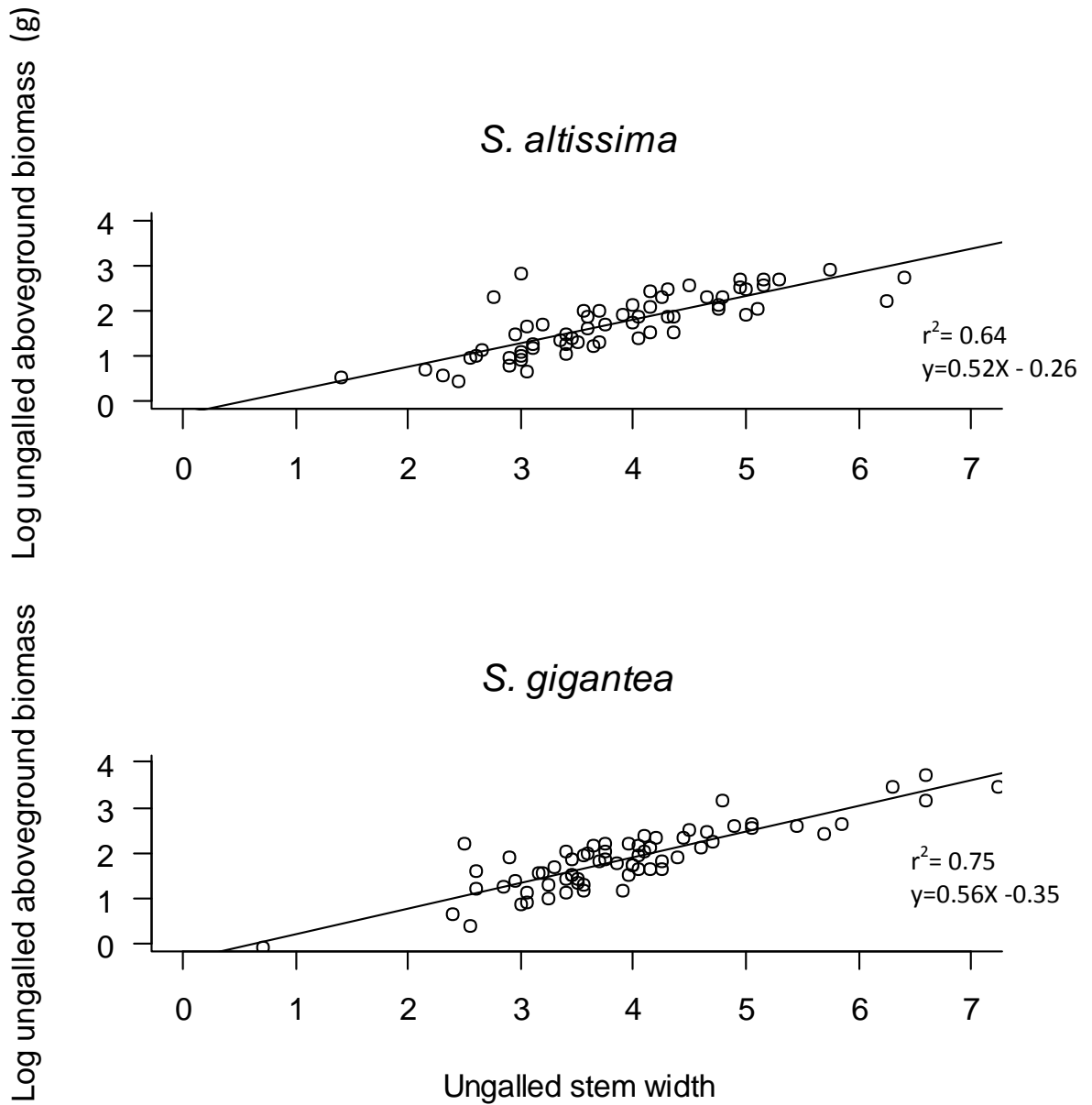


Figure 2.3: Exponential regression using a linear model of log transformed ungalloled aboveground biomass, and the corresponding ungalloled stem width from Iowa Kent Park 2012 collections.

Assuming that the differences in aboveground biomass (even after correcting for gallmaker preference) are only due to herbivore impact might be misleading, since environmental conditions and genotype will also influence the size of a ramet. By correcting for genotypic and environmental variability among triplets of goldenrods, we can get a more accurate estimate of hypothetical unattacked plant aboveground biomass. We estimated local environmental/genotype effects using residuals for each goldenrod triplet. We assume that if these are positive, this suggests either a microenvironment or a genotype that favors robust growth; if negative, the opposite is true. For the two ungalled plants within each triplet we established the residuals from stem-diameter/biomass regressions, reported in Table 2.3. We also constructed a regression of biomass on stem width for galled plants (separately for each site/species/year combination); these regressions are analogous to those in Table 2.3, but are not reported here. After we found the microenvironment and genotypic variations as residuals from regressions for each plant within the triplet, we averaged them, giving us the microsite effect. We added this average to our hypothetical unattacked plant size for galled ramets, to make sure that unattacked size would reflect any local/genotypic conditions.

Once we have the hypothetical unattacked biomass for each galled plant, we can compare it to that plant's actual (post-attack) biomass. The difference is our estimated herbivore impact. We then ask whether herbivore impact is significantly different between the ancestral host (*S. altissima*) and the novel host (*S. gigantea*) using a one-way ANOVA for each gallmaker at each site.

Results:

From the field and greenhouse damage experiments we found no evidence for differential response to damage in *S. altissima* and *S. gigantea*. This is evident as there is no significant difference between plant species in damage from lopping and defoliation (Table 2.4).

Table 2.4: One way ANOVAs comparing the impact of lopping and defoliating between *S. altissima* and *S. gigantea* on their stem width growth across field and greenhouse experiments.

Stem width growth		Df	MS	F	P
Lopped	Species	1	2.10	1.43	0.24
	Residuals	74	1.47		
Defoliated	Species	2	0.61	0.19	0.82
	Residuals	94	3.16		

In regards to Iowa goldenrod collections, the impact of *Rhopalomyia* attack was significantly higher (around 30%) in the novel host *S. gigantea*, compared to the ancestral host *S. altissima*, for all the years and all collection sites, except two. At Kent Park in 2014, impact was higher on the novel host (16%) but the difference was not significant, while at Kent Park in 2012, *S. altissima* had higher herbivore impact (36%) compared to *S. gigantea*. Herbivore impact from *Eurosta* gallmakers was significantly higher on the novel host only for Kent Park 2013(33%), although for Kent Park 2014 and Lakeside 2014 combinations, the trend was in the same direction (not significant). Only at Lakeside in 2013 did *S. altissima* experience higher herbivore impact (around 30%) than *S. gigantea* (and the difference was significant, see Table 2.5 and Figure 2.4).

Table 2.5: ANOVA table for analysis of *Eurosta* and *Rhopalomyia* herbivore impact, measured as the difference between galled and hypothetically ungalled ramets, on *S. altissima* and *S. gigantea* hosts . Aboveground biomass difference between novel and ancestral host, where negative values represent more damage on ancestral host.

			Df	MS	F	P	Aboveground biomass difference
KP 2012	<i>Eurosta</i>	Species	1	206.50	2.41	0.125	-16%
		Residual	70	85.76			
	<i>Rhopalomyia</i>	Species	1	865.20	14.73	0.0003	-36%
		Residual	55	58.73			
KP2013	<i>Eurosta</i>	Species	1	673.30	11.85	0.0008	33%
		Residual	87	56.83			
	<i>Rhopalomyia</i>	Species	1	722.90	7.41	0.0079	68%
		Residual	85	97.62			
L 2013	<i>Eurosta</i>	Species	1	80.88	9.27	0.0031	-30%
		Residual	86	8.72			
	<i>Rhopalomyia</i>	Species	1	173.30	15.29	0.0002	32%
		Residual	88	11.33			
KP2014	<i>Eurosta</i>	Species	1	80.61	0.83	0.364	1%
		Residual	87	96.92			
	<i>Rhopalomyia</i>	Species	1	84.81	1.13	0.291	16%
		Residual	87	75.20			
L 2014	<i>Eurosta</i>	Species	1	1.24	0.03	0.855	3%
		Residual	95	36.95			
	<i>Rhopalomyia</i>	Species	1	235.10	4.78	0.031	30%
		Residual	89	49.22			

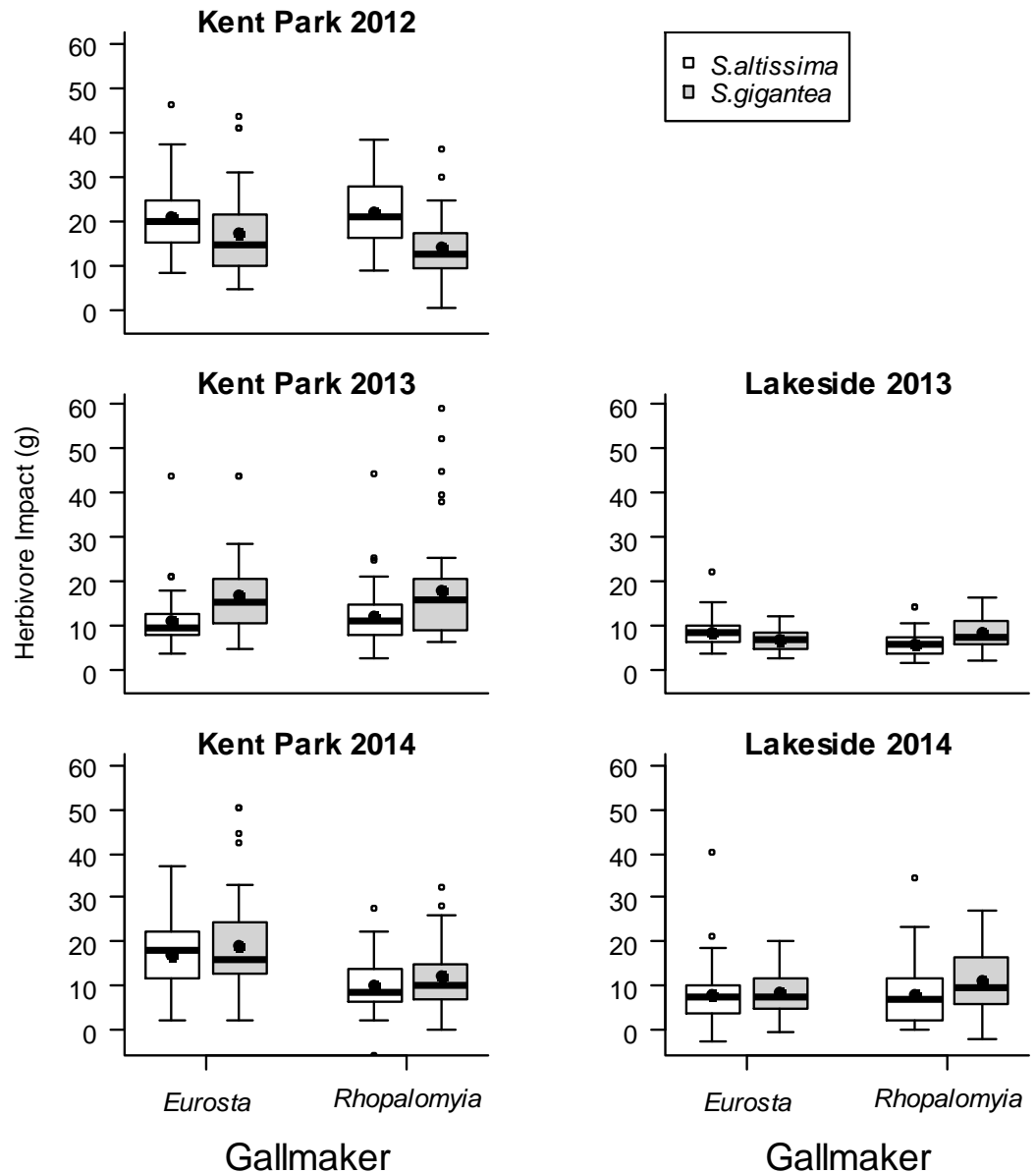


Figure 2.4: Effects of herbivore impact of *Eurosta* and *Rhopalomyia* gallmakers on the aboveground biomass of their novel and ancestral hosts *S. altissima* and *S. gigantea*. Data collected in Iowa through summers of 2012-2014, with two sites at Kent Park and Lakeside. Box plot summarizing the (25% and 75%) lower and upper quartiles, with whiskers representing the 5th and 95th percentile. Horizontal line represents the median and the point the mean.

Discussion

We studied the impact of specialist herbivores on their ancestral and novel host plants, predicting that the impact would be greater on the novel host compared to the ancestral one. As an overview, for each gallmaker we collected five data sets (10 in total); Kent Park 2012-2014 and Lakeside Laboratory 2013-2014. The results were not entirely consistent among our datasets: most noticeably, at Kent Park 2012, the results were opposite to our expectation, with the ancestral host *S. altissima* experiencing greater herbivore impact than the novel host *S. gigantea*, for both gallmakers (for *Eurosta*, the trend was not significant). We begin by asking what could have caused Kent Park 2012 to show a reversed pattern from the other data sets.

In 2012, Iowa experienced a dramatic drought, with temperatures 3.8 °C above average, and precipitation 22.8 cm below average, making it one of the warmest and driest years in the past 140 years (Hillaker, accessed August 31, 2015 <http://www.iowaagriculture.gov/climatology/weatherSummaries/2012/pas2012.pdf>). At Kent Park, furthermore, the two goldenrod species were unevenly distributed, with *S. altissima* collections made (mostly) from a well-drained hillside, but *S. gigantea* collected (mostly) from a moist bottomland adjacent to the hill. Hence the two goldenrod species did not experience drought equally, with *S. altissima* likely exposed to drier soils and experiencing greater stress. Therefore, in a separate study (Chapter 3), we tested the hypothesis that drought stress on *S. altissima* might decrease its tolerance of herbivory. We subjected *S. altissima* and *S. gigantea* to water stress and simulated herbivory in a greenhouse experiment, measuring growth and other performance related traits. A strong synergistic impact, with respect to aboveground biomass, from drought and herbivory, was observed for *S. altissima*, while an additive impact was found for *S. gigantea*. The

pattern at Kent Park in 2012 may reflect both greater exposure to drought for *S. altissima* than *S. gigantea*, and an increase of severity in herbivore impact by drought for *S. altissima* but not for *S. gigantea*. These effects have probably masked any results of coevolution between herbivore and host in this dataset. As a result, we believe our Kent 2012 results should be discounted with respect to the impact-coevolution hypothesis.

Removing our 2012 datasets, and pooling our remaining data with those of Heard and Kitts (2012) for the gallmaking moth *Gnorimoschema* on the same hosts, we have 10 data sets comparing impact of three different gallmakers on the same pair of novel and ancestral host plants (Figure 1 and Table 1 of Heard and Kitts 2012). Of these, 9 had greater impact on *S. gigantea*, the novel host (7 significantly so), and only 1 showed the reverse pattern (sign test, $P = 0.011$). We are certain that the difference in the herbivore impact between two plant species was based on the co-evolutionary history with gallmakers, because when we conducted a greenhouse mechanical damage experiment (presumably having a similar impact as gallers), both species tolerated mechanical damage in a similar way. Although we have individual cases where the pattern is not significant or reversed, these may be explained by other environmental factors.

Gallmaker impact on their hosts might depend on many other factors, and fluctuate between years. Therefore it is important to test coevolutionary hypotheses over multiple years and sites. In general we find strong support for the coevolutionary hypothesis of overall gallmaker impact decreasing through time. This suggests that either the ancestral host has developed higher tolerance, or the gallmaker has developed lower virulence over time (or both).

The host shift from *S. altissima* toward *S. gigantea* for *Eurosta* occurred no more than 200,000 years ago, and for *Rhopalomyia* at least 2 million years ago (Stireman et al. 2005). We might expect that there would be lower herbivore impact on the novel host from *Rhopalomyia* compared to *Eurosta*, since even the “novel” *Rhopalomyia-Solidago* association has had a relatively long time for coevolution. However, our data suggest that for both gallmakers, herbivore impact affecting the novel host is of similar strengths. This may imply that selection for increased tolerance and decreased virulence is relatively weak, such that novel associations take a long time to reach the same level of tolerance and virulence as on the ancestral host. One factor that might lead to a weak selection on such traits is the clonal reproductive method of *Solidago* plants. Goldenrods can reproduce through rhizomes, producing many new shoots of the same genotype the following spring. Even if there is a strong herbivore impact on a single ramet, the selection pressure on a genet alone may be relatively low. Gallmaker attack on ramets is relatively sparse, around 2% (CM Moffat, personal communication). Even once a ramet is attacked, the supporting genet network can segregate the infected plant by disconnecting from it through rhizomes. This in return will keep the genet as a whole almost unaffected.

Coevolution is an important evolutionary force that shapes many plant insect communities. When a novel host switch occurs we predict that the herbivore impact will be greater on the novel host, as evident from our data. However, this pattern might not always hold true and can be sensitive to environmental conditions. While we were able to explain why Kent Park was abnormal in 2012, it is important to address the issue of drought having such a strong effect on herbivore impact. Climate change, such as

increased temperatures, might negatively affect plant defense systems, making plants more susceptible to herbivore impact. This can further mask any coevolutionary relationships between ancestral and novel host-parasite pairs at a given year. Therefore, it is important to replicate collections through years and sites. In some cases environmental change might weaken or even reverse herbivore impact from expected trend based on host-parasite theory, hence it is important to consider this possibility.

With increasing global change there is also an increase in invasive species entering native plant-insect communities. Invasive insects, for example, will adapt to the novel plant hosts of the new geographic range. This is similar to a host shift, where herbivores attack a novel host plant. Thus, by studying coevolution on host shifts, we can apply the knowledge to invasive species adapting in novel plant communities. When it comes to invasive species, it is important from a biocontrol perspective to note how they coevolve with species around them, since they interact with the native and novel biota differently (Lau and Strauss 2005). Based on our results, we might expect to see heavy impact on novel hosts from herbivores. It is commonly believed that invasive species thrive in novel environments because of release from natural enemies (Keane and Crawley 2002; Maron and Vila 2001). However, multiple studies on herbivores attacking exotic plant species reveal that native herbivores, novel to the exotic species, actually impact them more strongly than exotic plant's native herbivores (Parker et al. 2006). Such knowledge of coevolutionary changes and their pace, as well as direct and indirect effects of herbivore damage to the novel host, can help us analyze and better understand the strength of selection on plants and how to apply biocontrols appropriately (Muller-Scharer et al. 2004). If these coevolutionary ties are ignored, biocontrols may be applied

incorrectly with the released agents, further attacking non-target species (Louda et al. 2003). Effective biocontrols can stabilize pest populations, however these targets are not evolutionary static, and one may have concerns that they may be prone to sporadic evolutionary breakdowns (Croft 1992). With time resistance and tolerance may evolve and lead to control release. Although there are few clear cut examples of coevolution leading to biological release (Channer and Gowen 1992), it is still very important to understand and take into account to avoid such instances in the future.

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**CHAPTER 3- SYNERGISTIC AND ADDITIVE EFFECTS OF WATER STRESS
AND CLIPPING ON *S. ALTISSIMA* AND *S. GIGANTEA***

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Abstract:

Understanding the combined effects of stressors on plants is important in understanding how they will tolerate herbivory and other damage under unfavorable conditions. Synergistic interactions are important because they can help us understand what seem like surprisingly severe impacts on plants under stress. We examined individual and combined water stress and clipping impact(simulated herbivory) on two species of *Solidago* (goldenrods). Aboveground biomass was reduced by 30% more from treatment interactions in *S.altissima*, while *S. gigantea* did not experience greater damage from the interaction of water stress and clipping. Synergistic effect was consistent among other plant tissues, such as inflorescence biomass, in *S. altissima*. The same stresses had additive impacts on *S. gigantea*. Our results suggest that even closely related plants may tolerate damage differently when under abiotic stresses. Reallocation of resources among above and below ground tissue also varies, and may be an important coping mechanism in the face of stress.

Introduction

In natural environments, plants are commonly exposed to multiple stresses that can compromise their fitness. Both biotic and abiotic stresses can reduce growth, reproduction, and survivorship of many plant species (Calow 1989; Jones and Jones 1989). Under extreme conditions, such as drought, salinity, or herbivory, plants' physiological functions and defense pathways may become disrupted (Serrano et al. 1999; Smirnoff 1998).

Studies of biotic or abiotic impacts on plants often focus on one stressor at a time (Collins and Smith 2006; Lotze and Worm 2002). However, under natural conditions,

plants endure abiotic stressors often coupled with biotic stressors. If the combined impact of two stressors is equal to the sum of the impacts of each stressor alone, the effect is said to be additive. In such cases, studies focusing on single stressors and their impacts are not misleading (Bansal et al. 2013). However, when the combined impact from two stressors is greater than the sum of the separate impacts, the effect is said to be synergistic, and studying such stressors independently cannot predict their joint impact. It is critical to understand in what manner stresses interact, since synergistic stressors may have particularly strong impacts on plant populations (Rand 2003; Trumble et al. 1992).

When plants grow in resource stressed conditions, their responses to herbivory may be altered, leading to unpredictable consequences for plant performance (Bansal et al. 2013; Paez et al. 1995; Trumbe et al. 1993). For example, in saline environments halophytic plant species also are exposed to herbivore damage (Ancheta et al. 2010). Often these stressors interact in unexpected and synergistic manner, potentially leading to even greater negative impacts on plants (Eyles et al. 2009; Rand 2003; Vierling and Kimpel 1992; Chapin et al. 1987).

Water stress and herbivory are two important ecological factors that could influence plant fitness (Holmgren et al. 2006; Hawkes and Sullivan 2001; Marquis 1992). With increasing global temperatures, drought conditions are becoming more common (Schär et al. 2004; Ayres and Lombardero 2000), and therefore it is important to understand how water stress and herbivory interact (Williams and Jackson 2007; Vinebrooke et al. 2004). A number of studies have suggested that under conditions of water stress, plant tolerance to herbivore damage is reduced (Atala and Gianoli 2009; Wise and Abrahamson 2007; Levine and Paige 2004; Hawkes and Sullivan 2001).

However, not all literature agrees on how the combined impact from stresses affect plants, and many studies ignore resource reallocation between various plant structures, such as aboveground and belowground tissues (e.g. March et al. 2013; Oosterheld and McNaughton 1991; Ackerson and Hebert 1981). This gives us an opportunity to study the impact of stresses on different plant tissues; for example, stress may reduce fitness and trigger changes in a plant's life history, such as sexual and asexual reproduction.

Knowing how these stressors interact is important in understanding how plants will respond to and tolerate damage (Niinemets 2010). Knowing if water stress and herbivory have a synergistic or an additive effect can lead to further appropriate actions in managing damaged or threatened plant populations. For example if an endangered species is more susceptible toward synergistic effects from herbivory and drought, then one possible conservation action would be to decrease water stress in drought like conditions.

We asked whether water deficit and clipping (simulated herbivory) inflict synergistic or additive effect on two species of goldenrod, *Solidago altissima* L. (Asteraceae), and its close relative *Solidago gigantea* Ait. Under natural conditions these plants grow in prairie-like environments (Abrahamson and Weis 1997), experiencing periods of drought. We examined the impact of water stress and clipping on various plant components (both aboveground and belowground) and resource allocation patterns. A plant's aboveground biomass, particularly stem and leaves, is an important fitness component because it determines the plant's ability to photosynthesize (Jones and Jones 1989; Patterson and Flint 1980); it also facilitates sexual reproduction and support for its structures. This portion of the plant is also highly susceptible to herbivore damage,

particularly by defoliators and browsers (Abrahamson and Weis 1997). Our objective was to also examine belowground tissues, since they are critical for asexual reproduction through rhizomes and resource gathering through roots.

Methods

Study System

Solidago altissima and *S. gigantea* are perennial plants that are abundant in prairies and disturbed habitats such as old fields and roadsides across much of eastern and central North America (Abrahamson and Weis 1997). In early spring these species may reproduce asexually by producing new shoots from overwintered rhizomes and/or sexually in early fall (Abrahamson and Weis 1997). These goldenrods are hosts to many generalist and specialist herbivores and may experience a variety of herbivore and environmental stresses.

Experimental Design

We haphazardly collected 51 *S. altissima* and 97 *S. gigantea* rhizomes from a field along the north side of the pedestrian bridge across the Saint John River in Fredericton, New Brunswick (45.57 N, 66.37 W). We collected ramets at least one meter apart to reduce the likelihood of collecting plants of the same genotype. All collections were made within a week of May 25th, 2014. In a greenhouse, rhizomes were immediately planted in 13x13x15 cm pots using a "Pro-mix BX" substrate from Premier Tech, Riviere-du-Loup, Canada. We fertilized the pots with a 14-14-14 (Nutricote) slow releasing fertilizer (5 mL per pot), and randomized them to bench positions. Once a quarter of plants within a species had reached 25 cm in height (with the smallest plants being 10 cm, and the tallest being 30 cm), we randomly assigned plants of each species to

four treatment groups: "water stressed and clipped", "water stressed and not clipped", "not water stressed and clipped", and "not water stressed and not clipped". Clipping involved removing one quarter of the plants' main shoot using pruning clippers, and was intended to simulate browsing herbivory or the damage to the apical meristems that often accompanies attack by gallmakers. Although artificial clipping is not an entirely accurate simulation of natural herbivore damage, the difference between the two methods tends to be small in studies dealing with plant growth and reproduction (Lehtila and Boalt 2004). We monitored plants on a daily basis, where regularly watered plants received water whenever their soil became dry, while water-stressed plants were not watered until they started to wilt. Once a plant in water stressed treatment showed signs of wilting, we watered it just enough for it to regain turgor pressure. The amount of water loss between treatment groups and plant size was not measured, and would have depended on the plant size. This type of pulsed drought is representative of natural water stressed conditions (Huberty and Denno 2004).

Data Collection

Around day 83 after planting, most plants showed signs of senescence, and we collected them by clipping the stems at the soil level and storing them in zip lock bags in a refrigerator. Pots still containing the belowground tissue in the soil were left unwatered and stored in a cold room for about 3-4 weeks, after which we separated roots and rhizomes by washing soil with high pressure water through a screen. Any fine root material that would have been lost would be equal across all treatments and therefore not pose any significant impact on the results.

We separated belowground tissue into roots and rhizomes, and above ground tissue into leaves, stems, and inflorescence (including phyllaries, peduncles, and associated tissues). Plant components were dried at 70C° for 48 hours and then weighed to the nearest 0.01 g.

Statistical analysis

We assessed the impact of water stress and stem clipping on total aboveground biomass by comparing treatment groups (fixed effects) with a two-way ANOVA for each species. The same analyses was conducted for separate plant components such as rhizomes, roots, and inflorescence. In all cases, we log-transformed biomass measurements in order to stabilize variances and also because biomass effects are likely to be multiplicative. In addition to the two-way ANOVA, presence/absence of inflorescence across treatment groups using a general linear model (GLM) with a binomial error distribution was also analyzed, since many plants never flowered.

Results

Above ground biomass

Water stress and clipping significantly reduced aboveground biomass for each species (Figure 3.1, Table 3.1). There was strong evidence for synergistic interaction of the two stresses in *S. altissima* (clipped*water stressed interaction significant; Table 3.1), but not in *S. gigantea*. *S. altissima* was more heavily impacted by clipping when water stress was also present (31% biomass reduction) (Figure 3.1; Table 3.6).

Table 3.1: Analysis of water stress and clipping impact on log of aboveground biomass in *S. altissima* and *S. gigantea*.

	df	MS	F	P
<i>a) S. altissima</i>				
Clipped	1	2.243	8.057	0.007
Water stress	1	9.643	34.64	<0.001
Clipped*water stress	1	1.676	6.021	0.018
Residuals	47	0.278		
<i>b) S. gigantea</i>				
Clipped	1	1.675	7.966	0.006
Water stress	1	17.18	81.69	<0.001
Clipped*water stress	1	0.435	2.070	0.154
Residuals	93	0.210		

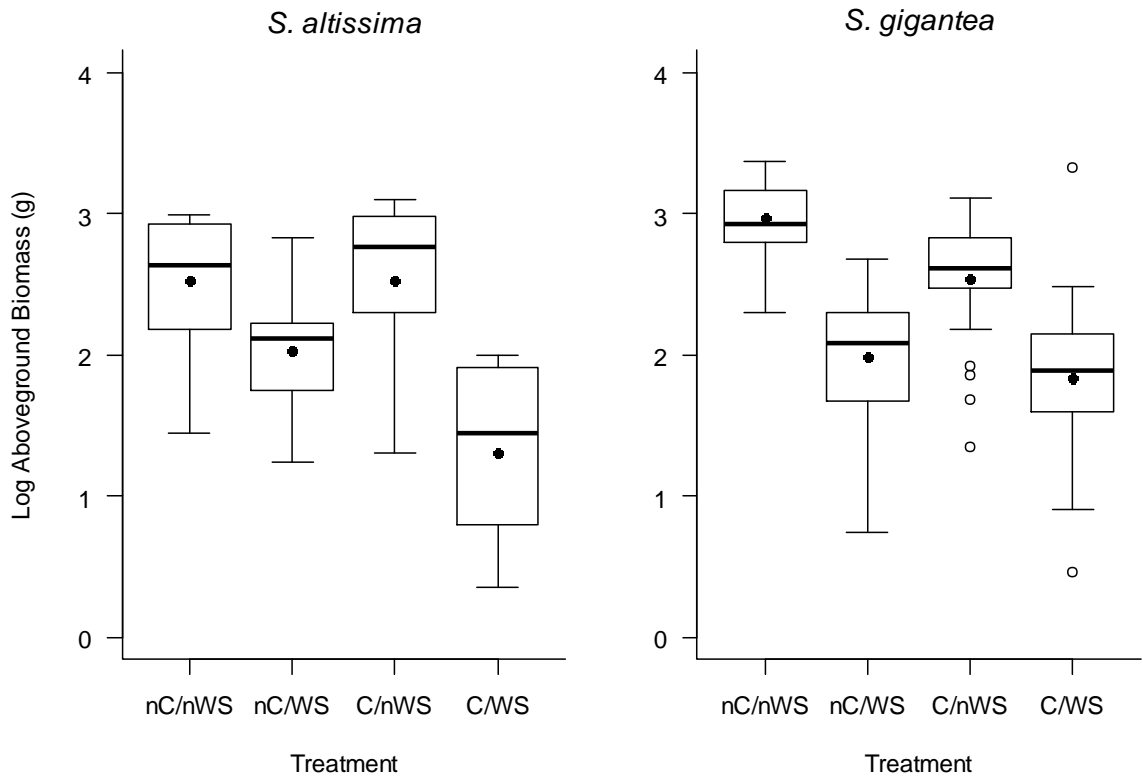


Figure 3.1 Effects of clipping and water stress on the log of aboveground biomass for *S. altissima* and *S. gigantea*. nC/nWS represent not clipped and not water stressed plants, nC/WS are not clipped and water stressed plants, C/nWS represents clipped and not water stressed plants, and C/WS are clipped and water stressed plants. Box plot representing the median of the data, with lower and upper quartiles (25% and 75%) and whiskers representing 5th and 95th percentile. The black points indicate treatment means.

Flowering presence/ absence

Clipping, that was applied before plants flowered, significantly reduced the number of individuals that flowered in *S. altissima* and *S. gigantea*, while water stress reduced the number of individuals that flowered only for *S. gigantea* (Figure 3.2, Table 3.2). There was no evidence of synergistic effects for either species (Table 3.2).

Table 3.2: Analysis of water stress and clipping impact on flowering in *S. altissima* and *S. gigantea*.

	Std.Error	Z-value	P
<i>a) S. altissima</i>			
Clipped	1.012	2.874	0.004
Water stress	0.876	1.099	0.271
Clipped*water stress	1.399	-0.620	0.535
<i>b) S. gigantea</i>			
Clipped	8.926	2.757	0.006
Water stress	12.64	2.001	0.045
Clipped*water stress	3.024	0.000	0.910

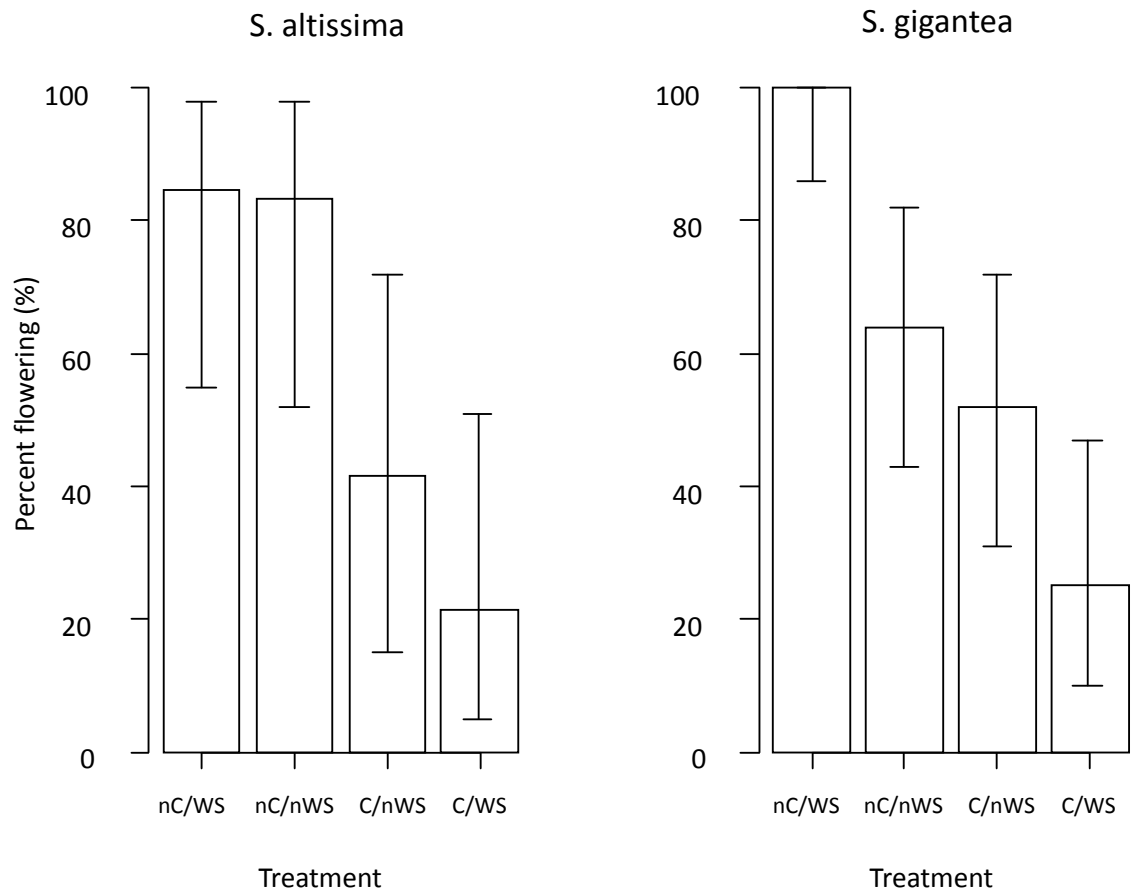


Figure 3.2 Effect of clipping and water stress on flowering in *S. altissima* and *S. gigantea*. Error bars represent 95% confidence interval on binomial fraction that uses proportion estimated in statistical sample allowing for sampling error.

Inflorescence biomass

Patterns in inflorescence biomass resembled those in total aboveground biomass. Inflorescence biomass was significantly reduced by clipping and water stress in *S. altissima* and *S. gigantea* (91% and 58% respectively) (Table 3.3, Figure 3.3 ; Table 3.6). There was strong evidence for a synergistic effect in *S. altissima* (Table 3.3), but not in *S. gigantea*.

Table 3.3: Analysis of water stress and clipping impact on log of inflorescence biomass in *S. altissima* and *S. gigantea*.

	df	MS	F	P
<i>a) S. altissima</i>				
Clipped	1	12.79	10.85	0.003
Water stress	1	12.61	10.70	0.003
Clipped*water stress	1	14.30	12.13	0.002
Residuals	25	1.179		
<i>b) S. gigantea</i>				
Clipped	1	8.926	8.128	0.006
Water stress	1	12.64	11.51	0.001
Clipped*water stress	1	3.024	2.754	0.103
Residuals	54	1.098		

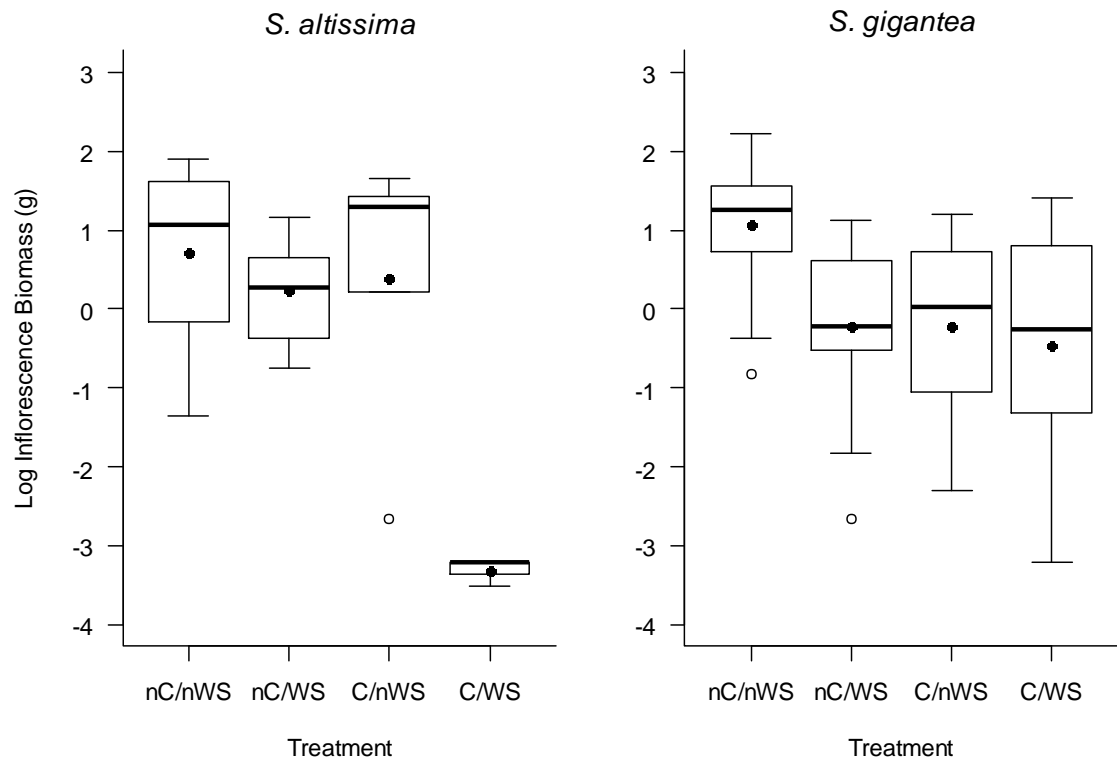


Figure 3.3 Effects of clipping and water stress on the log of inflorescence biomass for *S. altissima* and *S. gigantea*.

Rhizome biomass

Clipping did not affect rhizome biomass for either species, while water stress reduced rhizome biomass only for *S. gigantea* (36%) (Figure 3.4, Table 3.4; Table 3.6).

There was no evidence for synergy in either species.

Table 3.4: Analysis of water stress and clipping impact on log of rhizome biomass in *S. altissima* and *S. gigantea*.

	df	MS	F	P
<i>a) S. altissima</i>				
Clipped	1	2.578	2.640	0.113
Water stress	1	0.191	0.196	0.661
Clipped*water stress	1	0.301	0.308	0.583
Residuals	35	0.977		
<i>b) S. gigantea</i>				
Clipped	1	0.040	0.106	0.746
Water stress	1	7.081	18.58	<0.001
Clipped*water stress	1	0.137	0.358	0.551
Residuals	86	0.381		

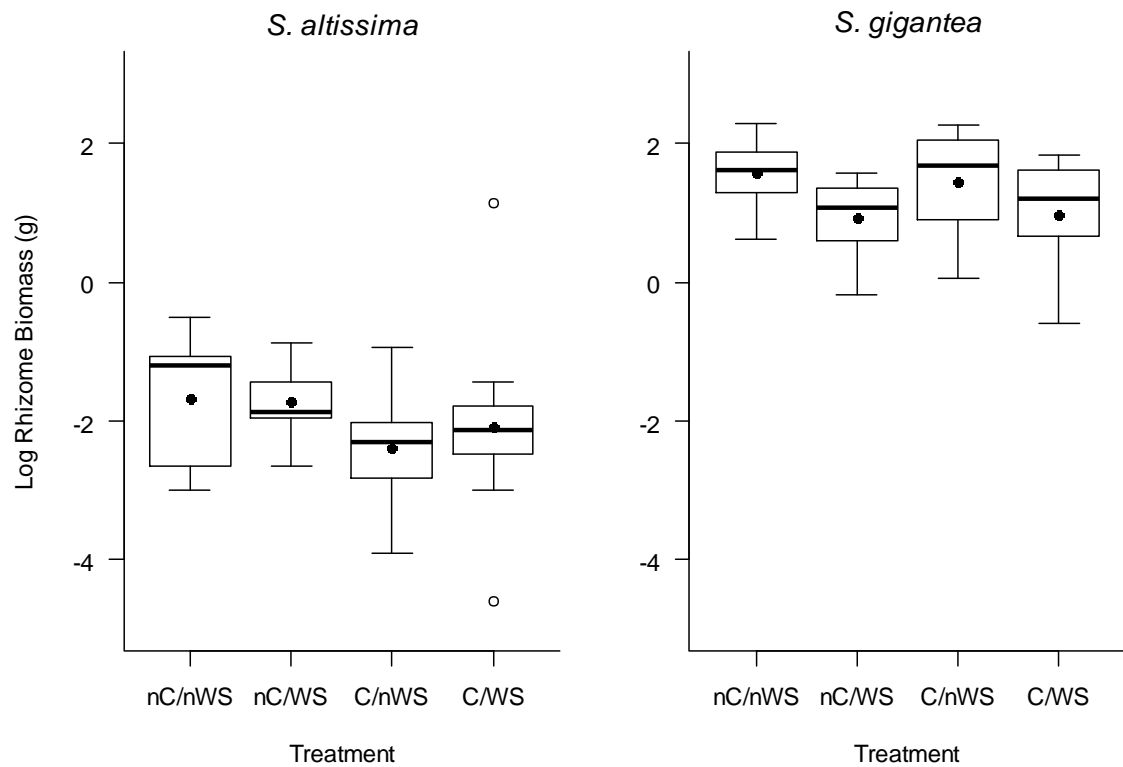


Figure 3.4 Effects of clipping and water stress on the log of rhizome biomass for *S. altissima* and *S. gigantea*.

Root biomass

Root biomass was significantly reduced by water stress in each species (49% and 68% respectively) (Figure 3.5, Table 3.5; Table 3.6). Clipping reduced root biomass only for *S. gigantea* (23%) (Table 3.5; Table 3.6). There was no evidence for synergistic effects in either species.

Table 3.5: Analysis of water stress and clipping impact on log of root biomass in *S. altissima* and *S. gigantea*.

	df	MS	F	P
<i>a) S. altissima</i>				
Clipped	1	2.331	3.463	0.069
Water stress	1	8.695	12.92	<0.001
Clipped*water stress	1	1.105	1.642	0.207
Residuals	45	0.673		
<i>b) S. gigantea</i>				
Clipped	1	2.369	7.329	0.008
Water stress	1	26.60	82.30	<0.001
Clipped*water stress	1	0.220	0.680	0.412
Residuals	86	0.323		

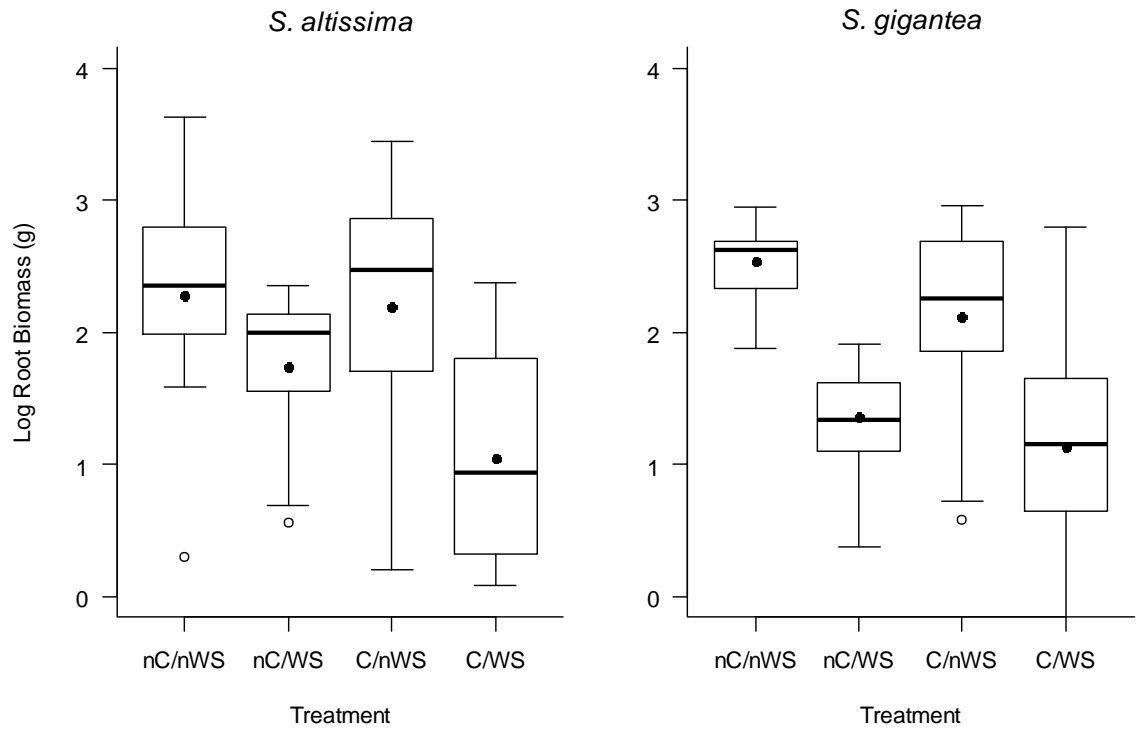


Figure 3.5 Effects of clipping and water stress on the log of root biomass for *S. altissima* and *S. gigantea*.

Table 3.6 Biomass (represented in grams) measured across four different treatment groups of the greenhouse experiment.

		Not clipped/not water stressed (g)	Not clipped/water stressed (g)	Clipped/not water stressed (g)	Clipped/water stressed (g)
Aboveground biomass	<i>S. altissima</i>	13.69	8.36	14.25	4.25
	<i>S. gigantea</i>	20.10	8.05	13.67	7.40
Inflorescence biomass	<i>S. altissima</i>	3.12	1.53	2.87	0.04
	<i>S. gigantea</i>	3.58	1.18	1.24	1.52
Rhizome biomass	<i>S. altissima</i>	0.19	0.19	0.08	0.34
	<i>S. gigantea</i>	5.19	2.81	5.12	3.31
Root biomass	<i>S. altissima</i>	12.69	6.54	12.61	3.77
	<i>S. gigantea</i>	13.09	4.17	10.10	4.08

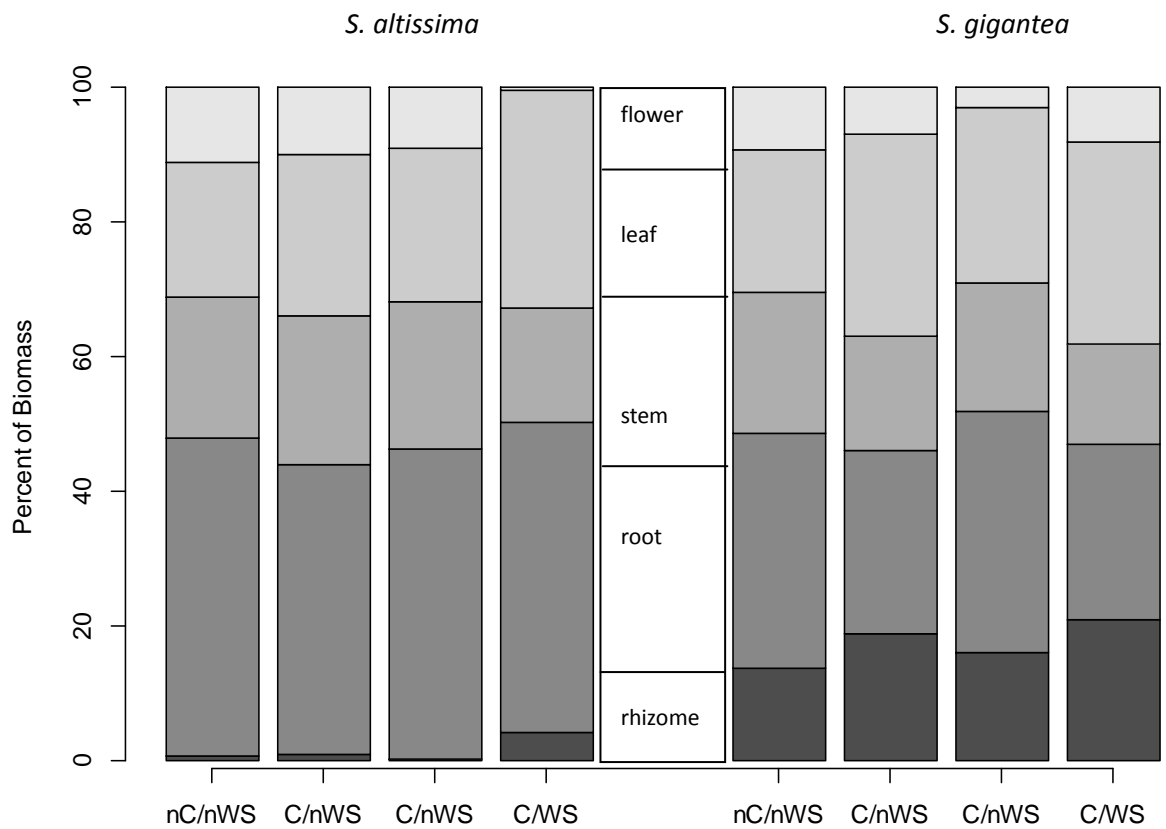


Figure 3.6 Allocation of dry plant components (flower biomass, leaf biomass, stem biomass, root biomass, rhizome biomass), expressed as a percentage form the plant biomass means in each treatment groups.

Discussion

We found that water stress and clipping were synergistic for most plant tissues in *S. altissima* but not for *S. gigantea*. Furthermore, responses to individual and combined stresses influenced resource reallocation across different plant tissues. As a consequence, stressed plants underwent major shifts in life history strategies, including dramatic reallocation of resources from sexual to asexual reproduction.

We began with analysis of aboveground biomass, because many plant population and community ecology studies focus on this variable (e.g., Bansal et al. 2013; Ancheta and Heard 2010; Grime 1989). If there is a negative impact on aboveground biomass, then reduced photosynthetic surface area will negatively affect plant fitness. As a general trend we found that for *S. altissima*, impact from clipping was greater in the presence of water stress. The synergistic effect might pose particular danger to *S. altissima* since with increasing drought episodes (expected under global climate change) and herbivory there will be more damage to the plant's aboveground biomass. *S. gigantea* on the other hand had an additive interaction, where tolerance to clipping was independent of water stress. Although *S. altissima* and *S. gigantea* are sister species, they possess many different traits that may allow them to respond to damage differently (Abrahamson and Weis 1997).

The manner in which a plant responds to herbivory might depend on how well it responds to water stress; in particular, plants that tolerate water stress well may experience lower impact from herbivory. Many traits can determine how well a plant can respond to water stress, and one of them is the outer cuticle permeability, which is important in preventing plants from losing water in water stressed conditions (Goodwin and Jenks 2005). Our study species have different cuticle properties: *S. gigantea* has a

glabrous and waxy stem, while *S. altissima* has more of a pubescent and scabrous stem (Abrahamson and Weis 1997). Cuticular waxes play a major role in reducing water permeability (Schönherr and Riederer 1989; Schönherr 1982). Although we have not directly measured water losses for our goldenrods, we suggest that *S. gigantea's* cuticle might be better adapted to tolerate water stress. On the other hand, *S. altissima* might be more prone to water loss during water stress, and with added clipping, the aboveground biomass will be reduced in a synergistic manner. By clarifying the nature of traits that facilitate such difference, we can learn what aspects can make a plant more tolerant to herbivory during water stressed conditions.

Belowground tissues were more tolerant than aboveground ones to damage from single or combined stresses. As for aboveground tissue, however, we found evidence for synergistic stress in *S. altissima* but not for *S. gigantea*. Difference in rhizome biomass between plant species are due to differences in rhizome structure: larger but shorter and fewer rhizomes grow in *S. altissima* plants, while *S. gigantea* has smaller but longer, and numerous, rhizomes. These species difference do not confound our results since we measured effects between treatment groups within each plant species. In general rhizome biomass did not decrease with damage and root biomass only decreased from water stress. This suggests that under conditions of stress, resources are reallocated to belowground biomass and asexual reproduction. Such reallocation might be adaptive because belowground tissues might not be as susceptible to water loss as aboveground tissues.

On the other hand, the combined stress had an additive effect on aboveground biomass of *S. gigantea*. However, the maintenance of aboveground biomass came at the

cost of reduced belowground biomass being significantly reduced with damage. When faced with water stress or clipping, rhizomes and roots were more damaged than those of *S. altissima*. The cost of maintaining aboveground tissue under water stress may be lower for *S. gigantea*, since the outer cuticle might reduce water loss.

S. gigantea and *S. altissima* both had a significant decrease in root biomass when faced with water stress alone. This is surprising, since one might think that root biomass would increase to tap into difficult to reach water sources during water stress conditions (Gianoli & González-Teuber 2005; Blum 1996; Fenner 1983). It is possible that root biomass that was present for the plant size was sufficient enough to acquire needed water and nutrients, and perhaps investing more into roots was unnecessary. Other studies have also found that not all plant species increase their root mass when under stress (Grace 1997 Schulze 1986;). Instead of spending resources for root expansion, they may be reallocated to other plant tissues, such as reproductive and photosynthetic structures, which might provide an adaptive advantage. However, there was no synergistic interaction between water stress and simulated herbivory for root biomass.

As a consequence of combined stresses, there was a shift from sexual to asexual reproduction. Resources were invested in maintaining rhizome biomass, while inflorescence biomass generally decreased. When looking at the ability for plants to flower, clipping alone significantly decreased plants that flowered for both *Solidago* species, while water stress significantly reduced plants that flowered only for *S. gigantea*. Past studies suggest that inflorescence production is correlated with plant size, indicating that many plants have a minimum size that allows them to flower (Schmid et al.1995; Weiner 1988). It is plausible that damage reduced *S. gigantea* plant size below such a

threshold, thus reducing the number of plants that could flower. Inflorescence biomass followed the same pattern as the aboveground biomass, *S. altissima* having a synergistic effect and *S. gigantea* an additive effect from water stress and simulated herbivory. Sexual reproduction was more affected by clipping when under water stress for *S. altissima*. It is also possible that clipping stressed plants and affected flowering structures. While inflorescence biomass decreased with damage, rhizomes were maintained and mostly unaffected for both species. This indicates a switch between reproduction modes from sexual to asexual when under stress.

These results were surprising to us because some clonal plant species increase their sexual and decrease their asexual reproduction when stressed (Van Zandt et al. 2003). Environmental stresses influence resource investments and the trade-offs between two reproductive modes (Pluess and Stocklin 2005). Many plants under unfavorable conditions switch to sexual reproduction to produce genetically diverse offspring, whereas when the habitat is favorable asexual reproduction helps plants to colonize faster (Van Zandt et al. 2003). Reproduction preferences may also depend on plant size since; as previously stated, many plants have a minimum size threshold in order to be able to produce inflorescence (Schmid et al. 1995). Pluess and Stocklin (2005) found that threshold in plant size toward clonal reproduction is smaller than for sexual reproduction. It is possible that *Solidago* plants get too small in size to reproduce well sexually when under stress, but are large enough to produce rhizomes.

Producing seeds compared to clonal structures such as rhizomes can be very costly, and when conditions are uncertain there is no certainty that seeds will establish in harsh environments (Prati and Schmid 2000). It might be evolutionary adaptive for plants

to invest more resource into rhizomes, which are already below ground and protected from some environmental stresses, instead of costly sexual reproduction. For some plant species rhizomes may stay dormant, allowing the plant to wait out water stressed condition and grow when more favorable conditions are present (Hutchings 1987; Mehrhoff 1984). Hence it might be beneficial to invest more resources into rhizome structures under water stress.

Conclusion

The combined effect of water stress and simulated herbivory through clipping was synergistic in *S. altissima* for aboveground biomass and inflorescence, and additive for *S. gigantea's* aboveground biomass and inflorescence. Such responses to individual or combined stresses further varied between certain plant tissues and two plant species. There was a switch between sexual to asexual reproduction strategies.

Knowing the impact on plants of combined multiple stresses is important, because it can help us understand how some plant species tolerate one stress while under pressure from any other stress. For example, applying a combination of water stress and herbivory can give insights into how plants tolerate herbivore damage while also under water stress. Since *S. altissima* and *S. gigantea* had different responses to these combine stresses, we can further study what traits make them respond differently. These two different plants species may have different stress-response pathways toward different biotic and abiotic stress conditions, as well as various physiological adaptations. Studying how they differ may give us a better understanding of what makes one plant less tolerant to herbivory under water stress conditions compare to the other plant.

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CHAPTER 4- GENERAL CONCLUSIONS

Coevolution is an important force in ecology and although research has been done on invasive species, not many studies investigate the relationship between coevolution and herbivore impact, such as from gallmakers, on sympatric plant hosts after a host shift. This is novel research that can bring insight into coevolutionary theory on how new plant-insect relationships evolve and old ones persist.

I examined how coevolution affects gall-forming herbivore impact on ancestral and novel host plants. I measured and compared the impacts of the specialist gallmakers *Eurosta* and *Rhopalomyia* on their ancestral *S. altissima* and novel *S. gigantea* hosts. From the difference of aboveground biomass between galled and ungalled plants (within plant triplets) I calculated gallmaker impact. A general trend of higher impact was found on the novel host than on the ancestral host. As a result of coevolution, plants may evolve higher tolerance to their herbivores, while gallmakers may evolve lower virulence. This pattern was observed for both *Eurosta* and *Rhopalomyia*, although not significant through all sites and/or years (Chapter 2).

My initial analysis of collections from Kent Park, Iowa in 2012 suggested that this was an unusual year in that there was a higher impact on the ancestral host than on the novel host for both gallmakers. On closer inspection, Iowa 2012 had higher than average temperatures and lower than average precipitation, inflicting drought conditions on the vegetation (Hillaker, accessed August 31, 2015 <http://www.iowaagriculture.gov/climatology/weatherSummaries/2012/pas2012.pdf>). This led me to speculate that perhaps extreme conditions such as drought and other stresses interact with herbivory in a synergistic manner. This synergy might mask any coevolutionary pattern in interaction strength that is already established. I conducted a

greenhouse experiment to test whether water stress and mechanical damage (representing simulated herbivore damage) had a synergistic or an additive effect on *Solidago* plants. I found that indeed, there was synergy with respect to drought and simulated herbivory, at least for *S. altissima* (the ancestral host) (Chapter 3). This gives a potential explanation for why the impact was higher on the ancestral host for the Iowa 2012 collections.

Detecting and removing this climate-driven anomaly of Iowa 2012 datasets produced a clearer coevolutionary trend. Heard and Kitts (2012) found that in a similar plant-insect system, where *Gnorimoschema* gallmaker attacks its ancestral host *S. altissima* and its novel host *S. gigantea*, herbivore impact was more severe on the novel host (Figure 1 and Table 1 of Heard and Kitts 2012). Pulling these datasets together (and excluding the anomalous Iowa 2012 dataset), I have 10 datasets comparing gallmaker impact on ancestral and novel *Solidago* host plants. Of these 9 showed greater impact on the novel hosts (7 significantly so), and only 1 showed greater impact on the ancestral host (sign tests, $P = 0.011$). I am certain that the difference from the galler impact between two plant species is from the co-evolutionary history with *Eurosta* and *Rhopalomyia*.

My findings are consistent with the conceptual model of coevolution leading to higher parasite impacts on novel hosts. Through evolution it is predicted that tolerance and virulence will have to coevolve between the host and its parasite, leading to reduced impact over time in some, but not all, study systems. A classic example where increased tolerance and/or decreased virulence was observed over time is with myxoma virus on Australia's wild rabbits (Fenner and Woodroffe 1953). Virulence and transmission of the virus were linked, and most virulent strains of virus killed their hosts before being able to

spread and infect new hosts. This linkage has led selection to favor less virulent virus strains, and evolution of more tolerant rabbits, leading to decreased impact from the virus over time (Fenner and Woodroffe 1953).

With inflicted damage, plants may re-allocate their resource to different plant tissues, depending on what will enhance their fitness during stress. In my greenhouse experiment, *S. altissima* and *S. gigantea* allocated their resource more towards belowground tissues than above ground tissues during water stress and mechanical damage (clipping). This suggests that investing resources towards rhizome maintenance compared to production of the inflorescence might be beneficial for these particular plants. Although this is slightly surprising, since in harsh conditions I would expect sexual reproduction to be favored, it might be adaptive because belowground tissue is less susceptible to water loss. Such allocation shifts may affect plant-insect communities, in that if stress leads to reduced aboveground biomass then fewer resources are available for insects that depend on it. This in turn may induce selection toward different insects within that community depending on the year and drought conditions.

Increasing global temperatures and erratic environmental fluctuations can disturb the relationships species already have evolved with each other, and can lead to the loss of biodiversity. This highlights the importance of how environmental conditions can shape the force of coevolution, and help us further understand ecological and evolutionary processes, which can be applied in conservation of biodiversity. In order to understand and study these interactions, we need to collect data through multiple sites and years, as the observed pattern may fluctuate depending on the environmental conditions. Previous studies on how climate change affects plant-herbivore interaction have shown significant

direct and indirect effects on plant defense mechanisms and herbivore abundance, as well as other life-history traits (Lindroth 2010; Valkama et al. 2007; Bale et al. 2002). Drought in particular influences changes in plant defense mechanisms and plant metabolism, potentially providing herbivores with higher quality food and altering their abundance (Gutbrodt et al. 2011; Herms and Mattson 1992; Mattson and Haack 1987). This relationship is unknown for specialists such as gallmakers and would be interesting to explore in future research. However, there have been cases where water stress led plants to be more susceptible towards specialist herbivore attacks (Gutbrod et al. 2011; Haugen et al. 2008). Environmental conditions are therefore very important to incorporate when studying coevolution, since they may alter community compositions and plant-herbivore interactions.

My findings indicate that coevolution can have an effect on herbivore impact towards plant hosts. Depending on the history and relationship between the host and the herbivore, the strength of the impact might differ. These findings of possible coevolution relationships may be applied towards future pest management and biocontrols. If the impact on plants from herbivores decreases over time, this might lead to potential biocontrols erosion over time. Although there are only a few clear-cut cases where biocontrols failed in long-term application due to coevolution, this process might play an more important role in targeting right species and using appropriate agents. Therefore there are clear applications of studying coevolution towards pest management.

Future research should focus on incorporating biotic and abiotic environmental factors when studying coevolution, such as mating systems and water stress. Also allocation of plant resources should be explored, as different plant species compensate for

damage in different ways. Measuring aboveground biomass, as I did for this study, was the most feasible way to measure herbivore impact, but since *Solidago* plants also reproduce through rhizomes, it is essential to measure below ground biomass too. Through my drought experiment (Chapter 3), I found that depending on the source of stress and plant species, resource allocation differs between aboveground and belowground tissues. In my case, drought and mechanical damage have led to resource reallocation into rhizomes, and this is an important portion of plant' fitness. Resource allocation to different plant tissues in response to gallmakers in *S. altissima* and *S. gigantea* is unknown. Manipulation of gallmakers in lab settings might be difficult, however if possible, it might help us better understand how plants respond to such specialized herbivore damage. Another important factor that should be incorporated in future studies is to tease apart tolerance from virulence, so it can be tested if both of these variables change in a predictable manner and how they interact through coevolution.

My research holds value in understanding how plant-herbivores interact through evolution, and how this knowledge towards controlling invasive species can be applied, as well as learning how environment shapes and interacts with such relationships and how the loss of biodiversity can be prevented.

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APPENDIX A- HYDROPONICS

Introduction

Measuring plant biomass is important in assessing plant fitness. Aboveground tissue is crucial for photosynthesis and other necessary plant functions. However, a plant consists of above and belowground tissues, with extensive root and (in some cases) rhizome networks. *Solidago* plants reproduce through inflorescences and rhizomes, and therefore both below and aboveground biomass are directly important to fitness. With damage, plants can reallocate their resources between different tissues in a way that is adaptive and beneficial for their fitness. In order to assess plant fitness, it is important to consider all plant tissues.

We were interested in gallmaker impact on *S. altissima* and *S. gigantea* plants, tying in with a broader question of coevolution and herbivore impact on ancestral and novel hosts (Chapter 2). We collected plants in the field and assessed herbivore impact based on aboveground biomass only. It was impossible to measure belowground tissue in the field because of the root and rhizome networks between neighboring ramets, which made it hard to tell where an individual began and ended. On a broader scale it is unknown if these plants respond to damage in a similar way when it comes to belowground tissues, or if they reallocate their resources in a different manner. This information can help us assess more accurately plant fitness and herbivore impact.

We thought that this restriction could be mitigated in a laboratory setting by imposing mechanical damage on *S. altissima* and *S. gigantea* and measuring their belowground biomass in addition to their aboveground biomass. In order to access the root and rhizomes easily, we decided to use hydroponics to grow our plants. Hydroponics is a system where one can grow plants in water rather than soil, to provide the all

essential nutrient and elements for a plant to grow (Harris 1997). This practice provides easy access to belowground plant biomass.

Methods

On July 10th, 2014, we haphazardly collected 45 *S. altissima* and 45 *S. gigantea* rhizomes from a field along the north side of the pedestrian bridge across the Saint John River in Fredericton, New Brunswick (45.57 N, 66.37 W). We collected ramets at least 1 m away from each other to avoid collecting ramets from the same genotype. In the greenhouse at CFS (Canadian Forest Service, Atlantic Forestry Centre) we placed rhizomes in 13x13x15 cm pots, filling only half of the pots with clay pellets as a substrate (bought from Scott's Nursery in Fredericton). We placed the pots into two 12 cm deep hydroponic tables. These hydroponic tables ran on a continuous-flow tank reservoir and pump system, with solution flooding once in the morning and once in the evening each day. Tables were flooded with a standard hydroponic solution, supplied by CFS, that mostly consisted of dissolved calcium, magnesium, potassium, nitrate, sulfate, and dihydrogen phosphate. Various other micronutrients were also added to the solution, such as iron, manganese, copper, zinc, boron, chlorine, and nickel. Nutrient concentration data were not supplied to us.

Discussion

On July 12th 2014, two days after we planted our rhizomes, we found that the water pumps were not functioning properly due to residue from the clay pellets. The pots contained large enough holes that some clay pellets spilled, further clogging up the system in one of the tables. This caused the rhizomes to be submerged in the hydroponics solution for two days with no drainage, leading to rhizome deterioration. The second

table had functioning drainage, but the clay pellets did not hold enough moisture, causing the rhizomes to dry out. In either case we ended up losing our rhizomes from both tables.

This procedure can be improved by using a different substrate, as the clay pellets neither held enough moisture nor worked well with the pumping/draining system. We can eliminate these problems by flooding the tables more often, using a mesh covering for the holes in the pots, or use a different substrate altogether. Alternatively, we can use high pressure water to wash the soil from belowground tissues, as we did with pot-grown material in our drought experiment (Chapter 2). This procedure was effective in achieving the end result of giving us access to belowground biomass. Better methodology for hydroponics should still be developed, since it might be a more accurate and easier technique in measuring belowground biomass.

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APPENDIX B- GENERALIST HERBIVORE EXPERIMENT

Introduction

Gallmakers and goldenrods can be used as a model system to study the evolution of herbivores' impact on their hosts. On several occasions gallmakers attacking goldenrods have undergone host switches between goldenrod species (Stireman et al. 2005; Abrahamson and Weis 1997). *Rhopalomyia solidaginis/capitata* (Diptera: Cecidomyiidae) and *Eurosta solidaginis* (Diptera: Tephritidae) each possess an ancestral and novel host-pair association with *Solidago altissima* and *S. gigantea* respectively (Stireman et al. 2005). The hypothesis that novel host associations should experience higher herbivore impact initially, compared to ancestral associations, can therefore be explored using these gallmakers and their appropriate goldenrod hosts.

However, it is unknown if the two species of goldenrods tolerate damage differently, regardless of the source. If one plant tolerates damage of all sorts better than the other, then this effect is not due to novel herbivore impact, but only due to species differences between the plants. While this question can be addressed with mechanical-damage experiments (Chapter 3), herbivore damage may induce responses (such as chemical defences in plants) that vary across species. Therefore it is important to know that both *S. altissima* and *S. gigantea* respond similarly to damage by a herbivore with which they have similar evolutionary histories. Whitemarked tussock moth, *Orgyia leucostigma* (Lepidoptera: Lymantriidae) (JE Smith), is a herbivore that does not possess any significant evolutionary history with either host plant. Whitemarked tussock moths do not commonly consume *Solidago*, however, they have a wide host range and feed as

generalists on many plant species. They can therefore act as novel defoliators for *S. altissima* and *S. gigantea*.

Methods

We used 30 ungalled plants of *S. altissima* and *S. gigantea*, and 30 previously galled plants of *S. altissima* and *S. gigantea*, from the Minnesota populations collected by Chandra Moffat (pers. comm.; September 24-26 2013). These plants were grown for one generation in the UNB greenhouse, from winter 2014 through spring 2014. During the week of April 21st 2014, rhizomes from senesced plants were put into a cold storage of around 4 °C for 10-12 weeks. After the cold treatment, pots containing rhizomes were brought to the CFS (Canadian Forest Service, Atlantic Forestry Centre) greenhouse, where each rhizome clump, still intact in its previous potting soil, was split by a knife into four separate rhizome clumps. One of clumps was put back into old pots and returned to UNB cold storage. The other three rhizome clumps were potted separately into 20x20x30 cm pots, using peat mixed with 25% perlite as a substrate. Pots were then randomized to bench position, and treated with a slow releasing 14-14-14 fertilizer (Nutricote) (5 mL per pot).

We ordered around 30 white-marked tussock moth egg masses (100-200 eggs in each), and rearing containers with pre-made artificial diet from Sylvar Technology Inc., Fredericton. On August 26, we placed the egg masses in containers with rearing diet (1 egg mass per a container). Diet containers were flipped upside down (for first couple of weeks) to avoid moisture from the diet interfering with egg hatching. All containers were under 12:12 light-dark cycle. Eggs hatched 12 days after, and we changed diet containers to new ones on a weekly basis. Once the caterpillars started reaching second and third

instars, we transferred them to new containers to decrease density, leaving around 100 individuals in each container for the first two weeks, and around 50 for the following weeks. By late September, once the larvae reached the fourth instar, we placed them on our goldenrod plants with densities of 5,10,15, and 20 larvae per plant. Our sample size consisted of 3 plants per species for each density. After 7 days we collected the larvae and the damaged plants by clipping them at the soil level.

Discussion

Despite our best efforts, we had trouble growing our plants. Also since the plants grew poorly and slowly, it was difficult to predict and synchronize the rearing of white-marked tussock moth so that they reached the fourth instar when plants reached 50 cm in height. This led us to reconstruct our initial experimental design to a simpler one where we tested if white-marked tussock moth larvae even consumed and damaged *Solidago* plants.

Defoliation damage from white-marked tussock moth larvae was evident on all *S. altissima* and *S. gigantea* plants that were treated. After larvae were placed on the plants, the caterpillars started to chew the leaves, with each passing day creating more noticeable damage. The degree of damage depended on the density of the larvae on the plant, however even with 5 larvae over 7 days, the damage was significantly noticeable. Although all the leaves were targeted, most damage was evident on the younger leaves at the top of the plant. This provides us with insightful information that a generalist herbivore with no close evolutionary history to *S. altissima* and *S. gigantea*, such as white-marked tussock moth, can consume and inflict damage to them. In future studies we can test if *S. altissima* and *S. gigantea* respond to herbivore damage in similar ways,

and the use of white-marked tussock moth can supplement mechanical damage in consecutive experiments.

The reason for poor plant growth was most likely inaccurate cutting and separating of the rhizomes into four components. This procedure probably left many rhizome clumps with too small a resource reservoir to produce a healthy ramet, or damaged the rhizomes altogether. Since we didn't separate rhizomes from soil beforehand, because we assumed prior plants were so healthy they produced many rhizomes, we couldn't have been really sure how well we separated them. In future studies, separating rhizomes from the soil and visually dividing them to needed components would be helpful. This has been our standard lab protocol for potting field-derived material, and should be adopted for greenhouse-propagated material too.

Another possible reason for the plants poor growth could be the condition they were reared in. Although these plant prefer disturbed habitats, the mix of peat and perlite might not have been right for *Solidago* plants. In future studies we should use soil from previous suppliers, such as Pro-mix BX from Premier Tech Rivière-du-Loup, Canada, since we know *Solidago* plants grew well in this soil from previous experiments.

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Stireman JO, III, Nason JD, Heard SB (2005) Host-associated genetic differentiation in phytophagous insects: general phenomenon or isolated exception? Evidence from a goldenrod insect community. *Evolution* 59:2573-2587

Curriculum Vitae

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EDUCATION

M.Sc Biology : University of New Brunswick, NB

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Thesis: Impact of attack by herbivores on their ancestral and novel plant hosts in a goldenrod-insect system

B.Sc Major in Ecology & Evolutionary Biology: University of Toronto, ON

2009-2013

Minor in Sociology, Minor in Buddhism, Psychology & Mental Health

PRESENTATIONS

Shibel Z., Heard S.B., & S. Hendrix. 2015. Impact of attack by *Eurosta* and *Rhopalomyia* on their ancestral and novel *Solidago* hosts. Acadian Entomological Society, Fredericton, NB.

Shibel Z. & S.B. Heard. 2015. Synergistic and additive effects of water stress and clipping on *S. altissima* and *S. gigantea*. Canadian Society of Ecology and Evolution Conference (CSEE), Saskatchewan, SK.

Shibel Z. & S.B. Heard. 2015. Synergistic and additive effects of water stress and clipping on *S. altissima* and *S. gigantea*. Graduate Research Conference (GRC), Fredericton, NB.

Shibel Z., Heard S.B., & S. Hendrix. 2014. Impact of attack by *Eurosta* and *Rhopalomyia* on their ancestral and novel *Solidago* hosts. Canadian Society of Ecology and Evolution Conference (CSEE), Montreal, QC.

Shibel Z. & J.E. Eckenwalder. 2013. Do Poplar Hybrids experience different levels of herbivory compared to their parental species? 43rd Annual Ontario Ecology, Ethology & Evolution Colloquium (OE3C), London, ON.

Austen E. J., Ison J., Weis A. E., & Z. Shibel. 2012. Does phenological assortative mating cause S-alleles to be structured by flowering time? University of Toronto research poster fair, Toronto, ON. Department of Ecology & Evolutionary Biology.

Austen E. J., Ison J., Weis A. E., & Z. Shibel. 2012. Does phenological assortative mating cause S-alleles to be structured by flowering time? 42nd Annual Ontario Ecology, Ethology & Evolution Colloquium (OE3C), Hamilton, ON.

Kutas C., Shibel Z., Della Mora L., & S. Dsouza. 2010. The Chemistry of Health and Beauty Products. Chemical Institute of Canada Conference, Toronto, ON.

AWARDS

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|---|------|
| -CSEE Poster 1st prize, Canadian Society of Ecology and Evolution | 2015 |
| -GRC Poster 2nd prize, UNB Graduate Research Conference | 2015 |
| -Acadian Entomological Society Oral Presentation 3rd prize, The Acadian Entomological Society Annual Meeting | 2015 |