Density Dependence and Clonal Integration in Common Milkweed, Asclepias syriaca

Mary Danielle Seward
Waverly, Virginia

Bachelors of Science, Christopher Newport University, 2014

A thesis presented to the Graduate Faculty
Of the college of William and Mary in Candidacy for the Degree of
Master of Science

Biology

The College of William and Mary
May 2016

APPROVAL PAGE

This thesis is submitted in partial fulfillment of
the requirements for the degree of

Master of Science

Mary D Seward

Approved by the Committee, April, 2016

Committee Chair
Assistant Professor Dr. Harmony J. Dalgleish, Biology
The College of William & Mary

Associate Professor Dr. Martha Case, Biology
The College of William & Mary

Assistant Professor Dr. M. Drew Lamar, Biology
The College of William & Mary

Assistant Professor Dr. Joshua Puzey, Biology
The College of William & Mary

ABSTRACT

The density of individuals in a population has the potential to affect growth and death rates of that population. This effect of density is called density dependence and can be negatively or positively related to a vital rate. In most populations, increasing density leads to lower growth rates, which is negative density dependence. Positive density dependence, or Allee effects, occurs when increasing density leads to an increase in a vital rate, such as increasing survival in large herds. However, in clonal plants, there is the potential for the effects of density to be ameliorated. Clonal plants produce genetically identical progeny and some maintain a physiological connection even after the progeny mature. Through this physical connection, resources may be shared through clonal integration and thus clonal plants ameliorate the effects of stressful environments by acting as one large plant, or a genet, rather than as many individuals. *Asclepias syriaca*, common milkweed, is a clonal species that maintains connections to its clonal progeny. Common milkweed has been a popular species of study because of its relationship with the monarch butterfly, *Danaus plexippus*, and other specialist herbivores and because it is a pest plant in agriculture. In spite of this research, little is known about how individuals, or ramets, interact with one another. Understanding if ramets are integrated is key to furthering our understanding of how this species functions.

My research on milkweed integration was conducted in two parts; a greenhouse experiment and a field study. The greenhouse experiment used pairs of milkweed to first determine if the defense chemicals of milkweed, known as cardenolides, are shared between damaged and undamaged ramets. A second portion of this experiment studied foliar concentrations of nutrients such as nitrogen and carbon and how they varied between connected and unconnected ramets. The third portion of this study focused on integration in a stressful environment, shade.

The field study used three years of data from 4 sites and 18 transects across the state of Virginia to determine how density changes across years and how that potentially changes the responses of the plants. Measures of survival, growth, reproduction, and herbivory were taken each year. These data were modeled with generalized mixed effects models to simultaneously take into account the effects of year, site, and transect on each plant response. These responses were predicted to follow patterns that would suggest integration, which could be seen as a lack of competition when it would normally be expected.

In the cardenolide study, all ramets responded to the damage treatment, indicating a volatile signal of herbivory. The foliar trait study showed connected ramets may be better at taking up nitrogen than unconnected individuals. Biomass at the end of the shade experiment showed signs of competition in unconnected but not in connected ramets, indicating integration. In the field study density only affected survival, height, leaf area, growth, and the number of inflorescences. At the patch scale, increasing density decreased survival, height, leaf area, and the number of inflorescences. Growth was not affected by density at the patch scale, but at the small scale it decreased with increasing density. Survival and height also decreased with increasing density at the small scale. The number of inflorescences was not affected by density at the small scale.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

This writer wishes to express his or her appreciation to Professor Harmony Dalgleish, under whose guidance this investigation was conducted, for her patience, guidance and criticism throughout the investigation. The author is also indebted to Professors Martha Case, Josh Puzey, and Drew Lamar for their careful reading and criticism of the manuscript. Additional thanks are due to the many undergraduate students in both the Dalgleish and Puzey lab for their assistance and unending energy while collecting the data that were used in this research.

This writer also wishes to express her appreciation to family and friends, especially Graham Bryant, for their never ending support and encouragement and for their occasional assistance with data collection.

Introduction

The density of individuals in a population has the potential to affect rates of growth and death of that population. This effect of density is called density dependence and can be negatively or positively related to a vital rate. In most populations, increasing density leads to lower growth rates, which is negative density dependence. Positive density dependence, or Allee effects, occurs when increasing density leads to an increase in a vital rate, such as increasing survival in large herds.

However, in clonal plants, there is the potential for the effects of density to be changed or avoided. Clonal plants produce genetically identical progeny through their vegetative structures, and some will maintain a physiological connection even after the progeny has matured. Through this physical connection, resources can be shared through a process called clonal integration. Through clonal integration, clonal plants can ameliorate the effects of stressful environments by acting as one large plant rather than many individuals. By functioning as an individual, it is possible that clonal plants respond to increasing density in a way that is different from that of non-clonal plants.

Asclepias syriaca, or the common milkweed, is a clonal species that maintains physiological connection to its clonal progeny. Common milkweed has been a popular species of study because of its relationship with the IUCN Red List near-threatened species monarch butterfly, *Danaus plexippus*, and other specialist herbivores. Common milkweed has also been studied because of its role as a pest plant in agriculture (Bhowmik, 1982). In spite of all this research, there is little understanding of how milkweed plants interact with one another, leaving a major gap in the knowledge about milkweed. Understanding how clones interact with one another is key to furthering our understanding of how this species functions.

My research on common milkweed integration was conducted in two parts; a greenhouse experiment study and a field density study. The greenhouse experiment used pairs of common milkweed plants to first determine how the defense chemicals of common milkweed, known as cardenolides, may be shared between damaged and undamaged individuals. A second portion of this experiment looked at foliar concentrations of nutrients such as nitrogen and carbon and how

they varied between connected and unconnected individuals. The third portion of this study focused on integration in a stressful environment: shade. Common milkweed does not tolerate shade well, and this experiment was established to determine if common milkweed shares photosynthates between unstressed and stressed individuals, and if this sharing depended on the age of the individual in the stressful environment.

The field density portion of my research used three years of field data from 4 sites and 18 transects across the state of Virginia to determine how density changes across years and how that potentially changes the responses of the plants measured each year. Measures of survival, growth, reproduction, and herbivory were taken each year at the beginning and the end of the growing season. These data were modeled using generalized mixed effects models to simultaneously take into account the effects of year, site, and transect on each plant response. These responses were predicted to follow patterns that would suggest integration, which could be seen as lower or a lack of competition when it would normally be expected.

Chapter 1-Clonal Integration in *Asclepias syriaca* – Foliar Chemistry and Shade Stress

Introduction

Clonal plants make up between 45 and 80 percent of the world's plant species (Abrahamson, 1980). Clonal reproduction through stolons, rhizomes, or roots is useful for plants that grow in harsh environments or are self-incompatible, allowing them to reproduce even without successful cross-pollination (Gerhardt, 1929; Moore, 1946). In clonal species, mature clones often remain connected to each other, but are capable of functioning fully on their own should they become disconnected from the rest of the clone (Stuefer, 1998). The potentially individual parts are considered ramets, while the entire body of genetically identical individuals is referred to as a genet (Bell & Tomlinson, 1980). When ramets remain physiologically connected, clonal integration may occur, meaning physically connected ramets share resources with one another (Stuefer, 1998).

Clonal integration can be advantageous because it ameliorates the cost of expanding ramets into less-than-ideal patches in the environment. When clonal plants spread through a heterogeneous environment, they will encounter areas of high and low resources, such that ramets may have a "spatial division of labor" with ramets specializing in acquiring and sharing the resource that is most abundant in their location (van Kleunen & Stuefer, 1999). For example, van Kleunen and Stuefer (1999) investigated such division of labor in the clonal plant *Potentilla anserina*. In genets where one ramet was water stressed but not shade stressed and the other was not water stressed but was shade stressed, connected ramets accumulated more biomass than unconnected ramets (van Kleunen and Stuefer 1999). In studies that investigated the effect of shade in clonal plants, such as *Fragaria chiloensis* and *Aegopodium podagraria*, connection to another ramet that was receiving full light significantly reduced the impact of shade stress (Friedman & Alpert, 1991; Nilsson & D'Hertefeldt, 2008). Friedman & Aplert (1991) used ramets of *F. chiloensis*, the beach strawberry, to determine the effects of shade, nitrogen limitation, and manual stolon severing on leaf mass and biomass. They found that ramets left intact accumulated 54% more biomass than did the severed ramets. Within pairs, the ramets with low

light and high nitrogen that were left connected to a high light, low nitrogen ramet had greater overall biomass than severed ramets (Friedman & Alpert, 1991).

In clonally integrated species, the strength of integration can differ based on the origins of the species. Nilsson & D'Hertefeldt (2008) conducted a shade stress experiment on *Aegopodium podagraria*, ground elder. They took northern and southern samples from forest and garden populations of ground elder and hypothesized that ramets from the harsher northern populations would be most integrated and that there would be more integration in the forest populations than the garden populations (Nilsson & D'Hertefeldt, 2008). They found that severing rhizomes significantly decreased biomass in all clonal fragments, shaded ramets had lower biomass, and there was an interaction between biomass and shading. The interaction between biomass and shading indicated a benefit of connection to connected shaded ramets without a negative impact on the unshaded ramets (Nilsson & D'Hertefeldt, 2008). Most importantly, they found that the forest ramets were more severely impacted by severing than garden ramets, though there was not a difference between northern and southern populations. Their results thus suggest that levels of integration can vary based on habitat but not geographic origin.

The age of ramets—that is, whether the ramet is a parent or offspring—can influence the level of clonal integration (Stuefer et al., 2004a; Lovett-Doust, 1981; Touchette, 2013). However, the impact of age on clonal integration is highly variable: in some species, only younger ramets benefit from physiological integration such as *F. chiloensis* and *P. ansernia*; in other species such as *Justicia americana* and *Ranunculus repens*, integration has little effect on the mother or daughter (Lovett-Doust, 1981b; Alpert 1991; Stuefer et al., 2004a; Touchette, 2013). For example, van Kleunen and Stuefer (1999) found in *P. ansernia* that integration was only observed when the younger ramet was shaded and well-watered indicating that older ramets share assimilate but not water. Similarly, Alpert (1991) found support for an age gradient in *F. chiloensis*. He found that only the connected ramets in low nutrient treatments were significantly affected by connection, and that they produced significantly more stolons and ramets than did the severed ramets. Differences between leaf mass in nitrogen-stressed younger ramets and older ramets indicated that nitrogen was shared with the younger ramets when stressed, but that older

ramets did not receive nitrogen. These results suggest an age effect on integration, with the older ramets giving, but not receiving, nutrients through their stolons. This age hierarchy may be driven by a parent-offspring relationship or simply by biomass.

Asclepias syriaca, common milkweed, is a clonal herbaceous plant found across eastern North America. Common milkweed is known to reproduce sexually as well as asexually through adventitious root buds and to maintain a physical connection between ramets; however, it is unknown to what extent ramets might be physiologically integrated. Physiological integration in milkweed may extend to both sharing resources such as water, nitrogen and phyotosynthate, and to the sharing of chemical defenses. When damaged by herbivores, common milkweed responds by exuding latex, which deters herbivory by gumming up insect mouthparts, and increasing production of cardenolides, a type of cardiac glycoside that is bitter tasting and toxic (Agrawal et al., 2008). There are about 23 known cardenolide compounds produced by common milkweed and other species such as *Adonis multiflora* and *Thevetia peruviana* (Jung et al., 2015; Tian et al., 2016). Cardenolides inhibit sodium and potassium ATPases (Tian et al., 2016; Malcolm & Zalucki, 1996). While toxic to insects, cardenolides have been used medicinally in humans for around 200 years (Gheorghiade et al., 2006). Cardenolides in common milkweed are produced at low constitutive levels and foliar levels of cardenolides increase after herbivory (Malcolm & Zalucki, 1996, Couture et al. 2013, Agrawal et al., 2014).

The latex and cardenolide production, as seen in common milkweed, are not the only forms of plant responses to herbivores writ large. Production of phenolic compounds, production of methyl jasmonate and reduction of leaf quality are just a few examples of responses to herbivory found in different plant species (Rhoades, 1983; Baldwin & Schultz, 1983; Karban et al., 2000). Responses such as these can attract predators of herbivores or make a plant less palatable to a subsequent herbivore (Wason & Hunter, 2014). Responses that attract predators can be volatile organic compounds that are released into the air after a plant experiences herbivory and can also elicit a response in neighboring conspecifics that are not experiencing herbivory. This conspecific response has been observed in clonal and non-clonal species such as tree species *Alnus rubra*, *Salix sitchensis*, *Populus X euroamericana*, and *Acer saccharum* and

herbaceous species *Nicotiana attenuata* and *Artemesia tridentata*. If a neighboring conspecific receives a signal, it responds just as if it had been damaged, and thus is better protected against a potential herbivore attack. However, some clonal species can produce induced systemic responses that are transferred through physiologically connected ramets and not to unconnected neighbors by volatiles (Gomez & Stuefer, 2006). Common milkweed is known to produce volatile chemicals that are used by ovipositing monarch butterflies (Bergstrom et al., 1995) and to attract predators of both above and belowground herbivores (Rasmann et al., 2011; Wason & Hunter, 2014). However, it is not known if these volatile signals are received by other milkweed ramets, which then increase production of defensive chemicals despite not receiving any direct damage themselves.

To further the understanding of clonal integration between ramets of common milkweed, I conducted two greenhouse experiments using connected and unconnected milkweed ramets. In the first experiment, one ramet in the pair underwent experimental herbivore damage. I measured cardenolide and foliar traits using reflectance spectroscopy to measure the levels of cardenolides and other foliar chemicals such as carbon, nitrogen, lignin, and fiber. In addition, stress was measured using PRI. Reflectance spectroscopy uses light at specific wavelengths ranging from ultraviolet to far infrared to predict chemical data (Couture et al., 2013). The predictions are then compared to reliable chemical measurements from traditional chemistry using multivariate models to create a prediction model (Couture et al., 2013). Reflectance spectroscopy allows for measurements to be taken on a leaf *in vivo* and to non-destructively take chemical data at multiple time points. In the second experiment, one ramet was experimentally shaded. The shade experiment used shade as a stressor that could potentially induce the sharing of resources between a shaded and unshaded ramet. Treatments in the shade experiment were crossed by age to determine if resource sharing is dependent on age in common milkweed.

Hypotheses

In the herbivory experiment, I did not expect cardenolides to be shared between ramets, or for a volatile signal to induce cardenolide production in nearby or connected ramets. I expected to see a sharp increase in cardenolide levels in response to the herbivory treatment by 24 hours

in the damaged leaves and that the cardenolides would then slowly drop back to constitutive levels, but no response would be seen in the undamaged ramet in both the connected and unconnected treatments. I expected foliar traits such as carbon, nitrogen, lignin, and fiber to be higher in the connected ramets because they would be larger and thus better able to assimilate nutrients. When stressed, plants reduce their photosynthetic activity and thus have an excess of energy, which is diverted from the chlorophyll to the xanthophyll cycle (Meroni et al., 2008). The PRI of stressed plants changes as a result of the switch to xanthophyll, and PRI becomes increasingly negative as plant stress increases and is usually a result of water or oxidative stress (Meroni et al., 2008). PRI has not been directly measured in relation to herbivory, but previous research has found that net photosynthetic rates were impaired only in the damaged leaves of *Asclepias curassavica*, with no photosynthetic impairment or compensation occurring in the other leaves (Delaney, 2008). Thus there is a possibility that herbivory can cause plant stress as measurable by PRI in *Asclepias* species. PRI was expected to remain the same through the experiment in undamaged ramets, and was expected to be higher in damaged ramets initially but to drop back to levels similar to undamaged ramets over time.

My hypotheses for the shade experiment were that photosythates would be shared between the connected ramets and that the shaded, connected ramets would grow more than the shaded, unconnected ramets. I also predicted that the sharing of the photosynthates would not differ between older and younger ramets.

Methods

Ramet collection

A greenhouse population of common milkweed was started in 2013. It was used for various experiments in a greenhouse at The University of Maryland and was moved to William & Mary in January 2015. By 10 April 2015, the ramets for the 2015 growing season emerged. Between 15 April and 1 May 2015, pairs of root buds that looked healthy and capable of sprouting were dug. Twenty-eight different clones were collected for this experiment. Root pairs were bagged in damp peat moss and kept in a refrigerator until the experiment started on 1 May. The

buds were potted in 14 inch pots. Half of the root samples were left connected; the other half were cut in half (Table 1). The plants were then left to grow for roughly 2 months. Root pairs were replaced by buds of approximately the same size from the same clone if they died, with this process continuing until all tubs had healthy pairs. The pots were monitored daily in order to mark the sprouts as “older” or “younger” depending on when they emerged. In marking the sprouts in this manner, there was a working assumption that the “older” ramet would have more biomass than the “younger” ramet. These designations were used in both experiments. Age for the purposes of these experiments was determined by emergence date of each bud.

Herbivory experiment

Beginning 6 July 2015, all plants with leaves large enough to be sampled for reflectance spectroscopy were used in the herbivory experiment. The determination of which ramet would be damaged, older or younger, was made randomly for each pot (Table 1). Plants were damaged by cutting off one eighth of total leaf tissue by removing one quarter of leaf mass from one leaf per leaf pair on the treated ramet, such that half the leaves were damaged per ramet (Figure 1). Leaf scans were made before cutting to create a baseline for each ramet. After cutting, leaves were scanned 0.25, 6, 24, 48, 72, and 120 hours after cutting. Both the damaged ramet and its partner were scanned at each time point. One leaf pair, or two leaves, was scanned per ramet, which were either the largest leaf pair or the topmost unfurled pair depending on the size of the plants and their leaves. One of the scanned leaves was damaged, the other was undamaged. Each leaf was scanned in three places and three scans were taken from the three locations. Each scan gave wavelength signatures of the light reflected off the sample and were modeled to create cardenolide content predictions. Scans were screened for any outliers, and were also visually inspected for any unusual or unlikely scans by our collaborator, John Couture, as he prepared the scans to be modeled for foliar trait and cardenolide concentration predictions.

Figure 1. Example of a plant after simulated herbivory. For each leaf pair, one quarter of one leaf was cut.

Shade experiment

The shade experiment was started on 31 July 2015 using the same plants from the cardenolide experiment. One ramet per pot was shaded, systematically chosen between younger and older as well as damaged and undamaged, in order to control for any effects caused by the cardenolide analysis. Therefore, half of the ramets used in the cardenolide experiment were shaded while the other half were not shaded. Shade cages were built by putting PVC piping into the tubs around the treated plant and then creating a structure of shade cloth around the plants using the PVC to support it. The shade cloth reduced the levels of light by an average of 34 percent. The plants were left for two weeks to grow and were then measured once a week for the three following weeks. The experiment was planned to run through September, but an early cold spell caused the plants to drop their leaves prematurely, thus experiment was stopped on 8 September and the plants were harvested. The aboveground and belowground portions of each plant were separated before drying. The number of buds per root mass were counted for each ramet. If the ramets were connected, the root mass was divided evenly between the two ramets and each part was counted independently for root buds.

Table 1. Treatments and Sample Sizes. There were 84 total tubs. At the start of the cardenolide experiment, only 40 pairs had both ramets large enough to be measured for reflectance spectroscopy. In the damaged and undamaged treatments, only one ramet per pair was damaged.

Treatment	Number of ramets	Number of Pairs
Connected	84	42
Unconnected	84	42
Damaged	40	20
Undamaged	40	20
Shaded	84	42
Unshaded	84	42

Analysis

Data were analyzed using general linear mixed effect models (GLMM) using lme4, lmerTest, and languageR in R (R core team, 2015; Bates et al., 2016; Kuznetsova et al., 2016; Bayeen, 2013). In these models R_i is a matrix containing the response of interest for ramet *i* (Equation 1, Zuur et al., 2009).

Equation 1.

$$R_i = X_i \times \beta + Z_i \times b_i + \varepsilon$$

$X_i \times \beta$ is the term for the fixed effects, which are the explanatory variables expected to have one true effect size. $Z_i \times b_i$ is the term for the random effects, which are explanatory variables that have many levels, each level with its own "baseline" or average. Including random effects allows the model to take the variation of each level into account, rather than using one average for all levels (Winter, 2014).

The responses for the cardenolide experiment were analyzed in ten different ways. The peak response was the highest predicted concentration of foliar cardenolides for a given plant, and trough was the lowest. I modeled the peak and trough response to determine if different ramets responded more strongly to the treatment than others. Time of peak and time of trough were modeled to determine if the timing of response varied between treatments. To determine if the absolute change in cardenolides was a better predictor of the response than peak alone, I modeled the difference between the peak and trough (peak-trough) as well, hereafter referred to as cardenolide response. Finally, normalized measures of cardenolides were calculated as peak divided by trough, as well as cardenolide response divided by peak or by trough.

The responses for the foliar trait models were carbon, nitrogen, C:N ratio, fiber, lignin, and PRI. The responses for the shade models were stem and root weight, change in height and diameter through the season, and number of buds. The fixed effects for both experiments were

connection treatment, and age with clone as the random effect, with the addition of damage treatment for the cardenolide experiment and shade treatment for the shade experiment.

A three way ANCOVA was run on the average cardenolide level for each time in each treatment to test for different effects of time, connection, or damage on average cardenolides (Equation 2).

Equation 2.

$$Y_{ijk} = \mu + \beta X_{ij} + \alpha_j + \epsilon_{ijk}$$

μ is the grand mean. β is the dependent variable in my models this was average cardenolides. α is the intercept for the model. X is the covariate term, which was height in my models. ε_{ij} is the error term.

Results

Herbivory Experiment - Foliar traits

The connection and damage treatments had no effect on any of the foliar traits. However, the traits responded differently to height. Nitrogen decreased with increasing height (Figure 2, Table 2). Carbon increased with increasing height (Figure 2, Table 2). The C:N ratio was increased with increasing height. Fiber followed a similar pattern, with an increase in fiber with increasing height (Figure 2, Table 2). Finally, PRI also increased with increasing height. Lignin did not have any significant relationships.

Table 2. Probability values from the GLMER models. The equation for these models was response ~ height * damage * connection + (1|clone). For all of these traits, none of the interactions were significant.

Chemical	Height (H)	Damage (D)	Connection (C)
Nitrogen	**0.0004**	0.78	0.63
Carbon	**0.0001**	0.94	0.15
C:N Ratio	**0.0001**	0.63	0.52
Fiber	**0.007**	0.76	0.13
PRI	**0.003**	0.8	0.79
Lignin	0.47	0.96	0.07

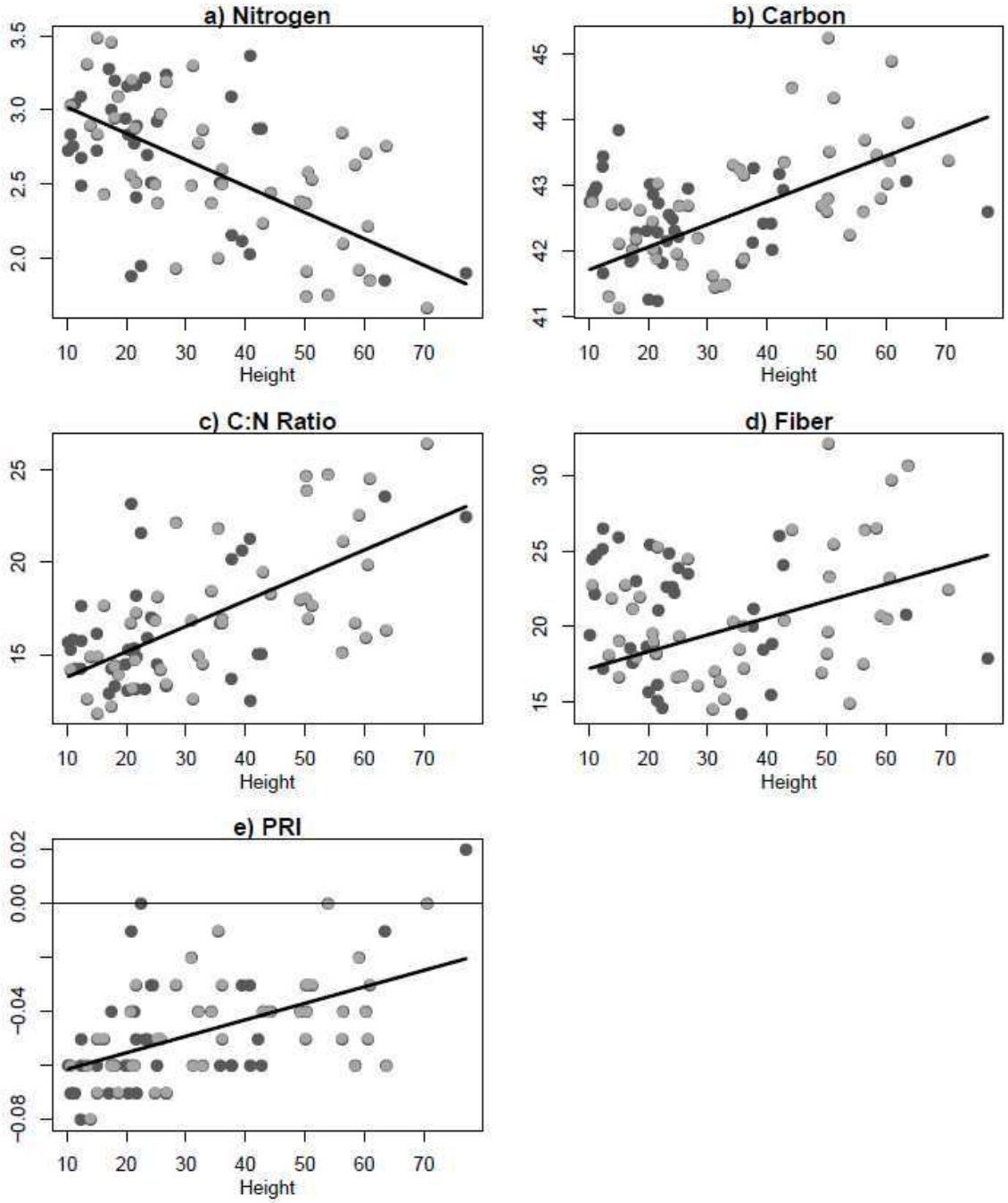


Figure 2. Height significantly affected nitrogen (*P*=0.0004), carbon (*P*=0.0001), the C: N ratio (*P*=0.0002), fiber (*P*=0.007), and PRI (*P*=0.003). The lighter weight horizontal line in the PRI graph marks zero. Dark grey circles represent unconnected ramets and light grey circles represent connected ramets and the black line is the prediction line from the GLMM. Nitrogen, carbon, C:N ratio, and fiber were measured as percent dry mass of the leaf. The equation for these models was response ~ height * damage * connection + (1|clone).

All ramets responded to the herbivory treatment, including the undamaged ramets. They all peaked at 24 hours and dropped back to near-constitutive levels by 72 hours (Figure 3). The

ANCOVA tests to on average cardenolides showed that only time had an effect, indicating that average cardenolides did not differ between ramets in the connection or damage treatments and that all ramets responded similarly to time (Table 3). There were no interactions between treatments. A Tukey Honest Significant Differences (HSD) test showed that all times were different from each other (Table 4).

Table 3. Results from the ANCOVA model.

	Sum of Squares	**Degrees of Freedom**	**F value**	***P* value**
Connection	0.312	1	1.3557	0.2454
Damage	0.187	1	0.8116	0.3685
Time	26.468	3	38.3392	< 0.0001

Table 4. Results from the Tukey HSD test to determine which times were different.

	Difference of Means	**Lower Interval**	**Upper Interval**	***P* value**
24-0	1.441	1.214	1.668	<0.0001
48-0	1.073	0.846	1.299	<0.0001
72-0	0.25	0.002	0.499	0.047
48-24	-0.368	-0.565	-0.172	<0.0001
72-24	-1.191	-1.412	-0.969	<0.0001
72-48	-0.822	-1.043	-0.601	<0.0001

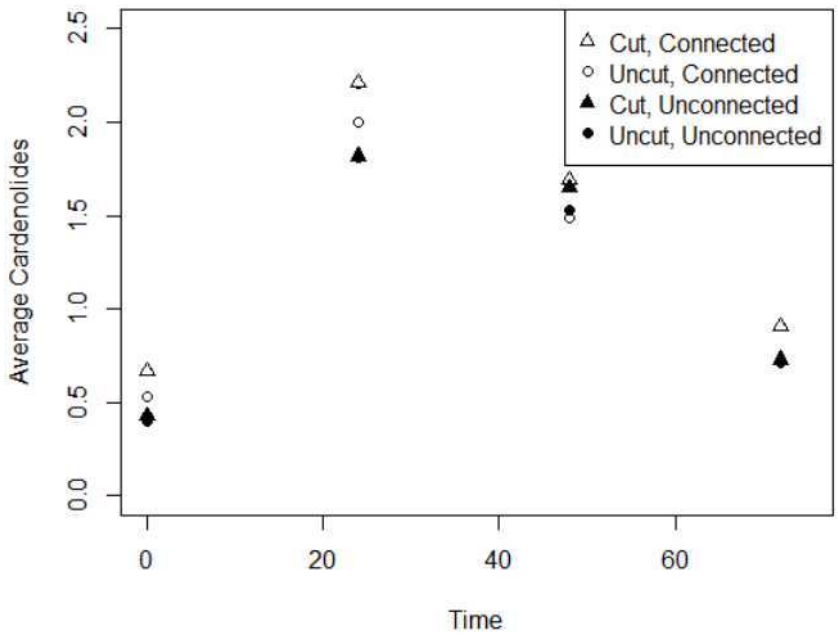


Figure 3. Average cardenolides for the four treatments. All peaked at 24 hours and dropped back to near constitutive levels by 72 hours.

Peak cardenolide levels had an interaction with height and damage (Figure 4, Table 4). In the damaged ramets, peak cardenolide levels decreased with increasing height (slope=-0.0086, SE=0.0064) but in the undamaged ramets there was no difference (slope=0.0026, SE=0.0051),

which indicates that height does not have an effect on the undamaged ramets. There was also a main effect of the connection treatment: connected ramets had higher peak cardenolide levels than the unconnected ramets (Figure 5, Table 4). The effects of height and connection contradict one another because the connected ramets were significantly taller than the unconnected ramets (*P*=0.005).

Table 4. Probability values from the GLMER models. Significant interactions give the two terms that were interacting, and then the *P* value from the interaction. The equation for these models was response ~ height * damage * connection + (1|clone).

	Height	**Damage**	**Connection**	**Significant interactions**
Peak Cardenolide	**0.02**	**0.007**	**0.009**	**Height*Damage, 0.02**
Cardenolide Response	0.12	**0.02**	0.09	**Height*Damage, 0.02**

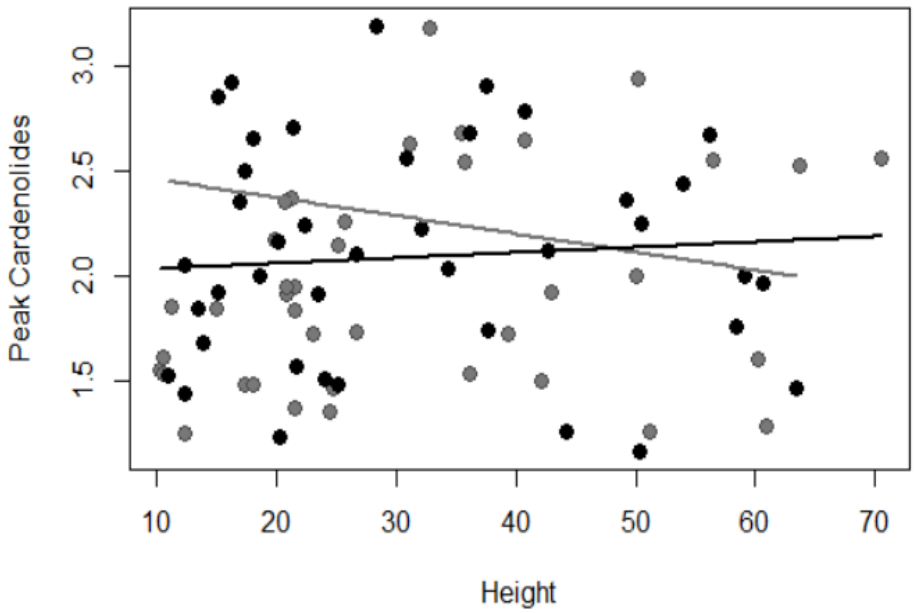


Figure 4. Peak cardenolides were affected differently by height depending on the damage treatment. Damaged ramets, represented by the grey points and grey line, had lower levels of cardenolides as they got taller (slope=-0.008642, SE=0.006390). Undamaged ramets, represented by the black points and black line, had higher levels of cardenolides as they got taller (slope=0.0026, SE=0.0051).

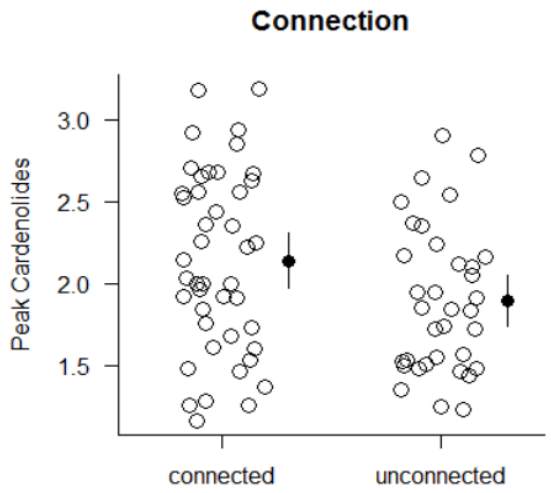


Figure 5. Peak cardenolides were higher in the connected ramets than unconnected ramets (*P*=0.036).

Cardenolide response, calculated as peak-trough cardenolide level, had an interaction between height and the damage treatment (Figure 6, Table 5). The damaged ramets cardenolide response decreased with increasing height (slope= -0.0047, SE=0.005), but in the undamaged ramets the response was not different (slope=0.005848, SE=0.005099). This indicates that height did not have an effect on the cardenolide response of the damaged ramets, which is the opposite of the results for peak cardenolide levels (Figure 6).

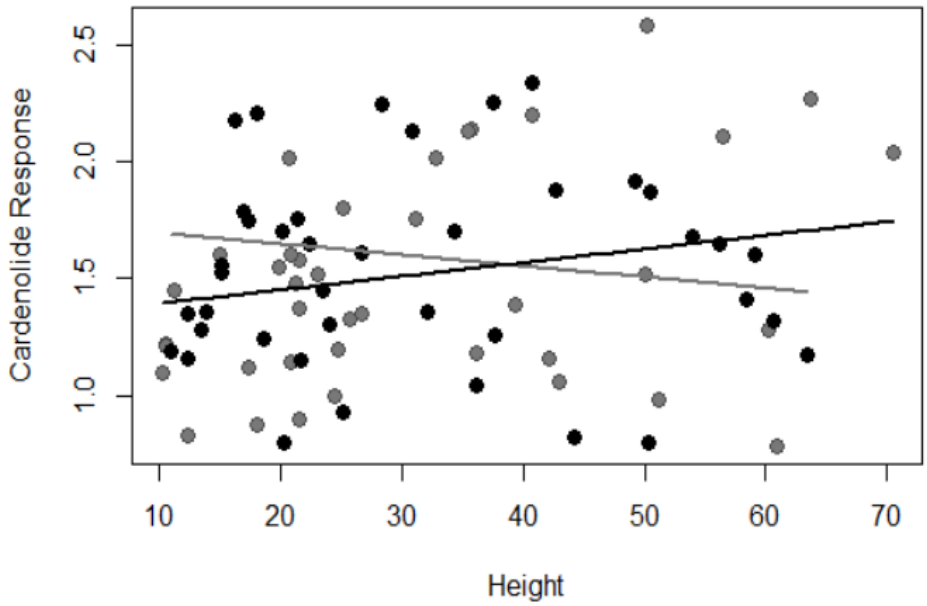


Figure 6. The cardenolide response was affected differently by height depending on the damage treatment. Damaged ramets, represented by the grey points and grey line, had a lower response as they got taller. Undamaged ramets, represented by the black points and black line, had a higher response as they got taller.

Shade Experiment

For the shade experiment, change in height, change in diameter, and number of buds were not affected by any of the treatments in the experiment. Both stem weight and root weight different depending on the age of the ramet, but there was not a difference between any of the other treatments. For stem weight, older plants had higher biomass than did younger (Figure 7, *P*=0.04). The same pattern was seen in root weight (Figure 7, *P*=0.017). Total biomass was higher overall in older ramets than younger (t=2.68, *P*=0.009). Total biomass was not significantly different when compared between the connected and unconnected ramets (t=1.24, *P*=0.22). However, when comparing the older and younger ramets within connection treatment, those in the connected treatment were not statistically different from each other in total biomass (t=1.3, *P*=0.19) while the unconnected ramets were statistically different (t=2.36, *P*=0.024).

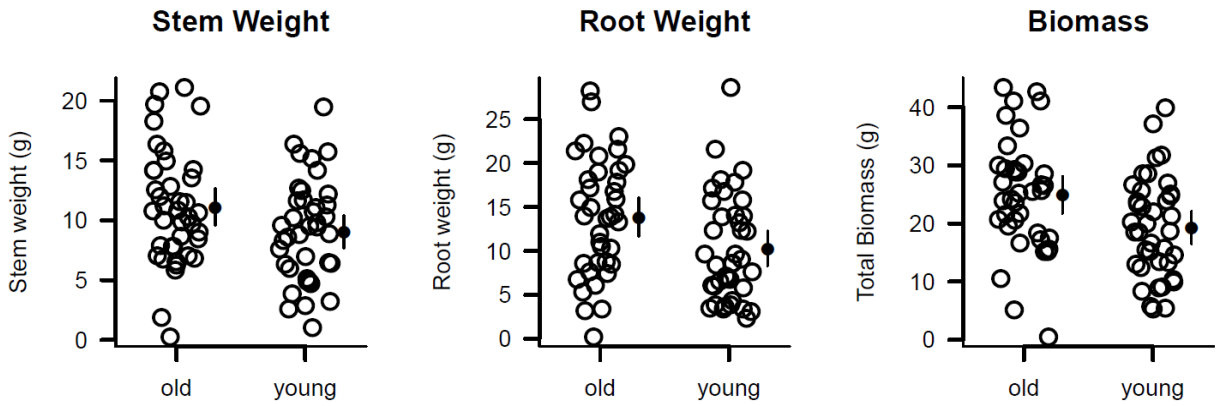


Figure 7. Strip plots of stem and root weight, as well as total biomass on average for the older and younger plants. These boxplots show that the old plants were, on average, larger than the young. Stem weight in older plants was significantly higher than in younger (t=2.03, *P*= 0.04), root weight was significantly higher in older plants than younger (t=2.43, *P*= 0.017). Total biomass in the older ramets was significantly higher than in younger ramets (t=2.68, *P*=0.009).

The random effect of clone in these models was not large and often overlapped zero, indicating that there was little variation between clones. Only in the models for C: N ratio and lignin did the effect of clone not overlap zero, but even with the effect of clone these models hardly explained more than 50% of the variance in the data (R^2 <0.5).

Discussion

The herbivory study showed that leaf traits were only affected by height but the peak cardenolide levels and cardenolide response were affected by an interaction between height and the damage treatment. Both cardenolide measures decreased with increasing height in damaged ramets but showed no effect of height on the undamaged ramets. All ramets, regardless of the damage treatment, showed an increase in cardenolide levels after 24 hours, suggesting that there is a volatile signal to increase cardenolide production that was received by the undamaged ramets. In the shade experiment, there were signs of competition in the differences of biomass in the unconnected ramets but not in the connected ramets, which suggest that there is clonal integration in *A. syriaca*.

If integration does occur in *A. syriaca*, it does not seem to have a direct effect on foliar traits because there was not a difference between connected and unconnected ramets. The foliar traits did not show any signs of integration because all foliar traits were only significantly related to height. PRI increased with increasing height, which indicates that the taller ramets had lower levels of stress. PRI can be affected by water or oxidative stress, and while the ramets should have been experiencing similar conditions, shorter ramets showed higher signs of stress which could be caused by competition for water between ramets (Meroni et al., 2008). Stress levels were not different between the damaged and undamaged ramets, indicating that PRI is not affected by herbivory in *A. syriaca*. The ability to measure PRI with reflectance spectroscopy could have applications in the field because it would allow researchers to detect stress *in situ*. A direct comparison of measured rates of photosynthesis and PRI in stressed and unstressed ramets would better validate this method.

In the cardenolide experiment, the response seen in all the ramets suggests that herbivory signals are shared through volatile signals, which is supported by the ANCOVA results that showed only time significantly affected average cardenolides. While the response in the undamaged ramets may have been caused by the leaf clamp, the peak in cardenolide levels followed by a slow decline over time suggest that the response was not caused by the leaf clamp. If the leaf clamp were eliciting such a strong response, there would have been either a steady increase in cardenolides over time or an increase with an asymptote at some maximum

cardenolide level. The responses seen here are similar to those found by Couture et al. (2013), where undamaged ramets of common milkweed showed a response that mimicked the response seen in the damaged ramets but at a lower magnitude. Though the experiment by Couture et al. (2013) did not study the effects of connection but only focused on changes in cardenolides, their results support the patterns seen in my unconnected ramet pairs. The potential for volatile signals from a damaged milkweed ramet to induce a response in an undamaged ramet adds to the list of ways that common milkweed uses volatiles. Volatiles in this species are known to be used in defense by attracting predatory insects of herbivores but also play a role in the oviposition choices of monarch butterflies (Bergstrom et al., 1995; Rasmann et al., 2011; Wason & Hunter, 2014). This experiment now indicates that common milkweed volatiles are used in plant communication as well.

For peak cardenolide levels, height and damage treatment had an interaction where damaged ramets showed decreasing levels of cardenolides with increasing height while height did not have an effect on the undamaged ramet's peak cardenolide level. The cardenolide response also had an interaction between damage and height, where damaged ramets had a lower response with increasing height and no effect of height on the undamaged ramets. What is interesting about these results is that the interaction between height and damage shows that damaged ramets have decreasing levels of cardenolides with increasing height while the connection results show that connected ramets had higher levels of cardenolides. These results seem to contradict one another because the connected ramets were tallest at the time of the experiment and should thus have lower levels of cardenolides. However, it seems that the connected ramets, on average, have higher levels of cardenolides overall and thus will always have higher levels of cardenolides in spite of the effects of height.

The shade experiment did not have many significant results because it started too late in the growing season to have an observable effect on the plants. By the time it started, most of the plants had already reached their maximum height. This experiment should be repeated for an entire summer, starting with all the plants at the same height to determine how *A. syriaca* ramets respond to shade. There was an effect of age on measures of biomass, but this effect was not

different between shade treatments. However, there are signs of integration because of the signs of competition in total biomass in the unconnected ramets. Because the unconnected ramets had had different weights but the connected ramets did not, it is possible that one ramet grew more and prevented the other from taking in more resources. Overall, older ramets were larger and had more overall biomass which shows a potential age gradient but also suggest that the first ramet to sprout in an area may have an advantage by being further along in development and thus larger or better able to take in nutrients from the soil. This type of "first come, first serve" resources use or asymmetric competition, where the first individual to emerge has an advantage over any subsequent individuals (Weiner, 1990; Roberts, 2000), has been modeled in *Agrostemma githago* and *Triticum aestivum* (Firbank & Watkinson, 1987) and shown in *Dactylis glomerata* and *Impatiens capensis* (Ross & Harper, 1972; Howell, 1981).

My experiments showed one sign of integration, which was the difference in biomass of the unconnected ramets but not the connected ramets. This difference suggests that the ramets in the unconnected treatment were competing for resources but the unconnected ramets were functioning as one ramet. There was also an interesting result from the cardenolide experiment, which was that it appears herbivory signals are sent via volatile signals to neighboring plants. Further investigation on the effects of shade and herbivory stress on ramets of common milkweed should be conducted, perhaps using reflectance spectroscopy to measure foliar traits through an entire growing season.

My results suggest that there is a volatile signal of herbivory that is released from damaged ramets and received by undamaged ramets. Further research needs to be done to determine if that is in fact what has happened. There are two experiments that could be used to confirm a volatile signal. The first would be a feeding assay using milkweed herbivores. A feeding assay would compare the growth weight and end biomass of insects feed leaves from damaged ramets, undamaged ramets near a damaged ramet, and undamaged isolated ramets. If the insects fed leaves from the undamaged isolated ramets grew the fastest or were the largest, there would be an indication that the other ramets had higher levels of cardenolides than did the isolated ramets (Rhoades, 1983). A second, more direct chemical test of the cardenolide levels

could also be conducted in which unconnected ramets would be grown in separate pots and one would be damaged while the other would not. Because it has been shown that common milkweed uses chemical signals in the soil to attract predators of their root herbivores (Wason & Hunter, 2014), the ramets would have to be in separate pots to determine if it is an aerosol. To determine if the response is sent through the air or through the soil, an additional treatment would be to pot ramets in the same pot or in different pots. The airspace around half of the ramets would be shared, but isolated from any other pairs while the other half of the ramets would also be isolated from each other. By comparing the response of the undamaged ramets that are with the damaged ramets and the response of the completely isolated ramets, one would be able to determine if the response of herbivory is received by undamaged ramets if the ramets that are sharing air with the damaged ramets show a response.

To further investigate integration in common milkweed, my shade experiment could be repeated for an entire growing season. To better determine how age may affect clonal integration, and to perhaps determine how age affects foliar traits, ramets could be grown and allowed to produce clonal offspring that would then remain connected or be cut apart before shading. Spectroscopy measurements as well as physical measurements could be made through the growing season to determine how resources could be shared between the ramets and how foliar traits of the ramets are affected by connection and shading. This would then show if there is a parent-offspring effect on integration.

These investigations of clonal integration and volatile signals in common milkweed further our understanding of common milkweed but also contribute to the literature that exists about volatile signals and clonal integration. Given then implications of these studies, there is reason to further investigate clonal integration and volatile signals in common milkweed. There are many avenues to expand upon the data presented here that will explore the effects of age and shade stress on clonal integration and the effects of clonal integration on herbivory responses. Further research can be conducted to confirm that there is a volatile signal of herbivory in common milkweed that can cause a response in undamaged ramets. These studies will contribute to a variety of bodies of literature on common milkweed, clonal integration, and volatile signaling.

Chapter 2 - Density Dependence in Natural Populations of *Asclepias syriaca*

Introduction

Density dependence is found in populations of organisms where density, in addition to the environment, influences the rates of reproduction, growth, and death (Hixon & Johnson, 2009). Density dependence can have either positive or negative effects. Negative density dependence is observed when population vital rates decline as density increases. Negative density dependence occurs when a populations size reaches a point where the resources available in the environment are no longer enough to support it, thus reducing the nutrients available per capita (Fowler, 1995; Piao et al., 2014). This reduction of nutrients can lead to lower survival or lower reproduction rates (Fowler, 1995). Plant size, fecundity, and probability of survival are often found to be negatively related to density (Shaw, 1987; Smith, 1983a, b, c). On the other hand, positive density dependence, or Allee effects, are a positive interaction between population density and growth rate and are more broadly defined as when individuals in a population benefit from the presence of conspecifics (Coron et al., 2013; Courchamp et al., 1999; Stephens & Sutherland, 1999). Positive density dependence is often found in harsh environments where the presence of one species ameliorates the effects of stress for other species (Goldenheim et al., 2008). While this positive relationship may sound beneficial to a plant population, Allee effects often occur in low density populations and put them at risk of extinction due to low fecundity or low genetic diversity leading to decreased population fitness (Courchamp et al. 1999).

Density dependence, both negative and positive, has been well studied in both plants and animals, though negative density dependence has received the most attention. Studies often focus on density dependence and its impacts on seedling survival or reproductive success (Duncan et al., 2009; Gustafsson & Erhlen, 2003; Keddy, 1981). Though some studies have attempted to explain the effects of density dependence on entire plant communities (e.g. Goldberg et al., 2001), many studies have one or two focal species. While smaller, less focused studies could ignore interspecific interactions and make it difficult to extrapolate patterns of density dependence to populations of mixed species, understanding the effects of density on one species is arguably still of use to the research community by simplifying interaction variables for one species before investigating density dependence in a community.

There are two main methods that have been utilized for understanding density dependence:

observational and experimental studies. Observational studies usually follow the vital rates of a study species to test for density dependence and usually involve long-term tracking studies (Watkinson & Harper, 1978). Such studies allow researchers to determine the effects of density in the conditions of the plant populations as they grow in the wild and usually focus on mortality and reproduction. For example, *Vulpia fasciculata* was studied by Watkinson and Harper (1978) who found that while death rates were slightly higher in the lower density plots, the risk of mortality was dependent more upon the characteristics of the individual plots than on density (Watkinson & Harper, 1978). Smith (1983a, b, c) conducted studies on *F. proserpinacoides* to investigate the effects of density on survival, growth rate as measured by number of nodes, rate of flower production, number of seeds per fruit, the proportion of successful seed set, and seedling survival. All of these were negatively related to density except seedling survival, which increased with increasing density (Smith 1983a, b, c). While studies like these give the effects of density *in situ* for a population, they may not be able to parse out the finer details of how varied densities or nutrient levels can affect density dependence.

Experimental studies, however, can be used to determine how different densities or environmental gradients affect density dependence and are most often field manipulation studies. Field manipulations artificially change the density of natural populations of the study plant such that density is controlled for while the abiotic environment is as close to natural as possible and usually focus on how mortality, growth, or reproduction rates change. These studies mostly involve the addition of seeds or seedlings and/or the removal of seedlings or adults (Clay & Shaw, 1981; Shaw, 1987; Fowler, 1995). For example, manipulated populations of the succulent *Diamorpha smallii* showed that the number of flowers and fruits produced per plant was negatively related to higher density while the number of seeds per fruit was not related to density (Clay & Shaw, 1981). Fowler (1995) manipulated field populations of bunch grasses *Bouteloua rigidiseta* and *Aristida longiseta* to determine both the intra- and inter-specific effects of density on survival and reproduction over 5 years. Fowler found weak indications of negative density dependence in *Bouteloua* but not in *Aristidia*, and not in a regular pattern across quadrats or years. Similar studies have found signs for negative density dependence in the survival, fecundity, and growth of *Salvia lyrata* (Shaw, 1987), negative density dependence in reproductive output of *Cakile edentula* (Keddy, 1981), and found no density dependent mortality but negative density dependence of the

reproductive effort of *V. fasciculata* (Watkinson & Harper, 1978). While these studies do not determine how density dependence is acting on a natural population, when paired with an observational study much can be learned about a species and its relationship with density.

While increasing density usually causes negative growth rates in plants, clonal species may show the opposite response. In clonal species, an individual's closest neighbors are likely to be offspring (ramets) of the same parent individual and collectively make up a genet. In some species, these ramets do not maintain clonal connections to each other, so ramets will experience competition from related conspecifics. However, in some species each ramet stays connected to the ramet that produced it. Through their connection, the two ramets are capable of sharing resources. Density will affect these integrated clonal species less because the ramets are not competing with many of their immediate neighbors but are instead sharing resources. It is also possible for a population of clonal plants to regulate ramet placing in such a way as to minimize intra-specific competition and maximize nutrient uptake (Hutchings, 1970).

Density dependence has been studied in a handful of clonal plants, many of which are grasses, such as *Elymus nutans*, or aquatic plants, such as *Potamogeton pectinatus* (Chu et al., 2008; Hidding et al., 2009). Chu et al. (2008) modeled and tested the effects of density on *E. nutans* and found that biomass was highest at intermediate densities, meaning that density dependence changed signs after reaching a critical density. The study by Hidding et al. (2009) on *P. pectinatus*, or sago pondweed, showed how herbivory in different seasons can change the effects of density. They found that herbivory in the summer helped alleviate the effects of density by thinning the populations (Hidding et al., 2009). With clonal plants, there is a further piece of the puzzle that should be considered when studying density dependence, and that is the effect of density at the ramet verses the genet level. To determine the difference between the ramets and genets, different spatial scales of density dependence could be investigated. At small spatial scales, one would expect to be investigating the effects of density at the ramet level, while at larger spatial scales one would expect to be investigating the effects of density at the genet level. Such a direct study of spatial scale at the ramet verses the genet level in clonal plants has yet to be conducted.

Density dependence has not been studied in *Asclepias syriaca*, common milkweed, but because

density levels in common milkweed patches range from 75 stalks to 10,954 stalks per square kilometer (Bhowmik & Bandeen, 1976), it is an ideal clonal species for studying density dependence. To understand how integration, and potentially optimal placement, could drive populations, this study investigated wild milkweed populations for density dependence. Understanding the effects of density on common milkweed will provide further research on density dependence in clonal plants, especially in species that are thought to maintain physical connections, and will also allow researchers to better understand how to maintain healthy milkweed patches for the threatened monarch butterfly, whose life cycle revolves around milkweed.

To detect the effects of density on vital rates of common milkweed, wild populations were tagged and then mapped and measured for three years. Each year, measures of growth, reproduction, survival, and herbivory were taken for each plant in 18 populations at four different field sites. After three years, the vital rates of the species were modeled using generalized linear mixed effects models. Included in these models were densities at varying spatial scales that were produced from the map data. These densities were used to determine if there is a critical scale at which the sign of the effect of density changes (i.e. goes from positive to negative).

My hypotheses assumed that clonal integration does occur in common milkweed. Height was predicted to increase with increasing density regardless of scale due to the shade-intolerance of common milkweed (Agrawal et al., 2012). Leaf area, number of inflorescences, percent leaves damaged, and presence of stem damage were all predicted to decrease as density increased at all scales because ramets were expected to be putting more energy into clonal reproduction than growth or sexual reproduction. The number of viable pods was predicted to increase with increasing density at the small scales due to a larger potential for cross-pollination, but to decrease with increasing density at the large scale due to a loss of pollinator effectiveness. A reverse pattern was expected for the number of aborted pods, assuming that the number of viable pods and aborted pods would be inversely related to each other.

Methods

Spring

There were four field site locations, each with varying numbers of transects (Figure 1). Two are located in northern Virginia, with one located at Blandy Experimental Farm and the other at Sky Meadows

State Park. A third site is south of Richmond, Virginia, just outside the City of Hopewell at Presquile Wildlife Refuge. The fourth is located in Yorktown Battlefield Park in Yorktown, Virginia. Site descriptions are found in Appendix 1.

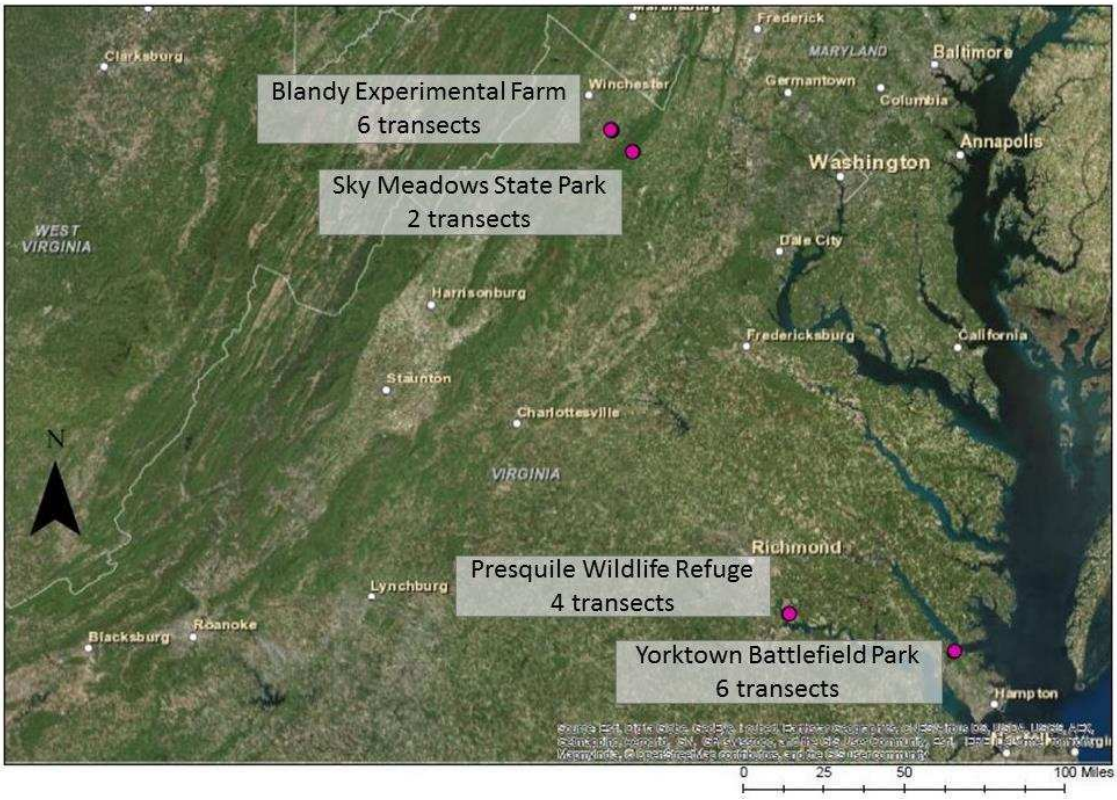


Figure 1. Map of Virginia with markers indicating the four sites from this study, with the total number of transects at each location included.

At the beginning of the field season in June, each transect was located and the tags from the previous year's plants were found. If any new milkweed shoots had emerged, they were given a tag with a new number. The spring data collection consisted of five different measurements: height, basal diameter, length and width of largest leaf, scoring of any inflorescences present, and mapping (Table 1). The height of each plant was measured to the apical meristem, which is nestled between the topmost pair of leaves. Basal diameter was measured with calipers, held level as close to the ground as possible. The largest leaf—usually found near the top of the plant—was identified, and its length and width were recorded. The number of inflorescences was recorded as a proxy of reproductive effort. In 2015, data on the number of pollinia insertions and removals per inflorescence were taken for select transect plants. Pollinia are sacs of pollen and it is easy to tell if they have been removed or inserted into individual *A. syriaca* flowers.

Table 1. Data collected per season

Spring (June)	**Fall (August)**
Height	Height
Basal diameter	Basal diameter
Leaf length and width	Seed pod presence and scoring
Mapping (density)	Density (2014)

After collecting all of the plant characteristic data, the plants were mapped. Map data were taken using a center point in the transect and a PosTex Laser Positioning System (Haglöf, Sweden). The PosTex uses ultrasound waves to triangulate the distance and angle of a given sample location from the center of a tripod. On the tripod are three sound transponders and a receiver is held by the measurer. The transponders allow for triangulation and give measurements with an accuracy of 0.1^{o} and 0.01 m. All of the plants were mapped in 2013 and 2015, but only some were mapped in 2014. In 2014, the mapping equipment malfunctioned; thus the mapping data were taken for only a few transects at sites Blandy and Presquile in September. To finish collecting density data, plant density measurements were taken by counting the number of milkweed stems that were within 1 m of each individual on the transect. This information gives similar density data for each transect and can be analyzed in similar manners.

There were three years' worth of data to analyze after the summer of 2015. There were only seven transects with map data from 2014, with only a manual density measure for the other ten transects. The density measure gives the number of plants within 1 m of each focal plant, and gives similar data to the mapping data, which allows an analysis of all transects that year but only at the 1 m scale.

Fall

On a return visit in either late August or early September of each of the study years, the height of each plant was measured again, as well as the number and status of pods. The height to apical meristem measurements were taken on as many of the plants as possible. This often was measured to the highest point of the stem because most of the milkweed had begun to senesce. A pod was considered viable if the pod was still green and not moldy or if it was open and the seeds were fully formed.

From the number of inflorescences and the number of viable pods, the measures reproductive effort and reproductive success were calculated. Reproductive effort was the number of inflorescences divided by the height of the plant. Reproductive success was the number of pods per inflorescence. These measures were created to give alternative measures of reproduction aside from the total number of pods.

Analysis

All analyses were performed in the program R using packages lme4, lmerTest, and languageR (R core team, 2015; Kuznetsova et al., 2016; Bates et al., 2015; Bayeen, 2013). In order to calculate densities, mapping data collected with the Postex was converted into an (X,Y) coordinate map, and the coordinates were exported as a data file for further use. With this file, the number of nearest neighbors was calculated for each plant using a radius of 10 cm to 1 m, increasing by 10 cm each time for a total of ten density windows, hereafter referred to as small scale windows. The density of the entire transect, calculated as plants/m^2, was used as the large scale, hereafter referred to as the patch scale.

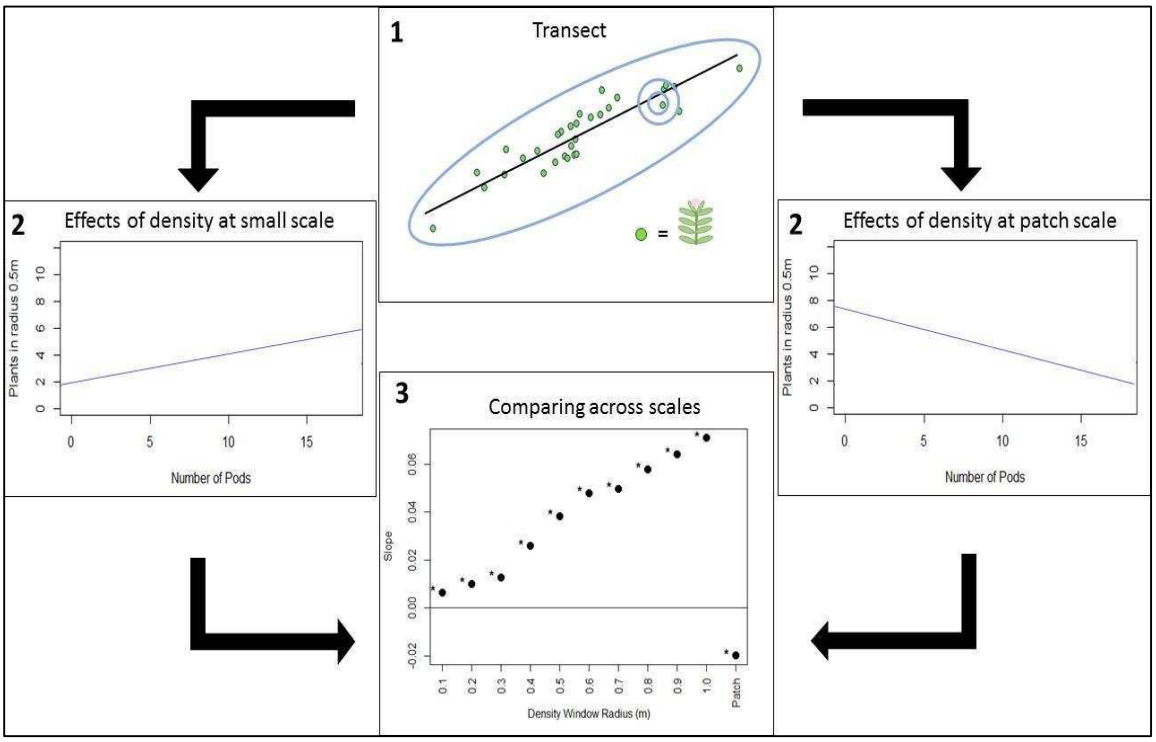


Figure 2. Illustration of the transect mapping process. Panel 1 shows the transect, with plants indicated by the filled circles. The open circles around a selected point and around the whole patch represent the windows that were used in analysis (explained below). Panel 2 shows the graphs produced after modeling. The effects of density at the small scale were analyzed using each plant as the focal point for calculating density. The effects of density at the patch level used the average density of the transect. Panel 3 shows the graphed results of the analyses at all scales, thereby allowing for a comparison across scales for each response.

Linear and generalized linear mixed models (LMM and GLMM, respectively) were used to analyze the data, with the type of model fit to the type of data: Gaussian, Poisson, or binomial (Zuur et al., 2009). LMMs were run on Gaussian data while GLMMs were run on Poisson or binomial data (Equation 1).

Equation 1.

$$R_i = X_i \times \beta + Z_i \times b_i + \varepsilon$$

In these models R_i is a matrix containing the plant vital rate of interest as the response variable for year *i* and in my model *i*= 1, 2, and 3. is the term for the fixed effects, which are the explanatory variables that contribute to the error, ε , in the models in ways that we do not understand. The fixed effect in my model was the small scale window. is the term for the random effects, which are explanatory variables that have many levels, each level with its own "baseline" or average. Including random effects allows the model to take the variation of each level into account, rather than using one average for all levels (Winter, 2014). In my models, I expected each year and each transect to have different linear relationships, so both were included as random effects. For each response, the slope of each model was plotted against density window (Figure 3). These graphs display how the strength of plant responses changed over density windows and provide a visual representation that aided interpretation.

In order to quantify the potential for each other the tree years to have different responses, overall temperature data were obtained, including growing degree days, Julian date of first field day, and day of last frost. Growing degree days are based on the average daily temperature subtracted from a base temperature, with the sum of each daily GDD value up to a given date (Equation 2).

Equation 2.

$$Daily\ GDD = \frac{Max\ temperature - Minimum\ temperature}{2} - Base\ temperature$$

The base temperature used for this calculation of GDD was 50° F, based on milkweed germination experiments by Baskin & Baskin (1977). Temperature data from the northernmost field site, Blandy Experimental Farm, were used for the calculation.

Results

Scale

The effects of density were significant in about half of the responses at the patch level. Survival, spring height, leaf area, and number of inflorescences were negatively related to density at the patch scale. Reproductive success as measured by number of pods per flower divided by number of inflorescences per flower was not statistically significant at the patch scale. Number of inflorescences and leaf area did not have any significant interactions at the small-scale window. Reproductive effort, pollinia

insertions, pollinia removals, and number of viable pods were not significant at any scales. The effects of density dependence at the small-scale windows were detected starting at 0.2 m^2 in four responses: survival, reproductive success, growth, and height. For the small scale *P*-values reported below, unless otherwise stated, they are for the model run at the 0.5 m^2 scale and α=0.05. The *P*-values were Bonferroni adjusted for multiple samples because of the large number of models run on the data. Appendix 2 contains results from each model.

Survival

Increasing density decreased survival at both the small scales from scales 0.5 to 0.9 m^2 (P=0.002) and patch scale (*P*=0.03, Figure 4).

Growth

Height in the spring decreased as density increased at the small and patch scales (Figure 4). This relationship was statistically significant at small scales 0.2 to 0.6 m^2 and at the patch scale (small P=0.001, patch *P*<0.001). This pattern was opposite from predictions that height would increase with increasing density. Leaf area decreased with increasing density at the patch scale (*P*<0.001) and was not affected by density at the small scale. Growth through the season also decreased as density increased, at small scales 0.5 to 1.0 m^2 (*P*=0.009). This is the reverse of the predicted pattern (Figure 4).

Herbivory

Density did not have a significant effect on percent leaves damaged at the small scale or patch scale after adjusting the P values for multiple measures (Figure 4).

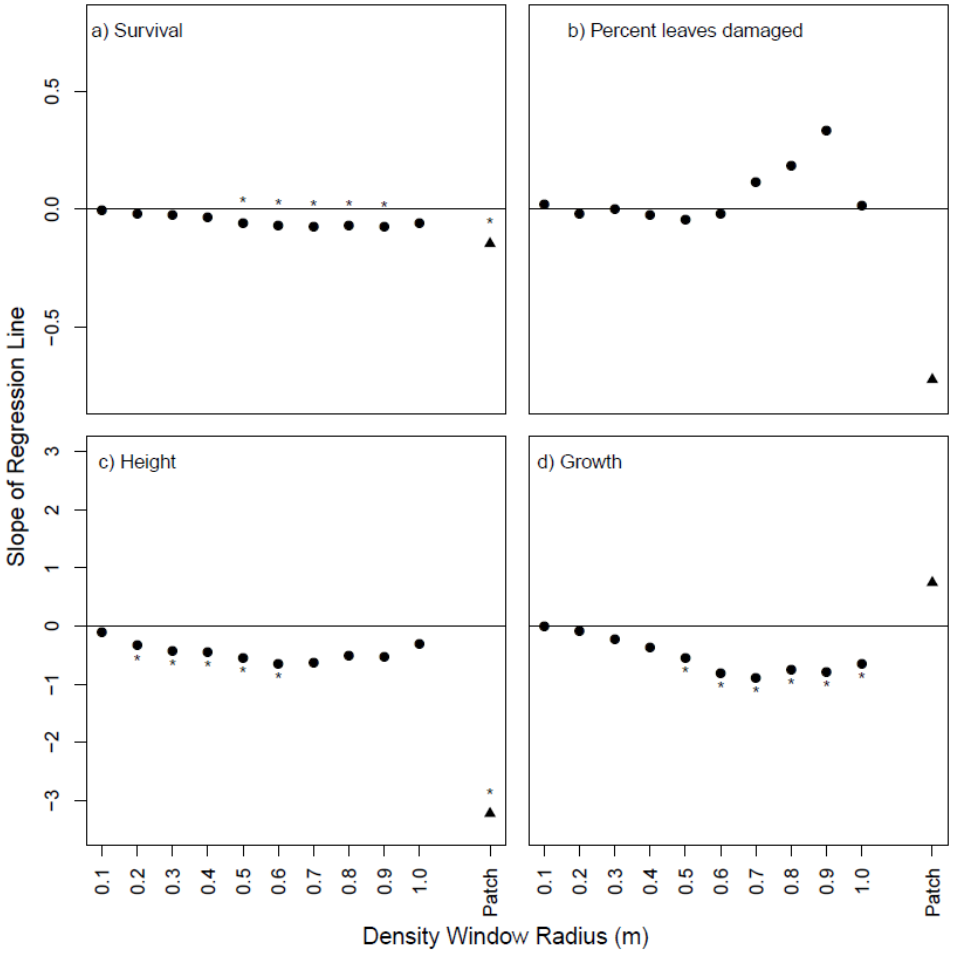


Figure 4. Results from the responses for survival, herbivory, and growth. Round points indicate the small scale windows while the triangle indicates the patch level. Points are the plotted slope for each model at each scale for the indicated response. Stars next to the point indicate a significant relationship with density ($\alpha=0.05$) and were Bonferroni adjusted for multiple samples. Note that panels a and b are on a smaller scale than panels c and d.

Reproduction

Increased density decreased the number of inflorescences at the patch scale (P=0.025), which was the opposite of expected (Figure 5). The relationship between density and viable pods, reproductive effort, pollinia insertions, or pollinia removals was not statistically significant after adjusting the P values (Figure 5). Reproductive success decreased with increasing density at various small scales (0.4, 0.7, & 0.9 m^2) but did not follow a clear pattern, thus these results may be spurious. Reproductive success was not statistically related to density at the patch scale (Figure 5).

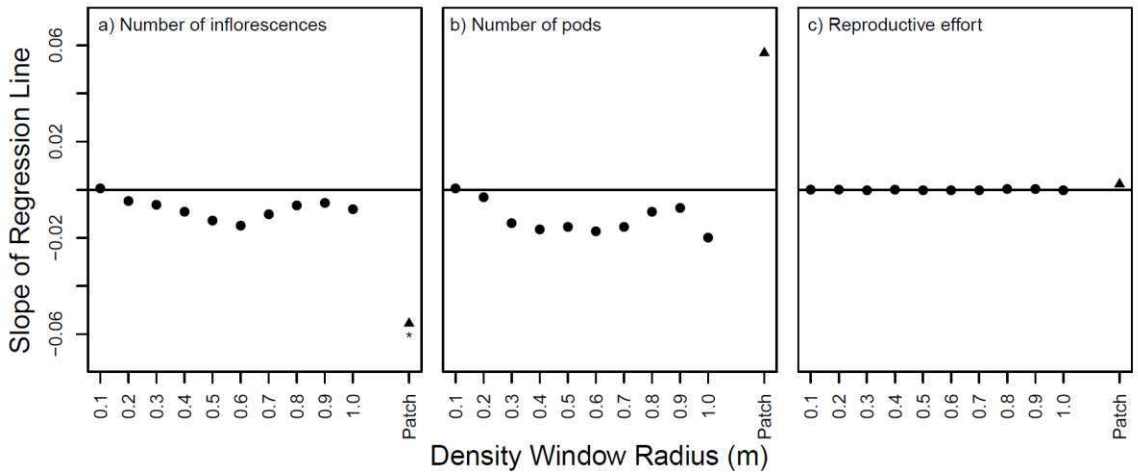


Figure 5. Results for the reproduction responses. Round points indicate the small scale windows while the triangle indicates the patch level. Points are the plotted slope for each model at each scale for the indicated response. Stars next to the point indicate a significant relationship with density (*α=0.05*) and were Bonferroni adjusted for multiple samples.

Year

The effects of year on height were further investigated separately to determine if the effects of density changed signs over years. There was a positive relationship between density and plant height in 2013, while 2014 and 2015 had negative relationships. A comparison of temperatures, densities, Julian date of first field day, and number of growing degree days (GDD) between the three years, both average temperatures and the last day of frost for the three years were similar. However, 2013 had the lowest average density and also had the fewest number of GDD. By the first day of fieldwork in 2013, only 951 GDD had accumulated (Table 2). By the first fieldwork day of 2014, 1004 GDD had accumulated, and by the first day of field work in 2015, 1233 days had accumulated (Table 2).

Table 2. Temperature and Date Data

Year	Julian Day	GDD	Last Frost	Density
2013	155	951	7 April	3.1
2014	157	1004	18 April	6.7
2015	163	1233	2 April	4.5

2013 had by far the fewest GDD of the three years, which could explain why that year was different from the other two. For height, leaf area, reproductive success, and survival, 2013 had a positive relationship between density and the plant response, while these responses were negative in 2014 and 2015 (Appendix 2). This pattern was not seen in other responses. For change in height through the season, reproductive effort, number of pods, and percent leaves damaged, 2015 had a sign opposite

from 2013 and 2014. Aside from starting about one week later, the only difference between 2015 and the other years was the greater number of GDD.

Discussion

There were three key results from this study. First if there was a significant relationship with density at the patch scale, it was always negative. At the small scales, half of responses had at least three statistically significant relationships with a density window. Second, of the small scales, most effects are seen at 0.4m^2 and are definitely seen by 0.5m^2 when there is a significant relationship at the small scales. Finally, density dependence was occasionally found at both scales, but was sometimes found in one scale but not the other. There was not a shift in signs across scales; density either had a negative effect or no effect at each scale.

Differences in effects of density at different scales have been found in other studies but have shown opposite signs at different scales. Fedriani et al. (2015) found positive density dependence in fruit production at small scales negative density dependence at large scales in Iberian pear, *Pyrus bourgaeana*. A seedbank experiment examining desert plants by Lortie et al. (2005) found that single scales did not capture the entire picture of what was occurring in the plant community because signs shifted as they observed the effects of density at different scales. Increased density decreased seedling emergence, final density of the plot, and mean plant size (Lortie et al., 2005). In the biennial *Sabatia angularis*, reproductive success as measured by seed count was negatively affected by density at the population scale and at the 1 m scale, but was positive at an intermediate 4 m scale included in a study by Spigler & Chang, again showing a change in signs at different scales (2008). Studies such as these demonstrate the importance of investigating the effects of density at different scales.

Overall, nearly every response was affected by density in a way that was opposite from what was first expected. Because these predictions were made assuming clonal integration has an effect, these data suggest that clonal integration does not occur in common milkweed and is therefore unable to ameliorate the effects of density or that there was very little clonal integration in these populations. It is also possible that there are different effects of density for different life stages in milkweed (i.e. seedling versus adult), which were not accounted for in this study and could cancel out the effects in an overall model (Elmberg et al., 2005). If there were more seedlings established than expected, there may be less

integration because there are fewer clonally produced ramets in the populations.

There are several studies that could further elucidate the effects of density in *A. syriaca*. The first experiment that could further elucidate the effects of density in *A. syriaca* would be to further investigate each year independently. Given the differences between GDD and last day of frost between the three years, and the differences in the slopes of the models, there could be different effects of density across the years that are not apparent in the aggregate models (Goldberg et al., 2001) A second area for further investigation would be the inclusion of abiotic factors that are not included in these models. Soil and temperature data are available for these sites and could be included as random effects in the models to see if they explain the variance better than do density or year. A third experiment to further this research would be to investigate the differences between the four sites, or to clump the two southern and two northern sites together. Spatial differences between, and even within, the four sites could also have an effect on density dependence. Both Nantel & Gagnon (1999) and Goldenheim et al. (2008) found different vital rates across a spatial gradient. Nantel & Gagnon (1999) compared populations of *Helianthus divaricatus* and *Rhus aromatica* across their natural ranges in Quebec and Ontario; both species exhibited different rates of mortality and *R. aromatica* showed different rates of stem sprouting between the southern and norther populations. Goldenheim et al. (2008) conducted a study on *Spartina alterniflora* and *Spartina linearis* along an elevation gradient a several beaches in Naragansett Bay, RI. Both species showed a positive relationship between seedling biomass at high densities at higher shore elevations, but a negative relationship at high densities at lower shore elevations (Goldenheim et al., 2008). The difference between scales in these two studies show how both small scale (within a small geographic region) and large scale (across territories) variation can have an effect on population vital rates. A third study that had similar results was conducted by Keddy (1981) on *Cakile edentula*.

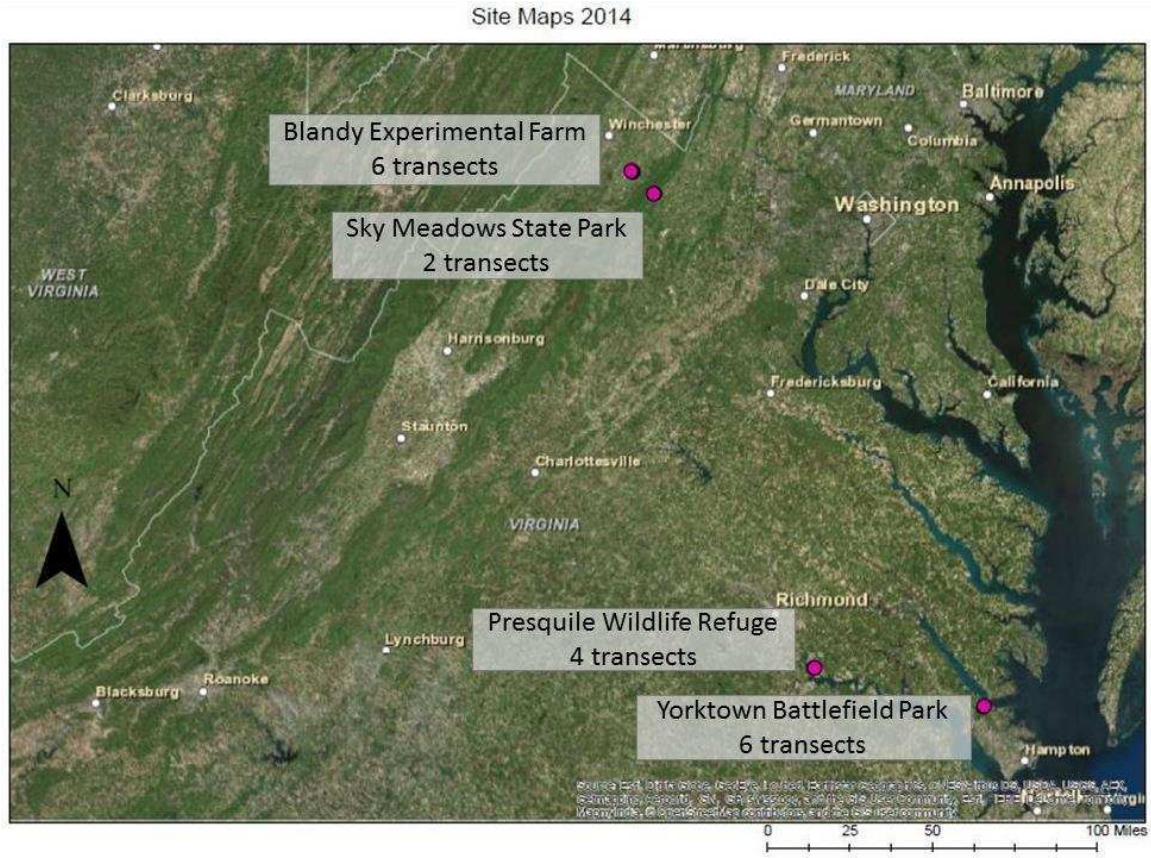
Another avenue of research would be to determine the effects of density at different life stages, from seedling recruitment and establishment to the reproductive adult stages. Such studies have been done in trees (Piao et al, 2014), in herbs (Shaw, 1987), and in large scale studies of seedbanks (Goldberg et al., 2001). The effects of density on different life stages varied in magnitude and direction in the study conducted by Goldberg et al. (2001), though the effects of density were most consistent in the seedling/emerging life stage. Piao et al. (2014) investigated the effects of density in two tree species,

Chamaecyparis obtusa and *Quercus serrata*, at two life stages, sapling and juvenile. They found signs for negative density dependence for saplings that diminished in the juvenile stage in both species and conclude that looking at individual life stages independently for signs of density dependence can show different signs at different stages that could otherwise be overlooked when focusing on overall population dynamics or on one particular life stage (Piao et al., 2014).

One final area of research that has yet to be addressed in common milkweed that may elucidate the population dynamics of this species is the clonal growth pattern. Plants can exhibit growth patterns that are described as phalanx or guerrilla. Phalanx clonal growth is characterized by highly branched growth with little space between ramets, creating dense patches (Lovet-Doust 1981, Benot et al, 2010). Guerrilla growth is characterized by long lateral expansions, with a large spatial distribution but low density of ramets (Lovet-Doust 1981, Benot et al, 2010). Common milkweed has yet to be classified in these terms, so a field study that excavated the root systems of common milkweed could be conducted to determine its growth patterns. This could inform further modeling efforts as to what spatial scale should be used based on how far one genet of common milkweed could spread and at what density ramets are formed.

In conclusion, the effects of density on vital rates of *A. syriaca* were variable across spatial scales but only significantly affected some vital rates. Density had the strongest effects on growth and height and weaker effects on reproductive success. There are ample ways to further investigate this species to determine what other factors could be affecting the vital rates of the species, and to perhaps begin parsing out the density-dependent and density-independent responses. There are many things left to learn about the population dynamics of *A. syriaca* that can be built off of this research. Learning more about how density dependence and clonal integration affect this species can better inform restoration efforts for *A. syriaca*.

Appendix 1



Blandy Experimental Farm

This study site is located near Boyce, Virginia, USA. It has two study locations within the farm property, both are open meadows. One meadow, called “thistle thicket” by the research team, has three transects and is bordered by a forest to the northeast and a study plot for tree of heaven (*Ailanthus altissima*) to the west. The other meadow, simply referred to as “the meadow”, has three transects and is open on all four sides. These meadows are burned approximately every 2 years, though there is not a consistent pattern.

Sky Meadows State Park

This park is located near Boyce, Virginia, USA and is less than half an hour from Blandy Experimental Farm. There are two transects here. Both are accessed only by foot along a hiking trail in the park. The first transect is in a flood plain of the creek that the path follows. The other study transect is further up the trail and is located on a hill.

Yorktown Battlefield

The Yorktown Battlefield Park is part of the National Historic Park of Virginia and is located on the Colonial Parkway. In Yorktown Battlefield Park, there is one meadow that is accessed through one of the Auto Tour paths. This meadow is where all six of our transects for the park are located. This meadow is mowed in the end of the summer each year.

Presquile Wildlife Refuge

This refuge is an island created in the 1900's when a canal was dug to cut an oxbow out of the James River near Hopewell and Chesterfield Virginia. Our study sites are on the northwest side of the island. All of our study sites are located in an area that is currently regrowing after being used as pasture.

Appendix 2

Model output for each model. R^2 conditional, or R^2 Cond, is an adjusted R^2 value specifically for linear mixed effect models that calculates the total R^2 for the fixed and random effects (Nakagawa & Schielzeth, 2013)

Linear Mixed Effect Models

Scale	Response	Slope	S.E.	t value	P-value	R^2 Cond
0.1 m	Growth	-0.0053	0.0268	-0.20	1	0.4312
0.2 m	Growth	-0.0725	0.0588	-1.23	1	0.4328
0.3 m	Growth	-0.2129	0.0858	-2.48	1	0.4377
0.4 m	Growth	-0.3549	0.1098	-3.23	0.124	0.4433
0.5 m	Growth	-0.5331	0.1361	-3.92	0.009	0.4488
0.6 m	Growth	-0.7972	0.1564	-5.10	3.93E-05	0.4599
0.7 m	Growth	-0.8865	0.1728	-5.13	3.32E-05	0.4632
0.8 m	Growth	-0.7350	0.1870	-3.93	0.009	0.4518
0.9 m	Growth	-0.7889	0.1995	-3.96	0.008	0.4529
1.0 m	Growth	-0.6475	0.1567	-4.13	0.004	0.4669
Patch	Growth	0.7474	0.3487	2.14	1	0.5108
0.1 m	Height	-0.0925	0.0281	-3.29	0.102	0.3632
0.2 m	Height	-0.3286	0.0583	-5.64	1.93E-06	0.3747
0.3 m	Height	-0.4160	0.0865	-4.81	0.0002	0.3738
0.4 m	Height	-0.4394	0.1121	-3.92	0.009	0.3716
0.5 m	Height	-0.5376	0.1353	-3.97	0.007	0.3736
0.6 m	Height	-0.6377	0.1607	-3.97	0.007	0.3754
0.7 m	Height	-0.6268	0.1821	-3.44	0.058	0.3726
0.8 m	Height	-0.5008	0.1993	-2.51	1	0.3696
0.9 m	Height	-0.5324	0.2124	-2.51	1	0.3697
1.0 m	Height	-0.2968	0.1654	-1.79	1	0.3611
Patch	Height	-3.2244	0.3811	-8.46	1.39E-09	0.4784
0.1 m	Leaf Area	-0.0305	0.0642	-0.48	1	0.2334
0.2 m	Leaf Area	-0.1854	0.1400	-1.32	1	0.2362
0.3 m	Leaf Area	-0.2405	0.2085	-1.15	1	0.2373
0.4 m	Leaf Area	-0.3053	0.2675	-1.14	1	0.2383
0.5 m	Leaf Area	-0.4419	0.3232	-1.37	1	0.2403
0.6 m	Leaf Area	-0.6085	0.3749	-1.62	1	0.2427
0.7 m	Leaf Area	-0.5516	0.4161	-1.33	1	0.2408
0.8 m	Leaf Area	-0.2058	0.4502	-0.46	1	0.236
0.9 m	Leaf Area	-0.0306	0.4811	-0.06	1	0.2342
1.0 m	Leaf Area	0.0464	0.3680	0.13	1	0.1994
Patch	Leaf Area	-4.1724	0.8809	-4.74	0.0003	0.2852
0.1 m	Percent Leaves Damaged	0.0219	0.0192	1.14	1	0.9115
0.2 m	Percent Leaves Damaged	-0.0178	0.0419	-0.42	1	0.9116
0.3 m	Percent Leaves Damaged	0.0029	0.0624	0.05	1	0.9116
0.4 m	Percent Leaves Damaged	-0.0220	0.0799	-0.27	1	0.9116

0.5 m	Percent Leaves Damaged	-0.0405	0.0965	-0.42	1	0.9117
0.6 m	Percent Leaves Damaged	-0.0177	0.1119	-0.16	1	0.9116
0.7 m	Percent Leaves Damaged	0.1184	0.1242	0.95	1	0.9114
0.8 m	Percent Leaves Damaged	0.1867	0.1348	1.38	1	0.9114
0.9 m	Percent Leaves Damaged	0.3327	0.1442	2.31	1	0.9113
1.0 m	Percent Leaves Damaged	0.0163	0.1237	0.13	1	0.8939
Patch	Percent Leaves Damaged	-0.7213	0.2955	-2.44	1	0.8928
0.1 m	Reproductive Effort	0.0001	0.0001	1.52	1	0.1835
0.2 m	Reproductive Effort	0.0001	0.0001	0.51	1	0.1834
0.3 m	Reproductive Effort	-0.0002	0.0002	-1.17	1	0.1898
0.4 m	Reproductive Effort	-0.0001	0.0003	-0.47	1	0.1869
0.5 m	Reproductive Effort	-0.0002	0.0003	-0.73	1	0.1888
0.6 m	Reproductive Effort	-0.0002	0.0004	-0.42	1	0.1873
0.7 m	Reproductive Effort	-0.0002	0.0004	-0.53	1	0.1885
0.8 m	Reproductive Effort	0.0002	0.0004	0.54	1	0.1817
0.9 m	Reproductive Effort	0.0004	0.0005	0.84	1	0.1801
1.0 m	Reproductive Effort	-0.0002	0.0004	-0.61	1	0.1982
Patch	Reproductive Effort	0.0023	0.0007	3.20	0.149	0.2384
0.1 m	Reproductive Success	-0.0005	0.0018	-0.26	1	0.2696
0.2 m	Reproductive Success	-0.0072	0.0040	-1.77	1	0.2733
0.3 m	Reproductive Success	-0.0187	0.0058	-3.21	0.136	0.2828
0.4 m	Reproductive Success	-0.0291	0.0075	-3.90	0.010	0.2908
0.5 m	Reproductive Success	-0.0299	0.0091	-3.28	0.109	0.284
0.6 m	Reproductive Success	-0.0364	0.0105	-3.46	0.057	0.2873
0.7 m	Reproductive Success	-0.0411	0.0116	-3.54	0.041	0.2871
0.8 m	Reproductive Success	-0.0410	0.0125	-3.29	0.105	0.2829
0.9 m	Reproductive Success	-0.0500	0.0133	-3.74	0.019	0.2884
1.0 m	Reproductive Success	-0.0320	0.0104	-3.09	0.207	0.2795
Patch	Reproductive Success	0.0100	0.0235	0.42	1	0.3222

General Linear Mixed Effect Models

Scale	Response	Slope	S.E.	t value	*P*-value	R^2 Cond
0.1 m	Number of Pods	0.0005	0.0011	0.48	1	0.5774
0.2 m	Number of Pods	-0.0032	0.0027	-1.17	1	0.5795
0.3 m	Number of Pods	-0.0139	0.0041	-3.43	0.060	0.5842
0.4 m	Number of Pods	-0.0166	0.0051	-3.25	0.112	0.5855
0.5 m	Number of Pods	-0.0155	0.0061	-2.53	1	0.5838
0.6 m	Number of Pods	-0.0174	0.0069	-2.52	1	0.5847
0.7 m	Number of Pods	-0.0155	0.0076	-2.05	1	0.5834
0.8 m	Number of Pods	-0.0092	0.0081	-1.13	1	0.5808
0.9 m	Number of Pods	-0.0077	0.0086	-0.90	1	0.5802
1.0 m	Number of Pods	-0.0200	0.0080	-2.52	1	0.6174
Patch	Number of Pods	0.0568	0.0216	2.63	0.847	0.6237
0.1 m	Number of Inflorescences	0.0005	0.0009	0.53	1	0.5235
0.2 m	Number of Inflorescences	-0.0048	0.0019	-2.57	1	0.5297
0.3 m	Number of Inflorescences	-0.0062	0.0027	-2.25	1	0.5308
0.4 m	Number of Inflorescences	-0.0093	0.0036	-2.58	0.985	0.5337
0.5 m	Number of Inflorescences	-0.0127	0.0043	-2.96	0.301	0.537
0.6 m	Number of Inflorescences	-0.0149	0.0051	-2.95	0.319	0.539
0.7 m	Number of Inflorescences	-0.0104	0.0057	-1.82	1	0.5336
0.8 m	Number of Inflorescences	-0.0065	0.0062	-1.05	1	0.5298
0.9 m	Number of Inflorescences	-0.0055	0.0066	-0.83	1	0.5286
1.0 m	Number of Inflorescences	-0.0080	0.0054	-1.48	1	0.5059
Patch	Number of Inflorescences	-0.0555	0.0152	-3.66	0.025	0.5532
0.1 m	Survival	-0.0019	0.0027	-0.71	1	0.3254
0.2 m	Survival	-0.0154	0.0056	-2.74	0.612	0.3251
0.3 m	Survival	-0.0239	0.0083	-2.89	0.379	0.3244
0.4 m	Survival	-0.0347	0.0107	-3.23	0.122	0.3254
0.5 m	Survival	-0.0549	0.0131	-4.20	0.003	0.3274
0.6 m	Survival	-0.0662	0.0153	-4.33	0.001	0.3282
0.7 m	Survival	-0.0705	0.0173	-4.07	0.005	0.3274
0.8 m	Survival	-0.0678	0.0190	-3.57	0.036	0.3245
0.9 m	Survival	-0.0729	0.0202	-3.61	0.030	0.3234
1.0 m	Survival	-0.0550	0.0158	-3.47	0.051	0.297
Patch	Survival	-0.1456	0.0411	-3.54	0.039	0.2327

Appendix 3

Coefficients for random effect of year for each response.

Height											
year	0.1 m	0.2 m	0.3 m	0.4 m	0.5 m	0.6 m	0.7 m	0.8 m	0.9 m	1.0 m	Patch
2013	2.94	2.78	2.88	2.94	2.84	2.78	2.75	2.87	2.81	3.22	-0.17
2014	-1.69	-1.76	-1.90	-1.87	-1.86	-1.80	-1.71	-1.70	-1.67	-1.53	1.91
2015	-1.24	-1.02	-0.98	-1.07	-0.98	-0.99	-1.04	-1.17	-1.14	-1.69	-1.75
Leaf Area											
year	0.1 m	0.2 m	0.3 m	0.4 m	0.5 m	0.6 m	0.7 m	0.8 m	0.9 m	1.0 m	Patch
2013	14.75	14.60	14.64	14.62	14.50	14.39	14.39	14.69	14.86	13.77	10.13
2014	-10.48	-10.43	-10.52	-10.52	-10.51	-10.45	-10.40	-10.48	-10.53	-7.48	1.32
2015	-4.27	-4.18	-4.12	-4.10	-3.99	-3.94	-3.99	-4.22	-4.33	-6.29	-11.5
Growth											
year	0.1 m	0.2 m	0.3 m	0.4 m	0.5 m	0.6 m	0.7 m	0.8 m	0.9 m	1.0 m	Patch
2013	5.06	4.95	4.81	4.70	4.54	4.34	4.20	4.33	4.23	4.75	8.30
2014	2.38	2.40	2.34	2.34	2.29	2.37	2.52	2.58	2.63	2.33	3.55
2015	-7.45	-7.36	-7.14	-7.04	-6.82	-6.70	-6.72	-6.91	-6.86	-7.08	-11.9
Reproductive Effort											
year	0.1 m	0.2 m	0.3 m	0.4 m	0.5 m	0.6 m	0.7 m	0.8 m	0.9 m	1.0 m	Patch
2013	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
2014	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00
2015	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01
Reproductive Success											
year	0.1 m	0.2 m	0.3 m	0.4 m	0.5 m	0.6 m	0.7 m	0.8 m	0.9 m	1.0 m	Patch
2013	0.43	0.42	0.41	0.41	0.41	0.40	0.39	0.39	0.38	0.39	0.50
2014	-0.14	-0.14	-0.15	-0.15	-0.15	-0.15	-0.14	-0.14	-0.14	-0.13	-0.08
2015	-0.29	-0.28	-0.26	-0.26	-0.25	-0.25	-0.25	-0.25	-0.24	-0.26	-0.42
Number of Inflorescences											
year	0.1 m	0.2 m	0.3 m	0.4 m	0.5 m	0.6 m	0.7 m	0.8 m	0.9 m	1.0 m	Patch
2013	-0.03	-0.04	-0.03	-0.04	-0.04	-0.04	-0.04	-0.03	-0.03	-0.01	-0.05
2014	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.07	0.19
2015	-0.06	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.06	-0.06	-0.06	-0.13
Number of Pods											
year	0.1 m	0.2 m	0.3 m	0.4 m	0.5 m	0.6 m	0.7 m	0.8 m	0.9 m	1.0 m	Patch
2013	0.37	0.36	0.34	0.35	0.35	0.35	0.35	0.35	0.36	0.37	0.67
2014	0.28	0.29	0.28	0.29	0.28	0.28	0.29	0.29	0.29	0.23	0.22
2015	-0.60	-0.59	-0.58	-0.58	-0.58	-0.58	-0.58	-0.59	-0.59	-0.56	-0.84
Percent Leaves Damaged											
year	0.1 m	0.2 m	0.3 m	0.4 m	0.5 m	0.6 m	0.7 m	0.8 m	0.9 m	1.0 m	Patch
2013	-32.40	-32.53	-32.49	-32.52	-32.53	-32.51	-32.39	-32.31	-32.16	-34.10	-34.7
2014	-21.31	-21.24	-21.26	-21.25	-21.25	-21.25	-21.29	-21.32	-21.37	-19.61	-19.9
2015	53.70	53.77	53.75	53.77	53.79	53.77	53.67	53.63	53.53	53.72	54.57
Survival											
year	0.1 m	0.2 m	0.3 m	0.4 m	0.5 m	0.6 m	0.7 m	0.8 m	0.9 m	1.0 m	Patch

2013	0.90	0.88	0.88	0.87	0.85	0.84	0.83	0.83	0.82	0.76	0.46
2014	-0.24	-0.25	-0.26	-0.26	-0.27	-0.27	-0.27	-0.26	-0.26	-0.15	0.06
2015	-0.72	-0.68	-0.67	-0.66	-0.63	-0.62	-0.62	-0.63	-0.62	-0.65	-0.55

Literature Cited

ABRAHAMSON W. 1980. Demography and vegetative reproduction. *In* Solberg O. [ed.], Demography and evolution in plant populations, 89-106. Blackwell, Oxford.

AGRAWAL A. A., M. J. LAJEUNESSE, AND M. FISHBEIN. 2008. Evolution of latex and its constituent defensive chemistry in milkweeds (Asclepias): a phylogenetic test of plant defense escalation. *Entomologia Experimentalis et Applicata* 128: 126-138.

AGRAWAL A., E. KEARNEY, A. HASTINGS, AND T. RAMSEY. 2012. Attenuation of the Jasmonate Burst, Plant Defensive Traits, and Resistance to Specialist Monarch Caterpillars on Shaded Common Milkweed (Asclepias syriaca). *Journal of Chemical Ecology* 38: 893-901.

ALPERT P. 1991. Nitrogen Sharing Among Ramets Increases Clonal Growth in Fragaria Chiloensis. *Ecology* 72: 69-80.

BAAYEN R. H. 2013. languageR: Data sets and functions with "Analyzing Linguistic Data: A practical introduction to statistics".

BALDWIN, J. AND SCHULTZ, J. Rapid changes in leaf chemistry induced by damage: evidence for communication between plants. *Science* 221: 277-279.

BASKIN J. M. AND C. C. BASKIN. 1977. Germination of Common Milkweed (Asclepias syriaca L.) Seeds.*Bulletin of the Torrey Botanical Club* 104: 167-170.

BATES, D., M. MÄCHLER, B. BOLKER, AND S. WALKER. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67: 1-48.

BATES D., M. MÄCHLER, B. BOLKER, S. WALKER, R. CHRISTENSEN, H. SINGMANN, B. DAI, G. GROTHENDIECK, AND P. GREEN. 2016. lme4: Linear Mixed-Effects Models using 'Eigen' and S4.

BELL A. D. AND P. B. TOMLINSON. 1980. Adaptive architecture in rhizomatous plants. *Botanical Journal of the Linnean Society* 80: 125-160.

BENOT, M. A. BONIS, AND C. MONY. 2010. Do spatial patterns of clonal fragments and architectural responses to defoliation depend on the structural blueprint? An experimental test with two rhizomatous Cyperaceae. *Evolutionary Ecology* 24: 1475-1487.

BERGSTRÖM G., M. ROTHSCHILD, I. GROTH, AND C. CRIGHTON. 1995. Oviposition by butterflies on young leaves: investigation of leaf volatiles. *Chemoecology* 5:147–158.

BERTNESS M. D. AND G. H. LEONARD. 1997. The Role of Positive Interactions in Communities: Lessons from Intertidal Habitats. *Ecology* 78: 1976-1989.

BERTNESS M. D. AND S. M. YEH. 1994. Cooperative and Competitive Interactions in the Recruitment of Marsh Elders. *Ecology* 75: 2416-2429.

BHOMIK P. AND. B., J. 1976. Biology of Canadian Weeds .19. Asclepias-Syriaca L. *Canadian Journal of Plant Science* 56: 579-589.

BHOWMIK P. AND J. BANDEEN. 1976. THE BIOLOGY OF CANADIAN WEEDS 19. Asclepias syrinca L. *Canadian Journal of Plant Science* 56.

CHU C., F. MAESTRE, S. XIAO, J. WEINER, Y. WANT, Z. DUAN, AND G. WANG. 2008. Balance between facilitation and resources competition determines biomass-density relationships in plant populations. *Ecology Letters* 11: 1189-1197.

CLAY K. AND R. SHAW. 1981. An Experimental Demonstration of Density-Dependent Reproduction in a Natural Population of Diamorpha smallii, a Rare Annual. *Oecologia* 51: 1-6.

CORON C., S. MÉLÉARD, E. PORCHER, AND A. ROBERT. 2013. Quantifying the Mutational Meltdown in Diploid Populations. *The American Naturalist* 181: 623-636.

COURCHAMP F., T. CLUTTON-BROCK, AND B. GRENFELL. 1999. Inverse density dependence and the Allee effect. *Trends in Ecology & Evolution* 14: 405-410.

COUTURE J. J., S. P. SERBIN, AND P. A. TOWNSEND. 2013. Spectroscopic sensitivity of real-time, rapidly induced phytochemical change in response to damage. *New Phytologist* 198: 311-319.

DOUST L. L. 1981. Intraclonal Variation and Competition in Ranunculus repens. *New Phytologist* 89: 495-502.

DUNCAN R. P., J. M. DIEZ, J. J. SULLIVAN, S. WANGEN, AND A. L. MILLER. 2009. Safe Sites, Seed Supply, and the Recruitment Function in Plant Populations. *Ecology* 90: 2129-2138.

ELMBERG J., G. GUNNARSSON, H. PÖYSÄ, K. SJÖBERG, AND P. NUMMI. 2005. Within-Season Sequential Density Dependence Regulates Breeding Success in Mallards Anas platyrhynchos. *Oikos* 108: 582-590.

FEDRIANI J. M., T. WIEGAND, G. CALVO, A. SUÁREZ-ESTEBAN, M. JÁCOME, M. ŻYWIEC, M. DELIBES, AND C. BROPHY. 2015. Unravelling conflicting density- and distance-dependent effects on plant reproduction using a spatially explicit approach. *Journal of Ecology* 103: 1344-1353.

FIRBANK, L AND WATKINSON, A. 1987. On the analysis of competition at the level of the individual plant. *Oecologia* 71: 308-317.

FOWLER N. L. 1995. Density-dependent demography in two grasses: A five-year study. *Ecology* 76: 2145.

FRIEDMAN D. AND P. ALPERT. 1991. Reciprocal Transport between Ramets Increases Growth of Fragaria chiloensis When Light andNitrogen Occur in Separate Patches but Only If Patches Are Rich. *Oecologia* 86: 76-80.

GERHARDT F. 1929. Propagation and Food Translocation in the Common Milkweed. *Journal of Agricultural Research* 39.

GHEORGHIADE M., D. J. VAN VELDHUISEN, AND W. S. COLUCCI. 2006. Contemporary Use of Digoxin in the Management of Cardiovascular Disorders. *Circulation* 113: 2556-2564.

GOLDENHEIM W. M., A. D. IRVING, AND M. D. BERTNESS. 2008. Switching from Negative to Positive Density-Dependence among Populations of a Cobble Beach Plant. *Oecologia* 158: 473-483.

GOMEZ, S. AND J. STUEFER. 2006. Members only: induced systemic resistance to herbivory in a clonal plant network. *Oecologia* 147: 461-468.

GUSTAFSSON C. AND J. EHRLEN. 2003. Effects of Range Position, Inter-Annual Variation and Density on Demographic Transition Rates of Hornungia petraea Populations. *Oikos* 100: 317-324.

HIDDING B., B. NOLET, T. DE BOER, P. DE VRIES, AND M. KLAASSEN. 2009. Compensatory Growth in an Aquatic Plant Mediates Exploitative Competition between Seasonally Tied Herbivores. *Ecology* 90: 1891-1899.

HIXON M. AND D. JOHNSON. 2009. Density Dependence and Independence. *Encyclopedia of Life Sciences (ELS)*.

HOWELL, N. The effect of seed size and relative emergence time on fitness in a natural population of Impatiens capesis Meerb. (Basalminaceae). *The American Midland Naturalist* 105: 312-320.

JUNG J., N. BAEK, J. HWANG-BO, S. LEE, J. PARK, K. SEO, J. KWON, E. OH, D. LEE, I. CHUNG, AND M. BANG. 2015. Two New Cytototoxic Cardenolides from the Whole Plants of Adonis multiflora Nishikawa & Koki Ito. *Molecules (Basel, Switzerland)* 20: 20823-20831.

KARBAN, R.; I. BALDWIN; K. BAXTER; G. LAUE AND G. FELTON. 2000. Communication between plants: induced resistance in wild tobacco plants following clipping of neighboring sagebrush. *Oecologia* 125: 66-71.

KEDDY P. A. 1981. Experimental Demography of the Sand-Dune Annual, Cakile Edentula, Growing Along an Environmental Gradient in Nova Scotia. *Journal of Ecology* 69: 615-630.

KLUTH C. AND H. BRUELHEIDE. 2005. Effects of Range Position, Inter-Annual Variation and Density on Demographic Transition Rates of Hornungia petraea Populations. *Oecologia* 145: 382-393.

KUZNETSOVA A., P. BROCKHOFF, AND R. CHRISTENSEN. 2016. lmerTest: Tests in Linear Mixed Effects Models.

LAU R. AND D. YOUNG. 1988. Influence of physiological integration on survivorship and water relations in a clonal herb. *Ecology* 69: 215-219.

LORTIE C. J., E. ELLIS, A. NOVOPLANSKY, AND R. TURKINGTON. 2005. Implications of Spatial Pattern and Local Density on Community-Level Interactions. *Oikos* 109: 495-502.

LOVETT-DOUST L. 1981. Population dynamics and local specialization in a clonal perennial (Ranunculus repens): I. The dynamics of ramets in contrasting habitats. *Journal of Ecology* 69: 743-755.

MALCOLM S. B. AND M. P. ZALUCKI. 1996. Milkweed latex and cardenolide induction may resolve the lethal plant defence paradox. *Entomologia Experimentalis et Applicata* 80: 193-196.

MICHELE MERONI, MICOL ROSSINI, VALENTINA PICCHI, CINZIA PANIGADA, SERGIO COGLIATI, CRISTINA NALI, AND ROBERTO COLOMBO. 2008. Assessing Steady-state Fluorescence and PRI from Hyperspectral Proximal Sensing as Early Indicators of Plant Stress: The Case of Ozone Exposure. *Sensors* 8: 1740-1754.

MOORE R. 1946. Investigations on Rubber-Bearing Plants: III. Development of Normal and Aborting Seeds in Asclepias syriaca L. *Canadian Journal of Research* 24.

NAKAGAWA S. AND H. SCHIELZETH. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution* 4: 133-142.

NANTEL P. AND D. GAGNON. 1999. Variability in the Dynamics of Northern Peripheral versus Southern Populations of Two Clonal Plant Species, Helianthus divaricatus and Rhus aromatica. *Journal of Ecology* 87: 748-760.

NILSSON J. AND T. D'HERTEFELDT. 2008. Origin matters for level of resource sharing in the clonal herb Aegopodium podagraria. *Evolutionary Ecology* 22: 437-448.

POTASH CORP. Growing degree day calculator. http://www.potashcorp-ekonomics.com/tools-to-calculate-fertilizer-needs/calculators/gdd/

R CORE TEAM. 2015. R: A Language and Environment for Statistical Computing. Vienna, Austria.

RAMULA S. AND Y. M. BUCKLEY. 2009. Multiple life stages with multiple replicated density levels are required to estimate density dependence for plants. *Oikos* 118: 1164-1173.

RASMANN S; A., ERWIN; R., HALITSCHKE; A., AGRAWAL. 2011. Direct and indirect root defenses of milkweed (Asclepias syriaca): trophic cascades, trade-offs and novel methods for studying subterranean herbivory. *Journal of Ecolology* 99:16–25.

RHOADS, D. 1983. Responses of alder and willow to attack by tent caterpillars and webworms: evidence for pheremonal sensitivity of willows. Plant Resistance to Insects, 55-68.

ROBERTS, J. 2000. Leaf Development and Canopy Growth. Eds. Marshall & Roberts. Taylor & Francis.

ROSS, M. AND HARPER, J. 1972. Occupation of biological space during seedling establishment. *Journal of Ecology* 60: 77-88.

SHAW R. G. 1987. Density-Dependence in Salvia Lyrata: Experimental Alteration of Densities of Established Plants. *Journal of Ecology* 75: 1049-1063.

SMITH B. H. 1983a. Demography of Floerkea Proserpinacoides, A Forest-Floor Annual: I. Density- Dependent Growth and Mortality. *Journal of Ecology* 71: 391-404.

SMITH B. H. 1983b. Demography of Floerkea Proserpinacoides, A Forest-Floor Annual: II. Density-Dependent Reproduction. *Journal of Ecology* 71: 405-412.

SMITH B. H. 1983c. Demography of Floerkea Proserpinacoides, A Forest-Floor Annual: III. Dynamics of Seed and Seedling Populations. *Journal of Ecology* 71: 413-425.

SPIGLER R. B. AND S. CHANG. 2008. Effects of Plant Abundance on Reproductive Success in the Biennial Sabatia angularis (Gentianaceae): Spatial Scale Matters. *Journal of Ecology* 96: 323-333.

STEPHENS P. A. AND W. J. SUTHERLAND. 1999. Consequences of the Allee effect for behaviour, ecology and conservation. *Trends in Ecology & Evolution* 14: 401-405.

STUEFER J., S. GOMEZ, AND T. VAN MOLKEN. 2004. Clonal integration beyond resource sharing: implications for defense signaling and disease transmission in clonal plant networks. *Evolutionary Ecology* 18: 647-667.

STUEFER J. F. 1998. Two types of division of labour in clonal plants: benefits, costs and constraints. *Perspectives in Plant Ecology, Evolution and Systematics* 1: 47-60.

TIAN D., H. CHENG, M. JIANG, W. SHEN, J. TANG, AND X. YAO. 2016. Cardiac Glycosides from the Seeds of Thevetia peruviana. *Journal of natural products* 79: 38.

TIEFENG PIAO, JUNG HWA CHUN, HEE MOON YANG, AND KWANGIL CHEON. 2014. Negative Density Dependence Regulates Two Tree Species at Later Life Stage in a Temperate Forest. *PLoS One* 9.

TOUCHETTE B., J. MOODY, C. BYRNE, AND S. MARCUS. 2013. Water integration in the clonal emergent hydrophyte, Justicia americana: benefits of acropetal water transfer from mother to daughter ramets. *Hydrobiologia* 702: 83-94.

VAN KLEUNEN, M. AND STUEFER, J. 1999 Quantifying the effects of reciprocal assimilate and water translocation in a clonal plant. Oikos, 85, 135-145.

WARREN R. J., V. BAHN, AND M. A. BRADFORD. 2012. The interaction between propagule pressure, habitat suitability and density-dependent reproduction in species invasion. *Oikos* 121: 874-881.

WASON, E. AND HUNTER, M. 2014. Genetic variation in plant volatile emission does not result in differential attraction of natural enemies in the field. *Oecologia* 174:479–491.

WATKINSON A. R. AND J. L. HARPER. 1978. The demography of a sand dune annual: Vulpia fasciculata: I. The natural regulation of populations. *Journal of Ecology* 66: 15-33.

WEINER, J. 1990. Asymmetric Competition in Plant Populations. *Trends in Evolution and Ecology* 5: 360-364.

WINTER B. Very basic tutorial for performing linear mixed effects analyses (tutorial 2). Website **http://www.bodowinter.com/tutorial/bw_LME_tutorial2.pdf**.

ZUUR. A. F., G. M. SMITH, E. N. IENO, A. A. SAVELIEV, AND N. WALKER. 2009. Mixed effects models and extensions in ecology with R. Springer-Verlag, DE.