THE PENNSYLVANIA STATE UNIVERSITY
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PHENOTYPIC PLASTICITY OF DISPERSAL CHARACTERISTICS OF THE INVASIVE THISTLE *CARDUUS NUTANS* IN RESPONSE TO MOWING AND FERTILIZER

JONATHAN SAPERSTEIN
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Reviewed and approved* by the following:

Katriona Shea
Professor of Biology
Thesis Supervisor

Bernhard Luscher
Professor of Biology and BMB
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Carduus nutans L. (Asteraceae) is a species of invasive thistle that is native to Europe and can be found in central Pennsylvania, as well as in many other places throughout the United States, Australia, and New Zealand. Because of its invasive nature, it competes with local flora for resources and often has a very detrimental effect on farmland and pastures. This can be crippling to agriculture and have major economic implications, which is why effective management strategies for this weed are crucial.

In this study we examined *C. nutans'* seed dispersal capabilities and how the use of varying treatment regimes in the field affects certain characteristics that are important for the dispersal and spread of the plant. We manipulated two factors in a fully-crossed experimental design: the addition (or lack) of fertilizer and the presence (or absence) of mowing. Plant height, terminal velocity, and seed production were analyzed. There was no significant effect of treatment on seed terminal velocity of the plants. However, across all treatments a relationship was seen between plant height and terminal velocity (taller plants had slower seeds). Moreover, mowed plants had significantly shorter final heights than those that were not mowed. Using a fluid dynamic model of seed dispersal by wind, the WALD model, we found that these mowed plants had a dispersal kernel that was significantly reduced compared to that of the unmowed plants. Reduced seed dispersal distance reduces spread, and our results suggest that mowing can be used as a way to manage the spread of this invasive and problematic thistle.

TABLE OF CONTENTS

LIST OF FIGURES

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Introduction

Carduus nutans L. (Asteraceae), known also as the “nodding thistle” or “musk thistle,” is a monocarpic perennial that is native to Europe, Asia, and North Africa (Moore and Frankton, 1974). While not native to the United States, its first appearance here can be traced back to Central Pennsylvania in the year 1853, and it can now be found across most of the country (Stuckey and Forsyth, 1971). In the U.S., along with other parts of the world such as New Zealand, Australia, and South America, this plant is considered both an invasive species and a weed. Invasive species are those that are introduced to an area and that compete aggressively with native organisms (Falk-Petersen et al., 2006). Often they have negative effects on their new habitat and do great harm to both the local biodiversity and economy (Wilcove et. al., 1998). Similarly, the term ‘weed’ is used to describe a plant that reproduces at the expense of other species around it and that is often found outside its natural habitat (Janick, 1979.)

C. nutans poses a large economic threat to farmers across the country because of its ability to outcompete native plants in fields and pastures (Thompson et al., 1987). Because nodding thistle is quite prickly, grazing animals cannot consume it or easily walk through it, thus reducing the productivity of the farmland it inhabits. Many management practices are already in place for this plant, such as the use of *Rhinocyllus conicus*, a biological control agent introduced into this country in 1969 (Gassmann and Louda, 2001). *R. conicus* females lays eggs on the bracts of the flowers, and the offspring eventually burrow through the flower head and feed on its seeds (Shorthouse and Lalonde, 1984). Mowing is also a common management strategy, and it has previously been shown that mowing significantly reduces reproduction in this species (Zhang and Shea, 2012).

In managing plant populations, studying the dispersal characteristics of a species is critical, especially when that species is considered invasive. While most management strategies focus on the reduction of local abundance, successful limitation of species spread is important as well. Spatial population spread is dependent upon two factors: the number of seeds produced and how far those seeds travel. *C. nutans* is a wind-dispersed plant that produces up to many thousands of seeds per individual (Skarpaas and Shea, 2007). When these seeds are released from a maternal plant, the distance that they travel from their original location can be predicted from two crucial factors: plant height and terminal velocity. This latter measurement is the maximum falling speed (typically in m/s) of a seed through still air and is accepted as an indicator of dispersal ability because studies have shown a strong inverse relationship between seed falling velocity and dispersal distance (Jakobsson and Eriksson, 2003). Thus, if a seed falls more slowly or falls from a tall plant, it can be taken farther by the wind than if it were falling more quickly or from a shorter height. Factors that affect terminal velocity include diameter of the seed pappus (a tuft of white hairs that acts as a dispersal structure) and seed weight. Another value that is of interest with respect to seeds is plume loading, which is a measurement relating a seed's weight to the area of its pappus. This value can explain why a large seed with a large pappus may fall at the same speed as a small seed with a small pappus.

These plant traits may respond to different environmental conditions if they are phenotypically plastic. Phenotypic plasticity is the capacity of an individual organism to make changes in morphology, physiology, phenology, or behavior in response to environmental conditions (Price et al., 2003). Thus, it is important to examine how plant success might be affected by environmental conditions or management strategies to improve management of this problem species. For example, farmers and land managers may be able to more easily reduce the spread of plants that grow under environmental conditions that limit their dispersal. In fact, previous work has shown that *Rhinocyllus conicus* works as an effective biological control agent for *C. nutans* not only by limiting seed production, and thus local species abundance, but also by impacting wind dispersal traits to effectively reduce its spatial spread (Marchetto et al., 2014).

In this study, we examined the effects of mowing and fertilizer application on the dispersal characteristics of *C. nutans*, including plant height, pappus dimensions, seed weight, terminal velocity, and seed production. Broadly, in this experiment, individuals were grown in the field and received one of four treatments that varied in both the addition of fertilizers and mowing. Fertilization and mowing were chosen as variables because *C. nutans* often encounters different levels of these variables in the invaded range. Fertilization is a result of both natural and human influences, namely the manure of grazing animals and runoff of fertilizers in soil, and mowing is a commonly used management practice.

After growing in the field, flower heads were harvested and their seed traits studied in order to determine if the various treatment combinations significantly affected dispersal traits among thistle individuals. We hypothesized that these treatments would have a measureable effect on seed dispersal in *C. nutans*. More specifically, we predicted that those plants receiving fertilizer would produce larger seeds that fall more quickly (and thus disperse shorter distances from the maternal plant) than their unfertilized counterparts. Moreover, we hypothesized that individuals receiving the mowing treatments would not grow as tall as those that remained uncut throughout the growing season and that their seeds would have a shorter distance to fall as a result. This too would be expected to limit dispersal.

Materials and Methods

At the start of the experiment in March 2012, there were a total of 35 *Carduus nutans* individuals that had successfully overwintered in the field nursery. The thistles had been germinated in the Biology greenhouse and then transplanted in four rows to the field in the previous fall and were originally planted 50 cm apart from one another. However, because not all of the original plants survived, some of these 35 thistles had a greater space than that between them. Each rosette was marked by a flag with its ID (row letter and individual number) on it. The young thistle rosettes were first measured for their plant diameter and longest leaf length in early spring. Based upon the longest leaf lengths, three outliers were removed so that the remaining number of plants (32) would be divisible by four, which was the number of different treatments being assigned. Longest leaf length was used to determine these outliers because it is typically a good indicator of whether or not a plant will bolt in the future. Two of these outliers were smaller than the rest, while one was larger than the rest. The remaining 32 thistles were stratified by longest leaf length and were then divided into eight blocks of four plants that were randomized based on initial plant size. Each plant within each block was assigned one of four treatments. These treatments were: fertilized/mowed (FM), fertilized/not mowed (FNM), not fertilized/mowed (NFM), and not fertilized/not mowed (NFNM). Mowing is a common management strategy, but as soil fertility can differ significantly between habitats we also wanted to test for any interaction. Once the treatments were assigned, 16 of the 32 plants received the fertilizer treatment on April 21, 2012. This involved adding two level teaspoons of fertilizer pellets (Osmocote 30-30-30) to the base of the rosettes. Also on this date, all grass within a radius of ten centimeters around the 32 rosettes was cut back, to reduce competition with surrounding vegetation.

Starting in the middle of May, measurements of the plants were taken two times each week. When plants were not yet bolting, meaning they had not yet produced a flowering stem, longest leaf length and diameter measurements were taken, and when the plants were bolting, height and longest leaf length measurements were taken. After it had been observed that some plants were not going to bolt during that growing season and that imbalance among treatments would arise as a result, certain plants were randomly reassigned treatments within blocks. Once a plant had bolted, it was tied with either a blue ribbon, dictating mowing, or a pink ribbon, dictating not mowing. Plants that were to be mowed were cut once to 5 cm when they had reached 40 cm in height and were then allowed to grow uninhibited. Once flower heads began senescing and were in the "paintbrush stage" (in which the seeds were mature but had not yet been released) starting at the end of June, individual heads were bagged with pollinator exclusion bags so that any loose seeds could be caught. When the florets and bracts had turned brown and the stem was slightly hollow, the heads were harvested. Ten inches of stem were cut off, and they were placed in labeled plastic boxes and kept in a dry storage room. The label included "JS12S" for Jon Saperstein Summer 2012, followed by the ID number of the flower head, a number counting the heads from the plant so far, the harvest date, and a "P" or an "S" for primary or secondary. Flower heads atop the main stems were designated as primary, while those branching off such stems were designated as secondary.

The flower heads required two to three days to dry, at which point some, but not all, of the heads had released seeds. The boxes were covered with lids (other plastic boxes) both to prevent pappi from escaping once seeds had released and to prevent pappus damage. Multiple flower heads were collected from a plant until there were none left, at which point the thistle was cut and completely removed from the field.

The data sheet that was completed on each census trip included the plants' ID numbers, the distance down the row at which they were located, their original diameters and longest leaf lengths, their assigned size blocks, their treatment number and type, the date, and that day's measurements of height, longest leaf length, and diameter (see App. B for data sheet sample). Once plants were cut and heads were harvested, these comments were included in a "NOTES" section of the data sheet.

At the beginning of the fall 2012 semester, once all the flower heads were dried, terminal velocity drops were performed on 10 healthy seeds from the first primary flower head collected from the field for each plant (180 seeds in total). These seeds were randomly selected from all of the seeds that could be pulled from the flower head with an attached pappus (using a grid and chart of randomly generated coordinates). Each seed was dropped down a 1.27 meter-long cardboard tube, topped by a short plastic tube, to minimize the effect of air resistance on falling times. The drop times were recorded using a stopwatch; drop times began as soon as the seed had passed through the plastic section of the tube to the cardboard (which is sufficient time for seeds of this species to reach terminal velocity) and ended when the seed landed upon the surface below. Drops were repeated for each seed until similar drop times, within 0.01 seconds, were achieved. The average of the times was then used. Each seed's pappus was then measured for height and width using calipers. Once all measurements were complete, the seeds were placed in individual labeled vials and returned to the dry storage room.

Dissections were performed on every flower head collected, 81 heads in total, in order to examine each treatment's effect on fecundity. Dissections consisted of removing all remaining seeds, both healthy and unhealthy, and counting them separately. *R. conicus* egg cases and emergence holes were also recorded, and all remaining plant material was saved in a paper bag. A data sheet was used to record all counts and measurements for each flower head. After their numbers were recorded, the healthy and unhealthy seeds were stored in coin envelopes that were labeled with flower head data and the date of the dissection. All material was then moved back to the dry storage room.

The mass of each seed that underwent a terminal velocity drop was measured in order to assess the effects of plume loading (which is dependent on both plume area and seed weight). Plume loading (PL) is calculated using the equation PL = 1000 * Seed Weight / Pappus Area (in which area is calculated by Area = π * (Diameter / 2)^2) (Skarpaas and Shea, 2007). This value has units in 10^{-6} g/m^2. Each seed's weight was taken after the removal of the pappus, and it was measured using a sensitive digital scale to the nearest thousandth of a gram. Once the masses were measured, the seeds were placed in a coin envelope, labeled, and moved back to the dry storage room.

Once all of the data was collected, it was entered into Excel. Linear-mixed effects models (lmer; Bates et al., 2011) were used in R (R Development Core team 2014) to assess relationships between treatment and terminal velocity, treatment and plant height, terminal velocity and plant height, treatment and seed weight, treatment and plume loading, and finally treatment and number of seeds produced per flower head and individual. The significance of the fixed effects of mowing, fertilizer, and their interaction combined with random effects of block (size-stratified as described above) were tested in all statistical models. If fixed effects were not significant ($p > 0.05$), they were removed from the models step-wise. Parameter estimates were then extracted from best-fit statistical models (Bolker et al, 2009).

Fitted parameter estimates of terminal velocity and height were then included in models of dispersal via the Wald analytical long-distance dispersal (WALD) model (Katul et al., 2005). This model has been shown to fit the empirical distribution of seeds in the field (Skarpaas and Shea, 2007) using dispersal kernels that describe the probability density of predicted seed dispersal distance from the maternal plant. It includes parameters for wind speed and turbulence, but seed plant height, which is assumed to be the release height of the seeds, and terminal velocity are the factors of interest for this particular study (Jongegans et al., 2008).

Results

The terminal velocity of the seeds of all four treatments were measured and compared to one another. While seeds of individuals receiving the various mowing and fertilization treatments displayed somewhat varying terminal velocities, none of these differences were statistically significant ($p > 0.05$). Thus, treatment had virtually no effect on seed terminal velocity (Figure 1).

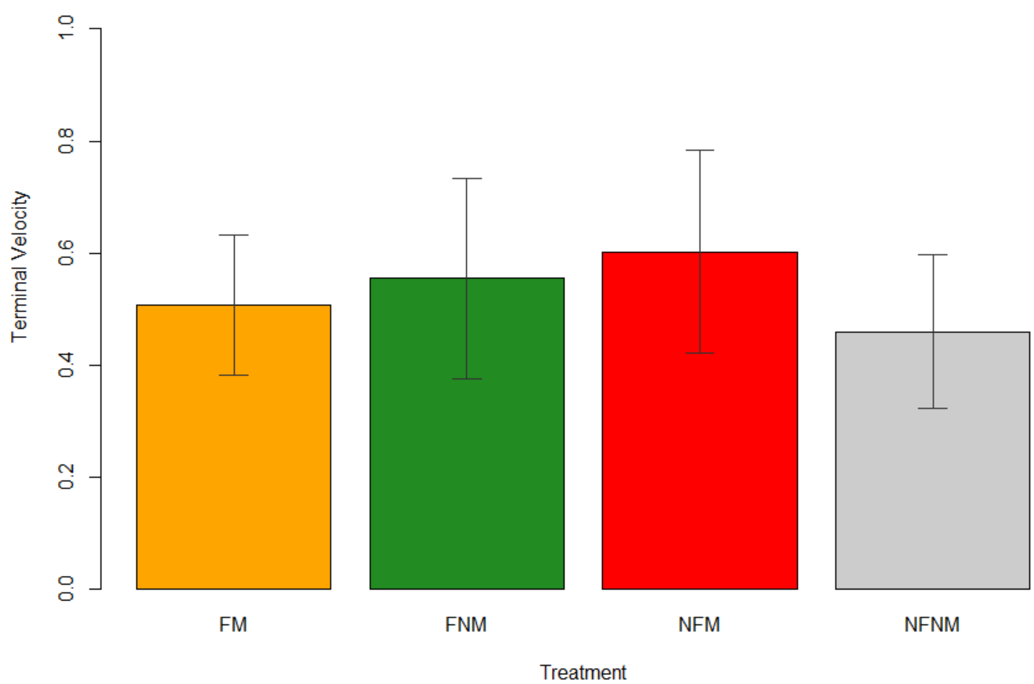


Figure 1. The effect of treatment on terminal velocity for *Carduus nutans*. The average terminal velocity (1.27 m / falling time) of the seeds of *Carduus nutans* measured for each treatment. There was no difference in seed terminal velocity between treatments. (N = 5, 6, 4, and 3 plants, respectively)

Treatment had a statistically significant effect on ultimate plant height (Figure 2); as expected, mowing versus not mowing had an enormous effect on final plant height. Those plants that received a mowing treatment (regardless of fertilization) were on average 40.5 cm shorter than their non-mowed counterparts. However, fertilization did have a significant effect on final height between the mowing treatments. NFM individuals were shorter than FM individuals.

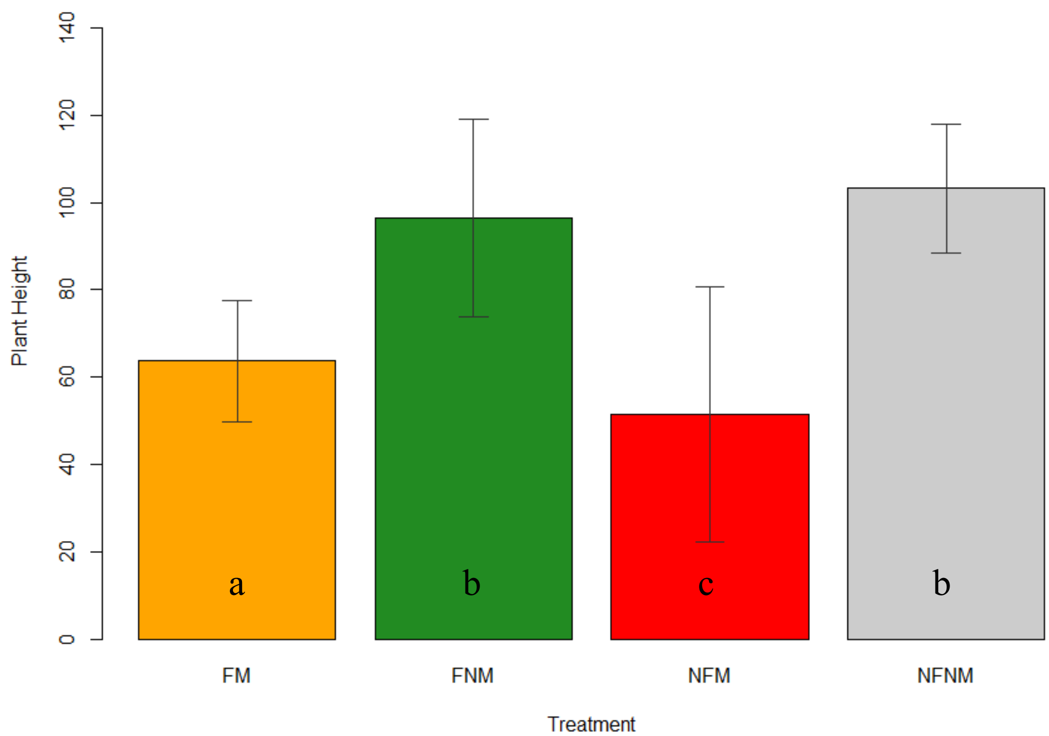


Figure 2. The effect of treatment on final plant height for *Carduus nutans*. The average final height of the *Carduus nutans* individuals measured for each treatment. Mowed individuals were significantly shorter. (N = 5, 6, 4, and 3 plants, respectively)

The relationship between seed terminal velocity and plant height was examined for all individuals in the four treatments. It was found that as plant height increases, terminal velocity decreases. This was significant for all treatments (Figure 3). Thus, as the plants get taller, their seeds tend to fall more slowly. However, this trend was not significantly different between treatments. That is, one treatment did not cause seeds to fall more slowly or quickly in relation to height than any other treatment.

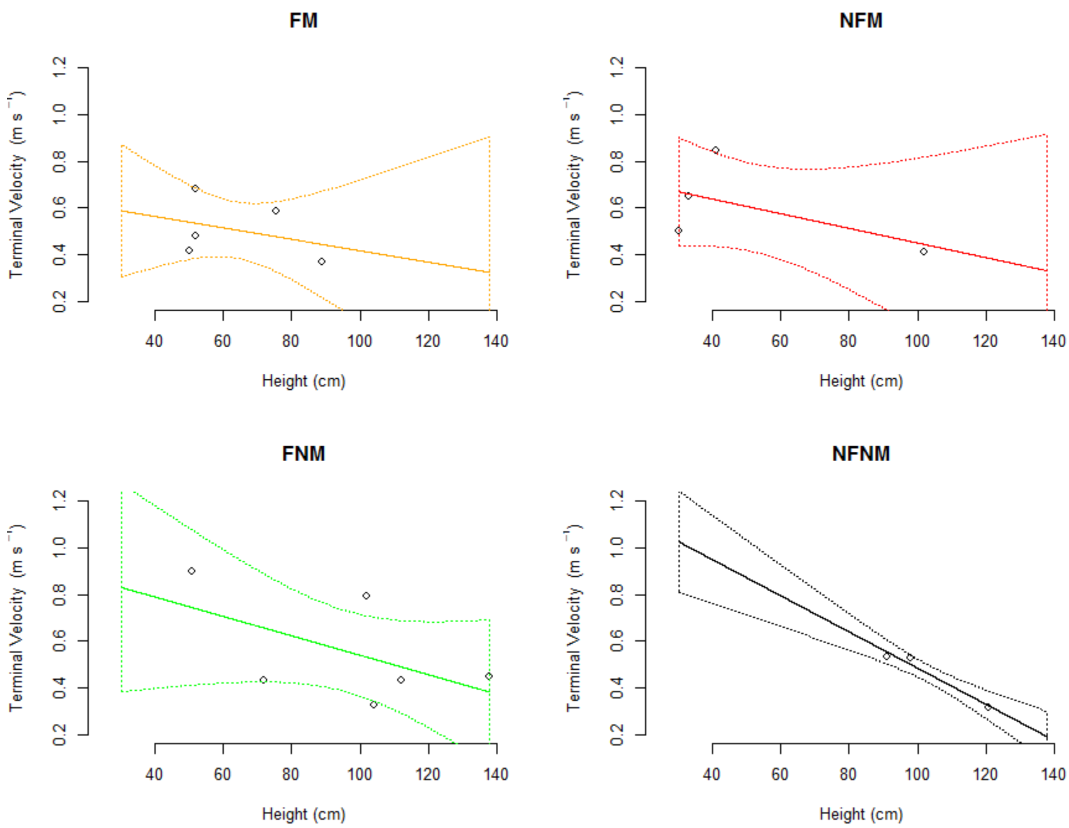


Figure 3. Terminal velocity vs. plant height for *Carduus nutans*. There is a significant relationship between plant height and the terminal velocity of seeds for *Carduus nutans*. There is not, however, any significant effect of treatment. (N = 5, 6, 4, and 3 plants, respectively)

Using the WALD model, we calculated the distance the seeds from each treatment were projected to travel from the maternal plant for each treatment using mean terminal velocity and plant height values. These dispersal kernels indicate that plants receiving the mowing treatments are projected to have seeds that travel a significantly shorter distance than seeds of those that were not mowed (Figure 4). That is, seeds have a much higher probability of traveling farther if the maternal plant was not mowed than if it was. According to the model, NFM seeds have the shortest median dispersal distance, while NFNM seeds have the longest median dispersal distance. The model shows the median predicted dispersal distances for the seeds are as follows: FM = 0.26 m, NFM = 0.14 m, FNM = 0.95 m, and NFNM = 1.06 m.

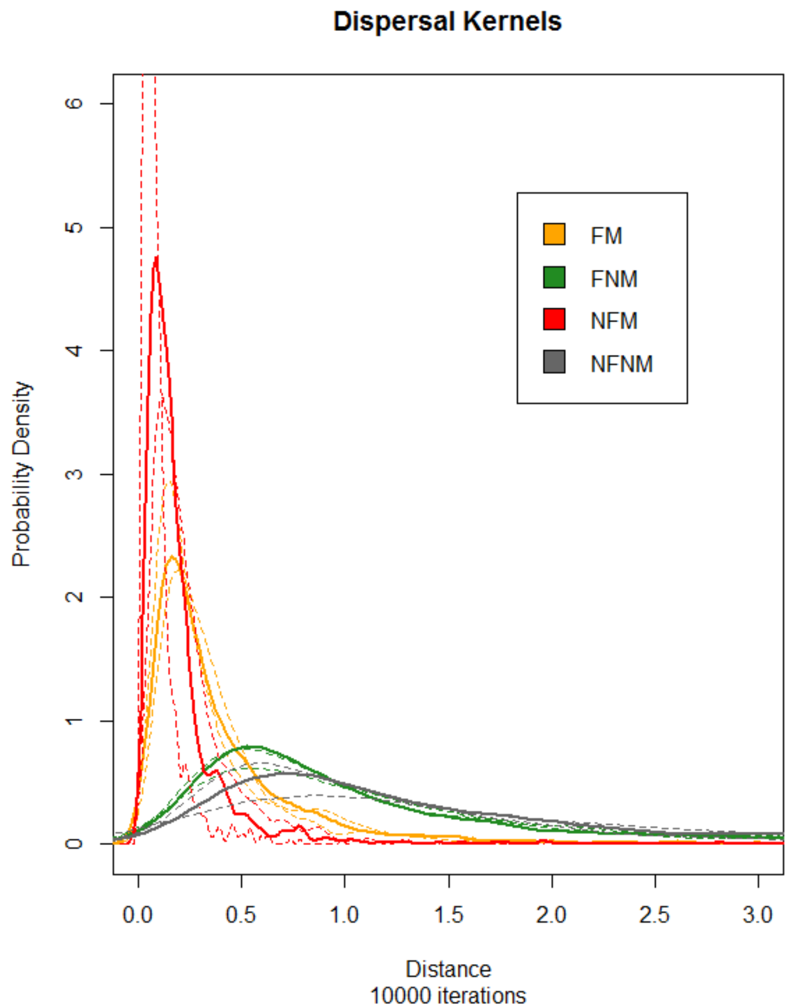


Figure 4. WALD model for *Carduus nutans* receiving four treatments. Using the WALD model, dispersal kernels were calculated for the four mowing/fertilization treatments, taking into account mean terminal velocity and plant height values. Mowed plants had much smaller predicted median dispersal distances than the other plants. Fertilization was not shown to have any significant impact on median seed dispersal distance. The dotted lines represent 95% confidence intervals.

Seed weight measurements were also taken of the seeds whose terminal velocities were found (180 in total). Treatment had no significant effect on average seed weight ($p > 0.05$) (Figure 5).

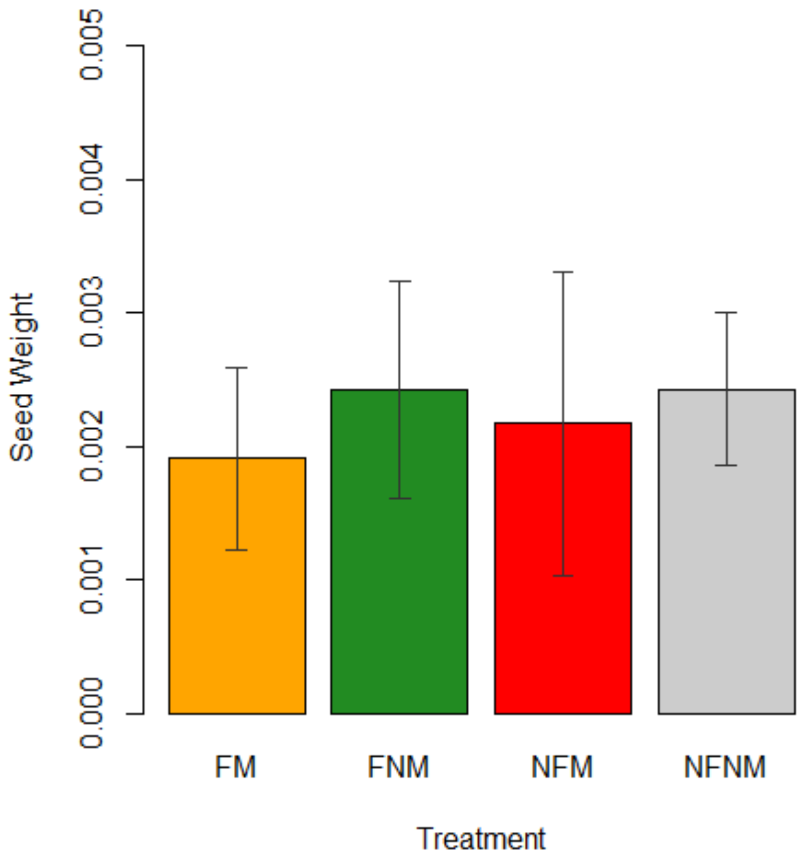


Figure 5. The effect of treatment on seed weight of *Carduus nutans*. The average healthy seed weight of *Carduus nutans* individuals measured for each treatment. Treatment did not significantly affect seed weight. (N = 5, 6, 4, and 3 plants, respectively)

Plume loading analysis was done using seed weight and plume area traits (determined by pappus length and width measurements). The square root of plume loading, which serves as good indicator of terminal velocity, was calculated for each treatment. Treatment had no significant effect on the average plume loading of the seeds of *C. nutans* individuals ($p > 0.05$) (Figure 6).

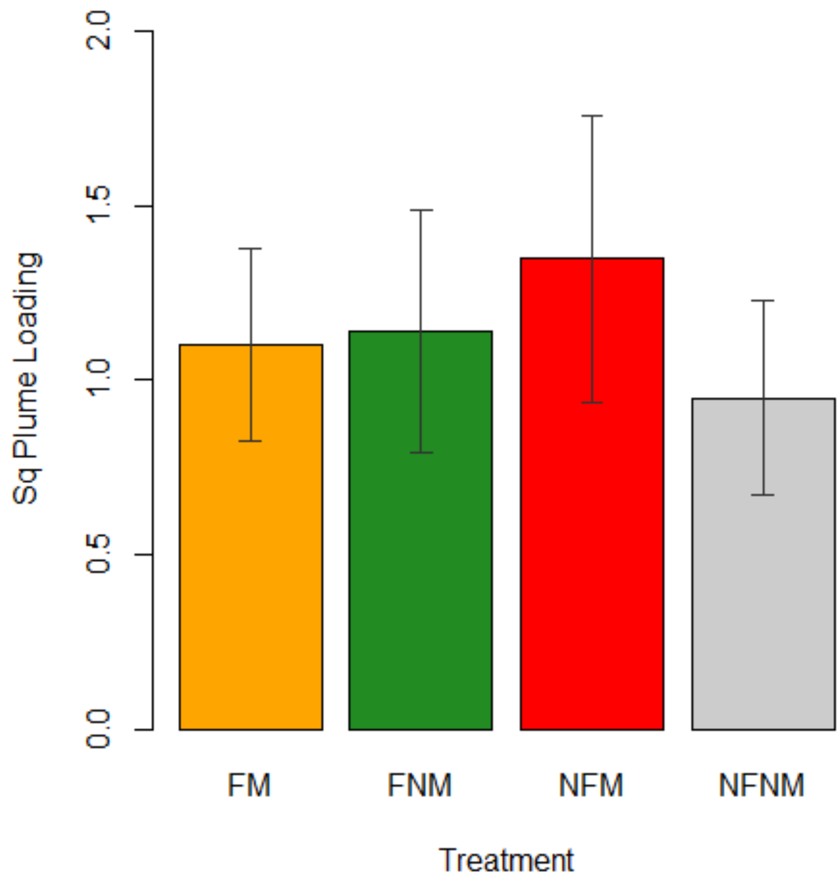


Figure 6. The effect of treatment on plume loading for *Carduus nutans*. There was no difference in the square root of plume loading (seed weight / pappus area) between treatments. (N = 5, 6, 4, and 3 plants, respectively)

Finally, fecundity was documented for each flower head and individual plant. The average number of unhealthy and healthy seeds per flower head and per plant was calculated for each treatment. Seed production per flower head was not significantly different across treatments ($p > 0.05$) (Figure 7). However, individual plants receiving the FM (fertilized and mowed) treatment had significantly fewer seeds than any of the other treatments ($p < 0.05$) (Figure 8).

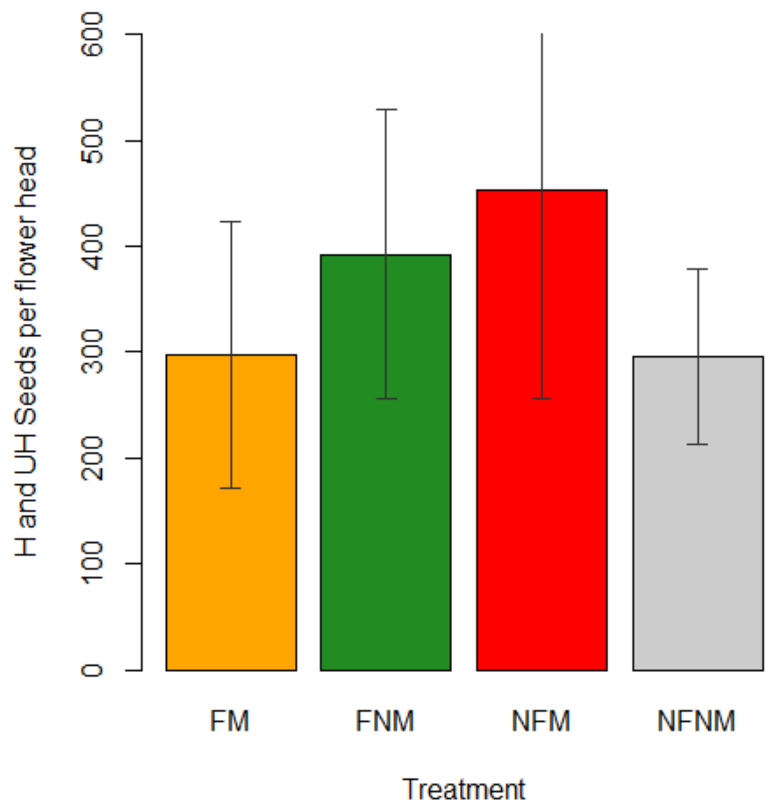


Figure 7. The effect of treatment on seed production per flower head for *Carduus nutans*. The average number of seeds per flower head was calculated for each treatment. There was no significant difference in the number of healthy and unhealthy seeds per flower head across treatments. (N = 5, 6, 4, and 3 plants, respectively)

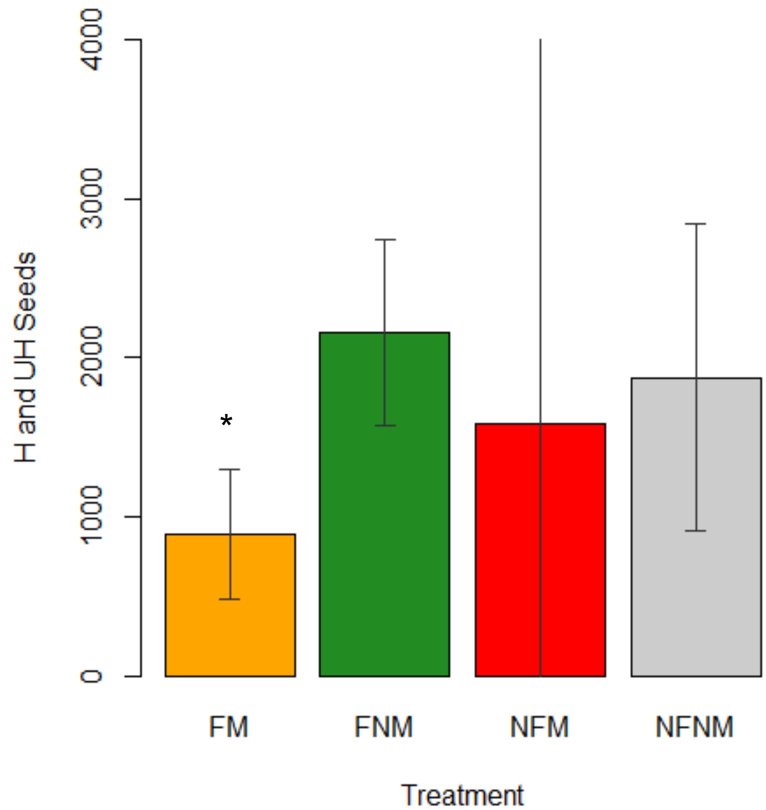


Figure 8. The effect of treatment on seed production per individual for *Carduus nutans*. The average number of seeds per plant was calculated for each treatment. Individuals with the FM treatment produced fewer total seeds than those receiving the other treatments. (N = 5, 6, 4, and 3 plants, respectively)

Discussion and Conclusions

In this study, we explored the hypothesis that mowing and fertilizer treatments affect the seed dispersal capabilities of nodding thistle individuals. According to the results, the different treatments did have a significant impact on the dispersal characteristics of *Carduus nutans*. The plants receiving the mowing treatments had significantly lower final heights than those that were not mowed. This result is important because it has major ramifications for seed dispersal. It is known that plant height at the time of seed release is an important factor in determining dispersal distance: the taller the plant, the farther its seeds can travel in the wind before hitting the ground (Jongejans et al., 2008). With the WALD model, we were able to show that the plants receiving the mowed treatments have dispersal kernels that are significantly shorter than those of plants that did not receive the mowing treatment.

This result is important for the purposes of species management. It is commonly accepted that mowing is a strategy for reducing local abundance and is practiced by farmers quite often when dealing with weeds. It has also previously been shown that mowing can reduce seed production in *C. nutans* (McCarty et al., 1975). However these new data show that mowing also negatively impacts the dispersal characteristics of this invasive weed. This in turn will reduce its spatial spread. To our knowledge, this study is the first to quantitatively estimate to what extent mowing reduces spread.

Terminal velocity was also examined in this study. The results show that mowing and fertilizer application had no statistically significant effect on the average falling velocity of seeds. A similar result has been seen in a previous study conducted by the Shea lab in which warming associated with climate change did not affect seed terminal velocity (Zhang et al., 2011). In that study, plant height was also the only dispersal characteristic that was significantly enhanced by warming (Zhang et al., 2011).

While treatment had no effect on seed terminal velocity, plant height was strongly affected by treatment. Furthermore, as plant height increases, average terminal velocity of the seeds decreased. This means that the tallest plants produced seeds that fell with the slowest speed for all treatments. This result has been found for drought-stressed plants in another experiment involving *Carduus nutans* (Teller et al., unpublished manuscript). This combination of tall plants and slow terminal velocities of seeds puts these individuals at an advantage in terms of dispersal according to the WALD model.

Average seed weight for each treatment was measured using the seeds that had undergone terminal velocity drops. Treatment was found to have no effect on seed weight. Moreover, plume loading was found not to be significantly different between treatments. These results strongly suggest that differences in mowing and fertilizing did very little, if anything, to affect the physical structure of the seed and its plume. All dispersal differences between treatments seen in the WALD model, therefore, are purely the result of differences in plant height.

Fecundity was also measured for each flower head and individual plant. Treatment did not significantly impact the total number of seeds (healthy and unhealthy) per flower head. However, individuals receiving the FM treatment had significantly fewer seeds than individuals receiving the other treatments. This result is significant for management and reduction of spatial spread, which is affected by both number of propagules and their dispersal. Because the FM plants showed both a significant decrease in height and a reduced number of total seeds, it would be expected that their potential for spatial spread would be highly reduced as compared to the other treatments if they had been left in the field long enough to release their seeds.

One caveat to this experiment is that the number of *C. nutans* that were studied was relatively low. While we started out with 32 plants in total, eight plants per treatment, only 18 of these individuals reached maturity and produced flower heads. This meant that by the end of the field season, only between three and six individuals were assigned to each of the four treatments. This small sample size, and thus low amount of replication, calls into question the reliability of our results. Further experimentation, with higher replication, may be necessary to confirm these findings.

As well as a larger sample size, future studies should use a greater number of treatment combinations and explore the role of other environmental variables such as competition. These additional factors may allow for enough phenotypic change among plants in different treatments to more significantly affect terminal velocity and fecundity in addition to plant height. Furthermore, because mowing had a significant impact on plant height and, consequently, seed dispersal, it may be of interest to conduct a study analyzing how dispersal is affected in plants that are cut to varying heights and/or at different times throughout the growing season.

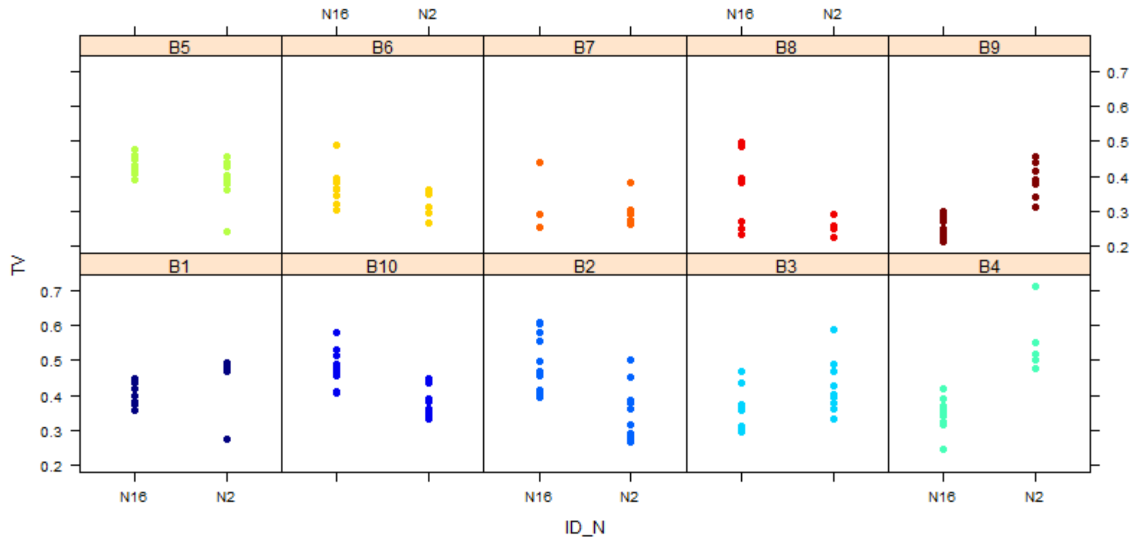
In conclusion, we have shown that mowing greatly reduces the dispersal and spread of *Carduus nutans*, in addition to simply reducing local abundance, by reducing plant height (and thus height of seed release). As such, mowing may act as an even better management strategy than was previously thought. Limiting the spread of this invasive species has many potential benefits for local agriculture and the economy. Further studies should be conducted to explore mowing as a method for curbing seed dispersal (as well as fecundity) in invasive species. If such studies support the findings of this experiment, mowing may increase in importance as a management strategy in the future.

Appendix A

Preliminary Study

This pilot experiment was based on an earlier study that was conducted using *Carduus nutans* individuals from one of Dr. Shea's field studies in which she analyzed plant phenotype changes in response to 16 different treatment regimes. The treatments included were ±mowing, ±competition, ±fertilizer, and ±weevil additions. We used single primary heads from plants from the treatment involving the best combination of variables (no mowing, no competition, fertilizer, and no weevil addition; treatment 16) and from the treatment involving the worst combination of variables that did not result in extreme mortality (mowing, no competition, no fertilizer, and weevil addition; treatment 2). Individuals had been divided into ten different blocks, and two flower heads (receiving either treatment) were taken from each block for a total of 20 flower heads/ We performed the same capitulum dissections and terminal velocity drops on 10 seeds from each head as we did in the more recent study.

Analysis of these data using R concluded that the treatments had no effect on terminal velocity. Our results indicated that in certain blocks, the average terminal velocity of the seeds from treatment 2 was faster than that of the other treatment, while in other blocks the exact opposite occurred and the average velocity of the seeds of treatment 16 was faster. These inconsistencies were unexpected and appeared to be the result of some other unforeseen factors.



We hypothesized that the inconsistent values in terminal velocity were likely the result of the both the plants' age and storage having negative impacts on the structural integrity of the flower heads. Unfortunately, the flower heads had been in storage in a cold room for nine years before they were dissected. During this time they had been compressed, which would have damaged the pappi. Previous research from this lab has actually shown that storage effects such as compression can have significant effects on seed characteristics and terminal velocity. Specifically, it was shown that compression during shipment and storage significantly increased terminal velocity in *C. nutans* (Marchetto et al., 2010).

As a result of the inconclusive data, we decided to redo the experiment on a smaller scale using fresh plants and a subset of the original treatments.

Appendix B

Data Sheets and Protocols

The following pages include data sheets and protocols that were used and followed throughout the experiment.

1. Data sheet used for census-taking in field
2. Dissection Protocol – Part 1
3. Dissection Protocol – Part 2
4. Data sheet used for terminal velocity drops
5. Data sheet used for dissections, seed counts, and weight measurements

Date: ____________________

ID	Transect	TransectNo	Distance	LLL	Diameter	Random	SizeBlock	TRTNO	FERT	MOW	TRT	HT	LLL	DIAM	NOTES
1	A	4	2.5	18.5	22	0.800737	SB8	4	NOTFertiliz	NOTMowe	NFNM				
2	A	4	3.5	15.5	17.5	0.29524	SB6	1	Fertilized	Mowed	FM				
3	A	4	9.5	9	13	0.722999	SB2	3	NOTFertiliz	Mowed	NFM				
4	A	4	15	14	17	0.861848	SB5	3	NOTFertiliz	Mowed	NFM				
5	A	4	16	8.5	8	0.96901	SB2	4	NOTFertiliz	NOTMowe	NFNM				
6	A	4	18.5	7.5	11	0.798965	SB1	3	NOTFertiliz	Mowed	NFM				
7	A	4	21	15.5	19	0.474497	SB6	2	Fertilized	NOTMowe	FNM				
8	A	4	22	17.5	24	0.579171	SB8	2	Fertilized	NOTMowe	FNM				
9	A	4	22.5	14	14.5	0.872813	SB5	4	NOTFertiliz	NOTMowe	NFNM				
10	B	3	0.5	18	18	0.43482	SB8	1	Fertilized	Mowed	FM				
11	B	3	1	14	19	0.629524	SB5	2	Fertilized	NOTMowe	FNM				
12	B	3	2	21.5	30	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	B	3	4	10	9	0.542367	SB2	1	Fertilized	Mowed	FM				
14	B	3	8	11	15	0.243903	SB3	1	Fertilized	Mowed	FM				
15	B	3	9	13	15	0.484474	SB4	2	Fertilized	NOTMowe	FNM				
16	B	3	12.5	4.5	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	C	2	0.5	16	19	0.643637	SB7	2	Fertilized	NOTMowe	FNM				
18	C	2	1	12.5	17	0.884573	SB4	4	NOTFertiliz	NOTMowe	NFNM				
19	C	2	1.5	15.5	28	0.875956	SB7	3	NOTFertiliz	Mowed	NFM				
20	C	2	2	14	17	0.530375	SB5	1	Fertilized	Mowed	FM				
21	C	2	2.5	16	16	0.922841	SB7	4	NOTFertiliz	NOTMowe	NFNM				
22	C	2	4	11.5	11	0.498292	SB4	3	NOTFertiliz	Mowed	NFM				
23	C	2	10.5	7.5	9	0.53814	SB1	1	Fertilized	Mowed	FM				
24	C	2	12	13	13.5	0.438913	SB4	1	Fertilized	Mowed	FM				
25	C	2	13.5	15.5	18	0.583542	SB6	3	NOTFertiliz	Mowed	NFM				
26	C	2	14.5	7.5	7.5	0.978111	SB1	4	NOTFertiliz	NOTMowe	NFNM				
27	C	2	21	8	11.5	0.562229	SB2	2	Fertilized	NOTMowe	FNM				
28	C	2	21.5	11	21	0.58437	SB3	3	NOTFertiliz	Mowed	NFM				
29	D	1	1	15	17	0.764001	SB6	4	NOTFertiliz	NOTMowe	NFNM				
30	D	1	1.5	16.5	24.5	0.157825	SB7	1	Fertilized	Mowed	FM				
31	D	1	2.5	10.5	10	0.572773	SB3	2	Fertilized	NOTMowe	FNM				
32	D	1	3	19	20	0.7262	SB8	3	NOTFertiliz	Mowed	NFM				
33	D	1	11	8	13	0.631228	SB1	2	Fertilized	NOTMowe	FNM				
34	D	1	13	10	14	0.603195	SB3	4	NOTFertiliz	NOTMowe	NFNM				
35	D	1	13.5	5	8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Honors project for Jon Saperstein: Dissection Protocol (Part 1) for Kat's invasion experiment plants and Jon's field study plants

If you count a zero for something, DO NOT leave the column blank or fill it in with a dash. Write "0" in the appropriate column.

If there are multiple heads in any pollination bag, DO NOT dissect them!

Notes: Bagged heads (*C. nutans* only to be investigated) were all primary, and usually in stage 5 (maturing seed (all florets brown, but not many or no pappi dispersed)) when collected.

For Kat's plants, start with steps 1-4
For Jon's plants, start with step 5-6
For both studies continue from step 7

1. For Kat's dissections only: Go down to the cold room and find the brown paper bags that have the flower heads for which you are looking.

2. For Kat's dissections only: Take out the papoose (the white bundle of heads in pollen bags). There will be up to 10 individually bagged heads in each papoose. If there are multiple heads in any pollination bag, DO NOT dissect them! Choose one bagged head (ideally the one in the best shape, with no pappi dispersed). The capitulum that is chosen should be the most intact, having a tear drop shape, and not "exploded".

3. For Kat's dissections only: Leave a note in the papoose of how many heads have been removed (dissected). Return the papoose to the original big brown bag.

4. For Kat's dissections only: Place flower head in a plastic box that is labeled with the flower head ID number. The ID will consist of Student Name, Cohort, Year, Block, Plot, Site, and Head numbers.

5. For Jon's dissections only: Randomly select the order of the flower heads to be dissected using a random number generator.

6. For Jon's dissections only: Go to the storeroom and find the box that has the primary flower head that was harvested first (look at dates) for the plant in question. Make sure the box is properly IDed. The ID will consist of Student Name, Year, Field ID number, Head number taken from that plant, Date harvested, and Type of head (primary).

7. Remove the head carefully from the box (for Jon's heads) or bag (for Kat's heads). If there are any loose seeds, store them in a separate box to be counted later.

8. Record the date, the flower head ID number (as listed in Steps 4 and 5 for Kat's and Jon's flower heads, respectively), head width, temperature and humidity of room, and harvest date of flower head on the data sheet.

9. Measure the diameter of seed head at the point where it is just barely held by the caliper. Record this number on the data sheet (rounding to the nearest 0.1 mm).

10. **Very** carefully, using tweezers and a microscope light, remove seeds (one-by-one) from the flower head, trying hard to keep the seeds and pappi attached. The goal is to collect 10 **healthy** seeds with the pappi still attached to use for terminal velocity drops. (Seeds may be taken from anywhere on the head, edge or center, but the general location must be recorded under the 'Notes' section on the data sheet if possible).

11. Place any seeds that separate from the pappus into the same box as the loose seeds so that they can be counted and recorded later. Continue pulling seeds until 10 TV-appropriate ones are found. Place any other plant material (pappi, chaff, etc.) into the same container. If more than ten seeds are found with pappi attached, choose 10 randomly (using a random number sheet and grid) for terminal velocity drops. The leftover ones will be added to the rest of the loose seeds.

12. For each terminal velocity seed:
    a. Repeat each drop (without flowers attached) until you get similar drop times within 0.01 seconds and record these numbers on the data sheet.
    b. Record the length and width of the pappi on the data sheet (see measurement instructions, below).
    c. Place the terminal velocity seeds, with pappi, into individual vials that are clearly labeled with the flower head ID number and seed number.
    d. **Try to do all TV drops on the same day the seeds are taken from the flower head so that the pappus does not detach before measurements are taken.**.
    e. The seeds will be dropped in a tube measuring 1.27 m in height. Timing starts when the seed passes the top of the cardboard section of the tube and ends when the seed hits the bucket at the bottom of the tube. If static occurs and the seeds stick to the side, drop a dryer sheet down the tube and try the drop again.

13. Place flower head back into its plastic box. Place this box, along with the box of seeds/plant material and the individual vials, into a gallon-sized plastic bag. Label this bag with your name, the proper flower head ID number (for either Kat's or Jon's flower heads), and the date. Store it in the cold room (Kat's samples) or the storeroom (Jon's samples).

Honors project for Jon Saperstein: Dissection Protocol (Part 2) for Kat's invasion experiment plants and Jon's field study plants

If you count a zero for something, DO NOT leave the column blank or fill it in with a dash. Write "0" in the appropriate column.

1. Collect plastic bag with flower head box, loose seeds and plant material box, and TV seeds (i.e. seeds for which terminal velocity has already been measured) from either cold room storage (Kat's flower heads) or storeroom (Jon's flower heads).

2. Record the date and flower head ID number (Student Name, Cohort, Year, Block, Plot, Site Number, and Head number for Kat's plants; Student Name, Year, Field ID number, Head number taken from that plant, Date harvested, and Type of head (primary) for Jon's plants) on the new data sheet.

3. Remove the flower head from its box and pull off as many remaining seeds as possible, adding them to the box of loose seeds/plant material. (This includes pulling seeds attached to pappi and agitating the chaff for a few minutes with tweezers.)

4. Count emergence holes on the bottom of flower head and record this in the data sheet.

5. Pull bracts one by one from the bottom of seed head and count the number of egg cases/emergence holes as they appear and record this number on the data sheet.

6. Break apart the whole seed head into sections (first cutting it in half with a box cutter) and record the number of cysts found.

7. Also at this time, sort through the seed head matter carefully to get any seeds you missed and add them to box of already loose seeds. Make sure to save all plant matter.

8. Separate the good seeds from the bad seeds and count the total numbers of each. (Good seeds are defined as seeds that have a potential to germinate - so they should have some volume; whereas bad seeds are the shriveled ones that we are sure won't germinate.) When counting seeds, remove pappi from any that are still attached.

9. Place good seeds in a clearly labeled coin envelope (Student Name, Cohort, Year, Block, Plot, Site Number, Head number, and Number of good seeds for Kat's plants; Student Name, Year, Field ID number, Head number taken from that plant, Date harvested, Type of head (primary), and Number of good seeds for Jon's plants).

10. Place bad seeds in a clearly labeled coin envelope (Name, Cohort, Year, Block, Plot, Site Number, Head number, and Number of bad seeds for Kat's plants; Name, Year, Field ID number, Head number taken from that plant, Date harvested, Type of head (primary), and Number of bad seeds for Jon's plants).

11. Individually weigh the terminal velocity seeds (from the vials) on an analytical balance, removing the pappus first. Record these values on the data sheet.

12. Under "Notes," write down anything different or unusual that you have noticed.

Date:_______________
Field ID number:_______________
Head number taken from plant:_______________
Harvest date:_______________
Type of head:_______________

Data Sheet for Terminal Velocity Drops

Head width:	Temperature:	Humidity:
______________________	______________________	______________________

Seed 1:
Times _________
____
____
____
____
____
____

Width _________
Length _________

Notes:

Seed 3:
Times _________
____
____
____
____
____
____

Width _________
Length _________

Notes:

Seed 5:
Times _________
____
____
____
____
____
____

Width _________
Length _________

Notes:

Seed 2:
Times _________
____
____
____
____
____
____

Width _________
Length _________

Notes:

Seed 4:
Times _________
____
____
____
____
____
____

Width _________
Length _________

Notes:

Seed 6:
Times _________
____
____
____
____
____
____

Width _________
Length _________

Notes:

Seed 7:

Times ________

____

____

____

____

____

____

Width ________

Length ________

Notes:

Seed 8:

Times ________

____

____

____

____

____

____

Width ________

Length ________

Notes:

Seed 9:

Times ________

____

____

____

____

____

____

Width ________

Length ________

Notes:

Seed 10:

Times ________

____

____

____

____

____

____

Width ________

Length ________

Notes:

JON SAPERSTEIN THISTLE DISSECTIONS		

Date ________

ID	
Number of Egg Cases	

Number of Cysts	

Total Number of Seeds	H	UH

TV Seeds	Weight
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

Notes

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ACADEMIC VITA

Permanent Address	**Jonathan Saperstein**	Local Address
302 Frazer Dr.	jonsaper@gmail.com	119 Locust Lane, Apt A4
Yardley, PA 19067	215-584-1879	State College, PA 16801

Education

- The Pennsylvania State University, University Park, PA
- B.S. Biology, Vertebrate Physiology option
- Minors in Spanish and Theatre
- Schreyer Honors College

Honors and Awards

- Schreyer Academic Excellence Scholarship, 2010 – Present
- Dean's list, Fall 2010 – Present
- Participant in FURP (Freshman Undergraduate Research Program), Spring 2011 – Fall 2012
- Schreyer Ambassador Travel Grant – 2012 and 2013

Professional Experience

- Undergraduate Researcher in Song Tan's Gene Regulation Lab, Fall 2010 – Spring 2011
- Volunteer at Mt. Nittany Medical Center, Spring 2011 – Fall 2013
- Mentor Schreyer Honors College Orientation, August 2011, 2012, and 2013
- Teaching Assistant for English 030, Fall 2011
- Undergraduate Researcher in Katriona Shea's Ecology Lab, Fall 2011 – Present

Association Memberships/Activities

- None of the Above (NOTA) A Cappella Group, Fall 2010 – Present
- The Penn State Thespians, Fall 2010 – Present
    - Marketing Chair, Fall 2011 – Spring 2012
    - THON Chair, Fall 2012 – Spring 2013
- No Refund Theatre, Fall 2012 – Present
- Penn State Global Human Rights Brigade, Fall 2011 – Spring 2013
    - Treasurer, Fall 2012 – Spring 2013