The Pennsylvania State University
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BIOLOGICAL INVASIONS IN A CHANGING WORLD: RESPONSES OF TWO
INVASIVE THISTLES TO DISTURBANCE AND CLIMATE CHANGE

A Dissertation in
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by
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ABSTRACT

Biological invasion has been recognized as a significant component of global change due to its detrimental consequences on biodiversity, ecosystem functioning and human health. Invasion can also interact with other elements of global change, such as human-mediated disturbances and climate change. Therefore, understanding the responses of invasive species to disturbance and climate change is crucial both for predicting future invasion risks, and in order to develop appropriate management strategies for the future. Using both experimental and theoretical approaches, I examined invasions of two congeneric thistles, *Carduus nutans* and *C. acanthoides* in the context of disturbance and climate change. I aimed to assess the invasion potential of these two species under different disturbance regimes (specifically, different levels of intensity, frequency, and timing of mowing) and under projected climates for the future (specifically, increased temperature and increased precipitation).

My disturbance results underline the importance of considering multiple aspects of disturbance in invasion studies, as interactions and interdependence of these aspects may lead to complex outcomes and potentially counter-intuitive conclusions (Chapter 2). Based on these empirical data, I demonstrate that the choice of management strategy may also depend on the time horizon of the management objective (Chapter 3). Strong tolerance of the two species to disturbance (i.e. regrowth ability after damage) is likely to contribute to their success in disturbed areas (Chapter 4). The two species also showed positive responses to climate change (Chapter 5). Besides enhanced demographic vital rates, dispersal of *Carduus* spp. was also enhanced under warming, mainly due to increased plant height. Consequently, population spread rates are expected to increase under future climatic scenarios. Phenology was affected by warming as well, altering both individual phenological dates, and durations of each developmental stage (Chapter 6). Uneven shifts in the phenological sequence of *C. nutans* led to extended growing duration,
which contributed to increased plant height at the dispersing stage. However, increased height of *C. acanthoides* appeared to be due to faster growth rates under warming. Furthermore, I found that for *C. nutans*, plant structural defense (i.e. prickle density) was reduced under warming (Chapter 7) and maternal warming significantly affected germination and seedling emergence of the offspring (Chapter 8). Results from this thesis suggest that invasions of *Carduus* spp. are likely to increase both in local abundance and spatial spread to become more problematic with ongoing global change.
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Chapter 1

Introduction

The movement of species from one place to another is a predominant feature of life on Earth (Elton 1958, Sax et al. 2005). Rapid growth of the human population, and improved transportation, however, have vastly increased the likelihood and spatial scale of species movement, especially the introduction of non-indigenous organisms into new ranges (Sharma et al. 2005, Pauchard and Shea 2006).

An invasive species is “a species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic or environmental harm or harm to human health” (The National Invasive Species Council, 2006). Invasions have been documented to cause detrimental consequences both economically and ecologically. Pimentel et al. (2005) reported that losses due to invasive species and associated control costs add up to almost $120 billion in the U.S. Many studies demonstrate that biological invasions also increasingly alter community composition and ecosystem function (D’Antonio and Vitousek 1992, Vitousek and Walker 1989).

Human activities continuously modify the appearance and functioning of the Earth. Direct human impacts, such as changed land use patterns and shifted disturbance regimes, are considered as major aspects of ongoing global change (Vitousek et al. 1997). Other aspects of global change include indirect human-mediated changes, such as elevated CO₂, global warming, shifted precipitation regimes, increased frequency of extreme weather events, and elevated nitrogen deposition, which immensely alter resource dynamics and species abundances and distributions (Dukes and Mooney 1999). Biological invasion, which is itself considered to be a substantial component of global change (Vitousek 1994, Vitousek 1997), is also affected by the
other aspects of global change described above. Hence the interactions between invasion and other aspects of global change (e.g. human disturbance and climate change) may generate complex effects on the structure and function of ecosystems.

Successful invasions are often found to take place in conjunction with anthropogenic changes (e.g. disturbance and land use change) in the environment (Vila et al. 2007). For example, establishment of six common invasive plant species were found to be closely related to soil disturbance in tall-grass mountain grasslands (Petryna et al. 2002). Grazing facilitated the invasions of *Taeniatherum asperum* (medusahead) and *Bromus rubens* (red brome) into grasslands which were previously dominated by grazing-intolerant native bunchgrass (Young 1971). Invasion of *Microstegium vimineum* (Japanese stiltgrass) was exacerbated along forest roadsides (Rauschert et al. 2010). On the other hand, disturbances can also be used to control invasions. For example, multiple mowing is an effective control that causes huge reductions in the biomass and seed production of invasive *Centaurea solstitialis* (yellow starthistle) (Benefield et al. 1999). Early summer prescribed burning effectively reduces cover of *C. solstitialis* (DiTomaso 2006). The varied responses of invasive species to disturbance may be due to different features in both the disturbance and the invader itself. Understanding how these features interplay with each other is essential for invasion management and conservation.

While direct human interventions (e.g. mowing, prescribed burning) often happen at the local scale and are usually considered as disturbances, or more specifically, pulse disturbances (Bender et al. 1984), most aspects of climate change (e.g. global warming, increased CO₂ level, but not storms) happen at the global scale and are considered as “trends” (IPCC 2007). These trends in climate, as well as manipulated warming using open top chambers or overhead infrared heaters, can also be considered as press disturbances (Bender et al. 1984), due to their ongoing and persistent occurrence in the life cycles of the organisms. Such aspects of global change have broadly affected species abundances and distributions (Walther et al 2002). Documented species’
responses to climate change include changes in physiology (Long et al. 2004), growth (Polle et al. 2001), reproduction (Sherry et al. 2007, McPeek and Wang 2007), phenology (Fitter and Fitter 2002), and distribution (Aert et al. 2006, Lenoir et al. 2006).

Many studies show that invasive species share certain physiological or life history traits that are favored by climate change, such as high resource acquisition ability, fast growth rate, large reproductive output, and strong dispersal ability (Nagel et al. 2004, Weltzin et al. 2003, Smith et al. 2000, Polley et al. 2003, Ziska 2003, White and Holt 2005, Moore 2004). For example, Smith et al. (2001) found that an invasive annual grass increased its productivity more than several native species under elevated CO₂ levels, suggesting enhanced relative success of this species in the future. Willis et al. (2010) demonstrated that invasive species are better at tracking climate change by adjusting their flowering dates than native species. Bradley et al. (2009) projected higher invasion risks for three invasive plants in Southeast U.S. in the future, using bioclimatic envelope models. This leads to an emerging hypothesis that ongoing climate change will facilitate invasions and exacerbate their impacts on ecosystems (Bradley et al. 2010).

Although predicting the effects of other elements of global change on biological invasion is a “daunting and complex” task (Dukes and Mooney 1999), such research is urgent and important because of its significant ramifications for both management and conservation.

In this thesis, I examine invasions of two congeneric thistle species *Carduus* spp. in the context of disturbance (Chapters 2-4, Appendix A) and climate change (Chapters 5-8, Appendix B), respectively. I aim to answer the following questions: 1. How do *Carduus* spp. respond to a human-mediated disturbance, specifically “mowing”? How do different aspects of a disturbance interplay to cause different responses of the species? Are *Carduus* spp. tolerant to frequently occurring disturbances? Is tolerance related to their invasion success in disturbed areas? 2. How does climate change affect *Carduus* spp.? If the two species’ responses are different, is it because of their different life histories? Are *Carduus* spp. likely to increase their population growth and
spread under future climate? What life history traits make them even better dispersers under climate change? Can responses to climate change be passed on to the next generation via maternal environmental effects?

I used both field experiments and modeling approaches to answer these questions, as I believe both empirical and theoretical approaches are crucial for understanding ecological processes, and the combination of the two provides a great tool to reach this goal. Therefore the thesis comprises experiment-oriented chapters (Chapters 2, 4, 7, 8), modeling-oriented chapters (Chapter 3), and chapters that involve both perspectives (Chapters 5, 6).

In Chapter 2 and Appendix A, I present a two-cohort study where multiple aspects (i.e. intensity, frequency, and timing) of a disturbance, mowing, were assessed with respect to their effects on the life histories of *Carduus* spp. Based on the results from Chapter 2, population demographic and spread modeling were used to explore the management implications for *C. nutans* in Chapter 3. Chapter 4 includes an independent case study where the tolerance of the two species to frequently occurring disturbance was examined – this chapter was inspired by results from Chapters 2 and 3 that frequent disturbances may not be beneficial for managing these species.

In Chapter 5 and Appendix B, I examine the responses of *Carduus* spp. to increased temperature and increased precipitation in a two-cohort field experiment, and project population growth and spread under climate change scenarios using spatial matrix modeling approaches. Using data from the same field experiment, in Chapter 6 I assess the relationship between phenology and growth of the two species under warming, focusing more on the comparison of the responses of the two species. Chapter 7 presents an auxiliary study on plant structural defense (i.e. thistle prickles) under warming. In Chapter 8, potential maternal climatic effects on early life histories of the following generation are evaluated.
In Chapter 9, I summarize conclusions from previous chapters and discuss possible future research directions.

**Study Species**

The species I studied are two congeneric invasive thistles, *Carduus nutans* L. and *Carduus acanthoides* L. (Asteraceae: nodding or musk thistle, and plumeless thistle, respectively). They rank second and fifteenth respectively in noxious weed lists in the USA (Skinner et al. 2000). Both species are native to Eurasia and widely invade pastures, croplands, roadsides, open fields and disturbed areas in North America (Allen and Shea 2006). Infestations of these two species have caused large productivity reductions in pastures and rangelands (Desrochers et al. 1988).

The native distribution of *C. nutans* includes Europe, western Siberia, Asia Minor, and North Africa (Moore and Frankton 1974). *C. nutans* was first recorded in North America around 1853 near Harrisburg, Pennsylvania (Allen and Shea 2006). The species has infested large areas in temperate rangelands in North America and is distributed across 46 continental American states and 9 Canadian provinces (NatureServe 2008). The species has also been introduced to Argentina (Mulligan and Frankton 1954), Australia and New Zealand (Doing et al. 1969). The native range of *C. acanthoides* covers Europe and Asia (Moore and Frankton 1974). The species was first discovered in the United States in Camden, New Jersey in 1878 (Frick, 1978). It has now been recorded in 31 continental American states and 5 Canadian provinces. *C. acanthoides* can be very abundant in crops and heavily grazed pastures (Feldman and Lewis 1990). A survey by Allen and Shea (2006) suggests that while the two species appear to coexist at the national scale, their distributions are segregated at finer scales.

Both species are short-lived monocarpic herbs and can be annuals, winter-annuals, biennials or short-lived perennials (Desrochers et al. 1988). Both species have spiny leaves, and
therefore deter livestock and other herbivores from grazing on the thistles themselves, and on other plants growing in their vicinity (Desrochers et al. 1988). Flowering requires vernalization and is age- and size-dependent (Shea et al. 2006). Seeds can survive many years in the seed bank and germinate under favorable environments to form flat rosettes, which bolt and flower one or more years after germination, producing a large number of wind-dispersed seeds (Desrochers et al. 1988).

In spite of their similarities in appearance and life history, the two thistle species differ in their morphology and phenology (Table 1-1, Figure A3-1, Figure A3-2). The height of *C. nutans* can vary between 20 and 200 cm while the height of *C. acanthoides* can vary from 20 to 150 cm (Desrochers et al. 1988). *C. nutans* produces terminal and solitary flower heads which are fewer and bigger (1.5 to 4.5 cm), compared to smaller (1.2-1.6 cm) and clustered flower heads of *C. acanthoides*. In *C. nutans*, earlier terminal capitula are developed at a taller height than later terminal capitula, whereas in *C. acanthoides* the reverse is true (Zhang, pers. obs.). Flowering of *C. nutans* occurs in late May or early June, while flowering of *C. acanthoides* starts a little later and is more continuous, lasting from June until November (Zhang, pers. obs.).

A wide range of management approaches has been developed for controlling the two invasive thistles, with variations in the effectiveness of each approach. Studies show that herbicides can be very effective in terms of controlling these two species (Desrochers et al. 1988, Beck et al. 1990). Mowing is also effective at controlling *C. nutans* when applied to flowering plants (McCarty and Hatting 1975). Cultivation is shown to be effective to control the two invasive species on pastures (Doing et al. 1969). Holst et al. (2004) found that goats can specifically graze on flowering *C. nutans* and therefore goat grazing can complement herbicides in integrated pest management. Three insects have been widely used as biocontrol agents for *C. nutans*, including: the receptacle feeding weevil *Rhinocyllus conicus*, the receptable feeding
gallfly *Urophora solstitialis*, and the root-crown weevil *Trichosirocalus horridus* (Shea et al. 2006).

I study the responses of two invasive thistles to disturbance and climate change, as I feel that they are good representatives of a generalized invader with fast growth, large reproductive outputs, and strong dispersal ability. Furthermore, the similarities and differences between the two species provide a useful comparative system, which can assist our understanding of divergent responses to environmental changes. Therefore, I expect the results of my research will have general implications for the fate of invasive species in a changing world.
References


University of Pennsylvania Press, pp194.


Table 1-1 Life history comparisons of *C. nutans* and *C. acanthoides*.

<table>
<thead>
<tr>
<th></th>
<th><em>C. nutans</em></th>
<th><em>C. acanthoides</em></th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowering phenology</td>
<td>May to August</td>
<td>June to October</td>
<td>(Rhoads and Block 2000)</td>
</tr>
<tr>
<td>Growth pattern</td>
<td>20-200cm tall fewer branches, branched at taller height, earliest capitula at the top</td>
<td>20-150cm tall more branches, branched at shorter height, later capitula at the top</td>
<td>(Desrochers et al. 1988; R. Zhang unpublished data, <em>pers. obs.</em></td>
</tr>
<tr>
<td>Capitulum</td>
<td>Solitary 20-60mm in diameter ~500 seeds/capitulum ~50 capitula per plant</td>
<td>Clustered 9-15mm in diameter ~100 seeds/capitulum ~150 capitula per plant</td>
<td>(Desrochers et al. 1988; Popay et al. 1990; Warwick et al. 1989; McCarty 1982; Feldman and Lewis 1990)</td>
</tr>
<tr>
<td>Seed traits</td>
<td>2-4mm, 4mg, less sensitive to moisture stress, able to emerge from great soil depth</td>
<td>1-3mm, 2mg, more sensitive to moisture stress, unable to emerge from great soil depth</td>
<td>(McCarty et al. 1969)</td>
</tr>
<tr>
<td>Life history strategies</td>
<td>Appear to be a more aggressive competitor</td>
<td>Appear to be more tolerant to disturbance</td>
<td>(Warwick et al. 1990; Zhang et al. 2011)</td>
</tr>
</tbody>
</table>
Chapter 2

Integrating Multiple Disturbance Aspects: Responses of an Invasive Thistle

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Abstract
Disturbances occur in most ecological systems, and play an important role in biological invasions. We delimit five key disturbance aspects: intensity, frequency, timing, duration and extent. Few studies address more than one of these aspects, yet interactions and interdependence between aspects may lead to complex outcomes. In a two-year case study, we examined how multiple aspects (intensity, frequency and timing) of a mowing disturbance regime affect the survival, phenology, growth and reproduction of an invasive thistle Carduus nutans L. (musk thistle). Our results show that high intensity and late timing strongly delay flowering phenology and reduce plant survival, capitulum production and plant height. A significant interaction between intensity and timing further magnifies the main effects. Unexpectedly, high frequency alone did not effectively reduce reproduction. However, a study examining only frequency and intensity, and not timing, would have erroneously attributed the importance of timing to frequency. Our results demonstrate the importance of a multi-aspect disturbance framework when seeking to understand and control biological invasions. Failure to consider possible interactions, and the inherent interdependence of certain aspects, could result in misinterpretation and inappropriate management efforts.

Key words: Carduus nutans; disturbance timing; frequency; intensity; invasive species; mowing
Introduction

Disturbances are ubiquitous in both natural and managed ecosystems. While there are many related definitions of disturbance (Sousa 1984, Grime 2001), most focus on any relatively discrete event that disrupts ecosystem, community, or population structure and changes resources, substrate availability, or the physical environment (White & Pickett 1985). Despite various origins and forms (White 1979, Sousa 1984), disturbances can be characterized using the following five aspects: intensity relates to the vigor of the perturbing force; frequency addresses how often a disturbance event happens; timing refers to when a disturbance happens relative to the life cycle of the disturbed organism; duration describes how long a single disturbance event lasts; and extent represents the spatial scale of the effects of a disturbance (Roxburgh et al. 2004, Shea et al. 2004, Miller et al. 2011). These aspects apply to both natural disturbances such as hurricanes and fires, and to management actions such as herbicide application and mowing, as all of these can be considered as disturbances (Buckley et al. 2007, Lockwood et al. 2007).

However, although there is a rich literature on disturbance, many studies are limited to just one aspect, for example only the frequency of the disturbance (Shea et al. 2004). Despite recent case studies where multiple aspects are considered (McCabe and Gotelli 2000, Laterra et al. 2006, Rinella and Hileman 2009), relatively few studies explicitly address the interactions between these aspects, and their possibly confounding natures. Therefore a multiple-aspect framework which considers these issues is needed when examining responses to natural disturbances or designing experiments to evaluate management outcomes. This framework should illuminate the relative importance of each main aspect and allow us to examine the potentially complex interactions between aspects (Collins et al. 1995, Shea et al. 2004). Furthermore, it should help tease out potential interdependence between aspects, which often happens in ecological systems but is easily overlooked. For example, infrequent floods are often associated with large extent and long duration; a key timing is more likely to be included in disturbances with higher frequency or
longer duration. In such cases, failure to consider multiple aspects may result in
oversimplification or misinterpretation of results.

Here we apply a multiple-aspect disturbance framework to a two-cohort mowing study. Specifically, we examine how intensity, frequency and timing of mowing interact to bring about differences in growth, survivorship, reproduction and phenology of an invasive plant species. Our results demonstrate the strengths of our multiple-aspect disturbance framework in interpreting results and guiding management practice.

Methods

Study Species

*Carduus nutans* L. (musk thistle: Asteraceae) is native to Eurasia, and has invaded North America, South America, Southern Africa, New Zealand and Australia (Allen & Shea 2006). Infestations of *C. nutans* cause serious problems in pastures, roadsides and natural areas both ecologically and economically (Desrochers *et al.* 1988). *C. nutans* is a short-lived monocarpic herb, and can complete its life cycle as a summer annual, winter-annual, biennial or short-lived perennial (Desrochers *et al.* 1988). Seeds germinate under favorable environments to form flat rosettes, which bolt and flower one or more years after germination, depending on vernalization and size (Shea *et al.* 2006). *C. nutans* flowers from May to August and die after producing wind-dispersed seeds (Desrochers *et al.* 1988).

Study site

The experiment was repeated for two independent cohorts (2006-2007 and 2007-2008) on two adjacent areas in an old pasture at The Russell E. Larson Agricultural Research Farm at Rock Springs, Pennsylvania (latitude 40.711°, longitude -77.942°). The pasture was dominated by *Arrhenatherum elatius,* *Dactylis glomerata,* *Elytrigia repens* and *Phleum pratense.* Other
common plant species included *Taraxacum officinale*, *Linaria vulgaris*, *Plantago* spp., *Trifolium* spp. and *Galium* spp.

The experimental site was prepared prior to planting each cohort, to mimic the high invasion success of these thistles in areas with disturbed soils. A Miller Offset Disk was applied twice to the area to kill aboveground vegetation. Then a Roller Harrow was applied to level the surface when the soil had dried.

**Experimental design**

Three-week old seedlings were transplanted to the field in 12 rows at spacing of 1.5 m in October 2006 and October 2007, respectively. To ensure that most of the thistles would bolt in the summer (and therefore would behave as winter annuals), we applied approximately 13g of slow-release fertilizer (Osmocote Flower/Vegetable Food, N-P-K: 14-14-14) to each individual in the following April.

In the May following transplanting, rosettes were measured, blocked by size, and randomly assigned to one of fifteen treatments (Table 2-1). We manipulated intensity, frequency and timing of the treatments, because these are the most meaningful aspects for mowing disturbance events. Extent and duration were excluded as the former does not apply to a single plant, and a mowing event is an instantaneous or pulse disturbance (Bender *et al.* 1984) so manipulation of duration is not possible. Each thistle was hand clipped at different intensities (cut to 5 cm or 20 cm aboveground), frequencies (never cut, cut once, twice or three times); and timings relative to when plants reached a height threshold of 40 cm (0, 2, or 4 weeks after reaching the threshold). Each treatment had 12 replicates, except for the control (never cut) which had 24 replicates to ensure the accuracy of the baseline for comparisons.

Treatments were initiated as soon as individual plants reached the height of 40 cm (when they had definitively commenced bolting). Surrounding vegetation in a 50×50 cm area was clipped to
the same height as the thistles. Capitula were bagged using pollen bags before seed dispersal to prevent new infestations.

Stem number, plant height, and key developmental dates, such as flowering date, were recorded in weekly field censuses from May until the end of the experiment. The experiments were terminated by removing all the aboveground biomass in early November, after the first frost in the second year of each cohort, when all plants had finished their life cycles. Capitula removed at each mowing event, and capitula from the final destructive census, were separated based on their developmental stages (especially whether they had set seed), and were then counted in the lab.

Data Analysis

We conducted our data analyses in R (R development core team 2008). Because *C. nutans* is monocarpic (plants died after reproducing), we define survival as the probability of surviving until reproduction. Plant reproduction, measured by capitulum production, is a direct measurement of plant fitness in this study. The capitulum production considered in this study included reproduction both before and after the treatments, as plants receiving late treatments produced mature capitula before the initiation of the treatments. We also analyzed flowering date, number of flowering stems and plant height at flowering, as they are either indirectly related to fitness (e.g. through interactions with pollinators or seed dispersal), or can help to explain different fitness results. Flowering date was analyzed using a non-parametric survival model (coxph function in the survival package) with cohort as a stratification variable (strata function). Plant survival, reproduction, height at flowering, and number of flowering stems were analyzed using Generalized Linear Mixed Models (GLMMs, Crawley 2007) in R (lmer function in the lme4 package). Log linear transformation was used for stem number and reproduction prior to fitting the models. Binomial error distribution was specified for survival. Initial rosette size was used as a covariate in all models because phenology and reproduction is strongly size-dependent
(Shea et al. 2006). We also considered between-year variation and spatial location as random effects in the models.

In our study, frequency and timing are inherently dependent on each other (i.e. high frequency treatments are more likely to include a late cut). To address this, we analyzed the effects of intensity and timing, and the effects of intensity and frequency, separately. We then compared Akaike Information Criteria (AIC) for the minimum adequate models for intensity and timing (specifically, with or without a late cut) with the comparable models for intensity and frequency, when we analyzed survival, plant height, and reproduction. We also compared the $R^2$ values in the models for flowering phenology. Based on the results from the AIC comparisons and $R^2$ comparisons (specifically, that late timing was more important than frequency, see results below), we examined the effects of intensity and frequency only for the treatments that included a late cut, in order to control for the strong effect of late timing. We then compared these results with the results when late timing is ignored.

**Results**

*Intensity and timing*

High intensity and late timing together significantly delayed flowering ($P<0.001$), leading to delays of up to 7 weeks. Intensity and timing both had significant effects on survival, plant height, and reproduction - treatments with high intensity or late timing caused larger reductions in the responses (*Figure 2-1*). The interaction between intensity and timing had significant effects on reproduction, indicating that the effects of late timing were further magnified by high intensity.

*Intensity and frequency*

Treatments with high intensity caused significantly larger delays in flowering, and larger reductions in survival, plant height, and reproduction than treatments with low intensity.
(P<0.001, Figure 2-2). However, the effects of frequency depended on whether late timing was considered, especially for treatments with high intensity (Figure 2.2). For example, increased frequency did not further delay flowering when not controlling for late timing, whereas it significantly delayed flowering when late timing was considered. Similarly, frequency appeared to have no significant effect on survival at high intensity level when we did not control for timing (Figure 2-2D). However, when timing was considered, high frequency treatments actually led to higher survival at high intensity level (Figure 2-2A, Figure 2-2B). Furthermore, although frequency appeared to have a significant negative effect on reproduction in treatments with high intensity (Figure 2-2E), this effect faded away when we accounted for the interdependent timing effect (Figure 2-2C).

Flowering stem number was positively correlated with reproduction (Figure 2-3A). While high intensity reduced number of induced flowering stems, high frequency significantly increased the number of flowering stems (Figure 2-3B).

**Timing or frequency**

In all cases, AIC values were lower (lower AICs indicate better fits) for models that included timing instead of frequency (Table 2-2). The R² values for non-parametric models for flowering date were an order of magnitude higher (higher R² values indicate better fit) in models with intensity and timing instead of intensity and frequency (0.438 vs. 0.047). This suggested that models which included timing instead of frequency fit better for all these responses, and the frequency results in the full data set were mostly driven by the effect of late timing.

**Discussion**

Using a multiple aspect framework for disturbance, we manipulated all three relevant aspects in our system - intensity, frequency, and timing, and examined their effects on the fitness of the invasive plant, *C. nutans*. Our results show that treatments with higher intensity or late timing
caused larger reductions in plant fitness, which broadly agrees with previous studies which addressed the importance of high intensity and relatively late timing in reducing the regrowth of monocarpic species (Lennartsson et al. 1998, Martinkova et al. 2004, Klimesova et al. 2007). This is likely because these treatments removed more biomass and impose harsher constraints (e.g. lack of inactive meristems, Huhta et al. 2000) on the post-injury plant recovery.

Furthermore, compared to previous investigations that only manipulated a single aspect of disturbance, our experimental design has two distinctive advantages. First, it enables us to examine the interactions between different aspects of a disturbance. Our results show that multiple aspects of a disturbance can interact with each other to produce complex outcomes. For example, higher intensity and late timing significantly interacted with each other to magnify decreases in capitulum production. On the other hand, the positive effects of high frequency on survival and plant height were only significant at the low intensity level. This indicates that the negative effect of high intensity on plant height was so strong that it masked the potential positive effect of high frequency (Figure 2-2B). Thus, considering these interactions provides a more thorough understanding of the disturbances.

Second, our multi-aspect experimental design allows us to clarify the tangled interdependence between disturbance aspects. In particular, our finding that timing has more explanatory power than frequency was only possible due to our factorial, multiple aspect design. Our results thus imply that high frequency may in fact take effect through late timing, because high frequency treatments are more likely than low frequency treatments to include key timings relative to the life history of the plants. For C. nutans, a late timing is crucial because late mowing likely depletes meristem and resource pools that are both critical for plant recovery. In our study, we controlled for the strong effect of timing when analyzing effect of frequency, and thereby addressed the distinction between the two (Figure 2-2A-C). However, if we had only considered frequency without addressing timing (Figure 2-2D-F), we would have attributed the
impact of late timing to high frequency mowing. Such limitations, as seen in many single-aspect studies, may not only conceal significantly positive effects (e.g. the positive effect of high frequency on survival at higher intensity and on plant height at lower intensity, Figure 2-2D, E), but also lead to false negative results (e.g. the false negative effect of high frequency on plant height and reproduction at higher intensity, Figure 2-2E, Figure 2-2F).

The confounding of disturbance aspects is likely to lead to potentially incomplete conclusions or wasteful management decisions unless both interdependent aspects are evaluated. For example, our work suggests that high frequency mowing treatments (requiring several individual mowing events throughout the growing season) can be replaced by fewer, well-timed mowing events. In our case, only after carefully controlling for the overwhelming timing effect, we were able to notice the ineffectiveness of high frequency in reducing plant fitness (Figure 2-2A-C) and further explore the underlying mechanism. This result is further verified in a study where this species managed to survive ten consecutive weekly cuts at 5cm (Zhang et al. 2011). The ineffectiveness of high frequency treatments is counterintuitive, and in contrast to previous studies which emphasized high frequency in weed management (Peters & Lowance 1978, Sullivan 2004, Nielsen et al. 2006). This is likely due to higher numbers of flower stems (Figure 2-3A) induced by frequent removal of apical meristems in these treatments, which was highly correlated with capitulum production (Figure 2-3B). In these high frequency treatments, the first cut happened before plants invested substantially in growth and reproduction, and therefore failed to remove much plant biomass or deplete meristem pools. Furthermore, the time intervals between two sequential cuts in the high frequency treatments were too short for plants to grow back significantly; thus subsequent cuts also failed to cause major damage to the plants.

Although aspects of a disturbance may be independent of each other (e.g. spring grazing every two years versus summer grazing every three years), they are often intertwined. Potential interdependence exists in disturbance aspects in that three (frequency, timing and duration) out of
the five aspects are inherently intertwined because all of them are temporal in nature. For example, disturbances of high frequency or long duration are prone to include key timings as well. This is especially true when disturbance return intervals are shorter than plant life cycles or seasonal cycles (e.g. multiple disturbance events happen at different times within a single growing season: Hogg et al. 1995, Nielsen et al. 2006, Schooler et al. 2008, Gao et al. 2009). In such cases, it is impossible to increase frequency without introducing effects on timing or duration. Moreover, disturbance aspects such as intensity and frequency may be biologically interdependent. For example, infrequent wild fires are often more intense because of fuel load accumulation (Keith et al. 2010). Therefore considering these interdependent aspects will help understand natural disturbances where correlations are difficult to avoid, and improve design of manipulative experiments for management purposes.

In conclusion, our study demonstrates the utility of our theoretical framework for multiple aspects of a disturbance. We show that interactions between these aspects can produce synergistic outcomes. Furthermore, some aspects are interdependent; this may lead to misinterpretation if they are not distinguished. Considering multiple aspects when examining disturbance can provide thorough understanding of undocumented interactions and interdependence, and hence a sound basis for management decisions.

**Acknowledgements**

RZ and KS conceived and designed the experiment. RZ conducted the experiment and analyzed the data. RZ wrote the first draft, and both authors edited this chapter. Many Shea lab undergraduates helped in the field and the lab. We are grateful for discussion and comments from Ottar Bjørnstad, Eric Post, David Mortensen, Stephen Roxburgh, Adam Miller, Suann Yang and Eelke Jongejans. We acknowledge the support of USDA-CSREES (Biology of Weedy and Invasive Plants) NRI grant #2002-35320-12289 and NSF grant #DEB-0815373.
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45*, 229-299.


Table 2-1. Treatment combinations of intensity, frequency and timing used in this study.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intensity*</th>
<th>Frequency</th>
<th>Timing†</th>
<th>Replicates</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>He</td>
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<td>1</td>
<td>e</td>
<td>12</td>
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<td>Le</td>
<td>low</td>
<td>1</td>
<td>e</td>
<td>12</td>
</tr>
<tr>
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<td>high</td>
<td>1</td>
<td>m</td>
<td>12</td>
</tr>
<tr>
<td>Lm</td>
<td>low</td>
<td>1</td>
<td>m</td>
<td>12</td>
</tr>
<tr>
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<td>high</td>
<td>1</td>
<td>l</td>
<td>12</td>
</tr>
<tr>
<td>Ll</td>
<td>low</td>
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<td>l</td>
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<td>2</td>
<td>em</td>
<td>12</td>
</tr>
<tr>
<td>Hel</td>
<td>high</td>
<td>2</td>
<td>el</td>
<td>12</td>
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<tr>
<td>Leml</td>
<td>low</td>
<td>3</td>
<td>eml</td>
<td>12</td>
</tr>
</tbody>
</table>

* For intensity, “high” represents cutting at 5 cm aboveground and “low” represents cutting at 20 cm aboveground. † For timing, “e” stands for an early cut (immediately after plants reached 40 cm); “m” stands for a middle cut (2 weeks after plants reached 40 cm); “l” stands for a late cut (4 weeks after plants reached 40 cm).
Table 2-2. Akaike Information Criteria (AIC) values for the minimum adequate linear mixed models fitted with intensity and timing (i.e. with or without a late cut) and comparable models fitted with intensity and frequency.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Timing and intensity</th>
<th>Frequency and intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Responses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>†Survival</td>
<td>267</td>
<td>282</td>
</tr>
<tr>
<td>Plant height</td>
<td>2120</td>
<td>2191</td>
</tr>
<tr>
<td>‡Capitulum production</td>
<td>762</td>
<td>777</td>
</tr>
</tbody>
</table>

* Initial rosette size was used as a covariate in all models. Cohort and spatial location of individual plants were considered as random effects.
†Binomial error distribution was specified for the survival data.
‡Results were based on log transformed data. The minimum adequate models include the interaction between timing and intensity or between frequency and intensity.
Figure 2-1. Effects of intensity and timing on the survival (A), plant height at flowering (B), and reproduction (C) of *C. nutans*. Treatments on the x-axes include control, treatment with high intensity but without late timing (HI), treatment with high intensity and late timing (HI+Late), treatment with low intensity but without late timing (LI), treatment with low intensity and late timing (LI+late). The letters denote the statistical difference between treatments.
Figure 2-2. Effects of intensity and frequency on the survival (A, D), flowering plant height (B, E), and reproduction (C, F) of *C. nutans* depends on whether the statistical model accounts for timing (A, B, C) or not (D, E, F). A, B, and C present results based on only treatments that included a late cut. D, E, and F present results of all treatments in the study (the full dataset). The effect of frequency at each intensity level is indicated to the right of the curves. “ns” denotes no significant effect. “+” denotes a significant positive effect. “−” denotes a significant negative effect. $\alpha=0.05$. 
Figure 2-3. The relationships between number of flowering stems and capitulum production (A), and between number of flowering stem and frequency (B). Number of flower stems is a significant predictor for capitulum production ($P<0.001$). Number of flowering stems significantly increases with frequency at each intensity level ($P<0.001$), indicating that high frequency treatments compensate for reproduction by increasing stem numbers.
Chapter 3

Objective-dependent management of an invasive thistle

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Abstract

The choice of management alternatives to control invasive species requires the statement of explicit management objectives, in terms of both the relevant time horizon and spatial scale. Here we show that the optimal management strategy for controlling an invasive thistle depends fundamentally on the management goals. Using data from a two-year mowing experiment, we assessed fourteen mowing treatments (differing in intensity, frequency, and timing) based on their effectiveness in i) reducing population density of the existing cohort, ii) decreasing long-term population growth, and iii) limiting population spread of an invasive thistle, Carduus nutans. The treatment with high intensity and a single late mow caused the largest reduction in plant survival and thereby in the density of existing adult plants; the treatment with high intensity and an early mow in addition to a late mow was most effective in reducing population growth rate and population spread. Furthermore, the most frequent mowing treatment did not provide the optimal management outcome for any stated objective. This study highlights the necessity of having clearly defined management aims; the term “control” is too vague to be truly useful. The results also provide important insights for the management of this invasive species.
Introduction

The choice of management strategy depends on management goals (Shea et al. 2010). Possible management goals include: to eliminate an existing weed population, to lower weed density to tolerable levels (i.e. below some economic damage threshold), to prevent or reduce weed reproduction, to reduce weed emergence after crop planting, to minimize crop damage caused by weeds, to shift weed community composition to easier-to-manage species, or to reduce population growth or population spread of the targeted weed (Liebman 2001, Buhler 2002, Wilkerson et al. 2002, Shea and Kelly 1998, Shea et al. 2010). Some of these objectives focus on short-term control outcomes, for example, immediate reductions in the density of existing populations, while others are aimed at long-term effects and are usually based on population projections such as growth rate. Although management should ideally focus on long-term outcomes rather than a single season or year (Jones and Medd 2000), management decisions are often constrained by the fact that managers typically face economic challenges and act on short time horizons (Hyder et al. 2008).

Because specific objectives are evaluated based on different life history traits or their resulting population outcomes, the best strategy to achieve an objective may vary. This is also true for objectives defined on different time horizons. For example, herbicide application may be an effective approach to achieve short-term management goals but may ultimately lead to induced resistance (Shaner 2000) and non-target effects (Crone et al. 2009). In contrast, biocontrol agents (available for only a small proportion of invasive species), are more effective in the long run despite time lags in their effectiveness (Hyder et al. 2008). Therefore, having a clearly stated time horizon is a prerequisite for making management decisions.

Besides different time horizons, management objectives, and hence their corresponding optimal strategies, can also vary depending on population spatial structure (Thomas et al. 1999). While many previous studies have focused on reducing local population abundance, only recently
has much attention been given to population spread rates in invasion control modeling (Bogich and Shea 2008, Dauer et al. 2009, Shea et al. 2010), in part because of recently developed modeling methodologies for coupling demographic and dispersal processes (Neubert and Caswell 2000, Jongejans et al. 2011). Consequently, optimal management strategies for controlling local population density and for controlling population spread on a regional scale may not be the same. For example, in New Zealand, the biocontrol agent *Rhinocyllus conicus* (which attacks the thistle receptacle) had a larger effect on population growth of *Carduus nutans* than another agent *Trichosiocalus horridus* (which attacks the plants’ roots), whereas the reverse was true for spatial spread of the population (Shea et al. 2010).

In this study, we included three management objectives – one short-term objective and two long-term objectives. Of the two long-term objectives, one focuses on local population dynamics while the other focuses on population spread. The target species is an invasive thistle, *Carduus nutans* L. (nodding thistle, Asteraceae). *C. nutans* is a monocarpic perennial weed that is native to Eurasia, but has become invasive in many other regions in the world (Allen and Shea 2006). Infestations by this species cause significant loss to pastures and rangelands, as the invader prevents livestock from grazing nearby and reduces productivity (Desrochers *et al.* 1988). *C. nutans* flowers from May to August, followed by a large production of wind-dispersed seeds (Rhoads and Block 2000). Mowing is a commonly applied management tool to control invasive weeds (DiTomaso 2000) and has been widely used to manage infestations caused by *C. nutans* (McCarty and Hatting 1975, Popay 1990, Tipping 2008). However, comparisons of management outcomes are often limited to only a few mowing regimes, and the evaluations of these regimes are mostly based on reductions in plant density or seed production. Therefore an integrated evaluation is useful to assess a spectrum of treatments (differing in management timing, intensity, and frequency) in the context of these different possible management goals.
We investigated the effects of fourteen different mowing regimes (Table 3-1) to control *C. nutans*. These regimes differed in intensity (i.e. mow at 5cm versus 20cm), frequency (i.e. mow once, twice, or three times within a growing season), and timing (i.e. mow before flowering, during early flowering, or at flowering peak). The effect of these regimes on the survival, growth and reproduction differed (see Chapter 2). We compared the mowing regimes based on their effectiveness in terms of realizing each of the following management goals: i. to reduce population density of the current cohort (i.e. to reduce adult plant survival); ii. to reduce population growth rate, \( \lambda \); iii. to reduce population spread rate, \( c^* \) (which integrates both population growth and propagule dispersal, Neubert and Caswell 2000). Given the differing effects of these management strategies on different vital rates, we predict that optimal management strategies for the three management goals should be different from each other.

**Methods**

Fourteen mowing treatments and a control treatment (no mow) were included in this study. Mowing treatments refer to the combination of one, two, or three separate mowing events (Table 3-1). Survival of adult plants was used to evaluate management effectiveness in terms of reducing density of the existing population (objective i). Evaluation of reductions in population growth rate (objective ii) was based on a population matrix model with modified demographic vital rates. Reductions in population spread (objective iii) were assessed using a spatial matrix model, which incorporates both modified demographic vital rates and modified dispersal-related traits.

Plant survival was defined as the probability of surviving to produce viable seeds in the same season. Plant height was defined as height of flowering plants at the end of the growing season. Plant lifetime capitulum production comprises two parts of reproduction: 1. capitula produced before the initiation of the mowing treatments (i.e. pre-mow reproduction); 2. capitula
produced after the completion of the mowing treatments completed (i.e. post-mow reproduction). No mature capitula developed between any two mowing events in the any of the mowing treatments.

Percentage changes in plant survival and post-mow reproduction of surviving plants (Table 3-1) were incorporated into a 4 x 4 size-structured baseline demographic matrix model. This model has four stages: seed bank, small rosettes, medium rosettes, and large rosettes. Baseline model parameters were derived from a previous field experiment conducted at the same field site (the Pennsylvania experimental population in Jongejans et al. 2008). We then modified reproduction based on significant percentage changes caused by the mowing treatments in the present study. In these models, we assumed that percentage decreases in reproduction caused by mowing did not depend on the size of the plants, and seed production per capitulum, as well as other vital rates, remained unchanged. Population growth rates $\lambda$ were calculated as the dominant eigenvalues of the demographic matrices.

The demographic matrices were then coupled with the Wald analytical long-distance dispersal (WALD) model (Katul et al. 2005) to project population spread rates, $c^*$, using integrodifference equations (Neubert and Caswell 2000, Skarpaas and Shea 2007, Jongejans et al. 2008, Shea et al. 2010). See chapter 5 for methodological details. Dispersal parameters were assumed to be the same as for the Pennsylvania experimental population in Jongejans et al. (2008), except for plant height, where plant heights significantly different from the control of the present experiment were used (Table 3-1). Surrounding vegetation height was assumed to be 5cm for all mowing treatments to mimic heavily grazed pastures, where this species is most successful. Mowing started the latest in treatment Hl and Ll, where pre-mow reproduction was significantly different from zero (1.9±0.3 and 2.3±0.4, respectively). Therefore these capitula could have dispersed at a taller height (than plant height at the end of the growing season) before being mowed. However, we did not consider differences in dispersing height between pre-mow
and post-mow capitula in the model, and thereby may underestimate c* for these two treatments in particular.

Results

Mowing generally decreased plant survival and reproduction (see also Chapter 2). Mowing treatments with higher intensity and mowing treatments including a late cut reduced survival and reproduction more than those with lower intensity and those without a late cut (Table 3-1). As a result, reductions in population growth rates $\lambda$ were larger in treatments with higher intensity and treatments including a late cut (Table 3-1, Figure 3-1b). Treatment Hl had the largest impact on survival of existing plants (a reduction of 71%, Table 3-1, Figure 3-1a). Treatment Hel caused the largest reduction in lifetime capitulum production (a reduction of 92%, Table 3-1) and hence on population growth rate (a reduction of 91%, Table 3-1, Figure 3-1b).

Reductions in plant height followed the same pattern as in survival and reproduction – treatments with higher intensity and treatments including a late cut caused a larger reduction than those with lower intensity and those without a late cut (Table 3-1). Treatment Hel caused the largest reduction in plant height (a reduction of 76%, Table 3-1), and therefore this treatment had the greatest impact on seed dispersal. Coupled with the largest reduction in population growth, treatment Hel therefore caused the largest reduction in population spread rate (a reduction of 91%, Table 3-1, Figure 3-1c).

Discussion

Generally, mowing treatments with higher intensity and late timing had a larger impact on survival, reproduction, plant height, population growth rate and population spread. However, the choice of the best mowing regime depends on the management objective under consideration.
Treatment Hl is optimal for reducing population density of existing adult plants (i.e. lowest survival) and therefore for attaining immediate weed control. A high intensity, late cut removed most biomass and caused most damage because it started late in the season when plants had already invested significantly in biomass.

In our study, we used reductions in population growth rate $\lambda$ and population spread rate $c^*$ to evaluate the long-term effectiveness of the treatments. The baseline matrix model parameters are from an experiment where thistles were grown with abundant resources and little competition (Jongejans et al. 2008). Therefore the high $\lambda$ values in the results represent the worst case scenario for an invasion, specifically fast population growth during the initial part of the invasion period in disturbed habitats. As the invasion continues, this invasive species will likely face strong interspecific competition, especially during seedling establishment, that can greatly affect its performance and hence reduce population growth (Peterson-Smith and Shea 2010; Ruggiero and Shea 2011). Nevertheless, projections of $\lambda$ and the corresponding $c^*$ (which integrates population growth and dispersal) assuming the status quo provide powerful tools for management decisions.

The optimal treatment for reducing population growth in the long run (i.e. lowest $\lambda$) and for reducing population spread in the long run (i.e. lowest $c^*$) is treatment Hel. This is because treatment Hel (which includes both an early and a late mow) more successfully reduced lifetime reproduction than treatment Hl, which was penalized by pre-mow capitula that could be released before treatment even started. Compared to a single late mow, an extra early mow as well as the late mow helps to remove early dispersing capitula. Therefore if we consider another short-term management goal, to reduce seed production in the current season, Hel is again the best strategy out of all fourteen treatments. Furthermore, the extra mow may have hastened depletion of the resource bank of the species (both nutrients and meristem pool, Huhta 2000) for later regrowth. Due to the large elasticity of the reproductive vital rates for the study system (Jongejans et al.
treatment Hel causes a greater reduction in population growth than treatment HI. In contrast to a previous study of biocontrol agents, where management strategy rankings differ for reducing local population abundance and reducing population spread (Shea et al. 2010), the optimal strategy for both longer-term objectives is the same mowing regime. This is because in the present study, the treatment reducing reproduction the most (Hel) also affected a dispersal parameter, plant height, immensely. Due to the significant contribution of plant height to spread (Zhang et al. 2011), treatment Hel thus causes the largest reduction in population spread rate. Furthermore, as the release height of pre-mow capitula in these treatments was assumed to be the same as plant height at the end of the season, the reductions for treatment HI may be overestimated. Therefore the advantage of treatment Hel over treatment HI in controlling spread could be even larger if such variations in dispersal height were taken into account.

Counter-intuitively, the treatment with the highest frequency and intensity (treatment Heml) was not the optimal management option for any of the three management goals. The treatment comprising an early and a late mow is more effective than mowing three times in terms of reaching all three management goals. This is probably because frequent removal of apical meristems in this treatment induced more flowering stems than the other treatments (see Chapter 2).

While we only considered the performance of the targeted weed species, *C. nutans*, management decisions also rely on a suite of other factors, such as forage yield, forage quality, and animal consumption patterns (Fulkerson and Donaghy 2001). For example, mowing only once late in the season results in large thistles at the time of the treatment, which may compromise the quality of forage. On the other hand, including an early mow as well as the late mow reduces the size of the thistles, and therefore may improve forage quality. Therefore future studies should include further examination of the effects of weed management practices on the rest of the forage producing plant community. Successful management of *C. nutans* also requires
appropriate handling of the cut-off plant parts. Because capitula have the potential to continue developing on cut-off stems (Zhang, pers. obs.), managers should carefully destroy the harvested biomass to prevent seed maturation, dispersal, and further infestations. As a consequence, managers intending to control this species should also consider practical implementation aspects in the decision process.

The choice of an optimal management strategy requires a clear statement of objective. While “weed control” is often used to describe the management action aimed to eliminate an existing weed population (Buhler 2002), a more precise statement is required, in terms of time horizon as well as spatial extent. For example, does the manager aim at a quick, short-term reduction in plant abundance, or long-term control whose effects may not be obvious during the first few years of management? Does the manager only care about the problem in a limited area where the weed is present, or is s/he also concerned about its spread into adjacent fields? Such questions are important for the appropriate choice of management option. Our study demonstrates that intense mowing both early (before flowering) and late (at flowering peak) is the safest, most effective, and most efficient management strategy of the fourteen mowing regimes we examined, for the long term control of C. nutans in terms of both local abundance and spatial spread. However, short-term management outcomes from a single intense late mow are acceptable, especially when managers are constrained by limited budgets and labor.

Acknowledgements

RZ and KS conceived and designed the experiment. RZ conducted the experiment and analyzed the data. RZ wrote the first draft, and both authors edited this chapter. Many Shea lab undergraduates helped in the field and the lab. We are grateful for discussion and comments from Ottar Bjørnstad, Eric Post, David Mortensen, Stephen Roxburgh, Adam Miller, Suann Yang and
Eelke Jongejans. We acknowledge the support of USDA-CSREES (Biology of Weedy and Invasive Plants) NRI grant #2002-35320-12289 and NSF grant #DEB-0815373.

References


Table 3-1 Summary of effects of different mowing treatments on plant survival, lifetime capitulum production, and plant height

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intensity*</th>
<th>Frequency</th>
<th>Timing†</th>
<th>Survival</th>
<th>Lifetime capitulum production</th>
<th>Plant height (cm)‡</th>
<th>Population Growth rate λ</th>
<th>Population spread rate c* (m/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>18.3 (100%)</td>
<td>130.5</td>
<td>363</td>
<td>85</td>
</tr>
<tr>
<td>He high</td>
<td>1</td>
<td>e</td>
<td>0.9n</td>
<td>7.8</td>
<td>38%</td>
<td>69.8</td>
<td>139</td>
<td>33</td>
</tr>
<tr>
<td>Le low</td>
<td>1</td>
<td>e</td>
<td>1n</td>
<td>12.9</td>
<td>70%</td>
<td>77.4</td>
<td>256</td>
<td>41</td>
</tr>
<tr>
<td>Hm high</td>
<td>1</td>
<td>m</td>
<td>0.67</td>
<td>5.7</td>
<td>31%</td>
<td>61.0</td>
<td>114</td>
<td>26</td>
</tr>
<tr>
<td>Lm low</td>
<td>1</td>
<td>m</td>
<td>0.83</td>
<td>8.2</td>
<td>45%</td>
<td>71.4</td>
<td>163</td>
<td>34</td>
</tr>
<tr>
<td>HI high</td>
<td>1</td>
<td>l</td>
<td>0.29</td>
<td>3.5</td>
<td>19%</td>
<td>41.3</td>
<td>69</td>
<td>14</td>
</tr>
<tr>
<td>Ll low</td>
<td>1</td>
<td>l</td>
<td>0.78</td>
<td>6.3</td>
<td>35%</td>
<td>38.5</td>
<td>126</td>
<td>14</td>
</tr>
<tr>
<td>Hem high</td>
<td>2</td>
<td>em</td>
<td>0.78</td>
<td>5.8</td>
<td>32%</td>
<td>59.9</td>
<td>116</td>
<td>25</td>
</tr>
<tr>
<td>Lem low</td>
<td>2</td>
<td>em</td>
<td>1n</td>
<td>14.5</td>
<td>79%</td>
<td>81.9</td>
<td>287</td>
<td>44</td>
</tr>
<tr>
<td>Hel high</td>
<td>2</td>
<td>el</td>
<td>0.54</td>
<td>1.5</td>
<td>8%</td>
<td>31.9</td>
<td>31</td>
<td>8</td>
</tr>
<tr>
<td>Lel low</td>
<td>2</td>
<td>el</td>
<td>0.95n</td>
<td>8.3</td>
<td>45%</td>
<td>54.6</td>
<td>164</td>
<td>23</td>
</tr>
<tr>
<td>Hml high</td>
<td>2</td>
<td>ml</td>
<td>0.65</td>
<td>3.5</td>
<td>19%</td>
<td>44.2</td>
<td>70</td>
<td>16</td>
</tr>
<tr>
<td>Lml low</td>
<td>2</td>
<td>ml</td>
<td>0.96n</td>
<td>10.8</td>
<td>59%</td>
<td>60.8</td>
<td>215</td>
<td>29</td>
</tr>
<tr>
<td>Heml high</td>
<td>3</td>
<td>eml</td>
<td>0.59</td>
<td>2.6</td>
<td>14%</td>
<td>38.6</td>
<td>52</td>
<td>12</td>
</tr>
<tr>
<td>Leml low</td>
<td>3</td>
<td>eml</td>
<td>0.87n</td>
<td>11.0</td>
<td>60%</td>
<td>61.9</td>
<td>218</td>
<td>31</td>
</tr>
</tbody>
</table>

* For intensity, “high” represents cutting at 5 cm aboveground and “low” represents cutting at 20 cm aboveground.
† For timing, “e” stands for an early cut (immediately after individual plants reached 40 cm in height); “m” stands for a middle cut (2 weeks after plants reached 40 cm); “l” stands for a late cut (4 weeks after plants reached 40 cm).
‡ Plant height in all mowing treatments is different from the control.
Bold numbers denote the lowest values for the fourteen mowing regimes. The superscript n denotes results that are not significant from the control (P > 0.05). For survival, treatments He, Le, Lem, Lel, Lml, and Leml were not different from the control. Numbers in brackets denote percentage modifications of reproduction used in the models. For lifetime capitulum production, treatment Lem was not different from the control.
Figure 3-1. Evaluation of effectiveness of mowing treatments (percentage reductions compared to the control) based on three management goals of different time horizons: a. to reduce density of adult plants in the present season (i.e. adult survival); b. to reduce the long-term population growth rate $\lambda$; c. to reduce the long-term population spread rate $c^*$. The black bars denote the optimal mowing treatments which resulted in the largest percentage reductions. Note that in c, the spread rates for treatment HI and LI may be underestimated, as the release height of pre-mow capitula in these treatments were assumed to be the same as plant height at the end of the season.


Chapter 4

Tolerance of two invasive thistles to repeated disturbance

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Abstract

Many invasive species have short life cycles, high reproduction, and easily dispersed offspring that make them good ruderal species under disturbance. However, the tolerance of these ruderal species to disturbance is often overlooked. In a two-year mowing study, we applied frequent intense disturbances to examine the tolerance of two congeneric invasive thistles, *Carduus acanthoides* and *Carduus nutans*, and potential differences in their responses. Our results show that both species can survive multiple mowing events, with *C. acanthoides* surviving repeated intense mowing through a whole season. Furthermore, *C. acanthoides* was found to adjust its growth form to the disturbance regime, and successfully overwintered and reproduced in the subsequent growing season if the disturbance was terminated. Our results support the idea that tolerance to disturbance should be considered when examining invasions by short-lived monocarpic species, since avoidance of disturbance via rapid life cycle completion and seed production, and tolerance of disturbance via regrowth can co-occur in these species. Consequently, management of short-lived invasives should take both life history strategies into account.

**Keywords:** disturbance; tolerance; invasive; *Carduus nutans*; *Carduus acanthoides*
**Introduction**

Ecosystems are continually exposed to a variety of disturbances. Natural disturbances, such as hurricanes and flooding, and human-mediated disturbances, such as land-use change and pollution, are becoming more common and severe in terms of frequency, intensity, extent, and duration (Vitousek et al. 1997; Foley et al. 2005; Jentsch et al. 2007). These disturbances may cause partial or total destruction of biomass, significant changes in substrate properties, resource levels, species interactions, and thereby the structure and function of ecosystems (White 1979; Sousa 1984; Hobbs and Huenneke 1992; Grime 2001).

Plants may respond to disturbance using two different strategies (Bellingham and Sparrow 2000). Plants either avoid disturbance via defense or by escaping in space or time, or tolerate disturbance by regrowing after being damaged, thus reducing biomass loss (Belsky et al. 1993). For example, in severe disturbances which remove most aboveground biomass, plants either regenerate from seed and rapidly complete reproduction (avoidance), or are able to survive and resprout from stem or root fragments to recover lost biomass (tolerance) (Bell 2001).

Although the trade-offs between disturbance avoidance and disturbance tolerance may lead to a perceived dichotomy in species responses (Bellingham and Sparrow 2000), the two distinct life histories can co-occur in many short-lived monocarpic species (Martinková et al. 2004, Klimešová et al. 2008; Latzel et al. 2009).

Invasive species are commonly believed to be disturbance adapted (Dukes and Mooney 1999). Many invasive species are classified as ruderals (Grime 2001), which are characterized by short lifespans, fast growth, large reproductive outputs, easily-dispersed offspring, and persistent seed banks (Prinzinger et al. 2002; Theoharides and Dukes 2007). These life history traits enable the invaders to avoid disturbance by regenerating rapidly from seed and completing their life cycles between disturbance events (Witkowski and Wilson 2001). Hence, numerous studies focus on seed germination, seedling emergence and survival, and seed bank dynamics of these species.
under disturbance as these short-lived species are commonly believed to regenerate only via seeds (Fumanal et al. 2008; Tipping 2008; Meiman et al. 2009). However, the tolerance of these invasive species to disturbance is often overlooked. Failing to include tolerance as a possible additional tactic in the face of disturbance may lead to underestimates of the spread and persistence of invasive species in disturbed areas. Consequently, we should consider plant tolerance to disturbance when examining invasions by these short-lived species.

Here we present a study to examine the tolerance of two congeneric invasive species Carduus acanthoides and Carduus nutans to a severe mowing regime. Both species are short-lived monocarps with rapid growth and massive production of wind-dispersed seeds (Desrochers et al. 1988), and colonize disturbed areas such as overgrazed pastures, roadsides, and railroad right-of-ways (Gassmann and Kok 2002; Kok and Gassmann 2002). The two species differ in their morphology and flowering phenology, with C. acanthoides having more stems, more but smaller capitula, a lower height at flowering, and a longer flowering period than C. nutans (Rhoads and Block 2000). The disturbance regime applied in this study was weekly clipping at 5 cm above ground after plant bolting. Mechanical management such as mowing is commonly applied to control thistle infestations (DiTomaso 2000). We used such a frequent and intense disturbance regime to mimic the commencement of weed eradication management, which often requires high levels of control activity (Panetta 2006). In addition, we used this severe mowing regime to magnify potential differences in the two species’ tolerance to disturbance because a previous study (Chapter 2) shows that both species can survive and produce viable seeds after three mows at 5cm above ground within a growing season. We hope to answer the following questions: How does this repeated disturbance regime affect the fitness of the plants in terms of their survival, reproduction, and height (which affects seed dispersal distances for the two species, Skarpaas and Shea 2007)? Do these two species differ in their tolerance to disturbance? The results from our study will improve our understanding of how short-lived invasive weeds
tolerate disturbance, and the role that tolerance plays in invasions that occur in frequently
disturbed habitats. Furthermore, such knowledge may also generate new insights to improve our
use of disturbance to control invasions.

Methods

Study Species

*Carduus nutans* L. (musk thistle: Asteraceae) and *C. acanthoides* L. (plumeless thistle),
usually occur in disturbed habitats in their native Eurasia, and commonly invade pastures,
rangelands, roadsides, and disturbed areas in North America (Allen and Shea 2006). The stems
and foliages of both species are prickly, and likely to deter herbivory (Desrochers et al. 1988).
Both species can grow up to 2m in height under favorable conditions and have single fleshy
taproots (Desrochers et al. 1988, Feldman and Lewis 1990). Both species are monocarpic
perennials, which can behave as summer annuals, winter annuals, biennials, or short-lived
perennials (Tipping 2008). Their germination occurs in spring and fall, followed by a rosette
stage for one or more years (Allen and Shea 2006). Flowering of the two species requires
vernalization. Plants bolt during late spring, flower in summer, and die after producing a large
number of wind-dispersed seeds (approximately 3000-7000 seeds per plant for *C. nutans* and
1000-10000 seeds per plant for *C. acanthoides*) (McCarty 1982, Feldman and Lewis 1990).
However, the two species have contrasting morphology and flowering phenology: *C. acanthoides*
has a lower branching pattern than *C. nutans*; *C. acanthoides* has a longer flowering period from
June to October, compared to *C. nutans* which flowers from May to August (Rhoads and Block
2000). *C. acanthoides* produces more, smaller capitula (1.2 -1.6 cm) which are sometimes
clustered, while *C. nutans* produces fewer, larger capitula (1.5 to 4.5 cm in head diameter) which
are solitary (Rhoads and Block 2000).

Experimental Site
The experimental site was a pasture at the Russell E. Larson Agricultural Research Farm, Rock Springs, Pennsylvania (latitude 40.711°, longitude -77.942°). The pasture was dominated by *Arrhenatherum elatius, Dactylis glomerata, Elytrigia repens, Phleum pratense, Taraxacum* spp., *Plantago lanceolata, Linaria vulgaris, Trifolium* spp., and *Gallium* spp.

The site was prepared prior to transplanting the thistle rosettes, to mimic the disturbed areas in which these thistles are commonly observed. A Miller Offset Disk was applied twice to kill all aboveground vegetation, and then a Roller Harrow was applied to level the surface when the soil was fairly dry.

**Experimental Design**

In the summer of 2007, seeds of the two species were collected from two naturally occurring populations in Pennsylvania. These seeds were sown and germinated in a greenhouse. In October 2007, we transplanted three-week-old seedlings of both species into the field, where they overwintered as rosettes. In early April 2008, we applied approximately 13g of slow-release fertilizer (Osmocote Flower/Vegetable Food, N-P-K: 14-14-14) per plant to ensure that most of the rosettes would bolt in the summer and therefore behave as winter annuals. At the end of May 2008, fifty bolting plants (with obvious elongated stems) of each species were randomly chosen from those available to be included in the experiment.

We used hand clipping to mimic mowing in farming practice. Clipping was initiated in May 2008 after thistles bolted – this was when thistles exceeded surrounding pasture species and would be likely to be noticed by managers. Forty plants of each species were randomly assigned to be weekly hand clipped to 5cm above ground together with surrounding vegetation in a 50cm × 50cm area (a maximum of twenty-five cuts were given by the end of the growing season in mid-November). The other ten plants remained uncut as controls. All *C. nutans* individuals, and the *C. acanthoides* controls, died before November 2008. However, twenty-nine out of the forty cut *C. acanthoides* were still alive in mid-November 2009, when we paused the experiment.
because of snow cover. Twenty-one of these twenty-nine \textit{C. acanthoides} survived the winter, and were included when the experimental treatments resumed in April 2009. Ten out of these remaining \textit{C. acanthoides} were randomly assigned to receive the same cutting treatment (weekly clipping to 5cm aboveground) as in 2008, while the other eleven plants were left uncut for the second growing season. The purpose of having uncut plants in 2009 was to examine the recovery of \textit{C. acanthoides} that had been intensely cut only in the previous growing season.

We conducted weekly censuses from May 2008 to November 2008 and from April 2009 to October 2009, which included plant stem number, plant height, and developmental status (e.g. flowering, setting seed, and dead). Flowering was defined as appearance of visible florets. Death was defined as total senescence of aboveground biomass with no green tissue left. Pollen bags were put onto capitula when florets turned brown in order to prevent seed dispersal. Plants were destructively sampled following their death. The capitula were separated and counted either as viable (contained developed seeds) or aborted (which had no seed set).

\textbf{Data Analysis}

We conducted statistical analyses on survival to reproduction, capitulum production, and plant height at flowering of the two species in R (R development core team 2008). A parametric regression model with Weibull distribution (survreg function in the package of “survival”) was used for the survival analysis of both species in 2008. A Kolmogorov-Smirnov test (Zar 1999) was used to test the difference in the survival of \textit{C. acanthoides} in 2009 because of the non-proportional hazard of plant death. Number of capitula and plant height were analyzed using Generalized Linear Models (GLMs, Crawley 2007). We specified the error distribution as Gaussian in the analyses for plant height, and we used a Quasipoisson distribution for number of capitula to account for overdispersion in the data. The ratio of aborted versus viable capitula was analyzed using a logistic regression model by specifying a binomial error distribution in the GLM.
To test how a plant’s recovery capability changed over time, we analyzed plant height before each clipping for the experimental groups using Generalized Linear Mixed Models (GLMMs, Crawley 2007) with time as the fixed effect and time nested in plant as the random effect. For the 2009 data, because treatment started only when plants exceeded the height of 5 cm, plants of height 5 cm or lower in the early season were excluded from the analysis.

**Results**

**Survival to reproduction**

In 2008, all controls flowered and then died by the end of the growing season. Twenty-four cuts were needed to kill all *C. nutans* ([Figure 4-1a](#), [Figure 4-2a](#)). However, 72.5% of the cut *C. acanthoides* were still alive at the end of the growing season (after twenty-five cuts) ([Figure 4-1a](#), [Figure 4-2d](#)), with 72.4% of these plants surviving the winter and resuming growth in the following spring.

In 2009, all *C. acanthoides* that were not cut in the second year flowered and died ([Figure 4-2d](#)). All *C. acanthoides* that continued to receive the repeated cutting died without flowering except one plant, which survived until the termination of the experiment in August (a total of twenty-one cuts in 2009, [Figure 4-1b](#)).

The survival analyses show that the frequent intense cuts delayed plant death for both species in 2008 (*P*<0.01). The Kolmogorov-Smirnov test shows that the two survival curves follow different trajectories in 2009 (*D*=0.78, *n*_1=9, *n*_2=11, *P*<0.01, [Figure 4-1b](#)).

**Reproduction**

For both species, all of the controls produced viable capitula. 30.0% cut *C. acanthoides* and 57.7% cut *C. nutans* had produced buds on their short stems between successive cuts in the first year. These buds were removed in the following cutting events. However, we believe they would have developed into viable capitula if cutting had ceased.
For *C. nutans*, all cut plants died without reproducing. For *C. acanthoides*, all plants that were cut in both years died without reproducing. The *C. acanthoides* controls produced significantly more capitula than those that were cut in the first year and uncut in the second year (123.3 ± 23.1 vs. 26.0 ± 5.8, d. f. = 20, *P* < 0.001, Figure 4-2e). Furthermore, controls had a significantly lower proportion of aborted capitula (10.1 ± 2.5 % vs. 39.1 ± 3.9 %, d. f. = 20, *P* < 0.001, Figure 4-2e).

**Plant Height**

Plant height at the beginning of the experiment did not differ between cut plants and controls (*P* > 0.1) for either species. The heights of living *C. acanthoides* as the experiment was resumed in the second year were not different between the treatments (*P* > 0.1).

Repeated removal of the shoot apex disrupted apical dominance, resulting in short plants with more than one induced stem. All continuously cut plants were below 5 cm when they died. The *C. acanthoides* controls were significantly taller than plants that were cut in the first year but not in the second year (123.0 ± 5.8 cm vs. 98.3 ± 8.4 cm, d. f. = 20, *P* = 0.028, Figure 4-2f).

Changes in heights of living plants before each week’s clipping (pre-cut height) are shown in Figure 4-3. Height of controls increased until mid-July, when it reached an asymptote. Recovered height in cut plants was greater early in the season, and then diminished towards the end of each growing season. A significant declining trend was found for recovered height of both species in both years (*P*<0.001).

The two species differed in their tolerance to the repeated disturbance regime. While no *C. nutans* survived the cutting in the first growing season to reproduce (Figure 4-2b), cut *C. acanthoides* individuals (even those that had developed buds between cuts) were found to stop stem elongation and instead grew new leaves to form rosettes near the plant base, thus escaping successive cuts. Twenty-one out of forty cut *C. acanthoides* survived the first year’s disturbance

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and successfully overwintered as rosettes. These *C. acanthoides* also had a 100% flowering probability if cutting ceased in the second growing season.

**Discussion**

Our study shows that two common invasive weeds in North America, *C. nutans* and *C. acanthoides*, have considerable tolerance to repeated severe disturbance. Although repeated mowing eventually caused large reductions in the survival, reproduction, and height of both species, these species were found to retain high recovery ability and survive numerous cuts (>10 cuts). Moreover, even though climatic differences between the two years may have affected the magnitude of plant recovery, our results in the second growing season nevertheless show that *C. acanthoides* individuals, after being repeatedly cut in the previous growing season, still had the potential to regrow and reproduce once cutting stopped.

The strong tolerance of *Carduus* spp. to repeated mowing is in contrast to expectations based on the trade-off between reproductive effort and regrowth capability (Bellingham and Sparrow 2000) and between plant defense and regrowth capability (Van der Meijden et al. 1988). Both species are typical representatives of the ruderal strategy: they produce a large number of easily dispersed seeds and have short life cycles and persistent seed banks. They also invest significantly in physical defense (i.e. prickles on stems and leaves). Their high tolerance is probably based on resources stored in their fleshy taproots. Previous studies show that root carbon storage plays an important role in resprouting ability of monocarpics (Klimešová and Klimeš 2003, Sosnová and Klimešová 2009). Although no study examines the root systems of these two species in particular, studies of other nonclonal thistles suggest that their deep taproots may be good at resource acquisition and storage (DiTomaso et al. 2003, White and Holt 2005, Eckersten et al. 2010). Additionally, disturbance timing also affects tolerance (Matinková et al. 2008). Since our cutting treatments started after bolting but before flower emergence, plants’
investment in reproduction (both resource reallocation and meristem differentiation) was not complete. Therefore, plants were possibly able to utilize remaining resources for regrowth.

The two species, though similar in their life cycles, differ in their tolerance to repeated disturbance. *C. acanthoides* was more tolerant than *C. nutans* as it maintained a higher survival and higher recovery ability after being damaged. Moreover, flowering in cut *C. acanthoides* was found to delay to the second growing season while all cut *C. nutans* died in the first growing season. This large phenological delay, although not expected for strict monocarpic species (Huhta et al. 2009, Piippo et al. 2009), has also been observed in other monocarpic species under severe disturbance (Klimešová et al. 2007). This strategy enables *C. acanthoides* to adjust its life history to disturbance and increases its chances of survival and reproduction in the future. The greater tolerance of *C. acanthoides* may be due to its lower branching pattern, later flowering phenology and longer flowering period. The lower branching pattern, and hence a lower shoot apical meristem position, minimized loss of meristems and preserved the meristem pool, which is crucial for later regrowth (Rosenthal and Kotanen 1994, Huhta et al. 2000, Rautio et al. 2005). This result agrees with a previous finding in *Centaurea solstitialis*, where a low-branching phenotype was less affected by mowing than a high-branching phenotype (Benefield et al. 1999). Furthermore, a longer flowering period ensures a slower increase in reproductive investment in *C. acanthoides*, which reduces biomass loss in the cutting treatment.

Our results provide new insights for management. More attention should be paid to the strong tolerance of *Carduus* spp when managing these invasive weeds. These species have a great potential for recovery after being damaged, and may cause further infestations if not completely eradicated, especially given their large reproductive outputs and significant dispersal ability (Desrochers et al. 1988; Skarpaas and Shea 2007). Furthermore, as suggested by this study and also noted in other studies (Zhang and Shea, in revision, Gao et al. 2009), purely increasing management frequency without attention to appropriate timing may not effectively reduce plant
fitness. This notion is especially important for management of *C. acanthoides*, given the findings of the present study.

In conclusion, some short-lived invasive species have considerable tolerance to repeated disturbance. This tolerance of disturbance may be especially important for their establishment at invasion fronts, where propagule pressure is low and Allee effects are likely (Wilson et al. 2009). Therefore tolerance to disturbance, in addition to rapid growth rate and large seed production, should be taken into account when studying invasions of ruderal species, as potential synergistic effects between disturbance tolerance and avoidance may accelerate invasion processes. Furthermore, management should be adjusted to acknowledge the tolerance of these species, especially when native counterparts are not as adapted to disturbance.

**Acknowledgements**

RZ conceived and designed the experiment. RZ, JMH, and EH conducted the experiment. RZ analyzed the data. All authors contributed to writing this chapter. We appreciate the help of Carrie Davila, Maria Stevens, Matthew Jennis, Jennifer Stella, Clayton Costa, Andrea Leshak, and Liza Senic in the field work. We are very grateful for comments from Suann Yang, Adam Miller, Katherine Marchetto, Ezra Schwartzberg, Eelke Jongejans, Laura Russo, Britta Teller, and two anonymous reviewers. We acknowledge the support of USDA-CSREES (Biology of Weedy and Invasive Plants) NRI grant #2002-35320-12289 and NSF grant #DEB-0815373 to KS.
References


Figure 4-1. Survival curves for *C. acanthoides* (black lines) and *C. nutans* (grey lines) in both growing seasons. (a) Solid lines represent cut plants and dotted lines represent controls. (b). The solid line represents *C. acanthoides* that received cutting in both years, the dotted line represents plants cut only in 2008. “+” indicates censored data.
Figure 4-2. Effects of weekly clipping on the fitness of *C. nutans* (a-c) and *C. acanthoides* (d-f). (a) and (d): survival to reproduction. In (d), survival to reproduction (represented by the second bar) was calculated as the product of survival until the following spring from the first year (21/40) and flowering probability of surviving plants that were not cut in the second year (11/11). (b) and (e): lifetime fecundity; the stripe pattern represents aborted capitula while the plain pattern represents mature capitula containing viable seeds. (c) and (f): plant height at flowering. The three treatments are on the x-axis: controls (uncut), plants that were cut only in the first year (1-yr-cut), and plants that were cut in both years (2-yr-cut). Note that the third treatment did not apply to *C. nutans* as all *C. nutans* died in the first year (NA); control *C. acanthoides* flowered and died in 2008 while *C. acanthoides* that were cut only in the first year flowered and died in 2009. The n values denote number of replicates for each treatment. Note that n=9 for the third bars in (d), (e), and (f), because one plant survived two years of cutting treatments to remain alive at the end of the experiment. Stars denote statistical significance (* for P<0.05, ** for P<0.01, and *** for P<0.001) for differences between controls and *C. acanthoides* that had been cut only in the first year.
Figure 4-3. Dynamics of plant height for *C. nutans* (a) and *C. acanthoides* in 2008 (b) and in 2009 (c). Error bars represent the standard errors for controls (hollow points). Standard errors for pre-cut height of cut plants (filled points) are too small to be represented in the graph. The dashed horizontal line represents the height of 5 cm to which plants were cut weekly. The arrows represent when the first death occurred in each group. Note that the last few data points of each curve are potentially biased because of limited sample size of living plants toward the end of the experiment.
Chapter 5

Warming increases the spread of an invasive thistle

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Abstract

Background

Global warming and shifted precipitation regimes increasingly affect species abundances and distributions worldwide. Despite a large literature on species’ physiological, phenological, growth, and reproductive responses to such climate change, dispersal is rarely examined. Our study aims to test whether the dispersal ability of a non-native, wind-dispersed plant species is affected by climate change, and to quantify the ramifications for future invasion spread rates.

Methodology/Principal Findings

We experimentally increased temperature and precipitation in a two-cohort, factorial field study (n=80). We found an overwhelming warming effect on plant life history: warming not only improved emergence, survival, and reproduction of the thistle Carduus nutans, but also elevated plant height, which increased seed dispersal distances. Using spatial population models, we demonstrate that these empirical warming effects on demographic vital rates, and dispersal parameters, greatly exacerbate spatial spread. Predicted levels of elevated winter precipitation decreased seed production per capitulum, but this only slightly offset the warming effect on spread. Using a spread rate decomposition technique (c*-LTRE), we also found that plant height-mediated changes in dispersal contribute most to increased spread rate under climate change.
Conclusions/Significance

We found that both dispersal and spread of this wind-dispersed plant species were strongly impacted by climate change. Dispersal responses to climate change can improve, or diminish, a species’ ability to track climate change spatially, and should not be overlooked. Methods that combine both demographic and dispersal responses thus will be an invaluable complement to projections of suitable habitat under climate change.

Key words

Carduus nutans; climate change; dispersal; invasive species; spread rate; vital rate; warming
Introduction

In the face of climate change, dispersal imposes a major limit on a species’ ability to keep pace with environmental shifts [1, 2], and thus determines the fate of individuals, population persistence, and species distributions [3, 4]. Species with low dispersal ability are more likely to suffer from range contraction and eventual extinction, particularly in fragmented habitats [5]. This is especially true for species with limited environmental tolerances and when evolutionary responses occur more slowly than climate change [6]. Invasive species, on the other hand, are often associated with high dispersal and may become more problematic under changing climate [7].

Although the importance of including dispersal in climate change studies has been raised repeatedly [8], dispersal is rarely examined in this context [9]. The lack of dispersal data often leads to unrealistic assumptions of unlimited dispersal, or no dispersal at all, when predicting future species distributions [8]. The disparate predictions resulting from these two extreme cases [10, 11] underline the necessity of including explicit dispersal information to reduce the significant uncertainties caused by spread when projecting species distribution shifts. Furthermore, even when dispersal under current climate is known, extrapolation of present dispersal abilities to the future remains a challenge. Factors affecting dispersal processes, such as the architectural features of maternal plants, the morphology of dispersal units [12], and dispersal vectors [7] may not stay constant over environmental gradients; thus, dispersal may also be altered by climate change. Therefore it is necessary to evaluate possible changes in dispersal when examining species’ responses to climate change. However, no study so far has examined dispersal-related plant responses to future climate scenarios. Neither does any study couple altered dispersal processes with altered population dynamics under climate change to assess future population performance, although theoretical approaches exist to reach this goal [13, 14, 15].
Here we present an experimental and theoretical study which addresses dispersal responses in addition to responses of survival, growth, and reproduction of a wind-dispersed invasive thistle, *Carduus nutans*, to climate change. Dispersal, although recognized as a critical component in invasion processes [16, 17], is generally overlooked when examining responses of invasive species under climate change. We divide the responses of *C. nutans* into two categories: i). responses in demographic vital rates, such as seedling emergence, rosette survival, bolting rate, capitulum production, seed production per capitulum; ii). responses that relate to dispersal characteristics, such as plant height at flowering (which affects seed release height), and propagule terminal velocity (which depends on seed weight and pappus morphology).

**Results**

We examined two independent cohorts in a former pasture in central Pennsylvania. We manipulated both temperature and precipitation. In half of our plots, fiberglass open top chambers (OTCs) passively increased daily average temperature on the soil surface by 0.58°C across seasons in the two years of experiment (corresponding to an annual increase of 97 degree days). We also achieved a 30% increase in winter precipitation with or without a 15% increase in summer precipitation by manually adding rain or snow – these percentages are the extremes from regional climate projections for this area [18, 19]. Other vegetation was suppressed in all plots to mimic the disturbed environments (e.g. overgrazed pastures) in which this species attains maximum invasion success.

Although our increase in temperature was mild compared to regional projections (increases of 2.9°C - 5.3°C in annual surface air temperature by the end of this century [19]), we found significant responses of *C. nutans* to warming. Seedling emergence in the fall was enhanced in warmed plots (25±3% versus 19±3%, mean±s.e., n=80, generalized linear mixed-effects model (GLMM) with quasibinomial error structure to account for overdispersion, n=80, *P*<0.001).
Warming also increased rosette overwinter survival (95±2% versus 87±3%, binomial GLMM, n=80, P=0.0085). Increased overwinter survival is due to both increased winter temperatures and enhanced fall rosette growth (and therefore bigger rosette sizes before winter). Bolting probability was not different between warmed and ambient plots for plants that survived the winter (86±4% versus 90±3%, binomial GLMM, n=79, P=0.70). Plant reproduction was significantly increased by warming. Both mature flower head production (49.84±5.60 versus 36.65±4.08, GLMM based on log linear transformed data, n=77, P=0.0028) and total (including buds) capitulum production (76.04±9.62 versus 57.41±6.75, GLMM based on log linear transformed data, n=77, P=0.028) were higher in warmed plots. Seed number per capitulum was not affected by warming (522±29 versus 476±31, GLMM, n=77, P=0.19). Precipitation addition in both winter and summer had no significant effect on any of the above vital rates. Increased precipitation in winter alone only reduced seed number per capitulum (427±50 versus 539±28, GLMM, n=77, P=0.0080).

We combined the percentage increases in seedling emergence (+29.53%), survival (+9.35%), and total capitulum production (+32.44%) due to warming, and decreases in seed number per capitulum due to increased winter precipitation (-20.79%) with a baseline 4×4 demographic matrix model (based on vital rates for an invasion experiment population at the same site from a previous study [14], see also SI, Table 5-S1, Table 5-S2) to calculate the long-term population growth rate $\lambda$ under three climate change scenarios: warming, winter precipitation addition, and warming with winter precipitation addition. We assumed that the proportional responses to warming are size-independent, the establishment rate of emerging seedlings is not changed under warming, and all buds counted at harvest would have continued to set seed. We projected an 87% increase in per capita population growth rate under warming (680 versus 363), a 20% decrease under winter precipitation addition alone (288 versus 362), and a 49% increase under both warming and increased winter precipitation (539 versus 362).
In our study, plant height, a dispersal-related trait, was enhanced in warmed plots by 11.88 cm, or 9% (151.75±4.94 cm versus 139.87±4.23 cm, GLMM, n=77, \( P=0.0033 \)). Warming did not have any significant effect on seed terminal velocity (0.75±0.04 m/s (ambient) versus 0.74 ± 0.04 m/s (warmed), GLMM, n=66, \( P=0.80 \)), seed weight (3.42±0.09 mg versus 3.36±0.12 mg, GLMM, n=66, \( P=0.80 \)), or pappus diameter (21.67±1.0 mm versus 21.58±1.0 mm, GLMM, n=66, \( P=0.81 \)). Increased precipitation did not have any significant effect on seed weight (n=66, GLMM, \( P=0.90 \)): 3.38±0.09 mg (ambient), 3.38±0.16 mg (winter precipitation addition), 3.46±0.18 mg (winter and summer precipitation addition). The effects of precipitation addition on pappus diameter and terminal velocity were not consistent for the two cohorts (Table 5-S1).

Therefore the only consistent and significant effects of climate change on dispersal are mediated via the enhanced plant height at flowering. We calculated dispersal kernels for both ambient and warmed conditions using the Wald analytical long-distance dispersal (WALD) model [20, 21]. We parameterized the model with terminal velocities and plant flowering height (assumed to be the same as seed release height) from our experiment, while horizontal wind velocity data from a nearby weather station collected during the main dispersal months were used to estimate mean wind speed and a turbulence parameter [14]. Increased plant flowering height under warming led to a distinct dispersal kernel compared to the control (Figure 5-1); each seed has a higher probability of travelling farther under warmed than under ambient conditions.

Spatial population spread results from a combination of 1) local demographic processes (which determine how many propagules are produced) and 2) the dispersal process itself (which determines how far each propagule moves). We coupled demographic models with dispersal models to project the population spread rate, \( c^* \), using integrodifference equations [13-15]. We used baseline data from an earlier invasion study [14] and modified the models based on our experimental results (Table 5-S1, Table 5-S2). The population spread rate increased by 27% under warming (66 m/year versus 52 m/year, the medians of 100,000 simulations under increased
or ambient temperatures, see simulations in Figure 5-2). In other words, it takes about 19 years for the invasion front to move 1km under ambient conditions, while only 15 years under warming. Increased winter precipitation alone, which affected seed production per capitulum, did not have a large impact on the spread rate (50 m/year versus 52 m/year). Increased winter precipitation and warming together led to a 23% increase in the spread rate (64 m/year versus 52 m/year). This implies that warming (with or without precipitation increases) is likely to cause more rapid spread of this invasive species once it establishes, even in the absence of other (e.g. human-mediated) dispersal pathways. We used a $c^*$-LTRE [14] approach to decompose the increase in $c^*$ under warming with increased winter precipitation into contributions by the parameter differences. Increased height made a much larger contribution to increased spread rate than did the demographic vital rates (Figure 5-3); warming-induced changes in plant height may have the strongest influence on population spread of C. nutans under climate change.

Discussion

As shown by our results, climate change affects many aspects of species life histories. While enhanced emergence, survival and reproduction directly contribute to local population dynamics [22], altered dispersal characteristics can affect the ability of offspring to arrive in suitable habitats and to buffer against harsh environments spatially. Here warming enhances the spread rate of an invasive wind-dispersed species through both demographic and dispersal processes, with enhanced dispersal contributing disproportionately to increased spread. This is because the invasion wave speed is very sensitive to plant height [14]. While we parameterized our model with data from an experimental population where growing conditions were optimal for invasion (i.e. little competition and abundant resources), we also assessed the spread rate using data from a naturally established population in Kansas. Though its contribution diminishes compared to the increased contribution of emergence (probably due to strong competition in
natural populations), increased dispersal still plays an important role in increased spread under warming (Figure 5-S1).

Our results suggest that the invasion of this species can be exacerbated by climate change, resulting largely from enhanced dispersal because of elevated plant height. Previous studies have documented that many invasive species are able to track local climate change, via adjusted phenology [23], improved establishment [24], and enhanced biomass and reproduction [25-28]. Our study demonstrates that some invasive species can also benefit from enhanced dispersal-related traits, which may increase their chance of reaching in newly suitable habitats. Furthermore, although possible changes in dispersal processes accompanied with climate change have been raised in recent reviews [7, 16], these studies consider extrinsic factors, such as altered transportation and deliberate introduction, rather than the traits of the plant itself. Our results, however, illustrate the possibility of altered plant dispersal traits due to climate change, which can interact with extrinsic factors involved in dispersal processes to produce synergistic or antagonistic outcomes.

Our experiment was conducted to maximize the invasion response of this species. In particular, plants were grown with very little competition from surrounding vegetation, thus mimicking scenarios in frequently disturbed areas, such as overgrazed pastures. Spread rates in such habitats are projected to increase under climate change, and our results suggest that management efforts should be focused more on the control of dispersal relative to demographic processes. While our study focuses on the maximal responses of the species, future studies should also address other biotic factors, such as the responses of surrounding vegetation to climate change and thereby possibly modified competition, and interference with dispersal. As well as biotic responses, future research based on this approach may also include environmental factors that affect dispersal processes, such as changes in wind speed [29] and turbulence [30] under future climate scenarios. For example, a recently published study by Nathan et al. [31] combined
demographic and dispersal processes with projected wind speed changes to project wind-driven spread of tree species under climate change. Such approaches will complement biotic envelope models, most of which do not address biotic processes such as population dynamics and dispersal [32]. Evaluations of species dispersal and spread abilities, and changes in these abilities, under climate change will provide important insights for both conservation of endangered species, which often lack efficient dispersal, and management of invasive species.

**Materials and Methods**

**Study species.** *Carduus nutans* L. (Asteraceae) is an introduced Eurasian weed, which causes major economic problems in many regions in the world [15]. *C. nutans* is a monocarpic, short-lived (winter annual in our experiment) thistle and produces a large number of wind-dispersed seeds [21].

**Field site descriptions.** We conducted our experiments at The Russell E. Larson Agricultural Research Farm at Rock Springs, Pennsylvania, USA (latitude 40.71°, longitude -77.94°). The field site was a former pasture, which was dominated by *Arrhenatherum elatius*, *Dactylis glomerata*, *Elytrigia repens*, *Phleum pratense*, *Taraxacum* spp., *Plantago lanceolata*, *Linaria vulgaris*, *Trifolium* spp., and *Galium* spp. The field site was disked and all aboveground vegetation was removed prior to planting each cohort, to mimic the high invasion success of this opportunistic species in disturbed habitats.

**Treatments.** Our climate manipulation incorporated both elevated temperature and increased precipitation according to the regional climate projections in the Northeastern U.S. Open top chambers (OTCs) were used to elevate temperature in the experiment. These OTCs were 0.4 m in height and 1.5 m in basal diameter, and were constructed according to the International Tundra Experiment Manual [33]. Cumulative degree-days for *C. nutans*, calculated based on McCarty [34], were significantly higher in plots with OTCs, with the largest increase in each spring
(81.40±11.65 degree-days). OTCs did not have a significant effect on soil moisture or snow depth based on field measurements. Therefore we assume that elevated temperature was the predominant abiotic factor altered by the OTCs. We manipulated both winter (December - February) and summer (June - July) precipitation. Passive precipitation collectors were used to collect natural precipitation in the field. Rain and snow were manually added after each precipitation event.

**Experimental design.** In each cohort, we set up ten blocks, each containing four plots (one control, one with warming, one with added precipitation, and one with both treatments). As most climate projections agree with increasing future winter precipitation in U.S. Northeast, whereas summer projections are highly variable [35], precipitation plots in half of the blocks received winter precipitation addition only (+30%), while precipitation plots in the other blocks also received summer addition (+15%). In each fall, we transplanted four three-week-old rosettes into 2m × 2m plots, either with or without OTCs. Field censuses were conducted weekly in the following growing season. Other plot vegetation was kept short by hand clipping. We terminated our experiments in the following late July by harvesting all aboveground biomass. Capitula were separated and counted based on developmental stage. One mature flower head per plot was used to examine seed production per capitulum, seed traits, and terminal velocities (i.e. the speed at which a propagule eventually falls in still air when drag equals the force of gravity; faster drops lead to shorter travel distances), following the methods of Marchetto et al. [36]. Fall seedling emergence was examined by sowing twenty-five seeds into the center of each plot in the October of 2008 and 2009. Emerged seedlings were recorded and killed every two days until December.

**Statistics.** Analyses based on plot averages were performed in R [37] using mixed linear models (lmer) with appropriate error structure specifications or transformations of the responses. Shapiro-
Wilks tests were used to verify normality. Full models started with fixed effects of warming, precipitation and their interaction (except for seedling emergence where precipitation was not relevant), and the random effects of cohort and block. Initial rosette size at transplanting was a covariate (except for seedling emergence). Stepwise model simplifications were based on lower AIC values. Bolting probability was analyzed as the conditional probability given rosettes survived the winter. Responses of reproduction and height were averaged for flowering plants in each plot before the analyses. The arithmetic means of seed weight and pappus diameter, as well as the geometric mean of terminal velocity of seeds within each flower head were used when analyzing effects of climate change on seed dispersal.

Models. We used intergrodifference equations to couple matrix models of population dynamics with a WALD model of dispersal to calculate spread rates (Text S1). We used the c*- LTRE technique to decompose the increase in spread rate into contributions of model parameter changes as in [14].

Acknowledgements

RZ and KS conceived and designed the experiments. RZ performed the experiments. All authors analyzed the data and wrote this paper. We are grateful for logistical assistance and/or comments from E. Post, S. Isard, S. Yang, A. Miller, L. Russo, S. Smiles, B. Teller, D. Mortensen, and many Shea lab undergraduates.

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Figure 5-1. Seed dispersal kernels for *C. nutans* grown in ambient and warmed conditions. The bands (grey for ambient; pink for warmed) illustrate the standard deviations based on bootstrapped plant height from the experiment, while the lines represent the mean of the bootstrapped curves (see Text S1).
Figure 5-2. Visualizations of invasion waves in 20 years based on two random simulations. A) Ambient condition. B) Warmed condition. Note that the front of the wave converged to a constant speed and shape after a few years.
Figure 5-3. Changes in demographic and dispersal-related traits and their contributions to increased spread rate. A) Mean percentage changes in demographic and dispersal-related traits. B) Their corresponding contributions to increased spread rate. Warming induced changes in fall seedling emergence, survival, capitulum production, and flowering plant height: W and in red. Mean percentage change in seed number per capitulum due to increased winter precipitation: P and in blue. Solid: demographic vital rates. Hatched: dispersal-related traits.
Supporting Information

Model details

Demographic model

The demographic models were based on existing demographic data for this species (e.g. [1], [2]), modified according to the proportional changes of vital rates based on our experimental results. We used vital rate-based matrix models for *C. nutans* as presented by Jongejans et al. [3], based on a four-stage classification (which represent annual demographic transitions from the end of autumn of one year to the end of autumn a year later):

<table>
<thead>
<tr>
<th>Seed bank (SB)</th>
<th>Small (S)</th>
<th>Medium (M)</th>
<th>Large (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε₁(1−γ₁−γ₄)</td>
<td>σ₂(1−σ₂)</td>
<td>σ₃(1−σ₃)</td>
<td>σ₄(1−σ₄)</td>
</tr>
<tr>
<td>σ₁(1−β₁)</td>
<td>σ₂(1−β₂)</td>
<td>σ₃(1−β₃)</td>
<td>σ₄(1−β₄)</td>
</tr>
<tr>
<td>(1−γ₁)(1−γ₄)</td>
<td>(1−γ₁)(1−γ₄)</td>
<td>(1−γ₁)(1−γ₄)</td>
<td>(1−γ₁)(1−γ₄)</td>
</tr>
</tbody>
</table>

We then incorporated into the models significant proportional changes in seedling emergence (ε₁, ε₄), survival (σ₂, σ₃, σ₄), and reproduction (π₂, π₃, π₄) caused by climate change, to project population growth rate λ. Bolting probabilities (β₂, β₃, β₄), transitions between stages due to growth (γ₃, γ₄, γ₃₂, γ₄₂, γ₃₄), potential seed escaping from floral herbivory (φ), and new seed entering the seed bank (ν) were assumed to remain the same.

Dispersal model

We modelled dispersal using the mechanistic Wald Analytical Long-distance Dispersal (WALD) model [4], which we had shown applies well for *C. nutans* in a major field experiment [5]. This model was derived from well-established models in fluid mechanics and predicts an inverse Gaussian (Wald) distribution of dispersal distances *r* [6]:

\[
\text{WALD}(r) = \frac{1}{\sqrt{2\pi r^3}} \exp\left(-\frac{r^2}{2r^3}\right)
\]
where location parameter $\mu'$ and the scale parameter $\xi'$ are given by:

$$
\mu' = \frac{H U}{F} \quad \text{and} \quad \xi' = \left( \frac{H}{\sigma} \right)^2
$$

where $H$ is the seed release height (mean plant height), $F$ is seed terminal settling velocity, $U$ is the hourly mean horizontal wind velocity between $H$ and the ground, and $\sigma$ is a turbulent flow parameter which reflects wind variation due to vegetation structure and weather conditions [4]. We used $H$ and $F$ calculated based on the present experiment, as well as $U$ and $\sigma$ estimated based on weather data collected in a nearby weather station, to parameterize the dispersal model.

We bootstrapped the plant height data 1000 times, while keeping the other parameters unchanged, and calculated the corresponding dispersal kernels. We then calculated the mean and standard deviation of the bootstrapping results, which were presented in Figure 5-1.

Spread model

The demographic and dispersal models were combined to allow the modeling of spatial spread using integrodifference equations [2, 3, 7]:

$$
H = M \circ A
$$

where $A$ is the demographic model, and $\circ$ signifies the Hadamard product (element by element multiplication), and $M$ is a matrix of the same size as $A$, which is comprised of elements containing the moment generating function of the dispersal kernel $k(r)$ (eqn 6). For population growth rates less than unity and for thin-tailed (exponentially bounded) dispersal kernels (e.g. the WALD model), the population spread rate converges to a constant speed, $c^*$:

$$
c^* = \min_{j > 0} \frac{1}{j} \ln \left[ \lambda M(j) \right]
$$
where $M(j)$ is the moment generating function of the dispersal kernel. The WALD model is integrated over terminal velocity and seasonal wind conditions to give the seasonal dispersal kernel (see also [3] for computational details):

$$k(r) = \int \int p(F)p(U)p(r)dFdU$$

(6)

where $p(F)$ and $p(U)$ are the probability density function of $F$ and $U$, respectively, and $p(r)$ is the WALD model. $k(r)$ gives the radial dispersal kernel in a two-dimensional landscape. To estimate the rate of spread in one dimension $x$, we obtained the moment generating function for the dispersal kernel marginalized for $x$ [8]. We estimated the empirical moment generating function by simulating a large number ($N = 100,000$) of random dispersal distances $r$ using $k(r)$ and random directions $\alpha$ using a uniform distribution (0-2$\pi$), and then calculated $x = r\cos(\alpha)$. We used the medians of these runs to exclude potential extreme outliers arising from the integrated WALD model. The empirical moment generating function $M^E(s)$ is:

$$M^E(w) = \frac{1}{N} \sum_{i=1}^{N} \exp(wx)$$

(7)

where $w$ is an auxiliary variable. This joint model then allowed us to project population spread ($c^*$) rates for all climate change scenarios.

$c^*$-LTRE Analyses

We used $c^*$-LTRE analyses to assess the contributions of changes in the vital rates and dispersal-related traits to changes in spread rates ($c^*$). We calculated the contributions of each altered parameters under climate change:

$$\frac{\partial c^*}{\partial \tau_q} = \sum m_{ij}(w^*) \frac{\partial \rho_i \partial h_{ij}}{w^* \rho_i \partial h_{ij} \partial \tau_q}$$

(8)

Where $\tau_q$ is the $q$th vital rate, $h_{ij}$ is the element at row $i$ and column $j$ of $H$, $w^*$ is the auxiliary value corresponding to $c^*$, and $\rho_1$ is the dominant eigenvalue of $H$. We calculated the
sensitivity of $c^*$ to plant height by introducing small perturbations [3]. We used the median of 1000 repeated calculations to avoid potential outliers.
**Table 5-S1.** Summary of the main results of the climate change study

<table>
<thead>
<tr>
<th>Responses*</th>
<th>Temperature</th>
<th>Winter precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ambient</td>
<td>Warmed</td>
</tr>
<tr>
<td>Fall emergence (%)</td>
<td>19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Winter survival (%)</td>
<td>87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bolting probability (%)</td>
<td>90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total capitulum production</td>
<td>57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Seeds per capitulum</td>
<td>476&lt;sup&gt;c&lt;/sup&gt;</td>
<td>522&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Demography**

| Height at flowering (cm) | 140<sup>a</sup> | 152<sup>b</sup> | 145<sup>c</sup> | 145<sup>c</sup> |
| Pappus diameter (mm)<sup>†</sup> | 21.7<sup>a</sup> | 21.6<sup>a</sup> | 18.1<sup>c</sup> (cohort 1) | 20.5<sup>c</sup> (cohort 1) |
| Seed weight (mg) | 3.42<sup>a</sup> | 3.36<sup>a</sup> | 3.38<sup>c</sup> | 3.38<sup>c</sup> |
| Terminal velocity (m/s) <sup>‡</sup> | 0.75<sup>a</sup> | 0.74<sup>a</sup> | 0.90<sup>c</sup> (cohort 1) | 0.81<sup>c</sup> (cohort 1) |

*The superscripts a and b denote the statistical significance between ambient temperature and warmed treatments, and the superscripts c and d denote the statistical significance between ambient precipitation and increased winter precipitation treatments. No significant interaction was found between temperature and winter precipitation manipulation.

†The effect of increased winter precipitation on pappus diameter is not consistent between the two cohorts. No significant effect was found for the first cohort (18.09 ± 1.00 mm (ambient), 20.49 ± 2.17 mm (winter precipitation addition), GLMM, n = 32, P>0.05). In the second cohort, precipitation addition only in winter led to a significantly shorter pappus diameter, while precipitation in both winter and summer was not different from control (26.83 ± 0.93 mm (ambient), 20.74 ± 1.99 mm (winter precipitation addition), 24.37 ± 1.27 mm (winter and summer precipitation addition), GLMM, n = 32, P<0.001 for the comparison of winter precipitation addition versus ambient, P=0.24 for the comparison of both winter and summer precipitation addition versus ambient).
The effect of increased precipitation on seed terminal velocity is not consistent for the two cohorts. No significant effect was found for the first cohort (0.90 ± 0.06 m/s (ambient), 0.80 ± 0.08 m/s (winter precipitation addition), 0.85 ± 0.07 m/s (winter and summer precipitation addition), GLMM, based on log transformation, n = 32, $P>0.05$), whereas in the second cohort terminal velocities were significantly higher (i.e. seeds drop more quickly) for treatments with increased winter precipitation than in the ambient condition (0.54 ± 0.03 m/s (ambient), 0.77 ± 0.07 m/s (winter precipitation addition), 0.66 ± 0.03 m/s (winter and summer precipitation addition), GLMM, based on log transformation, n = 34 , $P<0.05$). As this result is inconsistent for the two cohorts, we ignore the effect of increased winter precipitation on seed terminal velocity in the spread rate modeling.
**Table 5-S2.** Vital rates and dispersal-related parameters used in the demographic models and spread models

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental population*</th>
<th>Natural population†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ambient</td>
<td>warm</td>
</tr>
<tr>
<td>( \sigma_1 ) Survival of seed in seed bank</td>
<td>0.2597</td>
<td>0.2597</td>
</tr>
<tr>
<td>( \sigma_2 ) Survival of small rosettes (S)</td>
<td>0.2619</td>
<td>0.2864</td>
</tr>
<tr>
<td>( \sigma_3 ) Survival of median rosettes (M)</td>
<td>0.6761</td>
<td>0.7393</td>
</tr>
<tr>
<td>( \sigma_4 ) Survival of large rosettes (L)</td>
<td>0.8971</td>
<td>0.9810</td>
</tr>
<tr>
<td>( \gamma_3 ) Growth of establishing seed to M</td>
<td>0.2076</td>
<td>0.2076</td>
</tr>
<tr>
<td>( \gamma_4 ) Growth of establishing seed to L</td>
<td>0.0911</td>
<td>0.0911</td>
</tr>
<tr>
<td>( \gamma_{32} ) Growth of surviving, not-bolting S to M</td>
<td>0.8028</td>
<td>0.8028</td>
</tr>
<tr>
<td>( \gamma_{42} ) Growth of surviving, not-bolting S to L</td>
<td>0.1268</td>
<td>0.1268</td>
</tr>
<tr>
<td>( \gamma_{43} ) Growth of surviving, not-bolting M to L</td>
<td>0.3824</td>
<td>0.3824</td>
</tr>
<tr>
<td>( \rho_{23} ) Retrogression of surviving, not bolting M to S</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>( \rho_{24} ) Retrogression of surviving, not bolting L to S</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>( \rho_{34} )</td>
<td>Retrogression of surviving, not bolting L to M</td>
<td>0.0000</td>
</tr>
<tr>
<td>( \beta_2 )</td>
<td>Bolting of surviving S</td>
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</tr>
<tr>
<td>( \beta_3 )</td>
<td>Bolting of surviving M</td>
<td>0.7143</td>
</tr>
<tr>
<td>( \beta_4 )</td>
<td>Bolting of surviving L</td>
<td>1.0000</td>
</tr>
<tr>
<td>( \pi_2 )</td>
<td>Potential seed production by S</td>
<td>5443</td>
</tr>
<tr>
<td>( \pi_3 )</td>
<td>Potential seed production by M</td>
<td>6150</td>
</tr>
<tr>
<td>( \pi_4 )</td>
<td>Potential seed production by L</td>
<td>12446</td>
</tr>
<tr>
<td>( \varphi )</td>
<td>Potential seed escaping from floral herbivory</td>
<td>0.8500</td>
</tr>
<tr>
<td>( \nu )</td>
<td>New seed entering seed bank</td>
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</tr>
<tr>
<td>( \varepsilon )</td>
<td>New seed establishing seedling</td>
<td>0.2333</td>
</tr>
<tr>
<td>( \varepsilon_1 )</td>
<td>Seed from seed bank establishing seedling</td>
<td>0.2333</td>
</tr>
<tr>
<td>( F )</td>
<td>Seed terminal velocity</td>
<td>0.7107</td>
</tr>
<tr>
<td>( \sigma_F )</td>
<td>Standard deviation of ln F</td>
<td>0.3003</td>
</tr>
<tr>
<td>( H )</td>
<td>Plant height (m)</td>
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</tr>
<tr>
<td>( h )</td>
<td>Vegetation height (m)</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Wind speed (m s(^{-1})) at 10m</td>
<td>1.7257</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>(\sigma_U)</td>
<td>Standard deviation of ln U</td>
<td>0.7288</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>Projected population growth rate</td>
<td>363</td>
</tr>
<tr>
<td>(c^*)</td>
<td>Projected spread rate (m year(^{-1}))</td>
<td>52</td>
</tr>
</tbody>
</table>
Modifications on parameter estimates based on the present study are in bold. Others were assumed to be the same as in previous studies. Warm: plots that were warmed using OTCs. Water: plots that received a 30% increase in winter precipitation. Warm + Water: plots that received both warming treatment and winter precipitation addition.

* The estimations for the experimental population were based on a previous experiment conducted in Pennsylvania (K. Shea unpublished data 2002-2005), where competition and floral herbivory were suppressed by regularly weeding and application of insecticide after bolting. Please refer to Jongejans et al. [3] for more details about the experiment.

† The estimations for the natural population were based on a naturally occurring population in Kansas which has been described in Lee and Hamrick [9].
Figure 5-S1. Modelling results based on a natural population. Mean percentage changes in fall seedling emergence, survival, capitulum production, and flowering plant height due to warming (W, in red) and mean percentage change in seed number per capitulum due to both warming and increased winter precipitation (WP, in purple) (A) and their corresponding contributions to increased spread rate (B). Solid: demographic vital rates. Hatched: dispersal-related traits. The spread rate was 21 m/year for ambient treatments and 32 m/year for warmed treatments with increased winter precipitation. Spread rates were calculated based on a natural population in Kansas [3] using the c*-LTRE technique. The calculated percentage change in reproduction (+4.9%) combines both increased capitulum production and decreased seed production per capitulum, as the original data only contained the product of these two terms: seed production per flowering plants [9].
Supporting references


Chapter 6

Warming leads to divergent responses but similarly improved performance of two invasive thistles

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Abstract

Plants’ responses to climate change are complex. Even the same net performance changes may involve different responses of multiple life history traits. Here we show that two congeneric thistles, *Carduus nutans* and *Carduus acanthoides*, both grew taller under increased temperature, albeit following divergent response patterns. For *C. nutans*, warming advanced bolting more than flowering, leading to a longer growing period before flowering and ultimately taller plant height at the end of the growing season. *C. acanthoides* maintained the same length of growing period because of equally shifted events in the phenological sequence, however, post-flowering growth rate was increased, which also led to enhanced final plant height. As seeds from taller plants disperse farther, their responses imply that future invasion spread rates of these two species will increase. Furthermore, similar consequences due to divergent responses in life history traits, as demonstrated in this study, suggest that considering only ultimate performance outcomes is not enough to understand the impacts of climate change.

Key words
climate change, divergent responses, global warming, invasive species, life history trait, phenological sequence
Introduction

Plant life histories are usually characterized by a set of life history traits, such as age at first reproduction, number of offspring, survival and size (Molles 2006). All these traits act and interact with each other to affect plant performance. Natural selection generally operates on such life history traits to maintain high levels of fitness across environments (Richard et al. 2006). However, selection forces and constraints may vary for the same traits in different species and also for different traits within a species (Post et al. 2008). Therefore different plants may exhibit various levels of plasticity in their traits, which will consequently lead to different performance outcomes under environmental change. For example, Chapin et al. (1995) found that elevated temperature increased biomass production of shrubs but decreased production of nonvascular plants; Nepstad et al. (2007) found that lianas had much lower mortality than trees and palms in tropical forests under climate-change-driven drought; Tang et al. (2006) found biomass of C3 forbs was enhanced the most under elevated CO₂, followed by C4 grasses and C3 grasses; a recent meta-analysis by Way and Oren (2010) showed that warming enhanced growth of deciduous species more than that of evergreen species. However, many such generalizations are based on the ultimate performance outcomes (e.g. biomass production, survival, etc.), and therefore do not include information on changes in the life history traits leading to such performance consequences. For example, increased aboveground biomass accumulation could result from a prolonged growing season (Rammig et al. 2010), enhanced assimilation rates (Ziska 2003), or simply from shifts in resource allocations between storage and growth (Pendall et al. 2011). Therefore, assessing only the ultimate outcomes is far from enough to thoroughly understand species’ responses to climate change. Plant life history traits that are related to these outcomes should also be taken into consideration.

Phenology, a key life history trait, is a sensitive indicator of recent global warming (Sherry et al. 2006, Hoffmann et al. 2010). Many studies have documented shifts in plant
phenology due to elevated temperatures (Fitter et al. 1995, Sparks and Carey 1995, Bradley et al. 1999, Fitter and Fitter 2002, Menzel et al. 2006, Miller-Rushing and Primack 2008). For example, a recent global meta-analysis documented a mean advancement of spring events of 2.3 days per decade (Parmesan and Yohe 2003); Lesica and Kittelsen (2010) found significantly advanced flowering dates for 75% of 32 species in the period from 1995 to 2008, though conversely, warming was found to delay spring phenology of plants on Tibetan Plateau (Yu et al. 2010). However, studies typically consider single phenological events in the botanical literature (Primack 1987), for instance, first flowering date, and therefore are limited in their power to capture whole-life responses. Conclusions drawn based on isolated single events may be misleading (Rathcke and Lacey 1985).

Study of phenological sequences, which comprise sequential phenological events (e.g. emerging, flowering, fruiting, and senescence), is a more effective way to assess species’ responses to climate change (Post et al. 2008). Phenological sequences present not only shifts in single life history events, but also changes in the intervals between them. In particular, uneven shifts in phenological events may occur as a result of different plasticities at different life stages, leading to either extended or shortened durations in various developmental stages (Post et al. 2008, Steltzer and Post 2009). For example, later onset of flowers and earlier senescence leads to a compressed reproductive period, which can reduce reproductive success (Cooper et al. 2011). Advanced leaf unfolding and unchanged leaf fall in Tilia cordata and Fagus sylvatica lead to a longer growing season under warming, which can potentially alter the competitive outcomes with other species (Kramer 1995).

Although the framework of phenological sequences and aggregate life history was originally developed with reproductive phenology in mind (Post et al. 2008), and has been applied mainly to reproduction since then (Haggety and Galloway 2011, Cooper et al. 2011), it has general applications to any plant performance measure that contains phenological
components, for instance, plant height. Plant height plays an important role in survival, competition, and spread (Westoby 1998, Westoby 2002, Skarpaas and Shea et al. 2007). For wind-dispersed species in particular, plant height strongly affects plant population spread rate, primarily through wind dispersal – pollen and seeds from taller plants usually travel longer distances than those from shorter plants in windy conditions (Bullock et al. 2006, Sundberg 2010). Phenology influences plant height increases in that the growth process is bounded by early phenological events (such as emergence and bolting), and cessation, which usually occurs after flowering begins (Rathcke and Lacey 1985). As events in this phenological sequence may not respond equally to the same change in the environment, length of time for growth may also change (Kramer 1995), which can in turn affect plant height at the dispersing stage.

In addition to phenology, ultimate plant performance is also affected by the speed with which plant traits change over time. In particular, plant growth rate, which defines the relationship between time and size, is also an essential life history trait that should not be neglected (Arendt 1997). Faster growth ensures plants quickly reach a critical size threshold for survival and reproduction while slower growth extends plant lifespans because of slow depletion of limited resources (Grime and Hunt 1975). Under mildly increased temperatures, growth rate may accelerate in some species, mainly due to enhanced photosynthesis but also depending on resource allocation (Xiong et al. 2000). Therefore changes in growth rate under global warming are also expected to affect plant performance.

Here we examine plant growth in height of two wind-dispersed congeneric species *Carduus acanthoides* and *C. nutans* in response to warming. Plant height plays a critical role in seed dispersal of both wind-dispersed species (Skarpaas and Shea 2007, Jongejans et al. 2008). Previous results show that increased plant height under warming contributed significantly to increased population spread rates of *C. nutans* (Zhang et al. 2011; Chapter 5). Growth in height of *Carduus* spp. arises in relation to several key consecutive phenological events (specifically,
bolting, flowering, and growth cessation), and depends on growth rates at different developmental stages (i.e. before and after flowering). In this study, we not only assess the ultimate outcomes of warming on plant height for these two species, but also examine the responses of multiple height-related life history traits (specifically phenology and growth rate) that lead to these outcomes. We aim to answer the following two questions: 1) Are the ultimate plant heights of the two species altered by warming? 2) If so, is the altered height due to different response strategies: changes in growth rate (Figure 6-1a), growing duration (Figure 6-1b), or both (Figure 6-1c)? We thus demonstrate a novel application of the phenological sequence framework (Post et al. 2008), which was developed to address reproduction, to plant growth responses to climate change. We expect our results to improve our understanding of the responses of Carduus spp. to global warming in particular, and moreover, to provide important insights on the processes underlying such responses in the face of climate change.

**Methods**

**Study species**

*Carduus nutans* L. (musk or nodding thistle: Asteraceae) and *C. acanthoides* L. (plumeless thistle: Asteraceae) are native to Eurasia and have invaded rangelands, grasslands, roadsides, and disturbed habitats all over North America (Allen and Shea 2006). Both species are short-lived monocarpic perennials. Their seeds germinate in fall or spring to form flat rosettes, which bolt one or more years after germination, depending on rosette size and vernalization (Shea et al. 2005). Bolting occurs in early summer when the main stem elongates, followed by rapid growth in height (plants can grow up to 2m, Desrochers et al. 1988). Flowering occurs in order from the terminal capitulum to lower branches, followed by production of a large number of wind-dispersed seeds (Desrochers et al. 1988). In *C. nutans*, earlier terminal capitula are developed at a taller height than later terminal capitula whereas in *C. acanthoides* the reverse is
true (Zhang, *pers. obs.*). The two species have different morphologies. *C. acanthoides* has more stems, and more but smaller capitula than *C. nutans* (Desrochers et al. 1988). The phenology of the two species is also different. *C. nutans* bolts and flowers early and has a short growing season while *C. acanthoides* has a later phenology, but a longer growing season, with more continuous flowering (Rhoads and Block 2000). *C. nutans* also has larger, heavier, and more stress-tolerant seeds than *C. acanthoides* (McCarty et al. 1969). Seedlings of *C. nutans* can emerge from a greater depth than seedlings of *C. acanthoides* (McCarty et al. 1969). *C. acanthoides* has a better tolerance to frequent disturbances than *C. nutans* (Zhang et al. 2011).

**Field experiment**

We conducted a two-year field experiment for two independent cohorts (cohort 2007-2008 and cohort 2008-2009) in an old pasture at The Russell E. Larson Agricultural Research Farm at Rock Springs, Pennsylvania (latitude 40.711°, longitude -77.942°). Yearly temperatures for this area vary from -6.7°C (mean minimum temperature in January) to 28.1°C (mean maximum temperature in July), with an average precipitation of 933 mm (Nord et al. 2010). The soil on the experimental site was Hagerstown silt loam. Common species in the pasture included *Arrhenatherum elatius, Dactylis glomerata, Elytrigia repens, Phleum pratens, Linaria vulgaris, Plantago* spp., *Taraxacum* spp., *Trifolium* spp. and *Galium* spp.

We disked the soil on the experimental site to suppress all aboveground biomass before starting each cohort. For each cohort, we set up ten blocks, each of which contained eight 2m×2m plots (four plots for each species). We randomly assigned half of the plots to receive ambient temperatures, while the other half were warmed by Open Top Chambers (OTCs). These chambers were 0.4m in height and 1.5m in diameter, and were built and installed based on the International Tundra Experiment (ITEX) manual (Molau and Møgaard 1996). These chambers have been widely used to increase temperature in various ecosystems (Dorrepaal et al. 2004, Post and Pederson 2008, De Frenne et al. 2010). In our study, they successfully increased soil surface
temperatures by 0.58°C on average over the two-year period (corresponding to an annual increase of 97 degree days), which presents a mild increase based on regional climate projections (increases of 2.9°C - 5.3°C in annual surface air temperatures by the end of this century, Hayhoe 2007). We did not find any significant side effects of these chambers on soil moisture and snow depth, and therefore assume that warming was the predominant abiotic change caused by these chambers. The original experimental design also included precipitation addition treatments (Zhang et al. 2011), which however had little effect and is not a focus of the present study.

We grew our plants as winter annuals. The seeds we used for the two cohorts were collected from natural populations in Pennsylvania in 2007 and 2008. We germinated these seeds in the greenhouse, and transplanted four three-week-rosettes to each plot in October 2007 and October 2008. Initial rosette size was recorded immediately after transplanting. Rosettes overwintered in the field. In the following growing season, we conducted weekly censuses, which included phenological status (rosette, bolting, and flowering), rosette size and plant height. Bolting date was defined as when the main stems reached 10 cm. Flowering was defined as the first display of purple florets on flower heads at a census. Pollen bags were put onto flower heads before seeds dispersed in order to prevent further infestations. The experimental cohorts were terminated at the end of July in each year.

Model estimation of growth cessation

The growth of plant height follows a sigmoid curve, with little change in height towards the end of the growing season. In order to estimate when growth cessation occurs, we fit our weekly height data to a three-parameter sigmoidal model:

$$H(t) = \frac{H_{\text{max}}}{1 + e^{-at}}$$

(eqn 1),

where t is the number of weeks since bolting (t=0) and H(t) represents plant height at time t. $H_{\text{max}}$, a, and b are three parameters which control for asymptotic height, initial plant height, and the
speed approaching the asymptote, respectively. This function has been shown to successfully characterize plant growth functions in previous studies (Kaufmann 1981, Sun and Freligh 2011). We then estimated date of growth cessation for each flowering plant (222 C. acanthoides and 203 C. nutans) as the time when plants reached 95% of their asymptotic height, H_max.

**Data Analysis**

We included in the data analyses all flowering plants that had approached their maximum height by the end of the experiment (H_end ≥ 95%H_max). We excluded plants that did not have good model fits (r^2 < 0.8). We used plot averages (the average of all plants within a plot, to avoid pseudoreplication) to assess the effect of warming on: phenology (bolting, flowering, growth cessation); durations between phenological events; plant height at first flowering and at the end of the experiment; and growth rates before and after flowering.

We used generalized linear mixed models (GLMMs, Crawley 2006) to examine the effect of warming on phenology, durations between phenological events, plant height at first flowering and at the end of the experiment, as well as absolute growth rates (ΔH/Δt) before and after flowering. In these models, we used warming as a fixed effect, cohort and block as random effects, and initial rosette size as a covariate.

**Results**

Ultimate plant height at the end of the experiment was greater under warming than under ambient conditions for both species (P=0.004 for C. acanthoides; P=0.0016 for C. nutans). Warming significant increased plant height at first flowering for C. nutans (P<0.001, Figure 6-3c), but not for C. acanthoides (P=0.39, Figure 6-3a).

Generally, the phenology of C. acanthoides was later than that of C. nutans in both warmed and ambient plots (P<0.001, n=154). Furthermore, C. acanthoides had a longer total growing duration (between bolting and cessation) (7.80±0.12 weeks versus 6.96±0.15 weeks,
n=154, P<0.01), a shorter pre-flowering growing duration (between bolting and flowering) 
(4.22±0.09 weeks versus 4.59±0.10 weeks, n=154, P<0.001), and a longer post-flowering 
growing duration (between first flowering and growth cessation) (3.59±0.07 weeks versus 
2.37±0.09 weeks, n=154, P<0.001) than *C. nutans*. *C. acanthoides* also had a slower overall 
growth rate (17.02±0.35 cm/week versus 20.40±0.65 cm/week), a slower pre-flowering growth 
rate (19.83±0.49 cm/week versus 23.55±0.82 cm/week, n=154, P<0.001) than *C. nutans*. However, the post-flowering growth rates were not different between the two species (13.53±0.28 

cm/week versus 12.86±0.43 cm/week, n=154, P=0.28).

Warming advanced the phenology of both species (**Figure 6-2**). For *C. nutans*, the 
advance of bolting date was larger than the advance of first flowering, leading to an increased 
duration between the two phenological events (i.e. pre-flowering growing duration, 4.86±0.13 
weeks versus 4.33±0.14 weeks, n=75, P=0.003); post-flowering growing duration was unchanged 
(P=0.89). As a result, the total growing duration was longer under warming (7.22±0.24 weeks 
versus 6.69±0.19 weeks, n=75, P=0.03, **Figure 6-2**). For *C. acanthoides*, none of the durations 
were different between warming and ambient (n=79, P>0.05).

For *C. nutans*, the growth rates were not different between warmed plots and ambient 
plots (P>0.05, **Figure 6-4c**, Fig 4d). For *C. acanthoides*, the growth rate before flowering was not 
different between warmed plots and controls (P=0.39, **Figure 6-4a**), while the growth rate after 
flowering was significantly higher under warming (P=0.013, **Figure 6-4b**).

**Discussion**

In our study, two congeneric thistles both had enhanced ultimate height under warming. 
This effect increases seed dispersal and hence the overall plant performance under warming 
(Zhang et al. 2011). However, enhanced plant height in the two species clearly arises from 
different proximal causes: *C. nutans* extended the duration of its growing period while
maintaining the same growth rate, while *C. acanthoides* accelerated its post-flowering growth rate, but over the same duration of growing period.

Our results demonstrate that consecutive phenological events may not shift evenly under warming. Despite possible correlations between the dates of consecutive phenological events (Rathcke and Lacey 1985), these events may display different responses to temperature increases. This is because the selection force and constraints for each life stage may be different (Fenner 1998, Post et al. 2008) - in addition to temperature, other environmental cues such as light and moisture may also affect phenology (Rathcke and Lacey 1985, Bernier 1988, Fenner 1998, Korner and Basler 2010). In a phenological sequence where phenological events are constrained by different environmental cues, or by the same cues but to different degrees, unequal shifts are likely to occur, causing extended or shortened intervals in between. For *C. nutans*, bolting was advanced more than flowering under warming, suggesting that bolting is more sensitive to temperature increases than flowering. This result agrees with previous observations that the bolting dates of *C. nutans* vary a great deal from year to year while flowering date has little variations (Roeth 1979), which suggests that flowering relies more on photoperiod rather than on temperature. In contrast, bolting date of *C. acanthoides* and its subsequent events were evenly advanced, leading to an unaffected duration of growing period under warming. Reproduction of both *C. acanthoides* and *C. nutans* requires vernalization, suggesting that development of both species depends more or less on temperature. However, the extent of dependence varies between the two species, and also between different life stages for the same species, leading to the two species’ divergent phenological responses to warming.

It appears that the enhancements in performance for the two species occur at different life stages. For *C. nutans*, increases in plant height were already clear at first flowering, while for *C. acanthoides*, increases in height were not significant until the end of the growing season. The mechanisms for these different responses are not entirely clear, especially because the two species
experience different environments at particular phenological dates due to the offset in their
developmental timing (with *C. nutans* having earlier phenology). However, life history
differences between the two species could also play a role. *C. acanthoides* has a more continuous
growth pattern than *C. nutans*, and its flowering does not strictly limit its vegetative growth.
Compared to *C. nutans*, whose first capitulum is usually taller than later-developing capitula, *C.
acanthoides* starts to flower at lower heights and the lateral capitula usually grow past the primary
capitulum in the center. Therefore in *C. acanthoides*, warming predominantly enhanced the
height of later-developing capitula.

In response to the increase in temperature, the two thistles showed divergent response
patterns. Similar performance outcomes (specifically, enhanced plant height at the dispersing
stage) arose from these patterns, suggesting that only assessing the ultimate outcomes may
neglect important underlying processes. A similar case has been seen for two tropical vines which
both increased height under elevated CO$_2$ (Condon 1992). While one species increased its
resource allocation to shoots compared to roots, the other species maintained the same shoot: root
ratio under elevated CO$_2$.

In this study, we merged the phenological sequence framework developed by Post et al.
(2008) with an analysis of duration and rates of growth, to demonstrate that different plant
responses to warming can nevertheless result in similar performance outcomes. A similar analysis
of the duration and rates of reproductive effort would be a valuable future direction; enhanced
reproductive outputs under climate change may be due either to accelerated rates of reproduction,
or to an extended reproduction period (Dunne et al. 2003, Sherry et al. 2007). Direct estimates of
plant performance, for example, height, biomass, and reproduction, only reflect the ultimate
outcomes of complex responses of different life history traits. A deeper understanding of the
effects of climate change, and reliable predictions of species performance, will require a thorough
examination of related life history responses.
Acknowledgements

RZ and KS conceived and designed the experiment. RZ performed the experiment and data analysis. RZ wrote the first draft and all three authors helped to edit the chapter. We are grateful for logistical assistance and/or comments from S. Yang, A. Miller, L. Russo, S. Smiles, B. Teller, D. Mortensen, O. Bjørnstad, and many Shea lab undergraduates. We acknowledge the support of NSF grant #DEB-0815373 to K. S..

References


Figure 6-1. Different processes leading to enhanced plant height at the end of the growing season. A: growth rate is enhanced under warming while duration between bolting and growing cessation remain unchanged. B: duration is longer under warming while growth rate remains the same. C: both growth rate and duration are enhanced under warming.
Figure 6-2. Effects of warming on phenological events (bolting, flowering, and growth cessation) and durations between them. “ns”, “*”, “**”, and “***” denote the statistical significance between warmed plots and ambient plots. ns: P ≥ 0.05; * P < 0.05; ** P < 0.01; *** P < 0.001. The error bars for phenology are too small to be presented.
**Figure 6-3.** Effects of warming on plant height at first flowering and height at the end of the experiment.
Figure 6-4. Effects of warming on growth rates before and after first flowering.
Chapter 7

Decreased structural defense of an invasive thistle under warming

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Abstract

Plant structural defenses play a key role in preventing fitness loss due to herbivory. However, how structural defenses are affected by potential climate change is rarely examined. We examined how leaf morphological traits that relate to the structural defense of an invasive thistle Carduus nutans change in a warmer climate. We manipulated warming using open top chambers (OTCs) and examined the morphology of leaves at three different positions (the fifth, tenth, and fifteenth leaves counted from the top of the plant) in two destructive summer censuses. We found that structural defense traits were different under ambient versus warmed conditions. Prickle densities (both the number of prickles per leaf area and the number of prickles per leaf mass) were significantly lower in plants grown in a warmer climate. Our results suggest that plant structural defenses may be reduced under warming, and therefore should be considered when examining species responses to climate change.

Key words

Carduus nutans, structural defense, prickles, climate change, warming
Introduction

Plant structural defenses such as thorns, spines, prickles, and trichomes play an important role in preventing biomass loss to herbivores (Herms and Mattson 1992, Lucas et al., 2000). These physical armaments either deter herbivores from feeding, or reduce their feeding efficiency, and thereby increase plant fitness (Cooper and Owen-Smith, 1986). However, despite the prevalence and importance of structural defenses in a wide variety of ecosystems, structural defenses have been relatively ignored (Grubb, 1992, Gowda, 2003, Hanley et al., 2007), especially compared to their counterpart - chemical defenses.

Plant structural defenses, such as spinescence, are carbon-based defenses, and their production requires carbohydrates from photosynthesis (Myers and Bazely 1991). Because physical armaments are constructed from the same materials as plant biomass and therefore are limited by the same resource (Skogsmyr and Fagerström 1992), trade-offs between defense and growth are likely to occur (Read and Stokes 2006, Matyssek et al., 2002). For example, in suboptimal environments where growth is inhibited by limiting resources such as nutrients and water, accumulated carbohydrates are likely to be allocated to differentiation (i.e. defense), leading to increased defenses (Herms and Mattson, 1992). On the other hand, in optimal environments, growth processes receive resource allocation priority and reduce the relative carbon availability to support defenses (Herms and Mattson, 1992).

Ongoing climate changes such as increased temperature, elevated CO₂, and shifted precipitation regime may affect local resource availability (Vitousek, 1994, Dukes and Mooney 1999), which may in turn potentially change plants’ investments in defense. Researchers have recently started to examine alterations in chemical defenses under climate change (Coley et al., 2002, Zavala et al., 2008, Peñuelas and Staudt 2010). However, studies examining structural defense under climate change are rare (Ziska 2002). Here we present a study to examine how leaf morphology, specifically prickle density, of an invasive thistle changes under warming. Increased
temperature affects many physiological processes in plants, such as photosynthesis, respiration, and growth (Hughes, 2000); warming may also shift allocation between growth and defense. Therefore, changes in morphological defense traits due to warming may be expected, depending on the relative resource allocations to defense versus growth.

Our study species, *Carduus nutans* L. (nodding or musk thistle: Asteraceae) is an introduced Eurasian weed, which causes major economic problems in many regions of the world (Shea et al., 2006). *C. nutans* is a short-lived monocarpic species (Desrochers et al., 1988); plants can grow up to 2 m tall and form dense monocultures of up to 150,000 plants per hectare (Desrochers et al., 1988). Plants are prickly on both stems and leaves. Leaves are deeply pinnately lobed, elliptic to lanceolate, and the margins are tipped with stiff and sharp prickles (Uva et al., 1997). Spinescence of *C. nutans* prevents livestock from grazing, both on thistles and on other plants growing in the vicinity, reducing overall productivity in pastures and rangelands (Desrochers et al., 1988). Therefore potential changes in its structural defense may provide insights for mitigating its damage and controlling its invasion using large herbivores.

**Methods**

The experiment was conducted in an old pasture at The Russell E. Larson Agricultural Research Farm at Rock Springs, Pennsylvania (latitude 40.711°, longitude -77.942°). The climate of central Pennsylvania is continental, with mean annual precipitation of 975 mm and mean monthly temperatures ranging from 3°C in January to 21.6°C in July (Smith et al. 2009). The experimental site was on a gentle northwest-facing slope, bounded on two sides by unpaved roads. The soil on the experimental site was Hagerstown silt loam. Dominant species in the experimental site included *Arrhenatherum elatius*, *Dactylis glomerata*, *Elytrigia repens* and *Phleum pratense*. Other common species included *Linaria vulgaris*, *Plantago* spp., *Taraxacum* spp., *Trifolium* spp., and *Galium* spp.. The experimental field was disked prior to the experiment
to kill all aboveground vegetation to mimic the high invasion success of *C. nutans* in habitats with disturbed soils.

We used fiberglass open top chambers (OTCs, Marion et al., 1997) to elevate temperatures passively. These OTCs were 0.4 m in height and 1.5 m in basal diameter, and successfully elevated average daily soil surface temperatures by 0.58°C across seasons. This corresponds to a mild increase in temperature compared to the regional climate projections for this area (increases of 2.9°C - 5.3°C in annual surface air temperature by the end of this century, Hayhoe et al. 2007). Cumulative degree-days for this species (calculated based on McCarty, 1985), were significantly higher in plots with OTCs, with the largest increase in spring (an increase of 81.40 ± 11.65 degree-days).

We set up five blocks, with two 2 m x 2 m plots (a warmed plot and an ambient temperature plot) per block. An OTC was placed in the middle of each plot receiving the warming treatment. In October 2008, four 3-week-old seedlings (germinated from seeds collected from a natural population in Pennsylvania) were transplanted into the center of each plot. Initial rosette sizes were recorded after transplanting. Rosette size, plant height, and key phenological dates were recorded in weekly censuses starting in April 2009.

We collected leaf samples at two censuses; one in mid-June 2009 at the start of anthesis and the other in early July 2009 at the peak of flowering. Our repeated measurements account for potential ontogenetic effects (Hanley et al., 2007). One plant in each plot was chosen so that focal plants in each block had similar phenology. We collected leaves at three positions on the plants: the fifth, tenth, and fifteenth leaves from the apical inflorescence. These leaves are of different ages, with the youngest leaf on top. Thus, we carefully controlled for the possible effects of leaf age (Wilkens et al., 1996), which is confounded in studies using a complete random sampling method.
Leaf thickness was measured as the average thickness of three random locations (excluding veins) on a leaf. Prickles longer than or equal to 3mm were counted on each leaf sample, and the length of these prickles was also recorded. Leaf samples were then scanned with a printed ruler using an EPSON GT-15000 scanner (Epson America, Inc., Long Beach, California, USA) at a resolution of 150 dpi. To reduce overlapping leaf portions in scanned images, leaf samples were dissected prior to scanning. Leaf areas and leaf length were calculated after converting scanned images to 8-bit binary images in ImageJ 1.40g (Rasband, 1997-2009). Leaf samples were then oven dried at 70°C for 24h and weighed.

The first flowering dates of the plants were analyzed using a pairwise t-test to examine possible phenological differences between treatments. Leaf mass, leaf length, leaf thickness, leaf area, specific leaf area, number of prickles and prickle density (both number of prickles per unit leaf area and number of prickles per unit leaf dry mass) were analyzed with generalized linear mixed models (GLMMs, Crawley, 2007) in R (R development group, 2008). Log transformation was used for all responses in order to ensure normality. Leaf position, treatment (warmed or ambient), collection date, and their interactions were included in the full models as fixed effects. Initial rosette size at transplanting was included as a covariate as the growth and reproduction of these thistles are size dependent (Shea et al., 2006). Collection date nested within plant was used as a random effect for temporal repeated measures; leaf position nested within plant was used as a random effect for spatial repeated measures (to account for leaves sampled from the same individual plant). Stepwise model simplification was based on lower Akaike's information criterion (AIC) values.

Principal Component Analysis (PCA) was conducted based on the log transformed averages of leaf mass, leaf length, leaf thickness, leaf area, and number of prickles across two censuses and on all three leaf positions. A Linear Discriminant Analysis (LDA, Crawley, 2007)
was conducted to examine the contributions of these explanatory variables to the difference between plants grown under warmed and ambient conditions.

Results

The flowering dates of plants in warmed plots were not significantly different from control plots (169.0 ± 1.9 Julian day (warmed) versus 171.8 ± 1.3 Julian day (ambient), paired t-test, n = 10, $P = 0.18$). No obvious herbivory was found on the leaves. Two outliers of the very young leaves (leaf position 5) were excluded from the original dataset.

Effects of leaf position and warming treatment were consistent for both censuses. Leaf position had a significant effect on all leaf morphological responses ($P < 0.05$). Older leaves were bigger, longer, thicker, heavier, and had more prickles in total, but with lower prickle densities and higher specific leaf area. Initial rosette size was a significant covariate ($P < 0.001$) for all responses except for prickle densities and specific leaf area. Warming had a significant negative effect on prickle densities (Figure 7-1). However, the effect of warming is not significant ($P>0.05$) on other leaf morphological traits (Table 7-1).

The PCA shows that the first principal component explains 89.7% of the total variance. Number of prickles, leaf area, leaf length, dry weight, and thickness contribute equally to the total variance (Table 7-1). The second principal component explains 7.0% of the total variance, but explains more variation between the warmed treatment and the control (Figure 7-2). The LDA shows the disproportionate contribution of leaf thickness to the leaf morphological variation between warmed plants and the controls.

Discussion

Our study shows that structural defense (i.e. prickle density) of an invasive thistle *C. nutans* was reduced under manipulated warming. We ensured that this morphological change was
attributable to the treatment effect of warming by carefully controlling for plant phenology (by choosing phenologically comparable plants and confirming equivalent flowering dates), plant ontogeny (by using repeated measures) and leaf development (by collecting leaves from different positions on the plant). All these factors may confound with leaf morphology and have rarely been considered in earlier studies (Wilkens et al., 1996, Hanley et al., 2007). In particular, we found leaf age can strongly affect leaf spinescence, with younger leaves (in higher positions) having a higher density of prickles than older leaves (in lower positions). This agrees with previous findings on ontogenetic variations in structural defense (Wilkens et al. 1996, Sanyal et al. 2006) and suggests that a strict sampling approach which considers leaf development order may be more informative than a completely random, or loosely designed, sampling method to examine plant structural defenses.

Construction of plant physical armors requires excess carbon (i.e. photosynthates) to accumulate in plants in amounts exceeding the needs of growth, (Estiarte and Penuelas, 1999), and therefore depends on both photosynthesis and plant growth (Herms and Mattson, 1992). Factors that cause changes in photosynthesis and/or in growth are thus likely to affect plant investment in structural defenses. For example, Fisher et al. (2002) showed that elevated irradiance increased the thorn density of *Artabotrys hexapetalus* in shaded sites. Wilkens et al. (1996) found that *Lycopersicon esculentum* grown in high-light-high-water conditions generally had lower leaf trichome densities than those growing in low-light-low-water conditions. Ziska (2002) showed that *Cirsium arvense* developed more and longer prickles under elevated CO₂ concentration. However, to our knowledge, no study so far has examined warming effects on structural defense.

Our results demonstrate that increased temperature may affect leaf morphology and its related functions, such as defense. As weakened structural defense reduces the inhibitory effect on herbivore bite sizes and consumption rates (Cooper and Owen-Smith 1986), altered prickle
density of *C. nutans* may affect management effectiveness of this invasive species under climate change. Even though, generally, herbivory of *C. nutans* is rare, previous evidence found that large mammals like goats can graze on flowering *C. nutans* (Popay and Field, 1996, Holst et al., 2004). Thus the grazing efficiency of goats may be improved when plants develop prickles at a lower density, making goat grazing a more practical management approach to control this invasive species. Furthermore, reduced spinescence may increase the palatability of this invasive plant to other large herbivores such as cattle and sheep, reducing its economic cost, and improving control. Hence the potential impact of reduced structural defense on herbivore preference is worth pursuing in future studies.

Climate change affects many aspects of plant life history, among which structural defenses should not be overlooked. While we only considered abiotic warming effects on structural defense of an invasive species, other aspects of climate change such as elevated CO₂, increase nitrogen deposition, and shifted precipitation regimes may alter plant structural defenses as well. Furthermore, biotic factors such as herbivore pressure may also come into play. These factors could act synergistically or antagonistically with increased temperature to determine structural defenses. Nevertheless, our study does suggest potential changes in structural defense of some invasive species under future climate may occur. We hope further research will consider multiple abiotic environmental changes and biotic factors, as well as their interactions, as they may impose both challenges and opportunities for the control of invasive species under climate change.

**Acknowledgements**

RZ conceived and designed the experiment. AL scanned the leaf samples and measured leaf traits. RZ performed data analysis. All authors contribute to writing this chapter. We thank Liza Senic for her assistance in the field and Suann Yang, Adam Miller, Laura Russo, and Britta Teller
for their invaluable comments and suggestion. We acknowledge the support of USDA-CSREES (Biology of Weedy and Invasive Plants) NRI grant #2002-35320-12289 and NSF grant #DEB-0815373.

References


Ithaca, New York.
Table 7-1. Comparisons between ambient and warmed treatments based on generalized linear models (GLMMs), coefficients of the first two principal components based on the principal component analysis (PCA) and the linear discriminants based on the linear discrimination analysis (LDA).

<table>
<thead>
<tr>
<th></th>
<th>GLMMs *</th>
<th></th>
<th>PCA</th>
<th></th>
<th>LDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ambient</td>
<td>Warmed</td>
<td>PC1</td>
<td>PC2</td>
<td>LD</td>
</tr>
<tr>
<td>Leaf length</td>
<td>14.7 ± 0.9 cm</td>
<td>15.0 ± 1.4 cm</td>
<td>0.45</td>
<td>-0.35</td>
<td>7.18</td>
</tr>
<tr>
<td>Leaf area</td>
<td>61.0 ± 8.4 cm²</td>
<td>70.4 ± 14.7 cm²</td>
<td>0.45</td>
<td>-0.39</td>
<td>-1.42</td>
</tr>
<tr>
<td>Leaf mass</td>
<td>0.48 ± 0.06 g</td>
<td>0.52 ± 0.10 g</td>
<td>0.46</td>
<td>-0.29</td>
<td>6.03</td>
</tr>
<tr>
<td>Thickness</td>
<td>0.40 ± 0.04 mm</td>
<td>0.35 ± 0.04 mm</td>
<td>0.44</td>
<td>0.39</td>
<td>-16.75</td>
</tr>
<tr>
<td>Total prickles</td>
<td>107.0 ± 13.8</td>
<td>78.3 ± 12.1</td>
<td>0.42</td>
<td>0.70</td>
<td>-3.11</td>
</tr>
</tbody>
</table>

*None of the listed leaf traits is significantly different between ambient and warmed treatments (P>0.05); neither do specific leaf areas calculated based on leaf area and leaf mass (P>0.05).

Numbers are presented as mean ± s.e.
Figure 7-1. Effects of warming on standardized prickle densities (a. number of prickles per leaf area, b. number of prickles per leaf mass) of leaves of different positions (5th, 10th, or 15th leaf, counted from the top of the plant) of *C. nutans*. The bars represent the means of the two censuses where blank bars denote controls and grey bars denote the warming treatment. The error bars denote standard errors. Warming had a significant negative effect on both prickle density measures (mean ± s.e., 1.49 ± 0.1 prickles/cm² versus 1.96 ± 0.1 prickles/cm², t-value = -2.51, \( P = 0.027 \), Fig. 7-1a; 189.4 ± 12.8 prickles/g versus 243.8 ± 13.7 prickles/g, t-value = -2.24, \( P = 0.044 \), Fig. 7-1b).
Figure 7-2. Principal component analysis (PCA) using leaf morphological traits of number of prickles per leaf, leaf area, leaf mass, leaf thickness and leaf length. All variables were log transformed prior to the analysis. Leaves from warmed plots are represented by the solid dots while leaves from ambient plots are represented by the empty dots. The horizontal axis (PC1) and the vertical axis (PC2) accounted for 89.7% and 7.0% of total variation, respectively.
Chapter 8

Maternal warming affects early life stages of an invasive thistle

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Abstract

Maternal environment can influence plant offspring performance. Understanding maternal environmental effects will help to bridge a key gap in the knowledge of plant life cycles, and provide important insights for species’ responses under climate change. Here we show that maternal warming significantly affected the early life stages of an invasive thistle, Carduus nutans. Seeds produced by plants grown in warmed conditions had higher germination percentages and shorter mean germination times than those produced by plants under ambient conditions – this difference was most evident at suboptimal temperatures. Subsequent seedling emergence was also faster with maternal warming, with no cost to seedling emergence percentage and seedling growth. Our results suggest that maternal warming may accelerate the life cycle of this species via enhanced early life history stages. These maternal effects on offspring performance, together with the positive responses of the maternal generation, may exacerbate invasions of this species under climate change.

Key words Carduus nutans, warming, climate change, maternal environmental effects, early life stages, germination, seedling emergence
Introduction

Mother plants can affect offspring performance not only via genes inherited by the offspring, but also through the effects the maternal environment can have on resource allocation and reproductive output (Roach and Wulff 1987, Gallagher and Feurst 2006, Donohue 2009, Latzel et al. 2009). Previous studies have shown that the quantity and quality of resources during plant development, such as light, temperature, water, and nutrients, as well as disturbance regimes, can affect offspring fitness (Alexander and Wulff 1985, Agrawal et al. 1999, Lacey and Herr 2005, Sultan 2009, Latzel 2010). These maternal environmental effects are mediated via hormones and enzymes that are involved in all stages of ovule and seed development (Etterson and Galloway 2002), and hence may impact offspring early life stages (e.g. germination and seedling growth, Breen and Richards 2008). For example, long maternal day length decreased germination percentage of seeds of *Portulaca oleracea* (Gutterman 1974), but had an opposite effect on germination in *Polypogon monspeliensis* (Gutterman 1982a). Drought during seed maturation decreased dormancy of seeds of *Sorghum halepense* (Arnold et al. 1992), but increased dormancy in *Spergularia mariana* (Okusanya and Ungar 1983). Nutrient addition to maternal plants generally decreases seed dormancy of the offspring (Baskin and Baskin 2001), but can also increase seedling tolerance to nutrient deficiency (Aarssen and Burton 1990). Such maternal environmental effects are recognized as an important missing link in plant life cycles (Donohue 2009), population dynamics (Lacey and Herr 2000), and adaptive evolution (Galloway and Etterson 2007). However, maternal environmental effects can be relatively weak compared to the direct effects of offspring environment, and were not discernible in some cases (e.g. Olsson and Agren 2002, Monty et al. 2009).

Climate change, such as increases in ambient CO₂ levels and air temperatures, will not only likely affect plant fitness in the current generation (Hughes 2000), but may also influence offspring fitness via seed characters that are subject to changes in maternal environment. For
example, in many plants, even small differences in temperature during plant development and seed maturation can affect offspring germination (Gutterman 2000); elevated CO$_2$ in the maternal environment increased germination percentage of *Plantago lanceolata* (Wulff and Alexander 1985), but decreased germination percentage of *Arabidopsis thaliana* (Andalo et al. 1998). Many invasive plant species exhibit enhanced growth and reproduction under climate change (e.g. elevated CO$_2$ and warming, Dukes and Mooney 1999, Willis et al. 2010). Given the importance of early life stages in invasion success (Grotkopp et al. 2002), addressing the transgenerational maternal effects of climate change will be an important contribution to our understanding of invasions under climate change; here we specifically focus on the transgenerational maternal effects of the increased temperatures associated with climate change.

We examine how maternal warming during vegetative and reproductive development, affects subsequent seed germination and seedling growth of an invasive thistle - *Carduus nutans* L. (musk or nodding thistle, Asteraceae). This species is native to Eurasia and has invaded North America, Australia, and New Zealand (Shea and Kelly 1998, Shea et al 2010). It is a monocarpic short-lived perennial (Desrochers et al. 1988). Rosette survival, vernalization, flowering phenology, and life cycle of this species largely depend on rosette size (Lee and Hamrick 1983). Seedling recruitment mainly occurs in autumn and spring, followed by rosette development, which lasts for one or more years (Popay and Medd 1990). Flowering requires vernalization and occurs in the summer, beginning with the primary buds and proceeding towards the base of the plant (McCarty 1982). Both outcrossing and selfing occur in the species (Desrochers et al. 1988, Yang et al 2011). A large number of wind-dispersed seeds are produced in summer, and have little primary seed dormancy (Desrochers et al. 1988). Seed banks can persist for approximately 20 years (Kok 2001), probably because of environmental germination requirements associated with quiescence (Murdoch and Ellis 2000).
In our previous studies, we assessed the effects of warming on the maternal generation of *C. nutans*. We found that warmed *C. nutans* enhanced its survival, growth, reproduction, and dispersal while decreasing its physical defenses (i.e. leaf prickles density) (Zhang et al. submitted, Zhang et al. in press). In the present study, we examine the effect of maternal warming on offspring performance - seed germination and seedling growth. We expect invasion of this species to be further exacerbated if warming not only enhances the fitness of the current generation, but also improves offspring performance via maternal environmental effects. Our results thus complement the knowledge of responses of this species to climate change in particular, and provide general insights for incorporating maternal effects in evaluating invasion success under future climate.

**Methods**

*Field experiment with maternal plants*

The experimental field was located at The Russell E. Larson Agricultural Research Farm at Rock Springs, Pennsylvania (latitude 40.711°, longitude -77.942°). Yearly temperatures vary from an average minimum temperature of -6.7°C in January to an average maximum temperature of 28.1°C in July; average annual precipitation is 933 mm, approximately evenly distributed among months (Nord et al. 2010). The experimental field was an old pasture dominated by *Arrhenatherum elatius, Dactylis glomerata, Elytrigia repens* and *Phleum pratense*.

We used Open Top Chambers (OTCs) to passively increase temperatures in this study. These chambers were 1.5m in diameter, 0.4m in height, and constructed according to Molau and Mølgaard (1996). Such OTCs had been widely used to elevate temperatures in various ecosystems (Dorrepaal et al. 2004, Post and Peterson 2008, De Frenne et al. 2010). Using these chambers, we achieved an average increase in daily soil surface temperature of 0.58°C throughout the field experiment (Zhang et al. in press). Our warming treatment affected the whole plant
during the rosette stage, but was then limited to the lower part of the adult plant (average height
of bolting plants is approximately 1.5m). Although this increase of temperature is mild compared
to the regional projections of climatic models (increases of 2.9°C - 5.3°C in annual surface air
temperature by the end of this century, Hayhoe et al. 2007), we have found significant changes in
the life history of this species (Zhang et al. submitted, Zhang et al. in press).

We conducted our field experiments for two independent cohorts. For each cohort, we set
up 10 blocks, each of which included an ambient plot and a plot warmed by OTCs. We started
each cohort in fall by germinating in the greenhouse seeds from a natural population in
Pennsylvania. We then transplanted four 3-week-old rosettes into each 2 m × 2 m plot (with or
without an OTC) in the experimental field, where thistles overwintered as rosettes and reproduced
in the following summer. Thus maternal plants in this experiment were grown as winter annuals.
At the end of the following July, we randomly sampled one terminal mature flower head from
each plot. A total of 20 flower heads (10 in ambient plots and 10 in warmed plots) were collected
for each cohort. Seeds with intact embryos from each flower head were stored in a freezer (-
20°C) until the initiation of this experiment. Seed mass was also assessed for both warmed and
ambient conditions (n=40).

**Germination experiment**

We randomly chose 75 intact seeds from each flower head and plated them onto 3 Petri
dishes (25 seeds per dish) containing a 0.015% non-nutrient agar (Difco™, non-nutrient,
Maryland, USA). These Petri dishes were sealed using laboratory Parafilm M®, and then placed
into two SG30SC Controlled Environment Chambers (Hoffman Manufacturing Inc., Jefferson,
Oregon, USA). We tested seed germination under three different germination temperature
regimes (day/night) separately: 15C/15C, 20C/15C, and 25C/15C, with photosynthetically active
radiation (PAR) of approximately 42μmol·m⁻²·sec⁻¹. McCarty et al. (1969) demonstrates that
germination percentage of *C. nutans* increases between 15C and 28C (constant germination
temperature). Therefore we believe our 15C/15C treatment is suboptimal for germination of this species. We used these temperature regimes to simulate the range of temperatures during fall and spring germination in this area. Locations of Petri dishes were rotated every day. Seed germination (appearance of radical) was examined every day for two weeks. The viability of non-germinated seeds by the end of the experiment was assessed by pinching with a forceps - seeds with white-intact embryos were considered viable.

**Seedling emergence experiment**

Indicators of seedling vigor were measured on a subset of seeds from the same treatments evaluated for germination. Ten seeds were randomly chosen from each flower head. Two hundred cell planting flats (cell size =12.5cm³, ITML Horticultural Products Inc., Ontario, Canada) were filled with well-moistened soilless potting mix (SUNSHINE Pro Soil #2, Sun Gro Horticulture Canada Ltd., Vancouver, Canada, containing no added nutrients). Approximately 2 cm of the potting mix was placed between the cell tray and the main holding tray to help keep the soil in the cell moist. One seed per cell was planted at a depth of approximately 2mm. The flats were kept in the previously described growth chamber at temperatures set at 25C/15C (day/night) to simulate the suitable condition for seedling growth in fall and spring. We watered the flats regularly and recorded seedling emergence every day for two months. Aboveground seedling biomass was harvested when seedlings were two weeks old. Number of true leaves and the longest leaf length of each seedling were measured at the same time. Seedling biomass was determined after being oven-dried at 70C for 24 hours.

**Statistical Analysis**

Seed mass, germination percentage, mean germination time (MGT, calculated as the average date of germination, based on Battle and Whittington (1969)), number of dormant seeds, seedling emergence percentage, mean emergence time (MET, calculated similarly to MGT, as the average date of emergence, number of true leaves of two-week-old seedlings (plant average), and
biomass of two-week-old seedlings (plant average) were analyzed in R (R core team, 2009). We used Generalized Linear Mixed Models (GLMMs) to analyze our data. We specified quasibinomial error structure for germination percentage and emergence percentage, in order to account for overdispersion in the data. We specified Poisson error structure for number of dormant seeds and number of true leaves of two-week-old seedlings. We specified Gaussian error structure for seed mass, MGT, log-transformed MET (to ensure normality), seedling longest leaf length, and seedling biomass. For the germination experiment, we used germination temperature (15C/15C, 20C/15C, or 25C/15C), maternal treatment (warmed or ambient), and the interactions between the two as fixed effects. For the seedling growth experiment, we used maternal treatment (warmed or ambient) as the only fixed effect as only one germination temperature regime (25C/15C) was applied. For both experiments, initial rosette size (at transplanting) of maternal plants was used as a covariate in the full models, as the reproduction of this species is size dependent (Shea et al. 2006). We also included cohort and block as two random effects, in order to account for spatial and temporal variations. We then performed stepwise model simplifications based on lower Akaike's information criterion (AIC) values.

**Results**

Seed mass was not different between warmed and ambient conditions (3.57±0.11mg versus 3.48±0.10mg, warmed versus ambient, n=40, P>0.05). For the germination study, the interaction between maternal temperature and germination temperature was not significant (P>0.05). Although the overall effects of maternal warming were significant for both germination percentage (P=0.002) and MGT (P=0.01), differences within the same germination temperatures were not significant except for the lowest generation temperature (Figure 8-1). Higher germination temperature also led to more germinated seeds and shorter MGT than lower germination temperature (P<0.001) (Figure 8-1). Maternal warming and high germination temperature
temperature significantly reduced the number of dormant seeds at the end of the germination experiment (P<0.001). Maternal warming significantly shortened MET (P=0.04, Figure 8-2b), but did not have any effect on emergence percentage, number of true leaves, longest leaf length or biomass of two-week-old seedlings (P>0.05, Figure 8-2a, c, d). Initial rosette size of the maternal plant did not affect any of the responses (P>0.05).

**Discussion**

Our results demonstrate that maternal warming during plant development and seed maturation had a small but significant effect on the early life stages of *C. nutans*. Seeds from warmed maternal plants were less dormant and germinated faster than seeds from the ambient environment; this effect was most pronounced in the suboptimal germination temperature (15C/15C). This is probably because at optimal germination temperatures, where germination percentages are already very high, there is no additional advantage conferred by warming of the maternal generation. Consistent with faster germination speed, seedlings from warmed maternal plants also emerged faster than those from maternal plants grown in ambient conditions. Our results agree with previous studies on other monocarpic species, in which maternal plants were grown in controlled chambers or greenhouses. Schmuths et al. (2006) found *Arabidopsis thaliana* seeds from warm maternal environments germinated at higher percentages than those from ambient environments. Qaderi et al. (2006) found cypselas of another thistle, *Onopordum acanthum*, germinated faster when matured in warmer conditions. However, in a study where maternal plants were from natural populations at different altitudes, no maternal environmental effect was detected (Monty et al. 2009). In our study, maternal plants were grown in field conditions, similar to the plants’ natural habitats, which also minimizes variation of other environmental factors such as water and soil nutrients. Our results further confirm that maternal warming can accelerate development of such species, at least in their early life stages.
Furthermore, the size and biomass of two-week-old seedlings did not differ between treatments, suggesting that seedling vigor may not be penalized by the early emergence caused by maternal warming. However, unlike previous studies which found significant changes in seed mass due to maternal warming (Quaderi and Reid 2008), seed mass was not different between warmed and ambient conditions. This suggests that the observed differences in germination and emergence were probably due to other seed traits. For example, Lacey et al. (1997) found that maternal temperature affected the seed coat mass of *Plantago lanceolata*, and therefore its germination. Furthermore, seed chemical composition may also affect germination and seedling emergence (Baskin and Baskin 2001). The mechanism by which maternal warming changes seed germination and emergence is not clear, though likely systemic, and could be due to differences in resource mobilization between warmed and ambient plants (van Noordwijk et al. 1998).

Early life history influences the entire life cycle schedules of plants (Baskin and Baskin 2001). For *C. nutans*, seedling establishment is an important factor determining the population growth rate (Shea and Kelly 1998). The seedling establishment period is the most vulnerable stage in *C. nutans’* life cycles (Popay and Medd 1990), as seedlings are exposed to competition from surrounding vegetation and are sensitive to environmental stress. Because successful seedling recruitment of *C. nutans* largely relies on disturbance-created openings (Peterson-Smith and Shea 2010, Ruggiero and Shea in press), germinating and emerging rapidly can benefit the thistles in competition with other plant species. Furthermore, thistle seedlings that emerge early in the fall have a longer time to grow and therefore are more likely to reach a size sufficient to survive the winter. The resulting larger rosettes also have a better chance of flowering the following summer and behaving as winter annuals instead of biennials or perennials (Doing et al. 1969). Moreover, advances in the timing of seedling emergence may be magnified because flowering phenology of the maternal plants is also advanced by warming (Zhang et al. in prep). Therefore earlier seedling emergence not only improves plant survival, but also expedites the life
cycle of the species, which may lead to even more rapid population growth under global warming.

However, loss of dormancy at suboptimal temperatures in seeds from maternally warmed plants may have complex effects on population dynamics. For the fall-germinating cohorts, germinating under low temperatures, which is likely towards the end of the growing season, may lead to mortality caused by adverse environments such as early frosts. On the other hand, spring cohorts may benefit from germinating under low temperatures in early spring when competition from neighboring plants is low. Therefore future field experiments on the establishment of seedlings in both fall and spring are needed to draw further conclusions. In addition, studies with different amounts of warming, to investigate the effect size of maternal environment on offspring performance, may be worthwhile.

Previous studies demonstrated that *C. nutans* produced more seeds under warming, with unchanged seed dispersal ability (i.e. terminal velocity), seed morphology, and seed weight (Zhang et al. 2011). Our present study demonstrates that seedling emergence can be advanced by maternal warming, which may benefit the species without additional cost to other life history traits. This positive response of the offspring, together with the positive responses on the maternal level, may cause faster population growth and invasive spread of this species under consistently warmer climates. However, loss of dormancy at suboptimal germination temperatures may lead to complex outcomes for population performance if greater temperature variation eventuates. As many monocarpic plants demonstrate similar life cycle variation (i.e. life history flexibility as winter annuals, biennials, or perennials), which is limited by the timing of germination and early growth (Donohue 2009), global warming may modify population growth via maternal environmental effects on early life histories. We hope future studies will consider maternal environmental effects when assessing invasion of these species under global warming.
Acknowledgements

RZ designed and conducted the experiments, with assistance from RG. RZ analyzed the data. RZ wrote the first draft and all three authors helped to edit the chapter. We are grateful for field assistance from many undergraduates. We especially thank L. Ruth, S. Yang, and B. Bradley for their assistance and comments in developing this study. We acknowledge the support of NSF grant #DEB-0815373 to K.S.

References


Figure 8-1. Effects of maternal warming on percentage of seed germination (A) and mean germination time (MGT) (B) under three germination temperature regimes (day/night: 15C/15C, 20C/15C, and 25C/15C). Data are presented as mean ± SE. The letters on the bars denote statistical significance at $\alpha = 0.05$. 
Figure 8-2. Effects of maternal warming on percentage of seedling emergence (A), mean emergence time (MET) (B), longest leaf length (C), and biomass of two-week-old seedlings (D). Data are presented as mean ± SE. The letters on the bars denote statistical significance at α =0.05.
Chapter 9
Conclusions

As introduced in Chapter 1, this thesis includes studies that generally fall into two categories: 1. invasion and disturbance; 2. invasion and climate change. While the thesis mainly focuses on the responses of two invasive species, these species can be considered as a model system for general invaders, and therefore my work also generates important insights for invasion ecology, as well as for management and conservation. In this final chapter, I summarize the findings of previous chapters from both the perspective of the invasive plants and from the perspective of management. Furthermore, I discuss future possible research directions arising from findings in previous chapters.

From the thistle perspective: what makes a good invader under disturbance and climate change?

Since Charles S. Elton published his classic book on biological invasion in 1958 (Elton 1958), consistent interest has been focused on characteristics related to successful invaders (Kolar and Lodge 2001). Numerous case studies, comparative studies, and meta-analyses have been conducted to generalize life history traits that are associated with invasive species (Grotkopp et al. 2002, Daehler 2003, Hamilton et al. 2005, Lloret et al. 2005, Richard et al. 2006, Theohaides and Dukes 2007). These traits include fast growth rates, strong competitive ability, large reproductive outputs, easily-dispersed offspring, and persistent seed banks (Prinzing et al. 2002, Theocharides and Dukes 2007). Under ongoing global change, a successful invader has to be able to maintain its performance in the novel environment. Therefore, in the face of disturbance and climate change, as particularly examined in this thesis, a good invader should either have strong
in situ tolerance and plasticity, or have strong dispersal ability to buffer against adverse conditions spatially.

As seen from Chapter 2, Chapter 4 and Appendix A, *Carduus* spp. are very tolerant of a pulse disturbance, mowing. Even with frequently applied intense mowing, it was difficult to affect their survival. The strong tolerance of these species is contradictory to the expectation for classic "ruderal” species (Grime et al. 2001) and contradictory to the expectation for species that invest so heavily in structural defense. This suggests that the C-S-R classification of competitors (C), stress tolerators (S), and ruderals (R) (Grime et al. 2001) as well as the tolerance-defense dichotomy (Van der Meijden et al. 1988) are not very distinct. The strong tolerance of *Carduus* spp. to disturbance is likely due to resources stored in their taproots, and in the case of *C. acanthoides*, also because of its versatile growth pattern and flexible life cycle. Furthermore, the strong tolerance of the two species reflects their adaptation to grazed and mowed pastures.

It should be noted that different life history traits of invasive species can also result in different responses. *C. acanthoides* was more tolerant to disturbance than *C. nutans*. The survival, flowering period, and height of *C. acanthoides* were less affected by mowing than those of *C. nutans*. This is likely due to *C. acanthoides’* lower branching pattern and longer and more continuous flowering period. Lower branching patterns can result in unsatisfactory control by mowing (Benefield 1999). The longer flowering period of *C. acanthoides* ensures a slow increase in reproductive investment over a longer period, and therefore reduces the proportional loss caused by disturbance at any time before the completion of reproduction. Strong tolerance to disturbance helps to explain the fact that both species are successful in disturbed areas such as over-grazed rangelands and regularly mowed roadsides. The success of *Carduus* spp. in disturbed habitats can be also attributed to their strong dispersal ability, which is examined in Chapter 5.

As shown in Chapter 5, *C. nutans* not only responded to warming in its demographic vital rates (e.g. emergence, survival, and reproduction), but also in the dispersal-related trait, plant
height. These positive responses suggest that this invasive species is able to adjust various aspects of its life history to maintain its high invasiveness in changing environments. While enhanced demographic vital rates contribute to increased population growth locally, increased plant height leads to greater dispersal distances and potentially faster population spread rates. Improved dispersal ability of the two species suggests that they have a large potential to track climate change spatially. Such ability is especially important in heterogeneous environments where disturbances occur on the local scale – plants that disperse their offspring more widely are more likely to escape fatal disturbances and to establish in favorable habitats (Snyder 2010).

Negative responses of some life history traits are also observed under environmental change. Reduced leaf prickly density of *C. nutans* under warming (Chapter 7) suggests a possible shift in the growth-differentiation balance (Herms and Mattson 1992). The ability to adjust resource allocation along an environmental gradient can also help this invader to succeed under climate change.

The comparisons between responses of the two species demonstrate that both species are able to grow taller under warming; however, this results from different alternations in their life histories (Chapter 6). Uneven shifts in the phenological sequence of *C. nutans* resulted in an extended growing duration, which accounted for enhanced plant height at the dispersing stage. For *C. acanthoides*, however, enhanced plant height was the result of an increased growth rate. This result suggests that various strategies exist to enhance plants’ performance under climate change, and different species may adopt different strategies, depending on their life histories.

While most of the research I conducted was on one generation only, I do find that maternal climate can affect early life stages of the offspring as well (Chapter 8). This result, although preliminary, suggests that effects of climate change can last at least for two generations and may be not negligible for longer-term population dynamics.
In general, the responses of \textit{Carduus} spp. to disturbance and to climate change suggest that their invasions may worsen in the future. Their strong tolerance to disturbance, their flexible life histories, and their increased population growth and dispersal make them likely to become even more invasive under global change.

\textbf{From the management perspective: what can be done to improve management of invasive species in a changing world?}

Biological invasions are likely to worsen in the future, as many invasive species share life history traits that are favored by global change (Dukes and Mooney 1999, Weltzin et al. 2003, Bradley et al. 2009). Managers of invasive species face new challenges, and developing appropriate management strategies is an urgent task (Hulme 2005).

Under any circumstances, a clear management objective is a critical prerequisite; choice of management strategy strongly depends on the goal of the management. In Chapter 3, the optimal mowing regimes are different for different management goals: while reducing population density of the current cohort only requires one intense mow late in the season, obtaining long-term reductions in local abundance and regional spread requires an extra mowing event in the early season.

Once the management goal is clearly stated, the next task is to determine the optimal strategy. Because management practices can be also considered as disturbances (Lockwood et al. 2006, Buckley et al. 2007), they share the five aspects of a disturbance (i.e. intensity, frequency, timing, extent, and duration). Considering multiple aspects when evaluating the effectiveness of management regimes is crucial. This is mainly because interactions between these aspects can lead to synergistic outcomes, and interdependence between them can mask the actual effects and cause misleading conclusions (Chapter 2).
Successful management should also be tailored to the specific biological system. The management of Carduus spp. should strictly follow the timing of the development of the target species (Chapter 2) and additional attention should be paid to secondary contamination during the management process (Chapter 3). Climate change imposes both challenges and opportunities for controlling invasive species (Hellman et al. 2007). Enhanced dispersal (as a result of increased plant height) contributes the most to increased spread of C. nutans under warming, and therefore more effort should be made to constrain the dispersal of the species in the future. On the other hand, decreased structural defense of C. nutans provides opportunities to use goat grazing as a management approach to control this species. However, further tests are needed to scrutinize this management practice.

Future work

In the second part of this thesis, I examined the responses of the two thistles to warming and increased precipitation, with a focus on later life stages of the plants (i.e. rosettes and flowering plants). Although emergence was assessed in an auxiliary experiment, emerged seedlings were not followed throughout their establishment due to limited experimental space. However, seedling establishment is a crucial limiting factor in the recruitment of Carduus spp. (Peterson-Smith and Shea 2010) and it greatly affects their invasion success (Shea and Kelly 1998). Furthermore, since vegetative phase responses are not always well correlated with those of reproductive traits at adult stages, integrating plants’ responses through their whole life cycle is critical to our understanding of how invasiveness changes under changing climate (Jablonski et al. 2002). In future studies, seedling growth and establishment should be assessed to complete our understanding of the two species’ responses to climate change, and then updated vital rates estimates should be integrated into population models.
Another aspect of climate change, elevated levels of CO$_2$, should also be included in future studies. As previous work shows, increased CO$_2$ supply significantly enhances vegetative growth in many plants (reviewed in Long et al. 2004). For *Carduus* spp., early rosette growth in large part determines plant survival, bolting probability and hence their life cycle schedules (Shea et al. 2006). Therefore exploring effects of elevated CO$_2$, together with effects of temperature and precipitation, would be an interesting future direction. Because of the high costs of CO$_2$ enrichment, I suggest future work focus on early life stages of *Carduus* spp. first, because seedlings and rosettes are easy to handle in CO$_2$ chambers, and short experimental durations make experiments more affordable.

In the experiments described in Chapter 5 and Appendix B, surrounding vegetation was regularly mowed to mimic an overgrazed pasture where these species are most successful. In other words, interspecific competition was suppressed to evaluate the potentially maximal responses of the invasive species to climate change. However, previous work shows that competition from other pasture species is a critical factor impeding thistle invasions (Sindel 1991). Under climate change, competition from other existing plants may strongly affect performance of invaders (Johnson et al. 1993, Suttle et al. 2007). Evaluating responses of *Carduus* spp. in the community context will generate new insights for understanding biotic interactions between native species and invasive species under climate change. Furthermore, responses of other plant species in the community may influence dispersal of *Carduus* spp. under climate change. For example, increased height and density of surrounding vegetation may influence wind speeds when thistle seeds are released, and therefore the spread of the invaders projected by the models (Marchetto et al. 2010).

As shown in Chapter 6, the phenology of *Carduus* spp. was affected by warming. Not only the individual phenological dates, but also durations of each developmental stage were altered. Another interesting question would be whether these shifts in phenology cause any
phenological mismatch with other species. A flower head weevil, *Rhinocyllus conicus*, has been widely used as a biological control agent for *C. nutans* in the U.S.A., Australia, and New Zealand (Sheppard et al. 1994, Louda et al 1997). The success of this biocontrol agent largely depends on the synchronization of weevil emergence and thistle development (Surles et al. 1974). Thus, phenological mismatches between the thistle and the weevil, potentially caused by climate change, may affect population persistence of *R. conicus*, and therefore reduce its control effectiveness. Such studies will be invaluable for future management of the invasive species under climate change.

In Chapter 8, maternal climatic effects were examined for the early life stages of the offspring. Future research could extend this to cover the adult stage as well. Comparative analyses can be conducted on life history traits, such as plant height and reproduction, of both the maternal plants and their offspring. By doing this, we will be able to assess whether maternal climatic effects enhance offspring performance in similar climates.

While in this thesis I examine biological invasion under disturbance and under climate change separately, future research should try to combine both components, as they often occur together. Modern modeling techniques, such as Integral Projection Models (IPMs, Easterling et al. 2000) and Individual Based Models (IBMs, Judson 1994), can also be applied to help improve our knowledge in the field of invasion ecology, climate change ecology, and disturbance ecology.

Last but not least, in Chapters 3 and 5 when I examine the spread of this invasive species, I focus on local, wind-mediated, spread, which depends strongly on plant traits. The projected spread rates under climate change and disturbance can be validated in future experiments, perhaps using similar methodologies as in Bullock et al. 2008. However, biological invasion often involves plant dispersal on multiple spatial scales (Pauchard and Shea 2006). Spread of invasive species on the regional scale, or even the continental scale, arises from multiple dispersal vectors, among which humans should not be overlooked (Rauschert et al. 2010). For *Carduus* spp., the
most extreme long-distance dispersal events are likely related to agricultural practices and transport on vehicles. For example, movement of hay bales contaminated with thistle seeds can facilitate thistle invasion. Furthermore, the distribution of *C. acanthoides* in the U.S. is found to be significantly correlated to milk production (Leib 2009), suggesting the importance of human-mediated dispersal in the invasion of this species. Therefore a complete picture of the spread of invasive species under climate change and disturbance will require knowledge of different dispersal processes on multiple scales, and consideration of the total dispersal kernel (Nathan 2008), to incorporate the contributions of all these processes.
References


APPENDICES

The experiments described in Chapter 2 and Chapter 5 were also conducted for *Carduus acanthoides*. Because of the lack of baseline demographic parameter estimates for this species, I am not able to model local population growth and population spread, as I did for *C. nutans*. Nevertheless, I present the experimental results for *C. acanthoides* in the two appendices. In Appendix A, I present the responses of *C. acanthoides* to mowing regimes differing in intensity, frequency, and timing. In Appendix B, I present responses of *C. acanthoides* to climate change (i.e. increased temperature and precipitation), in both demographic vital rates and dispersal-related traits. Note that the experimental designs and data analyses in the appendices are the same as for *C. nutans* in Chapter 2 and Chapter 5.
Appendix A

Responses of Carduus acanthoides to different mowing regimes

I followed the same experimental design as described in Chapter 2 to examine the responses of *C. acanthoides* to mowing treatments with different levels of intensity, frequency, and timing. In general, results are similar to those for *C. nutans* and support the conclusions in Chapter 2 that multiple disturbance aspects need to be considered to assess interactions, and furthermore, to avoid misleading conclusions caused by interdependence between aspects. In particular, failure to account for the interdependence between frequency and timing can lead to either false negative results or can obscure potentially positive effects.

**Intensity and timing**

High intensity or late timing alone significantly delayed flowering (P<0.001), but the interaction between them was not significant (P>0.05). Approximately 5% (15 out of 303 cut plants) were still alive when the experiment was terminated at the end of November. Ten of these fifteen plants bore no capitula and apparently retrogressed to the rosette stage (leafing at a short height below 10cm). These plants could bolt in the following growing season given favorable environment, as suggested by Chapter 4. All of the ten plants were previously cut at 5cm and nine of them had received a late cut. Intensity and timing both had significant effects on survival, plant height, and reproduction - treatments with high intensity or late timing caused larger reductions in the responses (Figure A1-1). The interaction between intensity and timing was significant for reproduction, indicating that the effects of late timing were further magnified by high intensity.

**Intensity and frequency**
Treatments with high intensity caused significantly larger delays in flowering, and larger reductions in survival, plant height, and reproduction than treatments with low intensity ($P<0.001$, Figure A1-2). However, the effects of frequency depended on whether late timing was considered (Figure A1-2). For example, increased frequency did not further delay flowering when late timing was considered, whereas it significantly delayed flowering when late timing was ignored – this is contrary to the results for C. nutans (i.e. frequency effect was significant when considering late timing). Similarly, frequency had a significant negative effect on survival at low intensity and on reproduction at high intensity levels when not accounting for timing (Figure A1-2B,F), but this effect disappeared when timing was considered (Figure A1-2A,E). Furthermore, although frequency appeared to have no effect on plant height at low intensity when not controlling for timing (Fig. A1-2D), higher frequency actually led to taller plants at low intensity when timing was considered (Fig. A1-2C).

Flowering stem number was positively correlated with reproduction (Figure A1-3A). High intensity reduced the number of induced flowering stems. While high frequency significantly increased the number of flowering stems at low intensity level, no effect was found for high intensity (Figure A1-3B). This suggests that the negative effect of high intensity was so strong that it masked the potential positive effect of high frequency.

Timing or frequency

In all cases, AIC values were lower (lower AICs indicate better fits) for models which included timing instead of frequency (Table A1-2); this agrees with Chapter 2’s findings for C. nutans. The $R^2$ values for non-parametric models for flowering date were much higher (higher $R^2$ values indicate better fit) in models with intensity, timing and the interaction between the main effects, instead of intensity with frequency, and their interactions (0.468 vs. 0.183). This suggests that models which include timing instead of frequency have a better explanatory power for all
these responses, and that the frequency results in the full data set were mostly driven by the effect of late timing.

Table A1-1. Akaike Information Criteria (AIC) values for the minimum adequate linear mixed models fitted with intensity and timing (i.e. with or without a late cut) and comparable models fitted with intensity and frequency.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Timing and intensity</th>
<th>Frequency and intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Responses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>† Survival</td>
<td>245</td>
<td>276</td>
</tr>
<tr>
<td>Plant height</td>
<td>2206</td>
<td>2234</td>
</tr>
<tr>
<td>‡ Capitulum production</td>
<td>1115</td>
<td>1156</td>
</tr>
</tbody>
</table>

* Initial rosette size was used as a covariate in all models. Cohort and spatial location of individual plants were considered as random effects.
† Binomial error distribution was specified for the survival data.
‡ Results were based on log linear transformed data. The minimum adequate models include the interactions between timing and intensity, or between frequency and intensity.
Figure A1-1. Effects of intensity and timing on the survival (A), plant height at flowering (B), and reproduction (C) of *C. acanthoides*. Treatments on the x-axes include control, treatment with high intensity but without late timing (HI), treatment with high intensity and late timing (HI+Late), treatment with low intensity but without late timing (LI), treatment with low intensity and late timing (LI+late). The letters denote the statistical difference between treatments.
Figure A1-2. Effects of intensity and frequency on the survival (A, D), flowering plant height (B, E), and reproduction (C, F) of C. acanthoides depends on whether the statistical model accounts for timing (A, B, C) or not (D, E, F). A, B, and C present results based only on treatments that included a late cut. D, E, and F present results for the full dataset. The effect of frequency at each intensity level is indicated to the right of the curves. “ns” denotes no significant effect. “+” denotes a significant positive effect. “-” denotes a significant negative effect: $\alpha=0.05$. 
Figure A1-3. The relationships between the number of flowering stems and capitulum production (A), and between frequency and the number of flowering stems (B). Number of flowering stems is a significant predictor of capitulum production (P<0.001). Number of flowering stems significantly increases with frequency at low intensity level (P=0.03), but not at high intensity level (P>0.05), indicating that at the low intensity level, high frequency treatments compensate for reproduction by increasing stem numbers.
Appendix B

Responses of *Carduus acanthoides* to warming and increased precipitation

The same experimental design and statistical analyses as described in Chapter 5 were conducted for *C. acanthoides*. In general, *C. acanthoides* is not as sensitive as *C. nutans* in its responses to increased temperature and increased precipitation. Increased precipitation (either only in the winter or in both winter and summer) did not have any significant effect on any of the responses described below (P>0.05). Therefore the following results are presented mainly with respect to warming. Please also note that only the first cohort’s data were included for analyses on seed production per capitulum, seed terminal velocity, seed weight, and pappus diameter.

Demographic vital rates

*Seedling emergence*

In contrast to *C. nutans*, which had higher seedling emergence in the fall under warmed than ambient conditions, *C. acanthoides* had a lower emergence rate under warming (14.9±2.4% versus 19.3±2.6%, warmed versus ambient, n=80, P<0.001, Figure A2-1).

*Winter rosette survival*

Warming did not affect overwinter survival (93.8±2.5% versus 90.6±2.9%, warmed versus ambient, n=80, P=0.39). However, increased precipitation in the winter marginally decreased rosette survival (88.8±3.3% versus 95.6±1.8%, increased winter precipitation versus ambient precipitation, n=80, P=0.053).

*Bolting*
Given that rosettes survived the winter, warming did not affect percentage of rosettes bolting (89.8±3.3% versus 94.2±2.2%, warmed versus ambient, n=80, P=0.16).

**Reproduction**

Warming did not have any significant effects on total capitulum production (378.5±46.9 versus 405.3±45.9, warmed versus ambient, n=80, P=0.39), mature capitulum production (170.1±23.4 versus 161.2±19.4, warmed versus ambient, n=80, P=0.97), immature capitulum production (208.5±24.9 versus 244.1±27.8, warmed versus ambient, n=80, P=0.24). Furthermore, warming did not affect number of seeds per capitulum (115.3±10.7 versus 113.1±10.7, warmed versus ambient, n=38, P=0.97). In this study, we terminated the experiment at the end of July, when most *C. acanthoides* were still flowering. Given the fact that *C. acanthoides* usually flowers till very late in the season (sometimes until November), the estimates of reproduction and thereby the effect of warming assessed in the present study may not well represent the lifetime reproduction of the species.

**Dispersal-related traits**

**Plant height**

Height of *C. acanthoides* plants by the end of the experiment was significantly greater in warmed plots than in ambient temperature plots (148.7±3.7cm versus 137.1±3.6cm, warmed versus ambient, n=80, P=0.003, **Figure A2-2**).

**Seed terminal velocity**

Warming did not significantly affect seed terminal velocity (0.96±0.04m/s versus 1.03±0.04m/s, warmed versus ambient, n=30, P=0.36).

**Seed weight**

Warming did not significantly affect seed weight (1.5±0.1mg versus 1.6±0.6mg, warmed versus ambient, n=30, P=0.40).
Pappus diameter

Warming did not significantly affect seed pappus diameter (10.5±0.6mm versus 9.2±0.6mm, warmed versus ambient, n=30, P=0.17).

Figure A2-1. Effect of warming on seedling emergence in the fall.
Figure A2-2. Effect of warming on plant height by the end of the experiment.
Appendix C

Photo demonstrations of *Carduus nutans*, *Carduus acanthoides*, and open top chambers

**Figure A3-1.** *Carduus nutans* in the field. Photo by K. Shea.

**Figure A3-2.** *Carduus acanthoides* in the field. Photo by K. Shea.
Figure A3-3. An open top chamber (OTC) used in the field experiment described in Chapter 5. These fiberglass chambers are 40cm in height, 1.5 m in basal diameter, and 1 m in top diameter, and were constructed based on the International Tundra Experiment (ITEX) manual (Molau and Møgaard 1996).

Reference

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