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Invasion of *Centaurea nigrescens*, Tyrol knapweed, in North America

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Abstract of the Dissertation

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Centaurea nigrescens is a native plant in Europe that was introduced to North America in the 1800s. This dissertation focuses on the introduction and spread of *C. nigrescens* across North America using herbaria records and genetic differences between native and non-native populations to construct likely invasion routes. Herbarium records and the results of a Minimum Cost Arborescence model coupled with chloroplast DNA analysis support the hypothesis that there was an initial introduction of *C. nigrescens* near Worcester, Massachusetts in 1830. Significant haplotype differences between the populations from the northeastern and the northwestern US support the hypothesis that there was a second, independent introduction of *C. nigrescens* in the Pacific northwest, USA. To compare the performance of plants from the native and non-native range of *C. nigrescens,* seeds from 8 European (native) and 8 North American (non-native) populations were collected. Germination and early developmental stages were assessed by studying germination rates, time to germination, time to first leaf, and time to second and third leaf formation at 18°C and 28°C. Seeds from non-native populations had a greater germination rate than those from native populations at both temperatures. However, there was no difference in time to germination or developmental rates between plants from native and non-native populations at 18°C or 28°C. Three greenhouse experiments were used to assess traits that could affect the competitive ability of *C. nigrescens* when exposed to low and high light, low and high water, and low and high nutrients. Surprisingly there were relatively few traits that differed between plants from native and non-native populations in any of the three experiments. Plants from the non- native populations were capable of adapting to the different treatments by differences in resource allocation. For the non-native plants in the light and water experiments, leaf structure changed significantly without affecting specific leaf area, a trait associated with competitive ability. There was no difference in specific leaf area between native and non- native populations in any of the treatments in any of the experiments. By incorporating the history of invasion, early life history traits, and responses to resources, this dissertation research provides important knowledge about the introduction of an invader, and indicates the importance of studies that include many different factors that affect the introduction and success of new invaders.

I dedicate this dissertation to Larry Slobodkin

for believing in me and seeing my potential as a scientist.

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Chapter 1

Introduction

Introduction

The genus *Centaurea* (Asteraceae) has about 300 species (Garcia-Jacas et al. 2006), all of which are native to Eurasia, and many that have been introduced outside of their native range and are now considered invasive. In this dissertation, invasive plants are defined as those that have a negative impact on communities they invade and/or negative human impacts. In North America 34 species of *Centaurea* are reported to have been successfully introduced (http://plants.usda.gov/), and 14 of these are considered noxious weeds in one or more U.S. states. Species of *Centaurea* that have spread and become pests in areas outside their native range include spotted knapweed *C. stoebe*, star thistle *C. solstitialis*, diffuse knapweed *C. diffusa*, squarrose knapweed *C. virgata*, and Russian knapweed *C. repens* (Sheley and Petroff 1999; LeJeune and Seastedt 2000; DiTomaso 2000; Marrs and Hufbauer 2006; Hufbauer and Sforza 2008; Marrs et al. 2008). *Centaurea nigrescens* (Figure 1) is within the section Jacea-Lepteranthus of the Circum-Mediterranean and Eurosiberian Clade of *Centaurea* (Garcia-Jacas et al. 2006). *Centaurea nigrescens,* commonly known as tyrol-knapweed or short fringed knapweed, is poorly studied relative to its congeners. It is a weed that was introduced in the United States in the 1800s from Europe (Chapter 2). It is not yet known whether *C. nigrescens* will become an aggressive pest like other introduced species of *Centaurea* that are successful invaders.

Centaurea nigrescens is a rosette forming herbaceous perennial that generally grows 30-150 cm in height, and has 1 to 50 erect flowering stems (Efloras 2008). Populations of *C. nigrescens* are typically found along roadsides and in highly disturbed sites (personal observation). This species can spread both by clonal growth via rhizomes as well as by seeds. It germinates in the spring and summer and can flower in its first year. In general, it over winters as a rosette, bolts and sends up stalks with floral buds in the spring (June), flowers and fruits in mid-late summer, and dies back above ground by late autumn (personal observation). Flowers are self-incompatible and each plant can produce up to 25 seeds per capitulum when fertilized (Efloras 2008).

The native range of this species is in Europe (Figure 1). Introduced populations in North America are primarily in the east, with a few populations in the west (Figure 2). Other species of *Centaurea* are proposed to have been introduced into the US through soil in the ballasts of ships and contaminants in alfalfa seed and hay for cattle (Maddox 1979). It is reasonable to assume

that *C. nigrescens* was introduced in a similar manner. To date, investigations have not been conducted on the history, ecology, and evolution of Tyrol knapweed in North America.

The focus of this dissertation was to unearth the history of the introduction and spread, ecology and evolution of *C. nigrescens* in the United States. Herbarium archive records and a computer algorithm were used to reconstruct the most parsimonious likely invasion route for *C. nigrescens* (Chapter 2). To address the differences in growth and development between native and non-native populations, seeds were collected from 8 European sites and 8 North American sites. A germination study (Chapter 3) was used to assess whether there are differences in the time to germination and the timing different early developmental stages between plants grown from native and non-native seeds.

There are many factors that affect invasion success. A greenhouse study was conducted to compare the effects that the critical abiotic factors of light, water, and nutrients have on the growth and morphology of plants from native and non-native populations of *C. nigrescens* (Chapter 4).

Population genetic analysis coupled with ecological studies can be a powerful approach for studying biological invasions (Hufbauer and Sforza 2008). Therefore, questions regarding whether multiple introductions have occurred and if the genetic diversity in introduced populations is similar to those found in the native range can be addressed with chloroplast DNA (cpDNA) (Chapter 5). Sequence data were used to resolve haplotypes found in plants in the native and non-native range, and to infer the source of introductions in the non-native range.

Information obtained from a comprehensive study of the invasion of *C. nigrescens* will provide critical information needed for understanding the initial stages of invasion: introductory history, local adaptation and traits that may enhance local competition.

Figure 1. *Centaurea nigrescens* in flower in July.

Figure 2. US States where *C. nigrescens* has been found. Data for map obtained from the United States Department of Agriculture.

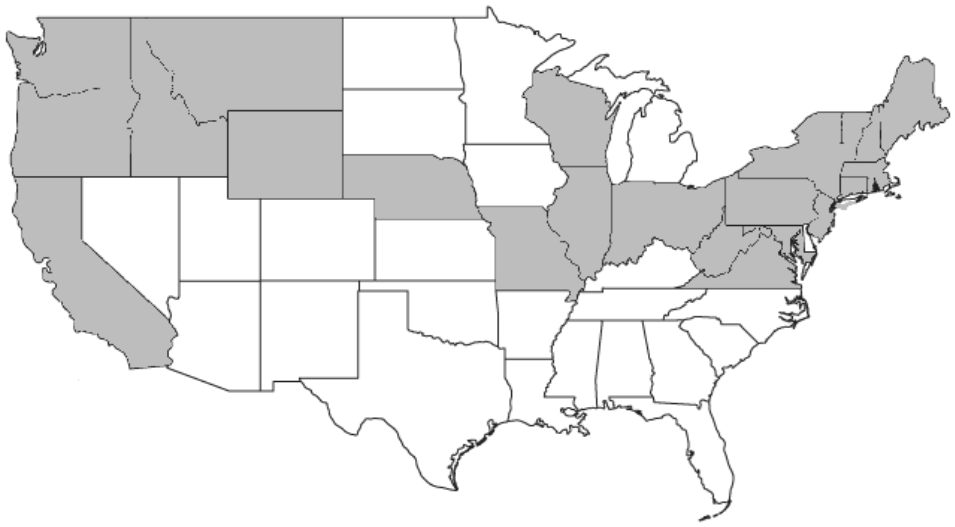

Figure 3. European countries where *Centaurea nigrescens* has been found. Data for map obtained from Flora Italia.

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Chapter 2

Reconstruction of Invasion Routes using the Minimum Cost Arborescence (MCA) Algorithm and herbarium records

Introduction

The genus *Centaurea* (*Asteraceae*) has about 300 species (Garcia-Jacas et al. 2006), all of which are native to Europe, and many of which have become invasive across the globe. In North America 34 species of *Centaurea* are reported to have been successfully introduced (http://plants.usda.gov/), 14 of which are currently considered noxious weeds in one or more U.S. states. Very little work has been done to document the early stages of invasion of any of the species of *Centaurea*.

Reconstructing the invasion pathways of introduced species represents a major challenge for the field of invasion biology. Many studies have used temporal and spatial information gleaned from herbarium specimens to infer invasion routes (Aikio et al. 2011; Delsile et al. 2003; Miller et al. 2009; Wu et al. 2010; Fonseca et al. 2006; Fuentes et al. 2008; Barney et al. 2008) and, although these efforts have some limitations, they often provide the only means for historical reconstructions (Delisle et al. 2008).

Recently, another approach, the Minimum Cost Arborescence (MCA) model, has been developed to predict likely spatial pathways of introduced species over time (Hordijk and Broennimann 2012). The MCA reconstructs the most parsimonious routes of spread given a directed graph, a unique root vertex, and a cost function (Figure 1). In a directed graph, vertices are connected using vectors and the root vertex, the initial point of introduction, is assigned. All other points come from the root vertex and a cost function is used to establish which point is most likely to be next. The cost function determines the minimum cost of traveling to the next point. The invasion route then follows the pathway of minimum cost. The MCA model uses locations and dates of collections from herbarium records as the points along a directed path. Therefore, with the use of an MCA algorithm, it is possible to reconstruct plausible invasion routes as well as the timing of spread. The goal of the present study was to use the MCA model to construct a plausible invasion route in North America for *C. nigrescens,* a close relative of the species, *C. stoebe*, spotted knapweed, which was the focus of the Hordijk and Broennimann (2012) study, that was introduced to North America about the same time. In addition, I asked if the spread of *C. nigrescens* corresponded with the introduction and spread of *C. stoebe.*

Methods

Centaurea nigrescens herbarium data

Data on the current distribution of *C. nigrescens* (Figure 3) were obtained from the USDA PLANTS database (http://plants.usda.gov/java/profile?symbol=CENI3). For states where *C. nigrescens* was reported to have been found, state herbaria were contacted to determine if they had records of *Centaurea nigrescens*. If online images of recorded specimens were available and accurate identification could be made, records were included in the study. For records that were not online, specimens were shipped or scanned and I reviewed specimens for accuracy and proper species identification (Table 1). Each record included the date and county where collected. If the record contained geographic coordinates, they were used. If geographic coordinates were not available on the voucher, the approximate center of the county where the plant was collected from (determine by using Google Earth Google Earth, Version 5.1.3533.1731, Mountain View, CA: Google Inc.) was used as the geographic coordinate for that specimen. If there were multiple records within a county, first occurrence in each county was used for the modeling of most likely route of invasion.

Counties were used as the unit of spatial resolution for the distribution and spread of this species. Before using the MCA model, I used ArcGIS (ArcGIS Desktop, ver.10) to plot specimen locations and the first date of collection for each herbarium record in each county. A total of 133 records were found, 52 of which were duplicate records within a county, leaving a total of 81 records used in the MCA model.

Minimum Cost Arborescence Model: MCA Algorithm

MCA (acquired from O. Broennimann, R 2.14, R Development Core Team 2012, Appendix I), was then used to construct a possible pathway of invasion for *C. nigrescens*. For the simulation 81 herbarium records from 55 counties in 18 states were used. The spatial resolution of the output was reported at the county-level. Euclidean distance between two points was used as the cost function *c(e)*. The vertex, the initial point and time of introduction, was assigned to be Worcester, MA, 1830, the first documented voucher of *C. nigrescens* in North America.

The model was run for 100 iterations, and a bootstrap value was assigned to each hypothesized path of travel. Paths with different Bootstrap values, with higher numbers

indicating the most support are plotted in Figure 5. I then overlaid the output for *C. nigrescens* onto the likely invasion routes of *C. stoebe* (provided by O. Broennimann).

Results

Most herbarium records for *C. nigrescens* were from herbaria in the northeastern and mid western US. The earliest record was from 1830 from Worcester County, Massachusetts, and therefore was used as the vertex for the MCA model. Figure 6 illustrates the year-by-year occurrence of *C. nigrescens* in the US based on first occurrence herbarium records. Figure 7 illustrates the counties within major geographic regions of the US where *C. nigrescens* was initially introduced and then spread to nearby counties.

After its initial introduction to Massachusetts in 1830, *C. nigrescens* did not appear to spread for 60 years. The results of the MCA model (Figure 5) was that the most likely path of spread from its initial introduction in Worcester MA was to the north to Maine (1890, 75-100% bootstrap support). Records later indicate the presence of *C. nigrescens* in Hartford County Connecticut in 1904 (75-100% bootstrap support). This species then spread south to Washington DC (1915, 75-100% bootstrap support), and then expanded to the midwest to Washtenaw County, Michigan in 1917 (75-100% bootstrap support), and into Lancaster County, Nebraska in 1933 (75-100% bootstrap support).. It appears that there was a second introduction into Douglas County, Oregon in the early 1900s (75-100% bootstrap support). From there, *C. nigrescens* spread to Klickitat, Washington State (1985) and Humboldt County, California (1961), but did not spread further east from there.

Discussion

Understanding the early stages of the spread of an invader following initial introduction, is of critical importance for developing a general understanding of invasion and preventing spread of unwanted species. Historical records, including herbarium records, can provide important data that allow us to reconstruct the spread of introduced species, and may help us in predicting the spread of future invaders. Previous methods that have used herbarium records to reconstruct invasion history require much more data than are available for *C. nigrescens* (Aikio et al. 2011; Delsile et al. 2003; Miller et al. 2009; Wu et al. 2010; Fonseca et al. 2006; Fuentes et al. 2008; Barney et al. 2008). The use of the MCA method, however, produces likely invasion routes when fewer herbarium records are available. *Centaurea nigrescens* appears to be in the

early stages of invasion. It has only been recorded from 55 counties in 18 states, but is likely to be more widespread in the future.

Many immigrants arrived in the early and mid 1800s with seeds to sow crops (Mack 2003). In addition to the deliberate introduction of agricultural species, it is likely that the seed stocks they carried were contaminated with unintentional species of European plants such as *C. nigrescens*. Another plausible mechanism of introduction for this species is through contamination of cattle feed (e.g., hay) during cattle importation. Although, a large majority of cattle were imported from Europe during the 1600's (Bowling 1942), cattle continued to be imported throughout the 1800s.

As *C. nigrescens* populations were becoming established in Massachusetts from 1830 to 1890, a rail system was being built to connect Worcester with ports in Boston (Boston and Maine Railroad, Boston and Lowell Railroad Corporation, 1904) and other northeastern cities. By 1890 *C. nigrescens* had spread to Maine followed by a much more rapid expansion to Connecticut in 1904, and Washington, D.C. in 1915 (Boston and Maine Railroad, Boston and Lowell Railroad Corporation, 1904). By the 1890's the rail system was more widespread, which likely allowed for the rapid expansion of range into the Midwest. It would be interesting to use the MCA model with the cost function determined by the distance along rail lines, to see if the same or different paths were more likely. Using Euclidian distances for the cost function assumes that spread occurs by natural dispersal or animal vectors, which are equally likely to move in all directions from a source. If, however, human transport was the most likely vector for transport, then the distances of travel would be along transportation routes, which at that time would be railroads or wagon routes, especially to the west.

The hypothesized route of invasion by *C. nigrescens* is very similar to that proposed for *C. stoebe* (Hordijk and Broennimann 2012). It is most similar for the northeast and midwest. However, *C. stoebe* has a greater range, particularly in the south, than *C. nigrescens* (Figure 8).

Understanding the characteristics and determinants of invasion routes has important practical applications, including the design and implementation of quarantine strategies, and anticipation of conservation actions such as preventing establishment of new focal populations or eliminating them, rather than just focusing on established invasion fronts (Miller et al. 2005; Handley et al. 2011). This study shows that MCA is a useful approach to reconstruct invasion routes when a limited number of records exit, making it a useful tool for many new invaders.

Additionally, the output of such models provides a framework for further studies on particular invasions, allowing a researcher to investigate causality of spread in an invasion and in so doing, furthering the development of our understanding of the processes by which biological invasions occur.

Table 1. List of herbaria providing records for the construction of invasion routes using a MCA model.

State	Herbarium
Wisconsin	Robert W. Freckman Herbarium Wisconsin State Herbaria
Missouri	Missouri Botanical Garden
Oregon	Consortium of Pacific Northwest Herbaria Intermountain Herbarium Western Illinois University
California	Consortium of California Herbaria
West Virginia	WVA (West Virginia University Herbarium)
Massachusetts	Amherst College at Massachusetts
Michigan	Michigan State University Bloomington Fields Herbarium WUD
Indiana	Kriebel Herbarium, Purdue University NHA
Connecticut	University of Massachusetts Academy of Natural Sciences in Philadelphia
Maine	Harvard University Herbaria
New Jersey	Rutgers University Chrysler Herbarium Chrysler Herbarium (CHRB)
Nebraska	United States Herbarium
Illinois	Morton Arboretum Herbarium
Virginia	Virginia Polytechnic Institute & State University VTB
Washington	New York Botanical Garden Otis Douglas Hyde Herbarium, University of Washington
Ohio	Smithsonian Institution
Idaho	Consortium of Pacific Northwest Herbaria
New York	Consortium of Pacific Northwest Herbaria
Montana	Consortium of Pacific Northwest Herbaria

Figure 1. Minimal spanning tree showing an initial point A, followed by sub graphs with successive nodes. A cost function (determined by Euclidian distance among points) determines the path at which the next point will be found. The cost function directs the algorithm to the most parsimonious location in a graph directed space. Point A on tree indicates vertex or origin t_i initial point for which all other points span. In this case, t_i represents the earliest recorded herbarium record with subsequent sites of travel (B-I).

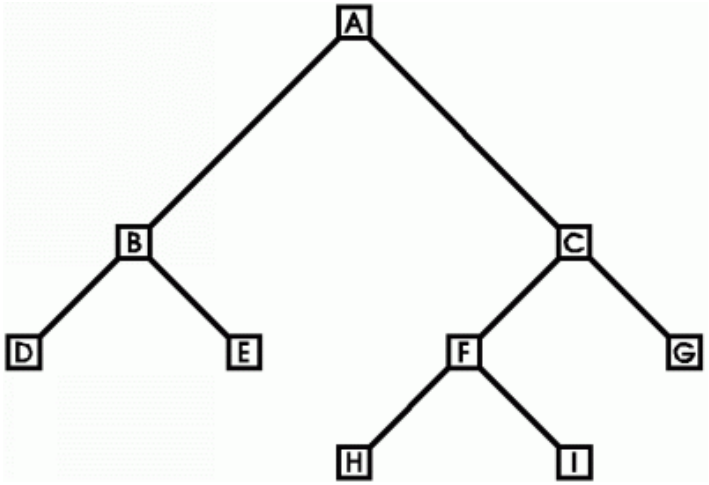


Figure 2. *Centaurea nigrescens* in flower, July 2011. Flower is approximately 3 cm in width.

Figure 3. Distribution of *C. nigrescens* in North America. Data for map obtained from the United States Department of Agriculture.

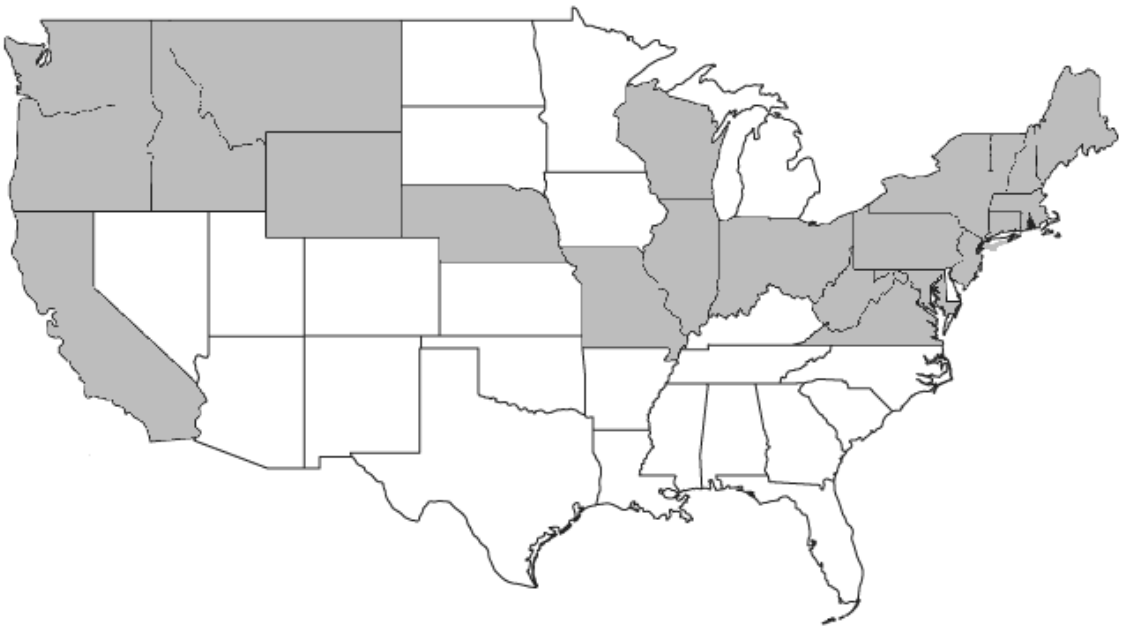


Figure 4. Records of *C. nigrescens* from state herbaria. The samples represented in the graph include multiple specimens collected in a given year. The records used to create this graph were obtained from the herbaria listed in Table 1.

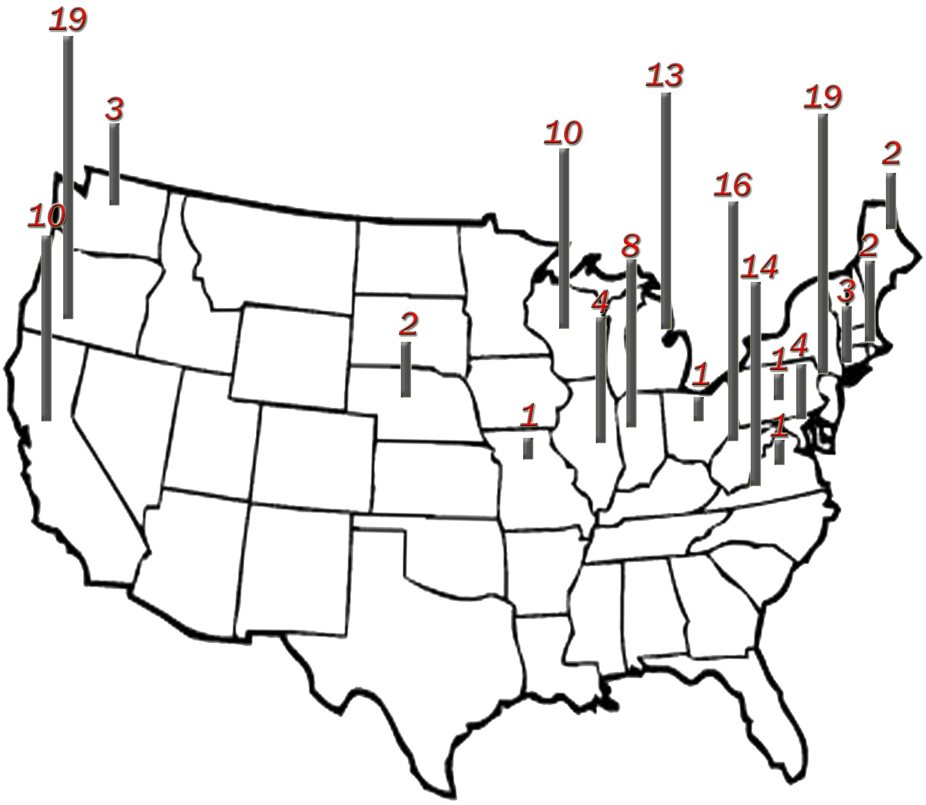


Figure 5. United States map illustrating invasion routes for *C. nigrescens* from MCA simulation in R 2.14. **A)** Initial node of introduction used for Algorithm is denoted by a black dot, t_i was 1830 located in Worchester County, Massachusetts. **B)** Suggested route for mid west introduction. **C)** Suggested route for Pacific Northwest introduction. Dark lines indicate high bootstrap support; lighter lines indicate lower level of support 75-100% (thick Line), 50-75% (thin line) and 25-50% (lightest line).

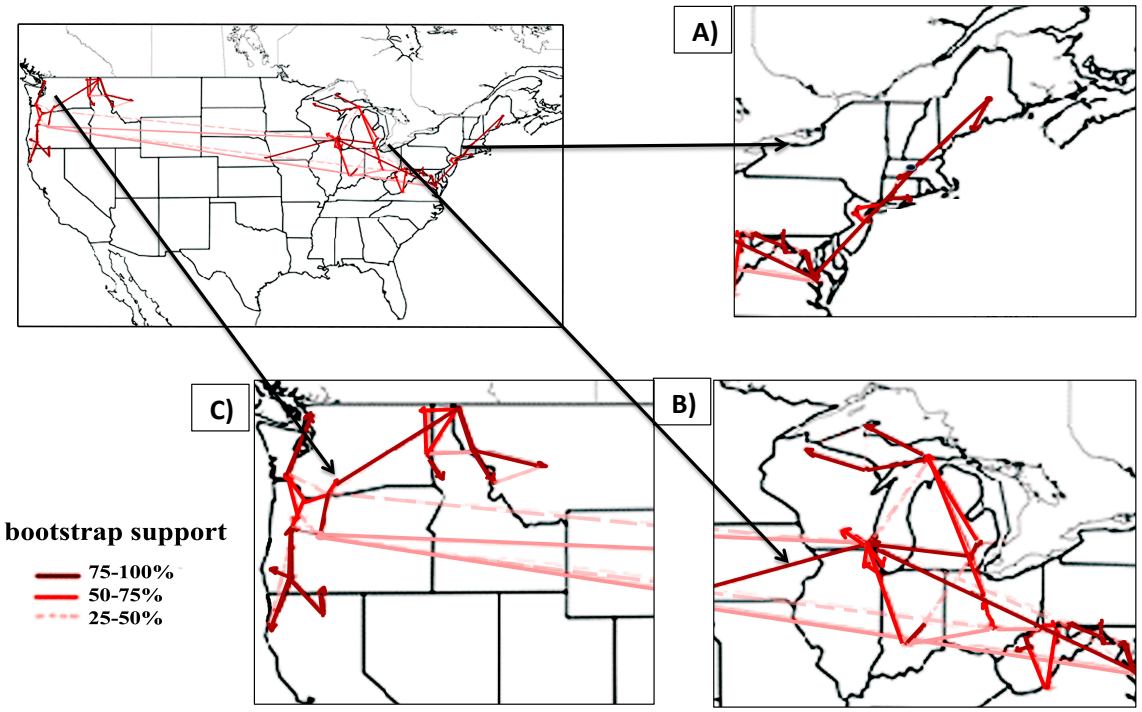


Figure 6. Year of first records of *C. nigrescens* within counties. Multiple dots in a county represent multiple records collected. For the MCA model only first occurrence reported for the county was used.

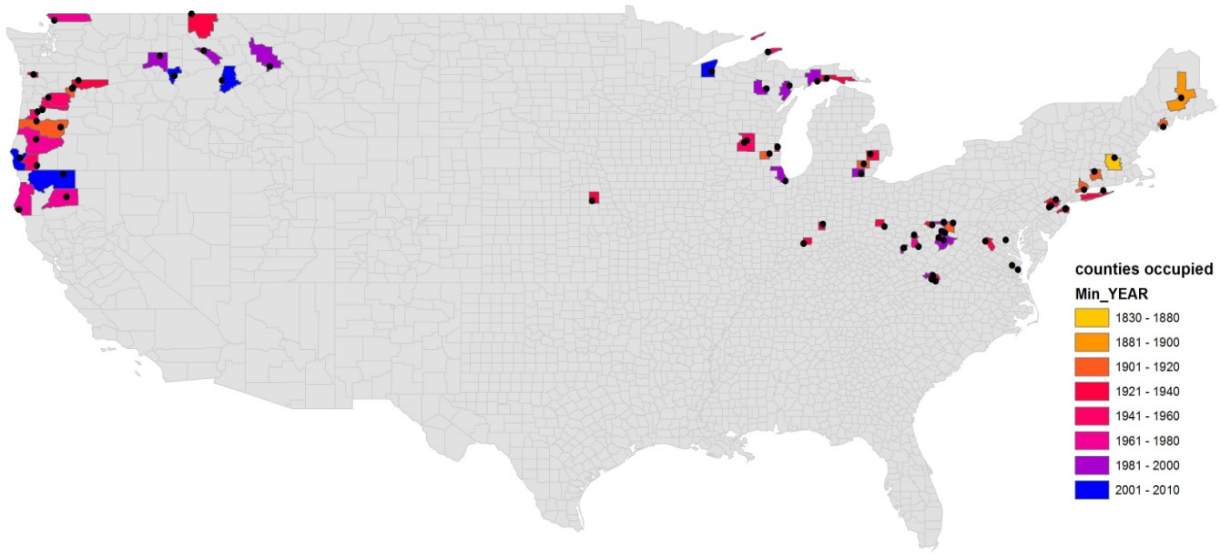


Figure 7. Counties within major geographic regions of the US where *C. nigrescens* was initially introduced and then spread to nearby counties**. A)** The initial point of introduction of *C. nigrescens,* Worchester, Massachusetts 1830. **B)** Midwestern invasion in Washtenaw, Michigan in 1917. **C)** Western introduction in Douglas County Oregon 1909. Pop outs show the counties with herbaria records of *C. nigrescens*

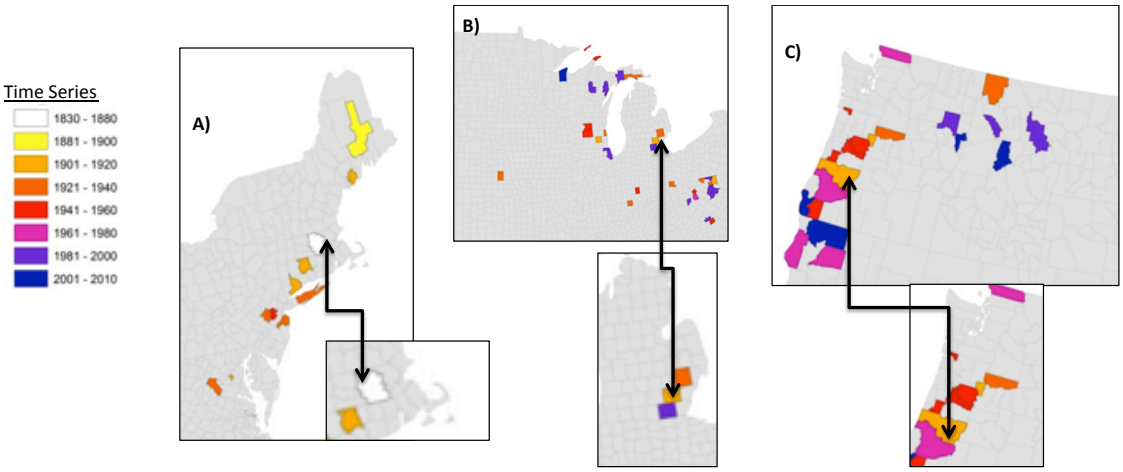
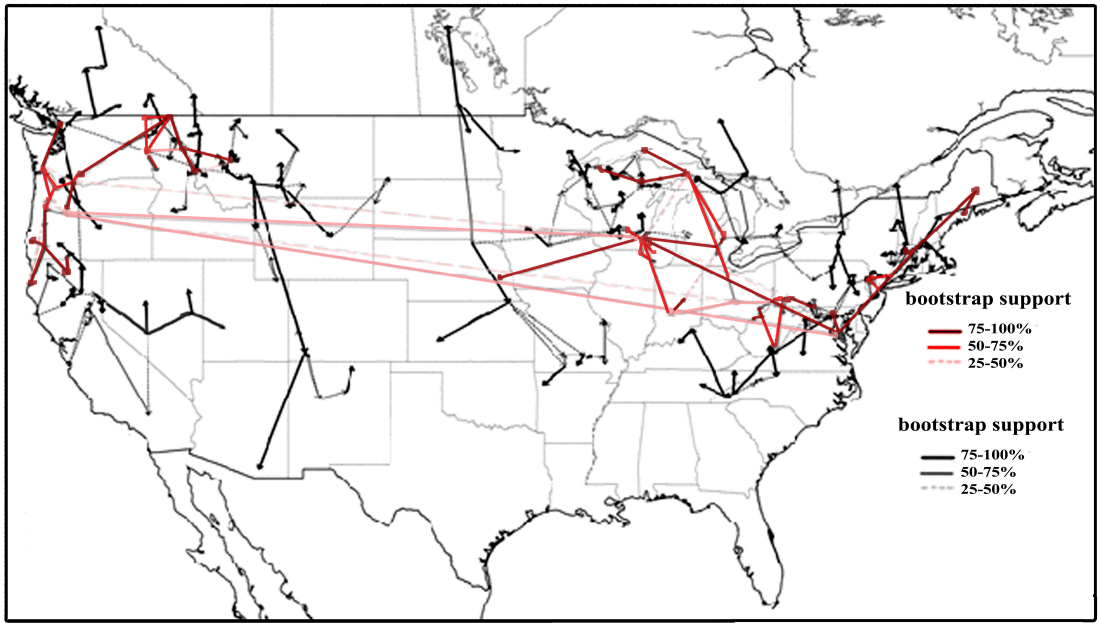


Figure 8. *Centaurea nigrescens* invasion pathway in red and the invasion of *C. stoebe* in black. Weak bootstrap support values are inferred by hashed lines. The hashed lines from the Midwest to the West coast have low support <25-50%.



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Chapter 3

The potential role of differences in germination in native and introduced *Centaurea nigrescens* (Tyrol Knapweed) for invasion success

Introduction

Biological invasions are considered to be a major threat to global biodiversity (e.g., Elton 1958; Williamson 1996; McNeely et al. 2001; Sakai et al. 2001; Mack 2005; but see Gurevitch and Padilla 2004; Davis et al. 2011). Characteristics of species that allow them to become important invaders has been one of the most studied topics in invasion ecology (reviewed in Rejmánek et al. 2005). However, to date, few studies have examined the initial stages of plant invasion, including establishment in a new environment even though it is crucial for invasion success (e.g., Williamson 1996; Kowarik 2003; Pyšek and Hulme 2005).

Germination and early seedling development are key to the establishment of a plant when introduced, and may determine the success of a plant species establishing in the new region (Moravcová et al. 2006). In some genera of plants, differences among congeners in germination characteristics separate species that have and have not had successful introductions outside of their native range (Forcella et al. 1986; Dreyer et al. 1987; Vilà and D'Antonio 1998; Mandák 2003).

Within species, seed mass frequently correlates with both germination characteristics and seedling traits (Vange 2004). Often, larger seeds have higher germination rates than smaller seeds (Schaal 1984; Simons and Johnston 2000), but the opposite has also been observed (Dolan 1984; Susko and Lovett-Doust 2000). Heavier seeds generally have a higher probability of emergence than lighter seeds within species (Dolan 1984; Stanton 1984; Winn 1991), and develop into seedlings with superior competitive ability (Stanton 1984; Houssard and Escarré 1991), higher survival (Simons and Johnston 2000), and better performance in later life stages (Wulff 1986; Vaughton and Ramsey 2001). Emerging early may provide a competitive advantage because early individuals can monopolize resources and attain sufficient biomass for successful establishment (Miller 1987; Wilson 1988; Verdú and Traveset 2005).

The genus *Centaurea* (Asteraceae) has about 300 species (Garcia-Jacas et al. 2006), many of which have been introduced globally and have become invasive (negatively impact humans or ecosystems) in areas where introduced (Clements et al. 2010). For over 50 years a variety of introduced species of *Centaurea* have posed problems for agriculture and grazing land in the United States (Marrs et al. 2008; Hufbauer and Sforza 2008; Clements et al. 2010). In North America 34 species of *Centaurea* are reported to have been successfully introduced, 14 of

which are currently considered noxious weeds (having negative impacts on livestock and agriculture) in one or more US states (USDA, NRCS 2010)

Seed germination studies have been conducted on several species of *Centaurea* (Figure 1). The earliest study was conducted by Schirman (1981), who suggested that a limited number of seeds were required to produce dense monocultures of *Centaurea*. Many additional studies on seed germination in a variety of species of *Centaurea* followed (Weiner et al. 1997; Pitccairn et al. 2002; Gerlach and Rice 2003; Young et al. 2005; Widmer et al. 2007; Turkoglu et al. 2009; Henery et al. 2010; Hierro et al. 2009; Clements et al. 2010). These studies have assessed how life history (Gerlach and Rice 2003), optimal germination temperatures (Pitccairn et al. 2002; Widmer et al. 2007; Turkoglu et al. 2009; Clements et al. 2010), and rates of germination differ for seeds from the native range and the non-native range of different species (Hierro et al. 2009), and the effects of predation on germination variability (Weiner et al. 1997).

The purpose of this study was to examine seed and early seedling characteristics in an emerging invasive species (just starting to spread widely and have negative impacts), *Centaurea nigrescens* Willd (Tyrol knapweed). *Centaurea nigrescens* was introduced to North America from Europe during the 19th century (Chapter 2). Although it is now becoming more common in many areas in the United States, and has been declared a class A noxious weed species in Colorado, and Washington state (USDA, NRCS 2010), ecological research on this species has been very limited. I conducted germination experiments at two temperatures (28°C and 18°C), representing the average maximum and minimum temperature in the northern and southern limit in both the native and introduced range during July-September seeds of *C. nigrescens* collected from plants from multiple populations within the native (European) and nonnative (North American) range of this species. I tested whether there were differences in seed mass between seeds from population in the native and non native rage, whether seed mass affected the time to germination, whether there were temperature-dependent differences in germination success between seeds from the two regions, and whether there were temperature-dependent differences in the timing of the production of first, second and third leaves during early development of *C. nigrescens*.

Methods

Study System

Centaurea nigrescens (Figure 2a*)* is a member of the section *Jacea-Lepteranthus* of the

Circum-Mediterranean and Eurosiberian Clade (Garcia-Jacas et al. 2006). *Centaurea nigrescens* is a herbaceous perennial, 30-150 cm in height with from 1 to 50 erect flowering stems (Efloras 2008). It was introduced into North America in the 1800s (Chapter 1), and is presently found across the northern U.S. (Chapter 1).

During the juvenile stage, *C. nigrescens* forms a rosette with a central crown and fibrous root. At maturity, *C. nigrescens* produces a single upright stem 0.3–1 m tall, with numerous spreading branches. Seed heads are solitary or born in clusters of 2–3 at the ends of branches. This species spreads by both clonal growth via rhizomes and by seeds. The flowering period for this species is from June through November. Flowers are self-incompatible and plants produce up to 25 achenes, or seeds (Figure 2b), per capitulum when fertilized (Efloras 2008).

Seed Collection and Experimental Setup

Seeds were collected from 8 native populations, 4 in Italy and 4 in Switzerland, and 8 non-native populations in the United States from August-November 2010. Populations were selected to cover a broad range of habitats where this species was found in both the native and non-native range, and where I had access to material (Table 1, Figure 3). Seeds were collected from fertile stems and leaf rosettes from a minimum of 20 plants per population. To insure that individual plants were not clones, collections were made from plants that were well separated (> 1 m) from other individuals. Seeds from each plant were sorted and stored at room temperature in coin envelopes.

Growth chamber experiments

A growth chamber experiment was used to test for differences in germination rates of seeds from native and non-native populations. Although it would have been ideal to test the effects of both temperature treatments at the same time, because of limited access to chambers, the 28°C and 18°C treatments were conducted at different times. The experiment at 28°C was conducted from June 26 to July 25, 2011, and the 18°C treatment from July 25 to August 24, 2011. Because the potential for germination and growth was not expected to change over such a short period of time, these two treatments were considered part of the same experiment. In total, seeds from 20 different plants in 16 populations were used. Chambers were lit with 160 Watt Phillips AgroGro bulbs (600 n*mol*) and kept at a 12/12 hr day/night cycle.

Seeds were weighed with a microbalance (+/- 0.0001 g) and placed systematically into 9 cm petri dishes containing 1% agarose such that observations throughout development could be

tracked for individuals. For each of the 16 populations, 80 seeds (20 parent plants from each population, 4 seeds from each parent) were plated onto a single petri dish. Seeds from each plant were placed in different petri dishes. The only exceptions to this experimental set-up were native populations 13, 15 and 16, for which seeds were collected from less than 20 different maternal plants (Table 1). Low collection numbers were the result of small population sizes in Switzerland. In total there were 64 plates (16 populations x 4 replicates) for each temperature treatment.

The number and location of each of seed germinating and germination stage of each seed in each petri dish was documented daily (Figure 4) for 31 days. The following data for each seed were collected over the course of the two experiments: 1) initial mass, 2) time from planting to radicle emergence (germination), 3) time from planting to formation of first leaf, 4) time from planting to formation of second leaf, and 4) time from planting to formation of third leaf.

Measurements and Statistical Analysis

Analysis of Variance (ANOVA; JMP ver. 10, SAS Institute 2012) was used to test for differences in seed mass between native and introduced populations. Data were checked for normality using a Shapiro-Wilk test. No deviations from normality were found. Mean population seed mass was used in the ANOVA because there were not 20 plants from each population available, creating an unbalanced design (Table 1). In this model, source was a fixed factor (i.e., native or non-native). Mean seed mass within populations was then regressed against mean time to germination at each treatment temperature.

Percent germination data were collected by population. Data were arcsin transformed and a two-way factorial ANOVA was used to determine differences among treatments. A post-hoc Tukey test was performed to determine differences among specific treatments.

To test for differences in time to germinate and each successive stage of development, failure time analysis was used (JMP version 10, SAS Institute 2012). The Survival platform was used to generate the product-limit survival estimates to examine the proportional hazards assumption, which assumes all groups have an identical hazard function (time to germination). Four failure time models were used to assess differences within and among groups. Because the major hypotheses that were of interest related to the associations of seed source and time to event, I used a similar approach for modeling each of the 'time to' stages with Cox regression modeling. For both within and among populations, the following two models were used: 1)

model 1, time to germination was assessed at each of the studied treatment temperatures, and 2) model 2, time to first leaf, second leaf and third leaf from planting was assessed at each of the studied treatment temperatures.

To graphically illustrate the associations between germination or developmental stages for plants from native and non-native populations grown at each of the two temperatures, Kaplan-Meir survival curves were plotted from the proportional hazards model for native and non-native populations for each growth temperature. All comparisons between the groups were made using failure time statistics. Kaplan-Meir product-limit survival curves were plotted for native and introduced populations at 18°C and 28°C. Wilcoxon Chi-square statistics were used to test for homogeneity of estimated survival functions across groups and were used to assess differences between groups.

Results

Seed mass

Seed mass was not significantly different between the non-native populations and the native populations (P = < 0.329, Table 2, Figure 5). Mean seed mass for non-native populations was 0.0019 g (+/-0.00002) and 0.0020 g (+/-0.00002)) for seeds from native populations (Figure 6, 7). Additionally, there was no significant correlation between the time to germination and mean seed mass for seeds from either native or non-native populations at each temperature (18°C - P = 0.465, r^2 =0.039, 28°C - P = 0.911, r^2 = 0.001, Figure 6).

Germination Rate

Results of full factorial analysis indicate that there was a significant difference in germination rate for seeds from native and non-native populations, but no effect of temperature on germination rate or a significant interaction term (Table 3). Germination rate was significantly lower for seeds from the native range at both temperatures, (Tukey Test, P =0.525, Figure 7). At 18°C, 39.48% (+/- 7.37) of the seeds from native populations (N = 8 populations) germinated, and at 28°C they had a mean germination rate of 35.05% (+/- 4.27). For seeds from the non-native populations (N = 8), the germination rate at 18°C was 56.56% (+/- 4.6) and 69.84% (+/- 5.32) at 28°C.

Time to germination, first and second leaf

Germination

There was a significant effect of temperature on time to germination for the non-native

populations. At 18°C, the mean time to germination was 8.51 (+/- 0.2786) days and at 28°C it was 7.44 days (+/-0.300) ($P < 0.001$, Table 4, Figure 8). There was no significant difference for the native populations in time to germination for the two temperatures ($P = 0.06$, Table 4). At 18°C the mean time to germination was 8.17days (+/- 0.278), and at 28°C it was 8.01 days (+/- 0.495). There was no significant difference in time to germination at 18°C between seeds from native and nonnative plants ($P = 0.337$, Table 4). Similarly, there was no significant difference in the time to germination between the seeds from native and non-native plants at 28°C ($P = 0.713$, Table 4).

Development times

Time to formation of first leaf: For populations from the native region, the time from germination to formation of the first leaf did not significantly differ between 18°C and 28°C (Table 5, Figure 9). Mean time to development at 18°C was 10.88 days (+/- 0.596) and 9.52 days (+/- 0.602) at 28°C ($P = 0.1656$, Table 5). For non-native populations, there was a significant decrease in time to formation of the first leaf at the higher temperature ($P = 0.003$). The mean time to formation of the first leaf was 10.77 days (+/- 0.382) at 28°C and at 12.10 days (+/- 0.357) at 18°C, (Figure 9, Table 5).

Time to formation of second leaf: For seeds from the native population, the time from germination to the development of the second leaf was significantly different between 18°C and 28°C ($P = 0.013$). Mean time to development of second leaf was 16.72 days (+/- 0.65) at 18°C and 15.05 days (+/- 0.75) at 28°C. For seeds from non-native populations, the time to the development of the second leaf was significantly different between 18°C and 28°C ($P = 0.048$, Table 6, Figure 10). Mean time to second leaf was 18.72 days (+/- 0.32) at 18°C and 16.22 days (± 0.29) at 28°C.

Time to third leaf: For native populations, the mean time from germination to the development of the third leaf was not significantly different between 18°C and 28°C ($P = 0.091$, Table 7, Figure 11). Mean time to the third leaf was 16.72 days (+/- 0.654) at 18°C and 15.05 days (+/-0.749) at 28°C. For seeds from non-native populations there was also a significant difference in the time to formation of the third leaf between the two temperatures ($P < 0.0001$). The time for formation of the third leaf was longer at 18°C (18.74 days +/-0.3235) than at 28°C (16.22 days +/- 0.293), (Table 7, Figure 11).

Discussion

Successful invasion may result from the combination of several mechanisms that lead to success at different stages in the invasion process (Seastedt et al. 2005). Early life stages, especially germination success, may play a critical role in allowing plants to successfully produce populations in new environments, and can influence ultimate competitive interactions with other plants (Schemske 1984; Baskin and Baskin 1981; Hierro et al. 2009). Additionally, for many species of plants, the proportion of seeds germinating among populations has been linked to specific characteristics of environmental quality and risk, including the total annual precipitation and the inter-annual variation in precipitation (Philippi 1993; Clauss and Venable 2000; Venable 2007; Hierro et al. 2009).

For *Centaurea nigrescens* germination rate was markedly higher for seeds from non-native populations than for seeds from native populations. This increased rate of germination may contribute to successful establishment of the non-native seeds in the novel environment.

There were observable differences in the timing of developmental stages between plants from native and non-native seeds at each of the treatment temperatures. For seeds from non-native populations there was a significant decrease in time to formation of the first leaf at the higher temperatures, but for plants from the native seeds, time to formation of the first leaf did not significantly differ between 18°C and 28°C. For plants from both the native and non-native region, the time to the development of the second leaf was significantly faster at 28°C. Development time of the third leaf was not affected by temperature for plants from the native region, but was affected for plants from the non-native region. For those plants, the third leaf developed faster with an increase in temperature.

The higher germination rate of non-native *C. nigrescens* at both 18°C and 28°C and the lack of difference between germination at the two temperatures imply that there are not stringent temperature requirements for germination.

In some species, large seeds have higher germination success than small seeds (Tripathi and Khan, 1990; Khan and Uma Shanker 2001), while for others, small seeds germinate at a higher rate than large seeds (Marshell 1986). In other cases, germination has been found to be independent of seed size (Gross and Kromer, 1986; Perez-Garcia et al. 1995). The production of seeds of different mass is commonly related to differences in other properties of the seeds, such as germination potential, competitive ability, dispersal or ability to emerge following burial

(Venable 1992). Although there were significant differences in germination rate and time to germination, the seeds of *C. nigrescens* from native and non-native populations did not differ significantly in mass, and there was no relationship between mass of a seed and time to germination. This lack of difference in mass between natives and non-natives could be due to a lack of genetic differences in traits that control the mass of seeds for plants from the native and introduced range (Stanton 1984b; Temme 1986). This lack of difference could also be due to selection to keep seed mass constant in *C. nigrescens* (Cohen 1966; Venable and Brown 1988; Evans and Cabin 1995; Donohue et al. 2005; Venable 2007), especially if there is a seed number – seed mass trade off (Stanton 1984a; Lalonde and Roitberg 1989; Venable 1992). It also suggests that any differences in the environments of the maternal plants did not result in consistent differences in seed mass between native and non-natives populations.

The post germination stages examined in this research may be important in understanding how establishment occurs in non-natives, and the role of early developmental stages for invasion success. The findings in this study contrast with those Turkoglu et al. (2009), who found that for three species of *Centaurea*, *C. virgata, C. iberca,* and *C. balsamita*, an increase in temperature had a positive effect on germination speed. Like the present study, Hierro et al. (2009) found that there was no observable difference in germination time for seeds collected seeds from native (Turkey, Georgia) and non-native populations (California) of *C. solstitialis*. Hierro et al. (2009) investigated local weather patterns variation at each of the collected sites and found that germination in *C. solstitialis* is lower in both native and non-native populations experiencing greater variation in winter precipitation. Seeds collected from those same populations from time periods exposed to drier conditions showed an increase in germination. It remains unknown how local environmental factors may affect the time to germination in *C. nigrescens*. Local differences in rainfall or environmental variability may be factors contributing to the observed increase in percent germination of seeds from the native range and should be explored.

Table 1. Locations of populations where seeds were collected in the United States (1-8, introduced range), Italy (9-12, native range) and Switzerland (13-16, native range). These sites were selected because of access and because they covered a wide range of habitat types where *Centaurea nigrescens* is found. The number of seeds (N) used per experiment at each temperature is noted. Four replicates were used for each population at each of the treatment temperatures.

Population	Location	Latitude	Longitude	N seeds at 18°C	N seeds at 28°C
1	Bronx, NY	40.51	73.49	80	80
2	Greenport, NY	41.05	72.23	80	80
3	Middlesex, NJ	40.25	74.31	80	80
4	Blacksburg, Virginia	37.13	80.26	80	80
5	West Anandale, PA	40.38	74.58	80	80
6	Route 66, Virginia	38.53	77.33	80	80
7	Bethpage, NY	40.46	73.26	80	80
8	Pauling, NY	41.33	73.35	80	80
9	Campetti Italy	45.79	9.97	80	80
10	Del Capo, Italy	45.79	9.98	80	80
11	Roco Pino, Italy	45.23	7.77	80	80
12	Valmagore, Italy	45.78	9.98	80	80
13	Giubiasco, Switzerland	46.10	9.03	66	72
14	Negrentino, Switzerland	46.15	8.49	80	80
15	Pree, Switzerland	46.31	8.49	50	56
16	Somazzo, Switzerland	45.52	8.99	18	24

Table 2. Results of an ANOVA used to test for differences in population seed mass between populations from the native and non-native range.

	SS	DF	MS	F	P
Status	8.44e-8	1	8.44e-8	1.045	0.3239
Error	1.13e-6	14	0.0267		

Table 3. A full factorial ANOVA was used to test for differences in the percent of seeds from native and non-native plants that germinated at 18°C and 28°C. Both temperature and status (native or non-native) were fixed effects.

	SS	DF	MS	F	P
Status	0.901	1	0.901	33.510	0.000
Temp	0.094	1	0.0938	3.486	0.0724
Status*Temperature	0.011	1	0.0111	0.414	0.525
Error	0.753	28	0.0267		

Table 4. The effect of temperature on time to germination and time to each developmental stage. Values shown represent the mean in days for 4 replicates for each of the 16 populations (8 non-native, populations 1-8, and 8 native populations 8-16, at 18°C and 28°C). Differences were assessed within native, non-native and between native and non-natives with Wilcoxon test.

Time to germination				
		Mean	**SE**	**P**
Native	**18°C**	8.17	0.037	0.0587
	28°C	8.01	0.495	
Non-native	**18°C**	8.51	0.279	<0.0001
	28°C	7.44	0.300	
Native vs. Non-native	**18°C**			0.337
	28°C			0.713
Time to first Leaf				
		Mean	**SE**	**P**
Native	**18°C**	10.88	0.596	0.166
	28°C	9.52	0.602	
Non-native	**18°C**	12.10	0.357	0.003
	28°C	10.77	0.382	
Native vs. Non-native	**18°C**			0.059
	28°C			0.076
Time to second leaf				
		Mean	**SE**	**P**
Native	**18°C**	12.14	0.638	0.008
	28°C	10.11	0.672	
Non-native	**18°C**	13.81		<0.0001
	28°C	10.94		
Native vs. Non-native	**18°C**			0.013
	28°C			0.048
Time to third leaf				
		Mean	**SE**	**P**
Native	**18°C**	16.72	0.654	0.091
	28°C	15.05	0.749	
Non-native	**18°C**	18.74	0.324	<0.0001
	28°C	16.220	0.293	
Native vs. Non-native	**18°C**			0.337
	28°C			0.713

Figure 1. Flowers of species of *Centaurea* that have been used for germination studies. A) *Centaurea stoebe* (Spotted knapweed), B) *C. diffussa* (Diffuse knapweed), C) *C. solistalsis* (Yellow star-thistle), D) *C. melitensis* (Maltese star-thistle), E) *C. balsmita* F) *C. sulphurea* (Sulfur Knapweed), G) *C. calcitropa* (mouse thorn).

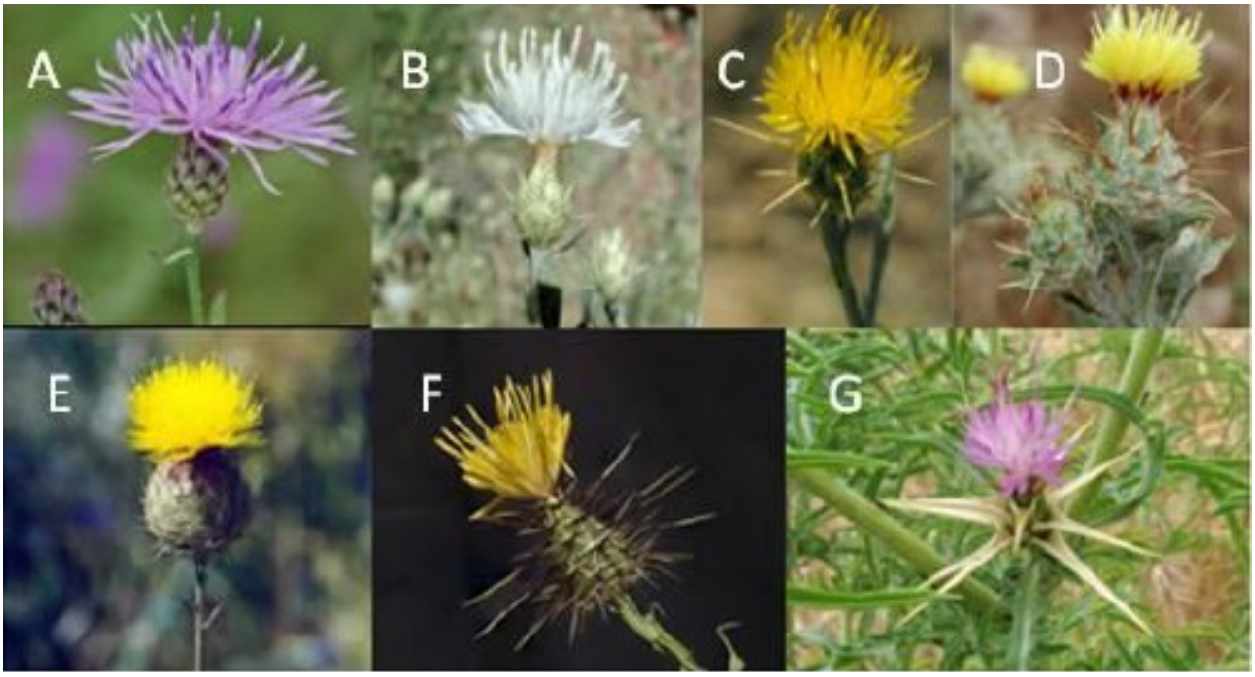


Figure 2. **A)** *Centaurea nigrescens* in flower July, 2011 Greenport, NY (Flower head approximately 3 cm). **B)** Achene (seed) harvested from flower head in an agar plate (scale bar = 2 mm).

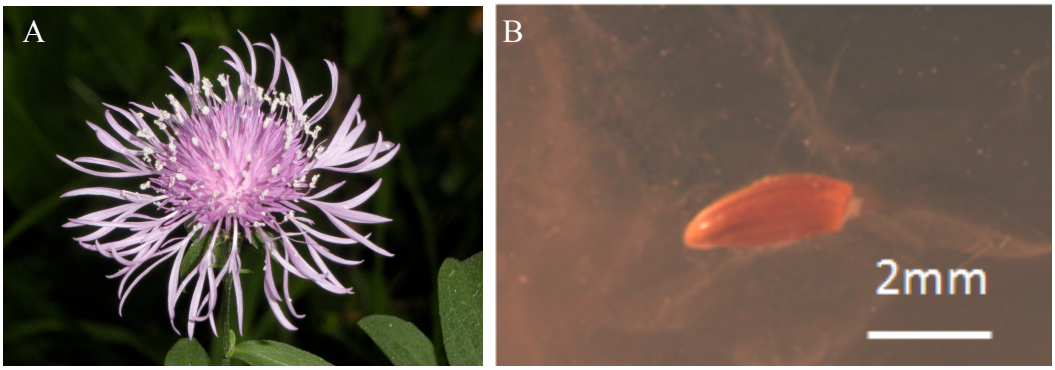


Figure 3. A) Collection locales in the United States (non-native) and **B)** Italy and Switzerland (native). At each location, seeds were collected from 20 maternal plants > 1m apart to avoid multiple samples from a single clone.

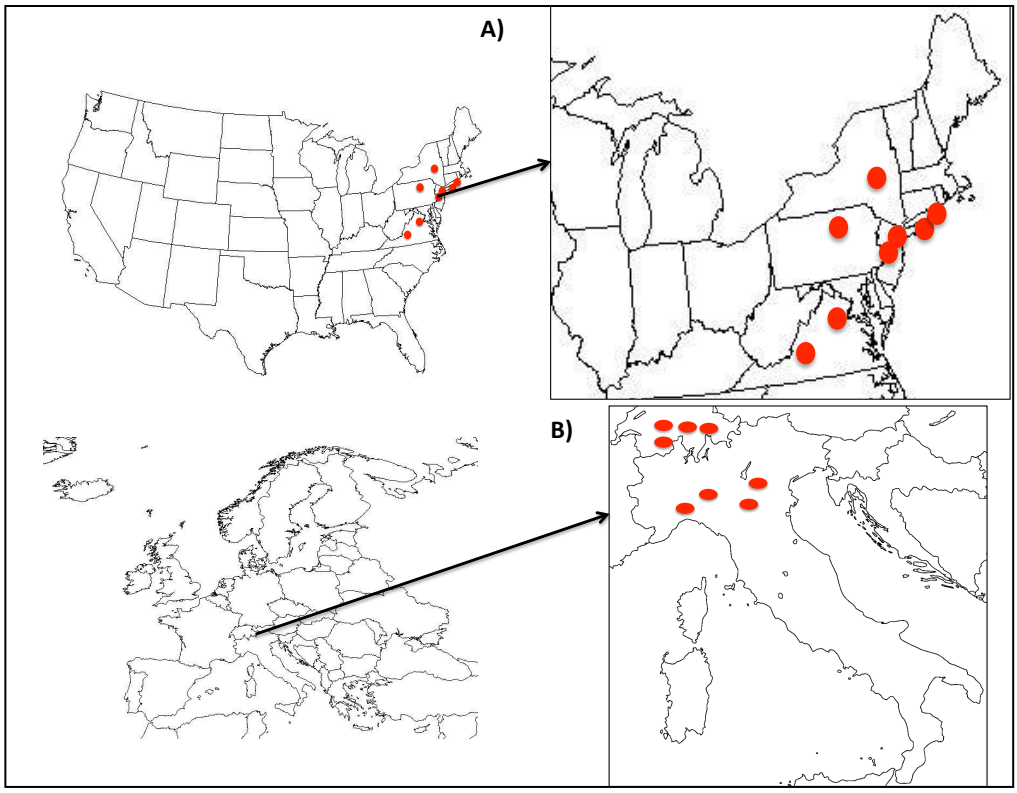


Figure 4. Stages of germination for *C. nigrescens*: **A)** radicle emergence (Germination), **B)** second leaf emergence, and **C)** elongation along axial lines emergence of cotyledons and formation of third leaf.

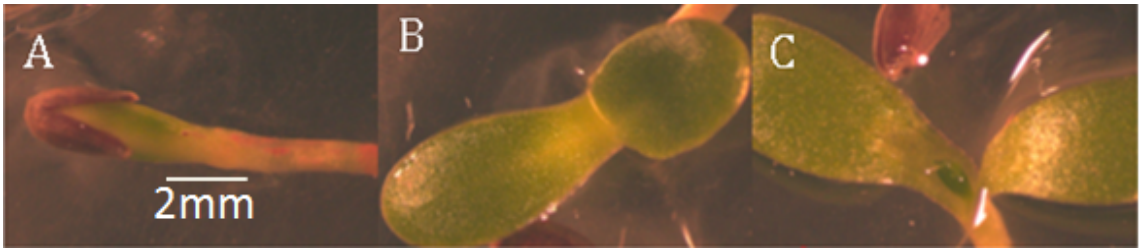


Figure 5. Mean seed mass from each of the sampled populations (1-16). Populations 1-8 are from the native range, and populations 9-16 are from the introduced range of *C. nigrescens*. Symbols are the means for each population and whiskers extend one standard error from the mean.

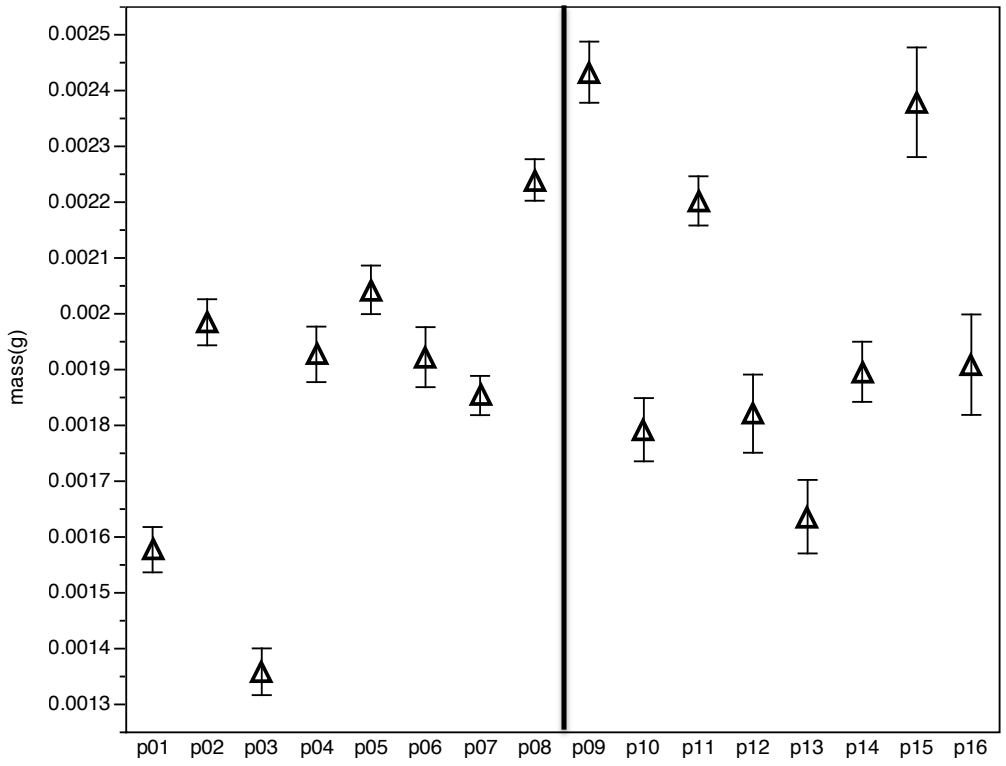


Figure 6. Linear regression of population mean time to germination versus seed mass for each treatment temperature: A) 18°C and B) 28°C. A line of best fit, using ordinary least squares, and r^2 values are reported on each of the graphs.

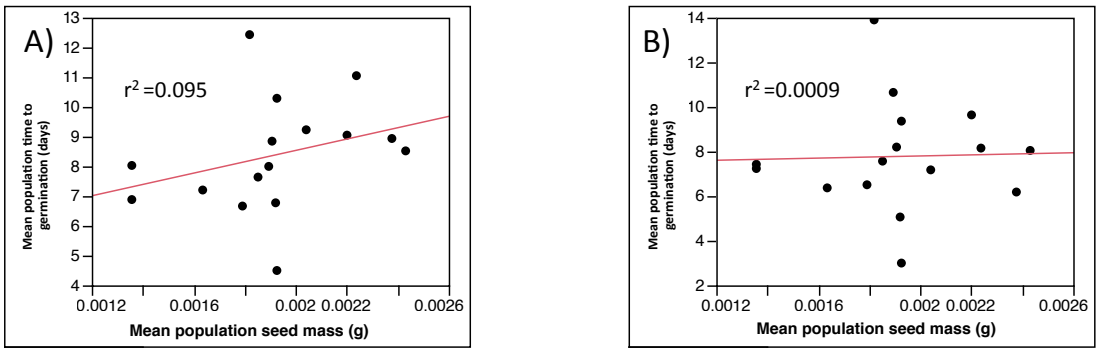


Figure 7. Effect of temperature on germination rates for seeds from native and non-native populations of *C. nigrescens*. The seeds from the non-native range had a higher germination rate at both temperatures than those from the native populations. There was no significant effect of temperature (18°C vs. 28°C) for seeds from either source populations (P = 0.525).

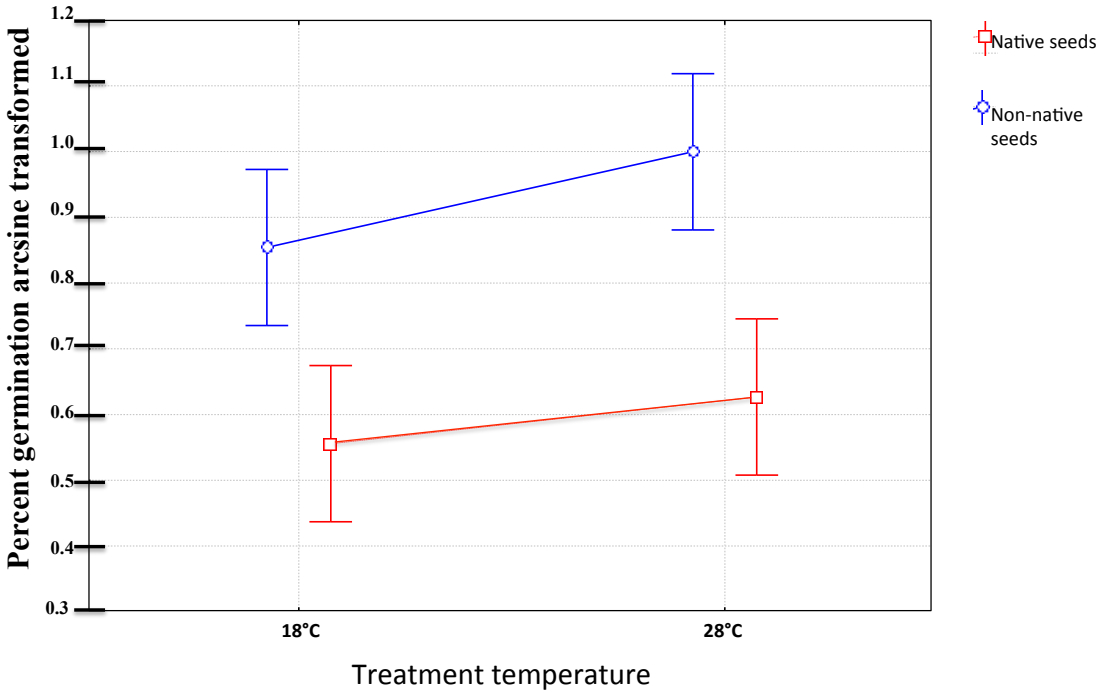


Figure 8. Failure plots for time to germination for **A**) native populations of *C. nigrescens* at 18°C and 28°C, **B)** non-native populations of *C. nigrescens* at 28°C. For each experimental temperature the number of plants reaching the studied stage are reported (N) and the number of days (mean) to each stage are reported with test statistics (Table 4).

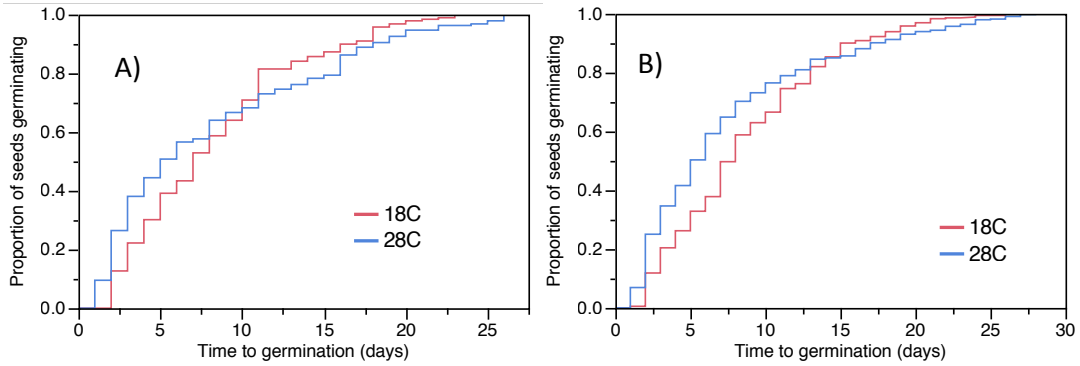


Figure 9. Failure plots for time to germination for **A**) native populations and non-native populations of *C. nigrescens* at 18°C, and **B)** native and non-native populations of *C. nigrescens* at 28°C. For each experimental temperature the number of plants reaching the studied stage are reported (N) and the number of days (mean) to each stage are reported with test statistics (Table 4).

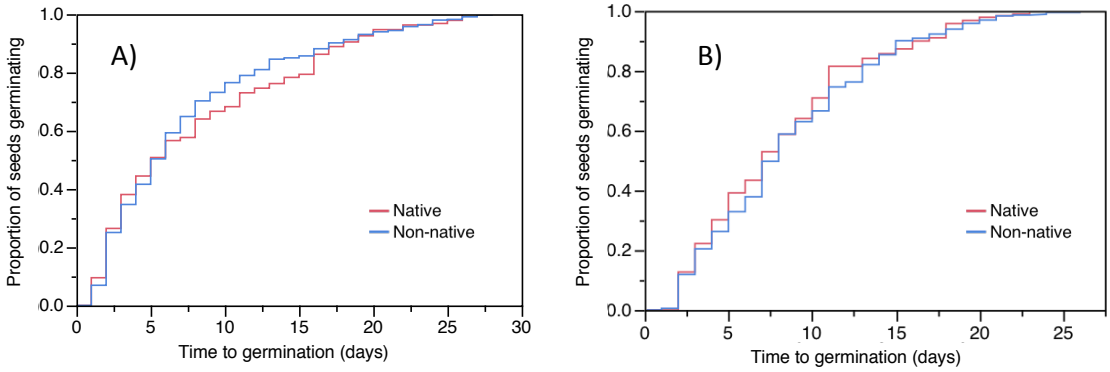


Figure 10. Failure plots for formation of first leaf, **A**) native populations of *C. nigrescens* at 18°C and 28°C, **B)** non-native populations of *C. nigrescens* at 18°C and 28°C. For each experimental temperature the number of plants reaching the studied stage are reported (N) and the number of days (mean) to each stage are reported with test statistics (Table 5).

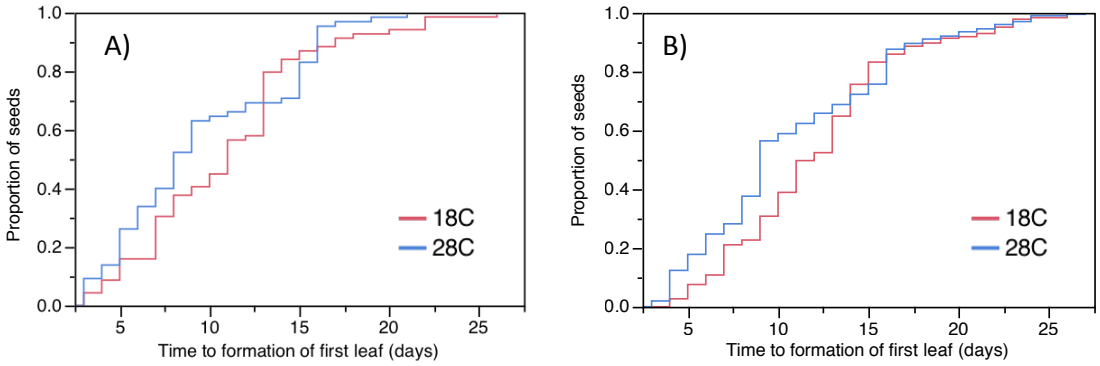


Figure 11. Failure plots for formation of second leaf, **A**) native populations of *C. nigrescens* at 18°C and 28°C, **B)** non-native populations of *C. nigrescens* at 18°C and 28°C. For each experimental temperature the number of plants reaching the studied stage are reported (N) and the number of days (mean) to each stage are reported with test statistics (Table 6).

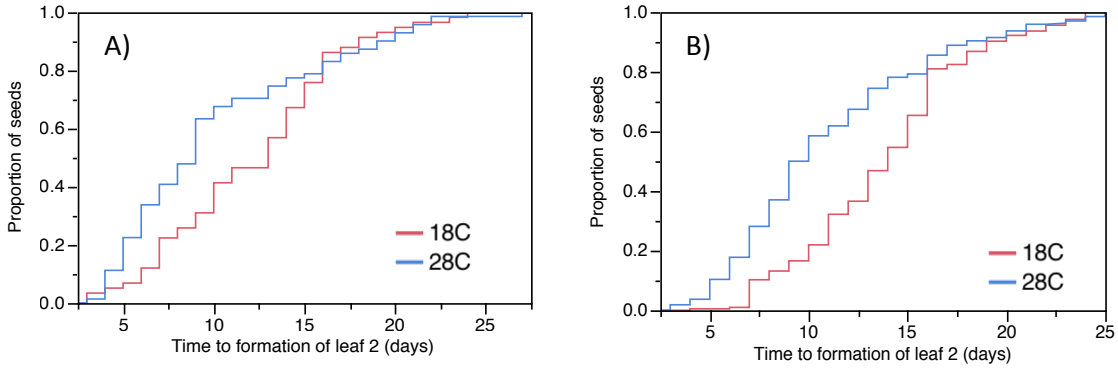
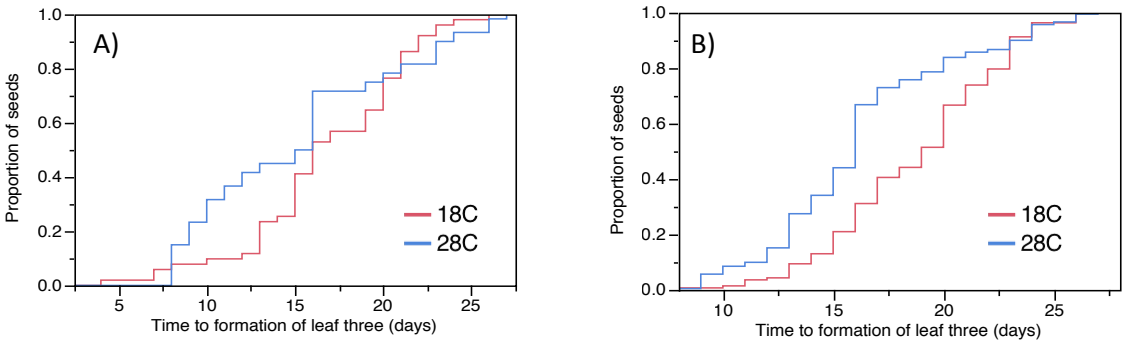


Figure 12. Failure plots for formation of third leaf, **A**) native populations of *C. nigrescens* at 18°C and 28°C, **B)** non-native populations of *C. nigrescens* at 18°C and 28°C. For each experiment the number of plants failed in total are reported and the number of days (mean) to each stage are reported with test statistics (Table 7).



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Chapter 4

Responses to light, water and nutrient enrichment in native and nonnative populations of the weed species *Centaurea nigrescens* (Tyrol Knapweed)

Introduction

An important topic in invasion ecology is whether certain traits of species are generally associated with invasion success and can therefore be used to predict and, ultimately, prevent biological invasions (Rejmánek et al. 2005b; Pyšek and Richardson 2007). The ability of successful invasive plants to attain higher densities in their introduced range compared to conspecifics in their native range suggests that they may grow more vigorously in their new environment, have fewer competitors, or fewer enemies (Elton 1958; Crawley 1987; Thebaud and Simberloff 2001; Leger and Rice 2003; Jakobs et al. 2004; Bossdorf et al. 2005). Some studies have demonstrated that when native or non-invasive aliens (here defined as introduced non-problematic species, species that have no demonstrated negative environmental or human impacts) are compared with invasive aliens (here defined as introduced problematic species, with demonstrated negative environmental or human impacts), the invasive plants grow faster, have higher leaf nutrients, higher specific leaf areas, shorter life cycles, devote more resources to reproduction and produce more seeds that disperse farther and germinate faster (Grotkopp et al. 2002; Grotkopp and Rejmanek 2007; van Kleunen et al. 2010; Thompson and Davis 2011).

High relative growth rate (RGR; the total mass increase per above ground biomass per day) has been found to be an important characteristic of invasive plant taxa in disturbed or open areas, especially in nutrient rich environments (e.g., Roy 1990; Rejmánek and Richardson 1996; Williamson and Fitter 1996; Reichard and Hamilton 1997; Pattison et al. 1998; Grotkopp et al. 2002). Rejmajek et al. (2005) found that for *Pinus* the RGR of invasive species is significantly higher than that of introduced non-invasive pines. Differences in RGR are primarily determined by leaf area ratio (LAR; total leaf area per total plant dry mass), and that LAR is primarily determined by specific leaf area (SLA; leaf area per leaf biomass). In *Pinus,* successful invaders have significantly higher specific leaf area than species that are not invasive, and there is a highly significant positive relationship between RGR and invasiveness in this genus (Grotkopp and Rejmanek 2007). Other work suggests that for introduced species, increased relative growth rates (RGR) is generally associated with lower root to shoot ratios (RSR; dry below ground biomass relative to dry above ground biomass), higher specific leaf areas (SLA), leaf area ratios (LAR) and higher net CO_2 assimilation (A) as well as lower respiration costs (RD) (Pattison et al. 1998; Baruch and Goldstein 1999; Durand and Goldstein 2001; Smith and Knapp 2001; Grotkopp et al. 2002; McDowell 2002; Ehrenfeld 2003; Wilsey and Polley 2006).

Greenhouse studies have been particularly useful for identifying the phenotypic traits that can give successful invaders an advantage and for testing whether there are functional trait differences between non-native invasive and native plants (Siemann and Rogers 2001; Leger and Rice 2003; Wolfe et al. 2004; Erfmeier and Bruelheide 2005; Güsewell et al. 2006). DeWalt et al. (2004) conducted a greenhouse study with the tropical shrub *Clidemia hirta,* which revealed that, contrary to expectations, introduced Hawaiian and native Costa Rican populations of this shrub displayed no significant differences in RGR, Amax (leaf maximum net photosynthetic rate) or SLA when grown under similar conditions. In studies with species in the Brassicaceae, experimental studies have found significant but not always consistent differences in growth and reproductive characteristics between plants from their native and introduced ranges (Buschmann et al. 2005). In *Solidago,* Güsewell et al. (2006) found that introduced European plants produced a greater number of shoots than their native counterparts in North America. However, other traits such as shoot size, leaf traits and litter decomposition were not different between native and introduced populations of different species of *Solidago*. In a 4-month greenhouse experiment with *Lythrum salicaria,* Bastlová and Kvêt (2002) found that total leaf area (TLA) and SLA were significantly greater for plants from invasive populations than from native populations, but found no significant differences in LAR or A.

Centaurea solstitialis is native to Europe, but has been introduced to California. Graebner et al. (2012) found that there were significant differences in a variety of traits between plants from populations in the non-native range of California versus those from the native range in Spain. Plants from the introduced range had greater competitive resistance, larger seed size, and larger seedling mass than plants from the native range, indicating that these differences may be the result of selection on traits that contribute to competitive success and affect growth and competitive ability. Graebner et al. (2012) also found that introduced populations of *Centaurea solstitialis* had the highest relative growth rates when grown in competition with other species within its introduced range.

Differences among the results from studies on traits that influence the success of invasives may reflect the fact that successful invasion could be the consequence of complex trait interactions between the invader and the environment rather than specific traits of plants that are successful invaders (Muth and Pigliucci 2007).

The purpose of this study was to compare a variety of traits and the performance of plants

from native and non-native populations of *Centaurea nigrescens* under a variety of conditions expected to influence invasion success. This plant was introduced into the United States in the 1800's (Chapter 1). It is uncertain if this species is in the incipient stages of invasion (i.e. rapid spread will occur in the near future), or if it will become an aggressive invader like other species of *Centaurea.* I conducted 3 separate greenhouse experiments with seedlings from 8 native and 8 non-native populations to test whether there are differences in traits that affect competitive ability in the introduced range of this species. In this study, I tested the hypothesis that non-native *C. nigrescens* from North America are better able to cope with drought, nutrient limitation and light limitation than native (European) populations of this plant species by examining differences in traits and the relative performance of plants. I compared a variety of morphological features expected to impact plant performance when plants were growth under low versus high nutrients, low versus high light, and low versus high water availability.

Methods

Study System

C*entaurea nigrescens*, Tyrol knapweed or short-fringed knapweed, is a flowering plant species introduced to North America from Europe in the 19th century. It is a herbaceous perennial, 30-150 cm in height with erect flowering stems numbering from 1 to as many as 50 (Efloras 2008). This species reproduces and spreads by both clonal growth via rhizomes and seeds. Populations are typically found along roadsides and in highly disturbed sites in Italy and Switzerland as well as in the United States (Wagenitz 1955; Chapter 1). The flowering period for this species is from June through November. C*entaurea nigrescens* forms a rosette during winter, bolts, and sends up stalks with floral buds in the spring, flowers and fruits in mid-late summer, and above ground biomass dies back by end of late autumn. Flowers are self-incompatible and plants produce up to 25 seeds per capitulum when fertilized (Efloras 2008).

Seed Collection

Achenes (referred to here as seeds), were collected from August-November 2010 from 8 native populations, 4 in Italy and 4 in Switzerland, and 8 non-native populations in the United States, from a broad range of habitats (Table 1). Seeds were collected from a minimum of 20 maternal plants per population. To insure plant selection included individuals that were not clones, seeds were collected from fertile stems and leaf rosettes that were well separated (> 1 m) from other individuals. Seeds from each parental plant were sorted and stored in coin envelopes

at room temperature.

Seeds were washed with distilled water and planted in 115 mm plastic pots. The top of the pots were filled with 1 cm of Sunshine mix No:1 (SUNGRO, Horticulture, Bellevue, WA 98008) and the rest of the pots were filled with Turface clay medium (PROFILE Products LLC, Buffalo Grove, IL 60089). Turface was used so that the roots could be completely recovered at the end of the experiments (e.g., Dudley 1996). Initially, plants were grown in a growth chamber at temperatures of 28°C with a maximum photosynthetic photon flux density (PPFD) of approximately 900 μm^{-2} s^{-1}. When plants were 28 days old they were transferred to a greenhouse, which was kept at approximately 28/25°C day/night air temperatures, and a 16-h photoperiod using supplemental lighting provided by (6) X 425W metal halide lamps (PPFD = 400 m*mol* photons m^{-2} s^{-1} at sun down). Plants were acclimated to greenhouse conditions for 16 days prior to the beginning of the experiments.

Pre-experimental measurements

To control for differences in initial plant size the following traits on each individual plant were measured prior to initiation of experiment: initial leaf number, initial length of longest leaf, initial plant height, and initial diameter of the basal rosette.

Greenhouse experiments

Three different experiments were conducted under greenhouse conditions to test for differences in the response of plants from native and non-native populations of *C. nigrescens* to light, water, and nutrients. Five maternal plants from each of the 16 collected populations were randomly selected for use in the experiment. For each treatment, five blocks were set up, and half of each block was assigned at random to either the control or to the experimental treatment. Plants were assigned at random from each population from each source area (native versus non-native range, 14-16 plants per treatment per block) to one of the two treatments in each of the five blocks. This was done for each of the three experiments (high versus low light, high versus low nutrient availability, high versus low water availability). Day length during the experiment was 16 h and light conditions in the greenhouse ranged from 400–600 m*mol* photons m^{-2} s^{-1} (LiCor LI-250A light meter, LiCor Biosciences) and the air temperature ranged from 18.3 to 28.0°C. There was a total of approximately 12-16 plants from each population in each treatment of each block.

Effects of High and Low Nutrient Conditions

The low and high nutrient treatments were designed to be a reasonable approximation of low and high soil nutrient levels that would be encountered under natural field conditions. In the low nutrient (LN) treatment 100 ppm of fertilizer was applied, whereas in the high nutrient (HM) treatment 400 ppm of fertilizer was applied. Stock solutions for nutrients were prepared using Jacks Water Soluble Fertilizer 15-5-15 (AM Leonard, 241 Fox Drive Piqua, OH 45356-9265), which is 15% total nitrogen, 5% phosphorous, and 15% potassium. Nitrogen was in the form of 12.00% nitrate and 3.00% ammonia. In addition, the solution contained minor nutrients: Calcium 4.00%, Magnesium 2.00%, Boron 0.0150%, Copper 0.0075%, Iron 0.0750%, Manganese 0.0375%, Molybdenum 0.0075%, and Zinc 0.0375%. All stock concentrations were prepared for a Dosatron injector (15 gallon upright, Dosatron, 2090 Sunnydale Blvd. Clearwater, FL 33765). Over the course of the two-month experiment, all plants were watered every other day to full capacity and the fertilizer nutrient solution was added to the treatments every 5 days.

Effects of high and low light

Plants in the low light treatment were placed inside 90 cm x 90 cm x 102 cm cages surrounded by shade cloth to simulate edge of forest habitat (K PRO Supply Co., Inc. Sarasota Florida). Light levels inside the shade cages were measured 15 times over the course of the experiment to calculate light levels in the cages and outside of the light cages. Light inside the shade cage varied from 195 ± 5.7 to 305 ± 4.8 m*mol* m^{-2} s^{-1} (mean ± SD, n = 15), depending on cloud cover on the day measurements were taken. Plants receiving the high light treatment were placed on the bench top 2.5 m from the light cage so that light was not obstructed by the shade case. Plants in the high light treatment experienced light levels ranging from 400 ± 4.5 to 600 ± 3.2 m*mol* m^{-2} s^{-1} (mean ± SD, n = 15). Greenhouse temperature records ensured that the plants in the high and low light environments experienced similar temperatures over the course of the experiment. Liquid nutrients were applied two weeks prior to the start of the experiment. All plants were watered every other day to full capacity, and no nutrients were applied over the course of the experiment.

Effects of High and Low Water Availability

The two water treatments were intended to represent continuously moist conditions (HW; pots watered every other day) and intermittent drought (LW; pots watered every five days, which was when soil medium was dry). The conditions of low water treatment with drought

represented roadside conditions. No plants were treated with nutrients over the experiment; liquid nutrients were applied two weeks prior to the start of the experiment. All plants had full light exposure throughout the experiment.

Plant Harvesting

For each of the three experiments all plants were harvested after 2 months of growth under treatment conditions (November 16, 2011, four months of total growth). Harvested plant material was divided into leaves, support tissues (stem and petiole) and roots. Leaf area was measured using a leaf area meter (Model LI- 3000, Li-Cor Inc. Lincoln, NE, with and conveyor belt assembly Model LI-3050, Li-Cor Inc. Lincoln, NE). All plant material was dried in an oven at 70°C for 48 hours. After drying, samples were weighed on a microbalance (± 0.0001 g). Specific leaf area (cm^2g^{-1}) was calculated for the longest leaf (LL), and a mid-sized rosette leaf and mid-sized stem (MS) if flowers were present on the plant.

Statistical Analysis

For each experiment, the effect of treatment on native and non-native plants was analyzed with a split-plot design. A mixed model with fixed and random effects was constructed in R 2.14 (R Development Core Team 2011) using the lme 4 package. The mixed-effects model was used to test for each response and assess differences between native and non-native source populations. Source effects, treatment effects and an interaction were assessed for 31 traits. The model constructed fits a regression between the response (e.g., biomass) as a function of the fixed effects (i.e., status and treatment), and accounts for the variance due to random effects (i.e., population and block). The model was fit using REML (restricted maximum likelihood). This method is recommended when there is an unbalanced design, in this case, due to many missing data points (Littell 2002). MCMC (Markov chain Monte Carlo method) was used to report p-values. All data were checked separately for nonlinearities. In no case did a nonlinear formulation improve the model fit significantly.

Results

Nutrient experiment

Assessment of plant randomization

At the start of the experiment, initial leaf number in native and non-native plants differed significantly between plants assigned to each treatment (P = 0.019, Table 2, Figure 2). At the start of the experiment, in high nutrient treatment, native population had 12.10 leaves (± 1.50) and non-native plants had 9.18 (± 0.51). In the low nutrient treatment, native plants had fewer leaves, 9.57 (± 0.91), as compared to non-native plants with 10.39 (± 0.52). There was no significant difference between plants from the two source regions (Table 2) for initial leaf length or initial plant height.

Post-treatment measurements

Area of leaf

A significant difference existed between non-native plants and native plants in area of the mid-sized rosette leaf (P = 0.014, Table 2, Figure 3). Non-native plants in high nutrient treatment had a larger area of mid-sized rosette leaves (avg. = 55.67 cm^2 ± 3.66) than native plants (avg. = 44.94 cm^2 ± 2.96). In the low nutrient treatment the non-native plants also had a larger leaf number (avg. = 56.84 cm^2 ± 2.41) as compared to native populations (avg. = 47.83 cm^2 ± 2.89). There was no significant difference in the area of the longest leaf on plants (P = 0.349) or the area of a mid-sized stem leaf (P = 0.932) for the different treatments.

Leaf width

A significant difference was found between non-native and native plants in the maximum width of longest leaf (P = 0.030, Table 2, Figure 4). Non-native plants in the high nutrient treatment had a larger length of longest leaf (avg. = 5.71cm ± 0.22) than the native populations in the high nutrient treatment (avg. = 5.05 cm ± 0.22). In the low nutrient treatment, non-native populations also had a higher width of longest leaf (avg. = 5.41cm ± 0.25) than native populations in the low nutrient treatment (avg. = 5.20 cm ± 0.18). Mid-sized rosette leaves were also significantly different between native and non-native populations (P = 0.001; Table 2, Figure 5). In the high nutrient treatment mid-sized rosette leaves were larger in non-native populations (avg. = 4.84 cm ± 0.23) compared to native plants (avg. = 3.82 cm ± 0.22). The non-native populations also had a larger maximum leaf width in the low nutrient treatment, (avg. =

4.78 cm ± 0.19) as compared to native populations in the same treatment (avg. = 4.01cm ± 0.19; Figure 5).

Leaf Mass

A significant difference was found between native and non-native plants in the mass of the longest plant leaf (P = 0.016, Table 3, Figure 6). In the high nutrient treatment, the mass of non-native plants was larger (avg. = 0.16g ± 0.01) than for native plants in that treatment (avg.= 0.12g ± 0.01). In the low nutrient treatment the mass of longest leaf was larger in non-native plants (avg.= 0.15g ± 0.01) than native populations (avg. = 0.12g ± 0.01).

A significant difference was also observed in the mass of mid-sized rosette leaves (P = 0.001, Table 3, Figure 6). In both high and low nutrient treatments mid-sized rosette leaves were larger in non-native populations. In the high nutrient treatment, non-native populations had a larger mass (avg.= 0.12 g ± 0.01) than native populations (avg. = 0.08g ± 0.01). In the low nutrient treatment the observed mass of mid-sized rosette leaves was also larger in non-native populations (avg. = 0.11g ± 0.01) than native populations in the same treatment (avg.= 0.08g ± 0.01, Figure 6).

There was no significant difference between native plants and non-native plants for mass of mid-sized stem leaves (Figure 6).

Additional trait measurements

There was no significant effect of the nutrient treatment, source region or light by source region interaction for the following traits: total leaf number, plant height, branch number, presence of flowers, capitula number, flower number, shoot length, root length, root to shoot ratio, area of longest leaf, area of mid-sized stem leaf, maximum width of mid-sized stem leaf, maximum length of longest leaf, maximum length of mid-sized rosette leaf, mass mid-sized stem leaf, specific leaf are of longest leaf, specific leaf area of mid-sized rosette, specific leaf area of mid-sized stem, root mass, shoot mass, total dry biomass (Table 2).

Light experiment

Assessment of plant randomization

At the start of the experiment, initial plant height was significantly larger in the native populations in the high light treatment (P = 0.027, Table 3, Figure 7). However, final plant height was not significantly different between the native and non-native plants in either treatment (P = 0.484, Table 3, Figure 7).

Post-treatment measurements

Branch number

Branch number in both high light and low light treatment was significantly greater in the non-native plants (P = 0.032, Table 3, Figure 8). Branching number in non-native populations in high light was greater (avg. = 1.56 ± 0.54) than native populations in the same treatment (avg.= 0.48 ± 0.24). In the low light treatment the non-native populations also exhibited greater branching (avg. 0.51 ± 0.20) than the native populations (avg. = 0.12 ± 0.12). The maximum number of branches observed was 16 branches on a non-native plant in the high light treatment. Excluding zeros, the minimum number of branches was 3, which was observed on a single native plant.

Leaf mass

Treatment effects were observed for native and non-native plant leaf mass (P = 0.004, Table 3). Source effects were also observed as the mass of the longest leaf was significantly larger in non-native populations (avg. = 0.16g ± 0.01) than native populations (avg. = 0.12g ± 0.01) in the high light treatment (P = 0.038, Table 3, Figure 9). The mass of mid-sized rosette leaves (avg.= 0.11g ± 0.01) was significantly larger in non-native plants (P = 0.039, Table 3, Figure 9) than native plants in the same treatment (avg. = 0.08g ± 0.01).

Maximum length of mid-sized rosette

There was a significant interaction between source and light treatment for the length of mid-sized rosette leaves (P = 0.019 Table, 3 Figure 10). In the high light treatment non-native plants had smaller rosette leaves (avg. = 102.12 cm ± 6.91) than native plants (avg. = 124.63 cm ± 2.69). In the low light treatment, non-native plants had a larger mid-sized rosette leaves (avg. = 129.27 cm ± 10.37) than the native plants (avg. = 105.86 cm ± 7.77).

Additional trait measurements

There was no significant effect of the light treatment, source region or light by source region interaction for the following traits: presence of flower(s), capitula number, flower number, root length, root to shoot ratio, area of longest leaf, area of mid-sized rosette, area of mid-sized stem, maximum width of mid-sized rosette, maximum width mid-sized stem, maximum length of longest leaf, maximum length of mid-sized stem leaf, mass mid-sized stem leaf, and specific leaf area mid-sized stem (Table 3).

Water Experiment

Assessment of plant randomizations

Initial plant height was significantly larger (P = 0.035, Table 4) for the native populations (10.65 cm ± 1.34) as compared to the non-native plants (10.06 cm ± 1.06) in the high water treatment. However final plant height was not significantly different between the groups (P = 0.272, Table 4, Figure 11).

Post-treatment measurements

Total leaf number

A significant difference was found for total leaf number between native and non-native populations at the end of the experiment (P = 0.011, Table 4, Figure 12). In the high water treatment non-native plants had a greater number of leaves (avg. = 40.54 ± 5.98) than native plants (avg. = 24.95 ± 3.52). In the low water treatment, non-native plants also had a greater leaf number (avg. = 24.05 ±3.02) than native plants in the same treatment (avg. = 19.0 ± 2.15).

Shoot Mass

Shoot mass was significantly greater in non-native populations in the high and low water treatments (P = 0.0007, Table 4, Figure 13). In the high water treatment non-native shoot mass was greater (avg. = 1.82 g ± 0.203) than for native populations in the same treatment (avg. = 1.10 g ± 0.17). Shoot mass was also greater in the low water treatment for non-native populations (avg.= 1.30g ± 0.12) than for native populations in the same treatment (avg.= 0.88g ± 0.11).

Leaf width

A significant difference in maximum leaf width for the longest leaf was found between native and non-native plants in both the high water and low water treatments f(P = 0.003), the maximum width of medium sized rosette leaves (P = 0.001) and the maximum width of mid-sized stem leaves (P = 0.043, Table 4, Figure 14). In the high water treatment, non-native populations had a larger maximum width of longest leaf (avg. = 5.30 ± 0.02) than native populations (avg. = 4.66 ± 0.27). In the low water treatment, leaf width was also larger for non-native (avg. = 4.70 ± 0.15) than native populations in that same treatment (avg. = 4.02 ± 0.26). Non-native populations in high water treatment had a larger maximum width of mid-sized rosette leaves (avg. = 4.59 ± 0.23) than native populations in the same treatment (avg.= 4.01 ± 0.019). In the low water treatment non-native mid-sized rosette leaves were also larger (avg. = 3.73 ± 0.15) than native populations (avg. = 3.29 ± 0.20). In high water mid-sized stem leaves were

larger in native populations (avg. = 3.82 ± 0.53) than non-native populations (avg. = 2.95 ± 0.21) in the same treatment. In low water the mid-sized stem the native populations was larger (avg. = 2.87 ± 0.30) than for non-native plants (avg. = 2.07 ± 0.13).

Mass of longest leaf

A significant treatment effect (P = 0.034) and source effect (P = 0.044) were observed in mass of longest leaf for both native and non-native plants in high water treatment (P = 0.0347, Table 4, Figure 15). In the high water treatment, leaf mass was greater for the non-native (avg.= 0.14g ± 0.01) than native populations (avg.= 0.12g ± 0.01). In low water treatment leaf mass was greater in non-native populations (avg. = 0.12 ± 0.01) than native plants (avg. = 0.09 ± 0.01) in the same treatment.

Additional trait measurements

There was no significant effect of the water treatment, source region or light by source region interaction for: initial leaf number, initial length of longest leaf, initial diameter, plant height, branch number, presence of flowers, capitula number, flower number, root length, area of longest leaf, area of mid-sized rosette, and area of mid-sized stem (Table 4).

There was no significant effect of the water treatment or the interaction, but there was a significant effect of source population for the mass of the shoot (Table 4).

There were significant treatment effects and a significant interaction for maximum width of longest leaf, maximum width of mid-sized rosette leaves, maximum width of mid-sized stem, and mass of longest leaf (Table 4).

There was a significant effect of the water treatment and source, but no significant interaction for specific leaf area of longest leaf, specific leaf area of mid-sized rosette (Table 4).

There was a significant effect of water treatment, but no effect of source region and no significant interaction for: shoot length, maximum length of longest leaf, maximum length of mid-sized rosette, maximum length of mid-sized stem, mass of mid-sized rosette leaf, mass mid-sized stem leaf, specific leaf area mid-sized stem, mass root, and biomass (Table 4).

Discussion

Although trait differences were expected between plants from the introduced range and native range of *C. nigrescens* in response to differences in light, water, and nutrient, I found relatively few differences between the native and non-native populations. In addition, there was no trait that consistently differed among the three experiments. However, some traits did differ

significantly in the direction one would expect for greater performance of plants from the introduced range of this species.

Nutrient Experiment

Past research suggests that among species, increased resource availability should favor the relative performance of non-native species (Harrison 1999; Smith and Knapp 1999; Smith and Knapp 2001: Knochel et al.2010a; 2010b). In this study, non-native plants had a significantly larger maximum leaf length and a greater maximum width. Additionally, mid-sized rosette leaves were also significantly wider in non-native populations in both treatment conditions. For each of these leaves, there was a significant increase in leaf mass in each of the treatments. Larger leaf area is a trait associated with faster growing species (Remkes 1990; Duru et al. 1996). Additionally, non-native plants had a significantly greater number of leaves in the low nutrient treatment, but not in the high nutrient treatment. Increased leaf number is also correlated with increased growth rate (Poorter and Lambers 1992), suggesting that *C. nigrescens* can allocate resources to the production of leaves even under lower nutrient conditions. Unlike other species of *Centaurea* that slow down the allocation of resources in resource-limited environments (*C. stoebe*; LeJeune et. al 2005), introduced plants of *C. nigrescens* do not appear to do so. In a competition experiment, He et al. (2012) showed that for *C. stoebe* simulated N deposition enhanced growth and relative competitive advantage over native North American plants. Additional experiments with *C. nigrescens* should be conducted to assess nutrient allocation when in an environment with competitors.

The combination of an increased leaf number and an increased area of rosette leaf make *C. nigrescens* an opportunistic capturer of solar energy. Opportunistic resource acquisition for growth and reproduction appears to be one of the key mechanisms important for invasion success (Davis et al. 2000; Burns 2004, 2006; Leishman and Thomson 2005; Blumenthal 2005). In order to confirm these findings it would be necessary to perform an experiment over a range of nutrient concentrations as it is possible that the treatment conditions of 100 ppm and 400 ppm did not represent a large enough range of nutrient availability.

Light experiment

The ability of a plant to develop extensive above ground branching allows it to produce many leaves for carbohydrate production during the growing season, and results in an increased number of flowers, thereby increasing fitness (Baker 1965). There was a significant increase in

branch number in non-native populations in both the high light and the low light treatments. Increased branching has been observed in other non-native species (Sasek and Strain 1991). In the present study, the change in branch number in response to light availability could give *C. nigrescens* and advantage over other plants and allow it to overtop neighboring plants. However, the lack of increased flower number is contrary to the findings of Gerlach and Rice (2003) who found differences in inflorescence number in invasive *C. solstitialis*. My findings with *C. nigrescens* support the study by Muth and Pigliucci (2006), which found no significant difference in inflorescence number between invasive and non-invasive species of *Centaurea*. My study, however, was relatively short term, so it is unknown if a difference would emerge had the plants been allowed to grow longer and complete flowering.

Interestingly, in the high light treatment non-native plants had smaller rosette leaves than native plants, however in the low light treatment, non-native plants had larger mid-sized rosette leaves. The shift in development of larger rosette leaves may be a critical factor in *C. nigrescens'* ability to grow under shaded conditions, unlike *C. stoebe,* which thrives in a variety of open, disturbed habitats (DeJeune et al. 2007). Additionally, the mass of the longest leaf was significantly larger in non-native populations than native populations under high light treatment. The mass of mid-sized rosette leaves was also significantly greater in non-native plants in high light. This again could indicate an opportunistic resource acquisition for growth and reproduction as well as an important shift in resource allocation in response to stress (Davis et al. 2000; Burns 2004, 2006; Blumenthal 2005; Leishman and Thomson 2005).

The ability of *C. nigrescens* to increase allocation of resources to the organs responsible for the uptake of whatever resource is limiting, e.g., the development of larger rosette leaves when light is limiting, may be an important mechanism allowing this invader to cope with changing conditions. It would be interesting to conduct a similar study with *C. nigrescens* grown in shade and then in light to simulate the opening of a gap forest, or suitable invasion habitat.

Water Experiment

In low water conditions, plants typically respond to drought by producing fewer leaves (Prasad et al. 2008). However, in this study the non-native plants in both the high and low water treatment had a significantly greater number of leaves and a greater overall shoot mass. The increased leaf number in each of these treatments could be an opportunistic adaptation to obtaining resources and providing protection during high stress conditions.

The width of the longest leaf and the leaf mass were greater in non-native plants in both the high and low water treatments. Additionally, the mid-sized rosette leaves and the mid-sized stem leaves were significantly larger in the non-native plants in both treatments. Other studies have also shown that some invasive species are more phenotypically plastic than native species in response to drought stress (Stratton and Goldstein 2001; Hill and Germino 2005).

The lack of response to water stress in *C. nigrescens* is intriguing. From other work it was predicted that *C. nigrescens* would reduce the number of leaves produced under water stress. Hill and Germino (2005) showed that physiological activity declined in *C. stoebe* when water stress was induced. Additionally, Wooley et al. (2011) found that when water-stressed, *L. minutus* also had reduced performance in physiological traits.

Conclusions

The overall behavior of native and non-native *C. nigrescens* in response to each of the treatments, light, water and nutrients, is crucial for uncovering whether there are in fact particular differences in morphology and resource allocation between native and non-native plants. The differences observed in the current study may provide further insight on whether stressors such as low nutrient, low light and low water availability are important for invasion success in other species of *Centaurea*. In future studies it will be imperative to focus on such attributes as leaf morphology and leaf shape. Additionally, it has been shown that *C. stoebe* polyploids respond differently to stress (Mraz et al. 2011). It is therefore important to assess ploidy in *C. nigrescens.* Tertraploids have been documented in *C. stoebe*, and this trait may be responsible for the large impacts seen in the invasive range.

My study with *C. nigrescens* focused on the traits of native and non-native species in a greenhouse experiment, providing an assessment of life history traits that may promote invasion success under controlled conditions. Further studies of this kind, testing many more contrasts, will be important to elucidate and understand differences in life-history patterns of introduced plant species. We need to learn more about the relationship between resource availability and invasion, especially resources that negatively impact invaders. Once we know more about extrinsic stressors, they may be used to control invaders, essentially becoming a form of "chemotherapy" for invaded habitats (Alpert et al. 2000).

Table 1. Locations of populations where seeds were collected in the United States (1-8, introduced range), Italy (9-12, native range) and Switzerland (13-16, native range).

Population	Location	Latitude	Longitude
1	Bronx, NY	40.51	73.49
2	Greenport, NY	41.05	72.23
3	New Jersey, NY	40.25	74.31
4	Blacksburg, Virginia	37.13	80.26
5	West Anandale, PA	40.38	74.58
6	Route 66, Virginia	38.53	77.33
7	Round Swamp, NY	40.46	73.26
8	Pauling, New York	41.33	73.35
9	Campetti Italy	45.79	9.97
10	Del Capo, Italy	45.79	9.98
11	Roco Pino, Italy	45.23	7.77
12	Valmagore, Italy	45.78	9.98
13	Giubiasco, Switzerland	46.10	9.03
14	Negrentino, Switzerland	46.15	8.49
15	Pree, Switzerland	46.31	8.49
16	Somazzo, Switzerland	45.52	8.99

Table 2. Table of P-values for treatment effects (high or low nutrients), source effects (native or introduced populations) and interactions for all measured traits in the nutrient experiment. Significant effects are in bold.

NUTRIENT EXPERIMENT			
Response Variable	**Treatment**	**Status**	**Interaction**
Pre-treatment			
Initial leaf number		**0.020**	
Initial length of longest leaf (cm)		0.722	
Initial plant height (cm)		0.081	
Initial diameter (cm)		0.876	
Post-treatment			
Total leaf number	0.924	0.960	0.115
Plant height (cm)	0.126	0.944	0.116
Branch number	0.579	0.484	0.105
Presence of flower(s)	0.269	0.237	0.241
Capitula number	0.333	0.168	0.096
Flower number	0.276	0.394	0.259
Shoot length (cm)	0.780	0.219	0.467
Root length (cm)	0.188	0.445	0.291
Root to shoot ratio	0.093	0.226	0.219
Area longest leaf (cm^2)	0.975	0.349	0.282
Area mid-sized rosette leaf (cm^2)	0.540	**0.014**	0.783
Area mid-sized stem leaf (cm^2)	0.227	0.932	0.283
Maximum width longest (cm)	0.654	**0.031**	0.303
Maximum width mid-sized rosette (cm)	0.567	**0.001**	0.563
Maximum width mid-sized stem (cm)	0.842	0.783	0.080
Maximum length longest leaf (cm)	0.439	0.862	0.060
Maximum length mid-sized rosette (cm)	0.271	0.819	0.969
Maximum length mid-sized stem (cm)	**0.033**	0.886	0.345
Mass longest leaf (g)	0.949	**0.016**	0.672
Mass mid-sized rosette leaf (g)	0.779	**0.002**	0.613
Mass mid-sized stem leaf (g)	0.840	0.093	0.063
Specific leaf area longest-leaf ($cm^2\ g^{-1}$)	0.721	0.872	0.869
Specific leaf area mid-sized rosette leaf (cm2 g^{-1})	0.968	0.219	0.491
Specific leaf area mid-sized stem leaf ($cm^2\ g^{-1)}$	0.576	0.169	0.058
Mass root (g)	0.728	0.424	0.706
Mass shoot (g)	0.965	0.207	0.752
Biomass(g)	0.796	0.263	0.893

Table 3. Table of P-values for treatment effects (high or low light), source effects (native or introduced populations) and interactions for all measured traits in the light experiment. Significant effects are in bold.

LIGHT EXPERIMENT			
Response Variable	**Treatment**	**Status**	**Interaction**
Pre-treatment			
Initial leaf number		0.406	
Initial length of longest leaf (cm)		0.807	
Initial plant height (cm)		**0.027**	
Initial diameter (cm)		0.180	
Post-treatment			
Total leaf number	**0.041**	0.440	0.749
Plant height (cm)	**0.005**	0.484	0.496
Branch number	0.496	**0.032**	0.339
Presence of flower(s)	0.256	0.475	0.643
Capitula number	0.061	0.521	0.950
Flower number	0.204	0.694	0.654
Shoot length (cm)	**0.010**	0.556	0.324
Root length (cm)	0.472	0.222	0.091
Root to shoot ratio	0.058	0.838	0.957
Area longest leaf (cm^2)	0.912	0.528	0.332
Area mid-sized rosette leaf (cm^2)	0.222	0.931	0.532
Area mid-sized stem leaf (cm^2)	0.599	0.588	0.174
Maximum width longest (cm)	**0.009**	0.141	0.641
Maximum width mid-sized rosette (cm)	0.159	0.371	0.799
Maximum width mid-sized stem (cm)	0.422	0.610	0.617
Maximum length longest leaf (cm)	0.367	0.924	0.881
Maximum length mid-sized rosette (cm)	0.199	0.099	**0.019**
Maximum length mid-sized stem (cm)	0.772	0.529	0.139
Mass longest leaf (g)	**0.004**	**0.038**	0.857
Mass mid-sized rosette leaf (g)	**0.001**	**0.039**	0.272
Mass mid-sized stem leaf (g)	0.748	0.922	0.634
Specific leaf area longest-leaf ($cm^2\ g^{-1}$)	**0.000**	0.588	0.380
Specific leaf area mid-sized rosette leaf (cm2 g^{-1})	**0.001**	0.502	0.839
Specific leaf area mid-sized stem leaf ($cm^2\ g^{-1}$)	0.200	0.644	0.618
Mass root (g)	**<0.001**	0.803	0.739
Mass shoot (g)	**<0.001**	0.211	0.497
Biomass(g)	**<0.001**	0.441	0.741

Table 4. Table of P-values for treatment effects (high or low water), source effects (native or introduced populations) and interactions for all measured traits in the water experiment. Significant effects are in bold.

WATER EXPERIMENT			
Response Variable	**Treatment**	**Status**	**Interaction**
Pre-Treatment			
Initial leaf number		0.966	
Initial length of longest leaf (cm)		0.493	
Initial plant height (cm)		**0.035**	
Initial diameter (cm)		0.865	
Post-treatment			
Total leaf number	0.476	**0.011**	0.239
Plant height (cm)	0.055	0.272	0.573
Branch number	0.703	0.343	0.801
Presence of flower(s)	0.127	0.683	0.833
Capitula number	0.380	0.533	0.575
Flower number	0.106	0.933	0.754
Shoot length (cm)	**0.020**	0.125	0.914
Root length (cm)	0.500	0.431	0.631
Root to shoot ratio	0.015	0.821	0.204
Area longest leaf (cm^2)	0.259	0.216	0.971
Area mid-sized rosette leaf (cm^2)	0.541	0.095	0.394
Area mid-sized stem leaf (cm^2)	0.639	0.646	0.913
Maximum width longest (cm)	**0.003**	**0.004**	0.703
Maximum width mid-sized rosette (cm)	**0.001**	**0.006**	0.951
Maximum width mid-sized stem (cm)	**0.043**	**0.035**	0.908
Maximum length longest leaf (cm)	**0.000**	0.238	0.853
Maximum length mid-sized rosette (cm)	**0.000**	0.910	0.073
Maximum length mid-sized stem (cm)	**0.020**	0.254	0.693
Mass longest leaf (g)	**0.035**	**0.044**	0.512
Mass mid-sized rosette leaf (g)	**0.001**	0.218	0.184
Mass mid-sized stem leaf (g)	**0.017**	0.169	0.309
Specific leaf area longest-leaf ($cm^2\ g^{-1}$)	**0.000**	0.173	**0.024**
Specific leaf area mid-sized rosette leaf (cm2 g^{-1})	**0.002**	0.841	**0.015**
Specific leaf area mid-sized stem leaf ($cm^2\ g^{-1)}$	**0.023**	.0.2127	0.549
Mass root (g)	**0.007**	0.232	0.308
Mass shoot (g)	0.151	**0.001**	0.524
Biomass(g)	**0.022**	0.117	0.930

Figure 1. Shade boxes constructed for low light treatment from ¼ inch PVC and covered with 50% shade cloth.

Figure 2. Initial leaf number at the start of the experiment differed for native and non-native plants in the treatment. There were significant differences between treatments for status (P = 0.019) and a significant interaction between status and treatment (P = 0.036). Error bars indicate one standard error from the mean.

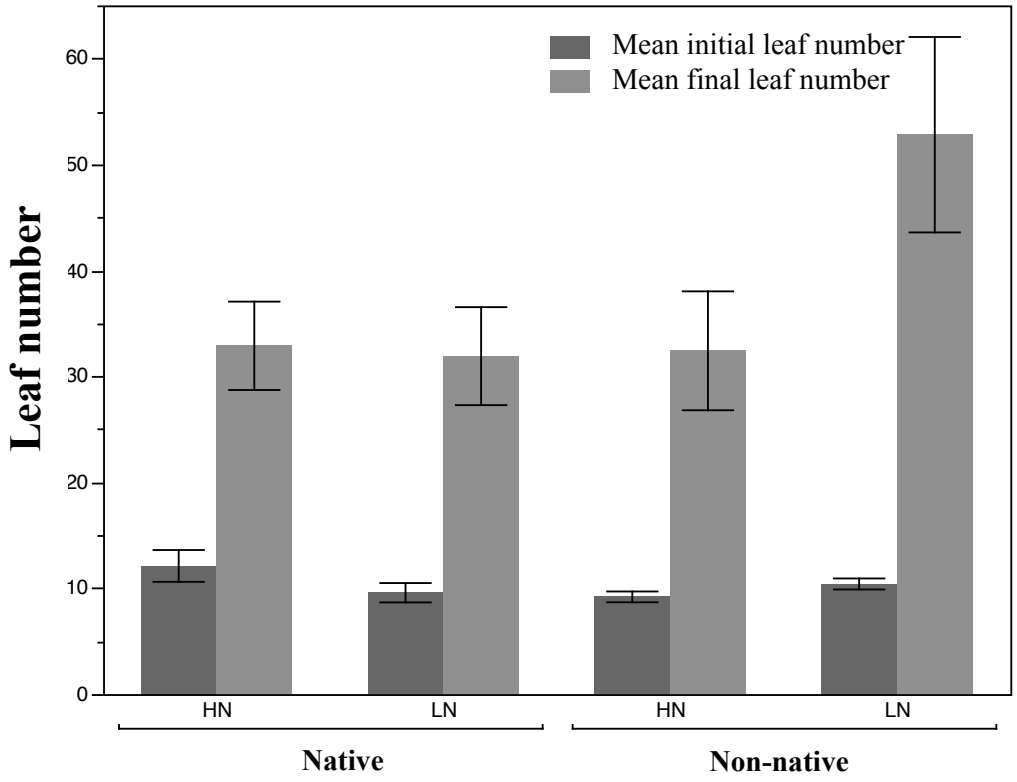


Figure 3. A significant difference existed between non-native and native plants in area of the mid-sized rosette leaf (P =0.014). Non-native plants in high nutrient treatment had a larger area of mid-sized rosette leaves (55.67 cm^2 ±3.66) than native plants in the low nutrient treatment (44.94cm^2 ±2.96). Error bars indicate one standard error from the mean.

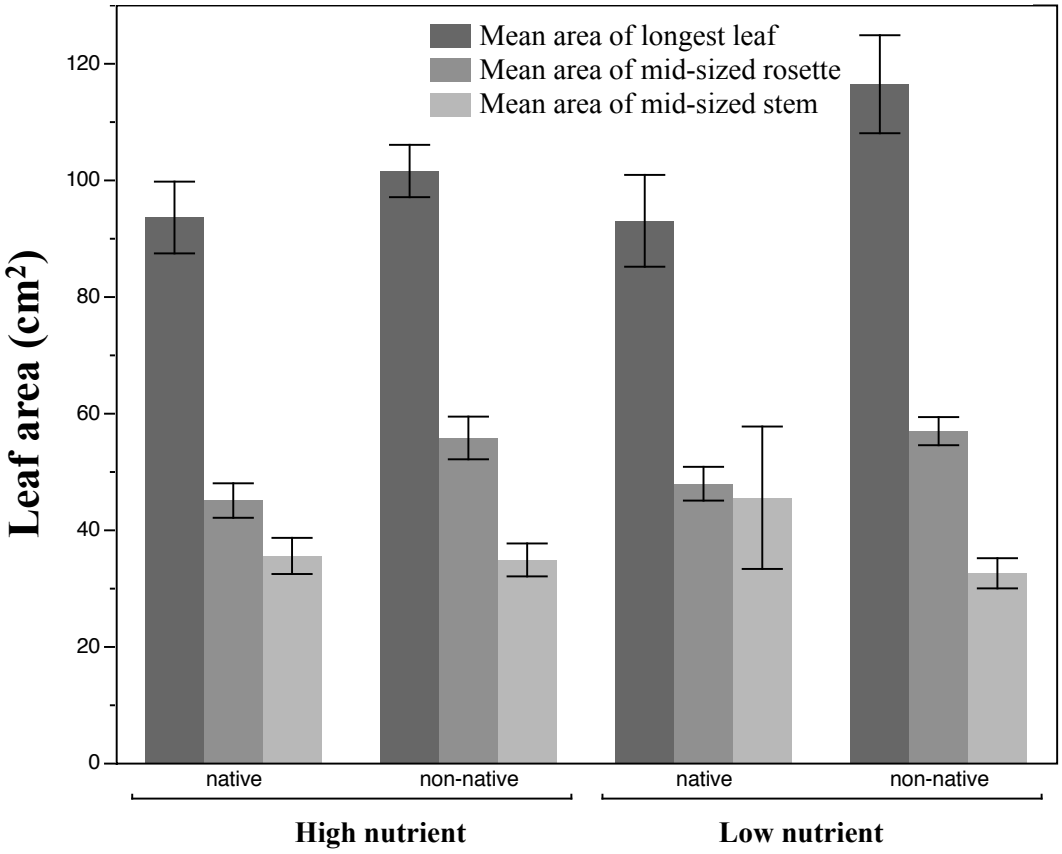


Figure 4. Maximum width of longest leaf was significantly larger in non-native plants in the low nutrient treatment (P = 0.031). Error bars represent one standard error from the mean.

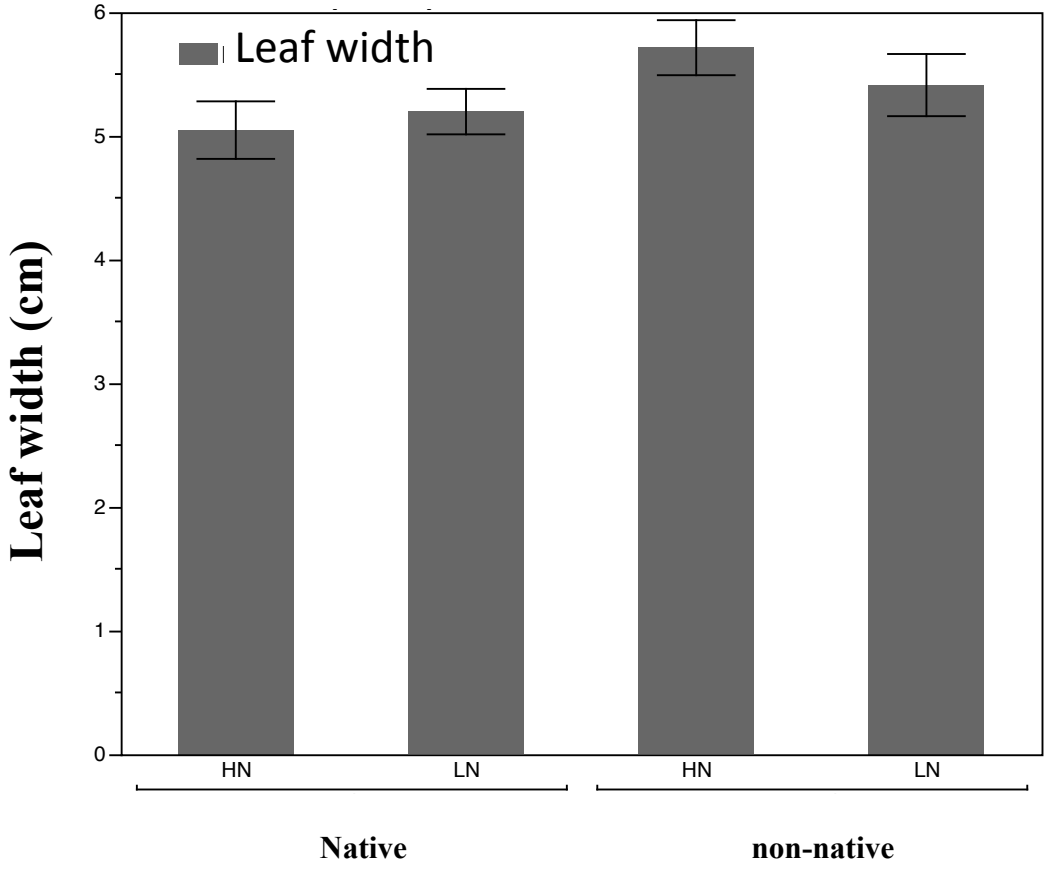


Figure 5. There was a significant effect of treatment on mid-sized stem leaf width (P = 0.033), in both native and non-native populations. Error bars indicate one standard error from the mean.

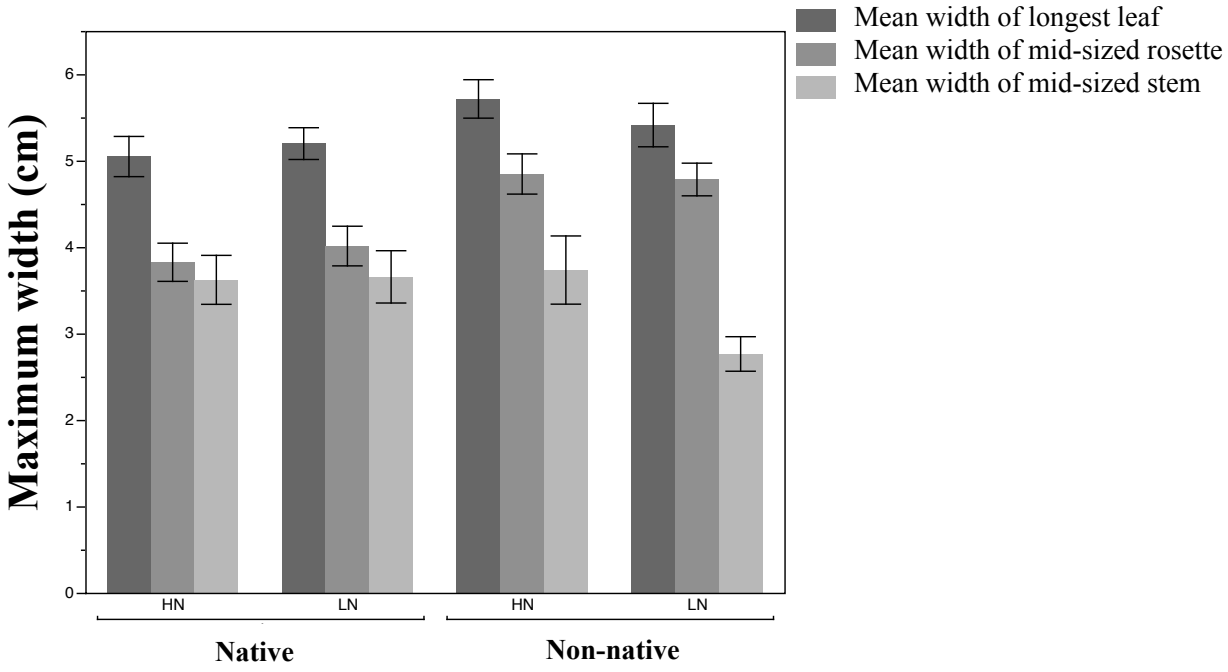


Figure 6. There was a significant difference between native and non-native populations for the mass of the longest plant leaf (P = 0.016). Error bars indicate one standard error from the mean.

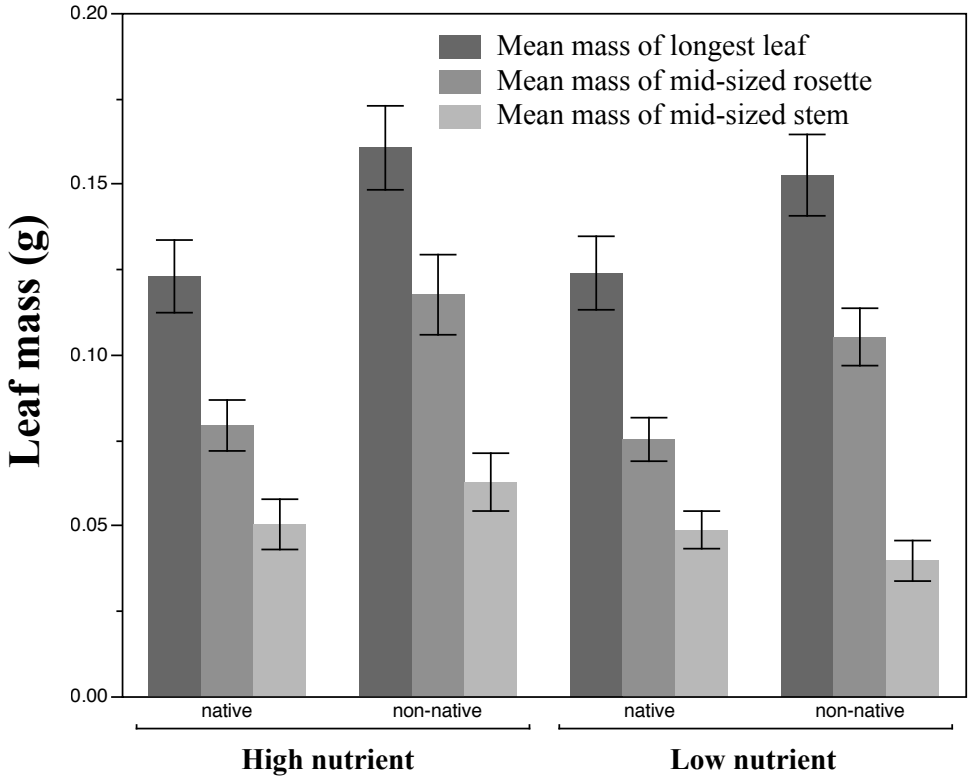


Figure 7. Plant height was significantly larger in native plants in the high light and low light treatment at the start of the experiment (P = 0.027). Final plant height was not significantly different between the groups (P = 0.484). A significant treatment effect was observed for both native and non-native plants exposed to high light (P = 0.005). Error bars indicate one standard error from the mean.

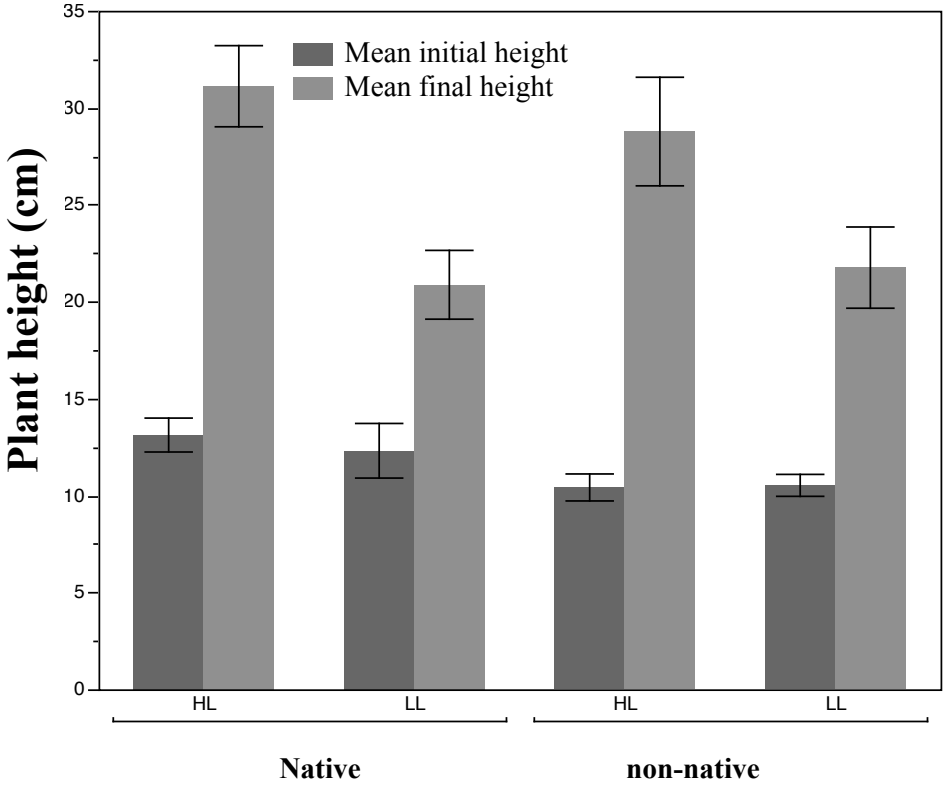


Figure 8. Increased branch number was observed in both high light and low light treatments for non-native plant populations (P = 0.032). Error bars indicate one standard error from the mean.

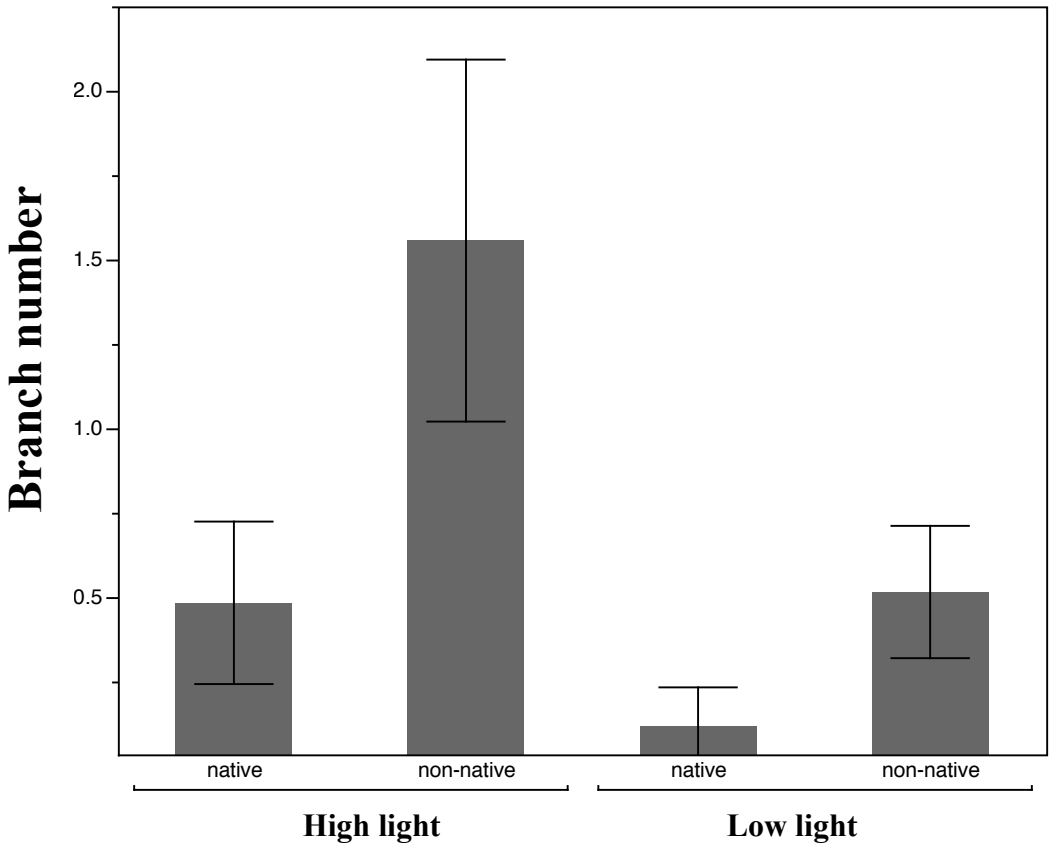


Figure 9. In both high light and low light treatments non-native plants had significantly larger leaf mass (P = 0.038). Significant treatment effects were observed for both native and non-native plants (0.004). Error bars represent one standard error from the mean.

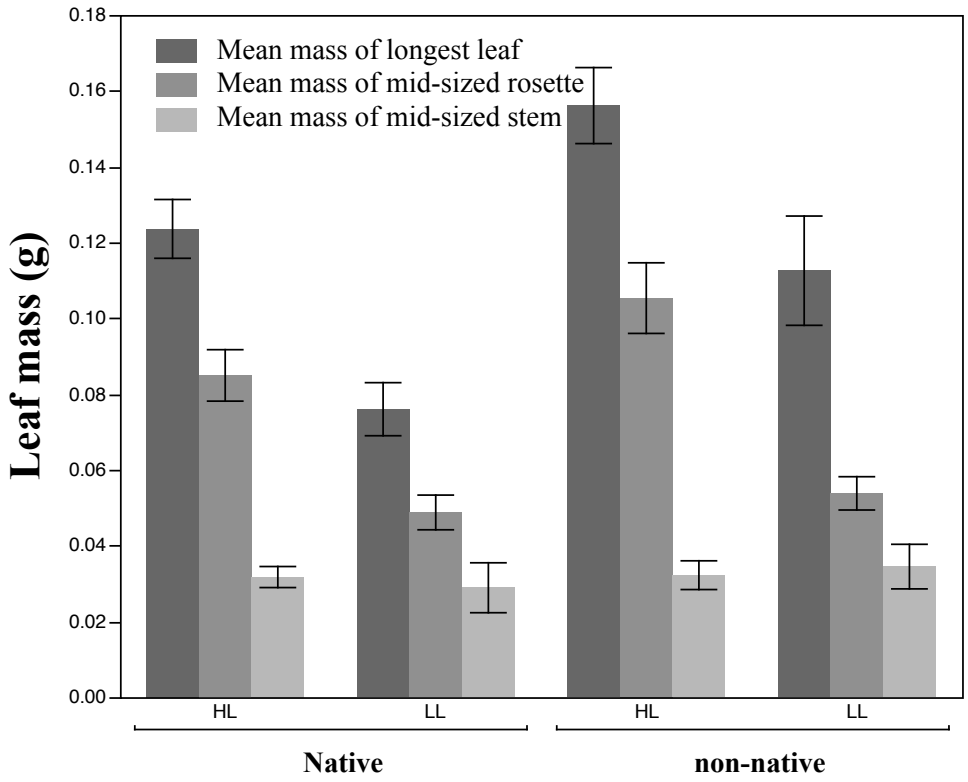


Figure 10. A significant interaction existed in the length of mid-sized rosette leaves between source and treatment (P = 0.019). In high light conditions native populations had a larger maximum length of longest leaf. In low light conditions, the non-native populations had a larger maximum length of longest leaf. Error bars represent one standard error from the mean.

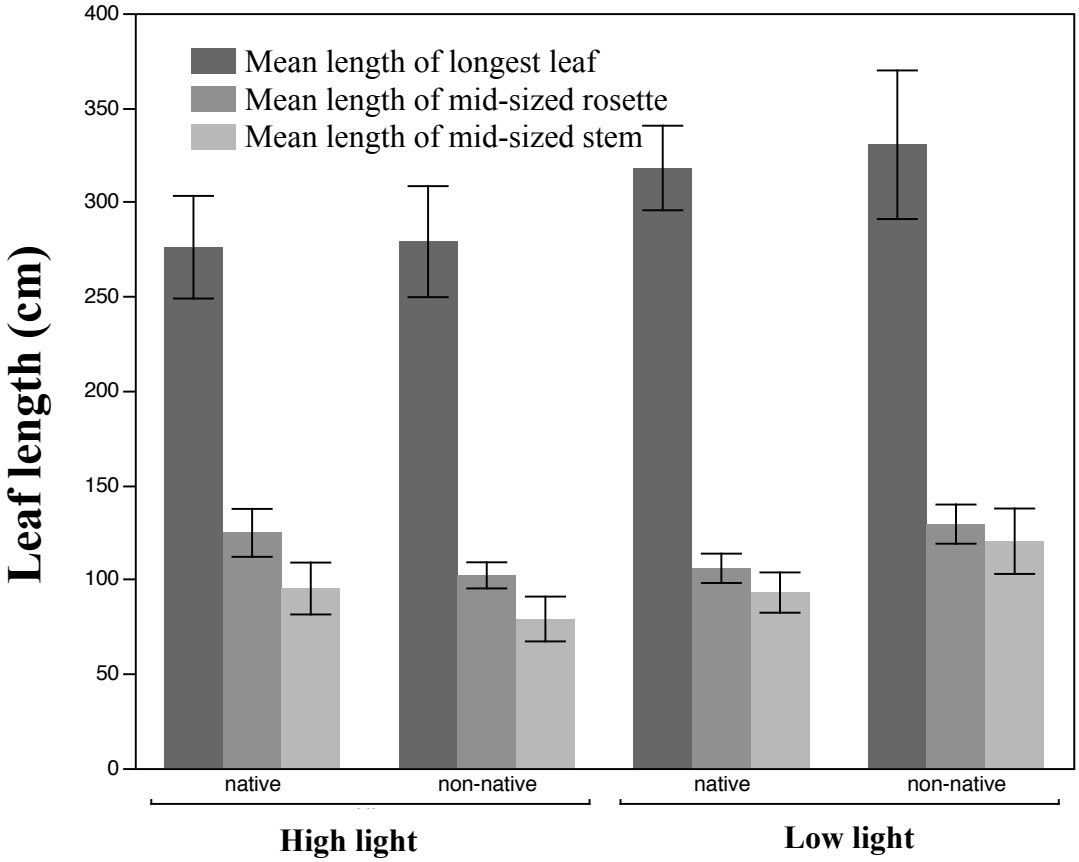


Figure 11. Initial plant height was significantly larger in native plants in high water experiment testing the effects of water availability (P = 0.035). Error bars indicate one standard error from the mean.

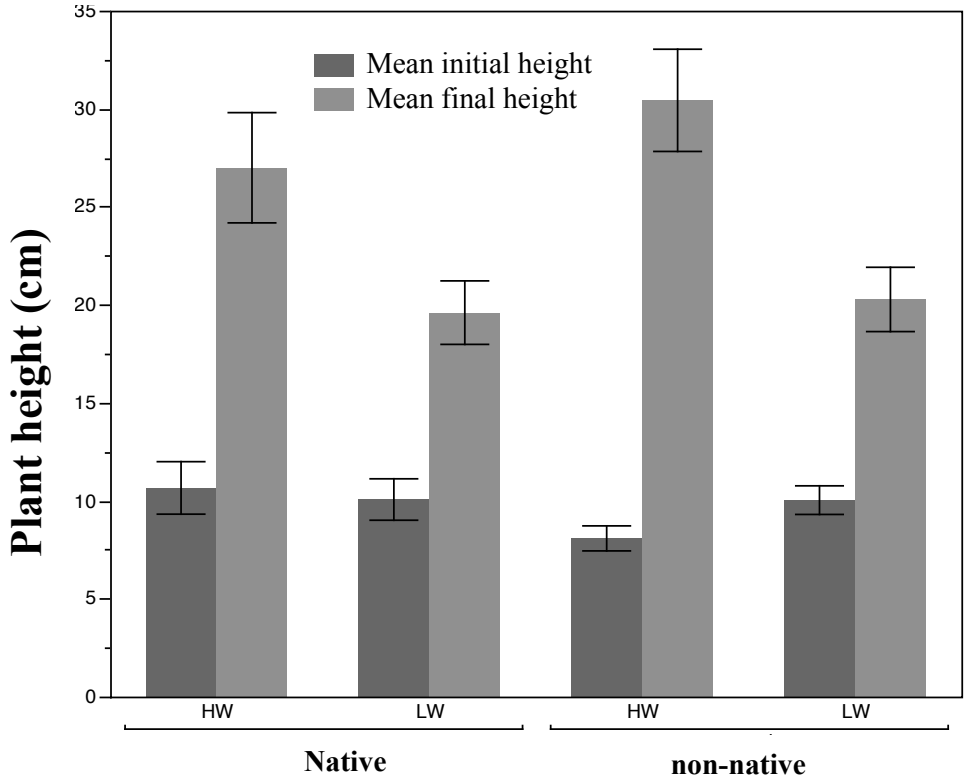


Figure 12. A significant difference was found for total leaf number between native and non-native at the end of experiment (P = 0.011). Plants from non-native populations had more leaves in both treatments. Error bars indicate one standard error from the mean.

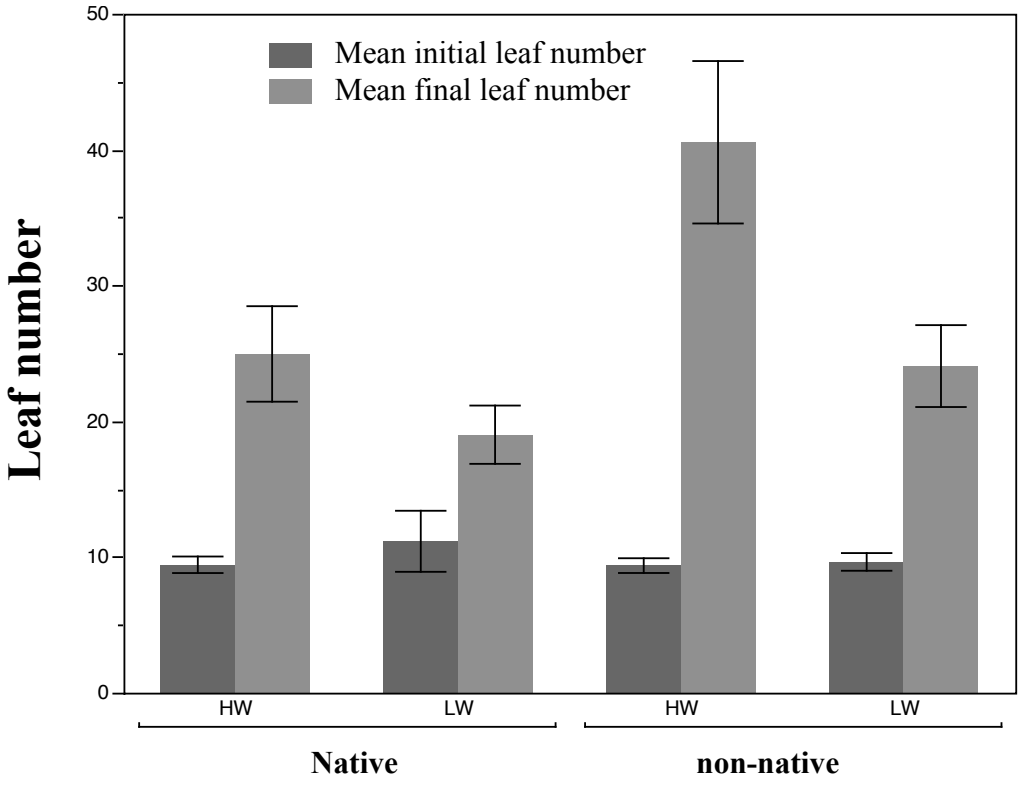


Figure 13. Shoot mass was significantly larger in native populations in both the high and low water treatments (P = 0.001). Error bars indicate one standard error from the mean.

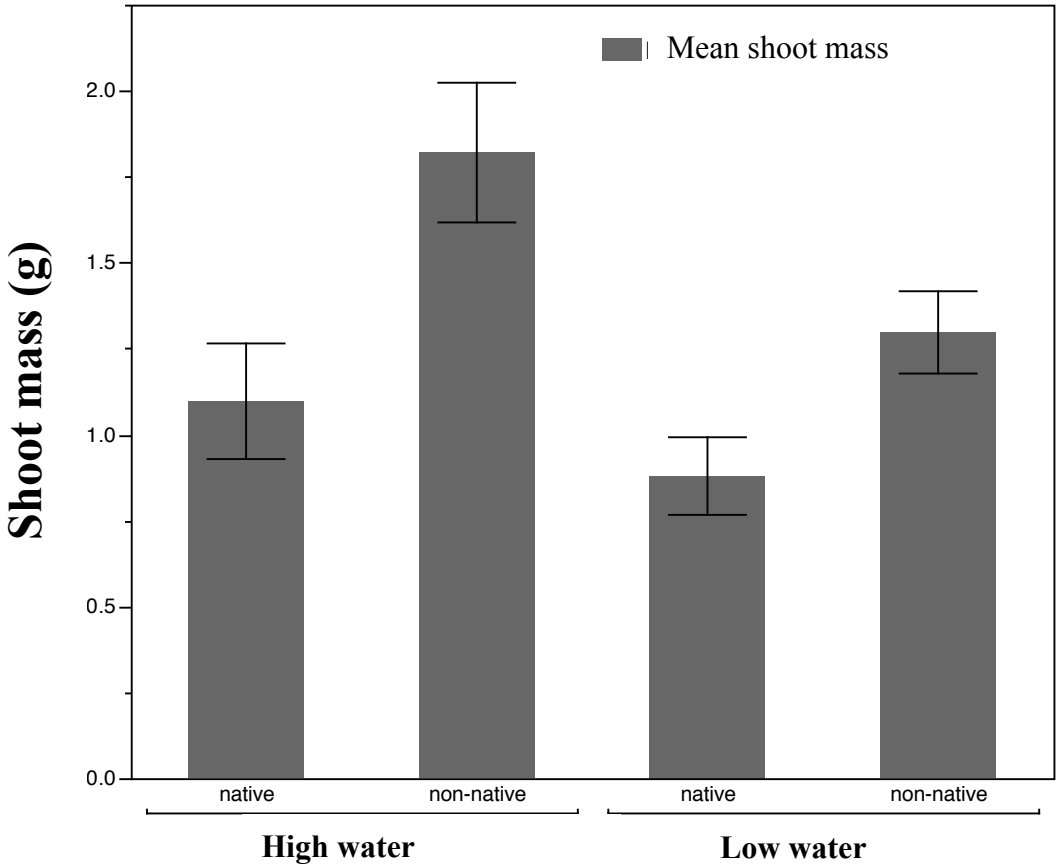


Figure 14. A significant difference in maximum leaf width was found between native and non-native in the high water and low water treatments (P = 0.003). Error bars indicate one standard error from the mean.

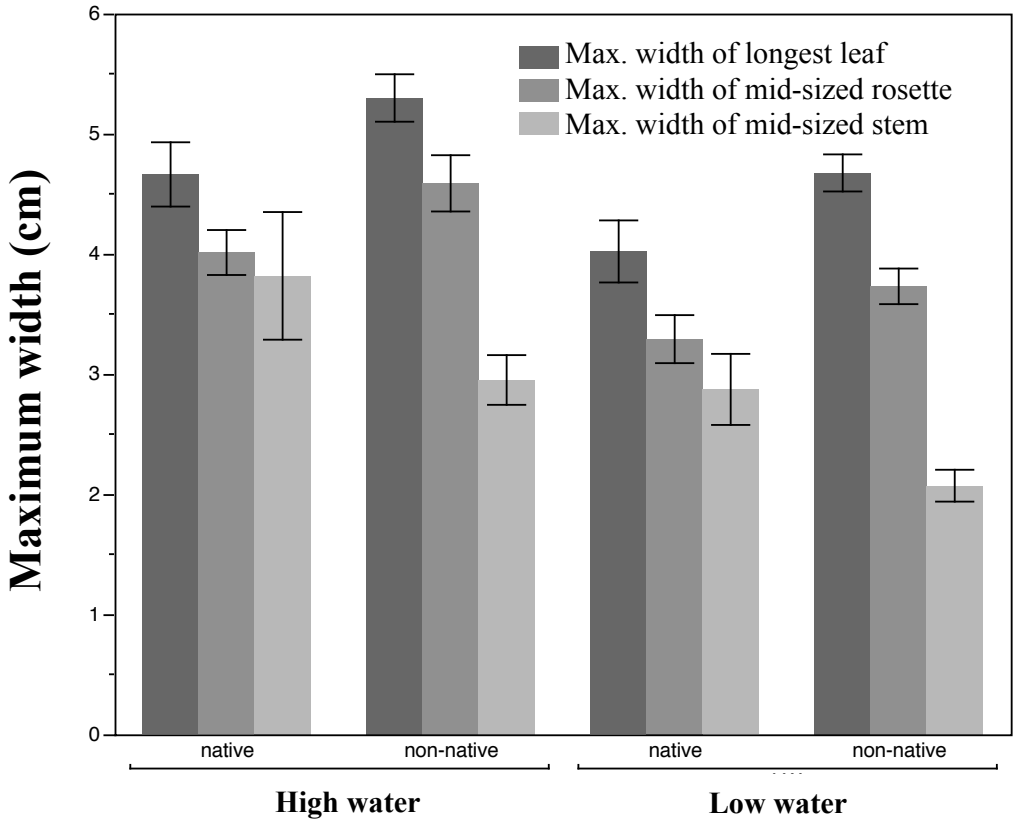
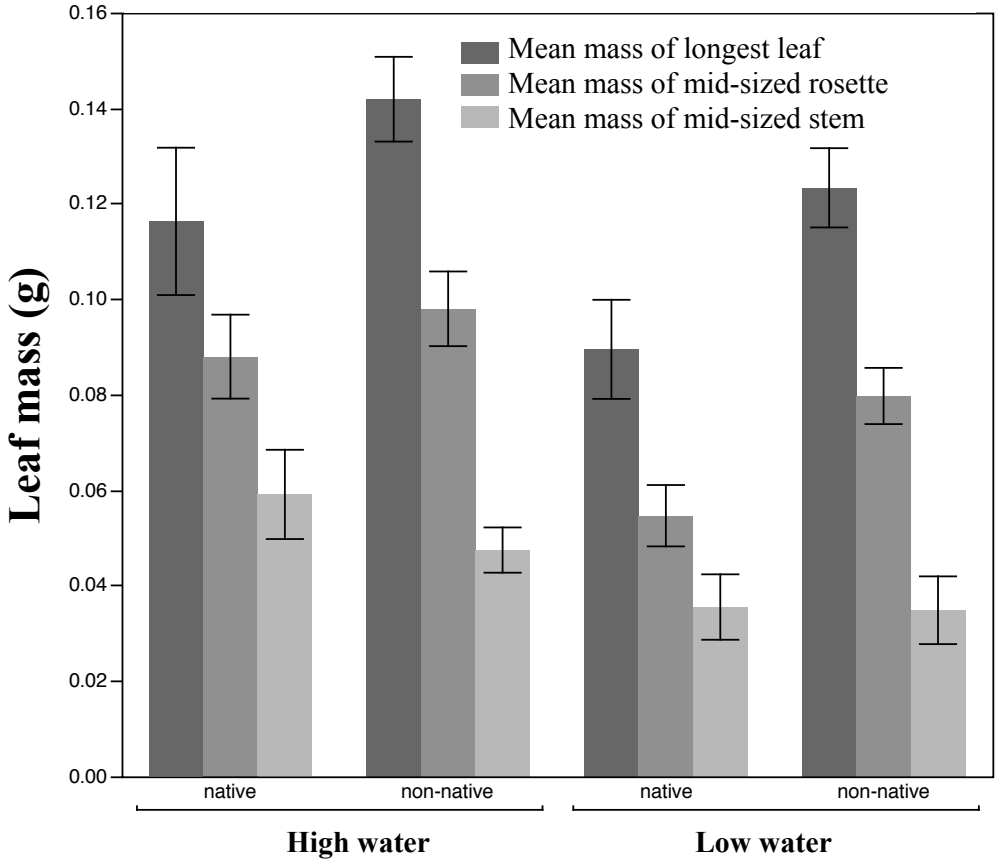


Figure 15. A significant treatment effect was found for the mass of longest leaf for both native and non-native plants in high water treatment (P = 0.035). Mass of longest leaf was significantly larger in non-native populations in both the high and low water treatments (P = 0.044). Error bars indicate one standard error from the mean.



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Chapter 5

Construction of a haplotype map for introduced populations of *Centaurea nigrescens*

Introduction

When a species is introduced and establishes in a new environment genetic variation can increase, decrease or remain the same. Genetic changes from source populations can occur for several reasons. Introductions can involve few propagules, resulting in a loss of genetic diversity relative to the native populations (Ellstrand and Schierenbeck 2000; Kolbe et al. 2004; Lockwood et al. 2005; Novak and Mack 2005; Lavergne and Molofsky 2007). Outcrossing and hybridization in the new environment can produce increased diversity (Allendorf and Lundquist 2003; Müller-Schärer et al. 2004 Nevo 1988; Williamson 1996; Lee et al. 2004). Genetic studies on plant introductions have found that when a species is transported to a new environment adaptation can occur in 20 generations or less, indicating that such evolutionary processes can influence invasion success (Prentis 2008). Multiple introductions from different source regions can also result in greater diversity in introduced ranges of plants relative to the diversity see in native populations (Abbott, 1992; Ellstrand and Schierenbeck, 2000; Hänfling and Kollmann, 2002; Callaway and Maron, 2006; Marrs et al. 2008). Some of these processes can result in performance differences for plants in the introduced range versus those from the native range as new combinations of genes not found in native populations arise. These changes can result in the ability of introduced plants to establish and spread into new habitats, including novel habitats not found in the native range. Alternatively, reduced genetic diversity could limit the ability of an introduced plant to adapt, or limit its ability to spread to new environments.

When introduction is the result of a small number of individuals, bottlenecks in population size, founder effects, and evolution via genetic drift can contribute to reductions in genetic variation (Nei et al. 1975; Husband and Barrett 1991). Species that have entered and quickly spread into a new area via a small number of introduction events may also show lower genetic diversity in the introduced range (Ellstrand and Schierenbeck 2000; Kolbe et al. 2004; Lockwood et al. 2005; Novak and Mack 2005; Lavergne and Molofsky 2007). Dlugosch and Parker's (2006; 2008) study on the invasive shrub *Hypericum canariense* demonstrates the severe genetic effects of a bottleneck in decreasing diversity during founder events. Data from these studies show a 50% decline in molecular genetic variation relative to native populations. Okada et al. (2009) showed that introduced populations of *Cortaderia jubata* have decreased levels of variation compared to populations in the native range, which is the result of a bottleneck. Meimberg et al. (2006) found reduced genetic variation in populations in the invaded

range of the invasive grass *Aegilops triuncialis* L. Their results showed strong evidence for an extreme bottleneck in this species as only three multilocus genotypes were detected in California compared to 36 genotypes in Eurasia.

Genetic variation can be maintained in introduced populations when there is outcrossing and hybridzation. Brown and Marshall (1981) found that populations of bull grass (*Bromus mollis)* do not suffer a decrease in diversity as a result of colonization. High levels of genetic diversity are proposed to be maintained by outcrossing. Interspecific hybridization is now recognized as a major mechanism of evolution in the plant kingdom, and such hybridizations between introduced species and related species have been implicated as a driving force of evolutionary processes in invasions (Abbott 1992; Ellstrand and Schierenbeck 2000; Hänfling and Kollmann 2002; Callaway and Maron 2006). Hybridization may increase genetic diversity in introduced taxa and provide the genetic material on which selection and genetic drift may act to promote population differentiation.

Multiple introductions are posed as the key factor in influencing genetic diversity in the introduced range of an invasive species (Warwick et al. 1987; Novak and Mack 1993, 2005; Meekin 2001; Maron 2004; Durka et al. 2005; Sun et al. 2005; Lavergne and Molofsky 2007; Marrs et al. 2008; Hufbauer and Sforza 2008). Multiple independent introductions of a species can introduce novel variants into the population, increasing genetic diversity and decreasing founder effects (Dlugosch and Parker 2008). Lavergne and Molofsky's (2007) study on the invasive wetland grass reed canary grass, *Phalaris arundinacea* L., illustrated that canary grass had higher genetic diversity and heritable phenotypic variation in its invasive range relative to its native range. Lavergne and Molofsky cite this as evidence of multiple and uncontrolled introductions into North America. In *Centaurea stoebe*, Marrs et al. 2008 found invasive *Centaurea stoebe micrathos* did not suffer from a severe demographic bottleneck or founder effects during its introduction. Hufbauer and Sforza (2008) found a similar pattern using cpDNA sequence data: haplotype diversity did not differ significantly between North America and Europe, supporting the notion that *C. stoebe micranthos* was not subjected to a severe genetic bottleneck when introduced to North America.

Centaurea nigrescens is a plant introduced from Europe in the 1800's (Chapter 1). This species is closely related to *C. stoebe,* a class A noxious weed (USDA). It is not certain if *C. nigrescens* is expanding or is limited to its current distribution. The purpose of this study was to

use chloroplast DNA (cpDNA) analysis to determine if there is reduced, increased, or unchanged genetic diversity in introduced populations, relative to native populations of this species.

Methods

Study species

The genus *Centaurea* L. (Asteraceae) contains approximately 300 species (Garcia-Jacas *et al*. 2006), many of which are indistinguishable morphologically (Ochsmann 2000). In North America, 34 species of *Centaurea* have been introduced (USDA Natural Resources. Conservation Service Plants Database), 14 of which are defined as noxious weeds in one or more states. C*entaurea nigrescens*, Tyrol knapweed, was introduced to North America from Europe in the 19th century. It is a herbaceous perennial, 30-150 cm in height with erect flowering stems numbering from 1 to as many as 50 (eFloras 2008). This species spreads by both clonal growth via rhizomes and seeds. Populations are typically found along roadsides and in highly disturbed waste sites in Italy and Switzerland as well as in the United States (Wagenitz 1955, efloras 2008; Chapter 1)

Sample collection and preparation

Samples were obtained from 9 European populations and 17 North American locations for cpDNA analyses. In all cases, plants that were sampled were at least 1 m apart to reduce the chance of sampling siblings, and increase the potential range of genetic variation present at each site. Leaf tissue samples were processed from 2-4 individuals from the 26 populations for a total of 96 samples (Table 1).

All leaf tissue samples were dried and stored with silica desiccant until DNA extraction, following Hufbauer and Sforza (2008). Genomic DNA was extracted from desiccated leaves with the Dneasy Plant Mini Kit from QIAGEN (Valencia, CA) according to manufacturer's instructions. Genomic DNA was stored at -20°C until PCR amplification was performed.

Methodology for amplifying and sequencing cpDNA

Using the methods of Taberlat et al. (1991) and Hufbauer and Sforza (2008), three regions of the cpDNA genome, trnL, trnT-trnL, and trnL-trnF, were amplified with the primers listed in Table 2, using standard protocols (Taberlat et al. 1991; Hufbauer and Sforza 2008). Polymerase chain reaction (PCR) products were then sent for purification and sequencing to the University of Washington High Throuput Genomics Facility (University of Washington, Seattle, Washington). Sequencing was performed on Applied Biosystems Sequencer primed with the

PCR primers (Table 2) for each corresponding cpDNA region. All sequences were exported into FASTA format for sequence analysis.

Multiple sequence alignment

Forward and reverse contigs were aligned from the three cpDNA regions using MySSP (Rosenberg, 2005) according to methods provided by Rosenberg (2005a). Sequences were trimmed and sequences below 90% quality threshold and less than 300 bp were deleted. Sequences were then visually inspected and exported to FASTA and NEXUS format for haplotype analysis and Genbank submission.

Sequence Analysis Statistics

DnaSP *ver* 5.0 (Librado and Rozas, 2009) was used to analyze polymorphic data in cpDNA samples. To detect the genetic differentiation among subpopulations DnaSP implements several statistics based on the number of haplotypes and nucleotide changes (sequence based statistics) (Hudson et al., 1992; Hudson 2000). A haplotype is simply the set of polymorphic alleles that co-occur on a chromosome. Haplotypes were estimated statistically for the SNPs in each gene using DNAsp, Nucleotide substitution rate Π was also reported.

Results

The sequences from the TrnL loci were not of a quality that was useable, or were missing a forward or reverse read. Therefore, these loci were not included in analyses.

Haplotypes of TrnT-trnL

trnT-trnL haplotype diversity in the Native European Range

Analysis of 35 native samples representing 5 populations from Italy and 4 populations from Switzerland yielded 8 distinct haplotypes of trnL-TrnF. Haplotypes 1 and 2 were dominant, representing 95% of all haplotypes in Italy and 93.3% of haplotypes in Switzerland (Table 3.). Overall, haplotype 2 showed the highest frequency distribution in the native range with 70% of populations from Italy and 93.3% of populations from Switzerland having that genotype. Haplotype 1 did not occur in the Swiss samples. Haplotype 4 occurred in 6.67% of the samples from Switzerland was not present in Italy (Table 3.) Figure 2 illustrates the distribution of haplotypes from the sampled populations in Switzerland, showing very little haplotype variation in this sampled range.

trnT-trnL haplotype diversity in the Non-Native North American Range

In the non-native range 7 distinct haplotypes were observed from the analysis of 56 samples representing 17 populations. The dominant haplotypes among this group were haplotype 1 and 2 with a combined frequency of 83.93%. As is shown in Table 3, Haplotype 1 was found in 48.21% of samples and haplotype 2 in 35.71% of the samples analyzed. Haplotypes 4 and 7 were each found in 5.36% of samples followed by Haplotypes 3, 5, and 8 which were each found in 1.79% of samples. The relative frequency of haplotypes across North America is shown in Figure 3.

For the trnT-trnL loci, haplotype diversity (h) was 0.601 plants from Europe and 0.598 for plants from North America. Nucleotide substitution (Π) was estimated to be 0.00157 for plants from Europe and 0.00148 for plants from North America.

Haplotypes of trnL-trnF

trnL-trnF haplotype diversity in the native European range

Nine distinct haplotypes of trnL-trnF were resolved from the 26 native samples that were tested (representing 5 populations from Italy and 4 populations from Switzerland). In both Swiss and Italian samples, the dominant haplotypes were found to be haplotypes 1 and 3. In Italy these two haplotypes combined to represent the genotype of 66.7% of the samples tested while in Switzerland the combined frequency was found to be 54.6% (Table 4). In Switzerland both haplotypes had similar frequencies of 28% for haplotype 1 and 36% for haplotype 3 while in Italy, haplotype 3 had a much higher frequency than haplotype 1 with frequencies of 60% and 6.7% respectively. Two other haplotypes of trnL-trnF were observed in the native samples, Haplotype 6 and 8. These haplotypes showed distinctly different frequencies in Switzerland versus Italy. In Italy, haplotype 8 had a frequency of 26.7% and haplotype 6 had a frequency of 6.7%, while haplotype 6 had the higher frequency at 32% in Switzerland compared to haplotype 8 with a frequency of 4.0% there. Figure 3 shows the distributions and the frequencies of various haplotypes in Switzerland and Italy.

trnL-trn F haplotype diversity in the non-native North American range

Among the 57 non-native samples tested (representing 17 populations), 9 distinct haplotypes were resolved having frequencies ranging from 4.55% to 22.72%, as seen in Table 3. Thus no truly dominant haplotype appears to be present in these samples. Just as seen in the native range, haplotypes 1 and 3 had the highest frequencies of 22.72% and 19.69% respectively.

The 7 other haplotypes observed all had similar frequencies of 4.55%-12.12%. The distribution of the various haplotypes and the frequencies of haplotypes are shown in Figure 4.

Haplotype diversity (h) was 0.76 for plants from Europe and 0.69 for plants from North America. Nucleotide substitution (Π) was estimated to be 0.00062 for plants from Europe and 0.00055 for plants from North America.

Discussion

The dominant haplotypes observed in the introduced range were found to be the same as in the native range, suggesting that the *C. nigrescens* populations sampled in the North America are genetically similar to those in the native range. The results for haplotype diversity of *C. nigrescens* parallel the findings for *C. stoebe* (Hufbauer and Sforza 2008). In *C. stoebe* several haplotypes are found in the introduced range and genetic variation is proposed to be maintained through multiple introductions. Unlike studies that indicate a loss of variation upon introduction (Ellstrand and Schierenbeck 2000; Kolbe *et al.* 2004; Lockwood *et al.* 2005; Novak and Mack 2005; Meimberg et al. 2006; Lavergne and Molofsky 2007; Dlugosch and Parker 2008; Okada et al. 2009), *C. nigrescens* has maintained genetic diversity in the introduced range like the that of *C. stoebe*, the aggressive rangeland invader.

Furthermore, haplotype analysis suggests that multiple introductions of *C. nigrescens* into North America have occurred. The samples from the Pacific northwest of the United States, although limited in number, appear to be an independent introduction as the haplotype variation is not consistent with samples from the east coast, but is more similar to the haplotype variation in Italy and Switzerland. This finding is consistent with herbarium records for the timing of introduction on the west coast (Chapter 2), which suggests an introduction in that region in the 1900's. These findings are similar to those for the introductions of *Centaurea diffusa* and *C. stoebe micranthos,* as they were also first recorded in the USA in Washington State in the early 1900s (Maddox 1979; Hufbauer and Sforza 2008). It is proposed that these species were introduced from Eurasia as contaminants of alfalfa seed or in ballast (Maddox, 1979). The patterns of haplotype diversity found by Hufbauer and Sforza (2008) for *C. stoebe* and *C. diffusa* is analogous to what is shown for *C. nigrescens.* Additionally the data found here support the herbarium reconstruction findings of *C. nigrescens* (Chapter 2) suggesting two separate introductions to North America; one in the Pacific northwest and the other in the northeastern United States. The reconstruction of invasion routes for *C. stoebe* by Hordjik and Broennimann

(2012) also support the findings of Hufbauer and Sforza (2008), suggesting multiple introductions for *C. stoebe*.

The greatest haplotype diversity for *C. nigrescens* is found in the northeast region of the United States. The high genetic variation associated with the haplotypes in the east coast region suggests that the region includes a sufficient number of large populations to maintain genetic variation. High haplotype diversity in concurrence with low nucleotide diversity has been linked to population growth after a period of low effective population size (Grant and Bowen 1998).

Taxonomically similar species are thought to invade in similar manners (Daehler and Strong 1993, Harris 2009), and rapidly evolving weeds are predicted to be successful invaders wherever they are introduced (Baker 1965). *Centaurea stoebe,* which is a close relative of *C. nigrescens,* is an important and detrimental invader in North America. Because the genetic diversity of *C. nigrescens* appears to remain the same as it does in the native range, it is still unknown if this species will become as noxious an invader as *C. stoebe*. Additionally, it remains unknown if ploidy levels have changed between the introduced and native range *C. nigrescens* like they have for *C. stoebe* (Treier et al. 2009). With *C. nigrescens,* maintenance of genetic diversity in the introduced range may also reflect a change in ploidy level (Treier et al. 2009). It has been suggested that polyploidy can increase the spread, and thus the success of alien plant species (Verlaque et al. 2002, Pandit 2006, Pandit et al. 2006). It remains unknown whether or not the samples in this study differ in ploidy level in the native and non-native range.

Insights obtained by studying the genetics of invasive species can provide evidence for the specific invasion patterns of introduced populations (e.g., of multiple introductions, Hufbauer and Sforza 2008) or of single founding events (Grapputo *et al.* 2005). Specific information on levels of variation in these populations can potentially guide control efforts as well as provide a better understanding invasion dynamics. Understanding variation in newly founded plant populations requires that we study the population genetics responsible for maintaining or eliminating variation in a population. Increasing efforts have been made at understanding the ecology of invasion history, however we still know relatively little about the post introduction evolutionary dynamics of the species themselves (Sakai *et al* 2001; Novak and Mack 2005; Lee 2002) as well as how these species change genetically. Studying invasion biology from historical, ecological and genetic perspectives represents an excellent opportunity to examine the multi-faceted nature of invasion. The present work supports the findings of reconstructing an

invasion route for *C. nigrescens* using Minimum Cost Arborescence, which suggested that there were two independent introduction events, one in the northeast US and the other in the west (Chapter 2). The answer to the question of how rapidly an invasive plant can evolve remains on the forefront of evolutionary ecological research into plant invasions. Coupling ecological studies such as seed germination and greenhouse studies with population genetic studies may provide us with the answers to some of these questions leading to future studies on incipient populations.

Table 1. Sampling locations of *Centaurea nigrescens* in both the native (European) and non-native (North American) range. N = the number of individuals sampled from each population.

Population Location	Code	N	Sample numbers
European Populations			
Giobasco, Italy	GB	4	1-4
Campetti, Italy	CP	4	5-8
Bergamo, Italy	BG	4	9-12
Rocolino, Italy	RO	4	14-17
Valmaggiore, Italy	VM	4	18-21
Claro, Switzerland	CL	4	22-25
Claro, Switzerland	C2	3	26-28
Biasco, Switzerland	B1	4	29-32
Biasco, Switzerland	B2	4	33-36
North American Populations			
Klickitat, WA, USA	KL	4	37-40
Alleghany County, NC	AC	5	41-45
RT 66, Virginia	RT	4	46-49
NJ US1, Middlesex	MI	4	50-53
Rhiner Farm, RT 8 NY	RH	4	54-57
Bay Shore, NY, USA	BS	2	58-59
Blacksburg, VA_1	BB	4	60-63
Blacksburg, VA_ 2	B2	4	64-67
Greenport, NY	GP	4	68-71
Anondale, PA	AN	4	72-75
East Setauket, NY	ES	4	76-79
Old Bethpage, NY	OB	4	80-83
Bronx, NY, USA	BR	4	84-87
Pauling, NY, USA	PA	4	88-91
Fishkill, NY, USA	FK	4	92-95
NY_55_2, NY, USA	N5	1	96
Nebraska	NE	1	13

Table 2. Primer codes and cpDNA region of study, primer A and B were used to sequence trnT-trnL, primer C and D were used for the trnL region and primer trnL-trnF was sequenced using primer E and F.

Name	Primer Code	cpDNA Region	Bp Size	Sequence 5'---3'
A	B48557	trnT-trnL	500	CATTACAAATGCGATGCTCT
B	A49291	trnT-trnL		TCTACCGATTTCGCTACG
C	B49317	trnL	485	CGAAATCGGTAGACGCTACG
D	B49855	trnL		GGGGATAGAGGGACTTGAAC
E	B49873	trnL-trnF	450	GGTTCAAGTCCCTCTATCCC
F	A50272	trnL-trnF		ATTTGAACTGGTGACACGAG

Table 3. Frequencies of haplotypes of trnT-trnL in non-native (North American) populations. In North America, 8 different haplotypes were found. N = the total number of individuals with each haplotype and Frequency is the % of individuals with that haplotype.

trnT-trnL	**North America**			**Italy**			**Switzerland**	
Haplotype	**N**	**%**		**N**	**%**		**N**	**%**
hap 1	27	48.21		5	25.00			
hap 2	20	35.71		14	70.00		14	93.33
hap 3	1	1.79						
hap 4	3	5.36					1	6.67
hap 5	1	1.79						
hap 6				1	5.00			
hap 7	3	5.36						
hap 8	1	1.79						

Table 4. Frequencies of haplotypes of trnL-trnF in nonnative (North American) populations. The frequency corresponds to the number of samples in the region sampled expressing that haplotype. In North America, 8 different haplotypes were found. N = the total number of individuals with each haplotype and Frequency is the % of individuals with that haplotype.

trnL-trnF	**North America**			**Italy**			**Switzerland**	
Haplotype	**N**	**%**		**N**	**%**		**N**	**%**
hap 1	**15**	**22.72**		**1**	**6.67**		**1**	**9.10**
hap 2	**5**	**7.55**						
hap 3	**13**	**19.69**		**9**	**60.00**		**5**	**45.50**
hap 4	**8**	**12.12**						
hap 5	**6**	**9.09**						
hap 6	**6**	**9.09**		**1**	**6.67**		**1**	**9.10**
hap 7	**5**	**7.58**						
hap 8	**5**	**7.58**		**4**	**26.67**		**4**	**36.40**
hap 9	**3**	**4.55**						

Figure 1. Positions and directions of universal primers used to amplify three non-coding regions of cpDNA, from Taberlat et al. (1991).

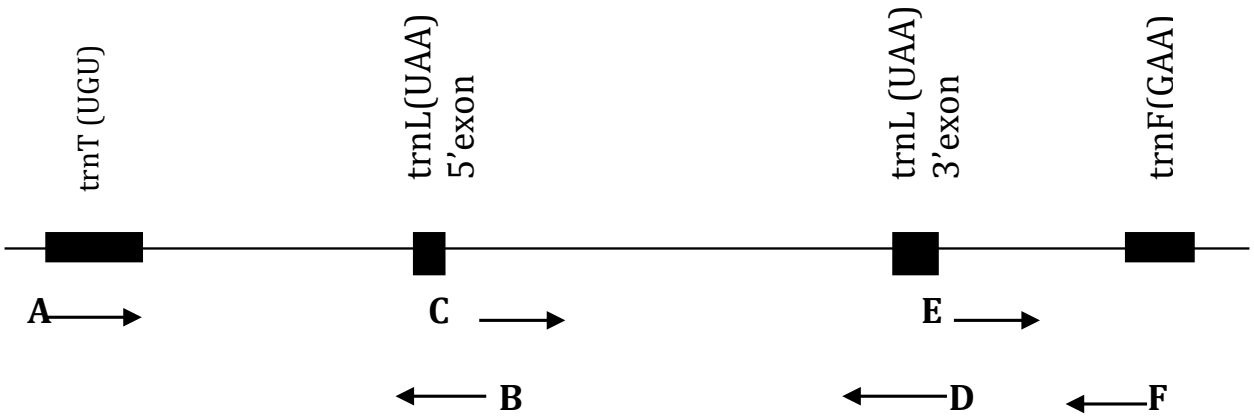


Figure 2. A) Haplotype distributions of trnT-trnL region in North America. B) Frequency distribution of haplotype (Table 3).

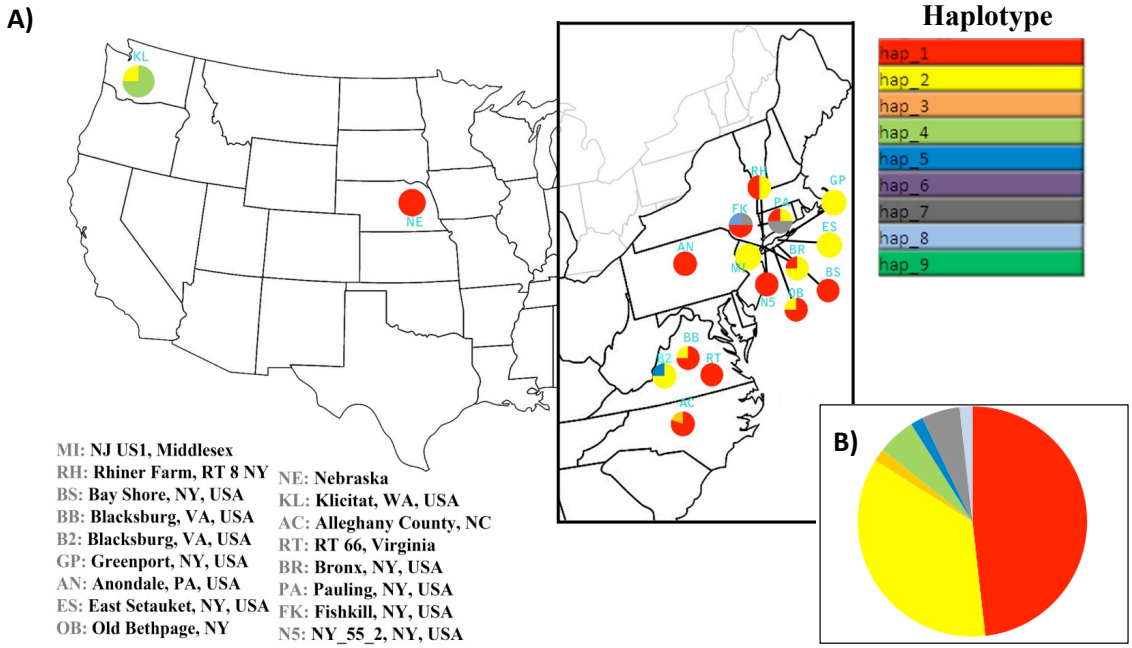


Figure 3. A) Haplotype distributions of trnT-trnL region in Switzerland, B) frequency distribution of haplotype in Switzerland (Table 3), C) haplotype distribution in Italy and D) frequency distribution in Italy.

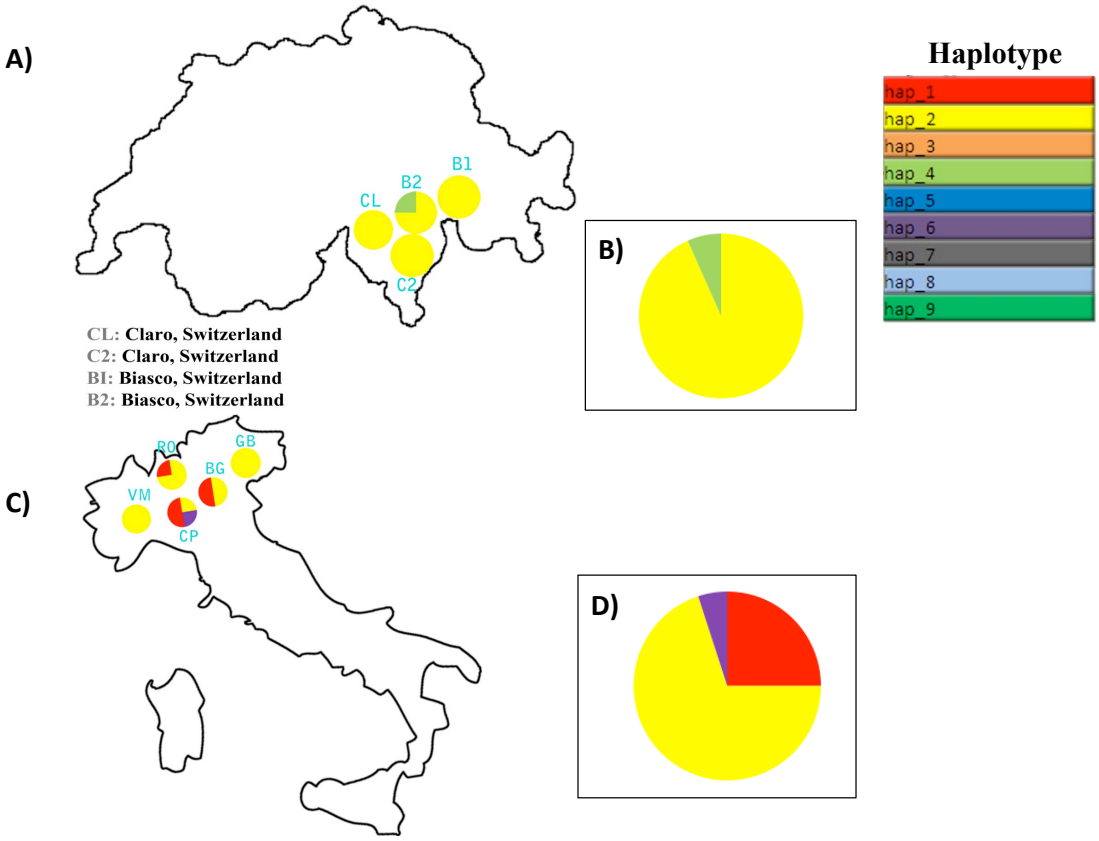


Figure 4. A) Haplotype distributions of trnL-trnF region in North America and B) frequency distribution of haplotype (Table 4).

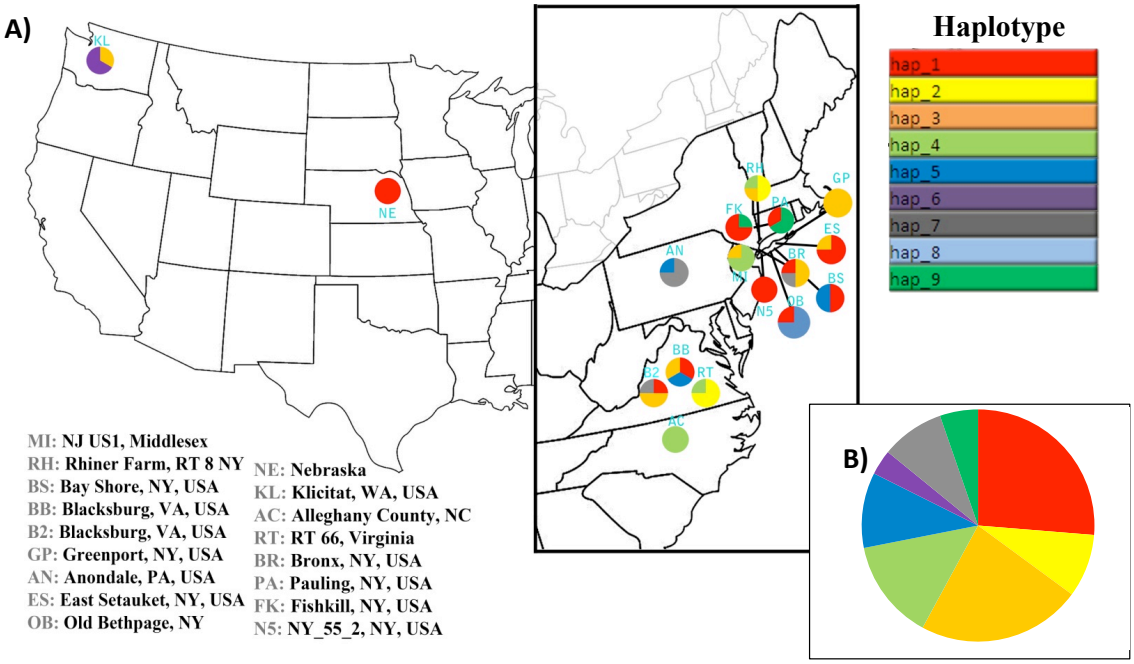
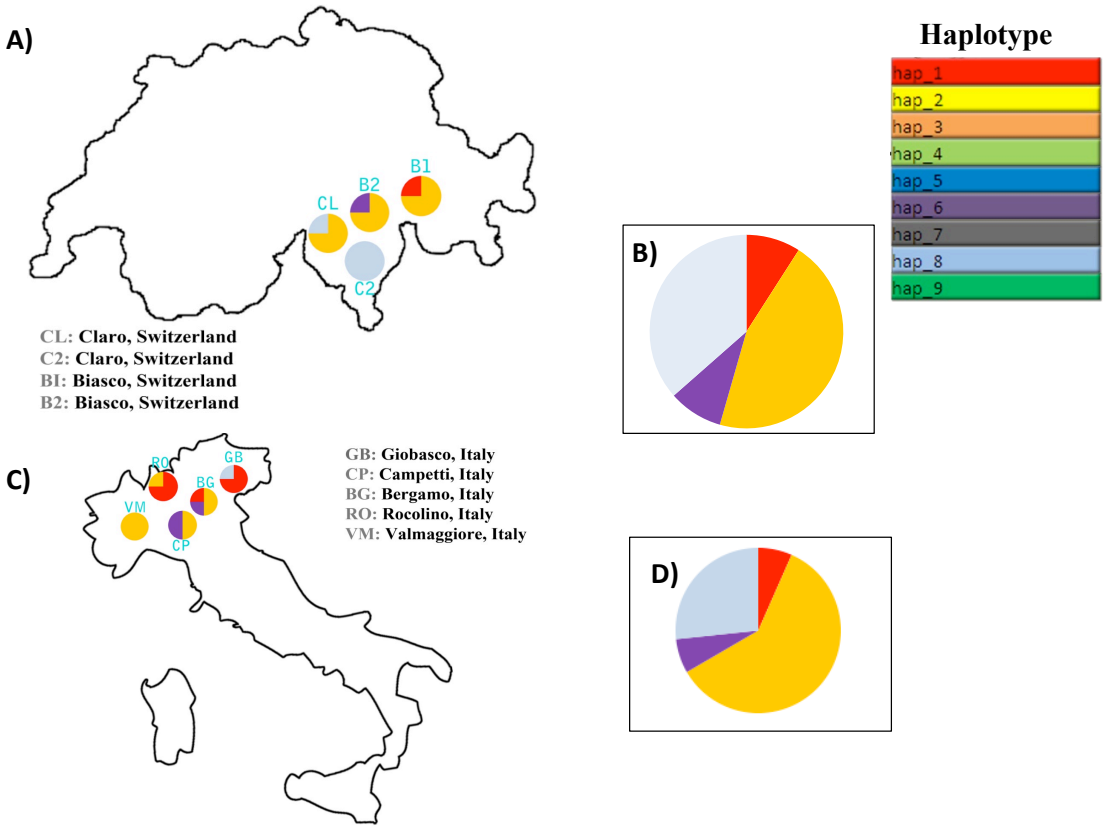


Figure 5. A) Haplotype distributions of trnL-trnF region in Switzerland, B) frequency distribution of haplotype in Switzerland (Table 4), C) haplotype distribution in Italy and D) frequency distribution in Italy.



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Chapter 6

Conclusions

Understanding the traits of what makes an invasive species successful in an introduced environment has been a central theme of research in invasion ecology. Although many hypotheses have been proposed to explain invasion success, a predictable set of characteristics of species that successfully invade new communities has yet to be developed (Lonsdale 1999; Williamson 1999; Gurevitch et al. 2011).

The results of the Minimum Cost Arborescence model of the likely routes of invasion for *C. nigrescens* in the United States are consistent with the findings from the chloroplast DNA. Both suggest that there were two independent introductions of *C. nigrescens* in North America, one in the northeast and one, later, in the northwest. The cpDNA haplotype diversity of *C. nigrescens* is very similar in the US compared to populations in the native range in Europe. However, there does seem to be some significant differences in plants in the invaded range as compared to those in the native range. North American *C. nigrescens* have a higher germination rate across different temperatures. In addition, in the greenhouse study I found that when under stress of low nutrients, low light or low water, plants from the introduced range had traits that are likely to influence competitive performance in acquisition of the resources that are limited. Future work should examine possible differences in branching and flower production between plants in the native range versus those that have been introduced. A longer greenhouse study, or better, a field experiment, would provide useful information about potential performance differences between plants from North America and those from European populations.

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Appendix

Appendix 1. Source code for Minimum Cost Arborescence model in R.

```
source("mdr.R")
library(maps)
library(raster)
mean.date.error<-0.01
fixed.sources.rows<-order(data$date)[1]
obs<-mdr(data=data,xcol=2,ycol=1,datecol=3,mode="observed",rep=100,mean.date
.error=0.01,fixed.sources.rows)
col<-gray(1-obs[[1]]$bootstrap.value)
x11();plot(obs[[1]][,3:4],type="n",xlab="longitude",ylab="latitude")
arrows(obs[[1]][,1],obs[[1]][,2],obs[[1]][,3],obs[[1]][,4],length =
0.05,col=col)
map(add=T)
points(data[fixed.sources.rows,2:1],pch=19,col="red")
title(paste("total routes length : ",round(obs[[2]],2)," D°","\n","median dispersal rate :
",round(obs[[3]],2)," D°/year","\n","number of outcoming nodes : ",obs[[4]]))
```