

ABSTRACT

Title of Document: ECOLOGICAL INTERACTIONS OF THE
CADMIUM- AND ZINC-
HYPERACCUMULATING PLANT, *THLASPI*
CAERULESCENS, AND THEIR
IMPLICATIONS FOR PHYTOREMEDIATION

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The success of invasive species can be attributed to a combination of abiotic factors, such as abundant resources and favorable climate, and biotic factors, such as low levels of competition and predation or herbivory, at the introduced location. While studies have demonstrated the effects of these factors on known invasive species, the degree to which these factors affect a non-native species can be used to predict its likelihood of becoming invasive. The metal-hyperaccumulating plant *Thlaspi caerulescens* (Brassicaceae) is potentially useful for remediating soils that are moderately contaminated with Cd and Zn, and has been experimentally introduced to contaminated sites outside of its native range for phytoremediation. To assess the ecological risks involved in introducing metal-hyperaccumulating plants for phytoremediation, including their potential invasiveness, I have performed three

studies to examine the abiotic and biotic factors that could influence the establishment of *T. caeruleascens* at three contaminated sites near the Rocky Mountain Biological Laboratory in Gothic, Colorado. In the first two studies, I test the effects of soil metal concentrations and interspecific competition on plant performance, and in the third study I examine the strength of herbivore pressure on this plant. Results from these studies show that the growth rate of *T. caeruleascens* in field conditions is generally low, but higher where there are high concentrations of soil Zn and low concentrations of soil Cu. Interspecific competition between *T. caeruleascens* and a native congener is weak overall, and herbivory pressure from a native Lepidopteran herbivore is also low. Therefore, abiotic conditions are more limiting to *T. caeruleascens* than biotic interactions, and would likely prevent *T. caeruleascens* from becoming invasive or spreading outside of contaminated soils at these sites. In the fourth chapter, I use a long-term dataset to describe the demography of *Frasera speciosa* (Gentianaceae), a long-lived monocarpic plant. Results show that the population is stable, and despite the low elasticity values for the reproductive stages, masting events must be observed to describe accurately the population dynamics of this species.

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HYPERACCUMULATING PLANT, *THLASPI CAERULESCENS*, AND THEIR
IMPLICATIONS FOR PHYTOREMEDIATION

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Introduction

Understanding the factors that determine invasive species success continues to be an important research focus because of the demonstrated negative economic and ecological impacts of invasive species. The ability of introduced species to establish invasive, or self-sustaining, dominant, and disruptive (Pimentel et al. 2005), populations depends upon the biotic and abiotic factors at the introduced habitats (Shea and Chesson 2002, Mitchell et al. 2006). Specifically, suitable abiotic conditions, such as abundant resources and favorable climate, can increase the likelihood of success for the introduced species, just as well as a lack of biotic resistance at the introduced site. Biotic resistance includes the presence of competitors and natural enemies that would reduce the fitness of introduced plants (Elton 1958; Mack 1996; Levine et al. 2004). The strength and outcome of these interactions with native species also depend upon the abiotic environment, so that the effects of competition and herbivory from native species may vary both temporally and spatially (Daehler 2003, Mitchell et al. 2006).

The intentional introduction of metal-hyperaccumulating plants for phytoremediation creates an excellent opportunity to test the effects of the interactions between biotic and abiotic factors on the establishment and success of an introduced plant. Metal hyperaccumulators are plants that can uptake extraordinarily high concentrations of heavy metals into the shoot tissues (Brooks 1977). They are potentially useful for remediating soils that are contaminated by heavy metals, as an alternative to costly and intrusive methods such as landfilling (Chaney 1983, Arthur et al. 2005, Pilon-Smits 2005, Pilon-Smits and Freeman 2006, Chaney et al. 2007).

However, the introduction of non-native metal-hyperaccumulating plants for phytoremediation may present an opportunity for plant invasions. In particular, it would be important to test the ability of these plants to establish successful populations outside of the contaminated area, on uncontaminated soils (Whiting et al. 2004, Angle and Linacre 2005). The major factors determining whether hyperaccumulators would become invasive at introduced sites include the suitability of the uncontaminated substrate for growth and reproduction of the plant, and the strength of biotic resistance against plants grown on that substrate. Findings from previous studies of metal-tolerant and metal-hyperaccumulating plants can be used to form predictions on how hyperaccumulators would be affected by these interacting factors at an introduced site. Specifically, metal-hyperaccumulating plants should have lower fitness and be less competitive on lower-metal soils if the hyperaccumulation trait is similar to the metal-tolerance trait (Cook et al. 1972, Hickey and McNeilly 1975), and hyperaccumulating plants should have lower herbivory damage on high-metal soils because of the demonstrated defensive role of heavy metals in leaf tissues (Boyd & Martens 1998, Boyd 2007).

The following chapters describe three studies designed to test each of these predictions, using the Cd- and Zn-hyperaccumulator *Thlaspi caerulescens* J. & C. Presl (Brassicaceae). I experimentally introduced this plant to three study sites containing Cd- and Zn-contaminated mine tailings, near the Rocky Mountain Biological Laboratory in Gothic, Colorado. The first chapter focuses on the effects of the abiotic conditions (soil metal concentrations) on the performance of this species, and describes the germination, survival, and growth rates of *T. caerulescens*

in the study plots over three growing seasons. In this study, I also included a vegetation removal treatment to examine how the presence of the native plant community as a whole would affect the performance of *T. caerulescens* at these sites. I found that while overall plant growth is slow, *T. caerulescens* has the highest survival and growth rates on the contaminated tailings at two of the three sites. At the third site, high soil copper concentrations induced phytotoxicity and the plants did not survive on those tailings. Additionally, the presence of the native vegetation facilitated the growth and survival rates initially, but these effects have decreased over time, and performance in the vegetated plots was not different from that in removal plots by the third growing season.

The second chapter describes an experiment that specifically tested the competition component of biotic resistance. In this greenhouse experiment, I tested whether *T. caerulescens* would compete with the native congener, *Thlaspi montanum* var *montanum*, which occurs at my study sites. Using field-collected substrate and a response surface design, I estimated the competition coefficient for each species. Results show that the effects of interspecific competition were not greater than effects of intraspecific competition for both plant species in most cases. There was one instance when *T. caerulescens* had a competition coefficient greater than one, meaning that its effects on *T. montanum* were greater than intraspecific effects among *T. montanum*. This occurred when plants were grown on the soil type with relatively high Zn but low Cu, where *T. caerulescens* plants grew well. Overall, results from this study indicate that the two species are not highly competitive, and soil metal concentrations have greater influences on their performance than competitive effects.

The third chapter focuses on the natural enemy, or herbivory, aspect of biotic defense, and describes a feeding choice trial experiment. Using a Lepidopteran herbivore that is native to my study sites, I asked whether it demonstrates a preference between the native food plant *T. montanum* and the non-native *T. caerulescens*, and whether this preference depends upon the leaf Cd and Zn concentrations. The results show that *Pieris napi macdunnoughii* (Pieridae) caterpillars prefer to feed on the native plant species, but it does not completely avoid the non-native *T. caerulescens*. Additionally, the herbivore is deterred by foliar Zn and/or Cd, with lower feeding on the plants with the highest concentrations of both metals, which had been grown on the tailings at one of my study sites.

Taken together, results from these three studies show that the biotic interactions between the introduced *T. caerulescens* and native species are less limiting to the success of *T. caerulescens* than the abiotic conditions at these study sites. The results from the first study support the prediction that *T. caerulescens* has the highest survival and growth on the high-metal tailings, except when there are phytotoxic levels of copper. Results from the second study provide evidence that *T. caerulescens* is more competitive on higher-Zn soils, but there is little competition between the native *T. montanum* and *T. caerulescens* overall. Finally, results from the feeding choice trials suggest that herbivory pressure from a native herbivore would be lower than that on native plants, and especially low for plants with high foliar concentrations of Cd and Zn. Based on these findings, *T. caerulescens* is unlikely to establish self-sustaining and dominant populations on uncontaminated soils, not because of biotic resistance from the introduced community, but because of

abiotic limitations. In addition to soil Zn and Cu concentrations, it is likely that other abiotic conditions such as the short growing seasons and subalpine climate have limited the growth of *T. caerulescens* at these high-altitude study sites. Therefore it is possible that biotic interactions with competitors and herbivores may have different outcomes if *T. caerulescens* is introduced to other contaminated sites, especially those at lower elevations. Further, although I did not observe *T. caerulescens* reproduction in the field because of its slow growth, the greenhouse experiment shows that it has the potential to produce large numbers of flowers and fruits. High propagule pressure can greatly increase the likelihood of non-native establishment, and is often associated with invasiveness (Rejmanek and Richardson 1996, Alpert et al. 2000, Lockwood et al. 2005). Therefore, similar studies performed at different field sites, over longer time periods, and using different species of hyperaccumulators are needed to assess fully the risks of introducing non-native metal-hyperaccumulating plants for phytoremediation.

Finally, the fourth chapter describes the population dynamics of the long-lived monocarp *Frasera speciosa* (Gentianaceae), using a long-term dataset on a population from the same study region as the previous studies. The uniqueness of both the *F. speciosa* life history and the dataset length makes this an interesting system for a demographic analysis. I used matrix projection models to examine the effects of masting on demography, and ask how many years of data are needed to obtain accurate estimates of population growth rate and elasticity values. Based on the full 34-year dataset, I find that the *F. speciosa* population is stable with a population growth rate not different from one, and that the transitions representing

stasis are most important to the growth of this population (i.e., they have the highest elasticity values). Results also show that even short datasets (3 years or more) would produce stochastic population growth estimates that overlap one, as long as they include a masting year. When ranking the importance of various transition categories by elasticity values, ten years or more of data are needed to consistently provide the same ranking as the full 34-year dataset. These results suggest that life history stages with low elasticity values can still be very important to population growth (i.e., reproductive stages of *F. speciosa*), and that capturing one reproductive event may be sufficient for studying the demography of long-lived monocarpic species.

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Chapter 1: Field germination, survival, and growth of experimentally introduced *Thlaspi caerulescens* (Brassicaceae) across a soil metal gradient

Abstract

Thlaspi caerulescens (Brassicaceae) is a model species for studying the genetics and physiology of metal hyperaccumulation. However, studies are needed to examine its growth and survival under varying environmental factors, which would help to assess its effectiveness and potential ecological impacts when used for phytoremediation. In this study, we experimentally introduced seeds and seedlings of the Cd- and Zn-hyperaccumulating *T. caerulescens* to three sites with contaminated mine tailings, to observe its germination, growth, and survival at these sites. By creating plots on, adjacent to, and 25m away from the tailings, we also asked whether *T. caerulescens* could establish populations outside of the contaminated area if introduced for phytoremediation. We found that plant performance varied by site and location. At one of the three sites, high soil Cu concentrations greatly reduced plant survival on the tailings. Survival and growth rates were highest at the tailings at the other two sites, and positively related to the plant-available concentration of Zn in the substrate. Overall, plant growth was slow and plants did not reach reproductive stage after three growing seasons, but the presence of native vegetation had a facilitative effect on growth and survival of germinated seedlings. Our results suggest that *T. caerulescens* is unlikely to become invasive if introduced to similar sites for

phytoremediation, but further research is needed to observe its reproductive potential, and its performance at other field sites.

Introduction

Metal-hyperaccumulating plants are those that not only tolerate high amounts of heavy metals in the soil, but actively uptake very high concentrations of metals to their shoots (Brooks 1977). The level of accumulation can be 50-100 times that found in normal plants, depending on the metal and plant species. For example, zinc (Zn) hyperaccumulation is defined as >10,000ppm, and cadmium (Cd) >100ppm in dry weight of plant biomass (Reeves 1988). Since the discovery of hyperaccumulating plants, much research has been devoted to understanding the physiological mechanisms and genetic basis of the hyperaccumulation trait (Pollard et al. 2002, Assunção et al. 2003, Yang et al. 2005, Milner and Kochian 2008), while ecological studies have mostly examined the role of accumulated metals in herbivory defense (Boyd and Martens 1998, Boyd 2007). Few studies have examined the ecological consequences of hyperaccumulation on the growth and survival of these plants across environmental gradients (Maestri et al. 2010).

In contrast, metal tolerance is a classical example of local adaptation in plant populations (McNeilly 1968, MacNair 1993, Linhart and Grant 1996), and its ecological trade-offs are well studied. In general, metal tolerant plants exhibit the suite of traits that characterize stress-tolerators (as in Grime 1977). They tend to have slower growth rates and reduced reproduction, because resources are allocated to tolerance mechanisms rather than plant functions such as growth or reproduction

(Ernst 1976, Baker 1987, Sambatti and Rice 2006, but see Harper et al. 1997b). Field experiments have also demonstrated that metal-tolerant plants exhibit lower competitive ability than non-tolerant plants when grown on low-metal soils (Cook et al. 1972, Hickey and McNeilly 1975). Additionally, highly tolerant plants may require an elevated concentration of soil metals to avoid micronutrient deficiency, due to their efficient sequestration mechanisms (Antonovics et al. 1971, Kazakou et al. 2008, but see Harper et al. 1997a). Together, these traits explain why metal-tolerant species or ecotypes are often limited to sites with high metal soils.

Metal-hyperaccumulating plants may not exhibit the same fitness trade-offs observed in metal-tolerant plants, because hyperaccumulation is associated with resource conservation strategies rather than resource re-allocation strategies (Maestri et al. 2010). Metal tolerance and hyperaccumulation have also been shown to be independent genetic traits (Macnair 2007, Maestri et al. 2010). Only one study has compared the survival and reproduction of hyperaccumulators grown on high- and low-metal soils in field conditions. These researchers were examining local adaptation in natural populations of *Thlaspi caerulescens*, a Cd- and Zn-hyperaccumulator (Deschamps et al. 2008). Results from their reciprocal transplant experiment show that plants from high-metal populations have higher individual growth rates and fitness than non-metal populations when grown on metalliferous soils. However, high-metal populations do not always perform better on metalliferous soils than on non-metalliferous soils, and the authors have concluded that it is possible for the high-metal population to colonize the non-metalliferous sites. Further studies are needed to examine whether these results also apply to

earlier life stages (such as germination rates and juvenile survival), and to examine the effects of competition from surrounding vegetation on hyperaccumulator growth.

Understanding the performance of metal hyperaccumulating plants on high- and low-metal soils has important implications for their use in phytoremediation. Phytoextraction is one form of phytoremediation, and involves planting metal-hyperaccumulators on contaminated soils to uptake and remove metal contaminants (Chaney 1983, Arthur et al. 2005, Pilon-Smits 2005, Chaney et al. 2007). Field tests using *T. caerulescens* have shown that it may be feasible for remediation of soils that are moderately contaminated with Cd and Zn (McGrath 1993, Brown et al. 1995, Hammer and Keller 2003, McGrath 2006). However, the introduction of a hyperaccumulating plant to a site for remediation may lead to the establishment and spread of a non-native species if it survives well on non-contaminated soils (Whiting et al. 2004, Pilon-Smits and Freeman 2006). Phytoremediation often involves the use of a non-native species, and the intentional introduction of non-natives is one method of assessing plant invasiveness that is rarely employed but potentially highly informative (Mack 1996). Because hyperaccumulators are expected to have slow growth rates and perform less well on normal soils, we hypothesize that they are unlikely to become invasive.

In this study, we experimentally introduced *T. caerulescens* seeds and seedlings to three sites containing metal-contaminated mine tailings, to compare their germination, survival, and growth on soils with varying metal concentrations. We performed this experiment in the context of phytoremediation, by creating plots on, adjacent to, and 25m away from the mine tailings to examine whether these plants

can establish populations at the introduced site away from the tailings. We also imposed a vegetation removal treatment to examine whether the presence of the native vegetation community may negatively affect the performance of *T. caerulescens* through competition.

Methods

Study species

Native populations of *Thlaspi caerulescens* J. & C. Presl (Brassicaceae; Alpine pennycress, formerly *T. alpestre* L.) range from the Scandanavian peninsula in the north, Switzerland to the south, and the United Kingdom to the west (Koch et al. 1998). Its life cycle can be annual, biennial, or short-lived perennial (Dubois 2002), and it flowers in early spring. These plants are constitutively metal tolerant (Escarré 2000), and exhibit Zn deficiency under normal substrate Zn concentrations (Li et al. 1995, Shen et al. 1997). The seeds used in this study were collected from Palmerton, PA, a Zn-smelter contaminated site that was used for a field test of *T. caerulescens* (Li et al. 1997). These seeds likely represent a mix of ecotypes commonly used in hyperaccumulation studies, including the Southern France and Prayon populations (Chaney et al. 2005).

Study sites

T. caerulescens was experimentally introduced at small abandoned mine sites near the Rocky Mountain Biological Laboratory (38° 57' 19.60" N, 106° 59' 28.32" W) in the Elk Mountains of the central Rocky Mountains, Colorado. This region was intensively mined for silver between 1879 and 1883 and for coal in the 1950s, and numerous mine tailings, or heaps of metal-contaminated mining wastes, remain in the

region. Three silver mine tailings sites were chosen for this experiment, representing a range in pH and total Cd and Zn concentrations in the tailings, from moderate to high contamination levels (Table 1). Substrate samples were analyzed for total metal concentration (Cd, Cu, Ni, Zn) using the Aqua Regia extraction method (McGrath and Cunliffe 1985) and flame atomic absorption spectrometry (AAS), and for plant-available metal concentrations (Cd, Zn) using strontium nitrate and AAS. Two of the three sites (Copper and Woods) are located at the Mexican Cut, a Nature Conservancy preserve at an elevation of 3,680m (39° 0' 57.54" N, -107° 3' 44.23" W). The third site (Cinnamon) is located 2km south of these sites, near Paradise Divide at 3,415m (38° 59' 49.86" N, -107° 3' 31.28" W).

Experimental plots

We examined the effects of vegetation presence and soil contamination level, represented by distance from the tailings, on the survival and growth of *T. caerulea* in the field. For the soil contamination factor, three plot locations were chosen: on the tailings ("tailing"), within one meter of the tailings ("adjacent"), and 25-30m away from tailings ("far"). The vegetation factor contained two levels: removal of all above-ground biomass of native plants (maintained throughout the experiment), or unmanipulated native vegetation. Because natural vegetation was absent on tailings, there were a total of five treatment combinations that were replicated at each site. For each treatment combination, we had separate plots planted with *T. caerulea* seeds and seedlings.

T. caerulea seeds were introduced to the study sites in July 2006. Fifty seeds were planted in 25x25cm plots for each treatment combination, with sets of five

seeds in 10 plastic rings to prevent scattering of the surface-germinating seeds. Each treatment combination was replicated three times at each site for the seed plots. Plants for the seedling plots were germinated on flats of potting mix in field conditions, and three-week old seedlings were planted into 1x1m plots for each treatment combination in August of each year from 2006-2008. Replication varied by site and year, depending on the number of germinated seedlings available (see Table 2 for sample sizes). In 2006, fewer seedlings were planted at Cinnamon and Woods sites because there were fewer seedlings of transplantable size than initially estimated. The survival of these seedlings through winter was low (< 9% average survival per plot), and these plots were cleared. New plots were established in 2007 and 2008, which were watered every other day for several weeks after planting. Vegetation removal treatments were not included to maximize replication using the number of seedlings that germinated in those years.

Germination rates were measured in the seed plots, and survival and growth (measured as the number of leaves and length of longest leaf) were recorded for each plant weekly throughout three growing seasons (2007-2009) in all plots. All surviving plants were collected in August 2009, and above-ground biomass was analyzed for mineral concentrations (Ca, Cd, Cu, Fe, K, Mg, Mn, Ni, P, Zn) using hot nitric digestion and Inductively coupled plasma atomic emission spectroscopy.

The effects of native vegetation and location on survival rates were analyzed using planned contrasts in an ANOVA because of the incomplete factorial design (there was no natural vegetation at the tailings). For the seed plots, the response variables were percent germination, and percent survival (out of number germinated)

after the first, second, and third winters, and at the end of the second and third growing seasons (called second and third years). Because site was a significant factor in the full model for all responses, seed plot data from each site were analyzed separately. For the seedling plots, percent survival (out of number planted) after the first and second winters, and at the end of the second growing season were used. Site was not a significant main effect for seedling plots, and location was analyzed as a nested effect within site. Percentages were arcsine square root transformed to conform to normality. To examine growth, a repeated measures ANOVA was used with the first principle component of the leaf measurements (length and number) as the response variable, and site, location nested in site, native vegetation treatment nested in site, and their interactions were the explanatory variables. For seed plots, one analysis excluded the tailings plots to test the effects of the vegetation treatment, while another analysis excluded the treatments with native vegetation to examine the effects of location including tailings. For seedling plots, only the effect of location on growth was examined because vegetation treatment was not included in our experimental design, and data from seedlings planted in 2007 were used because leaf measurements were recorded in these plots over the longest time period.

To examine the relationship between soil characteristics and plant performance, canonical correlation analyses were used. This method controls for correlations among the sets of variables: soil characteristics (pH and metal concentrations), arcsine square root transformed germination and survival rates, and square root transformed leaf number and leaf length at the end of the experiment. To control for the effect of vegetation treatment, separate canonical correlation analyses

were performed for the seed plots data, one including only the plots without vegetation (tailing, adjacent removal, and far removal plots), and another including only the unmanipulated plots (tailing, adjacent vegetation, far vegetation plots). Individual plant metal concentrations were analyzed using an ANOVA to test for effects of site, location, and their interactions. All analyses were performed using SAS software (version 9.2, SAS Institute Inc. 2008).

Results

In the seed plots, *Thlaspi caerulescens* germination rates did not differ by location or vegetation treatment, except at Cinnamon where germination at the tailing was significantly lower than that at far and adjacent (Table 3; Fig. 1). For Copper site, survival differed by location in the first winter and the second year, when tailings plants had lower survival than the other locations (this was marginally significant between tailings and far in year two; Table 3). After this, survival at the other locations decreased and was not different from the tailings. At Cinnamon site, survival was significantly higher on tailings than on other locations starting in the second year (this was marginally significant between tailings and far in the second winter; Table 3), while survival on tailings was highest at all times at Woods site (Table 3; Fig. 2 shows survival in the third winter). At Woods site, survival was also higher at far than at adjacent in the second year and the second winter. Vegetation treatment did not have an effect on germination, or survival of the first and third winters. The presence of native vegetation increased survival in the second year and the second winter at Cinnamon and Woods sites, and also in the third year at Cinnamon site (Table 3). In the seedling plots, there was a significant effect of

planting year on survival of the first winter ($F_{1,56} = 35.06$, $p < 0.0001$; Fig. 3); the seedlings planted in 2006 had significantly lower survival in the first winter than those planted in 2007 or 2008 ($t_{df=47} = -5.95$ and $t_{df=47} = 6.11$, respectively, $p < 0.0001$ for both). Year two and winter two survival did not differ by year, site, or location.

To assess growth in the seed plots, we first use the adjacent and far plots only to test the vegetation treatment effect on leaf measurements (because tailings plots did not support native vegetation). There was a significant interaction between time and vegetation treatment nested within site ($F_{6,1419} = 2.46$, $p = 0.023$). Leaf measurements were higher in the vegetation treatment than in the removal plots initially, but they decreased at a greater rate over time, such that there was no significant difference between vegetation treatments at the end of the experiment. There was also a significant effect of location nested within site ($F_{3,6} = 6.06$, $p = 0.030$; Fig. 4a - leaf length data were also used in the analyses but not shown; they followed the same trends as the leaf number data). When we examined only the no-vegetation plots (to include the tailings plots for comparison), the effect of location nested within site was again significant ($F_{6,12} = 6.18$, $p = 0.004$), as was the main effect of site ($F_{2,6} = 9.20$, $p = 0.015$). Comparing the leaf measurements across sites and locations, plants in the seed plots were largest at the tailings at Woods and Cinnamon, while plants at Copper were smallest at the tailings (Fig. 4a). Overall growth was very slow, however, and after three growing seasons only three plants in the seed plots had leaves longer than 1.5cm, and only one plant had more than 15 leaves. Growth measurements from the seedling plots did not differ by site, location, or time (Fig. 4b).

The canonical correlation analyses showed a significant correlation between soil characteristics and the survival and growth in seed plots, and survival but not growth in seedling plots (Table 4). Factor loadings and standardized canonical correlation coefficients can be used to interpret how each original variable contributes to the correlation between the sets of variables. The factor loadings are the correlations between each original variable and the canonical variate. They showed that in the seed plots, all survival variables were positively correlated with the total and available concentrations of Cd and Zn in the soil, while the growth measurements are positively correlated with pH only. For the seedling plots, the factor loadings showed that all survival variables were positively correlated with pH and total Ni in the soil, and negatively correlated to all other metal variables. The standardized canonical correlation coefficients describe the unique effects of each original variable when all other variables are held constant. The coefficients showed that available Zn concentration has the highest positive influence on the second year survival and leaf growth of *T. caerulea* in the seed plots, and on the first winter survival in seedling plots. The negative coefficients for total Zn indicate that when the available Zn concentration is held constant, any increase in total Zn actually decreases the leaf size of plants in seed plots and survival of plants in seedling plots. When other soil variables are held constant, higher available Cd decreases the growth and survival in seed plots and the survival in seedling plots. The coefficients also show that total Cu concentration has a slight negative correlation with seed survival and growth, and a slight but positive correlation with seedling survival.

Metals concentrations in plant tissues differed by location for Cd and marginally for Zn ($F_{2,8} = 10.27$, $p = 0.006$ and $F_{2,8} = 3.76$, $p = 0.071$, respectively; Fig. 5), and there was no significant effect of site. Across sites, plant Cd was higher at tailings than at adjacent or far ($t_{df=8} = 3.64$, $p = 0.007$ and $t_{df=8} = 3.93$, $p = 0.004$, respectively; Fig. 5). Cd concentrations reached hyperaccumulation levels (>100ppm) only at the tailings at Cinnamon. Plants did not hyperaccumulate Zn at our sites. Other plant metals and nutrients did not show significant differences between locations and sites.

Discussion

Overall the growth of *Thlaspi caerulescens* was slow at our study sites, and plants did not reach reproductive stage after three growing seasons. The abiotic environment at these high elevation sites were likely limited their growth, with shorter growing seasons and cooler mean and minimum temperatures. Other studies have found a slow growth rate for this species and cited this as one of major obstacles to its use in phytoextraction (Koopmans et al. 2008), though fertilization treatments have been shown to improve growth rates (Schwartz et al. 2003). Plant growth and survival across the locations (i.e., distances from the tailings) differed among sites. At Copper site, the Cu concentrations in the tailings were at phytotoxic levels, and plants were unable to survive. This did not occur at the other two sites, where *T. caerulescens* seeds had highest survival and growth at the tailings. The conditions on the tailings at Woods site were clearly best suited for *T. caerulescens*, because high survival and growth were also seen in the seedling plots there. Plant performance at this location may have been promoted by a combination of factors: moderate levels of

Cd and Zn contamination, low Cu concentrations, and a pH near 5.1 which was found to be optimal for growth in this species (Yanai et al. 2006). At Cinnamon tailings, germination rate was low in the seed plots, as was survival rate in the seedling plots. At first, these results seem contradictory to the high survival of germinated seeds at that location. However, high variance in the total metal concentrations in the soil (Table 1) suggests a patchy distribution of contaminants in those tailings, which could explain these findings. Because of the patchiness, the majority of planted seeds and seedlings were unable to survive, but the few seeds that landed in suitable microsites successfully germinated and had high survival rates.

There was no clear difference in performance of *T. caerulescens* planted at the edge of the tailings compared to those planted 25m away from the tailings. Although metal concentrations were higher at the adjacent than at the far location, the differences did not impact growth or survival. Our results are in agreement with an earlier study that found that the metal-adapted population of *T. caerulescens* grew well on high-metal soils, though they also found that it grew equally well on one of their two low-metal sites (Dechamps et al. 2008). Their findings and ours both show that *T. caerulescens* performance is highly site-specific, depending not only on soil Zn and Cd concentrations but also on soil nutrient concentrations and slope aspect (as in Dechamps et al. 2008), soil Cu concentrations (our study), and soil pH (both studies). These findings can be used to select sites that would be most suitable for phytoremediation using *T. caerulescens*. Specifically, sites with high concentrations of available Zn, low concentrations of Cu, and moderately low soil pH would best support *T. caerulescens* growth. Sites at lower elevations would also be likely to

support faster growth rates than we observed because of longer growing seasons, and plants that are larger may accumulate higher concentrations of heavy metals. Finally, seedling survival only varied depending on the year of planting. Seedlings planted in 2006 had low over-winter survival, and we found that watering for two weeks after transplantation improved seedling survival. We did not find significant results for growth in the seedling plots, perhaps because the number of transplanted seedlings in 2007 was too low. Seedlings intended for transplantation into seedling plots were germinated on flats and placed under field conditions, but germination rates were low in 2007, potentially because of the hot, dry weather that summer (based on SNOTEL data for Schofield Pass, CO, approximately 2km from the field sites). Both of these findings have implications for the success of using *T. caerulescens* for phytoremediation: watering or other treatments may be required initially, and germination or survival rates may depend on the year of introduction.

The plants in our study did not hyperaccumulate Zn (>10,000ppm) at our sites, but some plants did hyperaccumulate Cd (>100ppm). The lower Zn accumulation may be caused by low soil pH (< 5), which can lead to Al and/or Mn toxicity and consequently reduced root growth and metal uptake (Wang et al. 2006). The plants at Cinnamon tailings had the highest metal concentrations with 140ppm Cd and 4800ppm Zn, which is within the range of Zn values reported in previous field trials of *T. caerulescens* (McGrath 1993, Brown et al. 1995, McGrath et al. 2006). Results from the canonical correlation analyses showed a strong correlation between high available Zn and better growth and survival of both seeds and seedlings. This supports the Zn requirement previously shown in this hyperaccumulator (Li et al.

1995, Shen et al. 1997), which may be a cost to the hyperaccumulation trait similar to the micronutrient deficiency in metal-tolerant plants when grown on low metal soils (Antonovics et al. 1971, Harper et al. 1997b). However, the response of *T. caerulescens* to the presence of competitors was not the same as that found in studies of metal-tolerant plants, which performed more poorly on low metal soils when planted with competitors than when planted alone (Cook et al. 1972, Hickey and McNeilly 1975). In this study, the presence of native plant species had a facilitative effect, in both adjacent and far locations. This result suggests that the abiotic conditions at these sites presented a stressful environment for these plants (Callaway 1995), which was likely due to the subalpine climate rather than substrate characteristics. This facilitative effect was not significant after the second winter, however, suggesting that older plants would no longer benefit from the presence of native vegetation.

Our results indicate that *T. caerulescens* plants are unlikely to become invasive if introduced to these sites for remediation. Although not measured here, this species possesses some of the traits associated with invasiveness (small, easily dispersed seeds, self-compatibility) but lacks other traits (high growth rate; van Kleunen et al. 2010b). The very slow individual growth rate we have observed would likely limit the rate of population growth at these sites, but whether their reproductive traits would counteract these effects on population growth remains unknown. Previous studies have found that many invasive species undergo a lag phase, in which newly introduced plant populations persist in low numbers before a rapid expansion (Mack 1996, van Kleunen et al. 2010a). This is unlikely to be the case for our

species, because survival declined steadily over the three seasons in our seed plots. Finally, we cannot yet fully predict the potential growth and impacts of *T. caerulea* at other sites (especially at lower elevations), because individual contaminated sites would have different combinations of contaminants, pH, climate, and other environmental factors that may interact in different ways. There is a need to study further the ecology and population dynamics of metal hyperaccumulators, especially in field conditions and including other hyperaccumulator species. For example, *Alyssum murale* is a Ni-hyperaccumulator that has been shown to be economically viable for phytoextraction of Ni (Li et al. 2003, Chaney et al. 2007), and field assessments similar to the one conducted here would also be valuable for predicting its establishment potential at planned introduction sites.

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Tables

Table 1. Mean soil metal concentrations (± 1 SE, in ppm) at the three sites (Cinnamon, Copper, Woods) and three locations (tailing, adjacent, far) at each site. The numbers of samples analyzed (N) are higher for adjacent and far locations because samples collected for another experiment are also included. Total metal concentrations were measured using the Aqua Regia extraction method, and plant-available metal concentrations were measured using the strontium nitrate extraction method. For reference, the average ranges in total soil metal concentrations (upper and lower limits of the 98% confidence interval) found in North American soils are shown, from Smith et al. (2005).

	Cinnamon			Copper			Woods			Reference
	tailing	adj	far	tailing	adj	far	tailing	adj	far	
N	3	10	9	4	10	10	5	12	9	
pH	4.37 \pm 1.370	4.85 \pm 0.113	4.64 \pm 0.076	5.59 \pm 0.117	4.82 \pm 0.079	4.88 \pm 0.084	5.33 \pm 0.104	5.32 \pm 0.145	5.00 \pm 0.100	
Total Cd	89.2 \pm 78.00	1.23 \pm 0.137	0.416 \pm 0.039	50.9 \pm 11.40	2.31 \pm 0.445	1.02 \pm 0.108	4.46 \pm 0.540	2.09 \pm 0.285	1.02 \pm 0.073	<0.1 – 1.9
Total Zn	12800 \pm 10300	381 \pm 25.7	163 \pm 6.68	6680 \pm 1310	464 \pm 73.5	555 \pm 44.3	853 \pm 45.9	587 \pm 71.6	335 \pm 5.19	11 – 182
Avail. Cd	2.76 \pm 2.49	0.126 \pm 0.018	0.035 \pm 0.009	0.414 \pm 0.118	0.132 \pm 0.059	0.024 \pm 0.005	0.034 \pm 0.012	0.026 \pm 0.005	0.026 \pm 0.007	
Avail. Zn	453 \pm 407.0	6.19 \pm 1.64	1.24 \pm 0.355	43.5 \pm 13.5	7.59 \pm 3.22	2.08 \pm 0.361	3.5 \pm 0.443	2.11 \pm 0.318	2.44 \pm 0.483	
Total Ni	2.33 \pm 0.933	6.82 \pm 0.198	6.52 \pm 0.139	23.9 \pm 2.40	7.05 \pm 0.258	7.89 \pm 0.217	8.82 \pm 0.248	6.27 \pm 0.165	6.57 \pm 0.173	2.7 – 322
Total Cu	1920 \pm 1440	85.7 \pm 5.93	37.7 \pm 1.09	7080 \pm 2910	992 \pm 162	64.3 \pm 2.49	1410 \pm 169	1980 \pm 34.7	32.2 \pm 1.03	2.2 – 65

Table 2. Numbers of seeds and seedlings planted and surviving in each plot type, summed across replicates and grouped by planting year. Times shown are germination, winter 1, year 2, winter 2, year 3, and winter 3 (where appropriate).

Site	Loc	Trt	2006 seeds								2006 seedlings		
			Reps	Planted	Germ	W1	Y2	W2	Y3	W3	Reps	Planted	W1
Cinnamon	Tail	no	3	150	19	22	22	15	13	7	3	75	0
	Adj	no	3	150	61	33	0	0	0	0	3	75	0
	Adj	veg	3	155	68	34	25	24	15	7	3	75	0
	Away	no	3	150	87	47	6	4	1	1	3	75	0
	Away	veg	3	150	78	49	23	21	14	4	3	75	1
Copper	Tail	no	3	150	34	0	0	0	0	0	4	156	0
	Adj	no	3	150	49	42	16	5	1	1	4	156	21
	Adj	veg	3	150	53	40	21	20	17	7	4	156	40
	Away	no	3	150	69	34	15	13	11	6	4	156	10
	Away	veg	3	150	94	58	42	25	17	11	4	156	37
Woods	Tail	no	3	150	106	107	71	67	62	55	3	72	35
	Adj	no	3	150	80	62	1	0	0	0	3	72	0
	Adj	veg	3	150	95	66	25	19	10	5	3	72	0
	Away	no	3	150	88	52	28	20	12	8	3	72	0
	Away	veg	3	150	115	75	58	41	33	26	3	72	0

Site	Loc	Trt	2007 seedlings					2008 seedlings			
			Reps	Planted	W1	Y2	W2	Reps	Planted	W1	Y2
Cinnamon	Tail	no	1	10	1	1	1	2	56	1	1
	Adj	veg	1	10	3	1	0	2	56	5	7
	Away	veg	1	10	4	1	1	2	56	6	6
Copper	Tail	no	1	10	0	0	0	3	90	5	7
	Adj	veg	1	10	4	3	3	3	90	34	39
	Away	veg	1	10	1	1	0	3	90	31	29
Woods	Tail	no	3	30	23	15	7	2	60	42	33
	Adj	veg	3	30	7	4	3	2	60	28	24
	Away	veg	3	30	7	7	5	2	60	8	7

Table 3. ANOVA for the effects of vegetation treatment, location, and their interaction on the germination and survival rates of the *Thlaspi caerulescens* in the seed plots. The effects of site and/or site by location interaction were significant for all responses, and separate analyses were performed for each site. Because the experiment was an incomplete factorial design, the effects of location were tested using planned contrasts (the pair-wise comparisons between the tailings, and the vegetation removal plots at adjacent and far). The degrees of freedom for all survival responses were the same, and are only shown once in the table. Significance: ** = $p < 0.01$, * = $p < 0.05$, ` = $p < 0.1$.

	percent germination			winter 1 survival			year 2 survival		winter 2 survival		year 3 survival		winter 3 survival	
Source	df	F	p	df	F	p	F	p	F	p	F	p	F	p
Cinnamon														
vegetation	1,9	0.01	0.923	1,7	<0.01	0.950	7.92	0.026*	8.78	0.021*	10.95	0.013*	0.95	0.363
tail vs adj	1,9	5.19	0.049*	1,7	5.53	0.051`	17.87	0.004**	8.35	0.023*	13.05	0.009**	8.74	0.021*
tail vs. far	1,9	10.13	0.011*	1,7	4.20	0.080`	12.58	0.009**	5.42	0.053`	10.91	0.013*	6.89	0.034*
adj vs. far	1,9	0.82	0.389	1,7	0.18	0.682	0.97	0.359	0.67	0.441	0.21	0.664	0.22	0.653
Loc*veg	1,9	0.14	0.717	1,7	0.01	0.931	0.05	0.827	0.02	0.900	0.13	0.730	0.47	0.515
Copper														
vegetation	1,9	0.63	0.447	1,8	0.05	0.836	1.34	0.281	1.77	0.220	1.77	0.220	2.13	0.183
tail vs adj	1,9	1.01	0.341	1,8	13.92	0.006**	5.76	0.043*	2.66	0.141	0.19	0.676	0.38	0.557
tail vs. far	1,9	3.26	0.104	1,8	6.05	0.039*	4.94	0.057`	5.20	0.052`	1.56	0.247	1.45	0.263
adj vs. far	1,9	0.64	0.444	1,8	2.06	0.189	0.04	0.846	0.54	0.484	0.83	0.388	0.44	0.527
Loc*veg	1,9	0.29	0.604	1,8	0.44	0.527	0.57	0.472	0.40	0.546	0.92	0.365	0.68	0.433
Woods														
vegetation	1,9	2.80	0.129	1,9	0.02	0.899	13.57	0.005**	8.37	0.018*	2.62	0.140	2.99	0.118
tail vs adj	1,9	2.43	0.154	1,9	9.63	0.013*	48.66	<0.001**	36.74	0.023*	25.18	0.001**	26.54	0.001**
tail vs. far	1,9	1.30	0.283	1,9	26.11	0.001**	7.17	0.025*	7.42	0.023*	8.46	0.017*	12.46	0.006**
adj vs. far	1,9	0.17	0.687	1,9	4.03	0.076`	18.47	0.002**	11.13	0.009*	4.45	0.064`	2.63	0.139
Loc*veg	1,9	0.27	0.614	1,9	1.70	0.225	2.21	0.172	1.92	0.199	0.02	0.903	0.33	0.577

Table 4. Results from the canonical correlation analysis between the set of soil characteristics (pH through total Ni) and the set of survival rates (germination rate to winter 3 survival rate), or the set of leaf measurements at the end of the experiment (number and length of leaves). Separate analyses were performed for seed plots and seedling plots, and for the seed plots that received vegetation removal treatments, to eliminate any confounding treatment effects. Results shown here are for the seed plots without removal treatments, but those from the removal plots yield the same conclusions. The significant canonical variates from each analysis are shown with their eigenvalues and canonical correlation values. Under each variate, the factor loadings are listed on the left (“Loadings”), and the standardized canonical correlation coefficients are listed on the right (“Coeff.”). The largest coefficients in each set of variables are highlighted in bold. See text for interpretation of these values.

Plant type	Seeds		Seeds		Seedlings	
Test type	Survival / Soil metals		Growth / Soil metals		Survival / Soil metals	
Eigenvalue	8.92		2.48		16.94	
Canonical correlation	0.948		0.844		0.972	
F (ndf,ddf)	2.56 (42,55)		3.01 (14,36)		3.46 (21,15)	
p	0.0006		0.004		0.0087	
	Loadings	Coeff.	Loadings	Coeff.	Loadings	Coeff.
pH	-0.39	0.41	0.16	2.06	0.34	0.52
Total Cd	0.51	-2.43	-0.14	18.77	-0.24	-2.92
Avail Cd	0.78	-3.93	-0.05	-11.28	-0.23	-8.89
Total Zn	0.56	0.19	-0.12	-44.80	-0.23	-21.66
Avail Zn	0.83	7.13	-0.03	39.44	-0.21	33.38
Total Cu	-0.20	-0.75	-0.23	-0.34	-0.09	1.73
Total Ni	-0.43	1.54	-0.21	9.04	0.02	8.29
Germination	0.16	-0.05				
Winter 1	0.54	0.07			1.00	1.23
Year 2	0.81	2.19			0.89	-0.30
Winter 2	0.52	-1.44			0.43	0.10
Year 3	0.56	-0.35				
Winter 3	0.49	0.33				
# Leaves			0.98	2.03		
Leaf length			0.94	-1.07		

Figures

Fig. 1. Mean germination rate of *Thlaspi caerulescens* seed plots (± 1 SE) planted at three sites (Cinnamon, Copper, Woods), three locations (tailings, adjacent, and far), and two treatments (veg = with natural vegetation, or no = vegetation removal). A lack of error bars indicates only one plot contained any germinated seeds. Different letters indicate significantly different means tested within each site, when arcsine square root transformed response variables were analyzed.

Fig. 2. Mean survival rate (± 1 SE) of germinated *T. caerulescens* plants after the third winter in the seed plots, planted at three sites (Cinnamon, Copper, Woods), three locations (tailings, adjacent, and far), and two treatments (veg = with natural vegetation, or no = vegetation removal). A lack of error bars indicates only one plot contained any surviving plants. Different letters indicate significantly different means tested within each site, when arcsine square root transformed response variables were analyzed.

Fig. 3. Mean survival rate (± 1 SE) of *T. caerulescens* in seedling plots after the first winter, planted at three sites (Cinnamon, Copper, Woods) and three locations (tailings, adjacent, and far). A lack of error bars indicates only one plot contained any surviving plants. There is a significant main effect of planting year on survival of the first winter ($F_{1,56} = 35.06$, $p < 0.0001$), in which seedlings planted in 2006 had significantly lower survival than those planted in 2007 or 2008 ($t_{47} = -5.95$ and $t_{47} = 6.11$, respectively, $p < 0.0001$ for both).

Fig. 4. Mean number of leaves (± 1 SE) per plant at the end of the experiment, in seed plots (A) and in seedling plots (B), planted at three sites (Cinnamon, Copper, Woods) and three locations (tailings, adjacent, and far). For the seed plots, the mean number of leaves between the vegetation treatments (with natural vegetation or vegetation removal) are shown for the adjacent and far locations. Different letters indicate significantly different means tested across sites, when arcsine square-root transformed response variables were analyzed. There were no significant differences between sites and locations in the seedling plots.

Fig. 5. Mean cadmium (A) and zinc (B) concentrations (± 1 SE, in ppm) in the shoots of *T. caerulea* planted at three locations (tailings, adjacent, and far) and three sites (Cinnamon, Copper, Woods) after three growing seasons. A lack of error bars indicates samples had to be combined for analysis due to insufficient biomass. Across sites, plant Cd was higher at tailings than at adjacent ($t_{df=8} = 3.64$, $p = 0.007$) and far ($t_{df=8} = 3.93$, $p = 0.004$).

Figure 1

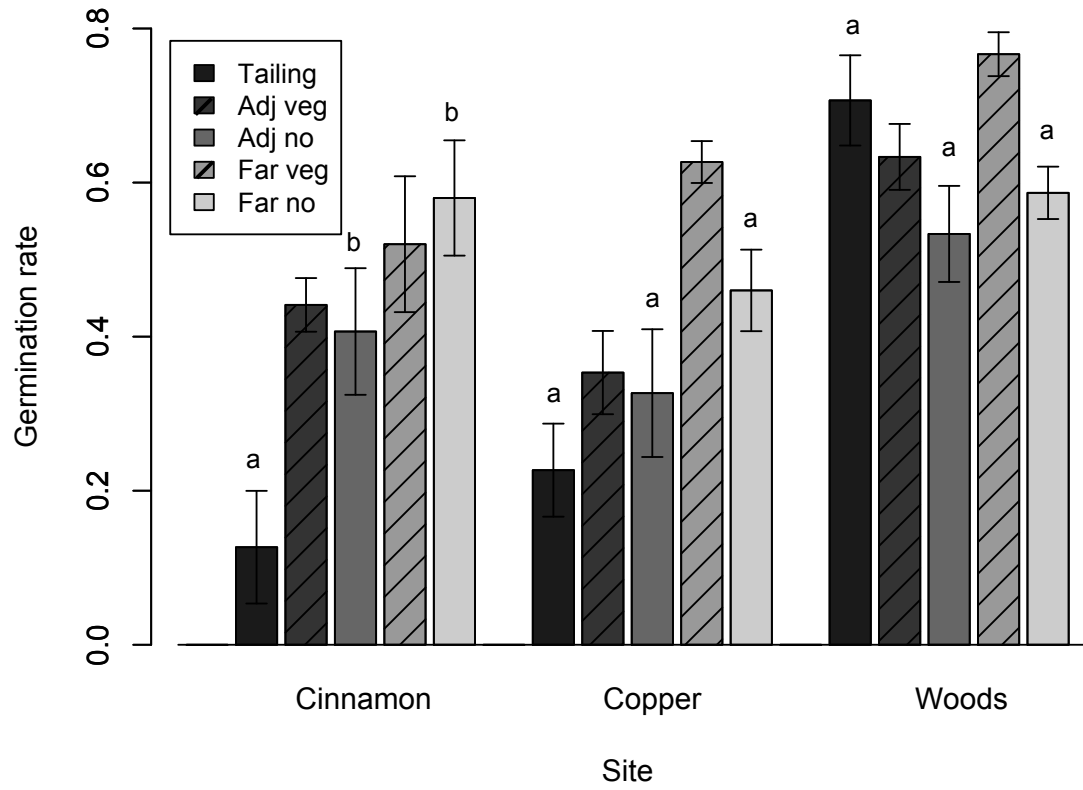


Figure 2

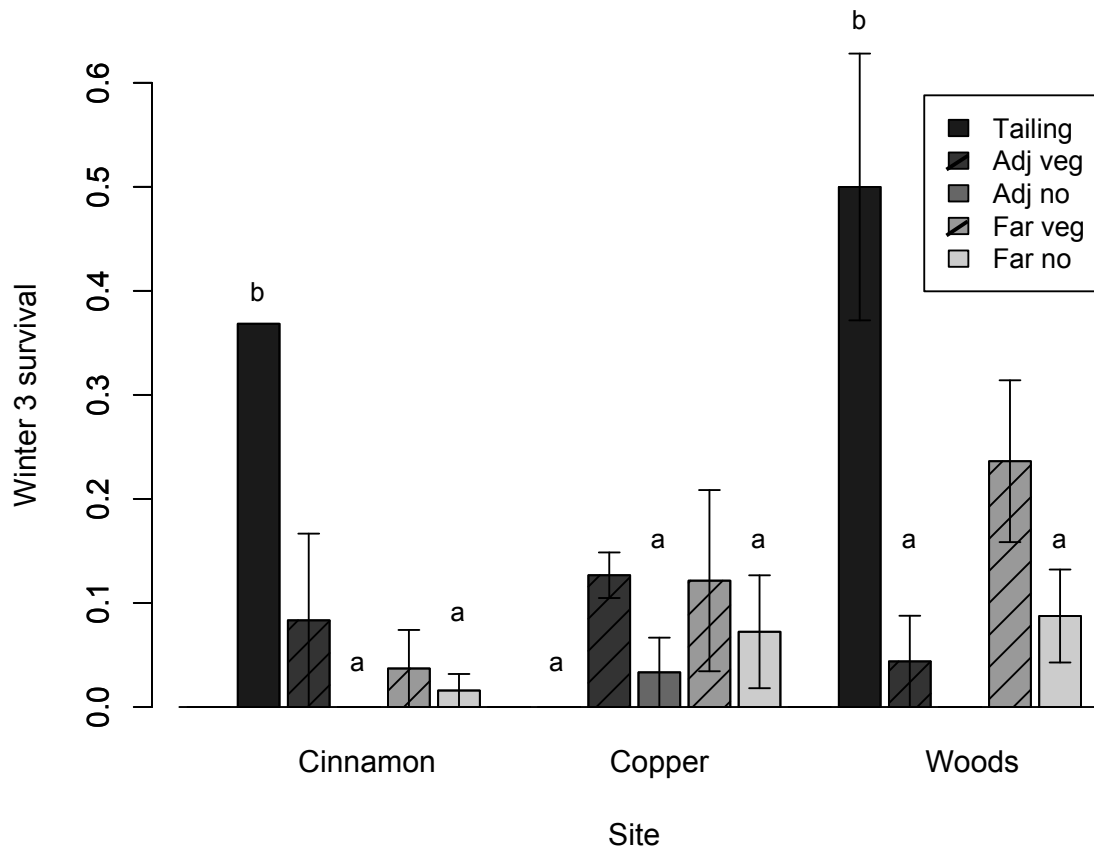


Figure 3

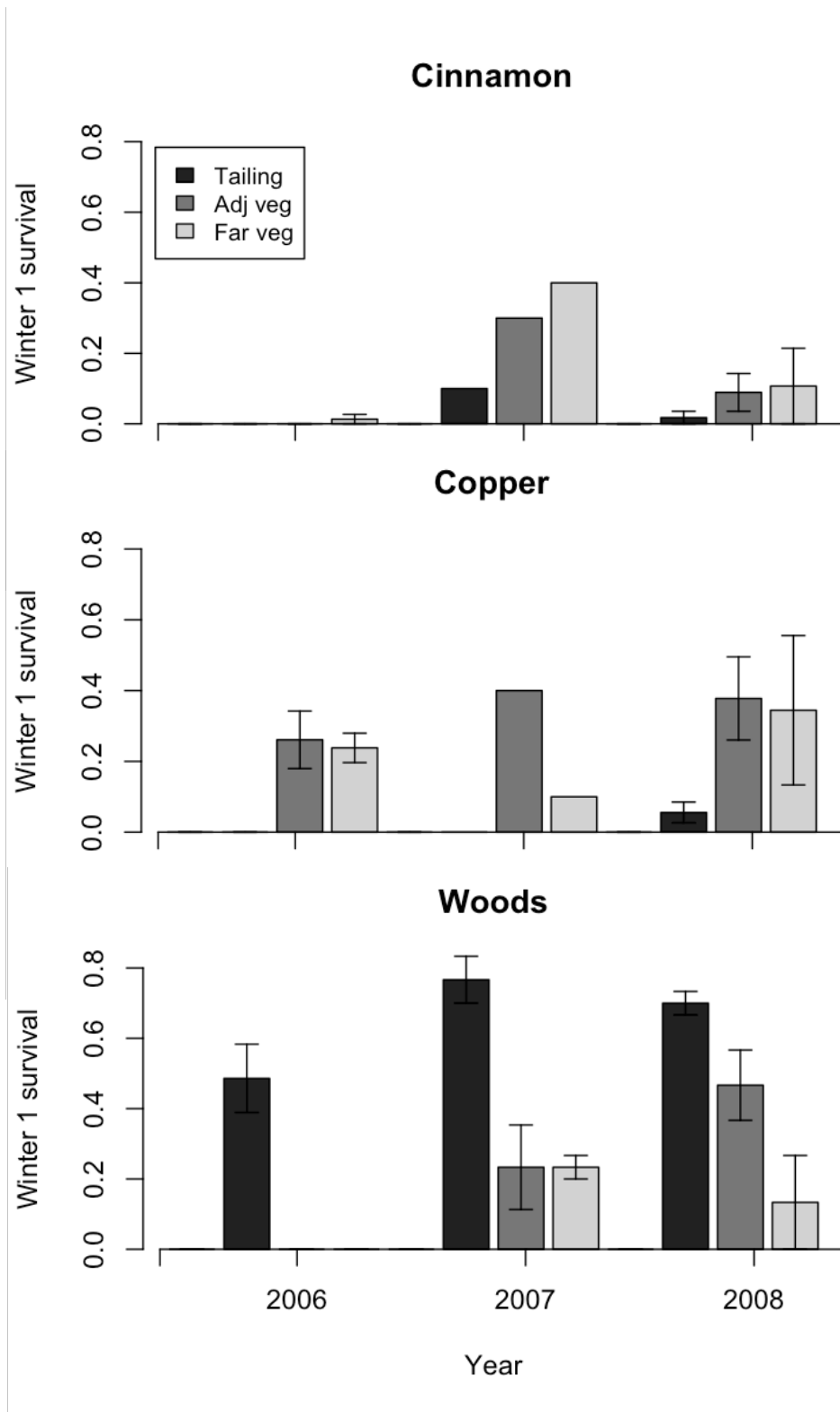


Figure 4

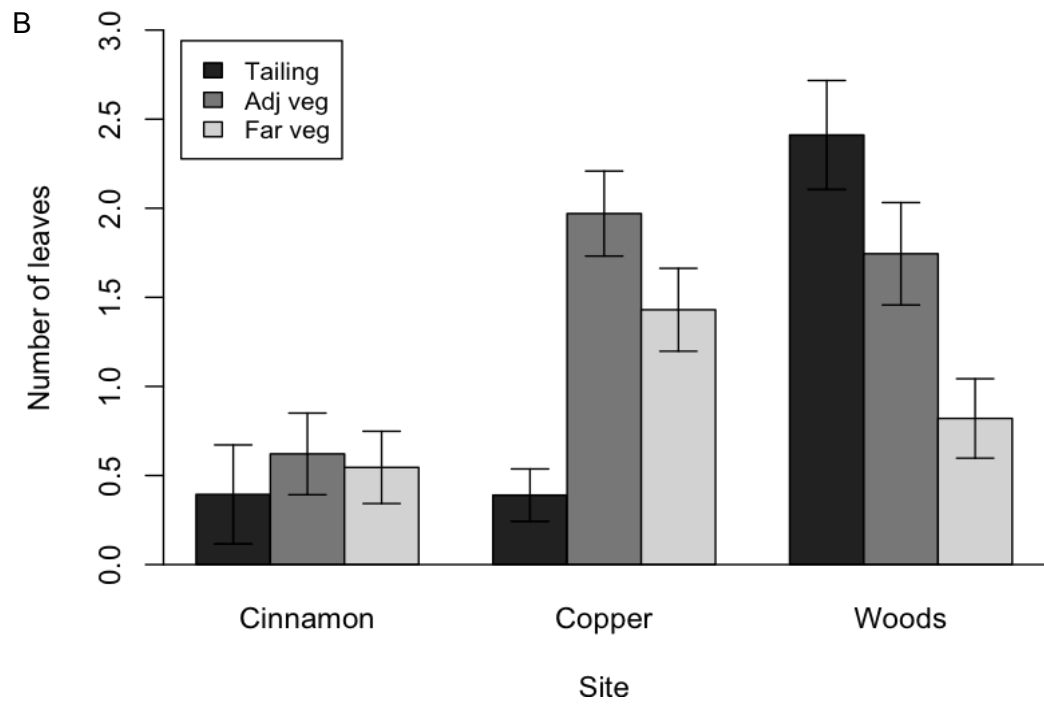
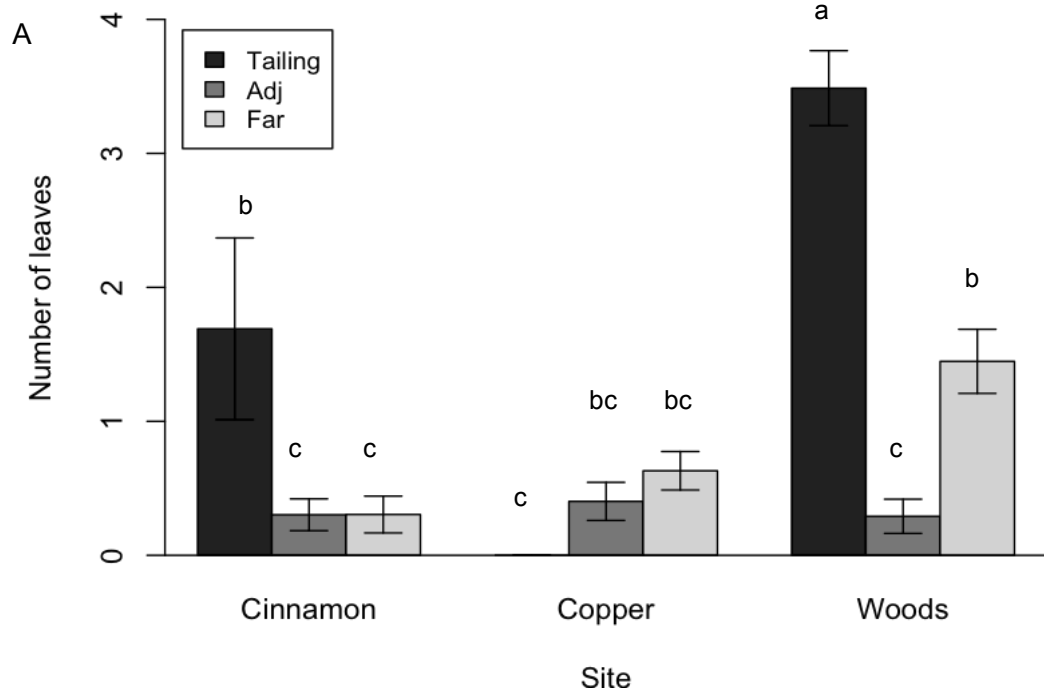
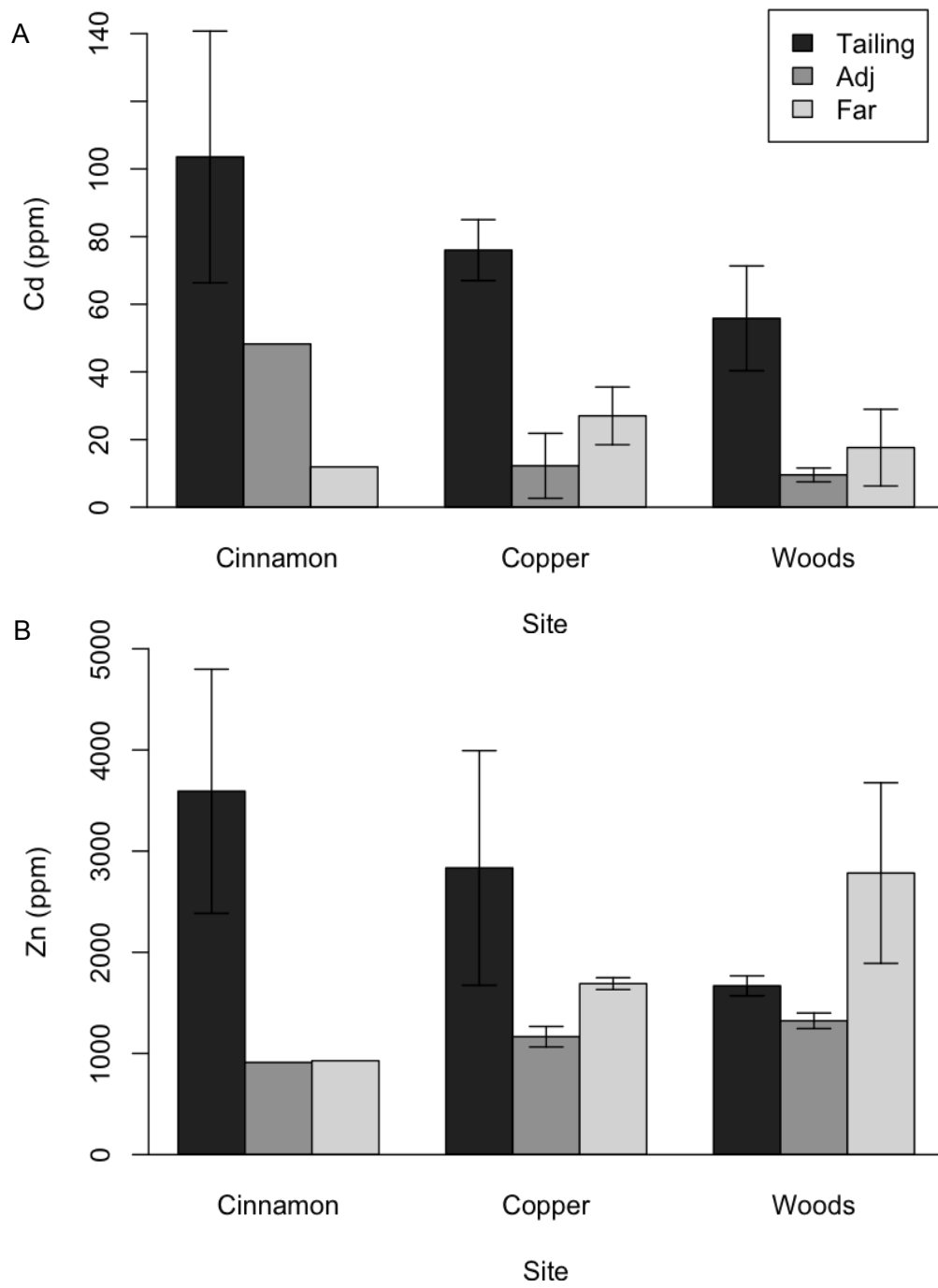


Figure 5



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Chapter 2: Interspecific competition between a non-native metal-hyperaccumulating plant (*Thlaspi caerulescens*, Brassicaceae) and a native congener (*T. montanum*)

Abstract

Experimental evidence shows that invasive plants often have higher competitive abilities than native plants in the introduced range, and therefore competitiveness should be examined when assessing the risks of introducing a non-native plant. One type of intentional introduction is the use of plants that can uptake and hyperaccumulate heavy metals in remediating metal-contaminated soils. In this study, we examined the competitive ability of a Zn- and Cd-hyperaccumulator, *Thlaspi caerulescens* (Brassicaceae), against a congener, *T. montanum*, that is native to metal-contaminated sites in southwestern Colorado. Using a greenhouse competition experiment with a response-surface design and field-collected soils, we show weak competition between *T. caerulescens* and *T. montanum* in five of the six soil types tested. Abiotic factors were more limiting to growth than competitive interactions, with both species performing better in soils with higher Zn and lower Cd and Cu concentrations. A comparable field test showed that both species perform less well in natural conditions. We conclude that *T. caerulescens* is unlikely to be highly competitive in the field, although the successful establishment and high reproduction rates observed in our trials suggest that caution should be taken when introducing these plants to new sites.

Introduction

Human activities such as horticulture and agriculture have led to the introduction of tens of thousands of non-native species in the United States (Reichard and White 2001, Pimentel et al., 2005). While many of these species have become naturalized, only approximately one-fifth have become invasive, which can be defined as self-sustaining, dominant, and disruptive to natural processes in the new habitat (Pimentel et al., 2005). A major factor determining whether non-natives become invasive is the degree of interspecific competition between the introduced plant and native species at the site of introduction (Crawley 1990, Alpert et al. 2000, Vilà and Weiner 2004). Studies to date have focused on comparing the competitive ability of plants that are known to be invasive with that of native plants. Results from these studies generally support higher competitive ability in the non-natives (Parker and Reichard 1998, Vilà and Weiner 2004), although this may be because they focus on known invasives, which are expected to be superior competitors. Previous studies also indicate that competitive outcomes between native and introduced plants often depend on resource availability (Alpert et al. 2000, Moen and Meurk 2001, Daehler 2003, Garcia-Serrano et al. 2007, Burns 2008). Specifically, environments that have high resource availability (such as water, nutrients, and light) tend to favor the non-natives, whereas native species can better tolerate conditions of environmental stress.

In contrast to studying known invasives, non-native plants can also be examined prior to introduction, to assess their likelihood of becoming invasive and potential effects on native species (Mack 1996). One situation involving the planned

introduction of non-natives is phytoremediation, in which a plant is introduced to remove, contain, or help degrade contaminants from soils (Chaney 1983, Arthur et al. 2005, Pilon-Smits 2005, Pilon-Smits and Freeman 2006). This includes a method called phytoextraction, in which metal contaminants are removed from soils using metal-hyperaccumulating plants, which can uptake and store unusually high amounts of heavy metals in their shoots (Brooks et al. 1977). Because of the rarity of the hyperaccumulation trait, the use of native plants in this form of remediation is often not possible.

In this study, we examine the potential invasiveness of an introduced metal-hyperaccumulating plant by examining its competitive ability against a native species. We chose three sites in southwestern Colorado that contain silver mine tailings, or mining waste heaps, which are contaminated with Pb (lead), Cd (cadmium), and Zn (zinc). We use *Thlaspi caerulescens* J. & C. Presl (Brassicaceae), which is a Cd- and Zn-hyperaccumulator (>100ppm Cd or >10,000ppm Zn in plant dry weight; Reeves 1988). This species has been shown in field trials to successfully extract Cd and Zn from moderately contaminated soils (Brown et al. 1995, Hammer and Keller 2003, McGrath 2006). The congener *Thlaspi montanum* var. *montanum* (Brassicaceae) is native to our study region. Because they are growing adjacent to and surrounding the mine tailings at our sites, they would interact with *T. caerulescens* if it were introduced for phytoremediation. Examining competition between these two congeners also controls for both phylogenetic history and growth form, which are factors that can affect the outcome of competition and likelihood of invasive success (Vilà and Weiner 2004, Garcia-Serrano et al. 2007, van Kleunen 2010; but see

Lambdon and Hulme 2006). Specifically, the presence of a native congener is expected to decrease the likelihood of non-native naturalization through more intense competition than less related native species.

We expect the two congeners to compete exploitatively for nutrients, with *T. caerulescens* potentially outcompeting *T. montanum* for Zn, which is a plant micronutrient (Broadley 2007). A superior ability to compete for Zn has been demonstrated previously in *T. caerulescens* when planted with other non-accumulating plants (Whiting et al. 2001, Meerts et al. 2003). To test for competitive interactions between these species, we performed a greenhouse experiment with a response surface design in order to examine independently the effects of intra- and interspecific competition on plant performance (Damgaard 1998, Inouye 2001). Data from a smaller-scale field experiment are also evaluated. Because competitive interactions can be influenced by resource availability, we used substrate collected at two distances from the tailings to represent different levels of contamination and substrate quality.

Methods

Study site and species

Three small abandoned mine sites (Woods, Copper, and Cinnamon) are chosen for this study near the Rocky Mountain Biological Laboratory (38° 57' 19.60" N, 106° 59' 28.32" W) in the central Rocky Mountains, Colorado. The mine tailings at these sites contain moderately high Cd (18.7 ± 5.24 ppm) and Zn (2860 ± 636 ppm), and are similar to sites where phytoremediation by *T. caerulescens* might be implemented.

Thlaspi caerulescens J. & C. Presl (formerly *T. alpestre* L.) is native to central and western Europe. It is not only a constitutive hyperaccumulator of Zn (Escarré et al. 2000, Assunção et al. 2003), but also requires Zn for optimal growth (Mathys 1977, Li et al. 1995, Shen et al. 1997). Populations differ in their ability to hyperaccumulate Cd (Chaney et al. 2005), which is not an essential nutrient, but has been shown to stimulate growth in one ecotype of *T. caerulescens* from southern France (Pongrac 2009). *Thlaspi montanum* var *montanum* (synonym: *Noccaea montana*) is a long-lived perennial native to the mountains of the western U.S.A. (Holmgren 1971). It is a known Ni-hyperaccumulator on serpentine soils and has also been shown to accumulate up to 3000ppm Zn in leaf dry mass (Reeves et al. 1983).

Greenhouse experiment

The competition experiment was conducted from Sept. 2007 to Dec. 2008 in a research greenhouse at the University of Maryland, College Park. The greenhouse allowed for greater control, higher replication, and a longer continuous growing period than in the field (field experiment described below). To address whether soil quality and soil metal content influence the competitiveness of the study species, substrate samples were collected at three field sites from two locations: the edges of the tailings (“adjacent”) and 25m away from the tailings (“far”). Substrate was taken at three spots per location per site and mixed and sieved prior to use. *Thlaspi caerulescens* seeds were obtained at Palmerton, PA, a site where *T. caerulescens* was tested for Cd and Zn accumulation in field soil (Li et al. 1997). These seeds likely contain a mix of ecotypes commonly used in hyperaccumulation studies, including

the Southern France and Prayon populations (Chaney et al. 2005). *T. montanum* seeds were collected haphazardly from at least 15 fruiting plants per location at our study sites in 2007. *T. montanum* seeds were stratified at 3°C for 3 months, and sown along with *T. caerulescens* seeds on potting soil for germination. Germination dates for each individual were recorded, and seedlings of the same age were matched in each pot to avoid size and age bias.

Seedlings were transplanted into 4" pots containing field substrate. A response surface design was implemented, in which the total density and proportion of the species were varied independently, so that the effects of inter- and intra-specific competition can be examined separately (Damgaard 1998). Each pot contained 0, 1, 2, or 3 plants of each species, for a total of nine competition treatments (given in the ratio of *T. montanum* to *T. caerulescens*): 1:0, 0:1; 2:0, 1:1, 0:2; 3:0, 2:1, 1:2, 0:3. These nine treatments were repeated at every level of the three sites and two locations for 54 treatment combinations. Pots with 1, 2, or 3 total plants had densities of 0.012, 0.025, and 0.037 plants/cm², respectively. Each treatment combination was replicated 4-6 times, depending on the availability of germinated seedlings and field soil, for a total of 267 pots and 597 plants. Plants were checked daily and watered as needed using deionized water. After 20 weeks, all pots were vernalized at 4°C for 12 weeks to induce flowering. The plants' initial size, growth (length of longest leaf and number of leaves), and reproductive effort (number of buds, flowers, stalks, and mature fruits produced per stalk) were measured weekly. Seeds were counted after all matured fruits were collected. Remaining shoot biomass was collected, dried, and analyzed for nutrient and metal contents (Ca, Cd, Cu, Fe, K,

Mg, Mn, Ni, P, Zn) using hot nitric digestion and inductively coupled plasma atomic emission spectroscopy. Total substrate metal concentrations (Cd, Cu, Ni, Zn) were analyzed before and after the experiment using Aqua regia extraction method (McGrath and Cunliffe 1985) and flame atomic absorption spectrometry (AAS). Plant-available metal concentrations (Cd, Cu, Ni, Zn) were also analyzed before the experiment using strontium nitrate and AAS.

The competition coefficient was estimated for each species using Damgaard's (1998) hyperbolic competition model:

$$y_i = D_i(a_i + b_i(D_i + c_{ij}D_j + e_iD_iD_j)^{d_i})^{-1/f_i}$$

where y_i is the yield of plant species i , D_i is the density (in plants/cm²) of species i , D_j is the density of the competing species j , and a_i , b_i , d_i , and f_i are shape parameters. The parameter e_i represents the interaction effect between the densities of the two species. Maximum leaf number was used as the measure of yield for both species. The competition coefficient of species j on species i (c_{ij}) indicates the relative effects of intra- and inter-specific competition on the yield of species i . If $c_{ij} > 1$, then interspecific competition from species j is greater than intraspecific competition in species i , and species j may outcompete species i . If $0 < c_{ij} < 1$, then interspecific competition is present, but is less than intraspecific competition. If $c_{ij} < 0$, then interspecific competition is not present, and the presence of the other species actually increases the fitness of species i . A simplified competition model, which assumes that the shape parameters d_i and f_i equal 1, was also fitted (Campbell and Snow 2007). Maximum likelihood parameter estimates were calculated using the normal, negative binomial, and Poisson residual distribution using the mle2 function

in the *bbmle* package in R (version 2.9.2, R Development Core Team 2009). The model with the best fit is chosen based on AIC values. One-sample t-tests are applied to each competition coefficient estimate to test explicitly whether it is significantly different from zero or one.

To test for differences in growth and fitness between species across sites, locations, and density treatments, a factor analysis was first performed on the growth response variables (longest leaf length, number of leaves, number of rosettes) to create a composite response variable. The resulting significant principal component was used as the growth response variable in an ANOVA to test the effects of species, sites, locations, density treatments, and their interactions. In a separate ANOVA, reproduction was examined using flower number as the response, because no *T. montanum* plants set seed in the greenhouse, likely because of self-incompatibility (Peer et al. 2006) and a lack of pollinators. Flower number was square root transformed to conform to normality. Because interspecific effects from the competition analysis were small (see below), overall density rather than density of each species was included as an explanatory variable in the analysis. To account for any effects of initial plant size, the first factor from a factor analysis of the initial leaf length, leaf number, and seedling age was used as a covariate in the models.

To examine relationships between plant performance and soil metal contents, canonical correlations analyses were used to quantify the correlations between the set of soil metal concentrations to the set of plant growth response variables (longest leaf length, number of leaves, number of rosettes) and to a set of reproductive variables

(number of buds, number of flowers). Because these relationships are expected to differ for each plant species, separate analyses were performed for each species.

Field experiment

In the summer of 2007, *T. caerulescens* seeds were germinated on flats of potting soil in field conditions. This component was carried out at one of the three study sites because of low germination rates that year. The germinated seedlings were then planted into 0.5m x 0.5m half-plots at one of the three sites (Woods), with similarly sized *T. montanum* seedlings transplanted from the field. Paired half-plots were planted with *T. montanum* only, in the same density as the mixed-species plots ($120 \text{ plants/m}^2 = 0.012 \text{ plants/cm}^2$; except 1 replicate at the far location unintentionally received fewer plants, $96/\text{m}^2 = .0096 \text{ plants/cm}^2$). Three replicates of paired plots were planted adjacent to the tailings and 25m from the tailings at one site, where substrate was collected for the greenhouse experiment. The number of leaves, length of the longest leaf, and any reproductive effort (number of buds, flowers, and fruits) were measured over the following two growing seasons. The first two factors from a factor analysis of these response measurements were used as the response variables in the analyses, and the initial leaf number and leaf length as the covariates. One ANOVA used only the *T. montanum* data, and tested whether location and presence of *T. caerulescens* affected its performance. The second ANOVA used only data from the mixed plots and tested for the effects of location and species. To compare plant performance between field and greenhouse conditions, the same analyses were performed on greenhouse data from the same site (Woods) and when plants were the same size as those in the field (~10 leaves, or after seven weeks in the

greenhouse). Density in the field plots was the same as the lowest density applied in the greenhouse (one plant per pot). However, because those pots only contained one species at a time, the measurements from the next lowest density were used. Specifically, pots with two *T. montanum* plants and pots with one of each species (treatments 0:2 and 1:1, respectively) were used. All analyses, except for the hyperbolic competition model, were performed using SAS software (version 9.2, SAS Institute Inc. 2008).

Results

Soil metal concentrations

Total soil Zn, Cd, and Cu (copper) were higher at adjacent than far locations in general, although the difference varied by site (Zn: site by location, $F_{2,67} = 21.61$, $p < 0.0001$; Cd: site by location, $F_{2,67} = 55.05$, $p < 0.0001$; Cu: site by location by time interaction, $F_{2,67} = 6.31$, $p = 0.003$; Table 1). Cu levels at the adjacent location of Copper and Woods were an order of magnitude higher than at other sites and locations. Soil metal concentrations changed during the experiment: Zn decreased at Cinnamon and Copper sites (site by time interaction, $F_{2,67} = 3.88$, $p = 0.025$), Cd decreased at Cinnamon and Woods (site by time interaction, $F_{2,67} = 8.10$, $p = 0.001$), Cu increased at the adjacent location at Copper and Woods sites (site by location by time interaction stated above), and Ni increased across sites and locations (time effect, $F_{1,67} = 4.35$, $p = 0.041$; Table 1). The increases in Ni and Cu concentrations may have been because these metals remain unused, while other soil nutrients were taken up by plants.

Competition coefficient estimates

For both species, the simplified model with a negative binomial error distribution, and including site and location effects, had the best fit to the data based on AIC values (Table 2). The maximum likelihood estimates of all but two of the competition coefficients were not significantly different from zero or one (Table 3), indicating that the effects of interspecific competition were absent or equal to intraspecific competition, but they were not greater than intraspecific competition. At Woods far, effects of interspecific competition from *T. montanum* on *T. caerulescens* were greater than zero, but not different from effects of intraspecific competition among *T. caerulescens*. At Copper far, effects of interspecific competition from *T. caerulescens* on *T. montanum* were significantly greater than effects of intraspecific competition among *T. montanum* (Table 3).

Plant performance

There was a significant site effect in both the analyses of the growth response factor and flower number, and both were then analyzed by site. The first factor from the factor analysis of plant growth variables was positively related to all three original variables (Table 4). Results using the growth response factor showed a significant species effect at all sites, with *T. montanum* having greater vegetative growth than *T. caerulescens* overall (Fig. 1, see Table 5 for ANOVA values). Density was a significant effect at all sites also, but its effects depended on other factors. At Cinnamon, there was a marginally significant interaction between density and location, in which the differences between densities were greater at adjacent. The significant interaction between density, location, and species at Copper was due to greater differences between densities at the far location, especially for *T. montanum*.

At Woods, greater differences between densities were seen for *T. montanum* than for *T. caerulescens* (Fig. 1). Examining the reproductive response, there was a significant interaction between location and species on flower number at all sites (Fig. 2, see Table 5 for ANOVA values). While *T. caerulescens* had more flowers than *T. montanum* in general, this difference was greater at the adjacent location at one site (Cinnamon), but at the far location at the other sites (Copper and Woods). Density effects were marginally significant at Copper, and differences between densities were greater at the far location than adjacent at Woods, and more so for *T. caerulescens* than for *T. montanum* (Fig. 2).

To examine the relationships between plant growth or plant reproduction variables and soil metal concentrations, canonical correlations analyses were used. The resulting factor loadings and standardized canonical correlation coefficients can be used to interpret how each original variable contributes to the correlation between the sets of variables (Table 6). The factor loadings, or correlations between each original variable and the canonical variate, showed that all soil metals were positively correlated with the growth of both species, except soil Ni was negatively correlated with *T. montanum* growth. *T. caerulescens* reproduction was positively correlated to soil Zn and Ni but negatively correlated to Cd and Cu, while *T. montanum* reproduction was negatively correlated to all soil metals. Canonical coefficients indicated how each original variable contributes to the correlation between the two sets of variates when all other variables are held constant. The coefficients indicate that higher soil Zn and lower soil Cd were positively correlated with better growth and reproduction of both species (Table 6).

Plant metal uptake

The highest levels of plant Zn were at Cinnamon adjacent and at the far location at Woods and Copper (site by location interaction, $F_{2,93} = 33.55$, $p < 0.0001$, Fig. 3a). Plant Cd was higher in *T. caerulescens* than *T. montanum* (site by species interaction, $F_{2,93} = 4.38$, $p = 0.015$; Fig. 3b), and plant Cu was high at Copper and at Woods adjacent (site by location interaction, $F_{2,93} = 9.34$, $p = 0.0002$; Fig. 3c). The very low survival of *T. caerulescens* at Copper adjacent, which is likely due to the high soil Cu, meant that no analyzable *T. caerulescens* biomass was collected there. There was no significant effect of plant density on plant metal accumulation. There were very few instances of Cd hyperaccumulation ($>100\text{ppm}$) by *T. caerulescens*: plants at Copper far in the 1:2 treatments contained 123.0 ± 19.2 SE ppm of Cd. No plants exhibited Zn hyperaccumulation ($>10,000\text{ppm}$), but the *T. caerulescens* in the Copper far 1:1 treatment (reps 2 and 4) had 8,800ppm of Zn and the *T. montanum* at Cinnamon adjacent 0:1 treatment (reps 1 and 3) had 8,900ppm of Zn in their shoots (plants were combined for metal analysis due to small biomass, thus only one measurement was made for each). Considering the relatively low contamination levels of our soils ($<1000\text{ppm}$ Zn for all substrates), these values support preferential uptake of Zn by these plants.

Comparing greenhouse and field results

Comparing the field data to the greenhouse data, plants performed better in the greenhouse overall. When planted alone, *T. montanum* performed better at the far location than adjacent in the field (Factor 1: $F_{1,262} = 11.2$, $p = 0.001$), and this difference was marginally significant in the greenhouse ($F_{1,21} = 3.21$, $p = 0.088$; Fig.

4). Both datasets showed that *T. montanum* grew better than *T. caerulescens* when planted together (field data Factor 2: $F_{1,178} = 73.99$, $p < 0.0001$; greenhouse: $F_{1,14} = 5.99$, $p = 0.028$). The field data also showed that while both species grew poorly at adjacent, *T. montanum* grew better than *T. caerulescens* at far (Factor 1: $F_{1,178} = 5.87$, $p = 0.016$; Fig. 4). Both datasets show no significant effect of the presence of *T. caerulescens* on *T. montanum* at this stage of growth.

Discussion

We found limited evidence for interspecific competition between a potentially introduced non-native plant and a native congener. Previous studies testing the competitive interactions between native – non-native pairs have clearly found a higher competitive ability in the non-native plant (Parker and Reichard 1998, Vilà and Weiner 2004). However, their results may be biased because only non-natives that are known to be invasive were examined. In contrast, our study tests whether *Thlaspi caerulescens* exhibits strong competitive ability associated with invasiveness, and we find that in most cases it does not. The only case in which the competition coefficient was greater than one occurred in soil from a location where *T. caerulescens* grew well. This indicates that conditions of the Copper far soils are highly favorable to the growth of *T. caerulescens* (and potentially also unfavorable to *T. montanum*), perhaps because of a combination of the relatively high Zn and low Cu in the soil, as well as mild greenhouse conditions. The low competition coefficient estimates in soil from the other tested locations indicate that the presence of the non-native has no greater negative impact on the native than intraspecific competition. The lack of competitiveness may be because both species are adapted to tolerate

abiotic stress (high metal, low nutrient soils for *T. caerulescens*; subalpine climate for *T. montanum*), and these stress-tolerant species are generally less competitive (Hickey and McNeilly 1975, Grime 1977, Linhart and Grant, 1996). However, we cannot rule out the possibility that we would find stronger competitive interactions with greater replication. The high uncertainty (indicated by large standard errors) in the competition coefficient estimates at Copper adjacent is due to the low survivorship on that soil, which led to fewer data for that location.

The use of a response surface design, which varies the ratio between species as well as total density, allowed us to examine independently the effects of intra- and inter-specific competition. Plant density (regardless of species) significantly affected the vegetative growth of *T. montanum* and the reproduction of *T. caerulescens*, but only at the sites and locations where the plants performed well (Figs. 1, 2). This indicates that substrate factors posed a greater limitation on the performance of these species than competition overall. Both species performed better on soils with higher Zn concentrations, but there was no evidence that *T. caerulescens* outcompetes *T. montanum* for Zn. Both species accumulated more Zn where Zn concentrations are higher (adjacent locations). Contrary to expectations, the native species did not perform better than the non-native under higher abiotic stress, which in this study occurred where the substrate contained higher Cu and lower Zn (at the Copper and Woods adjacent locations). However, we did find evidence that the native *T. montanum* is more tolerant to these abiotic stresses: soil Cu had a positive effect on the number of leaves in *T. montanum* (positive canonical correlation coefficient in

Table 6), and *T. montanum* at the high-metal Woods adjacent location grew as well as those at the far location (Fig. 1).

Based on our results, we predict that *T. caerulescens* is unlikely to outcompete *T. montanum* in the field. Natural conditions are usually more stressful than greenhouse conditions (for example in terms of water limitation and temperature fluctuations), and this was supported by the data from our field experiment showing that both species perform worse in the field (Fig. 4). Again, higher stress is expected to favor the natives in competition (Alpert et al. 2000, Moen and Meurk 2001, Daehler 2003, Garcia-Serrano et al. 2007, Burns 2008), and in fact a previous study found higher competitive ability of an invasive in the greenhouse but not in the field (Huenneke and Thomson 1995). On the other hand, a review study concluded that competition indices estimated from greenhouse experiments were similar to those from field experiments (Vilà and Weiner 2004), but this also suggests that competition is not stronger when tested in the field. The poor performance of the field-grown plants further suggests that natural environmental conditions would be highly limiting to the growth of both species, suggesting an even smaller role of biotic competition as a limiting factor. Thus, it is unlikely that *T. caerulescens* would exhibit higher competitive ability in the field and outcompete the native *T. montanum* at our sites.

However, factors other than differences between controlled and field conditions may also affect the competitive interaction between the two species. While the densities used in this study are similar to natural plant density adjacent to the tailings, they are lower than the natural density 25m away from the tailings at the

three sites (*pers. obs*). A higher density of native plants would present greater competition (Schoener 1983), perhaps leading to poorer performance of *T. caerulescens* at those locations, which would help to further prevent the spread of this introduced plant. Further, the effects of interspecific competition on *T. montanum* may be expressed in fitness measures that we did not record, specifically seed production and performance in future growing seasons. The lack of seed set in *T. montanum* in the greenhouse may have caused *T. montanum* to shift more resources into vegetative growth, though this increase was likely small because the plants still produced stalks, flowers, and fruits. Nevertheless, interspecific competition could have a larger effect on the long-term growth and reproduction of the long-lived *T. montanum*. Our study only spanned the equivalent of two growing seasons because *T. caerulescens* is likely biennial or a short-lived perennial (Dubois 2002). This life history difference between the species may also have implications for the long-term competitive interaction between the species, because *T. caerulescens* seeds and seedlings would have to compete for space and other resources with older, established *T. montanum* plants.

While *T. montanum* invested more in vegetative growth, *T. caerulescens* invested in reproduction, even under high-stress conditions. The production of many small seeds, and persistent reproduction, are some of the key traits of invasive plants (Rejmanek and Richardson 1996, Alpert et al. 2000, Daehler 2003, Gerlach and Rice 2003, Lockwood et al. 2005). The germination and establishment rates of these seeds would greatly affect the rate of establishment and density of the plants, which would again alter competitive interactions. Furthermore, the stage of invasion can also

affect the degree of competition between native and non-native species (Alpert et al. 2000, Vilà and Weiner 2004, Theoharides and Dukes 2007). In this study, we examined a period that might be considered the introduction stage, before the plant has established or become invasive, and indeed we found a low level of competition.

Our goal in this study was to examine whether the introduction of a non-native plant for phytoremediation would negatively impact native *T. montanum* performance. While our results indicated that population-level effects on *T. montanum* are unlikely, our results also varied among sites, indicating site specificity. It is possible that introduction to sites with higher levels of contamination would favor the highly Zn- and Cd-tolerant *T. caerulescens*, resulting in higher competitive ability. Additionally, our data show that even locations away from the tailings, where native plants naturally occur and the soil does not appear contaminated, could contain levels of Zn that favor the growth of *T. caerulescens*. Therefore we recommend that the viability and establishment of *T. caerulescens* be tested at each new site in advance, and carefully monitored throughout the process, if it is to be used for phytoremediation.

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Tables

Table 1. Mean values (\pm SD) for soil pH and the total concentrations of zinc, cadmium, copper, and nickel before (pre) and after (post) the experiment, by site and location. Substrate taken from the field experiment plots (“Woods - F”) was analyzed before the experiment only. “Av” indicates plant available metal concentrations measured using strontium nitrate, and these values for the greenhouse soils include data from other experiments that analyzed soil from the same locations, to increase sample size. Values without SDs indicate measurements that were only taken from one sample.

Site	Loc	pH	Av Zn	Zn-pre	Zn-post	Av Cd	Cd-pre	Cd-post	Av Cu	Cu-pre	Cu-post	Av Ni	Ni-pre	Ni-post
Cinnamon	adj	4.67 ± 0.04	6.19 ± 4.65	451.0 ± 7.22	383.0 ± 17.10	6.19 ± 4.650	1.45 ± 0.104	1.27 ± 0.100	0.070 ± 0.090	92.3 ± 1.12	82.4 ± 2.63	0.055 ± 0.031	6.41 ± 0.229	6.76 ± 0.56
Cinnamon	far	4.39 ± 0.94	1.24 ± 0.94	167.0 ± 5.66	139.0 ± 3.21	1.24 ± 0.940	0.403 ± 0.026	0.347 ± 0.042	0.010 ± 0.009	37.3 ± 1.62	33.5 ± 1.52	0.080 ± 0.023	6.17 ± 0.202	5.62 ± 1.83
Copper	adj	4.74 ± 0.02	7.58 ± 8.53	667.0 ± 36.20	571.1 ± 182.00	7.58 ± 8.530	2.96 ± 0.033	3.14 ± 0.201	1.190 ± 0.874	1320.0 ± 43.5	1630.0 ± 178.00	0.107 ± 0.061	7.40 ± 0.065	10.00 ± 3.57
Copper	far	4.81 ± 0.00	2.08 ± 1.02	604.0 ± 63.60	478.0 ± 17.50	2.08 ± 1.020	1.13 ± 0.133	1.26 ± 0.201	0.038 ± 0.024	63.5 ± 3.02	59.5 ± 3.65	0.025 ± 0.000	8.01 ± 0.371	10.45 ± 3.82
Woods	adj	5.35 ± 0.06	2.38 ± 0.95	745.0 ± 13.50	941.0 ± 155.00	2.38 ± 0.949	2.83 ± 0.051	2.51 ± 0.165	0.441 ± 0.151	1950.0 ± 26.20	2270.0 ± 64.30	0.025 ± 0.000	6.25 ± 0.089	7.75 ± 1.90
Woods	far	4.66 ± 0.03	2.45 ± 1.27	348.0 ± 7.79	298.0 ± 16.40	2.45 ± 1.270	0.915 ± 0.027	0.805 ± 0.075	0.018 ± 0.010	33.8 ± 1.26	34.9 ± 3.00	0.033 ± 0.015	6.02 ± 0.189	7.49 ± 1.34
Woods - F	adj	5.88 ± 0.49	2.44 ± 0.71	764.0 ± 38.90		0.037 ± 0.019	2.67 ± 0.153		0.487 ± 0.127	2000.0 ± 62.6		0.025 ± 0.000	6.72 ± 0.413	
Woods - F	far	5.06 ± 0.32	2.82 ± 0.35	325.0 ± 20.20		0.032 ± 0.012	0.881 ± 0.226		0.020 ± 0.006	30.6 ± 2.60		0.025 ± 0.000	6.57 ± 0.337	

Table 2. Differences in AIC values for the different hyperbolic competition models fitted to the dataset. Model name endings indicate which type of error distribution was used: “.nb” indicates negative binomial, “.p” indicates poisson, and no ending indicates normal. For both species (*Thlaspi caerulescens*, TC, or *T. montanum*, TM), the simplified model with negative binomially distributed residuals, and including both site and location effects, had the best fit to the data.

TM on TC				TC on TM			
Model	Effects	dAIC	df	Model	Effects	dAIC	df
simplified.nb	loc,site	0	30	simplified.nb	loc,site	0	30
full.nb	loc,site	32.3	42	full.nb	loc,site	4	42
simplified.nb	loc	214.2	10	simplified.nb	loc	89.1	10
full.nb	loc	217.5	14	full.nb	loc	96	14
full.nb		252.8	7	simplified.nb		129.5	5
simplified.nb		256.6	5	full.nb		130.7	7
full.p	loc,site	3603.9	36	simplified.p	loc,site	6879	24
simplified.p	loc,site	3741.4	24	full.p	loc,site	7048.9	36
simplified	loc,site	193922.6	24	simplified	loc,site	589603.4	24
full	loc,site	198242.2	36	full	loc,site	645496.9	36

Table 3. Competition coefficient estimates for the effects of *Thlaspi montanum* on *T. caerulescens* (left) and the effects of *T. caerulescens* on *T. montanum* (right) by site and location. Values presented are the maximum likelihood estimate and its standard error, and the calculated one-sample t-values testing whether the estimates are different from zero or one. For all tests, the critical t-value ($n = 30$, $p = 0.05$) is 2.045. With two exceptions (bolded), the majority of the estimated competition coefficients are not significantly different from zero or one, indicating that the effects of interspecific competition may be absent or equal to intraspecific competition, but they are not greater than intraspecific competition. At Woods far, the interspecific effects of *T. montanum* on *T. caerulescens* are present (coefficient is greater than zero), but they are equal to the intraspecific effects of *T. caerulescens* on itself. At Copper far, the interspecific effects of *T. caerulescens* on *T. montanum* are significantly greater than the intraspecific effects of *T. montanum* on itself.

		TM on TC				TC on TM			
Site	Loc	Estimate	SE	t (from 0)	t (from 1)	Estimate	SE	t (from 0)	t (from 1)
Cinn	adj	0.326	0.437	0.747	1.542	2.253	1.303	1.729	0.962
Copp	adj	230.39	365.350	0.631	0.628	-11.585	54.454	0.213	0.231
Woods	adj	2.572	2.970	0.866	0.529	0.277	1.798	0.154	0.402
Cinn	far	4.073	25.481	0.160	0.121	0.837	1.419	0.590	0.115
Copp	far	0.421	0.440	0.958	1.315	5.906	2.336	2.528	2.100
Woods	far	1.896	0.802	2.363	1.117	1.174	0.626	1.875	0.278

Table 4. The eigenvalue of the first factor from the factor analysis using the plant growth response variables, and its standardized scoring coefficients indicating how the factor relates to the original variables.

	Factor 1
Eigenvalue	2.326
Longest leaf length	0.340
Number of leaves	0.408
Number of rosettes	0.384

Table 5. Anova results for plant performance, using Factor 1 of the growth response variables (top) and flower number (bottom) as the response variable. Significance:

*** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, ` = $p < 0.1$

Effect	Cinnamon			Copper			Woods		
	NDF	DDF	F	NDF	DDF	F	NDF	DDF	F
covariate	1	197	1.56	1	146	3.35`	1	160	5.35*
Location	1	197	10.40**	1	146	81.12***	1	160	0.24
Species	1	197	31.88***	1	146	40.62***	1	160	77.00***
Loc*species	1	197	0.80	1	146	0.42	1	160	5.40*
Density	2	197	8.33**	2	146	4.72*	2	160	8.95**
Loc*dens	2	197	2.98`	2	146	5.72**	2	160	1.28
Species*dens	2	197	1.22	2	146	0.78	2	160	6.62**
Loc*spp*dens	2	197	0.84	2	146	3.65*	2	160	0.51
covariate	1	197	0.00	1	146	0.00	1	160	0.09
Location	1	197	16.98***	1	146	112.1***	1	160	22.37***
Species	1	197	149.2***	1	146	56.92***	1	160	66.06***
Loc*species	1	197	10.51**	1	146	63.65***	1	160	4.35*
Density	2	197	1.16	2	146	2.71`	2	160	3.60*
Loc*dens	2	197	2.08	2	146	2.59`	2	160	5.70**
Species*dens	2	197	0.86	2	146	0.27	2	160	2.11
Loc*spp*dens	2	197	0.78	2	146	0.12	2	160	3.05*

Table 6. Results from the canonical correlations analysis to examine the relationships between plant growth or reproduction and soil metal concentrations, for the *Thlaspi caerulescens* and *T. montanum* plants in the greenhouse experiment. Only the significant canonical variates from each analysis are shown, with their eigenvalue, correlation coefficient, and significance values. Under each variate, the factor loadings are listed on the left (“Loadings”), and the standardized canonical correlation coefficients are listed on the right (“Coeff.”). To facilitate comparison, the largest coefficients in each set of variables (those with the greatest unique influences on the correlations) are highlighted in bold. See text for interpretation of these values.

Species	TC		TC		TM		TM	
	Growth / Soil metals		Reprod / Soil metals		Growth / Soil metals		Reprod / Soil metals	
Eigenvalue	0.406		0.319		0.442		0.289	
Canonical correlation	0.537		0.492		0.554		0.473	
F (ndf,ddf)	2.03 (12,138)		2.36 (8,106)		2.94 (12,146)		2.25 (8,112)	
p	0.0260		0.0222		0.0011		0.0268	
	Loadings	Coeff.	Loadings	Coeff.	Loadings	Coeff.	Loadings	Coeff.
Leaf #	0.881	0.545			0.919	1.964		
Leaf length	0.840	0.426			0.305	-0.265		
Rosette #	0.599	0.272			0.757	-0.956		
Bud #			-0.966	-1.304			-0.967	-0.666
Flower #			-0.610	0.426			-0.904	-0.394
Total Zn	0.544	3.747	-0.077	-5.170	0.832	1.499	0.002	-3.569
Total Cd	0.339	-1.404	0.174	3.740	0.831	-1.183	0.229	2.501
Total Cu	0.022	-1.736	0.310	1.110	0.906	0.798	0.223	0.857
Total Ni	0.311	-1.690	-0.166	2.358	-0.027	-0.503	0.139	1.739

Figures

Fig. 1. Values for the first principal component from the factor analysis of all growth response variables, by site (Cinnamon, Copper, and Woods), location (adjacent or far), plant species (*Thlaspi caerulescens* or *T. montanum*), and plant density regardless of species (1, 2, or 3 plants per pot). Overall, *T. montanum* had greater vegetative growth than *T. caerulescens*, and the density effects were more prominent in Cinnamon adjacent, and Copper and Woods far locations, and only in *T. montanum*.

Fig. 2. Reproduction (measured as flower number) by site (Cinnamon, Copper, and Woods), location (adjacent or far), and plant species (*Thlaspi caerulescens* or *T. montanum*) in the left column, and by site, location, and plant density regardless of species (1, 2, or 3 plants per pot) in the right column. *T. caerulescens* had higher reproductive effort than *T. montanum* at most sites and locations. The difference between species was greater at the adjacent location at Cinnamon, but at the far location at Copper and Woods. The effects of densities were significant at Woods site, with greater differences between densities at the far location than at adjacent.

Fig. 3. Shoot metal concentrations for zinc (A), cadmium (B), and copper (C), by site (Cinnamon, Copper, Woods), location (adjacent, far), and plant species (*Thlaspi caerulescens* and *T. montanum*). *T. caerulescens* biomass at Copper adjacent was too low to analyze.

Fig. 4. Comparing the performance of *T. caerulescens* (TC) and *T. montanum* (TM) by experiment (field or greenhouse), location (adjacent or far), and competition treatment (TM alone, or TC and TM in 1:1 ratio). Data shown are the values for the first principal component of all reproductive and growth responses for the field experiment, and values for the first principal component of leaf length and leaf number after two months for the greenhouse experiment. Overall, plants performed better in greenhouse conditions. When grown alone, *T. montanum* plants grew better at the far than at adjacent location based on both datasets (black bars). When planted together, *T. montanum* grew better than *T. caerulescens* only at the far location in the field, while in the greenhouse *T. montanum* grew better overall (plant species was a significant main effect).

Figure 1

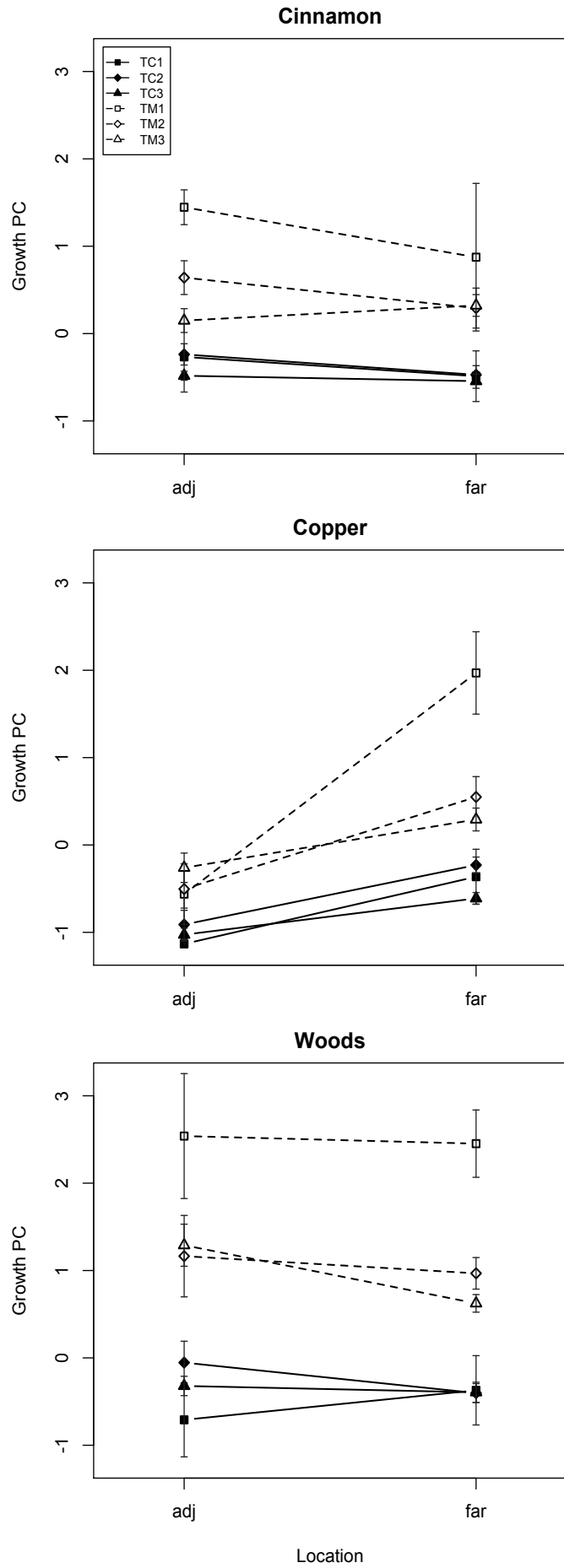


Figure 2

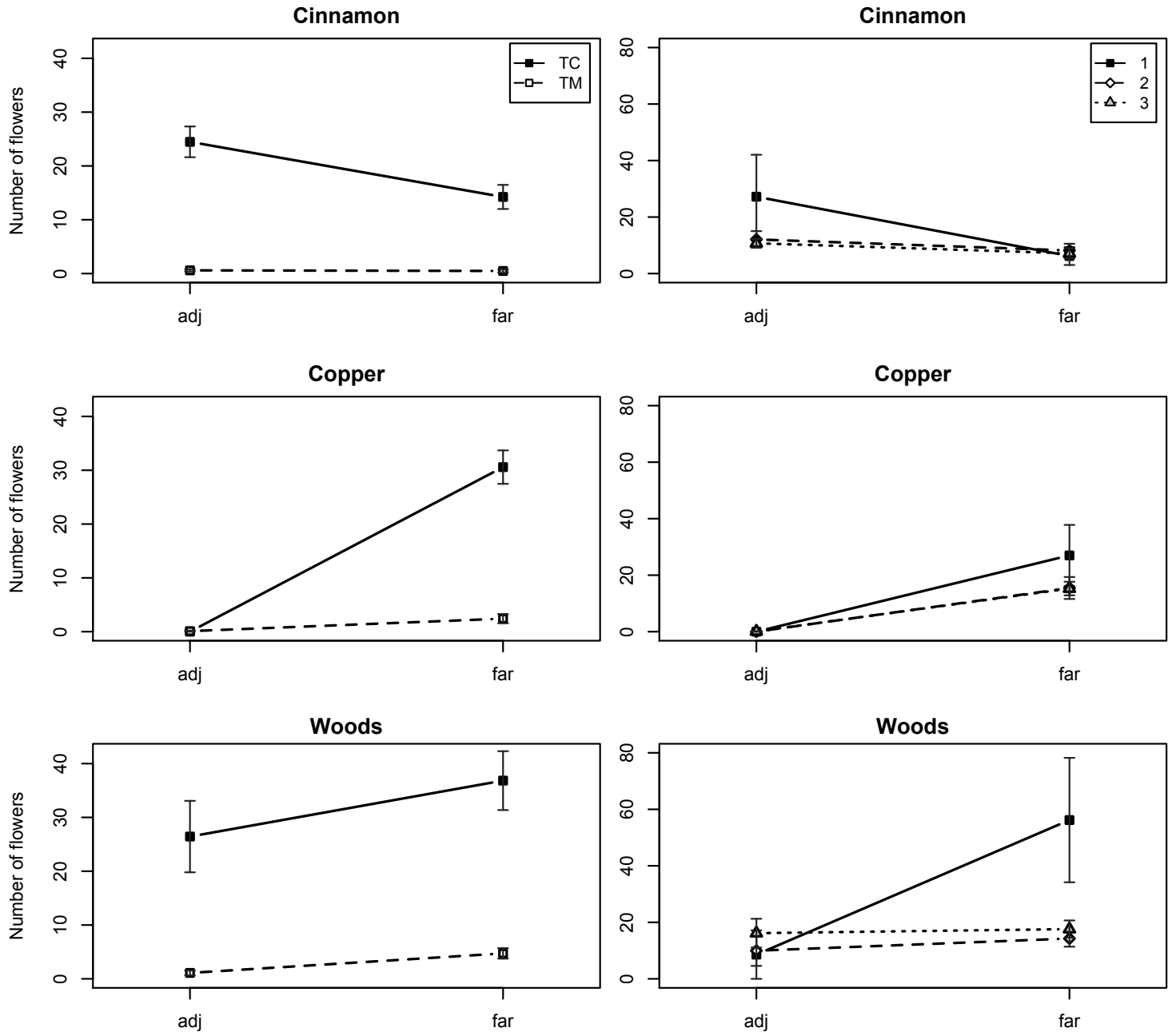


Figure 3

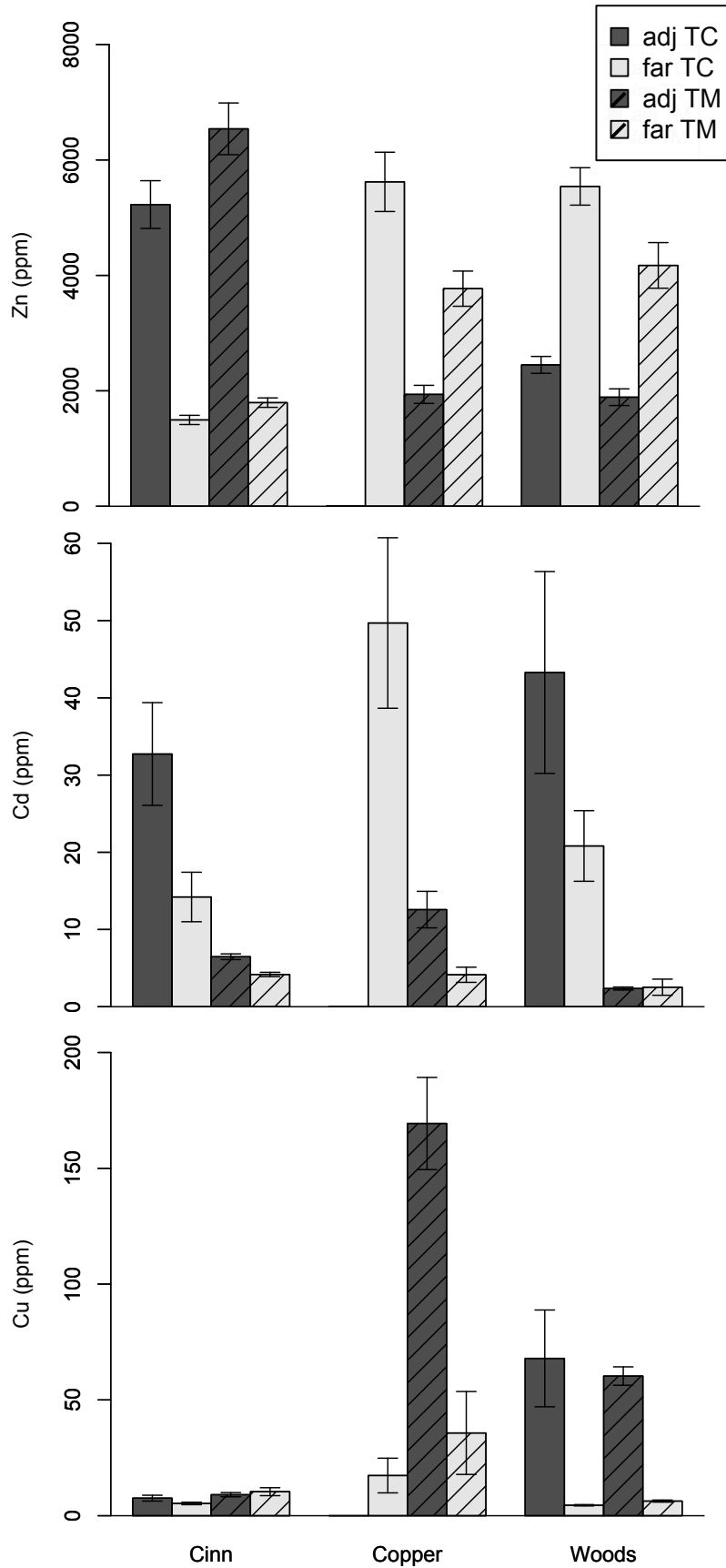
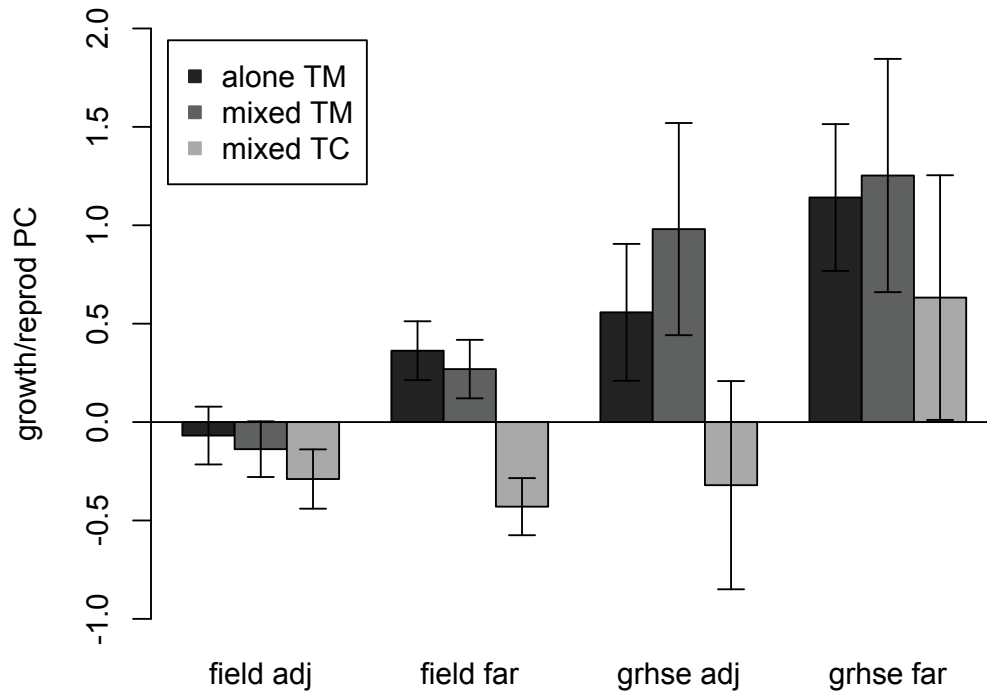


Figure 4



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Chapter 3: Foliar metal concentrations affect native herbivore preference between an introduced metal hyperaccumulating plant and a native congener

Abstract

Metal hyperaccumulation, or the uptake and storage of very high concentrations of heavy metals in above-ground plant tissues, is hypothesized to be an adaptation for herbivory defense, and the deterrence of herbivores by various heavy metals has been demonstrated in several species of metal hyperaccumulating plants. This defensive role of metals may confer a fitness advantage to a hyperaccumulator if it is introduced to a new site for phytoremediation (the extraction of heavy metals from contaminated soils). We performed an herbivore choice trial experiment to examine feeding preferences between the Cd- and Zn-hyperaccumulator *Thlaspi caerulescens* that had been introduced and grown at three mine tailings sites, and the native congener *T. montanum* from the same sites. We used the native herbivore *Pieris napi macdunnoughii* to test for 1) effects of plant species and 2) effects of plant metal concentrations on herbivore choice. Our results showed that *P. napi macdunnoughii* preferred to feed on the native over the non-native, though it did not completely avoid the non-native *T. caerulescens*, including individuals that contained high concentrations of Cd and Zn. We also showed that *P. napi macdunnoughii* is deterred by Cd and/or Zn, with reduced feeding on plants containing the highest Cd and Zn concentrations, which were grown at the tailings at

one of our three sites. These results provide evidence that a novel defense chemical can deter native herbivores from an introduced plant, and suggest an advantage to introduced hyperaccumulators that may translate to increased invasive success.

Introduction

Plants have evolved a variety of defenses to reduce their susceptibility to herbivores, including physical defenses such as waxy leaf coatings and trichomes, biotic defenses such as mutualistic ants, and chemical defenses such as the many secondary metabolites. Plant chemical defenses also include inorganic compounds, for example heavy metals that are extracted from soil and stored in leaf tissues. This has been termed elemental defense, and is unique to metal-hyperaccumulating plants (Boyd and Martens 1992, Boyd 2007). Metal hyperaccumulators, first described by Brooks (1977), can actively uptake approximately 100 times the average amounts of metals in their shoots. Metal defense is well demonstrated for Ni, which has been shown to reduce damage from leaf chewers, root chewers, and fungal pathogens on the hyperaccumulator *Streptanthus polygaloides* (Boyd 2007). Studies of the Zn- and Cd-hyperaccumulator *Thlaspi caerulescens* have shown a defensive role of Zn against leaf chewers (Pollard and Baker 1997, Jhee et al. 1999, Behmer 2005; but see Noret 2005) and Cd against sap feeders (Jiang et al. 2005). Selenium hyperaccumulation by *Stanleya pinnata* has been shown to deter arthropod and even mammalian herbivores in field settings (Galeas et al. 2008, Freeman et al. 2010). Overall, the elemental defense hypothesis has been supported across different metal types, plant species, and herbivore species (Boyd 2007), and the conclusion from a meta-analysis of feeding

trial studies is that herbivores, especially insects, tend to avoid high-metal plants (Vesk and Reichman 2009).

Plants that are well-defended and experience low herbivory pressure may be more likely to become invasive when introduced to a new site. While many studies have focused on the enemy release hypothesis, which proposes that invasiveness is enabled by the absence of coevolved natural enemies at the introduced site (Keane and Crawley 2002, Colautti et al. 2004), invasive success also depends upon whether the introduced plant can evade new enemies (Levine 2004, Mitchell et al. 2006).

Herbivores, pathogens, and competitors at the introduced site comprise the community-level biotic resistance against introduced species (Elton 1958), and meta-analyses have shown that native herbivores can negatively affect the establishment and performance of introduced plants (Levine et al. 2004, Parker et al. 2006). If introduced plants can evade these new enemies, their probability of invasion success would be higher. Indeed, plants categorized as highly invasive were found to have less herbivory damage than other non-natives, and the authors attributed this to chemical defenses in the invasives that are novel to herbivores at the introduced site (Carpenter and Cappuccino 2005). Because the hyperaccumulation trait is relatively rare, heavy metals would likely be a novel defense for introduced hyperaccumulating plants.

Phytoremediation is the intentional introduction of metal-hyperaccumulating plants to remove heavy metals from contaminated soils (Chaney 1983). Field trials of *T. caerulescens* have shown that this species would be feasible for remediating soils that are moderately contaminated with Cd and Zn (McGrath 1993, Brown et al. 1995,

Hammer and Keller 2003, McGrath 2006). Once introduced, however, these plants have the opportunity to establish populations in surrounding uncontaminated areas, as well as to alter local ecological interactions such as those between native plants and herbivores (Whiting et al. 2004, Angle and Linacre 2005, Pilon-Smits and Freeman 2006). Because herbivory has been shown to be important to the establishment of non-native species, testing the palatability of introduced hyperaccumulating plants to native herbivores would help assess the likelihood of these potential risks. Herbivory pressure is expected to be greater for plants growing outside of the intended remediation area, because plants that spread onto uncontaminated soils should be less well defended because of lower metal concentrations.

In this study, we examine the potential herbivory pressure on *T. caerulescens* that has been experimentally planted at three sites in southwestern Colorado. We conducted binary feeding choice trials using an herbivore that is native to the region, *Pieris napi macdunnoughii*. To assess the importance of plant metal concentrations on native herbivore preference, we used plants that were grown on soils with varying contamination levels (on mine tailings, adjacent to tailings, and 25m from tailings). In addition to the presence of defensive chemicals, another factor that influences whether an introduced plant would be consumed by native herbivores is its phylogenetic relationship to native species. Specifically, introduced species in the same family or genus as native species are more likely to be consumed than less related species, especially when examined at local scales (Agrawal and Kotanen 2003, Diez 2008, Hill and Kotanen 2009; but see Cappuccino and Carpenter 2005). At our study sites, the native *Thlaspi montanum* can be found naturally colonizing the

edges of mine tailings, and it is known to be a larval host plant for *P. napi macdunnoughii* (Chew 1977, Chew 1980). *T. montanum* is a known Ni-hyperaccumulator on serpentine soils (Boyd and Martens 1994), but they do not hyperaccumulate at our sites, and there are no other native hyperaccumulating plants at our sites. By varying the pair-wise combinations of plant species and plant source location (different distances from the mine tailings), we asked:

1. Does an herbivore native to these contaminated sites show a preference between the native plant and the introduced congener?
2. Does herbivore preference depend on the concentrations of Zn or Cd in the plant tissues?

Methods

The caterpillars of *Pieris napi macdunnoughii* Remington (Lepidoptera: Pieridae; green-veined white butterfly) were used as the native herbivore in our choice trials. Its larvae are known to feed on a variety of cruciferous plant species in the montane regions of the southern Rocky Mountains (Chew 1977). Adult females were collected and caged individually on their preferred host *Cardamine cordifolia* (Brassicaceae) in late June through early July of 2009 at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO (Gunnison County). Females were released once they had oviposited, or after three days if the female did not lay eggs. Sugar water was provided to the butterflies twice daily. The eggs from 12 females (approximately 250 eggs total) were collected and reared on *C. cordifolia* leaves in an incubator at $25 \pm 1^\circ\text{C}$ under incandescent lights. Third and fourth instar larvae were used for the choice trials.

Plants used in the choice trials were collected from three abandoned mine sites near the RMBL, where *Thlaspi caerulescens* J. & C. Presl (Brassicaceae) were experimentally planted for a related study. The soil metal concentrations at these sites and the field experimental design are described elsewhere (Che-Castaldo 2011, Chapter 1). *T. caerulescens* plants were collected early in their third growing season from experimental plots at three locations that vary in soil metal concentrations (tailings, adjacent to the tailings, and 25m from the tailings; called “tailings”, “adjacent”, and “far”, respectively) at each site, and *Thlaspi montanum var montanum* (Brassicaceae; synonym: *Noccaea montana*) plants were collected from native populations at the same locations and sites. Plants were maintained on field-collected substrate from their corresponding locations until used in the choice trials. The plant mineral concentrations (Ca, Cd, Cu, Fe, K, Mg, Mn, Ni, P, and Zn) in the experimentally grown *T. caerulescens* and field collected *T. montanum* were analyzed using hot nitric digestion and inductively coupled plasma atomic emission spectroscopy. The analyzed plant material included additional plants that were used in a different experiment; plants used for this experiment were randomly chosen from the field samples from each site and location described above. Plant Cd and Zn concentrations are shown in Table 1.

To determine whether herbivores showed a preference between the two plant species and between the plant source locations (tailings, adjacent, and far), three types of trials were set up consisting of six different pair-wise combinations of species and location (see Table 2 for trial types and replication levels). The “Species” type consisted of trials in which the herbivore had a choice between a *T. caerulescens* and

T. montanum from the same location (trials 1-3 in Table 2), to test for plant species preference. The “Location” type presented a choice between two *T. caerulescens* plants from different locations (trials 4-5 in Table 2), to test for a source location preference, which was meant to represent a metal concentration preference within species. The “Edge” type presented a choice between *T. caerulescens* grown on the tailings and *T. montanum* grown adjacent to the tailings (trial 6 in Table 2), which was meant to simulate the choice that would be presented at the tailings edges if *T. caerulescens* were planted at those sites for phytoremediation. In total, 60 pair-wise choice trials were run, and the replication level for each trial type was determined by plant availability (the number of *T. caerulescens* rosettes of a usable size differed between sites and locations). Trials were performed in a laboratory at the RMBL.

At the time of the trials, rosettes were transferred individually into vials containing a small cellulose sponge moistened with distilled water to prevent the plant from wilting. The vials were capped so that the sponge, roots, and any associated soil in the vial were not exposed to the herbivore. Paired rosettes in each trial were chosen for similarity of size, and if similar-sized plants were not available, the larger plant was trimmed (trimming was included as a factor in our models but had no significant effects). The choice trials were performed in 10cm petri dishes, each containing a moistened cellulose sponge. Caterpillars were placed individually into petri dishes with two rosettes from different sources. The treatment and placement of each plant (left or right side of the dish) were chosen randomly for each dish, and each caterpillar was only used in one trial. Between six and 10 trials were simultaneously run, each lasting 60 minutes. This period was sufficient for capturing

multiple bouts of sustained feeding, pauses between feedings, and switching between plants.

The mass of the plants (in their vials), number of leaves, length of the longest leaf, and the mass of the caterpillars were measured before and after the trials. The length of the caterpillars was measured before each trial, and the mass of the frass was measured at the end of each trial. During the trials, some feeding behaviors were also recorded: the rosette that was first touched, and the time and duration of feeding on each rosette.

Data from each test type (Species, Location, and Edge) were analyzed separately. The explanatory variables for the Species test were site, location nested within site, plant species, and all possible interactions. The explanatory variables for the Location test were analyzed two ways: first to test for the effects of site and location nested within site, and second the effects of location and trial number, which represented different pairs of source locations (the interaction effect could not be tested due to the different combinations of locations for each trial number). The explanatory variables for the Edge test were site, plant species, and their interaction. For all three analyses, MANOVA were used to test these treatment effects on all four feeding variables: difference in plant mass before and after the trial divided by initial mass (measured in grams, “proportion of mass eaten”), difference in leaf number divided by initial leaf number (“proportion of leaves eaten”), duration of feeding time on each plant (in minutes; “feeding time”), and difference in the longest leaf length divided by initial length (measured in mm; “proportion of leaf length eaten”). Because the plants form rosettes, the length of the longest leaf measures the radius of

the circular rosette and was used as an indicator of the total above-ground plant area. The proportion variables were first arcsine square root transformed to conform to normality. Plant trimming and trial date were included as covariates, the latter to account for potential differences due to the age of the herbivore because trials were performed over four consecutive days. Univariate analyses (ANOVA) were also performed on individual feeding responses if the MANOVA showed significant effects. Finally, multivariate regression analyses were performed to test the relationships between the four feeding response variables and the plant metal concentrations (Cd and Zn, separately) across all trials. All analyses were performed using SAS software (version 9.2, SAS Institute Inc. 2008).

Results

For the Species test, which paired plants of each species grown at the same location, there was a significant effect of species ($F_{4,43} = 3.26$, $p = 0.020$), with a greater amount of feeding on the native *Thlaspi montanum* than on *T. caerulescens* (Fig. 1). Univariate analyses showed that the species effect was significant for the proportion of mass eaten, proportion of leaves eaten, and the proportion of leaf length eaten ($F_{1,43} = 6.36$, $p = 0.015$, $F_{1,43} = 9.37$, $p = 0.004$, $F_{1,43} = 8.18$, $p = 0.006$, respectively; Fig. 1). The interaction between species and location was not significant, meaning that the preference for *T. montanum* was consistent at all three locations tested (although tailings location was only tested using plants from Woods). For the Location test, which paired non-native *T. caerulescens* plants grown at different locations, the response variables did not differ by site or location nested in site ($F_{8,42} = 0.89$ and $F_{12,56} = 0.77$, respectively, and both $p > 0.10$; Fig. 2). Responses

also did not differ by location or trial type ($F_{8,46} = 0.87$ and $F_{4,23} = 1.71$, respectively, and both $p > 0.10$). The covariate trial date was significant ($F_{4,23} = 3.41$, $p = 0.025$), with the proportion of leaves eaten increasing with later dates. For the Edge test, which paired the native *T. montanum* from adjacent with non-native *T. caerulescens* from tailings, there was a significant effect of site ($F_{4,13} = 3.49$, $p = 0.038$; Fig. 3). Univariate analyses showed that there was a greater proportion of leaf length eaten at Woods than at Cinnamon regardless of plant species ($F_{1,13} = 6.23$, $p = 0.024$), and this was marginally significant for the proportion of leaves eaten ($F_{1,13} = 3.53$, $p = 0.079$).

The regression analyses showed that the Zn and Cd concentrations in plant tissues have negative effects on three of the four feeding response variables: the plant mass eaten, proportion of leaves eaten, and proportion of leaf length eaten (Table 3, Fig. 4).

Discussion

Our results show a significant preference by *Pieris napi macdunnoughii* for the native plant, *T. montanum*, over the introduced congener, *T. caerulescens*. Previous studies using larvae of this species have found distinct preferences between food plants, but they did not avoid a naturalized crucifer (*Thlaspi arvense*) that is lethal to the larvae, because its glucosinolate profile is similar to that of their preferred food plant (Chew 1980). Our results agree with her findings in that the native plant is preferred, and *T. caerulescens* is not completely avoided. Even plants with the highest metal concentrations had some levels of feeding (Fig. 3). This result is consistent with the hypothesis that introduced plants with native congeners are more likely to be consumed by native herbivores than more distantly related species

(Agrawal and Kotanen 2003, Diez 2008, Hill and Kotanen 2009). Further studies would be needed to determine whether *T. caerulescens* has a similar glucosinolate profile to that of native plants. Interestingly, the preference for the native species did not depend on location, and there was no preference between *T. caerulescens* from different locations. This is likely because the Species tests did not include the use of plants that hyperaccumulated metals (>10,000ppm Zn or >100ppm Cd; 100 times the normal concentrations), and the Location tests only contained two trials with plants that hyperaccumulated metals (those from Cinnamon tailings). Nevertheless, these results indicate that herbivory pressure from *P. napi macdunnoughii* on *T. caerulescens* would be no different at the edges of the tailings compared to 25m away, and this pressure would be lower than that on the native *T. montanum*.

Our results also support the idea that native herbivores are deterred from feeding on introduced species if they have novel chemical defenses (Carpenter and Cappuccino 2005). Results from the Edge tests do not show a species preference, but herbivores responded to plants from the two sites differently. *T. caerulescens* grown on Cinnamon tailings have the highest Cd and Zn contents, and they were less preferred by the herbivores. The multivariate regression analyses also show that feeding was lower on plants with higher concentrations of Cd and Zn. We cannot ascertain whether just one metal is acting as the deterrent, because the avoided plants had the highest concentrations of both metals. The effect of Cd and/or Zn on feeding appears to be a threshold effect, with the minimum effective concentration between 2,300 and 4,800ppm for Zn and between 70 and 140ppm for Cd (Fig. 4). This range of Zn values is lower than that used in previous arthropod feeding trials of *T.*

caerulescens (7,400 and 5,770ppm in Pollard and Baker 1997 and Behmer et al. 2005, respectively), but is consistent with a previous report that suggests the threshold for Zn deterrence lies between 2,700-5,400ppm for gastropods (Noret et al. 2007). Our Cd concentrations are lower than the 1000ppm that has been shown to deter thrips from feeding on *T. caerulescens* (Jiang et al. 2005), but sap feeders may be less sensitive to plant metal concentrations because metals are stored in the leaf epidermis in *T. caerulescens* (Küpper et al. 1999, Cosio et al. 2005). It is likely that both Zn and Cd are detected and avoided by *P. napi macdunnoughii* in our trials.

Overall, our results indicate that if *T. caerulescens* were introduced to new sites for phytoremediation, herbivores would likely avoid the plants growing on mine tailings with high Cd and Zn concentrations. *T. caerulescens* introduced to other sites may accumulate higher levels of Cd that may further deter local herbivores. In the case that plants establish outside of the contaminated tailings and grow on uncontaminated soils, herbivory pressure would be present but lower than that on native plants. However, our results may be a conservative test of herbivory pressure on *T. caerulescens*, because we used plants that had grown over two seasons; herbivory rates may be higher for plants that are older and larger. The actual herbivory pressure exerted by *P. napi macdunnoughii* over time would also depend on whether feeding on *T. caerulescens* would negatively impact larval development, as was the case with *T. arvensis* (Chew 1980). Additionally, further studies would be needed to investigate the effects of herbivory on the reproductive fitness of *T. caerulescens*. It is possible that our observed feeding rates on *T. caerulescens* would have little effect on the growth and reproduction of these plants, and therefore

herbivory by *P. napi macdunnoughii* may actually confer little biotic resistance. The full measure of biotic resistance also depends upon the other herbivores, especially generalists, as well as pathogens and competitors at the introduced site. A related study has shown that the native *T. montanum* is not highly competitive against *T. caeruleascens* (Che-Castaldo 2011, Chapter 2), but other natural enemies must also be considered before we can conclude that biotic resistance against *T. caeruleascens* would be low at these sites. Nevertheless, our findings suggest that precautions to prevent the spread of hyperaccumulators (e.g. harvesting prior to seed set) are necessary when implementing phytoremediation, because their invasive success may be enhanced by their ability to evade the local natural enemies with novel chemical defenses.

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Tables

Table 1. Cd and Zn metal concentrations (ppm) in the above-ground tissues of plants used in the feeding choice trials, by species (*Thlaspi caerulescens*, TC, and *T. montanum*, TM), site (Cinnamon, Copper, Woods), and location (tailings, adjacent, or 25m from the tailings – “far”). *T. caerulescens* plants did not survive on the tailings at Copper site, and native *T. montanum* only occurred on the tailings at Woods site.

		Cinnamon			Copper			Woods		
		tailings	adjacent	far	tailings	adjacent	far	tailings	adjacent	far
TC	Cd	140.75	48.24	11.90		21.84	35.52	68.81	7.39	28.95
	Zn	4799	911	928		1267	1632	1853	1720	1891
TM	Cd		3.62	1.98		1.03	1.60	3.09	0.81	0.65
	Zn		2371	1230		580	564	534	665	972

Table 2. The six pair-wise combinations of plant species (*Thlaspi caerulescens*, TC, and *T. montanum*, TM) and location (tailings, adjacent to tailings, and “far” or 20m from edge of tailings) that were used in the choice trial experiments, with the level of replication for each of the three study sites (Woods, Cinnamon, and Copper). Trials were grouped into three test types based on whether they test for species preference, location preference, or preference at the tailings edge, which represents the likely scenario if *T. caerulescens* were introduced for phytoremediation. Replication levels were dependent on the availability of plants. For example, *T. caerulescens* did not survive when experimentally planted on the tailings at Copper, so we were not able to perform the Edge test using plants from Copper.

Test type	Trial #	Plants used	Woods	Cinnamon	Copper
Species	1	tail TC vs tail TM	13	0	0
Species	2	adj TC vs adj TM	3	3	2
Species	3	far TC vs far TM	7	2	3
Location	4	tail TC vs far TC	11	2	0
Location	5	adj TC vs far TC	0	0	3
Edge	6	tail TC vs adj TM	5	6	0

Table 3. Results from the multivariate regression between the four feeding response variables and the concentrations of Cd (top) and Zn (bottom) in the plant tissues. For each metal, the multivariate statistic (Wilks' Lambda), F value, degrees of freedom, and p-value for the full model including all four feeding responses are shown in the first row. Below this are the regression coefficients for each feeding response, with the adjusted R² values and t-tests values from testing whether each coefficient is different from zero. Plant Zn and Cd have significant negative effects on all of the feeding variables except for feeding time.

Cd	Estimate	F	R ²	t	df	p
Model	0.9167	2.50			4,110	0.047
Mass eaten	-8.86x10 ⁻⁵		0.03	-2.44	1	0.037
Leaves eaten	-4.22x10 ⁻³		0.07	-2.92	1	0.003
Leaf length eaten	-3.14x10 ⁻³		0.03	-2.56	1	0.027
Feeding time	-0.024		0.01	-1.69	1	0.114

Zn	Estimate	F	R ²	t	df	p
Model	0.9153	2.54			4,110	0.044
Mass eaten	-3.83x10 ⁻⁶		0.04	-1.86	1	0.016
Leaves eaten	-1.53x10 ⁻⁴		0.06	-2.82	1	0.004
Leaf length eaten	-1.34x10 ⁻⁴		0.05	-3.19	1	0.012
Feeding time	-9.62x10 ⁻⁴		0.02	-2.14	1	0.093

Figures

Fig. 1. Mean (± 1 SE) values for the four feeding response variables from the Species type feeding choice trials, shown by species (non-native *Thlaspi caerulescens*, TC, and native *T. montanum*, TM). These trials paired plants of each species that were grown at the same location. The response variables were: proportion of plant mass eaten, shown multiplied by a factor of 10 to match the other variables in magnitude; proportion of leaves eaten; proportion of leaf length eaten; and feeding time (in min). The MANOVA with all four variables showed a significant species effect, indicating that *P. napi macdunnoughii* preferentially fed on the native *T. montanum*. Asterisks indicate the responses that showed a significant species effect in a univariate ANOVA.

Fig. 2. Mean (± 1 SE) values for the four feeding response variables from the Location type feeding choice trials, shown by location (tailings, adjacent to tailings, and “far” or 25m from edge of tailings). These trials paired non-native *T. caerulescens* plants grown at different locations. The response variables were: proportion of plant mass eaten, shown multiplied by a factor of 10 to match the other variables in magnitude; proportion of leaves eaten; proportion of leaf length eaten; and feeding time (in min). *P. napi macdunnoughii* showed no feeding preference when presented with *T. caerulescens* from different locations, when they do not contain high metal concentrations.

Fig. 3. Mean (± 1 SE) values for the four feeding response variables from the Edge type feeding choice trials, shown by site (Cinnamon and Woods). These trials paired the native *T. montanum* from adjacent locations with non-native *T. caerulescens* grown on tailings, which represents the likely scenario if *T. caerulescens* were introduced for phytoremediation. *T. caerulescens* grown on Cinnamon tailings accumulated the highest concentrations of Cd and Zn. The response variables were: proportion of plant mass eaten, shown multiplied by a factor of 10 to match the other variables in magnitude; proportion of leaves eaten; proportion of leaf length eaten; and feeding time (in min). The MANOVA with all four variables showed a significant site effect, indicating that *P. napi macdunnoughii* preferentially fed on the plants from the Woods site. An asterisk indicates the response that showed a significant site effect in a univariate ANOVA.

Fig. 4. The relationships between plant Cd (left column) and Zn concentrations (right column) and the four feeding responses (proportions of mass, leaves, and leaf length eaten, and feeding time) across all trials. Filled symbols represent the non-native *Thlaspi caerulescens* (TC), open symbols represent the native *T. montanum* (TM), and source locations are represented by different symbols (squares = tailings, triangles = adjacent to tailings, and circles = “far” or 25m from the edge of tailings). Trend lines are shown if the regression between the metal and the individual feeding responses are significant (see Table 3). *T. caerulescens* grown on the tailings at one site (Cinnamon) had the highest Cd and Zn values, and were less preferred by *P. napi macdunnoughii*.

Figure 1

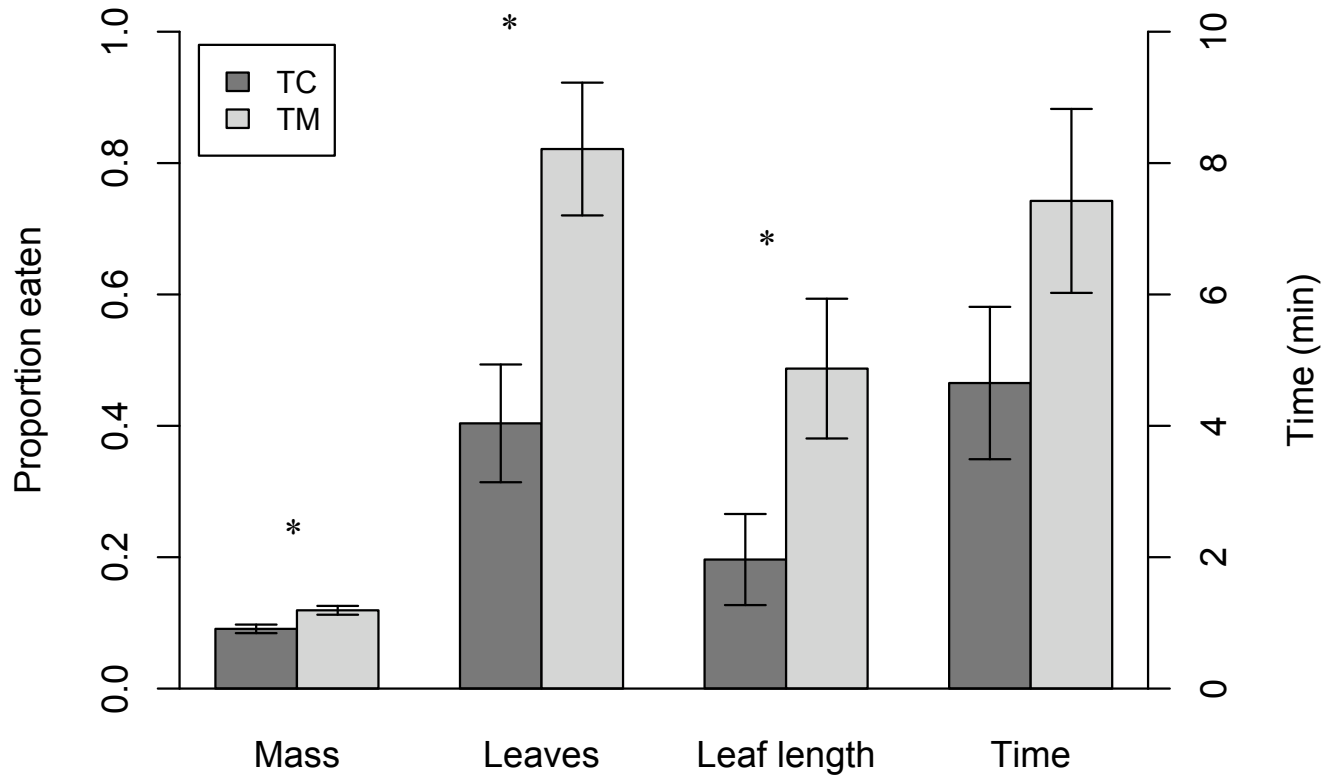


Figure 2

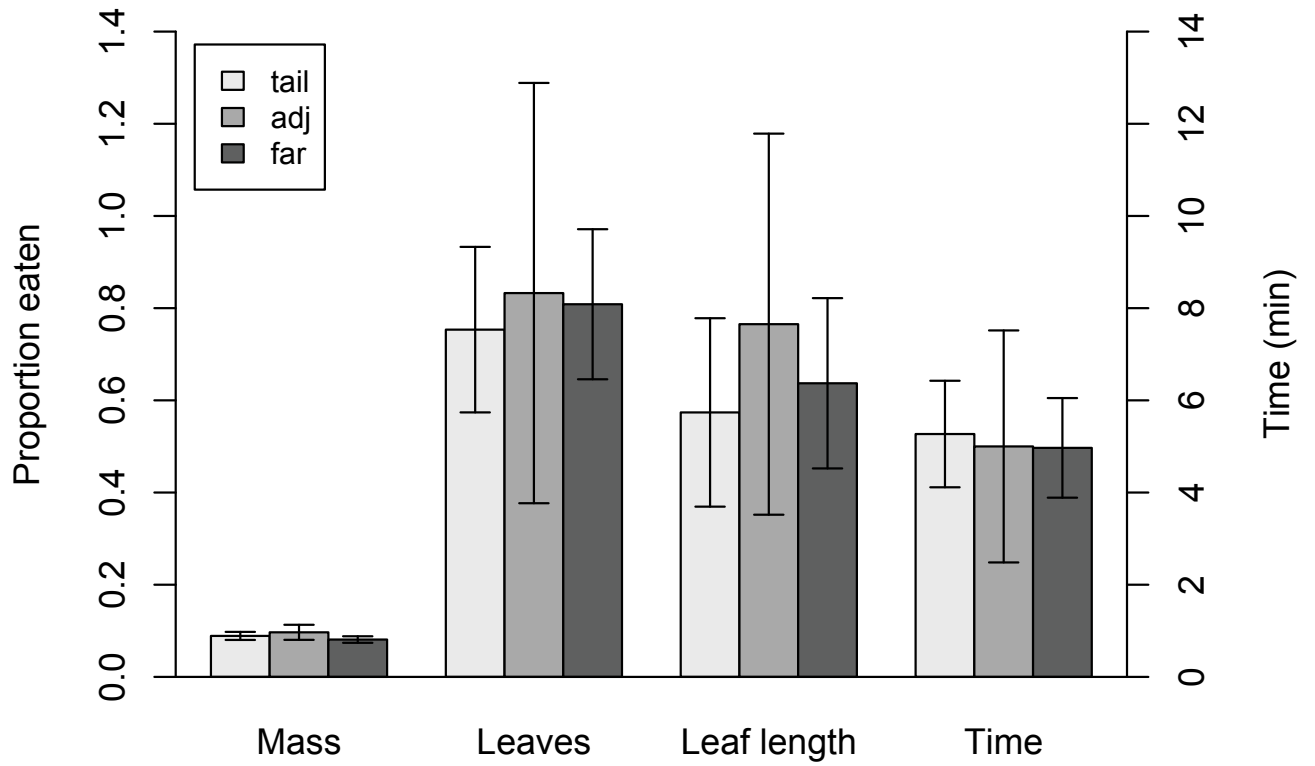


Figure 3

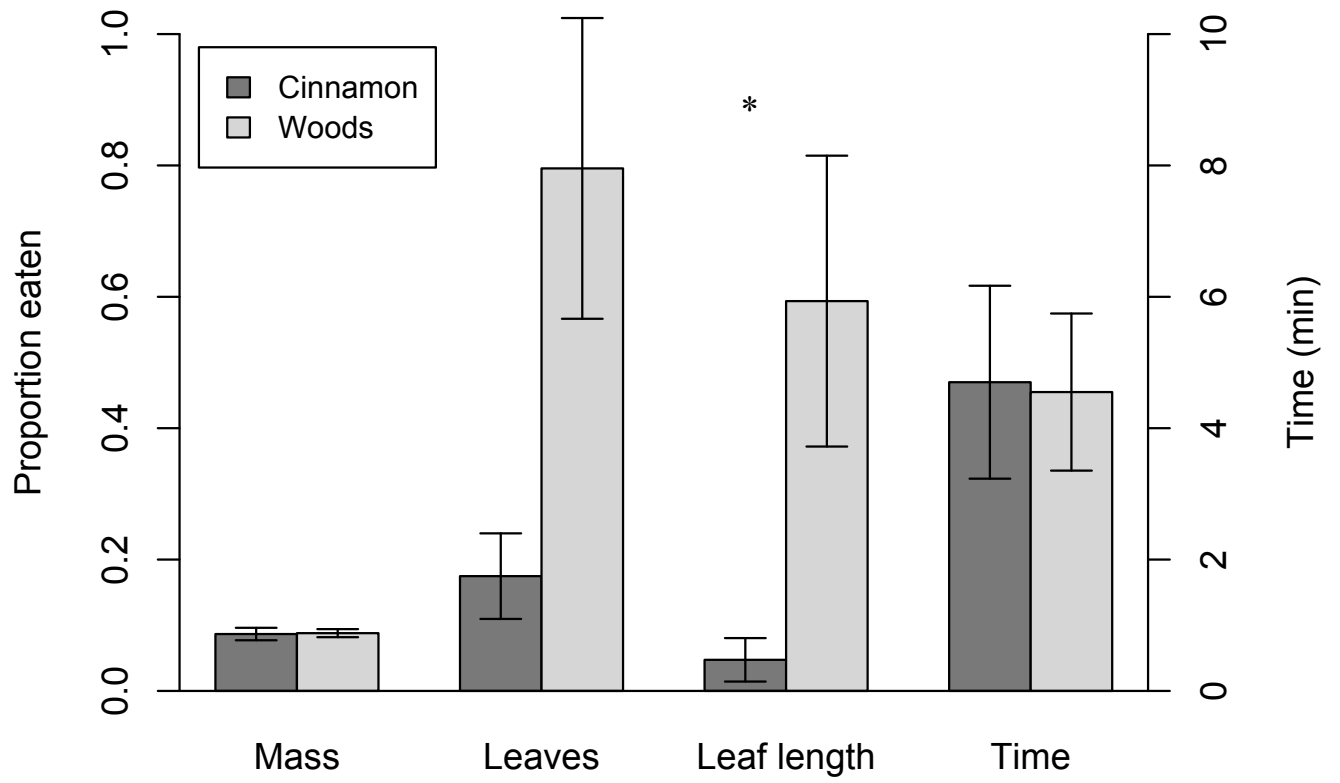
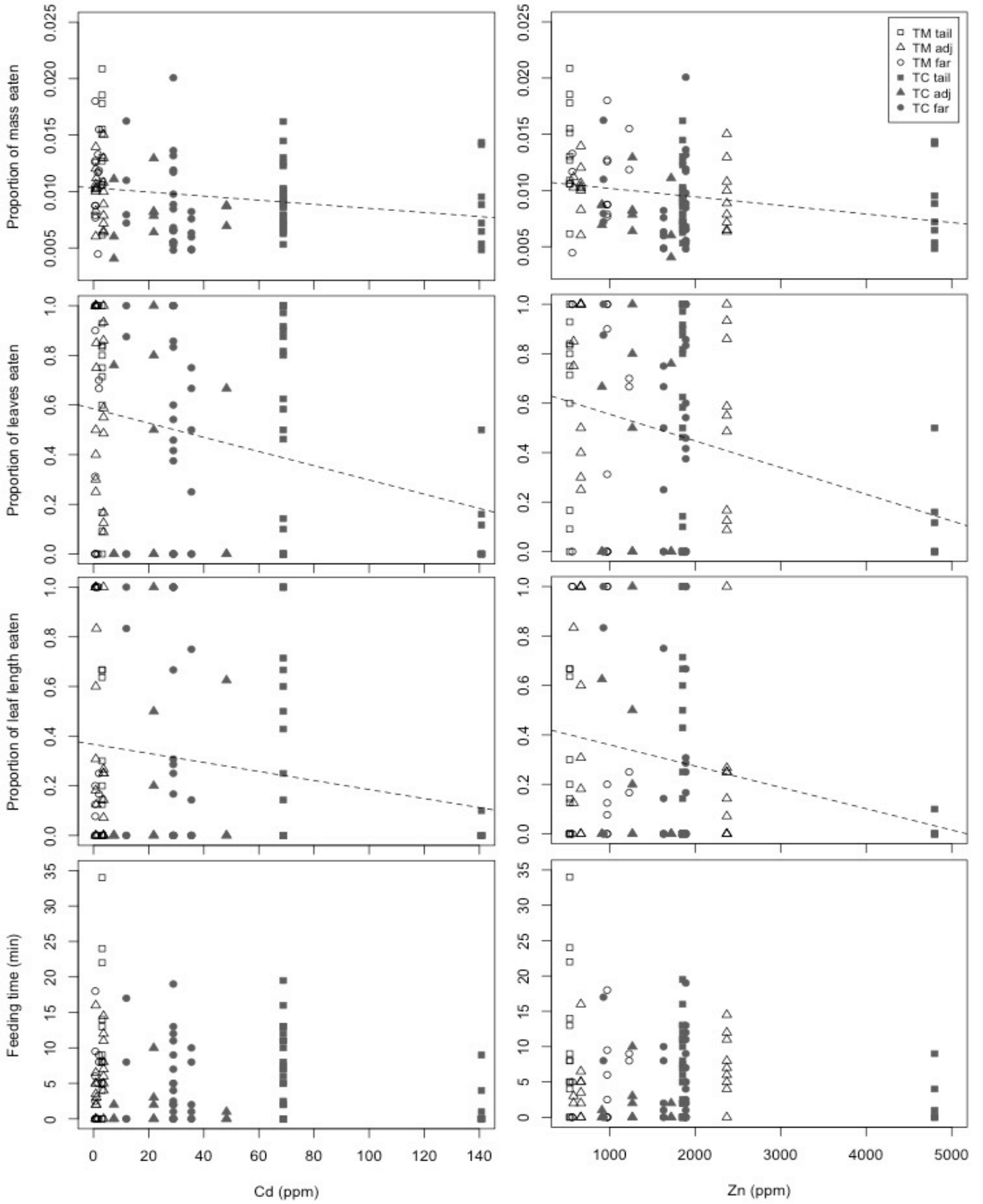


Figure 4



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Chapter 4: The effect of dataset length on demographic analyses,
illustrated with *Frasera speciosa*, a long-lived monocarp with mast
reproduction

Abstract

Frasera speciosa (Gentianaceae) is a long-lived monocarpic plant with mast reproduction, a type of plant whose demography is rarely studied. Using a 34-year dataset from an ongoing study, we describe the population dynamics of *F. speciosa* using matrix projection models, examine the effects of masting on demographic parameter estimates, and address how many years of data are needed to estimate population growth and elasticity values accurately. Our results indicate that our population is stable, with the annual population growth rate close to one ($\lambda = 0.954$, $\lambda_s = 0.997$ with 95% confidence interval of 0.940-1.057). Mean generation time is 36 years, and the mean estimated lifespan is 6.6 years with a very high variance of 173.5 years, consistent with observations of very long-lived individuals in the field. Elasticity values are highest for the transition stages representing stasis and lowest for reproduction, as found for other, non-masting perennial monocarps. Eighteen years of data are needed to yield lambdas that are within 1% of the long-term estimate, but even three-year datasets would yield qualitatively correct lambdas, as long as they include masting years. Finally, 10 years of data would rank correctly the different life history stages by their elasticity values, indicating that the full range of environmental variation experienced by our population can be captured within this time span. Our results demonstrate that life history stages with low elasticity values can still be very

important to population growth, and that capturing one reproductive event may be sufficient for studying the demography of long-lived monocarpic species.

Introduction

Long-lived monocarpic plants, which flower once at the end of their life, are rarely the subject of demographic studies (but see Young 1984, Augspurger 1985, Young 1990, Nobel 1992, Metcalf et al. 2003, Kuss et al. 2008), and even fewer have examined those with mast reproduction (but see Janzen 1976). While some studies have examined the evolutionary biology of masting in monocarpic perennials (Janzen 1976, Keeley and Bond 1999, Read et al. 2008), the population dynamics of these plants is not well-studied. *Frasera speciosa* (elk weed, Gentianaceae) is a long-lived monocarp with population-level mast reproduction that occurs in the Colorado Rocky Mountains and other western states in the USA. Its pollination (Beattie et al. 1973), life history (Taylor and Inouye 1985), and temporal pattern of flowering (Inouye and Taylor 1980) have been described previously, but a demographic study using matrix projection models has not been performed. Although the biology of this plant is unusual, predictions can be made about its population dynamics based on studies of plants with similar qualities. For example, plants with long life spans are associated with stable population growth (with the finite rate of increase, or λ , close to one) and low variability in fecundity (García et al. 2008). Additionally, two life history patterns involving vital rates (such as the rates of reproduction, survivorship, and growth) have been described for perennial monocarpic plants. Monocarpy (also known as semelparity) would be favored when juvenile survivorship and adult

mortality are high, because survival as an adult is uncertain, and energy is therefore allocated to reproduction rather than growth (Charnov and Schaffer 1973, Young 1990, Lesica and Shelly 1995, Williams 2009). Alternatively, monocarps may have high juvenile mortality and growth but high adult survivorship. Under this scenario, once an individual has survived to a certain age, delaying flowering is a small risk compared to obtaining more resources for their sole reproductive effort (Augspurger 1985, Metcalf et al. 2003, Kuss et al. 2008).

In addition to describing population growth and how vital rates vary with age, previous studies of monocarpic perennials have asked which life history stages (such as seeds, seedlings or recruits, non-reproductive juveniles, or reproductive adults) and which vital rates contribute the most to population growth, by using elasticity analysis. Elasticity values quantify how small changes in the vital rates, represented by transition probabilities from one stage to another in the matrix model, can affect the population growth rate. Elasticity values are standardized so that the different transition probabilities, which may vary greatly in magnitude, can be compared in their relative contributions to population growth and fitness (de Kroon et al. 1986, Silvertown et al. 1993, de Kroon et al. 2000, Caswell 2001). These studies have found that long-lived plants, and those living in stressful environments, tend to have low elasticities for the transitions representing reproduction and high elasticities for the transitions representing survival (Enright et al. 1995, Silvertown et al. 1996), which has been demonstrated in some monocarpic perennials (Forbis and Doak 2004, Kuss et al. 2008). However, while elasticity values indicate how small changes to transition probabilities would affect population growth, species with mast flowering

experience large variations in reproductive rates between years. Specifically, some years there could be no production of seeds or recruits, while other years there would be thousands of seeds and hundreds of recruits. Whether this large variability would increase the importance of fecundity and reproductive stages to population growth for masting plants is not known.

When vital rates and elasticity values vary greatly between years, long datasets are needed to obtain an accurate description of the long-term population dynamics and the relative importance of various life history stages (Horvitz and Schemske 1995, Benton and Grant 1996). The need for extensive datasets to study demography is a well-recognized limitation, especially for conservation applications of demographic models (Fieberg and Ellner 2001, Ellner et al. 2002, Morris et al. 2002). A few studies have examined how many years of data would be sufficient for demographic studies (Fagan et al 1999, Doak et al. 2005, Fox et al. 2006, Holmes et al. 2007), but they focus on a wide range of organisms and a variety of demographic parameters, and only one uses data from a natural population (Fox et al. 2006). This is because the actual value of the demographic parameter must be known, so that results from the shorter datasets can be compared against it to evaluate accuracy. One way to do this is to set the parameter at a known value and simulate datasets of various lengths. The other way is to have a sufficiently long dataset so that the parameter estimates converge upon one value, and subdivide the dataset into shorter subsets from which the parameter is also estimated; this is the approach we used. With a long-term dataset available, we also address the effects of dataset length on

estimating demographic parameters for a long-lived plant, which, when combined with the other studies, should help to establish more generalizable results.

We have monitored an alpine population of *F. speciosa* in the Elk Mountains of southwestern Colorado annually from 1973 to the present. Using this dataset, we built stage-based population matrix models to describe the demography of this long-lived monocarpic plant with synchronized reproduction. Additionally, we examine whether their population growth rate and elasticity values are similar to other monocarpic perennials, whether fecundity is more important to population growth for *F. speciosa* than plants without masting, and how many years of data are needed to produce accurate population growth and elasticity estimates. Because the length of the dataset has also been shown to affect whether deterministic or environmentally stochastic demographic models yield more accurate results (Doak et al. 2005), we apply both types of models to our data. The deterministic model uses mean transition probabilities that are averaged over the entire dataset, whereas the stochastic model incorporates the variance and correlation of the transition probabilities across years (Tuljapurkar 1990, Caswell 2001, Boyce et al. 2006).

Methods

Study site and study species

Beginning in 1973, ramets of *Frasera speciosa* were individually tagged in a study plot (now 18 x 29m) in a south-facing alpine bowl at an elevation of 3,740m near Cumberland Pass (Gunnison County), Colorado (38° 41.632, 106° 28.549). The plants grow in a matrix of tundra plants, at about the upper elevational limit of the distribution of the species in the Rocky Mountains. An aluminum tag with an

embossed number was placed next to each plant using a 16-d nail; in cases where plants were too close to tag individually, distances from a nail to each plant were recorded and used to identify individuals.

An annual census in July was made of all *Frasera* plants in the plot; newly discovered plants were tagged, the number of leaves in the basal rosette of tagged plants was counted, length and width of leaves of some rosettes were recorded, and the height and number of flowers were recorded for flowering plants. To facilitate relocation of tagged plants, the plot was marked into 1m squares with a grid of string, and plant locations were recorded to the nearest quarter square meter. Typically over 98% of plants were re-found each year (pocket gophers sometimes bury them, and there is some tag damage from grazing cattle, elk, or deer). A careful search was made for seedlings around each of the dead flower stalks for the next few years following flowering. When untagged 2-leaf plants are found near old stalks, indicating that they were missed for their first few years (during which their two leaves may only be a few mm long), we recorded them as offspring of the nearest old stalk. This method slightly underestimates recruitment, because while seeds are generally deposited within a meter or two of the parent stalk, strong winds may move a few seeds significant distances across the snowpack, and recruitment from those seeds would be missed. Mean height of the 63 plants that have flowered from 1995 – 2009 is 46.2 cm; range 25 – 76 cm.

The basal rosettes of *Frasera* plants typically have a fixed number of leaves each growing season, with on average 86% of each year's rosettes having exactly 2, 4, 6, 9, 12, 16, 20, 25, 30, 36, 42, or 49 leaves at Cumberland Pass. Plants at lower

altitudes sometimes have 56, 64, 72, 80, or 90 leaves (Taylor and Inouye 1985).

Leaves expanded in a particular season are preformed 2-3 years prior to appearing above ground, as are inflorescences (Inouye 1986). Thus, plants may be flowering in response to an environmental cue that occurred years previously.

Matrix construction

This study uses the *F. speciosa* data from 1974 to 2007 (Appendix A; 2007-09 data were used only for seed dormancy data, see below), during which period the average number of individuals observed per year was 823, ranging from 228 to 1687 individuals (recruits and larger). The demography of this population is described by: $\mathbf{n}(t+1) = \mathbf{A}(t)\mathbf{n}(t)$, where the matrix $\mathbf{A}(t)$ projects the population vector (\mathbf{n}) from the current year (t) to the next year ($t+1$). The stage-classified model distinguishes stages based on the observed pattern of rosette sizes described above. Plants are placed into the following leaf number categories: 2 (1-2 leaves), 4 (3-4 leaves), 6 (5-6 leaves), 9 (7-10 leaves), 12 (11-14 leaves), 16 (15-17 leaves), 20 (18-22 leaves), 25 (23-27 leaves), 30 (28-32 leaves), 36 (33-38 leaves), 42 (39-45 leaves), or 49 (46-64 leaves, but only 7 plants had more than 54 leaves). Plants can remain in the same category, regress one or more categories, or advance one or more categories between years. Plants have been observed to flower after reaching the 12-leaf stage, but the most common size before flowering is 25-36 leaves. Matrices were built following the methods of Horvitz & Schemske (1995). Transition rates (a_{ji}) were calculated as the percentage of the individuals starting at stage i that were in stage j the next year.

Reproduction: The matrix models are constructed based on post-breeding censuses so that the measure of reproductive output is the mean number of seeds or

recruits per individual. Fecundity values for production of seeds (f_{1i}) were calculated as the proportion of individuals starting in stage i and flowering in the next year, multiplied by the number of seeds produced on average by a reproductive individual in stage i (see Appendix B for details on how seed production were calculated).

Fecundity values for production of recruits (f_{2i}) were calculated as the mean number of recruits produced per individual starting in stage i . The proportion of recruits produced by each stage is taken to be the same as the proportion of seeds produced per stage, because the parents of the recruits are not always identifiable in the field.

Seed fates: Seeds have been observed to germinate up to the third year after flowering (new seedlings appearing around the base of an old flower stalk), and therefore two dormant seed categories were included (first year dormant, and second year dormant). Because no experimental data were available, seed dormancy and germination rates were estimated from observational data. We assumed a constant germination rate for that cohort of seeds, which was calculated by dividing the total number of recruits from the flowering event by the total number of seeds produced. This value was used as the transition probability from first year dormant to recruits in the next year's matrix, and as the transition probability from second year dormant to recruits in the third year after flowering. The number of seeds that go into dormancy was estimated as the number of recruits found in the second and third years following flowering, divided by the constant germination rate. Data from 2007-2009 were used to determine the seed dormancy rates for the final matrix from 2006 to 2007.

Deterministic models

For each starting year from 1974 to 2006, the number of individuals that made a particular transition from stage i to stage j in the next year was divided by the number of individuals that started at stage i , to obtain the transition probability (a_{ji}). To obtain datasets of different lengths, these transition probabilities for different lengths of consecutive years (from 2 to 34 years) were retained. For each subset of data, one matrix was constructed from the average transition probabilities across those years. In other words, for the dataset length of 34 years, one matrix was produced with the average of the 33 annual probabilities for each transition. For the dataset length of 33 years, two matrices were produced, one averaging over the years 1974-2006, and the other over the years 1975-2007. Thirty-three matrices were produced for the dataset length of 2 years (these 2-year matrices are archived online in the Digital Repository at the University of Maryland, DRUM, and can be found at <http://hdl.handle.net/1903/10050>).

To describe population dynamics, the annual population growth rate (λ) was calculated for each matrix produced following procedures from Caswell (2001, section 5.3.4). Generation time, or the mean age at reproduction, was calculated in two ways. First, the commonly used parameter T is calculated as $T = \ln(R_o) / \ln(\lambda)$, where R_o is the net reproductive rate. Secondly, the stage-specific generation time, μ , which is specifically derived for stage-based populations with multiple types of offspring is used. A vector of generation times estimated for individuals born in stage j is calculated as $\mu^{(j)} = \text{diag}(\mathbf{F}\mathbf{N}\mathbf{e}_j)^{-1} \mathbf{F}\mathbf{N}\mathbf{U}\mathbf{N}\mathbf{e}_j$, where \mathbf{U} is the transient matrix, \mathbf{F} is the fertility matrix, \mathbf{N} is the fundamental matrix, and \mathbf{e} is a vector of ones (Caswell 2009, section 4.5). The stage-specific lifespan, or the expected time to death for an

individual starting at each stage, and the variance in lifespan due to individual stochasticity, are calculated following the methods in Caswell (2009, sections 4.2-4.3). For μ and lifespan estimates, the values calculated for an individual starting life as a seed and as a recruit are presented.

Elasticity values, which quantify the proportional changes in the population growth rate resulting from small changes in each of the matrix elements, were also estimated for each deterministic matrix following procedures from Caswell (2001, section 9.1, 9.2). Because the elasticity values for a matrix sum to one, these values can be used to compare the relative importance of each transition to changes in the population growth rate (de Kroon et al. 1986, Caswell 2001). To facilitate the comparison of elasticity values between biologically meaningful categories, elasticities were summed in two ways. First, summed elasticities were calculated for these transition categories: reproduction (contributing to seeds and recruits in the following year), growth (growing to any larger stage), shrinkage (reverting to any smaller stage), and stasis (staying in the same stage). Second, summed elasticities were calculated for the fates of these reproductive categories: seeds, recruits, non-reproductive rosettes (between stages 2-12 leaves), and reproductive rosettes (greater than 12 leaves).

Stochastic models

To build environmentally stochastic models, one matrix was constructed for every two consecutive years of data, resulting in the same set of 33 matrices yielded from the deterministic analysis with dataset length of two years. The stochastic rates of population growth (λ_s), and their 95% confidence intervals, for different lengths of

this dataset (from 3 to 34 years, because at least 2 matrices are required) were estimated by simulation (Caswell 2001, section 14.3.6.1). This was done using R code modified from the popbio package version 1.1.8 (Stubben and Milligan 2007), with 10,000 iterations and equal probability of drawing each matrix within a sub-dataset.

To determine the dataset length needed to yield accurate estimates of population growth rates, the deviation of the lambda estimates from the “true” population growth rate, or that based on our full 34-year dataset, was calculated for the deterministic and the stochastic models. This accuracy parameter was calculated as the absolute difference between each lambda estimate for the sub-datasets and the long-term estimate, divided by the long-term estimate, and then averaged across the estimates for each dataset length. To determine the dataset length needed to yield correct rankings of life history stages by their contribution to population growth, elasticities summed by transition and by reproductive categories were ranked from highest to lowest for each subset of data. Each ranking was compared with the “correct” ranking that was produced by the full 34-year dataset. For each dataset length, the proportion of sub-datasets that yielded correct rankings was calculated.

To examine the importance of vital rates and their variability over time, the stage-specific rates of survivorship, growth, shrinkage, seed production, and recruit (or seedling) production were calculated. For each starting year, the proportion of individuals in each stage that survived to the next year (survivorship), proportion of surviving individuals in each stage that moved to a larger stage (growth), proportion of surviving individuals in each stage that moved to a smaller stage (shrinkage), the

number of seeds produced per individual in each stage (seed production), and the number of recruits per individual in each stage (recruit production) were tallied. The correlations across years among all pair-wise combinations of these vital rates were calculated. As an example of how the reproduction vital rates varied with the population growth estimates, average stage-specific rates of seed and recruit production were calculated for each subset of data with the length of 5 years. The correlations between these mean vital rates, and the population growth rates λ and λ_s , were calculated. Finally, the relationship between vital rate variability and the elasticity rankings was explored, to test whether the subsets of data that yielded correct elasticity rankings captured greater variability in the vital rates. The coefficient of variation ($CV = SD/\text{mean}$) for each stage-specific vital rate was calculated for each subset of data that was 3 years or longer (at least two vital rates were needed). The correlation coefficient between these CVs and whether that dataset produced a correct elasticity ranking (one that matches that from the full dataset, 0 for incorrect or 1 for correct) was calculated. This was done using the rankings from elasticities summed into both transition and reproductive categories. All described analyses were performed in R version 2.9.2 (R Development Core Team 2007).

Results

Population Dynamics

Based on the full 34-year dataset, the deterministic estimate of the population growth rate (λ) is 0.954 and the environmentally stochastic growth rate (λ_s) is 0.997 (95% confidence interval = 0.940 to 1.057; Fig. 1). Estimates of generation time, or

the mean age at reproduction, differ depending on the method of calculation. The traditional generation time, T , is 49.8 years, whereas the stage-specific generation time, μ , which is specifically derived for stage-based populations with multiple types of offspring, is 36.1 and 35.1 years for individuals starting as a dormant seed and as a seedling (also called a recruit), respectively. The stage-specific lifespan estimate for a seed is 1.02 years with a variance of 0.300 years, while the lifespan estimate for a recruit is 6.63 years with a variance of 173.487 years.

Ranking of the summed elasticity values by transition and reproductive categories indicates which type of vital rates (stasis, growth, shrinkage, or reproduction of seeds and recruits), and which type of life history stages (non-reproductive rosettes, reproductive rosettes, recruits, and seeds), contributes the most to the rate of population growth. Based on the transition categories, the summed elasticity value is highest for the transitions representing stasis (0.614), followed by those for growth (0.231), shrinkage (0.143), and reproduction (0.012). Summed by reproductive categories, elasticity values are highest for the fates of non-reproductive rosettes (0.658), reproductive rosettes (0.316), recruits (0.012), and seeds (0.003).

Examining the vital rates, all of the significant correlations for survival rates among different stages are positive (see Appendix C for a table of all survival rate correlations), indicating that there are “good years” in which survival is high for all stages. The same trends are seen with the reproductive vital rates of seed production and recruit production. Growth rates of recruits are negatively correlated with those of non-reproductive stages while the growth rates of other stages are positively correlated. Finally, survival rates of reproductive stages are negatively correlated

with the rates of seed and recruit production, which is due to the monocarpic life history.

Dataset length & importance of masting

Population growth rates and elasticity values calculated from subsets of data of different lengths are used to examine how many years of data are needed to obtain accurate estimates. For estimating the rate of population growth, 18 years or more of data would yield lambda estimates that are within 1% of the “true” estimate based on the full dataset, for both deterministic and environmentally stochastic models (Fig. 2). Alternatively, 10 years of data or more would yield lambda estimates that are qualitatively the same as the full dataset. That is, the deterministic model, which does not estimate confidence intervals, always yields lambda values that are less than one when the dataset is 9 years or longer (Fig. 1A). When using the stochastic model, datasets that are 10 years or longer would always yield lambda confidence intervals that overlap one (Fig. 1B), and this pattern is likely driven by masting years. Examining the stochastic lambda estimates from 3-year subsets of data, we see that datasets that contain no masting events produce lambdas that are below one (Fig. 3). This is true for any subset of data that only contain data from the non-masting years 1984-1991 and 1998-2002, which are the two longest observed periods without masting. Thus, the longest dataset that contains no masting event is the 9 years from 1984-1992, which includes transitions to 1992 but not the flowering event itself. The importance of including a masting event on lambda estimates is corroborated by the positive correlation between reproductive vital rates and the population growth estimates (for example, using dataset length of five years and the rate of recruit

production: $r > 0.487$, $t_{28} > 2.95$, $p < 0.01$ for λ ; and $r > 0.434$, $t_{27} > 2.50$, $p < 0.02$ for λ_s ; for all reproductive stages except 12-leaves; see Appendix D for all correlation coefficients).

Elasticity values calculated from each subset of data are summed by transition and reproductive categories and then ranked, and the rankings matching that based on the full dataset are considered “correct.” Nine and ten years of data are required to yield consistently correct rankings when elasticities are summed by transition and reproductive categories, respectively (Fig. 4). Stasis is the transition category with the highest elasticities for all dataset lengths, while reproduction has the lowest elasticities for datasets longer than six years. Nine years or more of data would consistently rank growth transitions higher than shrinkage transitions (Appendix E). When elasticities are summed by reproductive categories, seeds have the lowest elasticities for all dataset lengths, and the fates of non-reproductive rosettes are consistently ranked as more important than reproductive rosettes when 10 years or more of data are used (Appendix F).

Relating vital rates to elasticities, there is a positive correlation between whether a subset of data produced correct elasticity rankings and the variability of most stage-specific vital rates, including all of the reproductive vital rates (Fig. 5). This indicates that datasets that include a wider range of vital rates, especially seed and recruit production, are more likely to give correct elasticity values. However, correct elasticity rankings are negatively correlated with the variability of certain survival rates (2-6, 16, and 30 leaves when elasticities are summed into transition

categories, and 2-6, 16, and 49 leaves when elasticities are summed into reproductive categories), and with the shrinkage rate of the 4-leaf stage.

Discussion

Population dynamics

Our results indicate that our population of *Frasera speciosa*, a monocarpic perennial with synchronized reproduction, has a near-stable population growth rate similar to other long-lived herbaceous plants (García et al. 2008). The environmentally stochastic lambda estimates are higher than or equivalent to the deterministic lambda estimates, which is consistent with the theoretical prediction that negative correlations between vital rates reduces the overall effect of incorporating environmental variation on population growth (Tuljapurkar 1982, Nakaoka 1996, Caswell 2001). Because these monocarpic plants have high adult survival rates in non-masting years and lower survival in masting years, there is a negative correlation between the vital rates of survivorship and reproduction. Deterministic lambda estimates are thus based on mean survival and reproduction rates between masting and non-masting years, while stochastic lambda estimates are based upon some years that have high survival rates but no reproduction and other years with lower survival but high reproduction. Although our stochastic analysis did not specifically incorporate the periodicity of masting years, the relative frequency of masting events in comparison to the long adult lifespan means that it would likely have little effect on our results. In terms of life history patterns of monocarpic perennials, *F. speciosa* plants have low seedling establishment and high adult survivorship (Augsburger 1985, Metcalf et al, 2003, Kuss et al. 2008) rather than high

juvenile survivorship and high adult mortality (Young 1990, Lesica and Shelly 1995, Williams 2009). It seems likely that the latter is only associated with shorter-lived perennial monocarps.

Generation time for our population is estimated as either 50 years (T) or 35-36 years (μ), depending on the calculation method. The latter corresponds exactly with the mean age at flowering of 36 years reported for this population in an earlier study, which was estimated using observed leaf size at flowering and the estimated average time spent in each leaf stage (Inouye and Taylor 1980). When a population is at or very close to stability, such as in our case, the divisor in the calculation of T approaches zero, which would produce misleading estimates of generation time (Caswell 2009). Thus, according to the cohort generation time estimate, our 34-year dataset captures nearly one generation of the *F. speciosa* population.

The mean expected lifespan for individuals starting at the seedling or recruit stage is 6.6 years, which is lower than our generation time estimate because of the high mortality of juveniles. The very high variance in this lifespan estimate also indicates that some individuals of this population may live for decades. This is consistent with observations in the field, where 498 individuals have been observed for at least 20 years (19.7 years is the mean + 1SD of the estimated lifespan for recruits), and 29 plants observed for the entire length of the study, from 1974-2009, have not yet flowered.

Elasticity analysis indicates that stasis and survival contribute most to changes in long-term population growth, and reproduction contributes the least, which is similar to other long-lived monocarps (de Kroon et al. 2000, Enright et al. 1995,

Silvertown et al. 1996, Benton and Grant 1996). When grouped into reproductive categories, elasticities were highest for the fates of non-reproductive rosettes, as was found for a short-lived monocarpic perennial (Lesica and Shelly 1995). Both types of summed elasticities show low contribution from reproduction, which is found for some previously studied perennial monocarps (Forbis and Doak 2004, Kuss et al. 2008), but not others (Rees and Rose 2002, Jongejans et al. 2006). Our elasticity value for reproduction is nearly equivalent to those reported for other long-lived alpine tundra plants in the Colorado Rocky Mountains (~ 0.01 , Forbis and Doak 2004). While it is possible that the relatively large number of stages that we chose for our models inflated the elasticity value for stasis (Enright et al. 1995), we believe that, like other long-lived monocarps, stasis is the most important life history stage for *F. speciosa*, and interpretations of the elasticity results are not affected.

Dataset length & importance of masting

Using our *Frasera* dataset, both the deterministic and environmentally stochastic models require the same number of years of data to converge on population growth estimates. Based on our measure of accuracy, 18 years of data would yield stochastic and deterministic lambda estimates within 1% of the long-term estimate based on the full dataset. Deterministic models do provide more accurate lambda estimates than the stochastic model when using short datasets (3-6 years long, Fig. 2), in agreement with an earlier finding (Doak et al. 2005). Qualitatively, ten years of data or more would consistently yield lambda estimates that are the same as the full dataset, with confidence intervals overlapping one. However, this is because the longest interval within our study period that had no masting event lasted 9 years.

Even the shortest datasets (≥ 3 years), as long as they include a masting year, would correctly estimate the stochastic population growth rate as not different from 1.0 for our population. This demonstrates the importance of reproduction on the growth rate of this population, despite the low elasticity values for reproduction. The elasticity reflects the low probability that a seed or recruit will survive to reproduce compared to that for larger stages (Forbis and Doak 2004; the reproductive value, or the expected number of recruits produced by an individual in a given stage, is 0.0001 for a seed and 0.0720 for a recruit, compared to 1.37 for 42-leaf and 1.61 for 49-leaf stages), rather than the actual importance of reproduction. Therefore, as previously suggested (de Kroon et al. 2000), elasticity values must be combined with biological knowledge when determining the relative importance of various life history stages to population dynamics.

Datasets of 10 years or longer are needed to rank correctly the various stages by their importance to population growth using deterministic elasticity values. We show that the datasets that give correct elasticity rankings include greater variability in the stage-specific growth and reproduction vital rates. Because the masting life history is a primary cause of inter-annual variation in reproductive rates, the inclusion of masting and non-masting years in a dataset is important for estimating elasticity values as well as for lambdas. However, short datasets that contain masting events do not always yield the correct elasticity rankings (e.g., starting years 1993-1994 in Appendix E & F), indicating that is not the only cause of vital rate variability. The negative correlations between correct elasticity rankings and variability of some survival and shrinkage rates (Fig. 5) are due to a few early years in which unusually

low values for those vital rates were recorded (for example, shrinkage of the 4-leaf stage was 0.04 for the starting year of 1976, while the mean for the entire study period was 0.19). These low values increase the CV of the vital rates between years, and also correspond with higher elasticity values for shrinkage than for growth in those subsets of data. Elasticity values calculated from longer datasets are less affected by these extreme vital rates. In our case, 10 years of data are sufficient for obtaining vital rate values that match the long-term estimates, indicating that this period sufficiently captures the range of environmental variability experienced by our population.

Our results for data length requirements are comparable to those from other studies that have examined how the length of a dataset can affect various population parameter estimates. Using a long-term dataset on the survival rates of a Florida scrub-jay population, one study finds that even two-year datasets would correctly identify a significant effect of maternal family on survival. However, at least 10 years of data are needed to estimate the parameters representing how family and age affect survival (Fox et al. 2006). Our results for estimating population growth also show that short datasets can be informative, especially in comparative studies that may only require qualitative assessments. Using vital rates from real populations, another study finds that 10 years of data would yield a narrow confidence interval for lambda estimates for a desert tortoise population, while the confidence interval for a winter annual plant continues to narrow with increasing dataset length beyond 20 years, with no clear dataset length limit (Doak et al. 2005). A third study uses discrete population growth models and simulated data to categorize populations into

one of three types of extinction categories, which are based on a population's intrinsic rate of increase (r) and its variability. The authors state that extinction probability depends on the species' carrying capacity when r is low, on chance catastrophes when r is high but variation is low, and on assumption violations such as non-random spatial distributions when both r and variation are high. For populations in the latter two categories, 15 years of data are sufficient to correctly classify them 80% of the time, while the first type of population requires more than 50 years of data to achieve the same accuracy (Fagan et al. 1999). Another study of population viability models shows that 10 years of data are not sufficient for estimating quasi-extinction risk (defined as the probability of an 80% decline) using their model, producing confidence intervals that spanned the range from 0 to 1. They recommend the use of at least 20 years of data, which would produce more precise extinction estimates (Holmes et al. 2007). While these studies and ours examined different study organisms and estimated various parameters, their results seem to suggest that 15-20 years of data would provide reliable demographic parameter estimates in most cases. Unfortunately, datasets of this length are quite rare. According to Menges (2000), only 3% of studies modeling the extinction risk of plant populations obtained more than 10 years of data. A more optimistic result from our study is that data requirement also depends on the life history of the study organism. It is likely that a dataset spanning only one or two reproductive events would be sufficient to describe the long-term population dynamics of other long-lived, masting plants. However, demographic studies of short-lived species, whose population growth is more affected by environmental variability than long-lived species (Morris et al. 2008), would likely

be limited by the range of environmental conditions captured in the dataset rather than by reproductive events.

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Figures

Fig. 1. Lambda estimates and how they vary with dataset length. Deterministic (A, as circles) and environmentally stochastic (B, as triangles) population growth rate estimates calculated from all possible subsets of consecutive years of data, from 2-year subsets to the full 34-year dataset. Each symbol (circles or triangles) indicates the lambda estimated from one subset of data, and there are fewer subsets of data for longer dataset lengths. The 95% confidence interval of the stochastic lambda based on the full dataset is shown as a vertical error bar in B.

Fig. 2. To quantify how many years of data are needed to estimate population growth, the accuracy of the lambda estimates from the environmentally stochastic (open triangles) and the deterministic (solid circles) models are shown by the length of the dataset used. Accuracy is calculated as the absolute difference between each estimate and the long-term estimate (based on all 34 years of data), divided by the long-term estimate, and averaged across the estimates for a given dataset length. Deviation of 1% from the long-term mean is shown in dashed line. Eighteen years of data are sufficient to yield lambda estimates within this threshold for both the environmentally stochastic and the deterministic models. For the shortest datasets (3-6 years in length), stochastic lambda estimates are less accurate than deterministic estimates.

Fig. 3. Stochastic lambda estimates (with 95% confidence intervals) from subsets of data that are three years in length, based on whether the dataset included a masting event. The starting year for each subset of data is labeled on the x-axis (for example,

“74” indicates the dataset from 1974-1976). During this study, the longest consecutive years with no masting events were the years of 1984-1991, and 1998-2002. Thus, the short subsets of data that are nested within these years produce stochastic lambda estimates that are below one.

Fig. 4. To quantify how many years of data are needed for calculating elasticities, the proportion of correct elasticity rankings is shown by the length of the dataset used. Elasticity values, which represent the relative importance of each transition to the rate of population growth, are first summed by transition categories (stasis, growth, shrinkage, and reproduction; filled circles) or reproductive categories (the fates of non-reproductive rosettes, reproductive rosettes, recruits, and seeds; triangles). The summed elasticities are then ranked from highest to lowest for each subset of data, and each ranking is compared with the “correct” ranking based on the full 34-year dataset. For each dataset length, the proportion of rankings that are correct is shown. Nine or more years of data would consistently yield the correct ranking of the transition categories by their elasticity values, and 10 years or more of data are required for the reproductive categories.

Fig. 5. Correlation coefficients between the variability of the vital rates (measured as $CV = SD/mean$) within each subset of data, and whether the dataset produced an elasticity ranking matching that from the full dataset (0 for incorrect or 1 for correct). Elasticity rankings were calculated both when elasticities were summed into transition categories (A) and when summed into reproductive categories (B). Vital

rates include stage-specific rates of survival, growth into larger stages, shrinkage to smaller stages, seed production, and recruit or seedling production. The leaf-number stage for each vital rate is shown along x-axis (with “r” representing the recruit stage), and only the significant correlations are shown. The datasets that yielded correct elasticity rankings are those that included greater amounts of variability in all of the reproductive vital rates, and smaller variation in the survival rates of some stages.

Figure 1

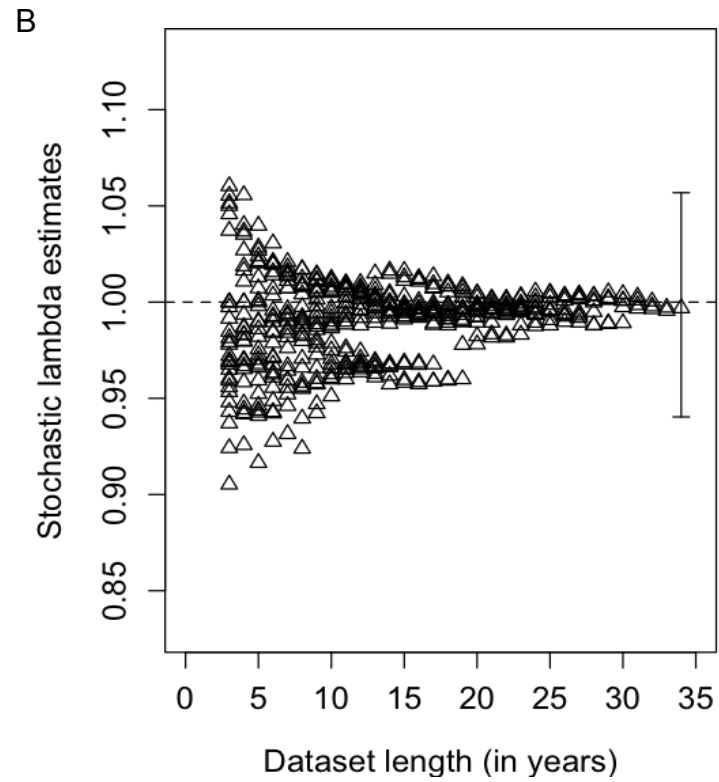
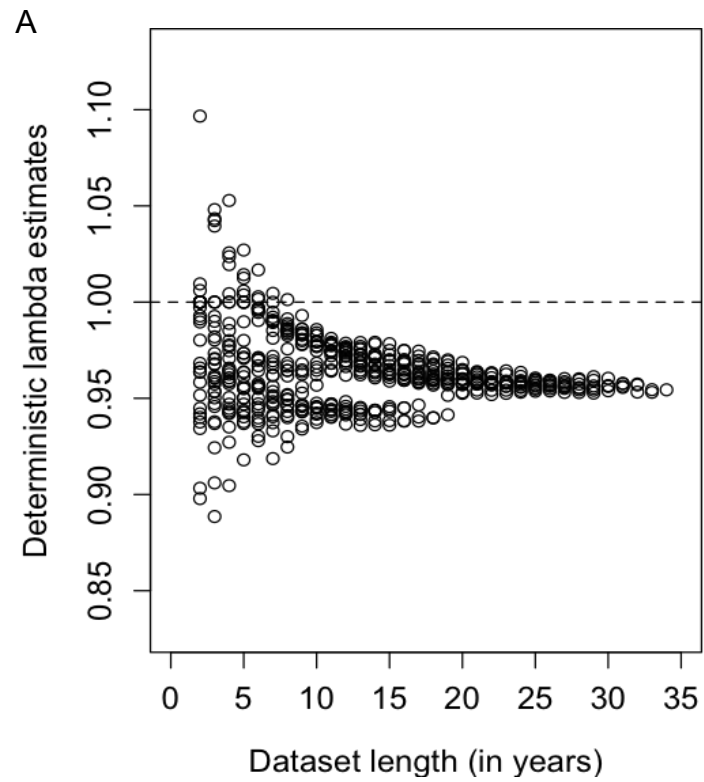


Figure 2

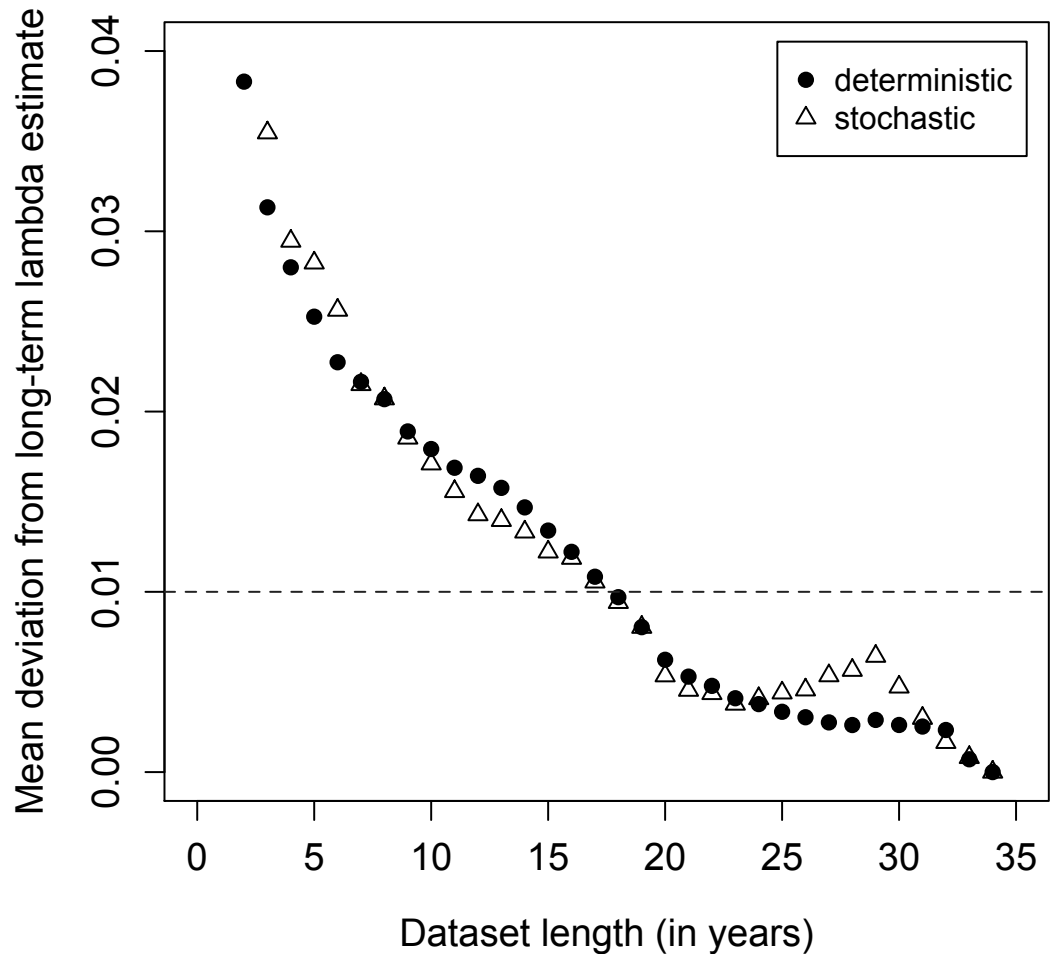


Figure 3

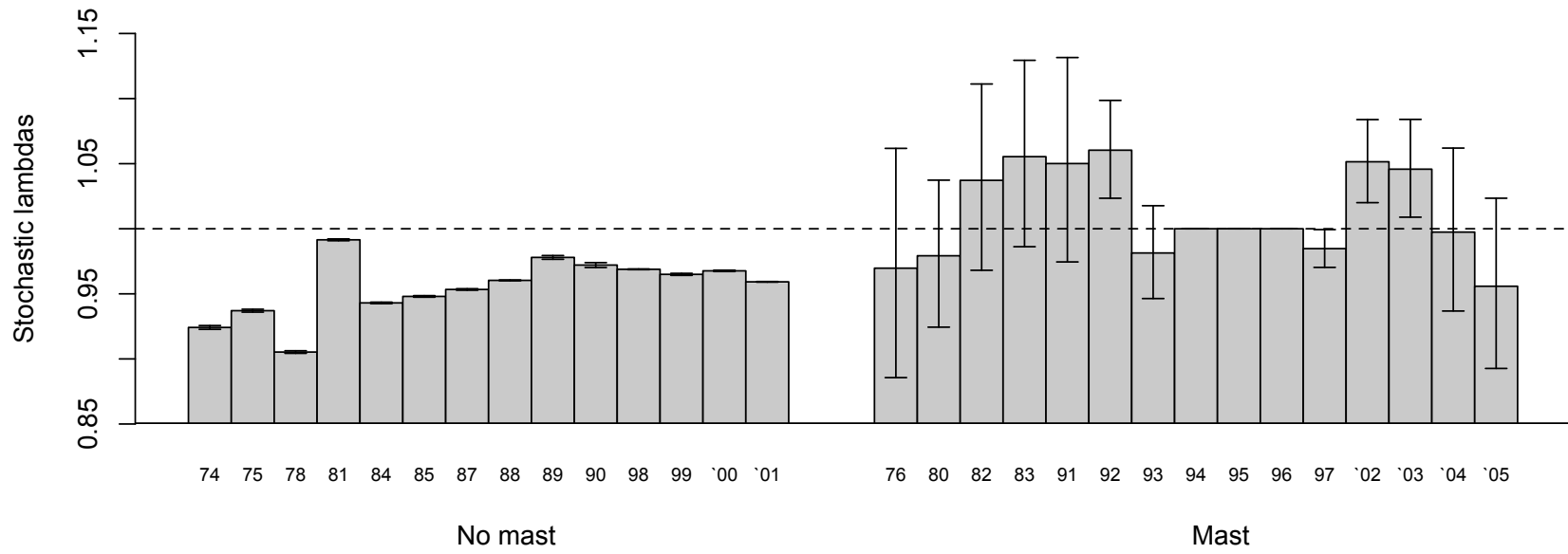


Figure 4

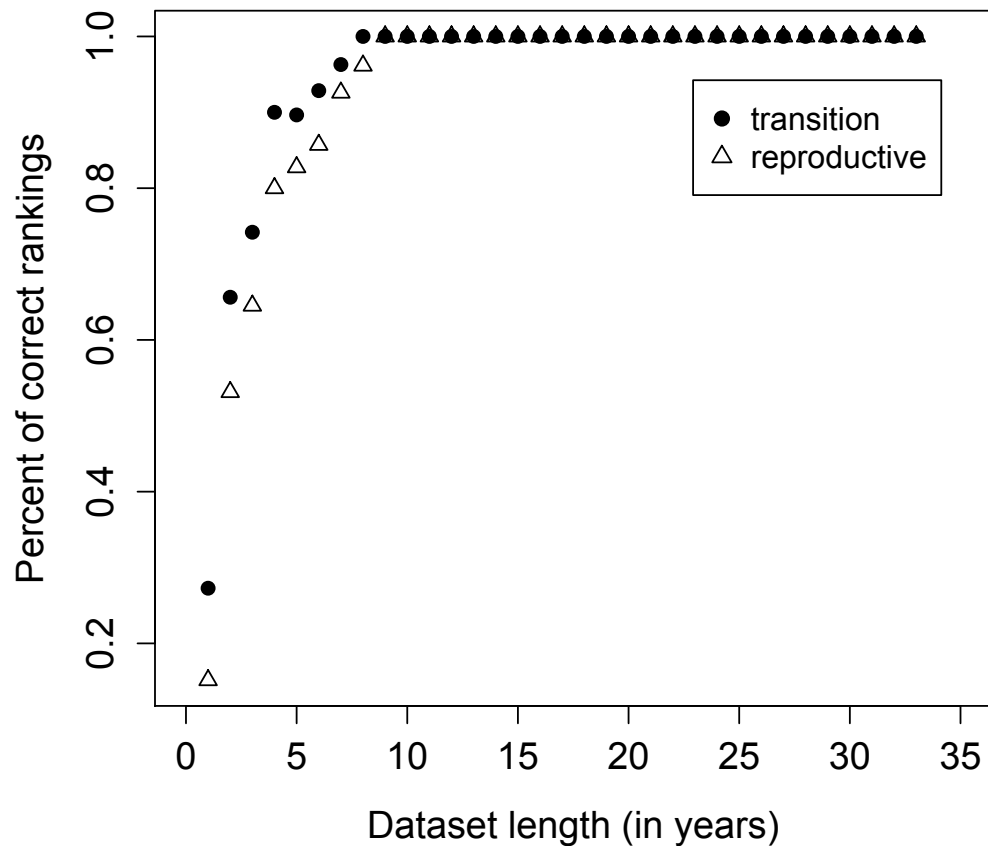
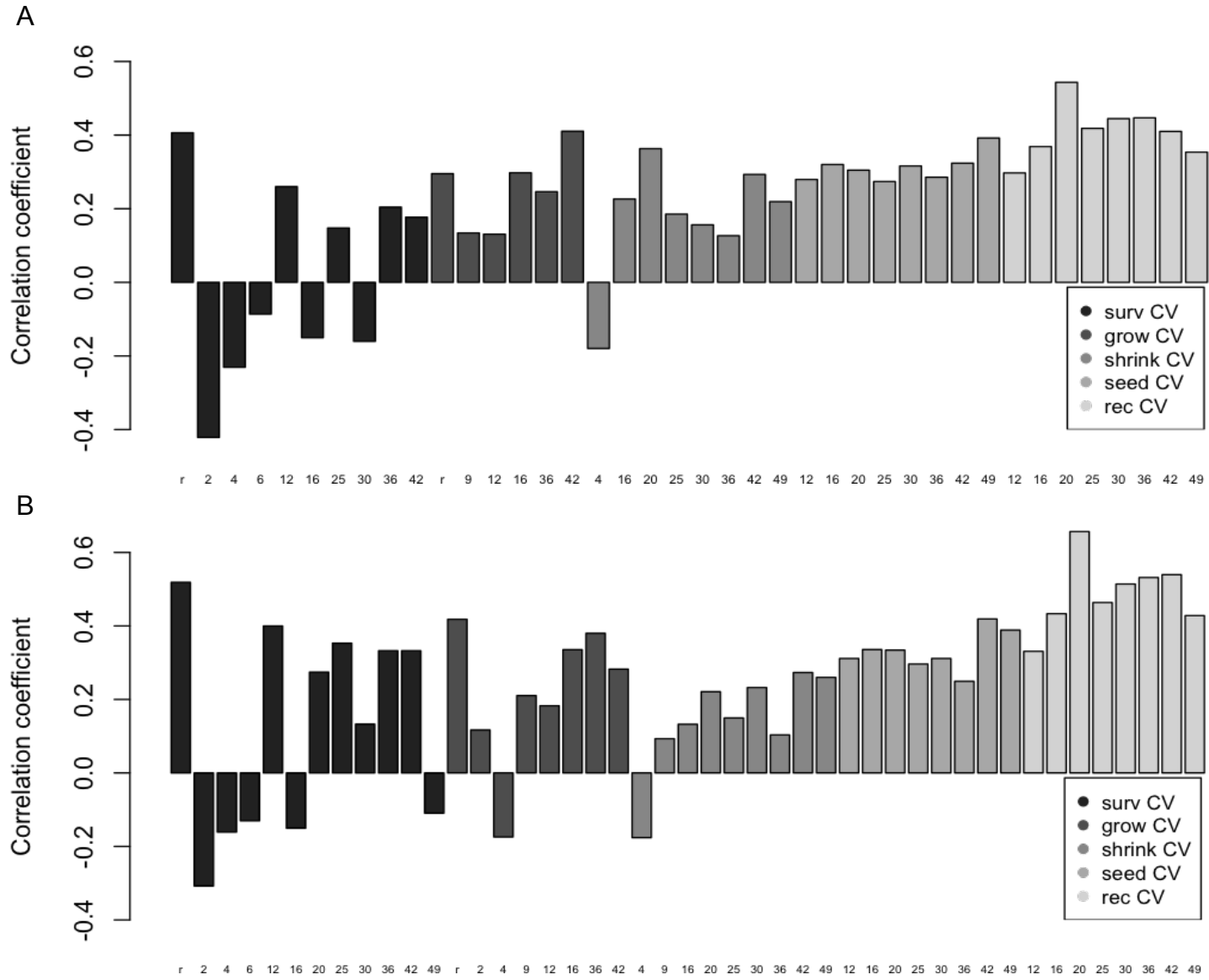


Figure 5



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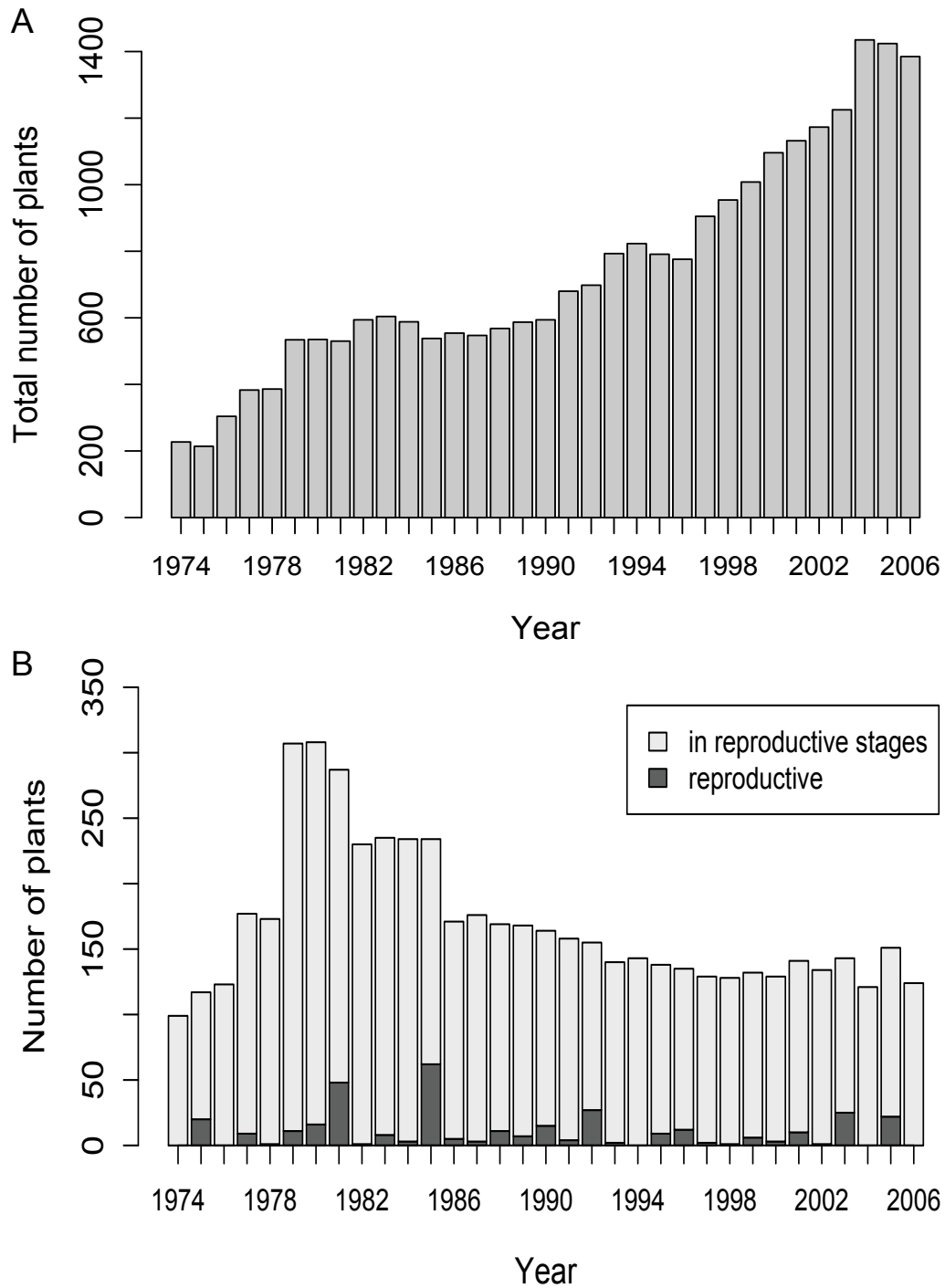
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Appendices

The following supporting figures and tables provide more detailed information to accompany the analyses and results presented in Chapter 4. Appendix A shows the population size and the number of reproductive individuals in the study population over the study period. Appendix B presents the details of how we estimated seed production by each flowering individual in our study population of *Frasera speciosa*. Appendix C contains a table of all pair-wise correlation coefficients between the stage-specific survival rates. Appendix D contains a table of the correlation coefficients between population growth estimates and the reproduction vital rates. Appendix E and Appendix F show the elasticity values summed by transition and reproductive categories, respectively, and how the rankings based on these values depend on dataset length.

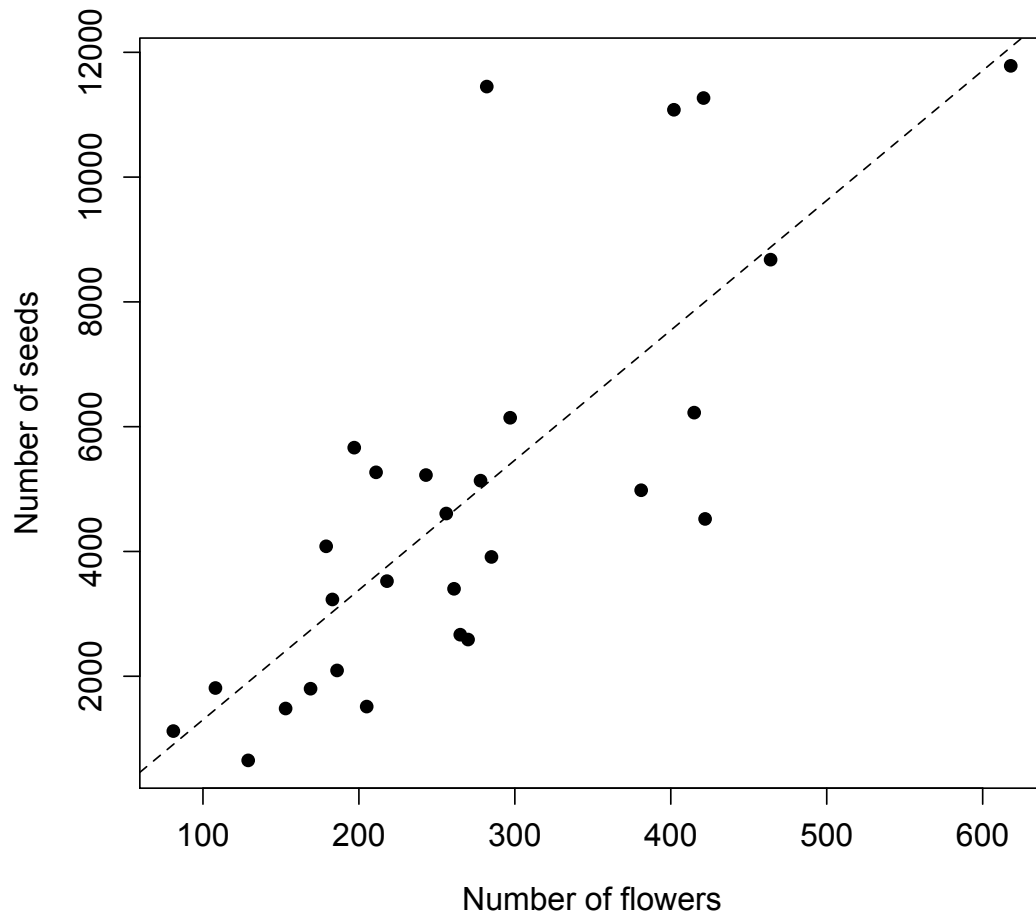


Appendix A. The number of *Frasera speciosa* individuals observed from 1974-2006

(A), which are the starting years used in the study, and the number of plants in reproductive stages (≥ 12 leaves) over the same period (B). In (B), the subset of

plants that actually flowered per year is shown as darker portions of the bars. The increasing number of plants shown in (A) reflects in part an increased effort over the years at finding very small plants in the plot and an increase in the size of the plot from 504 to 522 m² in the 1980s.

Appendix B. To estimate the fecundity values for production of seeds (f_{i_i}), we needed the average number of seeds produced per plant per stage. Although the number of individuals that flowered in each size class was recorded annually, the number of flowers produced per plant was not recorded for all of the flowering plants, and the number of seeds produced per individual in each size class was measured only in 1992. For the 344 flowering plants with missing data, when the flower number was recorded (135 plants), the number of seeds was predicted from the flower number for each reproductive individual using the regression equation derived from the 1992 data ($N_{\text{seeds}} = 20.809 * N_{\text{flowers}} - 778.8$, $R^2 = 0.60223$, number of observations = 28):



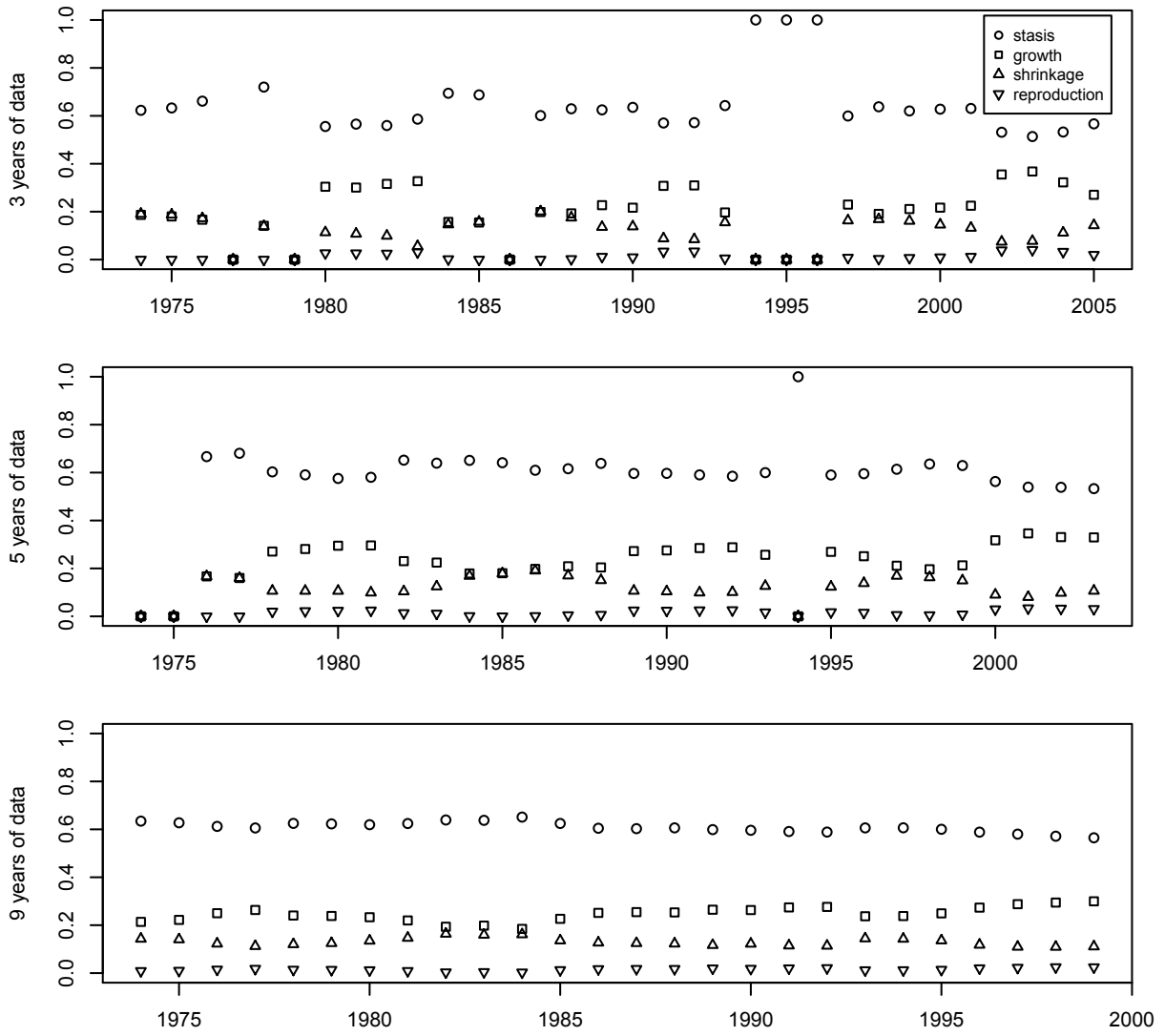
When neither the seed number or the flower number was recorded (209 plants), seed production was estimated as the average number of seeds produced per flowering plant in each leaf number stage in 1992. The use of estimated rather than observed seed numbers is unlikely to have affected our results, because the subsequent elasticity analysis reveals that seed production has a very small contribution to changes in population growth compared to other stages.

Seed Estimate by Stage

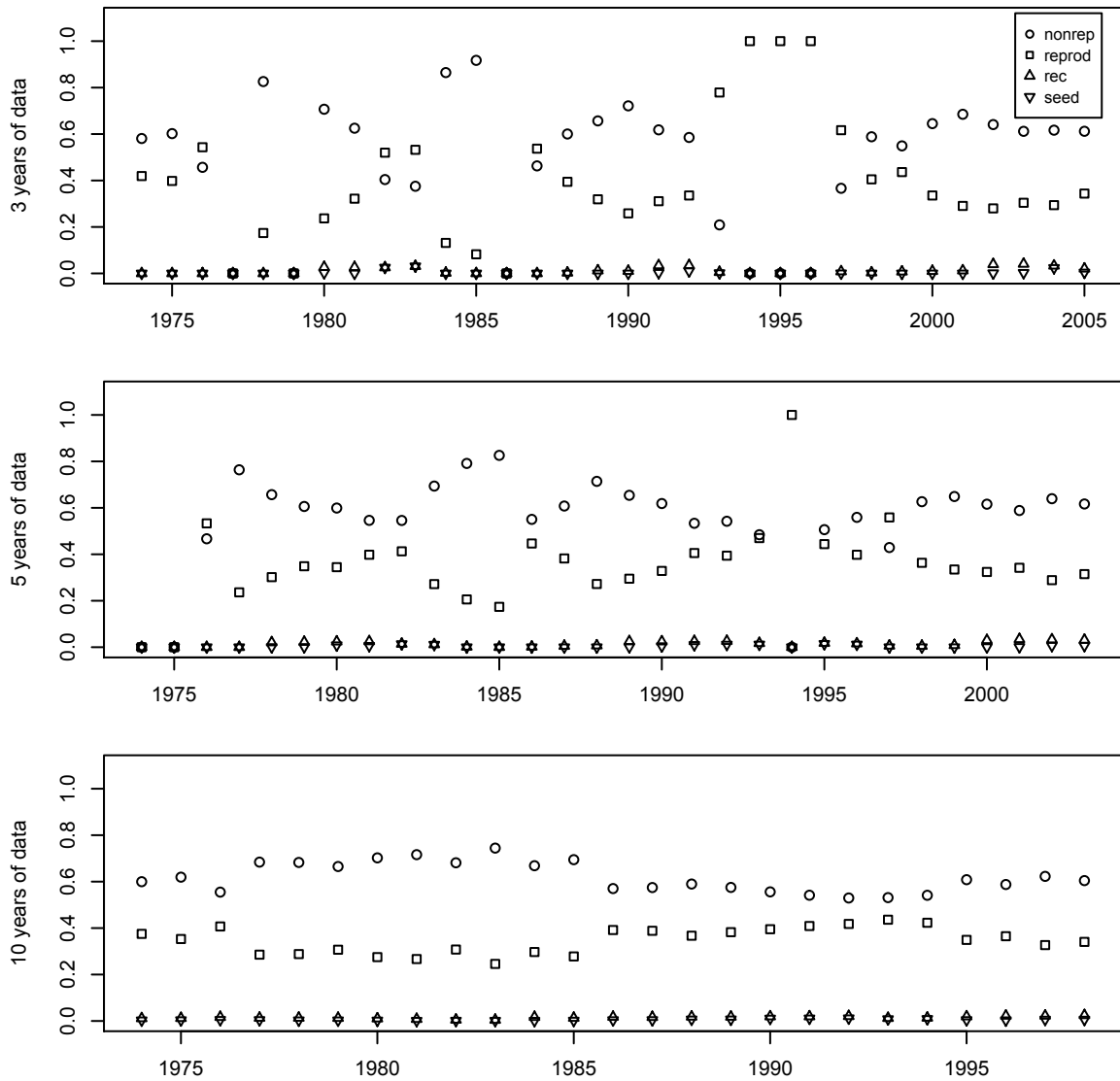
Stage	N_{seeds} per individual
12	1121
16	1121
20	651
25	2898
30	4035
36	6007
42	11431
49	8677

Appendix D. Pearson’s correlation coefficients (r) for the relationships between deterministic and stochastic lambda estimates and the average rates of stage-specific seed and recruit production, based on datasets of five years in length. Significant correlations are shown in bold, and only reproductive stages (12-leaves and above) are shown. In general, there is a positive correlation between reproductive vital rates and estimates of population growth.

Mean recruit production rates and deterministic lambda								
stage	12	16	20	25	30	36	42	49
r	0.495	0.183	0.833	0.734	0.862	0.843	0.487	0.703
t value (df=28)	3.017	0.986	7.967	5.710	9.012	8.278	2.954	5.232
p value	0.005	0.333	<0.001	<0.001	<0.001	<0.001	0.006	<0.001
Mean recruit production rates and stochastic lambda								
stage	12	16	20	25	30	36	42	49
r	0.434	0.190	0.781	0.700	0.807	0.782	0.440	0.671
t value (df=27)	2.503	1.007	6.507	5.091	7.102	6.510	2.546	4.704
p value	0.019	0.323	<0.001	<0.001	<0.001	<0.001	0.017	<0.001
Mean seed production rates and deterministic lambda								
stage	12	16	20	25	30	36	42	49
r	0.531	0.210	0.561	0.407	0.441	0.336	0.520	0.583
t value (df=28)	3.316	1.134	3.583	2.359	2.599	1.891	3.220	3.801
p value	0.003	0.266	0.001	0.0255	0.015	0.069	0.003	0.001
Mean seed production rates and stochastic lambda								
stage	12	16	20	25	30	36	42	49
r	0.473	0.279	0.520	0.411	0.435	0.335	0.475	0.539
t value (df=27)	2.787	1.512	3.161	2.344	2.510	1.844	2.803	3.329
p value	0.010	0.142	0.004	0.027	0.018	0.076	0.009	0.003



Appendix E. Elasticity values summed by transition categories (stasis, growth, shrinkage, reproduction), calculated from subsets of data of length 3 (top), 5 (middle), and 9 (bottom) years, shown by the starting year of the dataset used. Stasis tends to have the highest elasticities and reproduction the lowest elasticities, regardless of dataset length. Nine years or more of data would consistently rank growth transitions higher than shrinkage transitions in their importance to the rate of population growth.



Appendix F. Elasticity values summed by reproductive categories (fates of non-reproductive rosettes, reproductive rosettes, recruits, and seeds), calculated from subsets of data of length 3 (top), 5 (middle), and 10 (bottom) years, shown by the starting year of the dataset used. Ten years or more of data would consistently rank the fates of non-reproductive rosettes higher than those of reproductive rosettes in their importance to the rate of population growth. Recruits and seeds tend to have the lowest elasticity values.

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