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CAUSES AND CONSEQUENCES OF PROLONGED DORMANCY:

WHY STAY BELOWGROUND?

By

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B.S. in Environmental Studies, University of California, Santa Barbara, 2002

Dissertation

presented in partial fulfillment of the requirements
for the degree of

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Causes and consequences of prolonged dormancy: Why stay belowground?

Chairpersons: Dr. Anna Sala and Dr. Elizabeth E. Crone

Prolonged dormancy is a stage in which mature plants fail to resprout during the growing season and instead remain alive belowground. Though it is relatively common, the causes and consequences of this intriguing stage have remained elusive. In this dissertation, I investigate the causes and consequences of prolonged dormancy in a long lived perennial herb, *Astragalus scaphoides*.

First, I use a combination of demography and ecophysiology to study the proximate mechanisms associated with prolonged dormancy. Analysis of a long-term demographic dataset indicates that both endogenous factors (e.g. age, condition, and history) and exogenous factors (e.g. climate and spatial variation) are associated with dormancy. I then investigate the association between stored resources and dormancy. My results indicate that individual plants with low levels of stored available carbon are more likely to enter prolonged dormancy. Surprisingly, individuals increased their mobile carbon concentrations while dormant, presumably by remobilizing structural carbon into mobile forms. Since stored resources integrate past conditions and performance with current state, these results can explain why some individuals remain belowground while others emerge to grow and reproduce.

I used matrix models to examine the ultimate causes and consequences of prolonged dormancy. I found evidence that prolonged dormancy acts as a conservative strategy that allows plants to avoid the risk of a variable environment. Further, my results demonstrate that intermediate levels of dormancy result in the highest fitness advantage. Finally, I measured the trade-offs associated with emerging during times of environmental stress. Although plants showed remarkable physiological tolerance to stress, stress led to demographic costs. Therefore, prolonged dormancy is shown to be a beneficial strategy in a variable environment.

Together, my research identifies both the proximate causes of prolonged dormancy, as well as the ultimate consequences of remaining belowground during the growing season. Therefore, my research not only identifies why some plants go dormant while others emerge, but also explains the prevalence of this intriguing life stage in the life histories of so many perennial plants.

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GENERAL INTRODUCTION AND OVERVIEW

Life history delays have long fascinated evolutionary ecologists, because organisms postpone fitness-enhancing activities such as growth and reproduction. All else being equal, individuals that grow and reproduce now will have higher fitness than individuals that delay these activities. These delays, then, can have major impacts on fitness. However, life history delays are fairly common (Tuljapurkar 1990, Koons et al. 2008). Indeed, research over the last half century has revealed that life history delays may be adaptive depending on the ecological context in which they occur. In a classic paper, Cole (1954) asked why it is common for species to spread reproduction over time (iteroparity), when species that reproduce once (semelparity) could achieve the same fitness by increasing reproduction by one offspring. Charnov and Schaffer (1973) solved this apparent paradox by incorporating variable survival of different life stages, particularly adults versus juveniles. Cohen's pioneering work (1966, 1967) demonstrated that delayed germination can be adaptive in a variable environment, but would be costly in a constant environment. Since then, ecologists have explored the adaptive significance of life history delays, and how the environment mediates the relationship between life history and fitness. Their work has shown that understanding the evolutionary significance of life history strategies requires a comprehensive understanding of the ecological context in which it operates. In this dissertation, I explore both the proximate and ultimate causes and consequences for a particularly interesting, and poorly understood, life history strategy seen in herbaceous perennial plants, known as prolonged dormancy (Lesica and Steele 1994).

Prolonged dormancy is a stage in which individuals fail to re-sprout for one or more years, and instead remain alive below ground (Lesica and Steele 1994). Prolonged dormancy

was initially discovered as a nuisance parameter for long-term monitoring studies (Shefferson 2009). Since then, prolonged dormancy has been observed in 64 species and 14 plant families (Shefferson 2009, Reintal et al. 2010), but has remained poorly understood because of the challenges of studying such a cryptic life stage. Not only are dormant plants hidden belowground, but dormancy seems to be particularly common in long lived species (Hutchings 1987; Lesica and Steele 1994; Shefferson 2001; Shefferson 2003; Lesica and Crone 2007; Shefferson 2009), making lab and greenhouse studies challenging. However, combining short-term ecophysiological studies and experiments with long term demographic monitoring can allow for inference between mechanisms driving dormancy and the long-term consequences of remaining below ground while other plants emerge to grow and reproduce.

At first glance, prolonged dormancy seems costly, since plants not only delay growth and reproduction, but also must maintain mature plant parts while they are belowground (Lesica and Steele 1994). These costs could have large impacts on fitness. However, like other life history delays, prolonged dormancy may be an adaptation to increase fitness in a variable environment. If so, then plants could be trading off current reproduction and growth for increased survival, leading speculation that prolonged dormancy may act as a bet hedging strategy (Miller et al. 2004, Shefferson et al. 2005, Shefferson 2009, Childs et al. 2010). Though there is empirical evidence that prolonged dormancy may buffer plants from stress above ground (Morrow and Olfelt 2003, Shefferson et al. 2005), my dissertation is the first to test whether prolonged dormancy indeed functions as a bet hedging strategy. In order to do so, prolonged dormancy must occur at a cost to average fitness and must reduce variance in fitness. By doing so, it should increase geometric mean fitness (Seger and Brockman 1987). Thus, prolonged dormancy should be costly in a constant environment and advantageous in a variable one.

In Chapter 3, I test the predictions of bet hedging theory in a long-lived native perennial, *Astragalus scaphoides* using a long term demographic dataset. I use matrix models to compare average fitness, the variance in fitness, and geometric mean fitness between dormancy phenotypes. First, I compared fitness between individuals with the average dormancy phenotype for the population with a hypothetical phenotype in which I removed dormancy. This comparison met all of the predictions of bet hedging. Hypothetical plants without dormancy had lower average fitness, less variance in fitness, and higher stochastic fitness. I then compared fitness among observed dormancy phenotypes in the population. In this comparison, individuals with intermediate levels of dormancy had the highest fitness, and both average and geometric mean fitness were highest for this phenotype. Analysis of lifetime reproductive success confirmed this relationship. Therefore, low levels of dormancy clearly increase fitness in my system, but no cost to average fitness was detected for these phenotypes. This result is not entirely consistent with bet hedging, and suggests that some other mechanism may be at work in my system.

Throughout the rest of my dissertation, I investigate the mechanisms associated with prolonged dormancy. In Chapter 1, I use the long term demographic data to explore the proximate causes and consequences of dormancy for *A. scaphoides*. Results from this study indicate that rates of prolonged dormancy vary among individuals in the population, and are associated with differences in individual histories. Correlations between weather variables and dormancy suggest that dormancy is more common in years with warm, dry springs. Together, these results suggest that the benefits of dormancy depend both on individual state as well as ecological context. I further explore these patterns in subsequent chapters, by studying how

stored resource dynamics and environmental conditions relate to the costs and benefits of remaining belowground during the growing season.

In plants, stored resources integrate the effects of previous history and past environmental conditions with current state (Chapin et al. 1990, Wyka 1999, Crone et al. 2009). Further, stored resources are not only an important metric for individual condition, but can also be an indication of future performance (Chapin et al. 1990). Therefore, I investigated whether stored resources were associated with the entry into prolonged dormancy, and whether dormancy was costly in terms of stored resources. My results show that low levels of stored available carbon (nonstructural carbohydrates, NSC) are associated with entering dormancy, and that dormant individuals increase concentrations of NSC throughout the growing season, presumably by remobilizing structural carbon. Therefore, in the short term, prolonged dormancy does not seem costly in terms of resources. More importantly, these results explain why some individuals remain dormant while others do not, but also provide a mechanism for their return. Therefore, stored resources, particularly carbon, may act as a proximate cue to remain belowground. However, the consequence of remaining belowground should depend on environmental conditions above ground.

Finally, in Chapter 4, I investigate the costs and benefits of prolonged dormancy within an environmental context. So far, my research has indicated that the benefits of dormancy depend on individual state (Ch. 1 and Ch.2), and that dormancy may allow plants to avoid risks in an unpredictable environment (Ch.3). If dormancy allows plants to avoid risky conditions above ground, then I expected to see costs for emerging during unfavorable conditions. First, I measured performance of emergent plants during an exceptionally hot and dry year. Despite stressful conditions, emergent plants were able to maintain physiological performance during the

season and only marginal costs to demographic performance were detected (in terms of future flowering). I then measured performance of emergent plants in response to defoliation. In terms of stored resources, I did not detect a large cost to emerging and experiencing defoliation. However, defoliation resulted in significant costs to future survival and flowering. These results suggest that dormancy may allow plants to avoid risk, but episodic risk (such as defoliation) may be more costly than emerging during hot and dry conditions.

Understanding the evolution of life history strategies requires a comprehensive investigation of the associated trade-offs. Therefore, the study of life history strategies requires incorporation of plant physiology, population ecology, and life history evolution (Obeso, 2002). By using this integrative approach, my research has uncovered both proximate mechanisms influencing prolonged dormancy, as well as the fitness consequences of remaining belowground while other plants emerge. In the proximate sense, stored resources seem to act as a cue that integrates past environmental conditions, previous history, and current condition to determine whether a plant goes dormant or not. Then, the consequences of remaining dormant depend on environmental context. During unfavorable conditions, risking emergence can result in decreased flowering probabilities and even death. These results suggest that prolonged dormancy may be beneficial at times. Finally, my analyses of the lifetime fitness consequences of prolonged dormancy clearly demonstrate that intermediate levels of prolonged dormancy increase fitness. Overall, the significant benefits I have shown explain the prevalence of this stage in the life histories of perennial plants. Further, the combination of ecophysiology and demography, allows for an understanding of the ecological factors driving this life history stage. Such approaches can provide powerful tools to address a multitude of ecological questions, and can result in a comprehensive understanding of the patterns we see in nature.

Literature Cited

Chapin III, F. S., E. Schultze, and H.A. Mooney. 1990. The ecology and economics of storage in plants. Annual Review of Ecology and Systematics 21:423-447.

Charnov, E. L., and W. M. Schaffer. 1973. Life-History Consequences of Natural-Selection - Coles Result Revisited. American Naturalist 107:791-793.

Childs, D. Z., C. J. E. Metcalf, and M. Rees. 2010. Evolutionary bet-hedging in the real world: empirical evidence and challenges revealed by plants. Proceedings of the Royal Society B-Biological Sciences 277:3055-3064.

Cohen, D. 1966. A General Model of Optimal Reproduction in a Randomly Varying Environment. Journal of Ecology 56:219-228.

Cohen, D. 1967. Optimizing reproduction in a randomly varying environment when a correlation may exist between the conditions at the time a choice has to be made and the subsequent outcome. Journal of Theoretical Biology 16:1-14.

Cole, L. C. 1954. The population consequences of life history phenomena. Quarterly Review of Biology 29:103–137.

Crone, E. E., E. Miller, and A. Sala. 2009. How do plants know when other plants are flowering? Resource depletion, pollen limitation and mast-seeding in a perennial wildflower. Ecology Letters 12:1119-1126.

Hutchings, M. J. 1987. The Population Biology of the Early Spider Orchid, *Ophrys-Sphegodes* Mill .1. A Demographic-Study from 1975 to 1984. Journal of Ecology 75:711-727.

Koons, D. N., C. J. E. Metcalf, and S. Tuljapurkar. 2008. Evolution of Delayed Reproduction in Uncertain Environments: A Life-History Perspective. American Naturalist 172:797-805.

Lesica, P., and Brian M. Steele. 1994. Prolonged Dormancy in Vascular Plants and Implications for Monitoring Studies. Natural Areas Journal 14:209-212.

Lesica, P., and E. E. Crone. 2007. Causes and consequences of prolonged dormancy for an iteroparous geophyte, *Silene spaldingii*. Journal of Ecology 95:1360-1369.

Miller, M. T., G.A. Allen, and J.A. Antos. 2004. Dormancy and flowering in two mariposa lilies (*Calochortus*) with contrasting distribution patterns. Canadian Journal of Botany 82:1790-1799.

Morrow, P. A., and Joel P. Olfelt. 2003. Phoenix clones: recovery after long-term defoliation-induced dormancy. Ecology Letters 6:119-125.

Obeso, J. R. 2002. The costs of reproduction in plants. New Phytologist 155:321-348.

Reintal, M., K. Tali, M. Haldna, and T. Kull. 2010. Habitat preferences as related to the prolonged dormancy of perennial herbs and ferns. Plant Ecology 210:111-123.

Seger, J., and Brockmann, H.J. 1987. What is bet-hedging? Pages 182-211 in P. H. Harvey, Partridge, L., editor. Oxford Surveys in Evolutionary Biology. Oxford University Press, Oxford.

Shefferson, R. P., Brett K. Sandercock, Joyce Proper, and Steven R. Beissinger. 2001. Estimating dormancy and survival of a rare herbaceous perennial using mark-recapture models. Ecology 82:145-156.

Shefferson, R. P., Joyce Proper, Steven R. Beissinger, and Ellen L. Simms. 2003. Life history trade-offs in a rare orchid: The costs of flowering, dormancy, and sprouting. Ecology 84:1199-1206.

Shefferson, R. P., Tiiu Kull, and Kadri Tali. 2005. Adult whole-plant dormancy induced by stress in long-lived orchids. Ecology 86:3099-3104.

Shefferson, R. P. 2009. The evolutionary ecology of vegetative dormancy in mature herbaceous perennial plants. Journal of Ecology 97:1000-1009.

Tuljapurkar, S. 1990. Delayed Reproduction and Fitness in Variable Environments. Proceedings of the National Academy of Sciences of the United States of America 87:1139-1143.

Wyka, T. 1999. Carbohydrate storage and use in an alpine population

of the perennial herb*, Oxytropis sericea*. Oecologia 120:198-208.

CHAPTER 1

Patterns of prolonged dormancy in *Astragalus scaphoides*: Inference from long term monitoring

Introduction

Prolonged dormancy is a stage in herbaceous perennial plants in which individuals fail to re-sprout for one or more years, and instead remain alive, below ground (also known as vegetative dormancy and adult whole plant dormancy; Gill 1989; Lesica and Steele 1994; Shefferson 2009). Prolonged dormancy is relatively common and has been seen in many distantly or unrelated species (Lesica and Steele 1994; Shefferson 2009 and references therein), but the causes and consequences of this behavior remain unclear. One reason that prolonged dormancy remains mysterious is that this cryptic life stage can be difficult to study (Shefferson et al. 2001; Shefferson et al. 2003; Kery and Gregg 2003). Since it is impossible to observe plants below ground, distinguishing between dormancy and death is difficult. Further, excavating plants to directly observe them would be likely to have a large effect on plant performance. Finally, prolonged dormancy is most common in long lived perennials (Hutchings 1987; Lesica and Steele 1994; Shefferson 2001; Shefferson 2003; Lesica and Crone 2007; Shefferson 2009), making laboratory or greenhouse studies of prolonged dormancy unrealistic and, in some cases, impossible.

One proposed solution to the problem of understanding what happens below ground is to analyze fates of dormant plants using capture-recapture models (where detection of plants above ground is analogous to "captures" in plant populations; Alexander et al. 1997). Alexander et al.

(1997) used these capture-recapture models to account for prolonged dormancy in estimates of plant population size. Shefferson et al. (2001) then estimated survival and population size of dormancy-prone orchids using standard capture-recapture models that assume all individuals have the same survival probability. While these methods were statistically robust, the biological assumption that all plants, regardless of age or size, have the same survival probability is probably incorrect for many plant populations. Shefferson et al. (2003) later proposed using multistate capture-recapture models to estimate stage-dependent survival and transitions between life stages. Unfortunately, mathematical analysis showed that many of the parameters in these multistate models are not mathematically estimable, including survival of dormant plants (Kery and Gregg 2005). In addition, a recent paper evaluating multistate capture-recapture models for animals with unobservable life stages suggests that, even when parameters are algebraically separable, they may not be estimable for many demographic datasets (Bailey et al. 2010). Therefore, although capture-recapture studies are useful for studying some aspects of prolonged dormancy, available methods do not seem suitable to address many of the interesting ecological and life-history consequences of prolonged dormancy.

Another possible solution to studying prolonged dormancy is to work with very long term data sets. By using long term data, prolonged dormancy can be distinguished from death by detecting whether plants re-emerge in later years. This approach uses a different definition of dormancy than capture-recapture studies, because dormancy is only identified when plants re-emerge. However, the advantage of this approach is that dormancy events are less ambiguous, and it is more straightforward to distinguish dormancy from recruitment and death. Unfortunately, many studies do not have this option because they are short relative to the length of bouts of dormancy, and/or the lifespan of the species being studied (Shefferson et al. 2001;

Shefferson et al. 2003; Shefferson et al. 2005; Shefferson et al. 2006; Miller et al. 2007). However, this approach has been used successfully for longer term studies (Primack and Stacy 1998; Shefferson and Tali 2007; Hutchings 2010), and, with long-term data, can lead to similar conclusions as capture-recapture models (when the two approaches are used to ask the same kinds of questions; Lesica and Crone 2007).

Here, I use a 23-year dataset to explore the causes and consequences of prolonged dormancy in a long lived native perennial, *Astragalus scaphoides*. Through observing plants over long time periods, I have sufficient data to analyze factors associated with prolonged dormancy in this population, as well as the consequences of dormancy over the lifetime of individuals. Specifically I ask: 1) How long do bouts of dormancy last, and is the probability of emergence constant? 2) Does the probability of dormancy vary among individuals? 3) Does the probability of dormancy vary in space? 4) What climatic factors are associated with prolonged dormancy? 5) How does dormancy relate to age? 6) Does previous history affect dormancy transitions? and finally, 7) How does dormancy affect longevity and lifetime reproductive success? I discuss each of these issues in relation to previous research on dormancy, which comes largely from shorter studies, and in relation to the body of my dissertation research.

Field Methods

Study species

Astragalus scaphoides (Fabaceae) is an iteroparous legume with a long, narrow taproot, found on south-facing slopes in high-elevation sagebrush steppe communities in western Montana and eastern Idaho, USA. Median age to first reproduction is 3 years (Lesica 1995).

Plants flower approximately in alternate years (Lesica 1995; Crone and Lesica 2004; Crone et al. 2005). Plants that do not flower may produce leaves and be vegetative, or remain dormant during the growing season. *Astragalus scaphoides* emerges above ground in late April, flowers from late May to mid-June, and seeds usually dehisce by mid-July.

Demographic data collection

Demographic data collection follows the protocol developed by Lesica and Steele (1997). Two monitoring transects were installed by Peter Lesica in 1986 at Sheep Corral Gulch located in Beaverhead County, Montana (45°06'55" N, 113°02'58" W). Monitoring was conducted by P. Lesica from 1986-1998, by P. Lesica and E. Crone from 1999-2005, and by E. Crone and I from 2006 to the present. Data from 1986 to 2008 are analyzed in this chapter, though monitoring continues for this species. Established transects are 1 meter wide belt transects consisting of approximately 50 adjacent 1 m^2 plots. Surveys are conducted in early July of each year, during fruit maturation. Within plots, plants are mapped to the nearest decimeter, and classified into 3 vegetative stages (small, medium and large vegetative), and a flowering stage. The small stage is defined as plants that have less than 6 leaves, medium have more than 6 leaves but no above ground branching, large have more than 6 leaves and branching, and flowering plants have inflorescences. Transitions to and from all of these stages are possible once plants are established (Fig.1). Reproductive plants are also surveyed for number of intact, predated, or aborted inflorescences as well as the number of fruits produced. I ensure detection of all emergent plants by referencing a map of plants known from each plot (i.e., by searching carefully for senesced of small plants in locations where plants have been in the past). Individual plants are identified by overlaying maps through time.

Detecting dormancy

Unambiguous dormancy episodes can be determined by observing plants both before and after a year in which it was not detected above ground (Lesica and Steele 1994; Kery and Gregg, 2004; Lesica and Crone, 2007). For example, consider two five-year histories of plant fates. The first, abbreviated "MF0FM", indicates that in year one the plant was a medium vegetative, it flowered in year two, was not detected above ground in year three, flowered again in year four, and was a medium vegetative in year five. This plant was clearly dormant in year three. In contrast, a different plant history may be "MF0000." In this case, the first two years are the same, but then the plant is not detected above ground. From these data, I cannot determine whether this plant has gone into dormancy in year three and remains undetected, or whether the mortality occurred between years 2 and 3. However, most bouts of dormancy in *A. scaphoides* last less than three years (see below). Therefore, I did not analyze transition rates from the first and last three years of data. In this way, I can separate dormancy from recruitment at the beginning of the study, and mortality at the end of the study (Lesica 1995). By this definition, dormant plants always emerge and thus have perfect survival. The middle 18 years of data from the 23-year monitoring study include individual histories for over 350 plants.

Analyses and results

Length of dormancy and the probability of emergence over time

Life history analyses for plants are often analyzed using Markov models, which mean that the performance of plants depends on their current stage class, but not on previous states (Nichols 1992). In other words, for dormant plants, the probability of emergence would be the

same after one year below ground as after two or more years below ground. If emergence probability is constant, then the length of bouts of dormancy would follow an exponential distribution, which has a defined relationship between the mean and the variance. (It is defined by rate parameter λ, and has mean = $1/\lambda$, and variance = $1/(\lambda^2)$; Hilborn and Mangel 1997). However, if the variance is less than expected based on the mean, then the length of dormancy bouts is more uniform distribution than random (bouts tend to be closer than the average length), and if the variance is more than the expected, then dormancy bouts are more clustered than random (very short or very long bouts of dormancy, but fewer than expected near the mean).

To test this hypothesis, I compiled each dormancy event by length and compared the observed values with those from an exponential distribution using a χ^2 test. In this population, the majority of dormancy events last one year (67%, Fig. 5) and the probability of emergence differs significantly from an exponential distribution (χ^2=99.822, P<0.001). Further, the mean length of bouts of dormancy (1.56 years $\rightarrow \lambda$=0.64) implies greater variance ($1/\lambda^2$ = 2.4) than the observed variance (1.06), which suggesting that dormancy tends to be more uniform than expected by random chance.

Dormancy and previous history

Lack of constant emergence from dormancy suggests that the probability of dormancy depends on previous life stage. Although Markov models are most common, other studies have also shown that, in perennial plants, current condition and previous history may interact to affect vital rates (van Noordwijk and de Jong 1986; Ehrlen 2000; Horvitz et al. 2002). To investigate whether historical effects influence the transition into dormancy in *A. scaphoides*, I tested for the

direct effect of stage in the previous year (t-1), the lagged effect of stage two years previous (t-2), and the interaction of the two using logistic mixed models (function lmer in R, R Foundation for Statistical Computing, 2009), with individual plant as a random effect. I constructed models that nested these independent variables (previous and lagged stage class), and used likelihood ratio tests to assess whether individual history affected dormancy probability, and what aspects of history best explained that variation.

My analyses provide evidence for effects of plant history since both direct and lagged effects of previous stage were significant as well as the interaction of the two (likelihood ratio tests: direct χ^2= 57.852 P<0.001; lagged χ^2= 306.41,..P<0.001; interaction: χ^2= 34.121 P=0.005). Plants that had been dormant for two years were most likely to remain dormant (Table 2). Besides dormant plants, young plants were most likely to transition to dormancy the year after recruitment. Medium and, especially, large vegetative plants were least likely to transition to dormancy (Table 2).

Variation in dormancy among individuals

In prolonged dormancy, usually only a fraction of a population remains dormant, while the rest emerge above ground. This pattern suggests that factors acting at the individual level may be important in this life history stage. Therefore, I tested for variation in dormancy rates among individual plants. If rates of dormancy do not differ among individuals then the probability of dormancy should follow a binomial model, in which the variance is determined by the mean. If rates of dormancy vary by individual, then the probability of dormancy should

follow a beta-binomial distribution, in which dormancy is a binomial process, but the probability of dormancy differs among individual plants.

Therefore, to test whether dormancy varied across individuals, I compared the fit of a beta-binomial model to a binomial model. I fit the beta-binomial model using the "Kendall" function in the popbio package in R (Stubben and Milligan 2007, R Foundation for Statistical Computing, 2009; see Kendall 1998 and Appendix 1 of this dissertation for more details). Since these models are nested, I used likelihood ratios to test for the model that provided a better fit to the data.

Average *A. scaphoides* plants in this population spend almost a quarter of their time dormant (maximum likelihood estimate, MLE, mean = 0.240, 95% CI [0.206, 0.278], see Figure 6). Additionally, the probability of dormancy differs significantly among individuals, since the beta-binomial model provided a better fit (MLE variance = 0.030, 95% CI [0.020, 0.043], likelihood ratio: χ^2= 147.636, P<0.001).

Spatial patterns of dormancy

One possible explanation for variation in the probability of dormancy among individuals is spatial variation, such as differences in microsite conditions. Thus, I tested for spatial variation in dormancy using logistic regression. As measures of location, I included both transect and plot as well as the interaction of the two in these analyses. First, I analyzed the probability of dormancy as a function of plot (1-m^2 sampling quadrats; location in the East-West dimension) and transect (two parallel transect lines, location in the North-South dimension), in

an analysis where both were treated as categorical factors. Dormancy differed as a function of plot and transect (Transect: P_1=0.150; $Plot_{44}$: P<0.001, Plot*$Transect_1$: P=0.007).

To further explore this pattern, I fit polynomial logistic models to the probability of dormancy, using plot as a continuous variable, and testing for interactions between location and transect (still fit as a categorical variable, since there were only two transects). Using stepwise regression (stepAIC function in R, R Foundation for Statistical Computing, 2009) I identified the polynomial terms that best fit the relationship between probability of dormancy and location. This model indicated that dormancy has a non-linear relationship with location in this population (Fig.3). Plants at the western end of the transect were more likely to be dormant than those on the eastern end, and plants were more likely to be dormant in the south transect than in the north transect.

Dormancy and climate

Previous studies suggest that climate factors may be associated with prolonged dormancy, particularly temperature and precipitation (Epling and Lewis 1952; Boeken 1991; Lesica and Steele 1994; Vaughton and Ramsey 2001; Miller et al. 2004; Shefferson et al. 2001, Lesica and Crone 2007). I analyzed the relationship between climatic variables and prolonged dormancy using hierarchical partitioning methods. Climate variables are often correlated with each other, making it difficult to determine the relative importance of any one factor. Therefore, I used hierarchical partitioning to calculate the independent effects of climate variables on the proportion of dormant plants seen each year (Murray and Conner, 2009). For this analysis, I used climate data for 1989-2005 from the University of Montana Western station (NCDC 2010), which is approximately 25 miles from my study site. I tested the effect of monthly climate

variables, as well as the accumulated effect of growing year precipitation. Here, I define the growing year as July of the previous year to June of the current year (Crone and Lesica, 2006). I used an arcsine-square root transformation to normalize the proportion of plants dormant for each year, and removed variables that had low zero order correlations before the final analysis (Murray and Conner 2009).

The proportion of dormant plants was positively associated with warm, dry weather during the growing season (Fig.2). Climate variables with the highest independent effects were average May temperature, average June temperature, precipitation in June, and total growing season precipitation (Table 1). Generally, dormancy was positively associated with these temperature variables, and negatively correlated with precipitation.

Dormancy and age

In plants, size is much more often used as a metric to predict future performance than age (Menges 2000; Ehrlen and Lehtila 2002), so most studies on prolonged dormancy have focused on the relationship between above-ground size and dormancy. However, other studies have suggested that relationships may exist between prolonged dormancy and age (Shefferson et al. 2006; Shefferson and Tali 2007; Shefferson 2009). Therefore, I investigated the relationship between age and dormancy for *A. scaphoides*, using only data for plants that recruited during the study period. By doing so, I could unambiguously assign ages to these plants.

I used quadratic logistic regression to test for relationships with age. Prolonged dormancy initially declined with age, but then increased as plants get older (Fig. 4). Both the linear and quadratic effects of time were statistically significant (Linear effect: z=-4.292,

P<0.001; quadratic: z= 3.840 P<0.001), creating a u-shaped relationship between dormancy and age.

Relationship of dormancy with lifespan and reproductive success

Prolonged dormancy may be a way in which plants avoid stress (Shefferson 2009), but the delay of growth and reproduction that occurs during prolonged dormancy could have large effects on plant fitness (Tuljapurkar 1990; Shefferson 2009). Therefore, I explored how dormancy relates to longevity and lifetime reproductive success. To test these relationships, I used data for those individuals that completed their life cycle during the study. By using only those individuals that recruited and died during the study, I could analyze the total lifetime impact of dormancy on fitness components. Lifespan, then, was simply the number of years these individuals were present in the study. Lifetime reproductive success was calculated as the total number of fruits produced per individual. I tested for both linear and non-linear associations using a negative binomial distribution for both lifespan and reproductive success.

Prolonged dormancy had a significant non-linear relationship with reproductive success (Fig. 7; linear effect: z=2.01, P=0.044; quadratic effect: z=-3.375, P<0.001). Reproductive success increased with dormancy at low dormancy rates, declined once plants begin to spend more than 20% of their time in dormancy. The overall relationship between lifespan and dormancy was negative (Fig. 7), but not significantly so (z = -1.612, P=0.107).

Discussion

My analysis of a long term dataset reveals some causes and consequences of prolonged dormancy that could be inferred from short term studies, but many that are only possible to observe through long term monitoring. For example, the rate of emergence from prolonged dormancy seems to depend on how long plants have been dormant. In shorter term datasets, this would be difficult to detect. Further, it shows that the assumption of many demographic models, that dormant plants all have the same transition rates, does not hold. This assumption is central to analysis of prolonged dormancy with capture-recapture models, emphasizing that these models would not be appropriate for this species. Therefore, throughout this dissertation, I continue to define prolonged dormancy as a phenomenon in which plants remain alive below ground and later re-emerge.

My results indicate that the probability of dormancy differs among individual plants, and that previous history had significant effects on the probability of dormancy. These results are consistent with findings in Chapter 2 of this dissertation (Gremer et al. 2010), which indicates that plants that go dormant are a non-random subset of individuals that have low levels of stored resources, particularly stored available carbon (nonstructural carbohydrates, *NSC*). Therefore, individual variation that leads to differences in stored resources may be an important driver in this life history stage. Furthermore, Chapter 2 suggests that dormant plants increased concentrations of NSC while dormant, presumably by remobilizing structural carbon into available forms. If this remobilization takes approximately one growing season to occur, then it makes sense for most bouts of dormancy to last one year. Alternatively, it could be that dormancy bouts lasting more than one year are more costly than shorter bouts. Dormant plants must maintain metabolic costs to remain alive while belowground, and these costs may become

increasing difficult to offset if plants spend more than one year without emerging above ground. Therefore, it may be less likely for plants to emerge if they need to remain below ground for more than one year.

Analysis of the long-term dataset also shows that variance in the amount of dormancy among individual plants is related to differences in fitness. In *A. scaphoides*, reproductive success initially increased with amount of time spent dormant, but eventually declined. This pattern suggests that intermediate levels of dormancy maximize fitness. Other studies on prolonged dormancy have found both negative relationships (Shefferson et al. 2003) and positive relationships (Lesica and Crone 2007) with reproduction, usually defined as fruit set or probability of flowering, rather than lifetime reproductive success. I explore relationships between dormancy and fitness further in Chapter 3 of this dissertation, using stochastic demography. Results from Chapter 3 also indicate that intermediate levels of dormancy had the highest fitness benefit, since plants that spent approximately 20% of their time dormant had the highest total fitness. Thus, it seems that some level of dormancy is beneficial for *A. scaphoides*. However, at high levels of dormancy, the demographic cost of missing multiple seasons of growth and reproduction may outweigh those benefits. Therefore, intermediate levels of dormancy may confer the highest fitness advantage, and may represent the point at which the benefits of remaining belowground outweigh the cost of missing seasons of growth and reproduction.

A third result in this study is more consistent with other studies of prolonged dormancy. I found that dormancy was positively related to warm and dry temperatures during the growing season. Many other studies have found similar results (Epling and Lewis 1952; Boeken 1991; Lesica and Steele 1994; Vaughton and Ramsey 2001), though some others did not (Miller et al.

2004; Shefferson et al. 2001). For instance, Shefferson et al. (2001) found that low temperatures in the spring increased dormancy, and Miller et al. (2004) observed higher dormancy fractions in wetter years. If prolonged dormancy is a strategy to avoid environmental stress (Lesica and Steele 1994; Miller et al. 2004; Shefferson et al. 2005; Lesica and Crone 2007; Shefferson 2009; Gremer et al. *in prep,* Chapter 3 of this dissertation) then plants that remain below ground during unfavorable conditions should perform better than those that emerge. In Chapter 4 of this dissertation, I investigate this possibility by comparing the physiological and demographic performance of dormant and emergent plants during times of stress. My results support this hypothesis that dormancy is a mechanism for stress avoidance, since plants that emerged during unfavorable conditions suffered higher mortality following extreme stress.

My analysis of long-term demography also revealed two patterns that I do not address further in this dissertation. First, variation in prolonged dormancy among individuals was at least partly spatially correlated within monitoring transects. This pattern of dormancy may reflect differences in environmental conditions within the study site. However, if dispersal distances of *A. scaphoides* seeds are low, then individuals in closer proximity to each other may be more closely related. If so, then the spatial gradient in dormancy could reflect relatedness among individuals that are more prone to dormancy. Vaughton and Ramsey (2001) showed a genetic basis for non-emergence in *Burchardia umbellata*, but the genetic basis for prolonged dormancy in *Astragalus scaphoides* is not known. Distinguishing between genetic and environmental drivers for this spatial gradient is beyond the scope of this dissertation, but it could be an interesting area for future research. Second, I found a significant non-linear relationship between dormancy and plant age. In *A. scaphoides*, prolonged dormancy initially declines with age, but then increases as plants get older. Jakalaniemi et al. (*in revision*)

observed a similar pattern in a northern orchid, and Shefferson (2009) suggested that dormancy should be common when plants are young, become less frequent as plants mature, and eventually become more likely as plants age and reach senescence. Studies comparing dormancy in long and short lived species suggest that age may be an important factor in shaping patterns of dormancy in natural populations (Shefferson 2006; Shefferson and Tali 2007; Shefferson 2009). The relationship between dormancy and age could also partly explain the relationship between dormancy and lifetime reproductive success. If young plants are most likely to become dormant, then plants that die before reaching reproductive maturity would have high dormancy. Further study and experimentation may lend insight into the causal relationships among dormancy, age, and lifetime reproductive success.

Together, these results suggest that the benefits of dormancy are context dependent, and are caused by both individual and environmental variation. Further, my analyses indicate that both endogenous factors (such as age, previous history, and stage) and exogenous factors (such as climate and spatial variation) may interact to determine patterns of prolonged dormancy. Therefore, understanding the causes and consequences of this cryptic life stage requires consideration of both individual and environmental variation. Additionally, these analyses highlight both the strengths and limitations of inference from long term studies. In other chapters of this dissertation, I complement this demographic dataset with physiological and experimental studies. By doing so, I can gain a more comprehensive understanding of the role of this puzzling stage in the life histories of perennial plants. This type of integrative approach can lend insight into both the proximate and ultimate mechanisms leading to the patterns seen in nature, yet this approach is rarely used to address questions in ecology and evolution. Thus, I hope my research will not only inform the causes and consequences of prolonged dormancy *per*

se, but also to contribute to a framework for integrating mechanistic experiments focused on individual performance with long-term population and evolutionary dynamics..

Literature Cited

Alexander, H. M., N. A. Slade, and W. D. Kettle. 1997. Application of mark-recapture models to estimation of the population size of plants. Ecology 78:1230-1237.

Bailey, L. L., S. J. Converse, and W. L. Kendall. Bias, precision, and parameter redundancy in complex multistate models with unobservable states. Ecology 91:1598-1604.

Boeken, B. 1991. Aboveground emergence in the desert tulip *Tulipa-Systola* Stapf in the Negev Desert of Israel. Functional Ecology 5:705-712.

Crone, E. E. and P. L. 2004. Causes of synchronous flowering in *Astragalus scaphoides*, an iteroparous perennial plant. Ecology **85**:1944-1954.

Crone, E. E., L. Polansky, and P. Lesica. 2005. Empirical models of pollen limitation, resource acquisition, and mast seeding by a bee-pollinated wildflower. American Naturalist 166:396-408.

Crone, E. E., and P. Lesica. 2006. Pollen and water limitation in *Astragalus scaphoides*, a plant that flowers in alternate years. Oecologia 150:40-49.

Ehrlen, J. 2000. The dynamics of plant populations: Does the history of individuals matter? Ecology 81:1675-1684.

Ehrlen, J., and K. Lehtila. 2002. How perennial are perennial plants? Oikos 98:308-322.

Epling, C., and H. Lewis. 1952. Increase of the adaptive range of the genus *Delphinium.* Evolution 6:253-267.

Gill, D. S. 1989. Fruiting failure, pollinator efficiency, and speciation in orchids. Pages 458-481 in D. O. a. J. A. Endler, editor. Speciation and its consequences. Sinauer Associates Inc., Sunderland, MA USA.

Gremer, J. R., A. Sala, and E. E. Crone. 2010. Disappearing plants: why they hide and how they return. Ecology 91:3407-3413.

Hilborn, R., and M. Mangel. 1997. The ecological detective : confronting models with data. Princeton University Press, Princeton, NJ USA.

Horvitz, C. C., and Douglas W. Schemske. 2002. Effects of plant size, leaf herbivory, local competition, and fruit production on survival, growth, and future reproduction of a neotropical herb. Journal of Ecology 90:279-290.

Hutchings, M. J. 1987. The population biology of the early spider orchid, *Ophrys sphegodes* Mill II, temporal patterns in behaviour. Journal of Ecology 75:729-742.

Hutchings, M. J. 2010. The population biology of the early spider orchid *Ophrys sphegodes* Mill. III. Demography over three decades. Journal of Ecology **98**:867-878.

Jäkäläniemi, A., E. E. Crone, P. Närhi, and J. Tuomi. *unpublished manuscript*. Stage-dependent benefits of prolonged dormancy in a perennial orchid (in revision for submission to *Ecology*).

Kendall, B. E. 1998. Estimating the magnitude of environmental stochasticity in survivorship data. Ecological Applications 8:184-193.

Kery, M., and K. B. Gregg. 2003. Effects of life-state on detectability in a demographic study of the terrestrial orchid *Cleistes bifaria*. Journal of Ecology 91:265-273.

Kery, M., and K. B. Gregg. 2004. Demographic analysis of dormancy and survival in the terrestrial orchid *Cypripedium reginae*. Journal of Ecology 92:686-695.

Kery, M., K. B. Gregg and Michael Schaub. 2005. Demographic estimation methods for plants with unobservable life-states. Oikos 108:307-320.

Lesica, P., and B. M. Steele. 1994. Prolonged dormancy in vascular plants and implications for monitoring studies. Natural Areas Journal 14:209-212.

Lesica, P. 1995. Demography of *Astragalus scaphoides* and effects of herbivory on population growth. Great Basin Naturalist 55:142-150.

Lesica, P., and B. M. Steele. 1997. Use of permanent plots in monitoring plant populations. Natural Areas Journal 17:331-340.

Lesica, P., and E. E. Crone. 2007. Causes and consequences of prolonged dormancy for an iteroparous geophyte, *Silene spaldingii*. Journal of Ecology 95:1360-1369.

Menges, E. S. 2000. Population viability analyses in plants: challenges and opportunities. Trends in Ecology & Evolution 15:51-56.

Miller, M. T., G.A. Allen, and J.A. Antos. 2004. Dormancy and flowering in two mariposa lilies (*Calochortus*) with contrasting distribution patterns. Canadian Journal of Botany 82:1790-1799.

Miller, M. T., J. A. Antos, and G. A. Allen. 2007. Demographic differences between two sympatric lilies (*Calochortus*) with contrasting distributions, as revealed by matrix analysis. Plant Ecology 191:265-278.

Murray, K., and M. M. Conner. 2009. Methods to quantify variable importance: implications for the analysis of noisy ecological data. Ecology 90:348-355.

National Climate and Data Center (NCDC). http://cdo.ncdc.noaa.gov/ancsum /ACS?coban=242409. Accessed March 2010.

Nichols, J. D. 1992. Capture-Recapture Models. Bioscience **42**:94-102.

Primack, R., and E. Stacy. 1998. Cost of reproduction in the Pink Lady's Slipper Orchid (*Cypripedium acaule*, Orchidaceae): and Eleven-Year experimental study of three populations. American Journal of Botany 85:1672-1679.

Shefferson, R. P., B. K. Sandercock, J. Proper, and S. R. Beissinger. 2001. Estimating dormancy and survival of a rare herbaceous perennial using mark-recapture models. Ecology 82:145-156.

Shefferson, R. P., J. Proper, S. R. Beissinger, and E. L. Simms. 2003. Life history trade-offs in a rare orchid: The costs of flowering, dormancy, and sprouting. Ecology 84:1199-1206.

Shefferson, R. P., Tiiu Kull, and Kadri Tali. 2005. Adult whole-plant dormancy induced by stress in long-lived orchids. Ecology 86:3099-3104.

Shefferson, R. P., T. Kull, and K. Tali. 2006. Demographic response to shading and defoliation in two woodland orchids. Folia Geobotanica 41:95-106.

Shefferson, R. P., and K. Tali. 2007. Dormancy is associated with decreased adult survival in the burnt orchid, *Neotinea ustulata*. Journal of Ecology 95:217-225.

Shefferson, R. P. 2009. The evolutionary ecology of vegetative dormancy in mature herbaceous perennial plants. Journal of Ecology 97:1000-1009.

Stubben, C. J., and B. G. Milligan. 2007. Estimating and Analyzing Demographic Models Using the popbio Package in R. Journal of Statistical Software 22(11).

Tuljapurkar, S. 1990. Delayed reproduction and fitness in variable environments. Proceedings of the National Academy of Sciences of the United States of America 87:1139-1143.

van Noordwijk, A. J., and G. de Jong. 1986. Acquisition and allocation of resources - Their influence on variation in life-history tactics. American Naturalist 128:137-142.

Vaughton, G., and M. Ramsey. 2001. Variation in summer dormancy in the lilioid geophyte *Burchardia umbellata* (Colchicaceae). American Journal of Botany 88:1223-1229.

Table 1. The relative importance of climate variables in explaining variation in prolonged dormancy. The first two columns give the results of a hierarchical partitioning analysis that quantifies the independent effect of variables after accounting for correlations between variables (see Methods). The final two columns give the results from linear regressions, including the coefficient of determination (R^2) and p values. Variables that had zero-order correlations near zero were removed from the final analysis (Murray and Conner, 2009). Variables are listed in order of relative importance.

	Independent effect	Independent Percent	r	R^2	p
May Temperature	0.186	41.9%	0.520	0.270	0.027
June Temperature	0.125	28.3%	0.473	0.224	0.048
May Precipitation	0.075	17.0%	-0.363	0.168	0.139
Growing Year Precipitation	0.057	12.8%	-0.410	0.132	0.091

Table 2. The probability of dormancy as a function of previous stage. The first column is the state two years previous (t-2) and the second column is the stage in the previous year (t-1). I used 3 vegetative classes (S= small, M= medium, and L= large), a flowering stage class (F), and a dormant stage (D). Dormancy probability was estimated using generalized linear mixed models (see *Methods*). Stage class histories are listed in order of dormancy probability.

Statet-2	Statet-1	Probability of dormancy	Std. Error
Dormant	Dormant	0.596	0.541
Recruit	Flowering	0.514	0.809
Recruit	Small	0.372	0.542
Recruit	Medium	0.345	0.591
Small	Dormant	0.313	0.551
Recruit	Large	0.304	0.778
Large	Small	0.286	0.645
Large	Dormant	0.279	0.596
Flowering	Flowering	0.274	0.599
Medium	Dormant	0.249	0.573
Flowering	Dormant	0.235	0.603
Small	Large	0.230	0.608
Medium	Large	0.196	0.585
Small	Medium	0.181	0.562
Medium	Flowering	0.178	0.563
Dormant	Large	0.167	0.624
Medium	Small	0.161	0.580
Small	Small	0.156	0.552
Dormant	Small	0.148	0.581
Large	Large	0.139	0.621
Small	Flowering	0.136	0.603
Dormant	Medium	0.135	0.587
Flowering	Small	0.117	0.633
Dormant	Flowering	0.112	0.620
Large	Medium	0.104	0.632
Large	Flowering	0.103	0.595
Flowering	Large	0.099	0.609
Medium	Medium	0.089	0.585
Flowering	Medium	0.064	0.617

Figure Legends

Figure 1. Annual life cycle diagram for *Astragalus scaphoides*. I used 3 vegetative classes (S= small, M= medium, and L=Large), a flowering stage class (F), and a dormant stage (D). Magnitude of transitions are indicated by thickness of arrows. Recruitment is indicated by dashed line from F to S (line not to scale).

Figure 2. Distribution of dormancy bouts by length. Bars represent the observed lengths of dormancy bouts in the long term dataset. The dashed line represents values predicted under the exponential distribution. Most dormancy events last 1 year, and the probability of emergence varies with how long plants have been belowground.

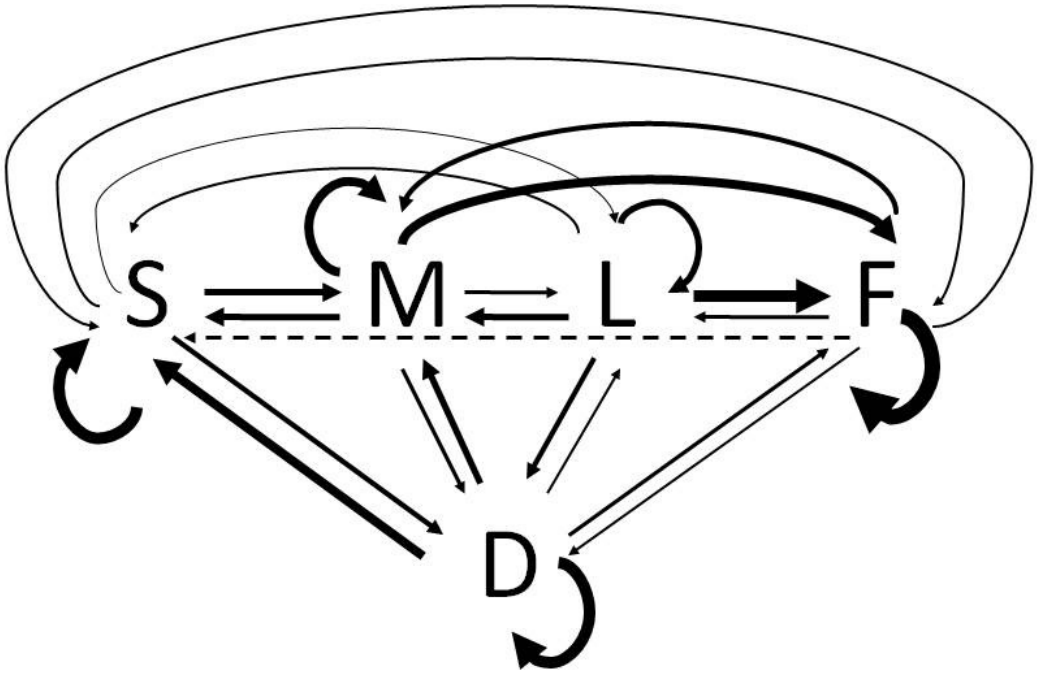
Figure 3. Histogram of proportion of time spent dormant. Solid bars represent observed values, while the dashed line represents values expected under a binomial distribution. The majority of plants spend 24% of their time in dormancy, but varied significantly among individuals.

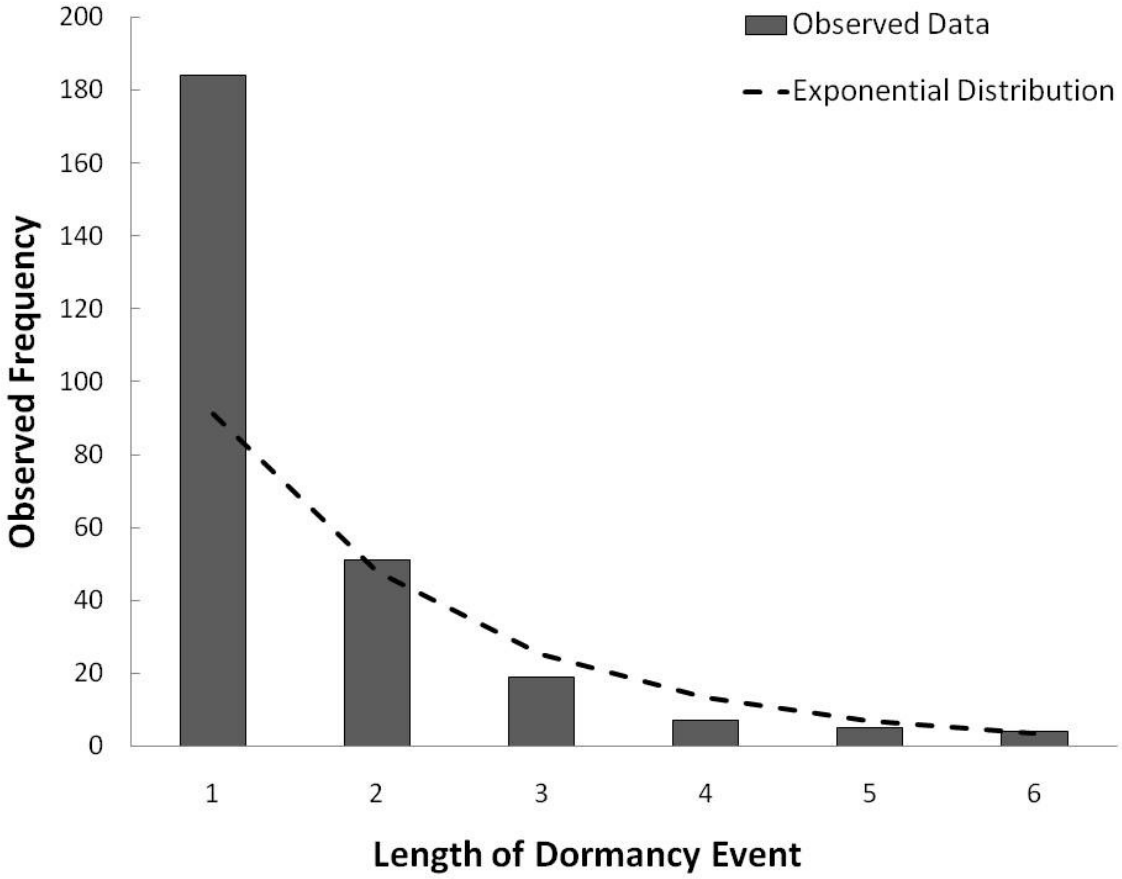
Figure 4. Spatial variation of prolonged dormancy by transect at Sheep Corral Gulch, MT. Lines represent the best fit models from stepwise regression. Dashed line indicates the probability of dormancy by plot on the upper transect while the solid line represents the lower transect.

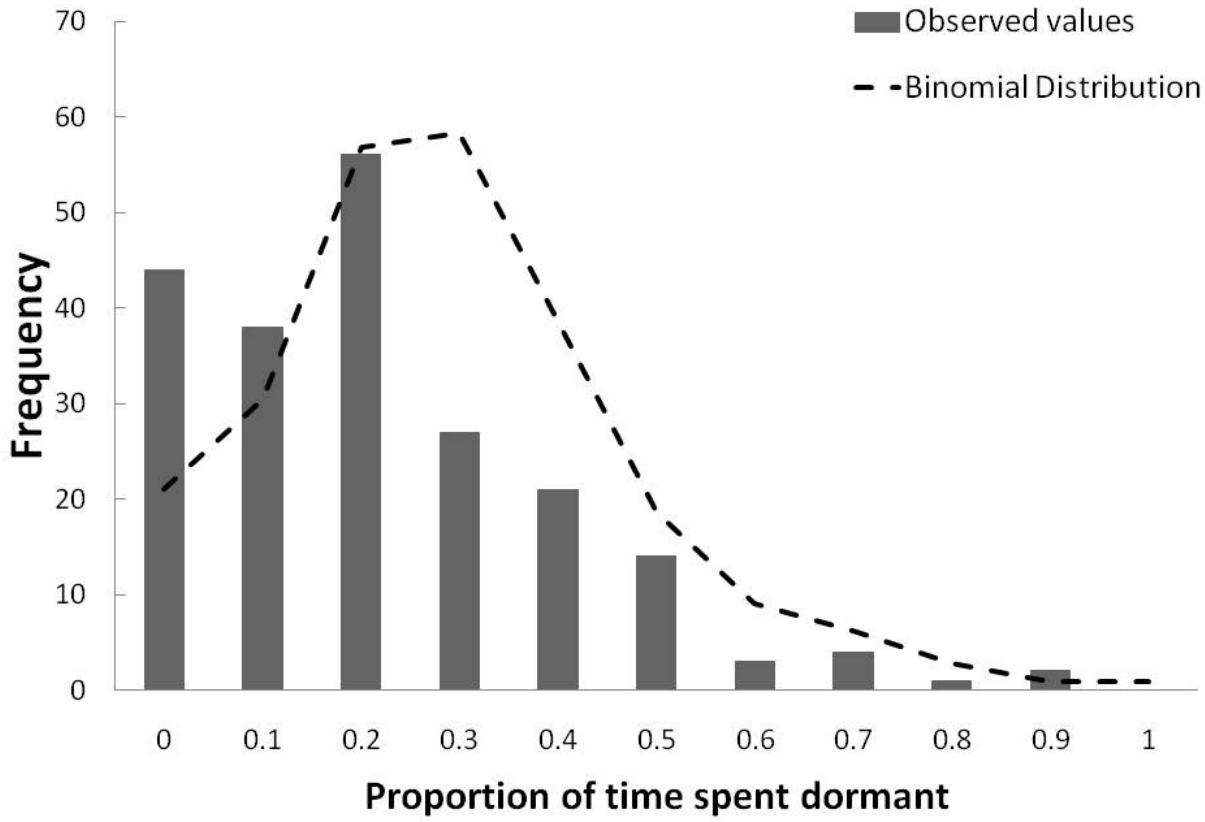
Figure 5. Prolonged dormancy in relation to climate variables. The proportions of dormant plants were arcsine square-root transformed to normalize the data. Correlation coefficients (r) and p-values are indicated on each graph. Dormancy is positively associated with temperature and negatively associated with precipitation.

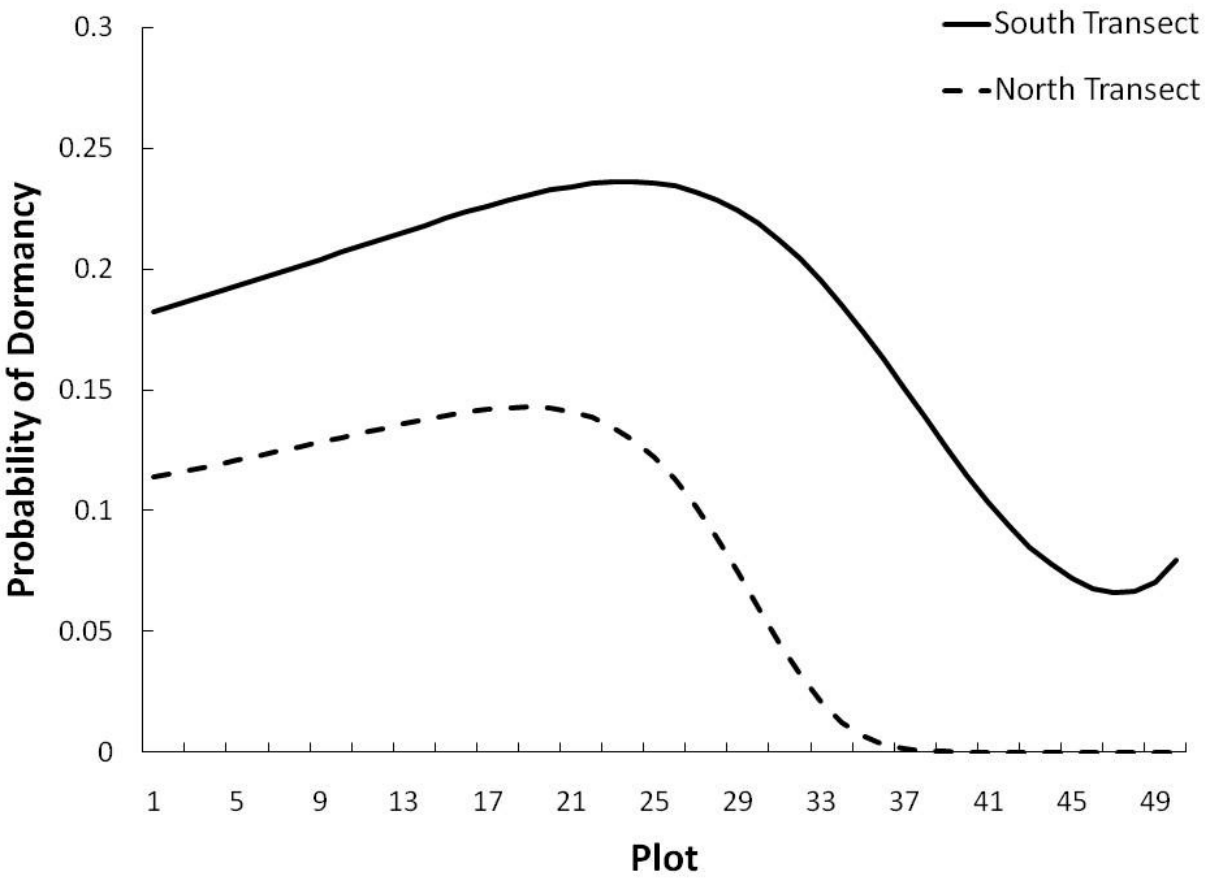
Figure 6. Prolonged dormancy as a function of age in *Astragalus scaphoides*. Dots represent the observed data while the solid line represents the predicted probability of dormancy by age from logistic regression. Younger and older plants are more likely to enter dormancy.

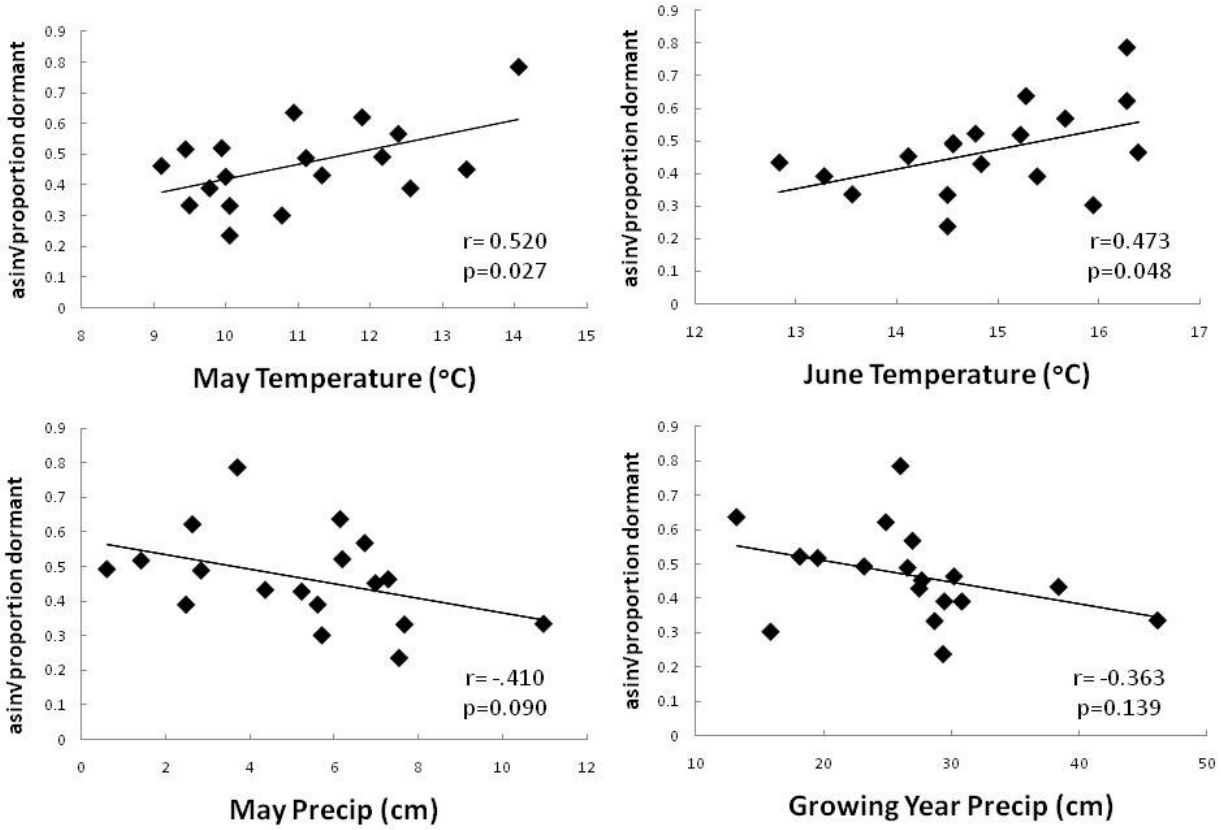
Figure 7. Fitness metrics in relation to prolonged dormancy. A) Lifespan as a function of prolonged dormancy and B) Total reproductive success, in terms of fruits produced, as a function of dormancy. Points represent observed data, solid lines represent regression lines. Dormancy is negatively associated with lifespan and reproductive success, but maximum values occur at intermediate levels of dormancy.

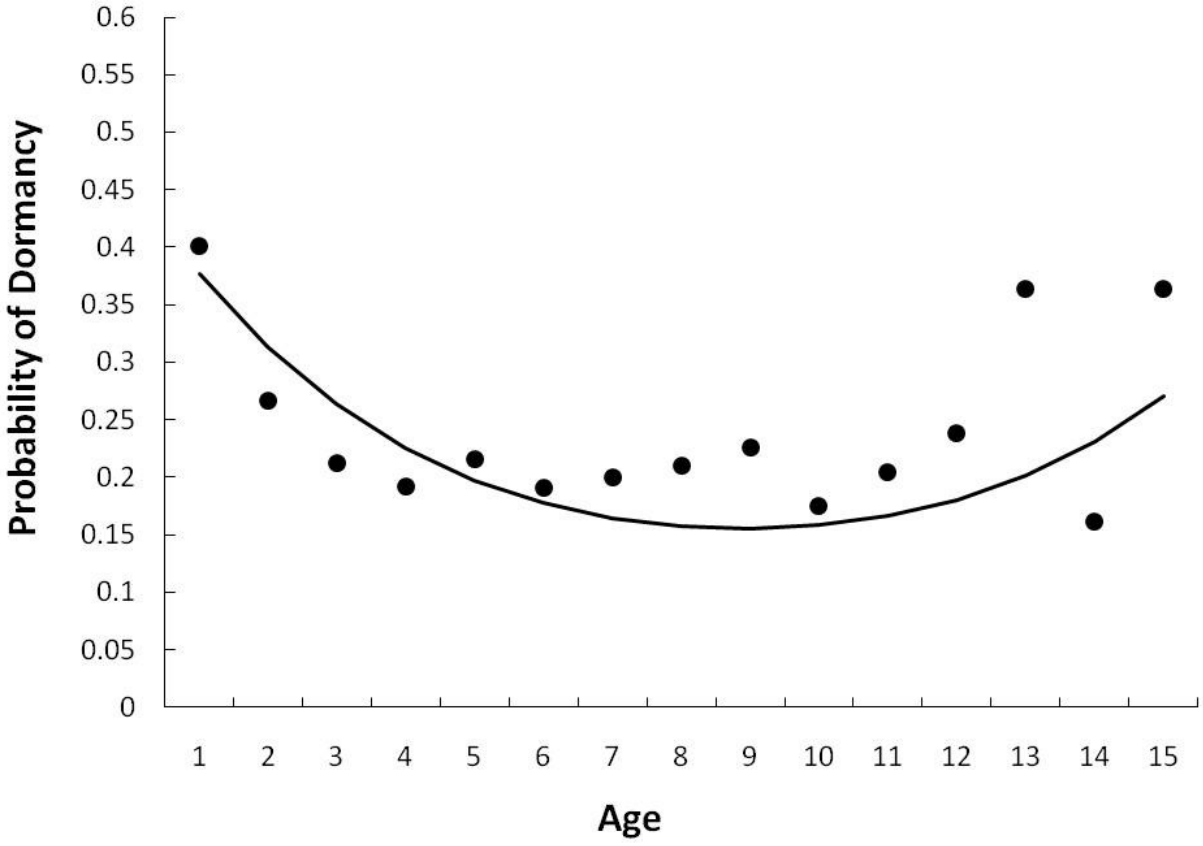

S
M
L
F
D

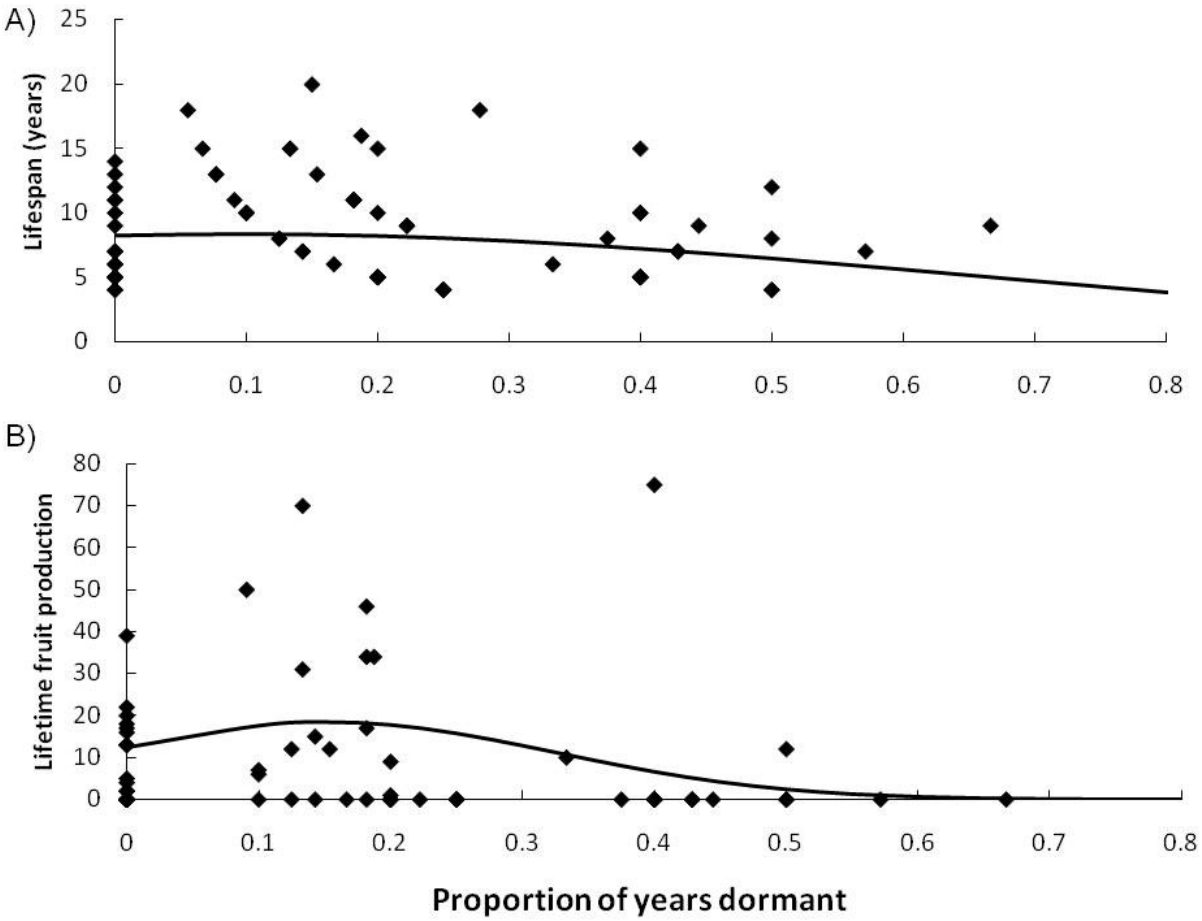

Observed Data
Exponential Distribution
Observed Frequency
200
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
Length of Dormancy Event


Observed values
Binomial Distribution
Frequency
70
60
50
40
30
20
10
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Proportion of time spent dormant


South Transect
North Transect
Probability of Dormancy
0.3
0.25
0.2
0.15
0.1
0.05
0
1
5
9
13
17
21
25
29
33
37
41
45
49
Plot


asinVproportion dormant
r= 0.520
p=0.027
May Temperature (°C)
r=0.473
p=0.048
June Temperature (°C)
r= -.410
p=0.090
May Precip (cm)
r= -0.363
p=0.139
Growing Year Precip (cm)


Probability of Dormancy
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Age


A)
Lifespan (years)
25
20
15
10
5
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
B)
Lifetime fruit production
80
70
60
50
40
30
20
10
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Proportion of years dormant

CHAPTER 2

Disappearing plants: Why they hide and how they return.

Abstract

Prolonged dormancy is a life history stage in which mature plants fail to resprout for one or more growing seasons and instead remain alive belowground. Prolonged dormancy is relatively common, but the proximate causes and consequences of this intriguing strategy have remained elusive. In this study, I tested whether stored resources are associated with remaining belowground, and investigated the resource costs of remaining belowground during the growing season. I measured stored resources at the beginning and end of the growing season in *Astragalus scaphoides*, an herbaceous perennial in Southwest Montana. At the beginning of the growing season, dormant plants had lower concentrations of stored mobile carbon (nonstructural carbohydrates, NSC) than emergent plants. Surprisingly, during the growing season, dormant plants gained as much NSC as photosynthetically active plants, an increase most likely due to remobilization of structural carbon. Thus, low levels of stored NSC were associated with remaining below ground, and remobilization of structural carbon may allow for dormant plants to emerge in later seasons. The dynamics of NSC in relation to dormancy highlights the ability of plants to change their own resource status somewhat independently of resource assimilation, and the importance of considering stored resources in understanding plant responses to the environment.

Introduction

Prolonged dormancy is a relatively common stage in herbaceous perennial plants in which mature plants remain belowground during one or more entire growing seasons instead of emerging to grow and acquire resources (Lesica and Steele, 1994). Prolonged dormancy has been most frequently observed in the Orchid family, but it has been reported in over 10 plant families and 52 species of plants, suggesting that it is a strategy that has evolved many times (Shefferson, 2009; Lesica and Steele, 1994). Despite the fact that prolonged dormancy is relatively common, the causes and consequences for this behavior remain unclear. Why do some plants forego the opportunity to grow and reproduce, while others resume seasonal activity? Here I investigate the proximate causes and consequences of prolonged dormancy in a long-lived native perennial, *Astragalus scaphoides*.

Prolonged dormancy (also known as vegetative dormancy, see Lesica and Steele, 1994 and Shefferson, 2009) is different from other, more extensively studied types of plant dormancy. The metabolic costs of maintaining mature plant parts below ground during prolonged dormancy are likely higher than costs of seed dormancy. Further, in contrast to seasonal dormancy, where all individuals go dormant, prolonged dormancy often involves only a fraction of individuals in any given year. The lack of photosynthesis and reproduction by individuals undergoing prolonged dormancy could have large negative fitness impacts. However, the prevalence of this strategy suggests either neutral or even positive effects. For instance, prolonged dormancy may allow individuals to avoid large resource demands or risks (e.g. biotic or abiotic stress) associated with growing above ground tissues (Shefferson, 2009). To date, little is known what causes certain individuals to remain belowground while others are able to grow and reproduce above ground, or about the costs and benefits of doing so.

Two leading hypotheses have been proposed to explain prolonged dormancy: a) it occurs in response to external cues, such as herbivory or drought (Morrow and Olfelt, 2003; Shefferson et al., 2003; Miller et al., 2004; Shefferson et al., 2005a; Lesica and Crone, 2007), and b) plants remain below ground because they lack resources to build leaves (Shefferson et al., 2005; Shefferson et al., 2006). If prolonged dormancy occurs in response to some critical limiting resource, then plants must gain this resource while dormant so they can emerge in later years. However, plants typically lose stored resources during the non-growing season due to metabolic demands and lack of photosynthesis (Wyka, 1999). Prolonged dormancy could incur a similar or even greater resource cost. Plants that are dormant during the summer miss a season of carbon gain through photosynthesis, and likely lose even more carbon to respiration in summer than in winter due to higher soil temperatures (Amthor, 2000). If so, prolonged dormancy would be a costly life stage because plants need remaining stored resources to survive another winter, as well as to initiate seasonal growth and reproduction. Such resource costs could have significant impacts on future performance and, ultimately, fitness. It is possible that metabolism during prolonged dormancy may fundamentally differ from dormancy during winter and drought, or that individuals that remain dormant during the growing season may gain resources through belowground processes. To date, no one has tested these alternatives by directly measuring the dynamics of stored resources in dormant plants.

I investigated the causes and consequences of prolonged dormancy by measuring stored resources in individual plants at different life history stages for a long-lived perennial wildflower, *Astragalus scaphoides.* I emphasized stored resources not only because they are a component of one of the leading hypotheses for prolonged dormancy but also because they reflect current condition as well as integrate past performance such as resource capture and

allocation to various life history functions (Chapin et al., 1990, Crone et al. 2009). Stored resources are critical for numerous plant functions, including plant growth following winter dormancy, reproduction, recovery from herbivory, and survival (Mooney and Hays, 1973; Ho and Rees, 1976; Chapin et al., 1990; Boyce and Volenec, 1992; Zimmerman and Whigham 1992, Van der Heyden and Stock, 1996; Kobe, 1997; Wyka, 1999). Furthermore, stored resources play critical roles in signaling pathways that control plant growth and development (Halford and Paul 2003, Rolland et al., 2006; Lee et al., 2007). Here, I asked: 1) are stored resources associated with the entry into prolonged dormancy? and 2) what are the resource consequences of remaining belowground during otherwise favorable conditions? If prolonged dormancy is associated with stored resources, I expect that dormant plants will be lacking in one or more stored resources at the beginning of the growing season. If prolonged dormancy is similar to other types of dormancy, such as winter dormancy, I expect dormant plants to deplete stored resources over the growing season. However, if prolonged dormancy differs fundamentally from other types of dormancy, dormant plants may be able to conserve or acquire stored resources during the growing season.

Methods

Study species

Astragalus scaphoides (Fabaceae) is an iteroparous legume with a long, narrow taproot, found on south-facing slopes in high-elevation sagebrush steppe communities (Lesica, 1995). It has an estimated life span of 21 years (Ehrlen and Lehtila, 2002), and does not reproduce vegetatively (Lesica, 1995). On average, 20% of the individuals in this population are dormant in any given year (Crone and Lesica, 2004) and dormancy events typically last one year.

Dormancy is weakly correlated ($0.2 < r < 0.3$) with warm, dry weather in the spring (J. Gremer, unpublished analyses). However, even in years of high dormancy, only a portion of individuals remain dormant while the rest emerge as vegetative or reproductive plants. Plants flower approximately in alternate years (Lesica, 1995; Crone et al., 2005), and this strategy is driven by fluctuations in stored resources rather than changes in climate (Crone et al., 2005; Crone et al 2009). Plants that do not flower may produce leaves and be vegetative, or remain dormant during the growing season.

If plants emerge aboveground, they initiate growth in April, and biomass senesces back to perennating roots in early July. Mature dormant plants can be located by dried flowering stalks that persist above ground for 2-3 years. Evidence of previous flowering events can be seen on root crowns, because the flowering stalks leave scars that are apparent even after several years.

Harvests

Sampling took place from 2006 – 2008 at Sheep Corral Ridge, located in Beaverhead County in southwestern Montana (45°06'55" N, 113°02'58" W). The climate is semi-arid; mean annual precipitation is 250 mm, with peak rainfall in May (Crone and Lesica, 2006). The 10 upper cm of taproot (closest to the soil surface) from randomly selected dormant, vegetative, and reproductive plants (n= between 5 and 7 per life stage) were destructively harvested in early May each year, as soon as the three stages could be clearly distinguished. In 2007 and 2008, roots were also harvested at the end of the growing season in July, after aboveground biomass of emergent plants had senesced. While I could not control for the age of dormant plants, I harvested only reproductively mature individuals (as evidenced on root crown, see *Study*

Species). Because it is not possible to sample these narrow tap roots non-destructively, the fate of harvested plants could not be followed through time.

Astragalus scaphoides plants emerge over several weeks in April. Dormant, vegetative, and reproductive plants can be clearly distinguished from each other in early May, when any plants that have not initiated growth will remain dormant. Since stored resource dynamics may not only vary in dormant plants, but are also expected to vary depending on whether plants are reproductive or vegetative, I conducted harvests in early May to compare stored resources among the three life stages. However, by early May, plants have already grown a bit and may have assimilated carbohydrates through photosynthesis. To account for potential changes in stored carbon from the time of early emergence to the time when reproductive plants start to develop flower buds, I conducted a "pre-season" harvest of plants starting to emerge in April of 2007 (hereafter, emergents). All samples were stored on ice for transport to the laboratory. Roots were analyzed for non-structural carbohydrates (NSC), nitrogen (N), and phosphorus (P). In 2007 and 2008, dormant roots were analyzed for total carbon, which includes both structural and mobile carbon compounds. Root tissue was analyzed for total carbon in 2008.

Immediately upon arrival in the laboratory, samples were heated in a microwave oven at 600 W for 60 seconds to denature enzymes. Samples were then oven dried to constant mass at 75ºC, ground to a fine powder and stored at 4ºC. Total nonstructural carbohydrates (NSC) analyses were performed, following methods described in Hoch *et al.* (2002). In brief, a subsample of extract from boiled and centrifuged ground material is treated with isomerase and invertase to convert sucrose and fructose into glucose. The total amount of glucose (total free sugars) is then determined photometrically in a 96 well plate reader, after enzymatic conversion to gluconate-6-P. The remainder extract is incubated with a dialysed crude fungal amylase to

break down starch to glucose. Glucose is then determined as above. Starch is the difference of NSC minus free sugars. Ground, dried roots were sent to the Stable Isotope Laboratory, University of California Davis, USA for analyses of N concentrations. There, samples were combusted at 1020°C in a reactor and nitrogen and carbon were determined by a continuous flow isotope ratio mass spectrometer (IRMS, Winooski, VT USA). The 2007 samples were sent to the Colorado State University Soil Plant and Water Testing Laboratory, Fort Collins, CO USA, where a nitric acid/perchloric acid digest was conducted to analyze P concentrations (Miller and Kotuby-Amacher, 1996). Because P did not appear to have any major role (see results) samples from 2008 were not sent for P analysis.

Since *A. scaphoides* has long narrow taproots (<1cm in diameter and >1 m deep), sampling the entire root system is excessively destructive, so I harvested only the top 10 cm of root. I tested whether this sample was a good indicator of stored resources in the entire root system by conducting a limited number of full root harvests. In 2007, I conducted two harvests (n=5, each) and excavated all root tissue. These samples were analyzed for NSC, N, and P. Total resource pools and concentration values for the top 10 cm of the root were highly correlated with those for the rest of the root tissue (R^2= 0.98 or higher). Further, root diameter was highly correlated with total biomass (R^2 = .982, p=.003) and I saw no significant differences among life stages in size at the beginning (ANOVA: $F_{2,23}$=1.195, p=0.32), or the end (ANOVA: $F_{3,30}$=2.08, p=0.14) of the season. Therefore, these harvests were representative of stored resources throughout the root, and not biased by plant size. These relationships allowed for calculation of total resource pools. Total resource pool data followed the same trends as concentration data and, for simplicity, are not presented here.

Statistical Analyses

All statistical analyses were conducted using R Statistical Software (R Foundation for Statistical Computing 2008). I used analyses of variance with year and stage as factors and their interaction to compare stored resources among life stages at the beginning of the season. I conducted the same analysis to compare stored resources at the end of the season. Tukey's honest significant difference (HSD) test was used as a post hoc comparison of mean resources between life stages at a given time. I then used general linear models with stage and time as independent variables to estimate the change in resource concentrations throughout the growing season for each life stage (normal distribution with identity link). Inspection of residuals confirmed that assumptions of general linear models were met.

Results

Nonstructural Carbohydrates

Dormant plants began the season with lower concentrations of NSC, but ended the season with NSC levels comparable to emergent plants. At the beginning of the season (in May), dormant plants had significantly lower concentrations of NSC, relative to vegetative and reproductive plants (Fig. 1A; ANOVA: Stage $F_{2,16} = 22.78$ $p<0.001$). NSC concentrations did not differ among years (ANOVA: Year $F_{2,16}= 1.49$, $p=0.24$). Lower NSC in dormant plants was not attributable to photosynthetic carbon gain by reproductive and vegetative plants from early emergence to May; NSC concentrations in emergent plants in April were not significantly different from those of reproductive or vegetative plants in May (Tukey's HSD, $p=0.84$ and $p= 0.18$ respectively). However, they were significantly higher than those of dormant plants in May (Tukey's HSD, $p=0.001$).

Over the growing season, NSC concentrations increased for all life stages (reproductive, vegetative and dormant) (Fig. 1A). Dormant plants gained an average 8.0% (95% CI [5.48-10.52]) an increase that was greater than vegetative and reproductive plants, although differences were not statistically significant (vegetative p=0.08, reproductive p=0.42). The increase of NSC in dormant plants was not associated with an increase in total carbon concentration (Fig. 2A), which did not significantly change over the season for any life stages (dormant: 95% CI [-0.92-2.73]; vegetative: 95% CI [-3.91- 0.95]; reproductive: 95% CI [-1.65-2.94]). There were no differences in total carbon concentrations among life stages (ANOVA: Stage: $F_{2,26}$=1.933, p=0.17; Time $F_{1,27}$=2.616, p=0.12; Time*Stage $F_{2,26}$=2.374, p=0.12). Increases in NSC were driven by sucrose concentrations, the main transport sugar in plants (Fig. 2B, average gain = 6.9%, 95% CI [4.13- 9.66]). At the end of the season, NSC concentrations did not differ among life stages (ANOVA: Stage $F_{2,35} = 2.54$, $p = 0.10$).

Nitrogen and Phosphorus

Dormant plants were not depleted in either nitrogen or phosphorus at the beginning of the season, and they did not gain mineral nutrients during the growing season. Dormant plants had higher N content than both reproductive and vegetative plants at the beginning of the season (Fig. 1B, ANOVA: Stage $F_{2,16} = 7.72$ $P < .001$). However, they did not gain N during the season (95% CI [-0.301, 0.300]) while reproductive and vegetative plants did (reproductive 95% CI [0.27, 0.82], vegetative 95% CI [0.43, 0.98]). These patterns were not affected by year (ANOVA: Year $F_{1,16} = .005$, $p = 0.94$). There were no differences in P content among life stages at the beginning of the season (ANOVA: Stage $F_{2,16} = 1.011$, p=0.37) and plants did not significantly gain P over the growing season (ANOVA: Stage $F_{1,55} = 1.364$, $p = 0.25$). No significant differences were detected among stages at the end of the growing season for either N

or P (N ANOVA: Stage $F_{2,34} = 1.923$, $p = 0.18$, Year $F_{1,34} = 1.99$, $p = 0.17$; P ANOVA: Stage $F_{2,18} = 0.016$, $p = 0.98$).

Discussion

My results indicate that stored nonstructural carbohydrates are associated with prolonged dormancy of mature plants. Although plants that remain dormant during the growing season do not have lower stored mineral resources (N and P) relative to plants that emerge above-ground, they do have lower nonstructural carbohydrates (NSC). Further, dormant plants do not gain or lose nitrogen or phosphorus while belowground. Surprisingly, despite being entirely belowground, dormant plants increase NSC concentrations during the growing season, and end with comparable concentrations to plants that had emerged. My results are consistent with the hypothesis that plants enter prolonged dormancy due to a lack of stored resources (Shefferson et al., 2005; Shefferson et al., 2006). In this case, dormant plants may remain belowground because they simply lack sufficient mobile carbon to construct leaves. Alternatively, low sugar concentrations may interrupt developmental pathways for above-ground growth. Recent work with model systems amenable to laboratory and greenhouse study has highlighted the pivotal role of carbon compounds in signaling pathways for growth and development (Halford and Paul 2003, Rolland et al., 2006; Lee et al., 2007). Thus, the shortage of NSC in dormant plants may reflect a shortage of the raw material to build tissue, or an interruption in signaling pathways that allow plants to emerge above ground.

In the past, the hypothesis that plants entered prolonged dormancy due to low resource stores seemed puzzling. If a plant lacks the resources to emerge above ground, how would it gain those necessary resources by merely staying belowground? In *A. scaphoides*, total carbon

concentrations remained constant, while the proportion of available carbon increased. I suggest that dormant plants remobilized cell wall compounds (e.g., hemicelluloses) into available forms, as suggested by Hoch (2007). In other systems, carbon starvation in tissues has been demonstrated to stimulate the degradation of cell wall compounds (Lee et al., 2007). Dormant plants begin the season with low NSC values, do not photosynthesize, and likely incur metabolic costs during the summer due to higher temperatures. These conditions are likely to lead to carbon starvation, which could then trigger cell wall degradation, and the subsequent release of sugars from cell wall materials. Further research in this area could provide more insight on the role of carbon metabolism, and not just assimilation, in the life history strategies of wild plants in the field.

I suspect that plants may enter dormancy because they lack key resources that they gain through remobilization. An alternative would be that plants gain resources from symbionts. In mycotrophic species such as orchids, plants may gain resources from mycorrhizal partners or other symbionts while they remain belowground (Gill, 1989; Shefferson et al., 2005b; Shefferson et al., 2007). *Astragalus scaphoides* does not have strong associations with mycorrhizal or rhizobial partners (E. Crone ,H. Addy and M. Rillig, unpubl. data), so it is not likely to be gaining carbon from below-ground symbionts. However, the potential for resource transfer in other species increases the plausibility of the idea that plants gain resources during prolonged dormancy. In mycorrhizal species, soluble carbon may be transferred either to mycorrhizae or from them (Gill, 1989; Lesica and Steele, 1994; Lesica and Crone, 2007; Shefferson, 2009 and references therein). Orchids have particular mycorrhizal associations that usually result in a net gain of carbon for the plant, even while dormant (Gill, 1989; Shefferson et al., 2005b; Shefferson et al., 2007; Shefferson, 2009). However, species with arbuscular mycorrhizal (AM)

associations (such as *Silene spaldingii*, see Lesica and Crone, 2007), typically allocate carbon to mycorrhizae in return for mineral nutrients. Such allocation could lead to low NSC and dormancy, if low NSC causes prolonged dormancy. Alternatively, if mineral nutrients, rather than NSC, limit emergence for AM plants, they may be able to gain those resources through mycorrhizal symbionts while dormant. The resource dynamics associated with dormancy for species with these symbiotic relationships deserve additional research.

My results strongly point to a causal link between NSC and prolonged dormancy. However, as with any observational study, the link between NSC and dormancy could be due to a spurious correlation with other driving factors. For example, both NSC and the tendency to remain dormant could be associated with plant age; however, dormancy in *A. scaphoides* declines very weakly with age ($r = -0.048$), whereas younger plants tend to have higher NSC concentrations (J. Gremer, unpubl. data). Alternatively, local low resource availability around sampled plants could reduce NSC and also stimulate dormancy. However, dormancy in *A. scaphoides* is not strongly associated with environmental resource availability. Previous work in this system has attempted to alter plant performance by adding supplemental water over three years (Crone and Lesica, 2006) and supplemental nitrogen and phosphorus in 2007 (E.E. Crone unpublished); neither affected the probability of prolonged dormancy (J.R. Gremer, unpublished analysis). As a third possibility, Morrow and Olfelt (2003) showed that *Solidago missouriensis* plants were most likely to be dormant after years of high herbivory, and it is plausible that herbivory would also affect NSC stores. At my field site, ~ 1% of plants are defoliated in any given year (J. Gremer pers. obs.) and other consumers are rare, but ~ 20% of plants go dormant in any given year. Therefore, I doubt that herbivory alone is the primary cause of prolonged dormancy in this species. Finally, I have no reason to suspect that some individual plants are

inherently more likely to remain dormant than others; most bouts of dormancy last only one year in this species (Lesica 1995), and most plants in this 25-year monitoring study have gone dormant at least once (E. Crone & J. Gremer, unpubl.). Furthermore, I harvested dormant plants with visible flowering stalks from previous years, indicating that these plants had flowered in the recent past. Overall, these results do not appear to be confounded by specific environmental factors or plant age. However, stored carbon resources reflect the integrated effect of plant performance over time, including NSC depletion after flowering (Crone et al. 2009) and combined responses to environmental factors (Chapin et al., 1990; Wyka, 1999; Crone et al., 2009). These results suggest that low NSC reflects this integrated effect rather than the effect of any single external environmental factor. Further research is needed to investigate the link between environmental factors, NSC stores, and prolonged dormancy.

Overall, I did not detect a large short term cost of dormancy in terms of stored resources; I suspect dormant plants remobilize existing resources. However, this remobilization of structural carbon could carry a long term cost. Studies that have investigated the demographic costs and benefits of prolonged dormancy have sometimes, but not always, reported long-term costs. For some species, prolonged dormancy decreased survival probability compared to plants that did not remain belowground (Hutchings 1987, Shefferson et al., 2003; but see counterexamples in Shefferson et al., 2005a; Shefferson, 2006; and Lesica and Crone 2007). Similarly, Shefferson et al. (2003) found that dormant *Cypripedium calceolus* plants were less likely to reproduce in the year following dormancy, while Lesica and Crone (2007) showed dormant *Silene spaldingii* were more likely than vegetative or flowering plants to reproduce the following year. It may be that, in some cases, remobilization of resources during dormancy carries a long-term cost in terms of future survival or reproduction, even if this remobilization is

better than death. Alternatively, prolonged dormancy may have different metabolic consequences in different plant species.

My results suggest that carbon storage may help explain why some individuals disappear belowground for one or more years, and provide the mechanism for their return. Low levels of mobile carbon are associated with remaining belowground, while remobilization of structural carbon during dormancy could be a mechanism that allows plants to come back up again. This suggests that the prolonged dormancy may not be exclusively under environmental control but it is also under strong internal control. Further work, such as experiments that include manipulation of carbon stores, is necessary to fully understand the relationship between stored carbon and dormancy. It may be that individual variation in carbon gain, allocation and metabolism can explain why some plants go dormant while others do not. Because the availability of mobile carbon compounds depends on environmental effects on carbon acquisition (e.g. drought or herbivory) as well as on internal carbon metabolism and subsequent effects on developmental pathways, my results can reconcile the two leading hypotheses for prolonged dormancy because carbon resources belowground may be under both external and internal control. To my knowledge, I document for the first time that carbon metabolism may be associated with life history strategies in a long-lived perennial plant in the wild. Because these strategies have important implications for population dynamics, my results may open exciting lines of research spanning from plant molecular biology to population ecology and evolution.

Literature Cited

Amthor, J. S. 2000**.** The McCree-deWit-Penning de Vries-Thornley respiration paradigms: 30 years later. Annals of Botany 86: 1–20.

Boyce, P. J., and J. J. Volenec. 1992**.** Taproot Carbohydrate Concentrations and Stress Tolerance of Contrasting Alfalfa Genotypes. Crop Science 32:757-761.

Chapin III, F. S., E. Schultze, and H. A. Mooney. 1990. The ecology and economics of storage in plants. Annual Review of Ecology and Systematics 21:423-447.

Crone, E. E., and P. Lesica. 2004. Causes of synchronous flowering in *Astragalus scaphoides*, an iteroparous perennial plant. Ecology 85:1944-1954.

Crone, E. E., L. Polansky, and P. Lesica. 2005. Empirical models of pollen limitation, resource acquisition, and mast seeding by a bee-pollinated wildflower. American Naturalist 166:396-408.

Crone, E. E., and P. Lesica. 2006. Pollen and water limitation in *Astragalus scaphoides*, a plant that flowers in alternate years. Oecologia 150:40-49.

Crone, E. E., E. Miller, and A. Sala. 2009. How do plants know when other plants are flowering? Resource depletion, pollen limitation and mast-seeding in a perennial wildflower. Ecology Letters 12: 1119-1126.

Gill, D. E. 1989. Fruiting failure, pollinator inefficiency, and speciation in orchids. Speciation and Its Consequences (eds. D. Otte & J. A. Endler), pp. 458-481. Sinauer Associates Inc., Sunderland, MA, USA.

Ehrlen, J., and K. Lehtila. 2002. How perennial are perennial plants? Oikos 98:308-322.

Halford, N. G., and M. J. Paul. 2003. Carbon metabolite sensing and signalling. Plant Biotechnology Journal 1:381-398.

Ho, L. C., and A. R. Reese. 1976. Re-mobilization and redistribution of reserves in the tulip bulb in relation to new growth until anthesis. New Phytologist 76:59-68.

Hoch, G., M. Popp, and C. Korner. 2002. Altitudinal increase of mobile carbon pools in *Pinus cembra* suggests sink limitation of growth at the Swiss treeline. Oikos 98:361–374.

Hoch, G. 2007. Cell wall hemicelluloses as mobile carbon stores in non-reproductive plant tissues. Functional Ecology 21:823-834.

Hutchings, M. J. 1987. The population biology of the early spider orchid, *Ophrys sphegodes* Mill II, temporal patterns in behaviour. Journal of Ecology 75:729-742.

Kobe, R. K. 1997. Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. Oikos 80:226-233.

Lee, E. J., Y. Matsumura, K. Soga, T. Hoson, and N. Koizumi. 2007. Glycosyl hydrolases of cell walls are induced by sugar starvation in *Arabidopsis*. Plant and Cell Physiology 48:405-413.

Lesica, P., and B. M. Steele. 1994. Prolonged Dormancy in Vascular Plants and Implications for Monitoring Studies. Natural Areas Journal 14:209-212.

Lesica, P. 1995. Demography of *Astragalus scaphoides* and effects of herbivory on population growth. Great Basin Naturalist 55:142-150.

Lesica, P., and E. E. Crone. 2007. Causes and consequences of prolonged dormancy for an iteroparous geophyte, *Silene spaldingii*. Journal of Ecology 95:1360-1369.

Miller, R. O., and J. Kotuby-Amacher. 1996. Western States Laboratory Proficiency Testing Program Soil and Plant Analytical Methods. Version 3.00. Utah State University, UT USA.

Miller, M. T., G. A. Allen, and J. A. Antos. 2004. Dormancy and flowering in two mariposa lilies (*Calochortus*) with contrasting distribution patterns. Canadian Journal of Botany 82:1790-1799.

Mooney, H. A., and R. I. Hays. 1973. Carbohydrate Storage Cycles in 2 Californian Mediterranean Climate Trees. Flora (Jena) 162:295-304.

Morrow, P. A., and J. P. Olfelt. 2003. Phoenix clones: recovery after long-term defoliation-induced dormancy. Ecology Letters 6:119-125.

Rolland, F., E. Baena-Gonzalez, and J. Sheen. 2006. Sugar sensing and signaling in plants: Conserved and novel mechanisms. Annual Review of Plant Biology 57:675-709.

Shefferson, R. P., J. Proper, S. R. Beissinger, and E. L. Simms. 2003. Life history trade-offs in a rare orchid: The costs of flowering, dormancy, and sprouting. Ecology 84:1199-1206.

Shefferson, R. P., T. Kull, and K. Tali. 2005**.** Adult whole-plant dormancy induced by stress in long-lived orchids. Ecology 86:3099-3104.

Shefferson, R. P., M. Weiß, T. Kull, and Taylor, D. L. (2005b) High specificity generally characterizes mycorrhizal association in rare lady's slipper orchids, genus Cypripedium. Molecular Ecology, 14, 613–626.

Shefferson, R.P. 2006. Survival costs of adult dormancy and the confounding influence of size in lady's slipper orchids, genus *Cypripedium*. *Oikos* 115:253-262.

Shefferson, R. P., T. Kull, and K. Tali. 2006. Demographic response to shading and defoliation in two woodland orchids. Folia Geobotanica 41:95-106.

Shefferson, R. P., D. L. Taylor, M. Weiß, S. Garnica, M. K. McCormick, and S. Adams, et al. 2007. The evolutionary history of mycorrhizal specificity among lady's slipper orchids. Evolution, 61, 1380–1390.

Shefferson, R.P. 2009. The evolutionary ecology of vegetative dormancy in mature herbaceous perennial plants. Journal of Ecology 97: 1000-1009.

Van der Heyden, F., and W. D. Stock. 1996**.** Regrowth of a semiarid shrub following simulated browsing: The role of reserve carbon. Functional Ecology 10:647-653.

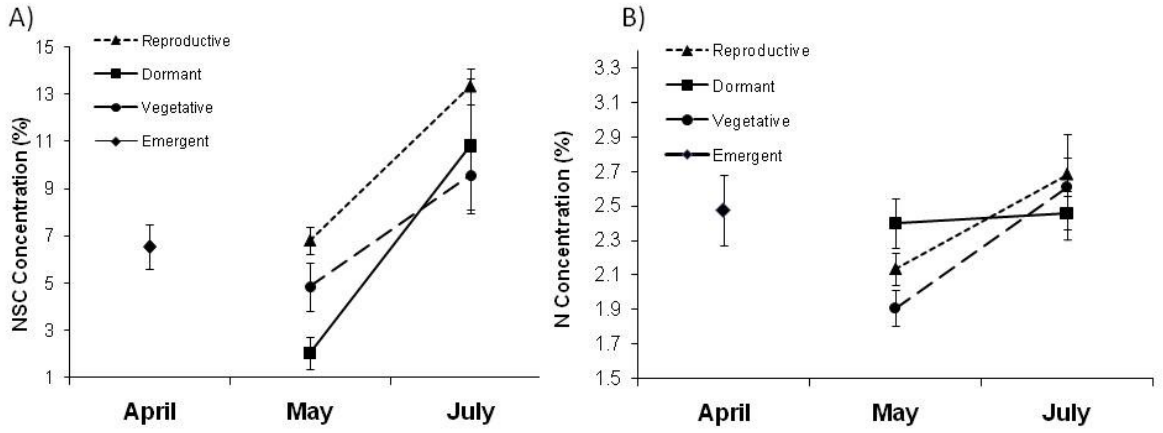
Wyka, T. 1999. Carbohydrate storage and use in an alpine population of the perennial herb, *Oxytropis sericea*. Oecologia 120:198-208.

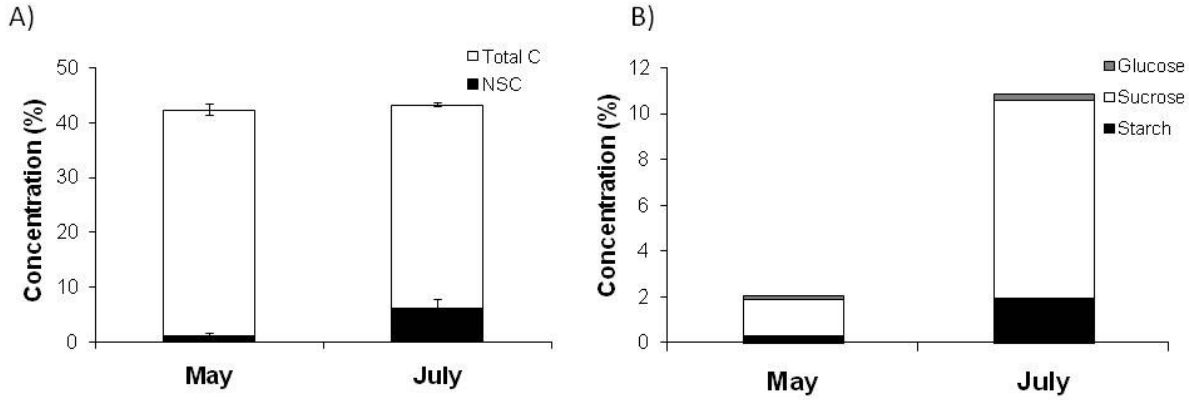
Zimmerman, J. K., and D.F. Whigham. 1992**.** Ecological functions of carbohydrates stored in corms of *Tipularia discolor* (Orchidaceae). Functional Ecology 6:575-581.

Figure Legends

Figure 1. **A)** Dynamics of stored nonstructural carbohydrates (NSC) for emergent (◆), reproductive (▲), dormant (■), and vegetative (●) plants (averages from 2006-2008). Dormant plants begin the season with low NSC concentrations and increase concentrations over the growing season. Error bars represent one standard error. **B)** Nitrogen (N) dynamics for all life stages (emergent (◆), reproductive (▲), dormant (■), and vegetative (●) plants), averaged from 2006-2008 data. Dormant plants had higher N concentrations at the beginning of the season, but did not gain N through the season. Error bars represent one standard error.

Figure 2. **A)** Change in nonstructural carbohydrate concentrations (NSC) in relation to total carbon concentrations for dormant plants. Averages of 2007 and 2008 seasons shown. Total carbon concentrations remained relatively constant as the proportion of carbon as soluble sugars (NSC) increased. Error bars represent one standard error. **B)** Nonstructural carbohydrate (NSC) dynamics by fraction for dormant plants. Averages of 2007 and 2008 seasons shown. Sucrose, the major transport sugar throughout plants, is the only fraction that significantly increased.


A)
Reproductive
Dormant
Vegetative
Emergent
NSC Concentration (%)
15
13
11
9
7
5
3
1
April
May
July
B)
Reproductive
Dormant
Vegetative
Emergent
N Concentration (%)
3.3
3.1
2.9
2.7
2.5
2.3
2.1
1.9
1.7
1.5
April
May
July


A)
Total C
NSC
Concentration (%)
50
40
30
20
10
0
May
July
B)
Glucose
Sucrose
Starch
Concentration (%)
12
10
8
6
4
2
0
May
July

CHAPTER 3

Are dormant plants hedging their bets?

Demographic consequences of prolonged dormancy in variable environments

Abstract

Prolonged dormancy is a stage in which some individuals in a population of herbaceous perennial plants remain belowground during the growing season, while the rest emerge to grow and reproduce. At first glance, prolonged dormancy (PD) may seem maladaptive, because plants remain below ground and delay growth and reproduction. However, PD may offer the benefit of safety while belowground, which could balance out the costs of missing a season of reproduction. This has led to the hypothesis that PD may be a conservative bet hedging strategy, in which mean fitness is sacrificed to reduce the variance in fitness. Here, I evaluated whether PD could function as a bet hedging strategy using a 23-year demographic study of *Astragalus scaphoides*. First, I compared fitness of plants with mean vital rates for this population to hypothetical plants without dormancy. This comparison showed all the signs of classical bet-hedging; relative to hypothetical phenotypes without PD, plants with observed phenotypes had lower deterministic growth rate (λ), higher stochastic λ, and lower variance in λ. I also compared fitness based on vital rates of three groups of plants, plants that never were dormant, plants that spent < 1/5 years in the dormant stage class, and plants that spent ≥ 1/5 years in the dormancy. In this case, dormancy reduced variance, but intermediate levels of dormancy led to increased fitness in constant and stochastic environments. Empirical patterns of lifetime reproductive success confirmed this relationship. My results suggest that dormancy could

function as a bet hedging strategy in principle, but that dormancy is also associated with variation in individual quality, and so may reflect plasticity, rather than classic bet hedging.

Introduction

Prolonged dormancy is a life history phenomenon in which some individuals in a population fail to re-sprout for one or more growing seasons and, instead, remain alive below ground (Lesica and Steele 1994; also known as vegetative dormancy *sensu* Shefferson 2009). Prolonged dormancy has been reported in at least 10 plant families, and over 52 species (Lesica and Steele 1994; Shefferson 2009) suggesting that this life history stage may have evolutionary significance. At first glance, prolonged dormancy seems maladaptive: Why stay belowground while others are growing and reproducing? The obvious cost of prolonged dormancy is that the individual foregoes photosynthesis and reproduction for one or more years. Delaying these activities incurs demographic costs, which, in turn can have large effects on individual fitness. However, it has been suggested that prolonged dormancy may allow plants to avoid stress, predation, or mortality above ground (Lesica and Steele 1994; Shefferson et al. 2005; Miller et al. 2004; Lesica and Crone 2007; Shefferson 2009). If so, plants in prolonged dormancy may be trading off current growth and reproduction in return for increased survival. This has led to speculation that prolonged dormancy may function as a bet hedging strategy (Miller et al. 2004; Shefferson et al. 2005; Shefferson 2009). In this study, I test the predictions of bet hedging theory for a long-lived native perennial in southwest Montana, *Astragalus scaphoides*.

In bet hedging, average fitness is sacrificed for a reduction in the variance of fitness, and this should maximize geometric mean fitness (Slatkin 1974; Seger and Brockman 1987; Philipi

and Seger 1989; Stearns 1992; Roff 2002; Evans et al. 2007). Therefore, three criteria must be met for prolonged dormancy to be bet hedging: the strategy must entail a cost to arithmetic mean fitness, reduce variance in fitness, and increase geometric mean fitness. Costs in terms of mean fitness are likely because, in addition to missing a year of photosynthesis, delaying growth and reproduction would also reduce fitness in most circumstances (Tuljapurkar 1990). Previous work on prolonged dormancy suggests that it can buffer plants from the negative effects of stochasticity (Morrow and Olfelt 2003; Miller et al. 2004; Miller 2004), suggesting that it may reduce variance in fitness. However, even if prolonged dormancy decreases variance in fitness, it is not clear whether this decrease is large enough to increase geometric mean fitness in stochastic environments. Here, I investigate these relationships quantitatively, to evaluate whether prolonged dormancy in *A. scaphoides* fits the three central criteria to function as a bet hedging strategy.

Bet hedging theory has a rich history in evolutionary ecology, because it seems to explain behaviors that at first seem maladaptive (Cohen 1966; Slatkin 1974; Stearns 1992; Roff 1992; Venable 2007; Evans et al. 2007). However, empirical demonstrations of bet hedging strategies are somewhat rare (Clauss and Venable 2000; Menu and Desouhant 2002). Many, if not most, well documented examples of bet hedging are of the "eggs in a basket" type, where fitness is maximized in a variable environment due to the outcome of collective events (Seger and Brockman 1987; Evans and Dennehy 2005). For example, clutch size and seed dormancy have been shown to reduce arithmetic mean fitness, as well as reduce variance in fitness, leading to higher fitness in variable environments (Oloffson et al. 2009; Crump 1981; McGinley et al. 1987; Einum and Fleming 2007; Menu and Debouzie 1993; Philippi and Seger 1989; Ellner 1985; Clauss and Venable 2000; Venable 2007; Evans et al. 2007). In seed dormancy, an

individual plant can produce seeds of variable dormant phenotypes, and can thus spread the risk of temporal variance in success over multiple years (Cohen 1966; Seger and Brockman 1987; Venable and Brown 1988; Clauss and Venable, 2000). However, it comes at an opportunity cost, because germination would result in higher fitness in good years (Seger and Brockman 1987; Pake and Venable 1996). Thus, seed dormancy can be seen as a "don't put all of your eggs in one basket" type of bet hedging (*sensu* Seger and Brockman 1987), where the success of such a strategy is averaged over multiple "baskets," or seed types. These types of bet hedging may lend themselves more directly to experimental perturbation, where clutch size or dormancy ratios can be manipulated and then observed.

In addition, bet-hedging can occur through a "bird in the hand" strategy (*sensu* Seger and Brockman 1987), where risk is avoided at the individual level. The saying that "a bird in the hand is worth two in the bush" implies that the probability of capturing two birds is uncertain, and one must release the bird already caught in order to pursue any more birds. Thus, the conservative strategy is to keep the bird already in hand if the benefit of catching two birds does not outweigh the disadvantage of ending up with none (Seger and Brockman 1987). This type of bet-hedging can be thought of as low yield (only one bird was captured) but also low risk (at least one bird was caught). Prolonged dormancy in plants could be an example of this kind of bet-hedging, where remaining below ground in many years is lower-yield but also lower-risk than emerging above ground. Though previous studies have demonstrated conservative bet hedging in semelparous plants (Rees et al. 2004; Rees et al. 2006, Simons and Johnston 2003), there are few, if any, examples of bet hedging strategies for plants with complex life cycles, such as long, lived iteroparous perennials (Childs et al. 2010).

Here, I analyze the fitness consequences of prolonged dormancy by comparing plants that spend different proportions of their life below ground. I compare plants in the population that do not exhibit prolonged dormancy with those that have intermediate and high levels of dormancy. I also use matrix models to conduct an experiment that is not possible in nature, namely, forcing dormant plants to emerge in order to determine whether prolonged dormancy meets the criteria for bet hedging. Though many matrix models include dormancy as a life stage, few have used these models to explore the fitness consequences associated with it (exceptions include Shefferson et al. 2003; Nicolé et al. 2005; Lesica and Crone 2007). Matrix models allow me to calculate the asymptotic rate of increase (λ) as a measure of individual fitness that integrates trade-offs between survival, growth, and reproduction throughout an individual's lifetime (McGraw and Caswell 1996; Roff 2002). Specifically, I use matrix models to ask: 1) How does the fitness of plants in this population compare to hypothetical plants that never go dormant? 2) For observed phenotypes, how does the time spent below ground affect fitness? Though many demographic studies of perennial plants include dormancy as a life stage, few have used these models to explore the fitness consequences associated with it (exceptions include Shefferson et al. 2003; Nicolé et al. 2005; Lesica and Crone 2007).

Methods

Study Species

Astragalus scaphoides (Fabaceae) is an iteroparous legume with a long, narrow taproot, found on south-facing slopes in high-elevation sagebrush steppe communities in western North America. It has an estimated life span of 21 years (Ehrlen and Lehtila 2002), and does not

reproduce vegetatively (Lesica 1995). Median age to first reproduction is three years (Lesica 1995). On average, 20% of the individuals in this population are dormant in any given year (Crone and Lesica 2004) and approximately 67% of dormancy events last one year (Gremer *in prep., Chapter 1 of this dissertation*). *Astragalus scaphoides* emerges above ground in late April, flowers from late May to mid-June, and seeds usually dehisce by mid-July.

Field sampling

Monitoring transects were installed by Peter Lesica in 1986 at Sheep Corral Gulch, located in Beaverhead County, Montana (45°06'55" N, 113°02'58" W). Methods for demographic data collection followed protocol developed by Lesica and Steele (1997). Transects are 1 meter wide belt transects consisting of approximately 50 adjacent 1 m^2 plots. Two monitoring transects are located at this site. Surveys were conducted in early July of each year from 1986 to 2008, during fruit maturation. Within plots, plants that emerged above ground were mapped to the nearest 0.1 cm and classified into flowering (F), or one of three vegetative stage classes: small (S), medium (M), and large (L). Small plants had less than 6 leaves; medium plants had 6 or more leaves; large plants had evidence of above ground branching (Lesica 1995). Number of inflorescences and fruits were recorded for flowering plants. Dormant plants were defined as those that disappear below ground for one or more years and re-emerge later (Lesica 1995). Transitions to and from all of these stages are possible once plants are established (Figure 1). Since most dormancy normally lasts three years or less, the first and last three years of data have been removed from this analysis. This allows me to separate dormancy from recruitment at the beginning of the study, and mortality at the end of the study (Lesica 1995). Therefore, I used

the middle 17 years of data to build matrix models. For the 23 years of this study, I collected data on individual histories for over 350 plants.

Transition Matrices

Vital rates were calculated for all stage classes (dormant, small, medium, large, and flowering) for 17 years of the study. Number of recruits was associated with the number of flowering plants in the previous year (t_1) and the number of flowering plants two years prior (t_2; linear model with quasipoisson error: $t_{-1,}$ coefficient, β_1=0.228, P=0.101; $t_{-2,}$ coefficient, β_2=0.426, P=0.026). Therefore, I updated Lesica's (1995) transition matrix for this species by adding a seedbank class to the transition matrices (b), which allows seeds to either enter the seedbank (transition from f to b) or germinate the next year (f_g) (but see Appendix 1 for details on how recruitment was modeled for simulations). These relationships lead to the following transition matrix (subscripts correspond to abbreviations for life stages seen in Figure 1):

$$A = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & f_{fb} \\ 0 & A_{dd} & A_{sd} & A_{jd} & A_{md} & A_{fd} \\ A_{bs} & A_{ds} & A_{ss} & A_{js} & A_{ms} & A_{fs} + f_{fg} \\ 0 & A_{dj} & A_{sj} & A_{jj} & A_{dmj} & A_{fj} \\ 0 & A_{dm} & A_{sm} & A_{jm} & A_{mm} & A_{fm} \\ 0 & A_{df} & A_{sf} & A_{jf} & A_{mf} & A_{ff} \end{bmatrix}$$

Transition probabilities sum to 1 and are thus negatively correlated (de Kroon et al. 1986). Therefore, the correlations between vital rates in a matrix model are a result of both mathematical restraints and the actual relationship between matrix elements (Morris and Doak 2002). Therefore, I transformed multinomial transition probabilities (i.e. transitions in which

there is more than one possible outcome) into binomial ones (with only one outcome; conditional vital rates *sensu* Morris and Doak 2002). By using conditional vital rates, I can separate these mathematical constraints from biological correlations (Morris and Doak 2002; Lesica and Crone 2007; Burns et al. 2010). I defined conditional vital rates as survival, emergence conditioned on survival, and growth conditioned on both survival and emergence as illustrated in Table 1 (for details see Appendix 1).

I calculated the means and variances for all of these conditional probabilities using a beta-binomial distribution (Kendall 1998; implemented as the "Kendall" function in the popbio package in R; Stubben & Milligan 2007, R Foundation for Statistical Computing, 2009). This procedure separates annual variation in mean vital rates from demographic stochasticity (Kendall 1998; Morris and Doak 2002). I estimated correlations between vital rates as well as autocorrelations and cross correlations among vital rates (hereafter, *serial correlations*, Tuljapurkar 1990; White et al. 1996; Halley and Kunin 1999; Ruokolainen et al. 2009; Morris and Doak 2002; see Crone & Gremer *in prep*, Appendix 1 of this dissertation). These correlations and serial correlations were multiplied by corrected variance estimates to calculate corrected variances and serial covariances (see Morris and Doak 2002, Ch. 8; Crone and Gremer *in prep*). Fecundity was calculated as described in Appendix 1 (Crone and Gremer *in prep*). Briefly, fecundity was decomposed into three terms: seed pods per flowering plant, the proportion that go into the seedbank, and the proportion that immediately recruit into the small vegetative stage class. Fecundity was then modeled as a function of these three terms using a negative binomial for seed pods per flowering plant and quasi-poisson distribution for annual variation in germination. Distributions were chosen to fit mean-variance relationships (see Hoef and Boveng 2007 & Appendix 1.)

Estimating fitness

Using these vital rates that were estimated from long term demographic data, I calculated deterministic lambda (λ_d), stochastic lambda (λ_s) and reproductive values. Reproductive value can be interpreted as conditional fitness, since it is defined as the expected lifetime reproduction of individuals of a stage class, based on probabilities of survival and reproduction, as well as generation time and population growth rate (Fiedler et al, 1998), and are found in the dominant left eigenvector of the average transition matrix (Morris and Doak 2002). Reproductive values are relative measures; here I scaled them to the small vegetative stage class. λ_d can be interpreted as fitness in a constant environment, and is the dominant eigenvalue of the average transition matrix; in this case I used the transition matrix calculated from mean vital rate estimates. λ_s, then, is fitness when variability is taken into account, and is calculated using stochastic simulations. For these stochastic simulations, I used methods as described in Crone and Gremer, *in prep* (see Appendix 1). Briefly, I generated vital rates using Kendall's method (Kendall 1998 as implemented by Morris and Doak 2002) and the full correlation/serial correlation structure (using methods as proposed by Morris and Doak 2002, their box 8.10). Kendall's method allows for separation of sampling error from variance that can be attributed to environmental stochasticity. In this way, I could explicitly model the effects of environmental variation in stochastic simulations. These estimated vital rates were input into matrix models using element selection, and I simulated growth of these phenotypes over several time steps (50 years) using matrix models. Stochastic lambda values were calculated as the geometric mean growth over these 50 times steps and simulations were replicated 1,000 times. These values of

λ_s represent the fitness of individuals with the phenotype described by their corrected means, variances, and serial covariances in a variable environment.

Testing the predictions of bet hedging

In order to function as a bet-hedging strategy, dormant phenotypes should have lower λ_d, but higher λ_s and lower variance in λ than non-dormant phenotypes. I compared the fitness of dormant and non-dormant phenotypes using five different scenarios. First, I used all individuals in the dataset to estimate the average phenotype of the population (*average dormancy* phenotype). I also grouped individuals in the demographic dataset by dormancy rates. To account for biasing estimates due to seedlings (plants that are younger than three years) or detection for dormancy (it takes at least three years of data to detect dormancy bouts), I used only those plants for which I had more than three years of data for these fitness comparisons. Within the long term data, I separated individuals into three groups: individuals that never went dormant in the study (hereafter *never dormant,* n=44), plants that spent less than 20% of their time in dormancy (*low dormancy* phenotypes, n=90); and plants that spent more than 20% of time in dormancy (*high dormancy* phenotypes, n=70). I then calculated vital rates for each of these phenotypes using the long term dataset. I also used a hypothetical phenotype (*hypothetical non-dormants*) in which I removed dormancy.

A classical problem in observational studies of life histories is that observed phenotypes may be confounded by other features of the environment or individual state. In this case, time spent dormant can be confounded with differences in microsite (for instance, soil moisture or nutrients) or individual differences (e.g. maternal effects, individual state). Therefore, I used this

hypothetical phenotype to account for these possibilities. To create this phenotype, I used the vital rates for the average dormant phenotype described above, except that I set the conditional probability of emergence (pE_x) to one. In this way, I could redistribute the transitions into dormancy among other stages without changing other vital rates, such as survival. Thus, for this hypothetical phenotype, I test how plants would perform if they could simply avoid the dormant stage and transition into other stages without penalty. I then calculated λ_d, λ_s, the variation in λ_s, and reproductive values for these 5 phenotypes in order to determine the fitness consequences of dormancy in a constant versus variable environment.

Results

Transition matrices

Not surprisingly, transitions to dormancy differed among the dormant phenotype classes (Table 2). Survival of medium plants differed significantly among dormancy groups, with the highest value for the low dormancy group and the lowest value for the plants that never went dormant. Other vital rates and vital rate variances varied among groups, but were not statistically different. The low dormancy phenotype was more likely to remain dormant than the average phenotype. The reproductive values for each phenotype indicate that the relative contribution to fitness for each stage varied by dormancy level (Table 2). As expected, the data show that the flowering stage had the highest reproductive value of all stages for all phenotypes, but was relatively higher for those without prolonged dormancy (hypothetical and never dormants). The dormant stage contributed more to fitness for average phenotypes than for low and high dormancy phenotypes.

Testing the predictions of bet hedging theory

Overall, phenotypes that exhibited dormancy had lower variance in fitness (λ) than plants without dormancy (Figure 3, Coefficient of variation for never =1.516; low= 1.507; high= 1.450; average =1.497; hypothetical = 1.517). In comparison to hypothetical plants without dormancy, the average dormancy phenotype had higher deterministic λ (average: λ_d= 1.006, hypothetical: λ_d=1.015). Estimates of λ_d are properties of the transition matrix, and therefore do not have associated error terms. However, in long lived organisms, small changes in growth rate can have large biological significance (Silvertown et al. 1993). Variance in λ was higher for hypothetical plants without dormancy, compared to the average dormancy phenotype. Accordingly, λ_s was higher for the average dormancy phenotype than the hypothetical phenotype without dormancy (average λ_s= 1.018, while hypothetical λ_s=1.009), and confidence intervals for these values did not overlap. For the observed dormancy phenotypes, high dormancy phenotypes had the lowest coefficient of variation, with never dormants having the highest variation. However, variance in fitness was not sufficient to make up for differences in mean fitness. Plants that had intermediate levels of dormancy had the highest λ values, both deterministic and stochastic (1.069 and 1.037 respectively), plants that never went dormant had the lowest values for both deterministic and stochastic λ (0.851 and 0.858 respectively), and high dormancy phenotypes had intermediate λ_d and λ_s (1.003 and 1.001).

Discussion

Our results indicate that prolonged dormancy would function as a bet hedging strategy if dormant plants could avoid the dormant stage and perform like emergent plants. Hypothetical non-dormants had higher deterministic λ, higher variance in λ, and lower stochastic λ than the average dormancy phenotype in our population. These results display the classic traits of bet hedging: prolonged dormancy came at a cost to average fitness, but resulted in a decrease in the variance of fitness that translated into higher stochastic fitness. Therefore, it is clear that low levels of dormancy increase fitness of plants in this system. In the past, dormancy was considered to be maladaptive (Hutchings 1987; Shefferson et al. 2003), but our results are consistent with the hypothesis that dormancy is beneficial in a variable environment (Miller et al. 2007; Shefferson 2009). However, differences among observed dormancy phenotypes in our long term dataset are not entirely consistent with bet hedging since no cost to average fitness was detected for low dormancy phenotypes.

A related study in this system (Gremer et al. 2010, *see Chapter 2 of this dissertation*) suggests that *A. scaphoides* individuals may be constrained to dormancy by low levels of stored available carbon (nonstructural carbohydrates, NSC). Plants recover NSC while dormant presumably by remobilizing structural carbon into nonstructural carbon. If so, then prolonged dormancy may represent a plastic response to low NSC stores. While plasticity is not strictly bet hedging (Seger and Brockman 1987), it is possible that both bet hedging and plasticity may occur within a system (Evans et al. 2007; Evans and Dennehy 2005; Cohen 1967). For example, if conditions at emergence are not predictive of growing conditions later in the season, then variation in emergence as a result of plasticity could still lead to bet hedging (Evans and Dennehy 2005). Further, since environmental conditions may have aspects that are more or less

predictable, it may be possible to see both bet hedging and plasticity occurring (Evans and Dennehy 2005; Evans et al. 2007; Cohen 1967). Evidence for both plasticity and bet hedging has been found for other systems. For example, a study of seed dormancy by Clauss and Venable (2000) demonstrated that germination fractions of *Plantago insularis* in the field were consistent with bet hedging predictions. However, when they conducted experimental studies of germination for *P. insularis*, they found evidence for phenotypic plasticity in response to water availability. Similarly, Evans et al. (2007) found evidence for both bet hedging and phenotypic plasticity in annual and perennial *Oenothera* populations. In these examples, as in my system, it may be that some level of plasticity can confer the benefits of bet hedging, though the mechanisms in which it is achieved are not strictly probabilistic in nature. Further research in this area could investigate the degree to which plasticity overlaps with bet hedging, and whether both mechanisms commonly occur in natural populations.

For this analysis, I defined prolonged dormancy as plants that disappear for one or more years and later emerge. Reduced variation in fitness of plants that spend more time below ground is due in part to the fact that, by this definition, dormant plants must survive (since by definition, they re-emerge), and there is no variance in the survival of dormant plants. Variance in other vital rates did not differ among dormancy groups (Table 3). Therefore, the benefit of prolonged dormancy as a bet hedging strategy is likely due to safer conditions (and, hence, higher survival) belowground (Lesica and Steele 1994; Miller et al. 2004; Lesica and Crone 2007; Miller et al. 2007; Shefferson 2009). Recently, researchers have attempted to estimate survival of plants that remain alive below ground for one or more years but eventually die without emerging, using capture-recapture models (Shefferson et al. 2003; Shefferson et al. 2005; Kery and Gregg 2004; Kery et al. 2005). By this definition, dormant plants have an

associated probability of mortality. More recent studies suggest that survival estimates for unobservable plants are usually not statistically robust (Kery et al. 2005; Link et al. 2010). Mortality during prolonged dormancy could affect the balance between the costs and benefits of this life stage. Lalonde and Roitberg (2006) found that changing survival rates of dormant seeds affected the range of dormancy phenotypes that were maintained in populations, though some level of dormancy was still favored. Similar processes could function for prolonged dormancy, though obvious differences between these two types of dormancy exist. Conversely, Lesica and Crone (2007) found minimal differences in the consequences of dormancy between analyses with perfect and imperfect survival of dormant plants.

Though it has been suggested that prolonged dormancy is a conservative bet hedging strategy (Lesica and Steele 1994; Shefferson et al. 2005; Miller et al. 2004; Lesica and Crone 2007; Shefferson 2009), this study is the first to directly test this hypothesis. Other studies of prolonged dormancy have investigated the fitness consequences of prolonged dormancy, but most have looked at fitness components such as survival and reproduction, instead of total fitness (Hutchings 1987; Shefferson et al. 2003; Miller et al. 2004; Shefferson et al. 2005; Shefferson 2006; Lesica and Crone 2007; but see Miller et al. 2007; Jakalaniemi et al. *in revision*.). For some species, growing season dormancy may decrease survival relative to plants that did not remain belowground (Hutchings 1987, Shefferson et al. 2003, Shefferson et al. 2005), while other studies have not detected a significant cost to dormancy in terms of survival (Lesica and Crone 2007; Shefferson et al. 2005; Shefferson 2006). Similarly, Shefferson et al. (2003) found that dormant *Cypripedium calceolus* plants were less likely to reproduce in the year following dormancy, while Lesica and Crone (2007) showed dormant *Silene spaldingii* were more likely than emergent plants to reproduce the following year. Ultimately, fitness relies on both survival

and reproduction, as well as other growth and transition rates within an individual's lifetime. This is particularly relevant in long lived species that trade-off the costs and benefits of survival and reproduction over many seasons. Therefore, using λ as my fitness metric provides a measure that integrates trade-offs that occur throughout the whole life cycle (McGraw and Caswell 1996) to understand long term fitness consequences of prolonged dormancy. Further, current performance and ability to respond to environmental conditions is likely to be a product of current state as well as past environmental conditions and the previous history of the individual (Ehrlen 2000; Doak et al. 2005). Here, I included serial correlations, allowing for incorporation of these historical effects into this analysis. I suggest that this life cycle approach, that accounts for historical effects, may reconcile the results of previous studies and provide a better understanding of how prolonged dormancy functions in the life histories of perennial plants.

Prolonged dormancy is, at first glance, a puzzling life stage because individuals remain belowground while neighboring plants emerge to grow and reproduce. These results suggest that prolonged dormancy has the essential elements to function as a bet hedging strategy, although some degree of individual variation is also at work in this system. Further, my results suggest that spending some proportion of time in prolonged dormancy is associated with fitness advantages in a variable environment. Precisely how individual quality, environmental variation, and prolonged dormancy interact to affect fitness deserves further study. Nonetheless, this study indicates that foregoing one or more seasons of growth and reproduction in favor of remaining in prolonged dormancy can confer fitness advantages in a variable environment.

Literature Cited

Burns, J. H., S. P. Blomberg, E. E. Crone, J. Ehrlen, T. M. Knight, J. B. Pichancourt, S. Ramula, G. M. Wardle, and Y. M. Buckley. 2010. Empirical tests of life-history evolution theory using phylogenetic analysis of plant demography. Journal of Ecology 98:334-344.

Clauss, M. J., and D. L. Venable. 2000. Seed germination in desert annuals: An empirical test of adaptive bet hedging. American Naturalist 155:168-186.

Cohen, D. 1966. A General Model of Optimal Reproduction in a Randomly Varying Environment. Journal of Ecology 56:219-228.

Cohen, D. 1967. Optimizing reproduction in a randomly varying environment when a correlation may exist between the conditions at the time a choice has to be made and the subsequent outcome. Journal of Theoretical Biology 16:1-14.

Crone, E. E. and P. Lesica. 2004. Causes of synchronous flowering in *Astragalus scaphoides*, an iteroparous perennial plant. Ecology 85:1944-1954.

Crone and Gremer. *In prep*. Costs of reproduction and stochastic population dynamics.

Crump, M. L. 1981. Variation in Propagule Size as a Function of Environmental Uncertainty for Tree Frogs. American Naturalist 117:724-737.

Ehrlen, J., and K. Lehtila. 2002. How perennial are perennial plants? Oikos 98:308-322.

de Kroon, H., J. van Groenendael, and J. Ehrlen. 1986. Elasticities: A Review of Methods and Model Limitations. Ecology 81:607-618.

Doak, D. F., W. F. Morris, C. Pfister, B. E. Kendall, and E. M. Bruna. 2005. Correctly estimating how environmental stochasticity influences fitness and population growth. American Naturalist 166:E14-E21.

Ehrlen, J. 2000. The dynamics of plant populations: Does the history of individuals matter? Ecology 81:1675-1684.

Einum, S., and I. A. Fleming. 2007. Of chickens and eggs: Diverging propagule size of iteroparous and semelparous organisms. Evolution 61:232-238.

Ellner, S. 1985. Ess Germination Strategies in Randomly Varying Environments 1. Logistic-Type Models. Theoretical Population Biology 28:50-79.

Evans, M. E. K., and J. J. Dennehy. 2005. Germ banking: Bet-hedging and varlable release from egg and seed dormancy. Quarterly Review of Biology 80:431-451.

Evans, M. E. K., R. Ferriere, M. J. Kane, and D. L. Venable. 2007. Bet hedging via seed banking in desert evening primroses (*Oenothera*, Onagraceae): Demographic evidence from natural populations. American Naturalist 169:184-194.

Fiedler, P. L., B.E. Knapp, and Nancy Fredricks. 1998. Rare plant demography: Lessons from the Mariposa Lilies (*Calochortus*: Liliaceae). Pages 28-48 in P. L. Fiedler, and Peter M. Kareiva, editor. Conservation Biology: For the coming decade. International Thomson Publishing, New York.

Gremer, J.R. *In prep*. Patterns of prolonged dormancy in *Astragalus scaphoides*: Inference from long term monitoring.

Gremer, J. R., A. Sala, and E. E. Crone. 2010. Disappearing plants: why they hide and how they return. Ecology 91:3407-3413.

Halley, J. M., and V. E. Kunin. 1999. Extinction risk and the 1/f family of noise models. Theoretical Population Biology 56:215-230.

Hoef, J. M. V., and P. L. Boveng. 2007. Quasi-poisson vs. negative binomial regression: How should we model overdispersed count data? Ecology **88**:2766-2772.

Hutchings, M. J. 1987. The population biology of the early spider orchid, *Ophrys sphegodes* Mill II, temporal patterns in behaviour. Journal of Ecology 75:729-742.

Jäkäläniemi, A., E. E. Crone, P. Närhi, and J. Tuomi. *unpublished manuscript*. Stage-dependent benefits of prolonged dormancy in a perennial orchid (in revision for submission to *Ecology*).

Kendall, B. E. 1998. Estimating the magnitude of environmental stochasticity in survivorship data. Ecological Applications 8:184-193.

Kery, M., K. B. Gregg and Michael Schaub. 2005. Demographic estimation methods for plants with unobservable life-states. Oikos 108:307-320.

Kery, M., and K. B. Gregg. 2004. Demographic analysis of dormancy and survival in the terrestrial orchid *Cypripedium reginae*. Journal of Ecology 92:686-695.

Lalonde, R. G., and B. D. Roitberg. 2006. Chaotic dynamics can select for long-term dormancy. American Naturalist 168:127-131.

Lesica, P., and Brian M. Steele. 1994. Prolonged Dormancy in Vascular Plants and Implications for Monitoring Studies. Natural Areas Journal 14:209-212.

Lesica, P. 1995. Demography of Astragalus scaphoides and effects of herbivory on population growth. Great Basin Naturalist 55:142-150.

Lesica, P., and E. E. Crone. 2007. Causes and consequences of prolonged dormancy for an iteroparous geophyte, Silene spaldingii. Journal of Ecology 95:1360-1369.

Lesica, P., and B. M. Steele. 1997. Use of permanent plots in monitoring plant populations. Natural Areas Journal 17:331-340.

McGinley, M. A., D. H. Temme, and M. A. Geber. 1987. Parental Investment in Offspring in Variable Environments - Theoretical and Empirical Considerations. American Naturalist 130:370-398.

McGraw, J. B., and H. Caswell. 1996. Estimation of individual fitness from life-history data. American Naturalist 147:47-64.

Menu, F., and D. Debouzie. 1993. Coin-Flipping Plasticity and Prolonged Diapause in Insects - Example of the Chestnut Weevil *Curculio-Elephas* (Coleoptera, Curculionidae). Oecologia 93:367-373.

Menu, F., and E. Desouhant. 2002. Bet-hedging for variability in life cycle duration: bigger and later-emerging chestnut weevils have increased probability of a prolonged diapause. Oecologia 132:167-174.

Miller, M. T. 2004. Demographic perspectives on the rarity and persistence of two Mariposa lilies (*Calochortus)* from Southern British Columbia. Dissertation. University of Victoria, Canada.

Miller, M. T., G.A. Allen, and J.A. Antos. 2004. Dormancy and flowering in two mariposa lilies (*Calochortus*) with contrasting distribution patterns. Canadian Journal of Botany 82:1790-1799.

Miller, M. T., J. A. Antos, and G. A. Allen. 2007. Demographic differences between two sympatric lilies (*Calochortus*) with contrasting distributions, as revealed by matrix analysis. Plant Ecology 191:265-278.

Morris, W. F., and Daniel F. Doak. 2002. Quantitative Conservation Biology: Theory and Practice of Population Viability Analysis. Sinauer Associates, Sunderland, MA USA.

Morrow, P. A., and Joel P. Olfelt. 2003. Phoenix clones: recovery after long-term defoliation-induced dormancy. Ecology Letters 6:119-125.

Nicole, F., E. Brzosko, and I. Till-Bottraud. 2005. Population viability analysis of *Cypripedium calceolus* in a protected area: longevity, stability and persistence. Journal of Ecology 93:716-726.

Olofsson, H., J. Ripa, and N. Jonzen. 2009. Bet-hedging as an evolutionary game: the trade-off between egg size and number. Proceedings of the Royal Society B-Biological Sciences 276:2963-2969.

Pake, C. E., and D. L. Venable. 1996. Seed banks in desert annuals: Implications for persistence and coexistence in variable environments. Ecology 77:1427-1435.

Philippi, T., and J. Seger. 1989. Hedging Ones Evolutionary Bets, Revisited. Trends in Ecology & Evolution 4:41-44.

Roff, D. 2002. Life History Evolution. Sinauer, Sunderland, MA.

Ruokolainen, L., A. Linden, V. Kaitala, and M. S. Fowler. 2009. Ecological and evolutionary dynamics under coloured environmental variation. Trends in Ecology & Evolution 24:555-563.

Seger, J., and Brockmann, H.J. 1987. What is bet-hedging? Pages 182-211 in P. H. Harvey, Partridge, L., editor. Oxford Surveys in Evolutionary Biology. Oxford University Press, Oxford.

Shefferson, R. P., Joyce Proper, Steven R. Beissinger, and Ellen L. Simms. 2003. Life history trade-offs in a rare orchid: The costs of flowering, dormancy, and sprouting. Ecology 84:1199-1206.

Shefferson, R. P., Tiiu Kull, and Kadri Tali. 2005. Adult whole-plant dormancy induced by stress in long-lived orchids. Ecology 86:3099-3104.

Shefferson, R.P. 2006. Survival costs of adult dormancy and the confounding influence of size in lady's slipper orchids, genus *Cypripedium*. *Oikos* 115:253-262.

Shefferson, R. P. 2009. The evolutionary ecology of vegetative dormancy in mature herbaceous perennial plants. Journal of Ecology 97:1000-1009.

Silvertown, J., M. Franco, I. Pisanty, and A. Mendoza. 1993. Comparative Plant Demography - Relative Importance of Life-Cycle Components to the Finite Rate of Increase in Woody and Herbaceous Perennials. Journal of Ecology **81**:465-476.

Slatkin, M. 1974. Hedging Ones Evolutionary Bets. Nature 250:704-705.

Stearns, S. C. 1992. The Evolution of Life Histories. Oxford University Press, Oxford.

Stubben, C. J., and B. G. Milligan. 2007. Estimating and Analyzing Demographic Models Using the popbio Package in R. Journal of Statistical Software 22(11).

Tuljapurkar, S. 1990. Delayed Reproduction and Fitness in Variable Environments. Proceedings of the National Academy of Sciences of the United States of America 87:1139-1143.

Venable, D. L. 2007. Bet hedging in a guild of desert annuals. Ecology 88:1086-1090.

Venable, D. L., and J. S. Brown. 1988. The Selective Interactions of Dispersal, Dormancy, and Seed Size as Adaptations for Reducing Risk in Variable Environments. American Naturalist 131:360-384.

White, A., M. Begon, and R. G. Bowers. 1996. Explaining the colour of power spectra in chaotic ecological models. Proceedings of the Royal Society of London Series B-Biological Sciences 263:1731-1737.

Table 1. Matrix of conditional probabilities for *Astragalus scaphoides*. s_j= the probability of survival for stage j; pE_j= probability of emergence for stage class j, conditioned on survival; pF_{ij}= the probability of flowering for stage class j, conditioned on emergence; pM_{ij}= the probability of transitioning to the large stage class for stage class j, conditioned on emergence and not flowering; pS_{ij}= the probability of transitioning to the small stage class for stage class j, conditioned on emergence, not flowering, and not being in the large class; pods= seed pods, fruits produced by flowering plants; g= probability of germinating the following year. B_1= recruitment from seed pods the previous year, B_2= recruitment from seed pods two years previous. For clarity, only the first two and last columns are shown.

0	0	...	$pods*B_2$
0	$s_d(1\text{-}pE_d)$	...	$s_f(1\text{-}pE_f)$
$pods \times g$	$s_d(pE_d)(1\text{-}pF_d)(1\text{-}pL_d)pS_d$	...	$s_f(pE_f)(1\text{-}pF_f)(1\text{-}pL_f)pS_f + pods*B_1*g$
0	$s_d(pE_d)(1\text{-}pF_d)(1\text{-}pL_d)(1\text{-}pS_d)$	...	$s_f(pE_f)(1\text{-}pF_f)(1\text{-}pL_f)(1\text{-}pS_f)$
0	$s_d(pE_d)(1\text{-}pF_d)pL_d$	...	$s_f(pE_f)(1\text{-}pF_f)pL_f$
0	$s_d pE_d F_d$	...	$s_f pE_f F_f$

Table 2. Conditional vital rate estimates for the average phenotype, hypothetical non-dormants, and three dormancy levels calculated using Kendall's method and serial correlations. 95% confidence limits are in parentheses. Rates that differed significantly among groups (as inferred from 95% confidence intervals) are indicated by letters and bold text. Note that survival of dormant plants is 1 because dormancy is defined as plants that re-emerge after some years below ground.

	Means				Variances			
Vital Rate	**Average dormancy**	**Never dormant**	**Low dormancy**	**High dormancy**	**Average dormancy**	**Never dormant**	**Low dormancy**	**High dormancy**
F Survival	0.903 [0.838,0.95]	0.806 [0.661,0.914]	0.941 [0.854,0.981]	0.869 [0.719,0.963]	0.002 [0,0.019]	0.000 [0,0.066]	0.002 [0,0.037]	0.003 [0,0.085]
M Survival	**0.908**[a] [0.873,0.939]	**0.768**[b] [0.652,0.862]	**0.972**[c] [0.939,0.99]	**0.912**[a,c] [0.84,0.959]	0.000 [0,0.005]	0.002 [0,0.047]	0.000 [0,0.003]	0.000 [0,0.016]
S Survival	0.814 [0.74,0.868]	0.567 [0.438,0.683]	0.942 [0.816,0.98]	0.896 [0.785,0.954]	0.007 [0.001,0.025]	0.019 [0,0.07]	0.007 [0,0.078]	0.009 [0,0.06]
L Survival	0.953 [0.877,0.983]	0.923 [0.713,0.992]	0.965 [0.915,0.99]	0.979 [0.805,0.999]	0.002 [0,0.031]	0.029 [0,0.166]	0.000 [0,0.01]	0.000 [0,0.137]
FtoD	**0.143**[a] [0.069,0.271]	**0**[b] [0.01,0.228]	**0.148**[a] [0.069,0.296]	**0.298**[a] [0.124,0.524]	0.013 [0.002,0.063]	0.010 [0,0.186]	0.011 [0,0.074]	0.037 [0,0.14]
MtoD	**0.134**[a] [0.081,0.21]	**0**[b] [0.01,0.162]	**0.109**[a] [0.047,0.234]	**0.285**[c] [0.169,0.436]	0.006 [0.001,0.028]	0.010 [0,0.143]	0.014 [0.002,0.077]	0.021 [0,0.079]
StoD	**0.217**[a,b] [0.144,0.321]	**0**[c] [0.01,0.17]	**0.168**[a,c] [0.1,0.264]	**0.423**[b] [0.296,0.555]	0.016 [0.005,0.049]	0.010 [0,0.149]	0.008 [0,0.038]	0.022 [0,0.073]
DtoD	**0.500**[a] [0.392,0.621]	**n/a**	**0.188**[b] [0.082,0.392]	**0.600**[a] [0.513,0.691]	0.025 [0.005,0.069]	n/a	0.034 [0.001,0.137]	0.006 [0,0.041]
LtoD	**0.159**[a] [0.088,0.272]	**0**[a] [0.01,0.188]	**0.125**[a] [0.05,0.277]	**0.384**[b] [0.224,0.566]	0.010 [0,0.052]	0.010 [0,0.162]	0.017 [0,0.097]	0.000 [0,0.075]
FtoF	0.434 [0.199,0.709]	0.246 [0.077,0.551]	0.310 [0.11,0.619]	0.146 [0.034,0.471]	0.153 [0.048,0.212]	0.080 [0,0.212]	0.124 [0.02,0.212]	0.018 [0,0.212]
MtoF	0.264 [0.136,0.447]	0.238 [0.098,0.434]	0.308 [0.158,0.507]	0.206 [0.084,0.393]	0.073 [0.031,0.147]	0.063 [0.016,0.153]	0.086 [0.036,0.165]	0.040 [0.006,0.135]

	Means				Variances			
Vital Rate	**Average dormancy**	**Never dormant**	**Low dormancy**	**High dormancy**	**Average dormancy**	**Never dormant**	**Low dormancy**	**High dormancy**
StoF	0.108 [0.045,0.244]	0.086 [0.025,0.23]	0.112 [0.041,0.257]	0.110 [0.034,0.305]	0.020 [0.005,0.093]	0.013 [0,0.099]	0.023 [0.005,0.105]	0.021 [0,0.147]
DtoF	0.162 [0.071,0.31]	n/a	0.198 [0.077,0.377]	0.144 [0.065,0.28]	0.028 [0.007,0.1]	n/a	0.036 [0.006,0.124]	0.012 [0,0.074]
LtoF	0.323 [0.144,0.548]	0.436 [0.192,0.697]	0.321 [0.134,0.559]	0.388 [0.138,0.691]	0.131 [0.066,0.201]	0.133 [0.007,0.212]	0.140 [0.067,0.212]	0.154 [0.013,0.212]
FtoL	0.331 [0.205,0.453]	0.387 [0.19,0.626]	0.340 [0.203,0.461]	0.298 [0.103,0.522]	0.009 [0,0.062]	0.022 [0,0.14]	0.005 [0,0.061]	0.004 [0,0.133]
JtoL	0.244 [0.136,0.402]	0.202 [0.081,0.396]	0.282 [0.141,0.488]	0.222 [0.097,0.408]	0.040 [0.013,0.105]	0.033 [0,0.132]	0.064 [0.015,0.153]	0.028 [0,0.116]
StoL	0.089 [0.051,0.154]	0.065 [0.023,0.14]	0.108 [0.059,0.184]	0.074 [0.018,0.221]	0.002 [0,0.021]	0.000 [0,0.019]	0.000 [0,0.025]	0.008 [0,0.102]
DtoL	0.156 [0.095,0.247]	n/a	0.190 [0.097,0.31]	0.131 [0.063,0.268]	0.001 [0,0.025]	n/a	0.000 [0,0.041]	0.003 [0,0.061]
MtoL	0.403 [0.265,0.569]	0.457 [0.181,0.79]	0.408 [0.241,0.611]	0.389 [0.124,0.671]	0.016 [0,0.086]	0.072 [0,0.212]	0.030 [0,0.114]	0.000 [0,0.16]
FtoS	0.370 [0.217,0.553]	0.366 [0.132,0.615]	0.413 [0.242,0.608]	0.223 [0.057,0.534]	0.029 [0.004,0.098]	0.007 [0,0.149]	0.031 [0.001,0.105]	0.021 [0,0.195]
MtoS	0.419 [0.3,0.55]	0.447 [0.236,0.621]	0.362 [0.188,0.562]	0.439 [0.267,0.621]	0.020 [0,0.07]	0.005 [0,0.143]	0.060 [0.007,0.15]	0.006 [0,0.101]
StoS	0.631 [0.487,0.758]	0.629 [0.449,0.769]	0.630 [0.463,0.778]	0.620 [0.418,0.774]	0.043 [0.015,0.098]	0.026 [0,0.113]	0.054 [0.016,0.121]	0.034 [0,0.119]
DtoS	0.565 [0.45,0.687]	n/a	0.529 [0.383,0.673]	0.579 [0.404,0.739]	0.010 [0,0.053]	n/a	0.000 [0,0.041]	0.036 [0,0.111]
LtoS	0.367 [0.196,0.591]	0.273 [0.05,0.679]	0.451 [0.226,0.698]	0.125 [0.01,0.511]	0.030 [0,0.147]	0.000 [0,0.212]	0.049 [0,0.183]	0.116 [0,0.212]

Table 3. Reproductive values for average phenotype, hypothetical phenotype without dormancy, and three dormancy levels (no dormancy, low=≤1/5 years dormant, high= >1/5 years dormant). Vital rates were estimated from 1989-2005 data using Kendall's method and incorporating serial correlations. Reproductive values are the left eigenvector of the transition matrix, and can be interpreted as conditional fitness. Reproductive values are scaled to the small stage, hence reproductive value of the small stage=1.

Stage	Average Dormancy	Hypothetical Non-Dormants	Never Dormant	Low Dormancy	High Dormancy
Dormant	1.283	n/a	n/a	1.114	1.132
Small	1	1	1	1	1
Medium	1.273	1.315	1.766	1.16	1.074
Large	1.395	1.46	2.616	1.154	1.226
Flowering	2.02	2.078	2.902	1.693	1.656

Figure Legends

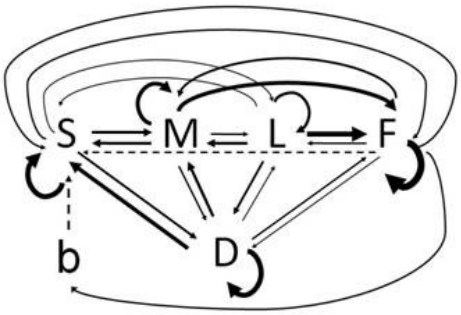
Figure 1. Life cycle diagram for *Astragalus scaphoides* and transition matrices for 5 dormancy phenotypes. A) Mean annual life cycle diagram for *Astragalus scaphoides*. I used 3 vegetative classes (S= small, M= medium, and L= large), a flowering stage class (F), a seedbank stage (b) and a dormant stage (D). Magnitude of transitions are indicated by thickness of arrows. Recruitment is indicated by dashed lines from F to S and b to S (lines not to scale). Average transition matrices for the average dormancy phenotype (B), hypothetical non-dormants (C), never dormant (D), low dormancy (E), and high dormancy phenotypes (F). See *Methods* for description of phenotypes. Vital rates were calculated using Kendall's method and incorporating correlations and serial correlations from 1989-2005 demographic data.

Figure 2. Deterministic λ (λ_d) for the average phenotype, hypothetical phenotype without dormancy, and three dormancy levels. Values for λ_d are the dominant eigenvalue of the average matrix for each phenotype.

Figure 3. Stochastic λ (λ_s) for the average phenotype, hypothetical phenotype without dormancy, and three dormancy levels. Averages of λ_s were calculated from 1,000 replicate simulations. 95% confidence intervals of the mean are enclosed by the variable markers and do not overlap. Dashed error bars represent prediction intervals.

Figure 4. Coefficient of variation for λ (CV_λ) for the average phenotype, hypothetical phenotype without dormancy, and three dormancy levels. Solid error bars represent 95% confidence intervals of the mean, dashed error bars represent prediction intervals.

a)



b) Average Dormancy

$$\begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0.422 \\ 0 & 0.499 & 0.177 & 0.122 & 0.151 & 0.129 \\ 1 & 0.200 & 0.327 & 0.184 & 0.119 & 0.331 \\ 0 & 0.154 & 0.191 & 0.254 & 0.205 & 0.185 \\ 0 & 0.065 & 0.051 & 0.141 & 0.219 & 0.145 \\ 0 & 0.081 & 0.069 & 0.208 & 0.259 & 0.336 \end{bmatrix}$$

c) Hypothetical Non-Dormants

$$\begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0.422 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0.417 & 0.212 & 0.141 & 0.349 \\ 0 & 0 & 0.244 & 0.294 & 0.244 & 0.215 \\ 0 & 0 & 0.065 & 0.163 & 0.260 & 0.169 \\ 0 & 0 & 0.088 & 0.240 & 0.308 & 0.392 \end{bmatrix}$$

d) Low Dormancy

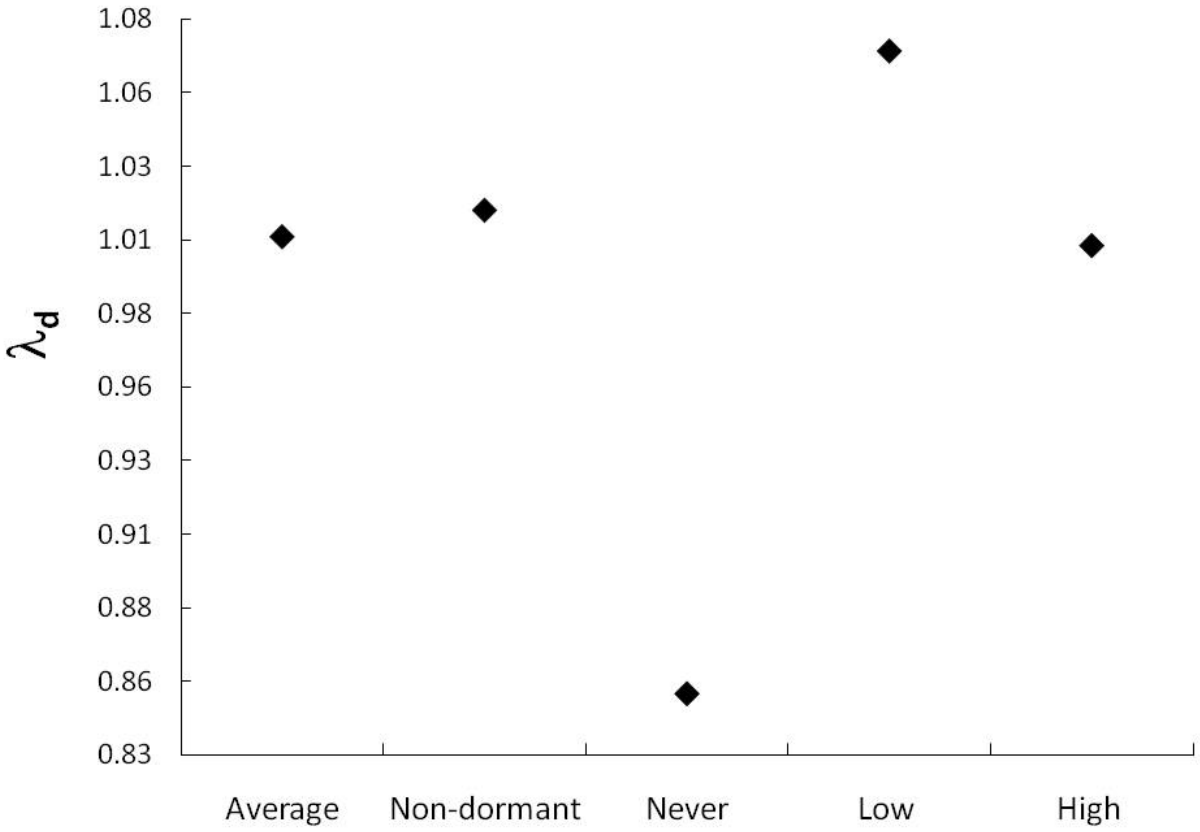
$$\begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0.422 \\ 0 & 0.188 & 0.158 & 0.106 & 0.121 & 0.139 \\ 1 & 0.279 & 0.391 & 0.156 & 0.153 & 0.373 \\ 0 & 0.248 & 0.229 & 0.275 & 0.186 & 0.214 \\ 0 & 0.124 & 0.075 & 0.169 & 0.234 & 0.188 \\ 0 & 0.161 & 0.088 & 0.267 & 0.271 & 0.249 \end{bmatrix}$$

e) High Dormancy

$$\begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0.422 \\ 0 & 0.599 & 0.379 & 0.260 & 0.376 & 0.259 \\ 1 & 0.172 & 0.264 & 0.177 & 0.028 & 0.304 \\ 0 & 0.125 & 0.162 & 0.226 & 0.198 & 0.284 \\ 0 & 0.045 & 0.034 & 0.115 & 0.144 & 0.155 \\ 0 & 0.058 & 0.057 & 0.134 & 0.234 & 0.089 \end{bmatrix}$$

f) Never Dormants

$$\begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0.422 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0.302 & 0.207 & 0.076 & 0.358 \\ 0 & 0 & 0.178 & 0.256 & 0.203 & 0.234 \\ 0 & 0 & 0.034 & 0.117 & 0.235 & 0.233 \\ 0 & 0 & 0.048 & 0.181 & 0.398 & 0.196 \end{bmatrix}$$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λ_d
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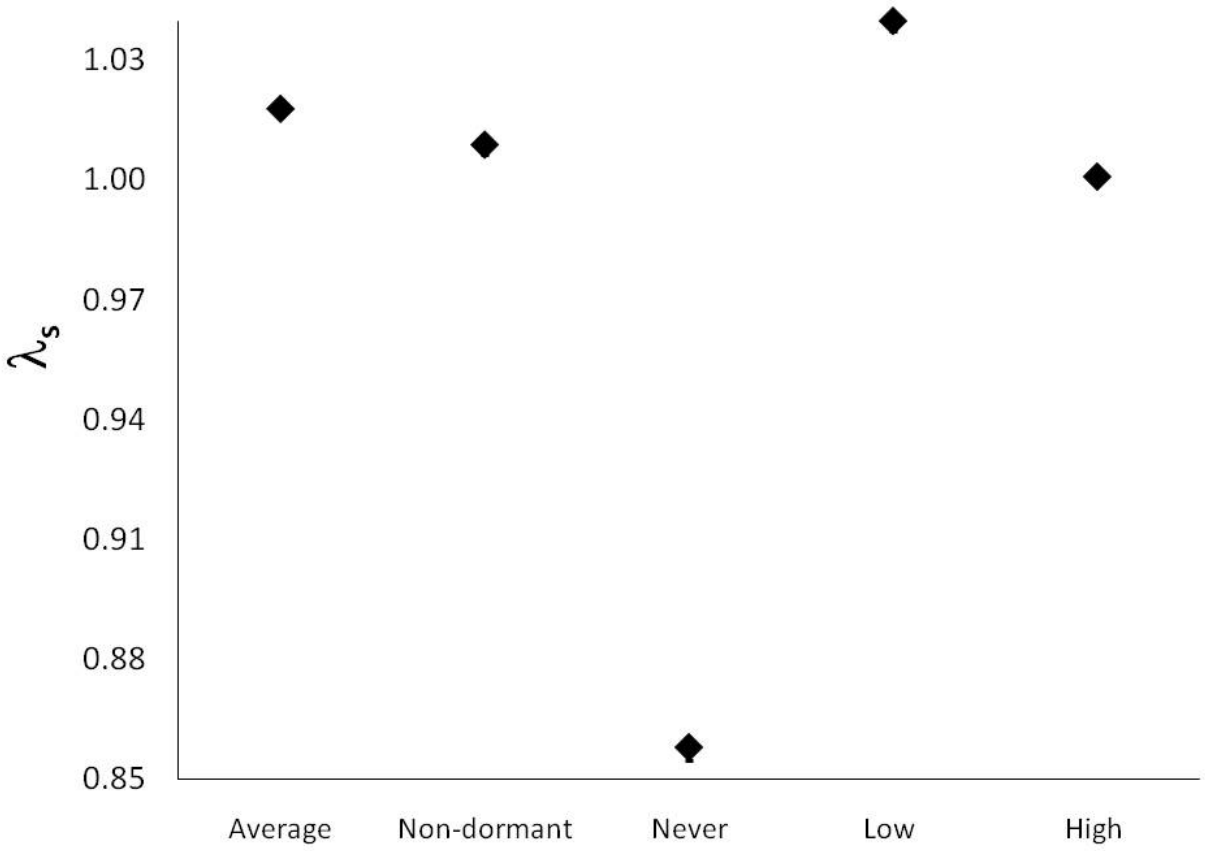
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λ_s
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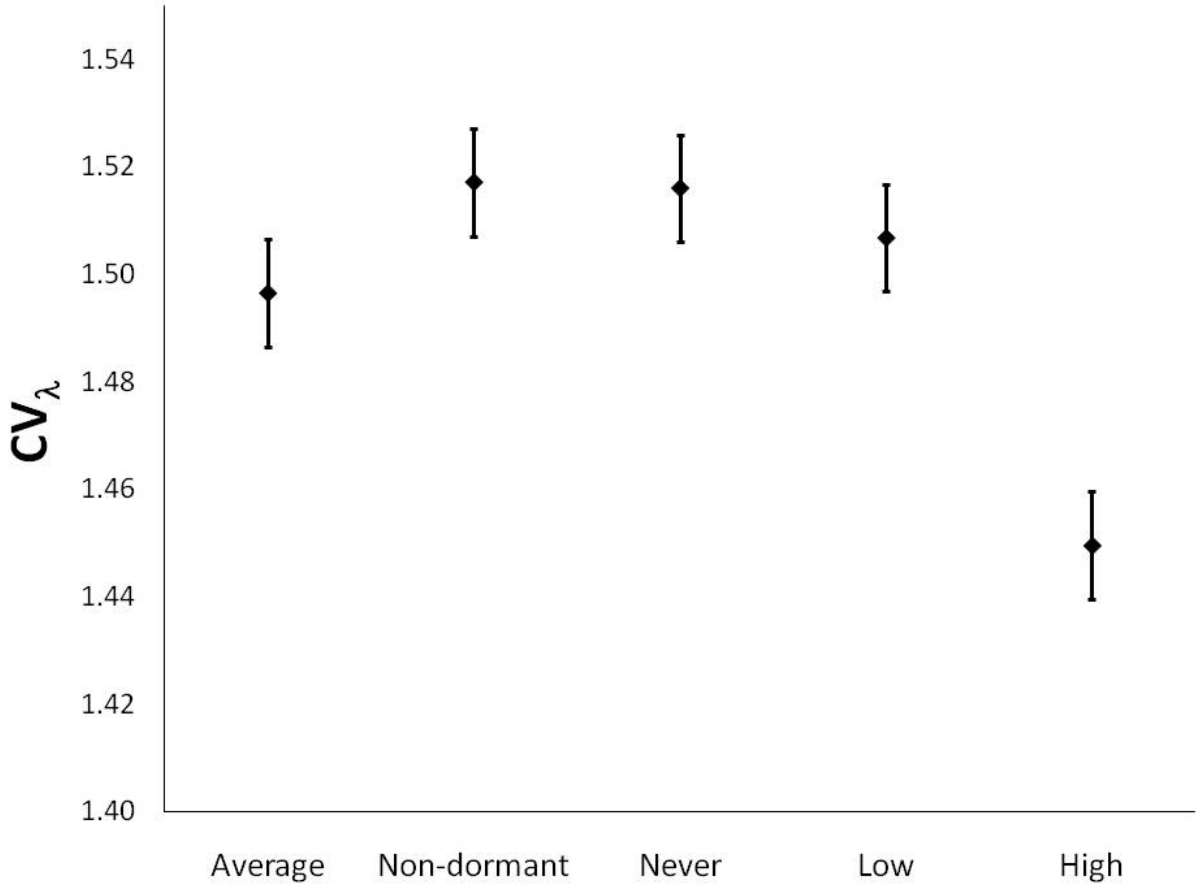
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1.46
1.44
1.42
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CV_λ
Average
Non-dormant
Never
Low
High

CHAPTER 4

It is risky out there: The risks of emergence and the benefits of prolonged dormancy.

Abstract

Prolonged dormancy is a stage in herbaceous perennial plants in which some individuals in a population fail to resprout for one or more growing seasons and instead remain alive, below ground. Prolonged dormancy is puzzling, because foregoing one or more seasons of growth and reproduction seems costly. However, it has been suggested that prolonged dormancy may benefit plants by allowing them to avoid risk above ground. If so, then the benefits of dormancy depend on the performance of above ground stages. Here, I measured physiological and demographic consequences of emerging during times of stress. Specifically, I asked: 1) How do emergent plants respond to stress imposed by high temperatures and low soil moisture? 2) What are the consequences for losing above ground tissue to defoliation? and 3) Do the risks of emergence outweigh the benefits during times of stress? Plants showed remarkable tolerance to stress in the short term, as high temperatures and low moisture did not have a strong effect on physiological performance and defoliation did not significantly impact stored resource dynamics. However, environmental stress did result in demographic costs. In particular, defoliation significantly increased mortality rates for emergent plants. These patterns suggest that the costs of emerging during times of stress may outweigh the benefits, but only during extreme stress. Further, my results suggest that prolonged dormancy is a beneficial stage, allowing plants to avoid the negative effects of a variable environment.

Introduction

In both plant and animal taxa, dormancy is a stage in which growth stops, metabolism slows, and the organism enters a state that is more resistant to environmental hazards (Harper 1977; Campbell and Reece 2005). Dormancy is very common in plants and animals during predictable stressful conditions, such as extreme temperatures or drought during the winter or summer when success at growth or reproduction is unlikely. In these cases, dormancy provides safety under extreme stress (high benefit) at the cost of maintaining basal metabolism to ensure survival (low cost). The expected net benefit of this strategy explains why all individuals opt to remain dormant at those times. In plants, a much more intriguing type of dormancy is prolonged dormancy, in which some individuals in a population fail to resprout during the growing season and instead remain belowground while others emerge to grow and reproduce (Lesica and Steele 1994). Though prolonged dormancy is quite common (Lesica and Steele 1994; Shefferson 2009), the costs and benefits of this type of dormancy are not well understood.

At first glance, prolonged dormancy seems costly. Usually only a fraction of plants remain below ground while others emerge to grow and reproduce (Lesica and Steele 1994; Shefferson 2009). Therefore, conditions appear to be suitable for photosynthesis and reproduction. Further, prolonged dormancy differs from seed dormancy in that metabolic costs of maintaining mature plant parts below ground are likely higher than costs of surviving as seeds below ground. Thus, prolonged dormancy entails demographic costs of missed resource gain and reproduction, as well as physiological costs of maintaining metabolism below ground.

Given these costs, the prevalence of prolonged dormancy in many unrelated species (Lesica and Steele 1991; Shefferson 2009) indicates that it also provides some benefits. Most obviously, prolonged dormancy may allow individuals to avoid unfavorable conditions above

ground (Lesica and Steele 1994; Miller et al. 2004; Shefferson et al. 2005; Lesica and Crone 2007; Shefferson 2009; Gremer et al. *in prep., Chap. 3 of this dissertation*). In a study on *Solidago* clones, Morrow and Olfelt (2003) found that prolonged dormancy allowed plants to escape future herbivore attack. Shefferson et al. (2005) simulated stress in the form of herbivory and shading for two orchid species, and found that prolonged dormancy seemed to buffer individuals from the risks of mortality. Other studies have shown that prolonged dormancy may buffer individuals and populations from the negative effects of environmental stochasticity (Miller et al. 2007; Gremer et al. *in prep. Chap. 3 of this dissertation*). Together, these studies suggest that prolonged dormancy may allow plants to avoid risk above ground, thereby providing the benefit of safety. If so, the benefits of dormancy depend on the degree to which encountering stress above ground results in negative consequences.

Here, I combine long term demographic data with comparative physiology and field experiments to measure performance of dormant and emergent plants during times of stress in *Astragalus scaphoides*, a perennial herbaceous plant. By doing so, I can understand the risks that plants face if they come above ground instead of remaining dormant below ground , and evaluate whether those risks may favor prolonged dormancy, and the safety that it provides. Environmental conditions such as high temperatures and low water availability can interfere with resource capture as well as result in decreased performance and mortality (Larcher 2001). Although plant responses to herbivory in nature can be complex (Quentin et al. 2010), tissue loss through actual and simulated herbivory has been shown to alter plant performance leading to physiological and demographic costs (Tiffin 2000; Ehrlen 2002; Ehrlen 2003; Knight 2004; Leimu and Lehtila 2006; Shefferson et al. 2006). Therefore, I evaluated the physiological and demographic responses of emergent plants to stress (heat, drought, and defoliation), and

determined the extent to which stress in emergent plants influences stored resource dynamics relative to dormant plants. Specifically I asked: 1) What are the physiological and demographic consequences for emerging during an extremely hot and dry season? 2) How do stored resource dynamics compare between dormant and emergent plants during times of environmental stress?, and 3) How does defoliation of emergent plants influence future performance? If prolonged dormancy is a strategy to avoid unfavorable conditions, then I expect to detect physiological or demographic costs for emerging during times of environmental stress.

Methods

Study species and long term monitoring

Astragalus scaphoides (Fabaceae) is a long lived, iteroparous legume with a long, narrow taproot, found on south-facing slopes in high-elevation sagebrush steppe communities (Lesica 1995). It does not reproduce vegetatively (Lesica 1995). Plants flower approximately in alternate years (Lesica 1995; Crone et al. 2004; Crone et al. 2005; Crone and Lesica 2006). Plants that do not flower may produce leaves and be vegetative, or remain dormant during the growing season. If plants emerge aboveground, they initiate growth in April, and biomass senesces back to perennating roots in early July. Evidence of previous flowering can be seen on root crowns, because the flowering stalks leave scars that are apparent even after several years. Mature dormant plants can be located by dried flowering stalks that persist above ground for 2-3 years.

On average, 24% of the individuals in this population are dormant in any given year and dormancy events typically last one year (Gremer, *in prep*., *Chap 1 of this dissertation*).

Dormancy is correlated ($0.3 < r < 0.5$) with warm, dry weather in the spring (Gremer, *in prep*., *Chap 1 of this dissertation*). However, even in years of high dormancy, only a portion of individuals remain dormant while the rest emerge as vegetative or reproductive plants (Gremer, *in prep*. *Ch 1 of this dissertation*). Dormancy in *A. scaphoides* does not seem to be strongly associated with resource availability. Two previous studies attempted to alter plant performance by adding supplemental water over three years (Crone and Lesica, 2006), and adding supplemental nitrogen and phosphorus in 2005 (E. Crone unpublished). I also added supplemental nitrogen in 2007 (Gremer, unpublished), but none of these treatments affected the probability of dormancy (Gremer, unpublished analysis).

Long term demographic data on *A. scaphoides* were collected along monitoring transects from 1986 to 2010. See Gremer (*in prep*., *Chap. 1 of this dissertation*) for details on data collection. For the 23 years of this study, data on individual histories for over 350 plants were collected. This demographic data includes information for 3 vegetative stage classes, a flowering stage, and a dormant stage. However, for this study, I combined the vegetative classes for simplicity and because differences in physiological performance among vegetative classes were not detected (J. Gremer *unpublished analyses*), resulting in 3 stages: vegetative, reproductive (flowering plants), and dormant. Dormant plants were defined as those that disappear below ground for one or more years and re-emerge (Lesica 1995). Since most dormancy bouts last three years or less (Gremer *in prep*., *Chap. 1 of this dissertation*), transitions were not estimated for the first and last three years of data. This allows for separation dormancy from recruitment at the beginning of the study, and mortality at the end of the study (Lesica 1995). Therefore, the middle 17 years of data were used to estimate average survival and transition rates for each of the 24 years.

Response to heat and drought stress

Response to stress in the field was measured under natural conditions as plants developed during an exceptionally hot year (National Climatic Data Center, hereafter NCDC, 2010; see *Results*). Conditions in the sagebrush steppe range from cool and moist early in the growing season to hot and dry later as the season progresses. In 2007, I measured the physiological performance of emergent plants as conditions changed during the season by measuring photosynthesis, water potential, and chlorophyll fluorescence. Seasonal changes in temperature and moisture were measured by using dataloggers and by sampling soil moisture. Hobo dataloggers (Hobo 4-Channel External datalogger, Onset Computer Corporation, Bourne, MA USA) recorded temperatures 6 cm below the soil surface every two hours throughout the growing season. Soil moisture was sampled using a soil moisture probe (Hydrosense Soil Water measurement system, Campbell Scientific, Logan UT USA) every 7 to 10 days. Because performance was expected to vary according to life stage (Crone et al. 2009; Gremer et al. *in press, Chapter 2 of this dissertation*), physiological measurements were conducted on both vegetative and reproductive plants.

Physiological Measurements

Leaf area based photosynthesis was measured on both vegetative and reproductive plants (n=between 8-10 for each life stage) every 7 to 10 days throughout the growing season 2007 using a Li-Cor 6400 Photosynthesis System (Li-Cor Biosciences, Lincoln, Nebraska USA). For all photosynthesis measurements, temperature and relative humidity in the leaf chamber were set to match ambient conditions. Photosynthetically active radiation (PAR: 400-700 nm) in the chamber was supplied by an internal light source. PAR was above saturation (J. Gremer,

unpublished analysis) and was set to reflect at ambient conditions on the day of measurement (from 1000 to 1800 μmol m^{-2} s^{-1}). Carbon dioxide concentration was kept at 400 μmol mol^{-1} and relative humidity in the leaf chamber was kept near ambient. Leaf area in the cuvette (*A. scaphoides* leaves are composite) was measured using an image analysis system (Leaf Area Measurement version 1.3, University of Sheffield 2003) and these areas were used to calculate leaf area based gas exchange values.

Conditions such as high light and high temperatures may damage photosynthetic apparatus (Larcher 2001; Maxwell and Johnson 2000; Germino and Smith 2000). Therefore chlorophyll fluorescence measurements were conducted throughout the growing season in 2007. Dark adapted fluorescence measurements indicate the functioning of photosystem II (PSII), which can give an estimate of photosynthetic performance (Maxwell and Johnson 2000). Perhaps more importantly, PSII is often the first point of damage in a leaf, so fluorescence measurements can indicate if stress has impaired the photosynthetic function of a leaf (Larcher 2001; Maxwell and Johnson 2000). Variable to maximum fluorescence (Fv/Fm) was measured on 2 leaves per plant (n= 6 to 10 for each life stage). Measurements followed protocol in Germino and Smith (2000), using a modulated fluorometer (Opti-Sciences, Inc., Hudson NH USA, Model OS-100). Leaves were dark-adapted using leaf clips for at least 1 hour before measurement. Both pre-dawn and mid-day measurements were conducted using the same leaves. For most plant species, optimal values for Fv/Fm are around 0.83 (Johnson et al. 1993; Maxwell and Johnson 2000). Measurements below that value indicate reduced photosynthetic capacity, and photoinhibition. Comparisons between mid-day and pre-dawn values can indicate whether photoinhibition is occurring, whereas comparisons of Fv/Fm values throughout the season allows distinction between dynamic (during the day only) and chronic (entailing damage

and lack of recovery through time) photoinhibition. Low xylem pressure potentials (XPP) may indicate water stress (Larcher 2001), predawn XPP measurements were conducted four times throughout the season to investigate water status of emergent plants. XPP measurements were conducted for both reproductive and vegetative plants(n=6 to 10 per life stage). Two leaves were cut from each plant, and were immediately sealed in a plastic bag and transported in a portable cooler to a work station for measurement within 15 minutes. XPP was measured using a pressure chamber (PMS instruments, Corvallis, OR).

I used generalized linear mixed models to compare physiology data (leaf area based photosynthesis, fluorescence, and XPP) between life stages and through time. Stage and time in the growing season were included as fixed factors, as well as the interaction between the two. Individual was included as a random factor, since measurements were repeated on the same individuals throughout the season. Association between photosynthesis rates and available soil moisture were analyzed using Pearson's product moment correlation coefficient.

Stored resources

To determine whether the degree of heat and drought stress influences stored resource levels in emergent plants relative to dormant plants, I harvested dormant, vegetative, and reproductive plants at the beginning and end of the growing season in 2007 (an unusually hot, dry year) and 2008 (a typical year; n= between 5 and 7 for each life stage at each harvest time in each year). The first harvest was conducted early in the growing season, and was timed such that all life stages could be identified. This timing does not affect conclusions about stored resource dynamics (see Gremer et al. *in press, Chap. 2 of this dissertation*). The late season harvest occurred at the end of the season after aboveground biomass of plants had senesced. Plants were randomly selected for harvest and the 10 upper cm of taproot (closest to the soil surface) was

destructively harvested. All samples were stored on ice for transport to the laboratory. Roots were analyzed for non-structural carbohydrates (NSC) and nitrogen (N), since these two nutrients because they are important in other life history transitions for *A. scaphoides* (Crone et al. 2009; Gremer et al. 2010, *Chapter 2 of this dissertation*). See Gremer et al. (2010*, Chapter 2 of this dissertation*) for details on NSC and N analyses.

I used analyses of variance with time and stage as factors and their interaction to compare stored resources between life stages at the beginning of the season. The same analysis was conducted to compare stored resources at the end of the season. Tukey's honest significant difference (HSD) test was used as a post hoc comparison of mean resources between life stages at a given time. General linear models with stage and time as independent variables were used to estimate the change in resource concentrations throughout the growing season for each life stage (normal distribution with identity link, R Foundation for Statistical Computing 2009). Inspection of residuals confirmed that assumptions of general linear models were met.

Demographic response to heat and drought stress

The long term monitoring dataset described above was used to compare whether vital rates after the 2007 season differed significantly from the long term average, specifically, survival and flowering probabilities in the subsequent season (2008). Logistic regression (R Foundation for Statistical Computing 2009) was used to determine whether survival or flowering after the 2007 season significantly differed from other years, and if there were differences between stage classes. Because *Astragalus scaphoides* tends to flower in alternate years (Crone and Lesica 2004; Crone et al. 2005; Crone and Lesica 2006; Crone et al. 2009), I used generalized linear models with fruits in the previous year and stage in the previous year as main

factors as well as year and individual as random factors (family = binomial, link=logit, R Foundation for Statistical Computing 2009).

Response to defoliation

In this population, individuals may experience complete defoliation by insect or mammalian herbivores (J. Gremer, *pers. obs*). This type of herbivory seems to affect a small proportion of plants (~2%, J. Gremer, *pers. obs*), but may constitute a large risk for those individuals that lose all above ground tissue to herbivory. Further, loss of leaf tissue through herbivory directly interferes with one of the benefits of emergence: resource gain through photosynthesis. Therefore, herbivory was simulated using two treatments: 1) A worst-case scenario in all leaf tissue was repeatedly removed right after full leaf expansion and upon any subsequent regrowth, so that the plant pays the full construction cost of leaves without the benefit of resource gain through photosynthesis(hereafter *press plants*), and 2) a typical-herbivory treatment in which all leaf tissue was removed only once in the season, simulating a more common situation in which an herbivore defoliates a plant only once (hereafter *pulse plants*). These treatments were implemented for both reproductive and vegetative stage classes, and were compared to a control group.

In May of the growing season 2008, vegetative and reproductive plants were marked in the field and randomly assigned to one of two treatment groups, or a control group (n=50 for each treatment group and life stage). For the severe herbivory treatment (hereafter, *press plants*), all above ground tissue was removed using small scissors approximately every 10 days throughout the growing season. The more typical herbivory treatment (hereafter, *pulse plants*) was implemented by removing all of the above ground tissue once in the season (on May 31, 2008).

Stored resource dynamics

In order to determine the consequences of defoliation, I compared stored resource dynamics between treatment, control, and dormant plants. A subset of treatment and control plants for each stage was harvested (n= between 5 and 7 per life stage and treatment), as well as dormant plants at two times during the growing season (n=3 at beginning of season, n=5 at end). The first harvest was conducted early in the growing season, before treatment, and again at the end of the season after aboveground biomass of plants had senesced. Plants were randomly selected for harvest. See *Stored Resources* above for more information on these harvests and analyses.

I used analyses of variance with treatment, time, and stage as factors and their interaction to compare stored resources between life stages at the beginning of the season. The same analysis was conducted to compare stored resources at the end of the season. Tukey's honest significant difference (HSD) test was used as a post hoc comparison of mean resources between life stages at a given time and general linear models were used to estimate the change in resource concentrations throughout the growing season for each life stage (normal distribution with identity link, R Foundation for Statistical Computing 2009).

Demographic consequences of herbivory

In order to quantify the demographic consequences of defoliation, the fates of treatment and control plants were followed for two seasons after treatment (2009 and 2010). Plant stage (flowering, or small, medium, or large vegetative) as well as fruit set for those plants that flowered were recorded. No treatment plants (press or pulse) had successful inflorescences in

2009 (no fruit set), so these data were not included in analyses. In addition, because plants that were not seen aboveground could be either dead or dormant, individuals were excavated to distinguish between dormancy and mortality events. Fortunately, dormant plants are easily distinguishable from dead plants, since plants lose turgor and color upon mortality. Chi squared tests were used to determine whether treatments resulted in differences in mortality rates and flowering (conditioned on survival) and then used logistic regression to estimate differences between each treatment group (R Foundation for Statistical Computing 2009). Tests were performed separately for vegetative and reproductive plants.

Results

Response to drought stress

Physiological performance

2007 was the 10th warmest year on record for the United States (since 1895) and the 47th driest (NCDC 2010). Montana was hotter and drier than average and July 2007 was the warmest on record. In addition, 2007 was a record year for days with temperatures above 37 ^{o}C (100 ^{o}F) in Montana. Soil temperatures were above 26^{o}C for the second half of the growing season (Fig 1). Soil moisture declined throughout the season, ranging from 24% early in the season to 9% towards the end of the season (Fig. 2).

Predawn pressure potentials decreased throughout the growing season (χ^2 =50.449, P<0.001), indicating reduced water availability to (Fig. 2), but did not differ between above-ground life stages (Stage: χ^2 =2.065, P=0.151; Time*Stage: χ^2 =1.1223, P=0.772). I detected marginally significant differences in photosynthetic rates over time (Time: χ^2=16.013, P=0.099), and photosynthetic rates were correlated with soil moisture (Pearson's correlation coefficient

=0.29, P=0.013). Photosynthesis did not differ significantly between above-ground stages (Fig. 3; Stage: $\chi^2 = 6.1897$, P=0.402; Time*Stage: $\chi^2 = 6.153$, P=0.292). Midday Fv/Fm differed among sampling dates, but did not consistently decrease (Fig. 4; Date: $\chi^2 = 8.983$, P=0.030). Predawn Fv/Fm values increased over the growing season (Fig. 4; $\chi^2 = 74.583$, P<0.001). Neither differed between above-ground life stages (midday: Stage: $\chi^2 = 1.347$, P=0.246; Time*Stage: $\chi^2 = 0.569$, P=0.451); predawn: Stage: $\chi^2 = 0.883$, P=0.643; Time* Stage: $\chi^2 = 0.558$, P=0.455). However, mid-day Fv/Fm values were lower than those at predawn suggesting dynamic photoinhibition (Time of day: $\chi^2 = 80.855$, P<0.001; Date: $\chi^2 = 29.549$, P<0.001; Time* Date: $\chi^2 = 7.285$, P=0.063).

Stored Resources

All plants gained NSC during the growing season 2007 (Table 1; ANOVA: Season: $F_{1,82}$=78.680, P<0.001; Stage*Season: $F_{2,32}$=1.290, P=0.289), but NSC gain did not differ between years (ANOVA: Season*Year: $F_{1,73}$=1.086, P=0.343). Nitrogen dynamics varied by life stage (Table 1; ANOVA: Stage*Season: $F_{2,32}$=2.548, P=0.093). Dormants did not gain N during the season (95% CI [-0.301- 0.300]) while reproductive and vegetative plants did (reproductive 95% CI [0.27- 0.82], vegetative 95% CI [0.43- 0.98]). Surprisingly, above ground stages gained less nitrogen in 2008 than in the hot, dry year of 2007 (ANOVA: Season* Year: $F_{1,73}$=4.833, P=0.031).

Demographic consequences of drought stress

The hot and dry conditions in 2007 did not have strong effects on survival or flowering (Fig. 5). Survival was not significantly lower than expected from long-term dynamics (z=0.139,

P=0.889), so the hot and dry conditions did not increase mortality. Flowering probability was slightly lower than expected in 2008 (following hot dry conditions in 2007), but this effect was only marginally significant (reduced flowering rates by 0.165 (logit scale), CI of change [0.025-0.606], z=-1.547, P=0.122).

Response to defoliation

Leaf removal

For the initial treatment, approximately 8 leaves per plant for vegetative plants (pulse treatment mean = 7.7 leaves, press mean = 8.7) and 19 leaves per plant for reproductive plants (pulse mean = 18.6, press mean = 19.4) were removed. Pulse plants regrew leaves, at an average of 3 leaves per plant (mean of reproductive pulse= 3, vegetative pulse = 2.8). Reproductive press plants regrew an average of 5.9 leaves per plant, while vegetative press plants regrew an average of 4.3 leaves. (Note that all of the regrowth leaves were removed from press plants.) In contrast, reproductive control plants had a mean of 22.2 leaves per plant and vegetative controls averaged 9.8 leaves per plant.

Stored resources

Defoliation treatments did not significantly affect NSC concentrations at the end of the growing season (Fig. 6; reproductive plants: $ANOVA_{2,24}$: F=1.548 P=0.233; vegetative plants: $ANOVA_{2,21}$: F=1.564 P=0.233). In vegetative plants, NSC tended to increase during the growing season (change in % NSC from early to late harvest: control: 6.796, t=2.397, P=0.04; pulse: 12.17, t=4.292, P=0.001; press: 6.788, t=2.271, P=0.0343) but did not differ significantly among treatments (control 95% CI [0.652-12.939]; pulse 95% CI [6.03-18.314]; press 95% CI [0.930-12.646]. Reproductive plants in control and pulse treatments increased NSC (change in % NSC

from early to late harvest: control: 5.247 P=0.04; pulse: 5.545 P=0.04) but reproductive plants in press treatments did not (press: 0.402, P=0.18). At the beginning of the season, dormant plants had lower NSC concentrations than reproductive plants (ANOVA: $Stage_{2,12}$: F=6.818, P=0.016; Tukey's HSD between reproductive and dormant P=0.013), and somewhat lower NSC concentrations than vegetative plants, though this difference was not statistically significant (Tukey's HSD between reproductive and vegetative P=0.220). At the end of the growing season, NSC concentrations did not differ between stages ($ANOVA_{2,14}$: F=1.285 P=0.307). Although NSC concentrations were lower in dormant plants at the beginning of the season, the seasonal increase in dormants was not statistically different from any other treatments (95% CI: 0.306,14.643). In summary, all plants increased NSC concentrations throughout the season, except for reproductive plants that received the press treatment.

Defoliation treatments also did not affect N concentrations (Fig 6). Vegetative plants began the season with the lowest nitrogen concentrations, while dormant plants had the highest N concentrations ($Stage_{2,12}$: F=15.586, P=0.001). However, all life stages ended the season with similar N concentrations ($Stage_{2,14}$: F=0.053, P=0.949). Reproductive plants did not gain nitrogen (Control: 0.208%, P=0.262; pulse: 0.0545%, P=0.779; press: 0.125%, P=0.720), but vegetative plants did (Control: 0.599%, P=0.021; pulse: 0.7%, P=0.008; press: 0.726%, P=0.009), regardless of treatment. Dormant plants seemed to lose N throughout the season (0.421%, p=0.072).

Demographic consequences of defoliation

Defoliation treatments increased the likelihood of mortality for both vegetative and reproductive plants (Fig. 7). Control plants had low mortality (vegetative: 0.12 and reproductive:

0.08) while treatment plants had significantly higher mortality (Veg: pulse mortality= 0.35 press mortality = 0.43, χ^2 = 7.922 P=0.005; Reproductive: pulse mortality = 0.28, press mortality = 0.36, χ^2 = 8.051 P=0.005). For both vegetative and reproductive plants, the press treatment had a stronger effect (vegetative: z=2.441, P=0.015; reproductive: z=2.456, P=0.014) than the pulse treatment (Vegetative: z=1.791, P=0.073; Reproductive: z=1.896, P=0.058). Neither defoliation treatment affected flowering probability in 2009 (Fig. 7; Veg: χ^2 = 0.974 P=0.324; Repro: χ^2 = 1.943, P=0.584). No plants in this experiment successfully set fruit in 2009. Defoliation treatments in 2008 did not affect survival from 2009-2010 (Veg: χ^2 = 3.319, n=44, P=0.344; Repro χ^2 = 4.046, n=59, P=0.257) or flowering in 2010 (Veg: χ^2 = 1.797,P=0.616; Repro χ^2 = 1.429, P=0.699).

Discussion

Prolonged dormancy may provide the benefit of safety below ground, but this benefit depends on the performance of plants that emerge. If prolonged dormancy functions to buffer plants from risky conditions above ground, then there should be costs to emerging during times of environmental stress. In this study, plants did not suffer large physiological costs in response to stress, yet the demographic costs were significant. Here, I found marginal effects of hot and dry conditions, as evidenced by slightly lower flowering probabilities in the following season. More importantly, my results show large consequences of defoliation on future survival. Considering that population persistence in *A. scaphoides* is strongly associated with survival (Lesica 1995), this constitutes a significant risk for emergent plants. Therefore, these costs of

emerging during times of stress may be enough to favor the dormant stage, in which plants can avoid these types of risk by remaining belowground.

My results suggest that, in the short term, *A. scaphoides* is quite robust to extreme temperatures and reduced moisture availability. Soil moisture was significantly correlated with photosynthetic rates but, with the exception of reproductive plants, photosynthetic rates did not seem to drastically decline as the season progressed. It may be that this correlation was driven by the June peak in photosynthesis, which coincided with high soil moistures. This is consistent with the fluorescence results, since Fv/Fm values remained stable throughout the season. Either way, the hot and dry conditions of 2007 did not seem to have significant impacts on the short term physiological performance of emergent plants. *Astragalus scaphoides* inhabits sagebrush steppe communities which exhibit high variability in rainfall and temperatures (Paruelo and Lauenroth 1998), and my physiological measurements suggest that this species is remarkably well adapted to the system. However, instantaneous physiological measurements, such as photosynthesis, may be poor indicators of overall plant status (Arntz et al. 1998). For instance, in a study on a semi-arid perennial, Casper et al. (2006) found that drought depressed gas exchange and leaf water potentials, but changes in leaf level physiology did not translate into differences in growth. This may be particularly true in long lived species that can use stored resources to buffer the effects of short term changes in resource assimilation.

In this study, neither drought nor defoliation influenced stored carbon concentrations at the end of the season. Emergent plants were able to increase concentrations of stored available carbon (NSC) despite slight decreases in photosynthesis with drought and leaf removal. This is surprising, since plants use stored resources to replace leaf tissue (Ho and Rees 1976; Chapin et al. 1990; Zimmerman and Whigham 1992; Van der Heyden and Stock 1996; Wyka 1999), and

press treatments would prevent further photosynthesis. In a previous study (Gremer et al. *in press, Chap. 2 of this dissertation*), it was shown that dormant plants were able to increase concentrations of NSC while completely belowground, presumably by remobilizing structural carbon into available forms. It may be that this remobilization occurs in response to low carbon stores, or reduced carbon input from photosynthesis. If so, then the physiological costs of defoliation may be initially masked by internal resource allocation. However, this internal reallocation of resources may carry a long term cost that is manifest demographically. In the case of drought, demographic costs were marginal, with only a marginal reduction in flowering probability, but for defoliation they were large. Future studies that explore the mechanisms and consequences of internal resource allocation and remobilization in plants may lead to a better understanding of plant response to environmental stress.

For *A. scaphoides*, prolonged dormancy has been shown to buffer individuals from the negative effects of environmental stochasticity (Gremer et al., *in prep., Chap 3 of this dissertation*), suggesting that the benefits of prolonged dormancy depend on environmental conditions. Here, I show that stressful conditions, particularly defoliation, can carry demographic costs, even when those conditions are episodic (i.e. occurring in one season). These negative impacts could be much larger if such conditions are either more extreme, or occur over longer time periods. For instance, in a study on a perennial woodland herb, Gustafsson (2004) demonstrated that only extreme defoliation treatments (complete and repeated leaf removal treatments) affected reproduction. Similarly, Whigham (1990) showed that only extreme defoliation treatments effectively decreased flowering rates. These studies suggest that there may be threshold response to stress, such that extreme stress or continued stress may be necessary to elicit responses in some herbs. If so, then it may take an accumulation of stressful

conditions or events to affect plant performance. Nonetheless, my study shows that episodic events can have large demographic costs.

Prolonged dormancy may not only provide a stage in which plants can avoid stress; it may also be a response to stress. Previous studies have shown dormancy rates to increase after defoliation (Morrow and Olfelt 2003; Shefferson et al. 2005; Ehrlen 2003) and drought (Epling and Lewis 1952; Boeken 1991; Lesica and Steele 1994; Vaughton and Ramsey 2001). Shefferson et al. (2005) speculated that dormancy reduced mortality rates following defoliation in an orchid species. In a study of *Solidago* clones, Morrow and Olfelt (2003) suggested that dormancy allowed plants to escape the risk of future herbivore attack. In this case, the herbivore in question (a leaf beetle) laid larvae in *Solidago* clones, and defoliation in one year increases the risk of defoliation in later years. Thus, plants could avoid further damage by remaining dormant. If stress in one year is a good indication that stress will occur in adjacent years, then dormancy may be both a response to stress and a strategy to avoid future stress. In the present study, press plants had significantly higher rates of dormancy than controls (Reproductive: χ^2=4.59, P=0.03; Vegetative: χ^2=7.57, P=0.006). Press plants also had significantly higher risks of mortality in the following year. Here, I cannot say definitively whether prolonged dormancy may have buffered these individuals from the risk of death, and mortality rates may have been higher without dormancy. However, if prolonged dormancy does reduce mortality rates following environmental stress, it may buffer populations from extinction risk due to environmental stochasticity as suggested by previous work (Gremer et al. *in prep., Chap 3 of this dissertation*).

Throughout their lifetime, plants may suffer damage from a variety of sources, including heat, drought, and defoliation (Nilsson et al. 1996). However, in prolonged dormancy, plants may benefit from avoiding those risks (Lesica and Steele 1994; Miller et al. 2004; Shefferson et

al. 2005; Lesica and Crone 2007; Shefferson 2009; Gremer et al. *in prep., Chapter 3 of this dissertation*). If so, then the benefits of this cryptic life stage depend on the consequences of encountering stressful conditions above ground. Here, plants showed remarkable physiological tolerance to short term stress. However, environmental stress, in particular defoliation, resulted in significant demographic costs, since defoliated plants had much higher risks of mortality. Thus, remaining dormant may be safer than coming up above ground during defoliation events. It is noteworthy that defoliation, but not drought and heat stress, caused such large demographic costs. In *A. scaphoides'* steppe habitats, risks of herbivory and subsequent defoliation are much more stochastic than risks of drought, which is a much more predictable form of stress. The large costs of such unpredictable events are consistent with previous studies (Gremer et al., *in prep Chap 3 of this dissertation*; Shefferson et al. 2005; Miller et al. 2007), suggesting that prolonged dormancy can buffer plants from the risks of environmental stochasticity. Overall, results here and elsewhere help explain the prevalence of this stage in the life histories of perennial plants.

Literature Cited

Boyce, P. J., and J. J. Volenec. 1992. Taproot Carbohydrate Concentrations and Stress Tolerance of Contrasting Alfalfa Genotypes. Crop Science 32:757-761.

Campell, N. A., and Jane B. Reece. 2005. Biology, 7th edition. Pearson, New York, NY.

Casper, B. B., I. N. Forseth, and D. A. Wait. 2006. A stage-based study of drought response in *Cryptantha flava* (Boraginaceae): Gas exchange, water use efficiency, and whole plant performance. American Journal of Botany 93:978-987.

Chapin III, F. S., E. Schultze, and H.A. Mooney. 1990. The ecology and economics of storage in plants. Annual Review of Ecology and Systematics 21:423-447.

Crone, E. E. a. P. L. 2004. Causes of synchronous flowering in *Astragalus scaphoides*, an iteroparous perennial plant. Ecology 85:1944-1954.

Crone, E. E., L. Polansky, and P. Lesica. 2005. Empirical models of pollen limitation, resource acquisition, and mast seeding by a bee-pollinated wildflower. American Naturalist 166:396-408.

Crone, E. E., and P. Lesica. 2006. Pollen and water limitation in *Astragalus scaphoides*, a plant that flowers in alternate years. Oecologia 150:40-49.

Crone, E. E., E. Miller, and A. Sala. 2009. How do plants know when other plants are flowering? Resource depletion, pollen limitation and mast-seeding in a perennial wildflower. Ecology Letters 12:1119-1126.

Dahlgren, J. P., and J. Ehrlen. 2009. Linking environmental variation to population dynamics of a forest herb. Journal of Ecology 97:666-674.

Ehrlen, J. 2002. Assessing the lifetime consequences of plant-animal interactions for the perennial herb *Lathyrus vernus* (Fabaceae). Perspectives in Plant Ecology Evolution and Systematics 5:145-163.

Ehrlen, J. 2003. Fitness components versus total demographic effects: Evaluating herbivore impacts on a perennial herb. American Naturalist 162:796-810.

Germino, M. J., and W. K. Smith. 2000. Differences in microsite, plant form, and low-temperature photoinhibition in alpine plants. Arctic Antarctic and Alpine Research 32:388-396.

Gremer, J.R. *In prep. (Chapter 1 of this dissertation).* Patterns of prolonged dormancy in *Astragalus scaphoides*: Inference from long term monitoring.

Gremer, J. R., A. Sala, and E. E. Crone. 2010. Disappearing plants: why they hide and how they return. Ecology 91:3407-3413.

Gremer, J.R., E.E. Crone, and P. Lesica. *In prep (Chapter 3 of this dissertation).* Are dormant plants hedging their bets? Demographic consequences of prolonged dormancy in variable environments.

Harper, J. L. 1977. Population Biology of Plants. Academic Press, London.

Ho, L. C., and A.R. Reese. 1976. Re-mobilization and redistribution of reserves in the tulip bulb in relation to new growth until anthesis. New Phytologist 76:59-68.

Knight, T. M. 2004. The effect of herbivory and pollen limitation on a declining population of *Trillium grandiflorum*. Ecological Applications 14:915-928.

Kobe, R. K. 1997. Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. Oikos 80:226-233.

Kolb, P. F., and R. Robberecht. 1996. High temperature and drought stress effects on survival of *Pinus ponderosa* seedlings. Tree Physiology 16:665-672.

Larcher, W. 2001. Physiological Plant Ecology, Fourth edition edition. Springer, New York NY USA.

Leimu, R., and K. Lehtila. 2006. Effects of two types of herbivores on the population dynamics of a perennial herb. Basic and Applied Ecology 7:224-235.

Lesica, P., and Brian M. Steele. 1994. Prolonged Dormancy in Vascular Plants and Implications for Monitoring Studies. Natural Areas Journal 14:209-212.

Lesica, P. 1995. Demography of *Astragalus scaphoides* and effects of herbivory on population growth. Great Basin Naturalist 55:142-150.

Lesica, P., and B. M. Steele. 1997. Use of permanent plots in monitoring plant populations. Natural Areas Journal 17:331-340.

Lesica, P., and E. E. Crone. 2007. Causes and consequences of prolonged dormancy for an iteroparous geophyte, *Silene spaldingii*. Journal of Ecology 95:1360-1369.

Maxwell, K., and G. N. Johnson. 2000. Chlorophyll fluorescence - a practical guide. Journal of Experimental Botany 51:659-668.

Miller, M. T., G.A. Allen, and J.A. Antos. 2004. Dormancy and flowering in two mariposa lilies (*Calochortus*) with contrasting distribution patterns. Canadian Journal of Botany 82:1790-1799.

Miller, M. T., J. A. Antos, and G. A. Allen. 2007. Demographic differences between two sympatric lilies (*Calochortus*) with contrasting distributions, as revealed by matrix analysis. Plant Ecology 191:265-278.

Mooney, H. A., K. Fichtner, and E.-D. Schulze. 1995. Growth, photosynthesis, and storage of carbohydrates and nitrogen in *Phaseolus lunatus* in relation to resource availability. Oecologia 104:17-23.

Morrow, P. A., and Joel P. Olfelt. 2003. Phoenix clones: recovery after long-term defoliation-induced dormancy. Ecology Letters 6:119-125.

National Climate and Data Center (NCDC). http://cdo.ncdc.noaa.gov/ancsum /ACS?coban=242409. Accessed March 2010.

Paruelo, J. M., and W. K. Lauenroth. 1998. Interannual variability of NDVI and its relationship to climate for North American shrublands and grasslands. Journal of Biogeography 25:721-733.

Shefferson, R. P., Tiiu Kull, and Kadri Tali. 2005. Adult whole-plant dormancy induced by stress in long-lived orchids. Ecology 86:3099-3104.

Shefferson, R. P., T. Kull, and K. Tali. 2006. Demographic response to shading and defoliation in two woodland orchids. Folia Geobotanica 41:95-106.

Shefferson, R. P. 2009. The evolutionary ecology of vegetative dormancy in mature herbaceous perennial plants. Journal of Ecology 97:1000-1009.

Tiffin, P. 2000. Mechanisms of tolerance to herbivore damage: what do we know? Evolutionary Ecology 14:523-536.

van Kleunen, M., and M. Fischer. 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. New Phytologist 166:49-60.

van Der Heyden, F., and W. D. Stock. 1996. Regrowth of a semiarid shrub following simulated browsing: The role of reserve carbon. Functional Ecology 10:647-653.

Wijesinghe, D. K., and D. F. Whigham. 1997. Costs of producing clonal offspring and the effects of plant size on population dynamics of the woodland herb *Uvularia perfoliata* (Liliaceae). Journal of Ecology 85:907-919.

Wyka, T. P. 1999. Storage, growth and reproduction in an alpine herbaceous plant, *Oxytropis sericea*. Ph.D. University of Missouri, Columbia.

Zimmerman, J. K., and D.F. Whigham. 1992. Ecological functions of carbohydrates stored in corms of *Tipularia discolor* (Orchidaceae). Functional Ecology 6:575-581.

Table 1. The change in resource concentrations over the growing season for 2007 and 2008. Values are differences in resource concentrations from the changes in resource concentrations, in parentheses are percent changes in resource concentrations (calculated as the change in the

concentration of the resource, divided by the initial concentration). No differences in nonstructural carbohydrate (NSC) dynamics were detected across years, but plants gained more nitrogen (N) in 2007 than 2008.

	Nonstructural Carbohydrates (NSC)		Nitrogen (N)	
Stage	**2007**	**2008**	**2007**	**2008**
Reproductive	6.33 (0.92)	5.25 (0.64)	0.64 (0.29)	0.21 (0.09)
Dormant	9.15 (4.67)	8.89 (5.22)	0.17 (0.073)	-0.42 (-0.15)
Vegetative	4.96 (1.75)	6.80 (1.29)	0.79 (0.41)	0.60 (0.32)

Figure Legends

Figure 1. Soil temperature throughout the growing season in 2007. Temperatures are 6 cm below the soil surface. The solid line represents daily averages while the dashed line with circles represents daily maximum temperatures.

Figure 2. Volumetric soil moisture and predawn xylem pressure potentials throughout the growing season in 2007. Top panel is soil moisture, where points represent averages of values collected using a soil moisture probe (n=20). Bottom panel is xylem pressure potentials (XPPs) for reproductive (open diamonds) and vegetative plants (solid squares) throughout the season. XPPs declined throughout the season, but no significant difference between stages were detected. Error bars represent +/- 1 standard error of the mean.

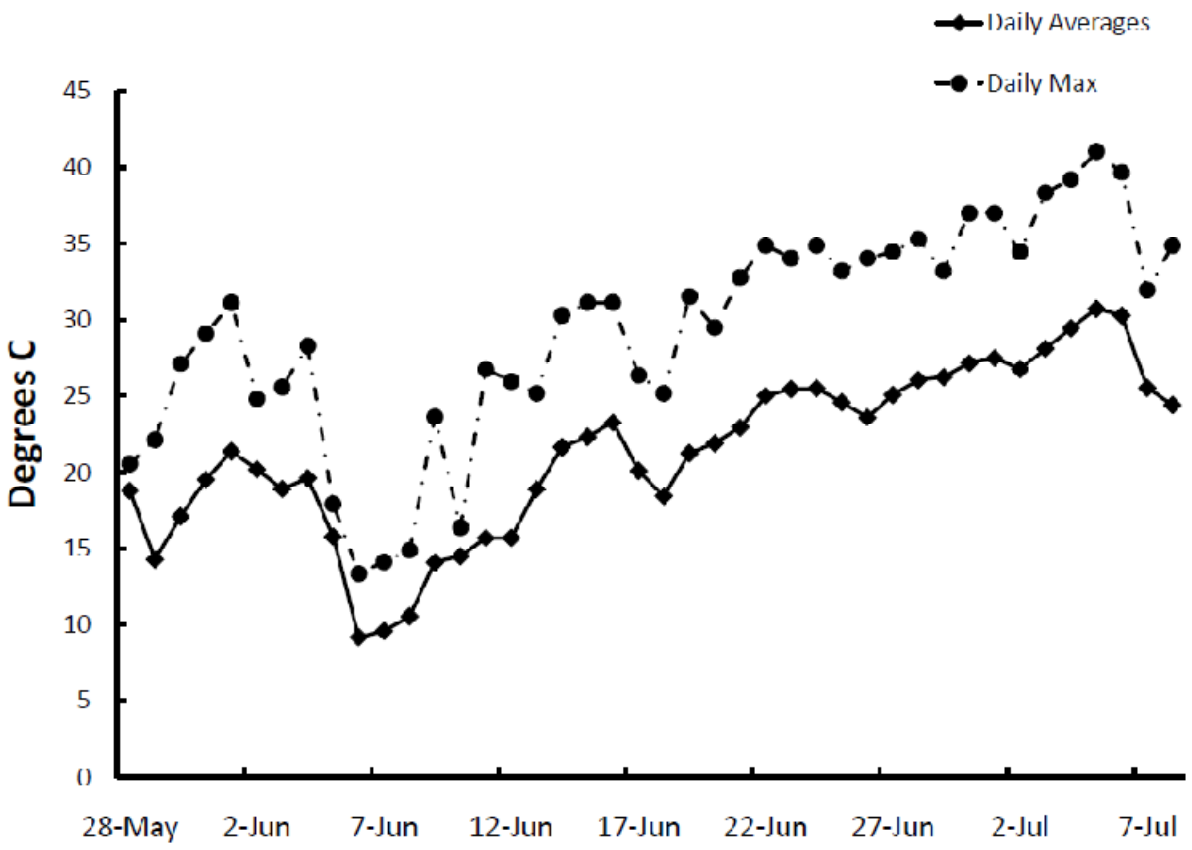
Figure 3. Leaf area based photosynthesis measurements for reproductive and vegetative plants throughout the growing season in 2007. Dashed lines with open triangles represent reproductive plants; while the filled circles with solid lines represent vegetative plants. Error bars represent one standard error.

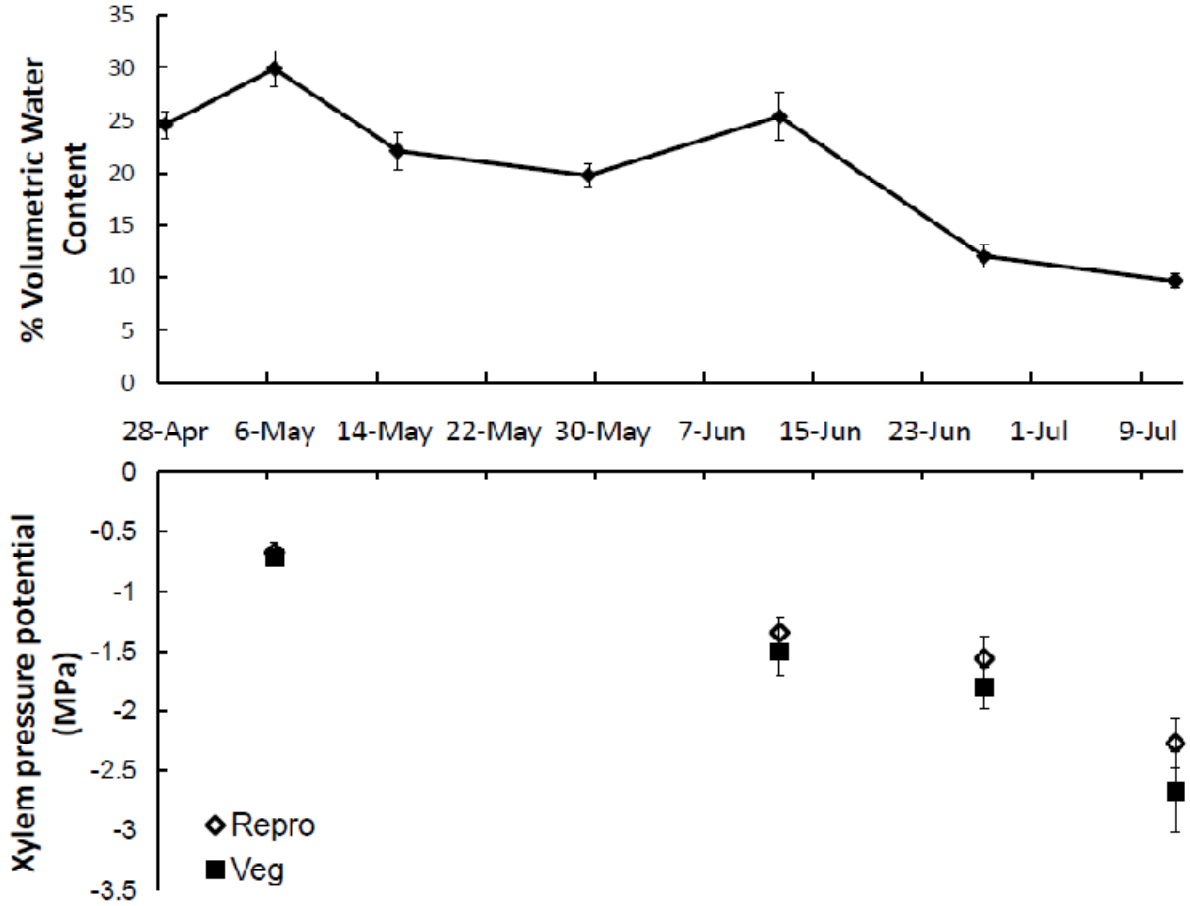
Figure 4. Variable to maximum fluorescence (Fv/Fm) for vegetative and reproductive plants throughout the growing season in 2007. Predawn measurements (AM) are shown on the left, afternoon (PM) measurements on the right. Error bars represent one standard error.

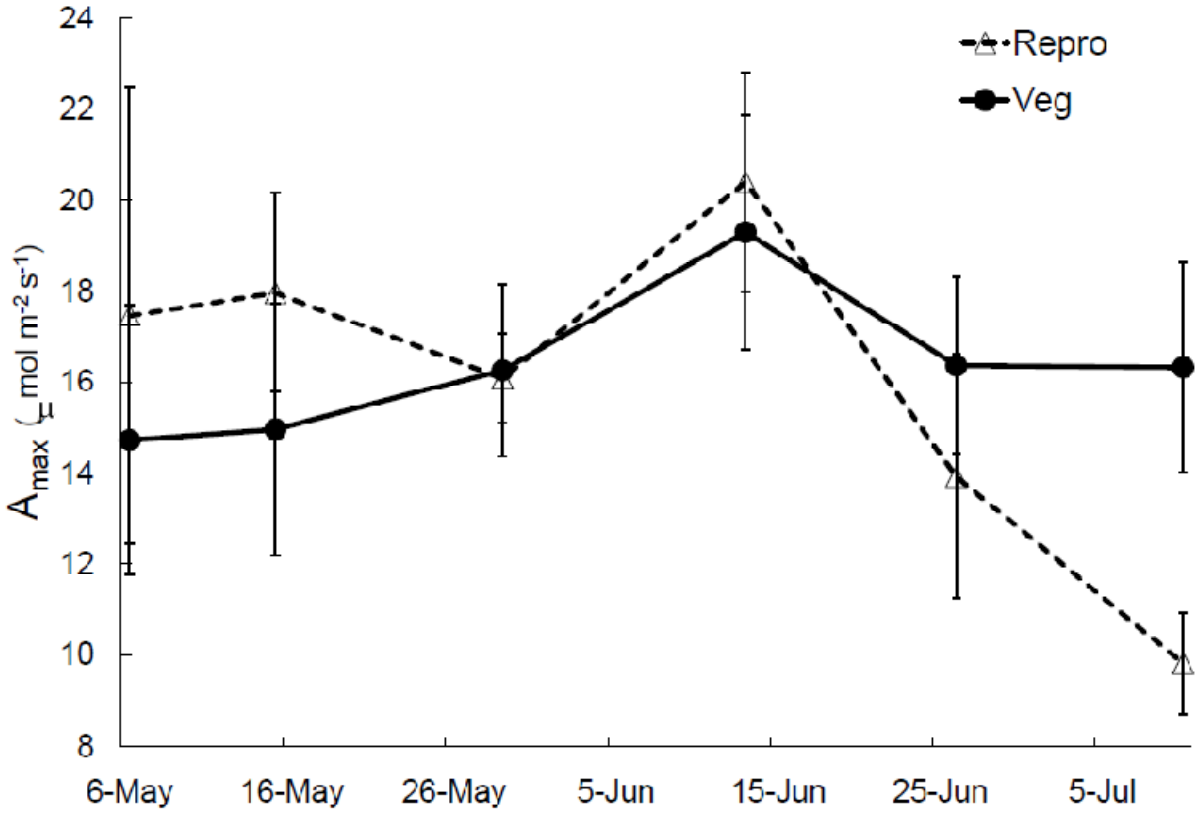
Figure 5. Number of flowering plants and fruit set for 1986-2009. The solid lines with filled circles represent number of flowering plants, the dashed lines with open diamond represent total number of fruits.

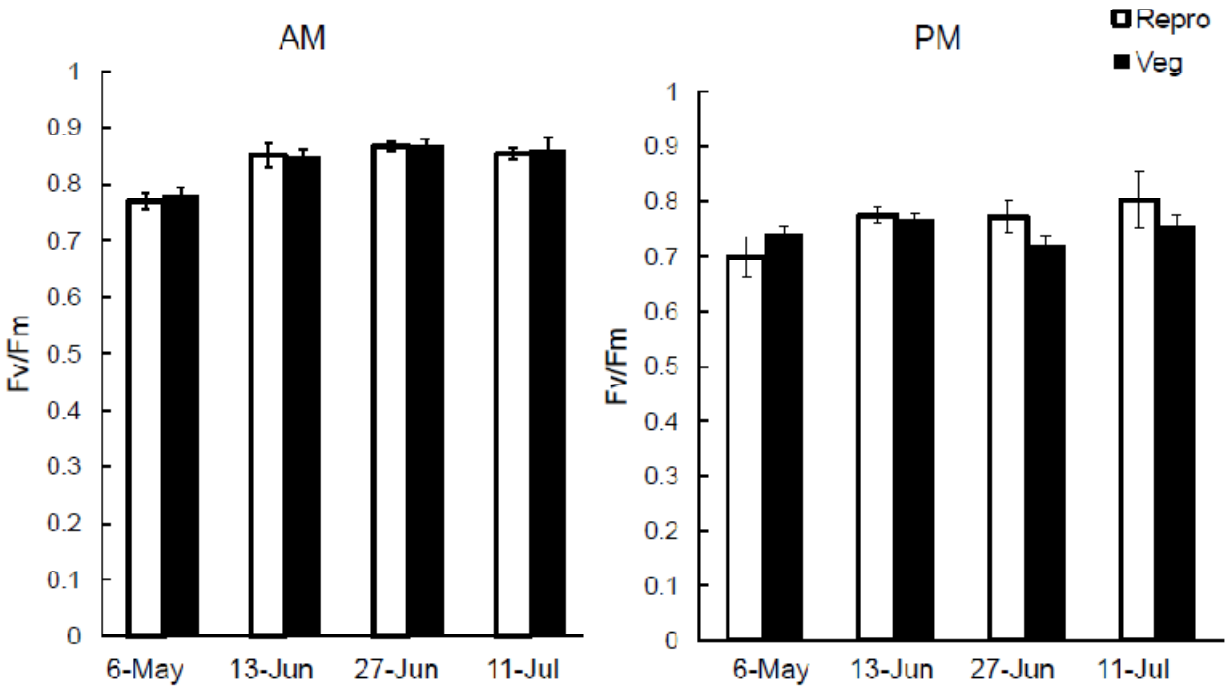
Figure 6. Stored resource dynamics for defoliation treatments and control plants in 2008. NSC (A) and N (B) dynamics for vegetative plants, NSC (C) and N (D) dynamics for reproductive plants. Data are average concentrations (n = 5-7 per treatment). Tissue was removed several times through the season for press plants (open squares, dashed lines), while pulse plants (gray diamonds, dashed lines) were defoliated only once. Dormants are solid lines with black triangles (no treatment).

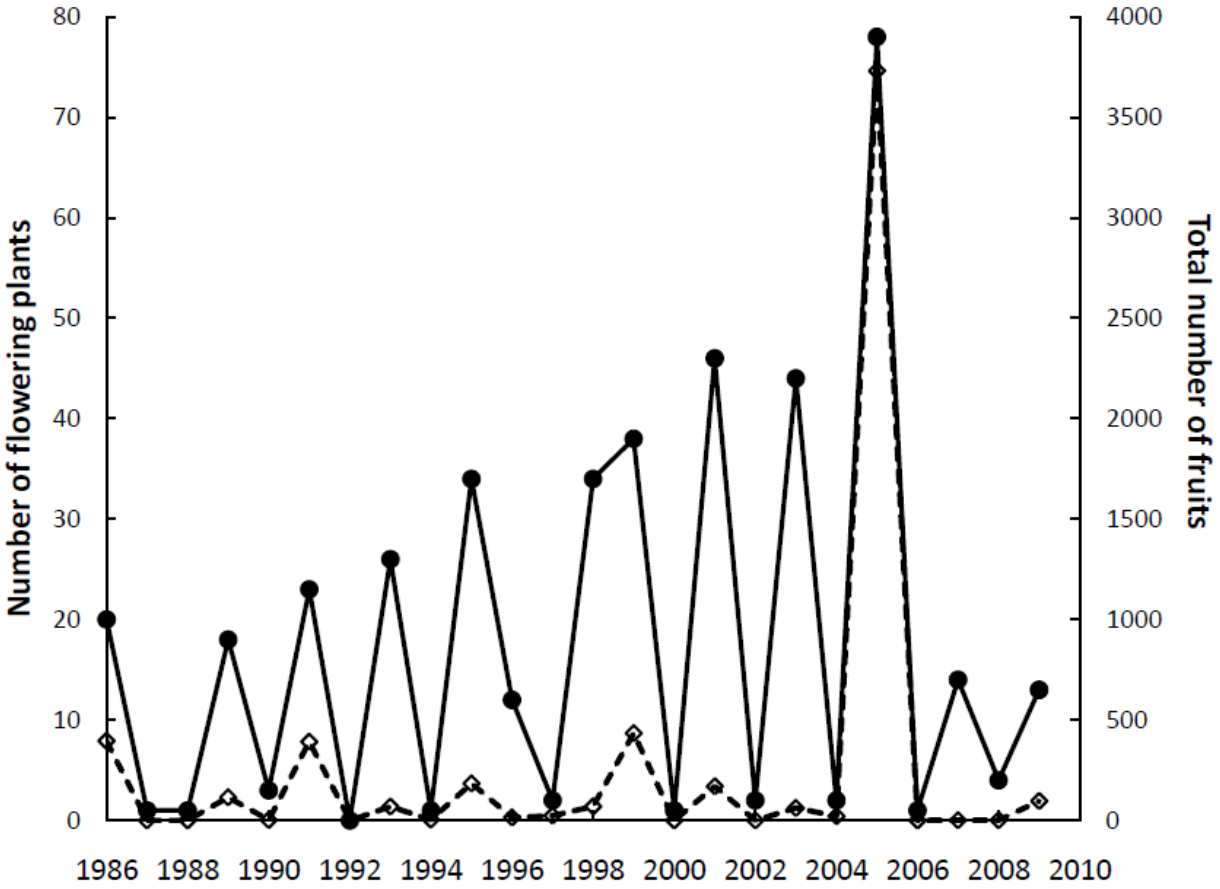
Figure 7. Mortality and flowering probabilities for treatment and control plants for the defoliation experiment. Flowering probabilities were conditioned on survival. Data for reproductive plants are represented by open bars; solid black bars represent data for vegetative plants. Press plants were defoliated several times; pulse plants were defoliated only once. Error bars represent 95% confidence limits.

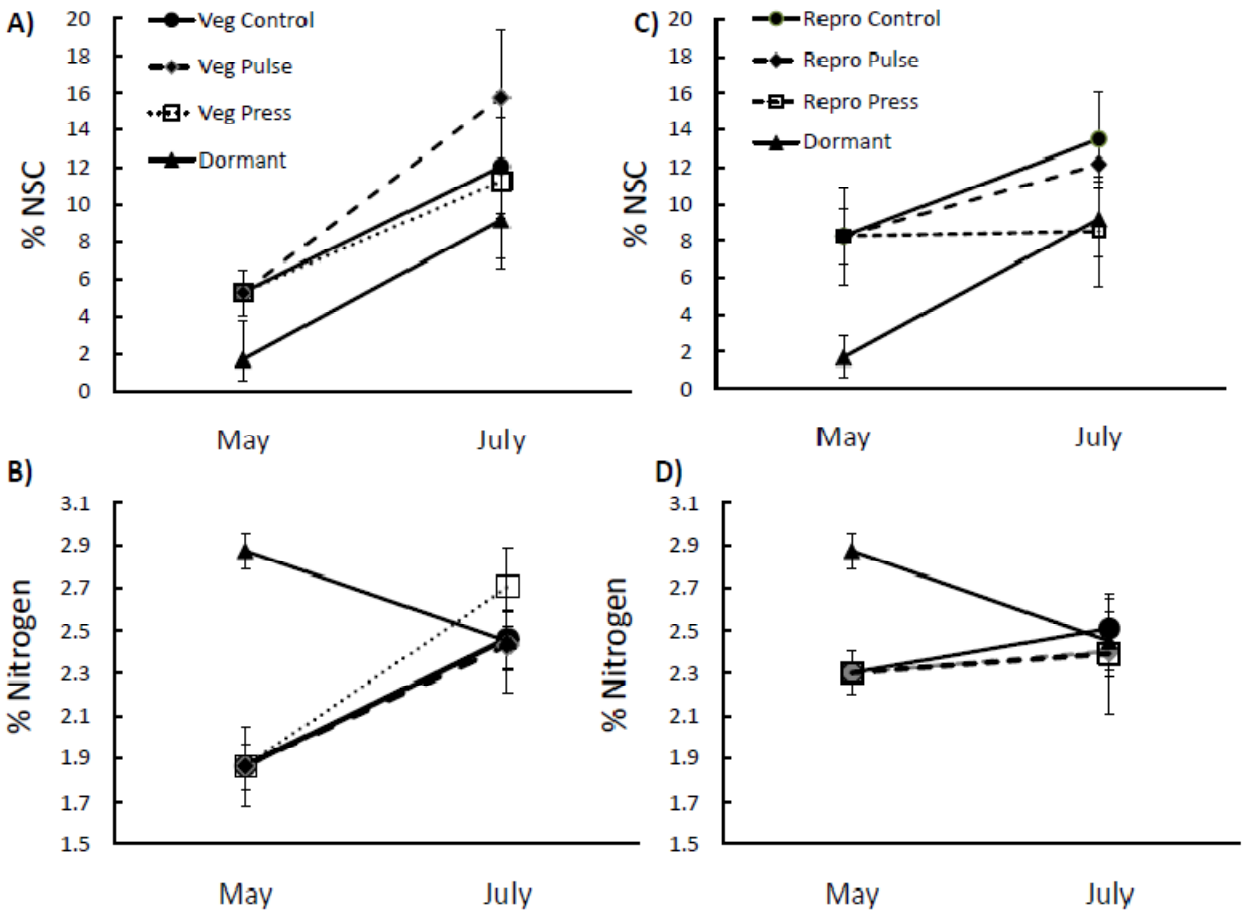

Daily Averages
Daily Max
Degrees C
45
40
35
30
25
20
15
10
5
0
28-May
2-Jun
7-Jun
12-Jun
17-Jun
22-Jun
27-Jun
2-Jul
7-Jul

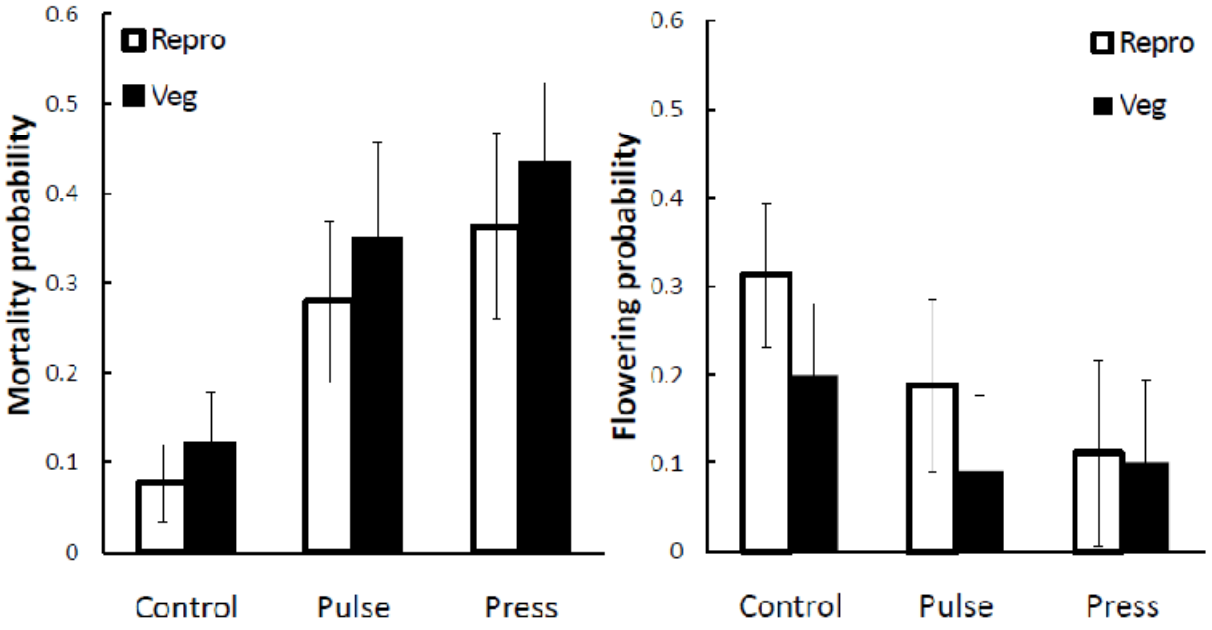

% Volumetric Water Content
35
30
25
20
15
10
5
0
28-Apr
6-May
14-May
22-May
30-May
7-Jun
15-Jun
23-Jun
1-Jul
9-Jul
Xylem pressure potential (MPa)
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
Repro
Veg


Repro
Veg
A_{max} (μmol m^{-2} s^{-1})
24
22
20
18
16
14
12
10
8
6-May
16-May
26-May
5-Jun
15-Jun
25-Jun
5-Jul


AM
PM
Repro
Veg
Fv/Fm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
6-May
13-Jun
27-Jun
11-Jul


Number of flowering plants
Total number of fruits
0
10
20
30
40
50
60
70
80
0
500
1000
1500
2000
2500
3000
3500
4000
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
2006
2008
2010


A)
% NSC
Veg Control
Veg Pulse
Veg Press
Dormant
May
July
C)
% NSC
Repro Control
Repro Pulse
Repro Press
Dormant
May
July
B)
% Nitrogen
May
July
D)
% Nitrogen
May
July


Mortality probability
0.6
0.5
0.4
0.3
0.2
0.1
0
Repro
Veg
Control
Pulse
Press
Flowering probability
0.6
0.5
0.4
0.3
0.2
0.1
0
Repro
Veg
Control
Pulse
Press

APPENDIX : Matrix model construction and simulation

To estimate growth of a phenotype using matrix models, it is critical to know the mean values for vital rates, the variability of each vital rate, and the covariance and correlations for each of those vital rates (Morris and Doak 2002). I directly estimated survival and transition rates from the demographic data spanning 1989 to 2005. As stated above, all transitions between the four visible stages (small, medium, and large vegetatives and the flowering stage) and the dormant stage are possible. This means that each vital rate is multinomial. For instance, small plants can grow to the medium stage, or they can go dormant, flower, or skip the medium stage and grow to the large vegetative stage. Thus, if they survive, there are 4 possible fates for these plants, making it more difficult to use matrix models that explicitly incorporate different sources of variation (Morris and Doak 2002). These rates are also mathematically constrained, since matrix elements sum to unity. Therefore, I transformed these vital rates into functions of binomial rates, also called conditional vital rates. I calculated vital rates from matrix elements as a sequence of conditional binomial probabilities, defined in the same way for all stage classes as shown in Table 1. Using "X" to represent the stage class in year t, these are: (1) s_x – survival; (2) pE_x - probability of emergence, conditioned on survival; (3) pF_x - probability of dormancy, conditioned on emergence; (4) pL_x - probability of being a large vegetative next year, conditioned on being in some vegetative stage class; (5) pS_x - probability of being a small vegetatiave next year, conditioned on being M or L.

The variation in the data is a product of both true variation in survival and growth rates as well as variation from sampling error. Kendall (1998) developed a method to estimate and remove sampling error from these estimates of variance, as well as to correct for bias due to unequal sample sizes or sampling variation (Morris and Doak 2002). In essence, this method

estimates probabilities for binary rates using a beta binomial model, and separates the variability due to sampling from that due to true environmental stochasticity. Therefore, I estimated environmental stochasticity using Kendall's method (Kendall 1998; implemented as the "Kendall" function in the popbio package in R; Stubben & Milligan 2007, R Foundation for Statistical Computing, 2009). Autocorrelations reflect the sequential relationships within vital rates across years and cross correlations are those relationships between different vital rates across time steps (Morris and Doak, 2002). I estimated correlations and serial correlations from the raw vital rate estimates (acf and cor functions in R), then converted these to corrected covariances and serial covariances by multiplying them by the corrected variances (see Morris and Doak 2002, their Ch. 8).

I used generalized linear models to estimate recruits in year t as a function of seed pods (pods) produced in years t-1 and t-2. In both cases, I used identity link functions, so that coefficients would relate naturally to per capita rates in density-independent matrix models. Initially, I tried both negative binomial and quasipoisson models. The negative binomial model vastly overestimated recruitment after years of high seed production (Figure 1). Presumably, this effect occurs because variance increases as a function of the square of the expected value in a negative binomial model (Ver Hoef and Boveng 2007), which means observations with high expected values have relatively little influence on the likelihood. Therefore, I chose the quasipoisson model, which produced less biased predictions.

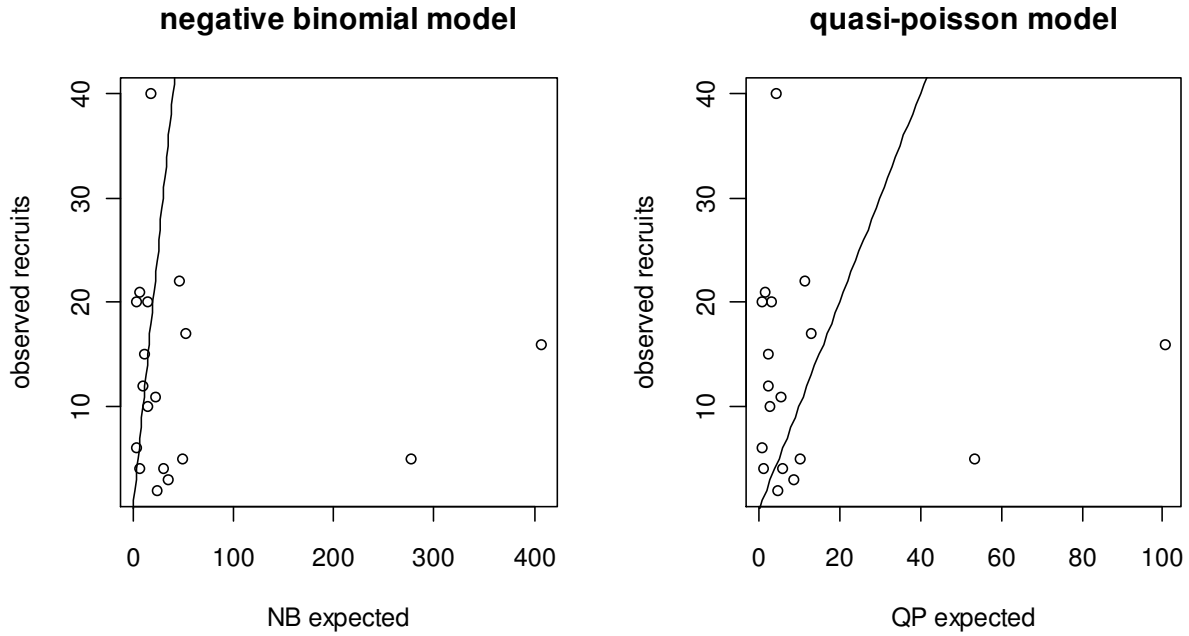


Figure A1. Expected versus observed values for the negative binomial and quaisi-poisson models of fecundity.

Within the quasipoisson error structure, I compared two models for recruitment: Recruitment only from seed pods in the previous year ($E[recruits[t]] = B_1 \times pods[t-1]$), and recruitment from the previous year, plus a one-year seed bank ($E[recruits[t]] = B_1 \times pods[t-1] + \times B_2 \times pods[t-2]$). There was one year in which I observed recruitment even though there were no seed pods produced in the previous year. Therefore, I modified the no-seed-bank model to: $E[recruits[t]] = B_1 \times (pods[t-1]+0.5)$. I compared these models using Analysis of Deviance, which adjusts the likelihood ratio using the estimated overdispersion factor, θ, from the quasipoisson model:

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
fruits_tm1	0.02870	0.03050	0.941	0.361

fruits_tm2 0.05439 0.04154 1.309 0.209

(Dispersion parameter for quasipoisson family taken to be 86.77735)

Analysis of Deviance Table
Model 1: recruits_t ~ -1 + I(fruits_tm1 + 0.5)
Model 2: recruits_t ~ -1 + fruits_tm1 + fruits_tm2

	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
1	17	1785.41				
2	16	592.98	1	1192.4	13.741	0.001914 **

Note that, although the quasipoisson/seedbank model is the best of the models I explored, it does not provide a particularly good fit to the data, suggesting that further investigation, possibly including empirical studies, into mechanisms of recruitment would be warranted. At the same time, this model seems sensible in that it is biologically motivated (unlike constant recruitment, i.e., recruits[t] = B_0), and at least captured the average number of recruits across the data set.

For a quasipoisson model:

$$\sigma^2 = \theta\mu = \theta(B_1 pods_{t-1} + B_2 pods_{t-2})$$

Therefore, I modeled stochastic variation in recruitment as:

$$recruits_t \sim Poisson\big(g_t(\, B_1 pods_{t-1} + B_2 pods_{t-2})\big)$$

$$g_t \sim LogNormal(\mu = 1, \sigma^2 = \theta)$$

I calculated estimates of recruitment from pods[t-1] and pods[t-2] in each year (for analyses of correlations and autocorrelations), using coefficients from the fitted regression model and as follows:

$$g_t = \frac{recruits_t}{(B_1 pods_{t-1} + B_2 pods_{t-2})}$$

After conducting these analyses described above, I had estimates of mean vital rates, including fecundity, as well as estimates of the variance in those vital rates. I used these estimates to assemble matrices for each of the 5 dormancy phenotypes (average, hypothetical non-dormants, never dormants, low, and high dormancy types) as shown in Figure 1. These matrices are the average transition matrices, since they include the mean values for all vital rates, and were used to calculate deterministic lambda and reproductive values. Deterministic lambda can be interpreted as fitness in a constant environment, and is the dominant eigenvalue of the average matrix. Reproductive values can be found in the dominant left eigenvector of the average transition matrix, and were scaled to the value in that vector for the small stage. I used the function eigen.analysis in R to calculate these values.

Using the corrected vital rates and serial covariances, I simulated population dynamics for 50 years, starting with the mean proportion of plants observed in each stage class from 1988-2007, with the seed bank set to the value from the stable stage distribution. Results were nearly identical from a range of starting values (E. Crone, unpubl. analysis.) I generated correlated and serially correlated vital rates using the methods proposed by Morris and Doak (2002, their Box 8.10), with beta-distributed survival and transition rates, and log-normal variance in fecundity (seed pods per plant) and g_t (recruits per seed pods). Log-normal distributions approximate

gamma-distributed vital rates from negative binomial models; and are more easily implemented using Morris and Doak's algorithm. Consistent with statistical estimation models, realized plant fates were generated from annual vital rates using binomial distributions for survival and growth transition rates and Poisson distributions for fecundity.

Literature Cited

Kendall, B. E. 1998. Estimating the magnitude of environmental stochasticity in survivorship data. Ecological Applications 8:184-193.

Morris, W. F., and D.F. Doak. 2002. Quantitative Conservation Biology: Theory and Practice of Population Viability Analysis. Sinauer, Sunderland, MA.

Ver Hoef, J. M. V., and P. L. Boveng. 2007. Quasi-poisson vs. negative binomial regression: How should we model overdispersed count data? Ecology 88:2766-2772.

Stubben, C. J., and B. G. Milligan. 2007. Estimating and Analyzing Demographic Models Using the popbio Package in R. Journal of Statistical Software 22(11).

Table 1. Matrix of conditional probabilities for Astragalus scaphoides. s_j= the probability of survival for stage j; pE_j= probability of emergence for stage class j, conditioned on survival; pF_{ij}= the probability of flowering for stage class j, conditioned on emergence; pM_{ij}= the probability of transitioning to the large stage class for stage class j, conditioned on emergence and not flowering; pS_{ij}= the probability of transitioning to the small stage class for stage class j, conditioned on emergence, not flowering, and not being in the large class; pods= seed pods, fruits produced by flowering plants; g= probability of germinating the following year. B_1= recruitment from seed pods the previous year, B_2= recruitment from seed pods two years previous. For clarity, only the first two and last columns are shown.

0	0	...	$pods*B_2$
0	$s_d(1\text{-}pE_d)$	...	$s_f(1\text{-}pE_f)$
$pods \times germ$	$s_d(pE_d)(1\text{-}pF_d)(1\text{-}pL_d)pS_d$	...	$s_f(pE_f)(1\text{-}pF_f)(1\text{-}pL_f)pS_f + pods*B_1*germ$
0	$s_d(pE_d)(1\text{-}pF_d)(1\text{-}pL_d)(1\text{-}pS_d)$	...	$s_f(pE_f)(1\text{-}pF_f)(1\text{-}pL_f)(1\text{-}pS_f)$
0	$s_d(pE_d)(1\text{-}pF_d)pL_d$	...	$s_f(pE_f)(1\text{-}pF_f)pL_f$
0	$s_d pE_d F_d$	...	$s_f pE_f F_f$