University of Alberta

Persistence of the moth – yucca mutualism at the northern edge of range

by

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CHAPTER 1

Introduction

Mutualism

Mutualisms are interspecific interactions in which the fitness of two (or more) partners increases due to the association (Addicott 1995). The increased fitness typically arises by the exchange of products or services not easily obtained or made by the other partner (Nöe and Hammerstein 1995, Schwartz and Hoeksema 1998). These interactions, however, are not cooperative as each species acts in its own selfish best interest, and perhaps are more appropriately thought of as mutual exploitations that result in net benefit (Janzen 1983, Herre et al. 1999). Mutualisms can range from obligate (e.g. Riley 1892, Bronstein 1989) to more generalized interactions that are facultative (e.g. Waser 1979), and include interactions between free-living species (e.g. Cushman 1989, Poulin and Grutter 1996) or symbionts (where one species lives in or on another) (e.g. Douglas and Smith 1989, Margulis 1992).

Some of the fundamental questions in the study of mutually beneficial interactions among species are how mutualisms evolve (Connor 1995), remain beneficial (Pellmyr and Huth 1994, Holland and DeAngelis 2001, Jousselin et al. 2001) and respond to the complex physical and biological environments in which they occur (Cushman 1991, Bronstein 1994). Of particular interest is how mutually beneficial interactions arise and persist given the inherently selfish nature of organisms (Axelrod and Hamilton 1981, Connor 1995, Frank 1995); selection favours the maximization of reproduction of each mutualist, often at a cost to its partner species.

Conditionality and Variability of Mutualisms

The strength (i.e. level of benefits acquired) and outcome (e.g. mutualism, commensalism etc.) of interspecific interactions can be highly variable over space and time, which can have profound effects on the maintenance and persistence of those interactions (Thompson 1988, Cushman and Addicott 1991). Beneficial interactions can shift in outcome from mutualism to commensalism or parasitism, or vary in the strength of benefits reaped by either partner (Bronstein 1994). The benefits achieved via mutualistic interactions may be conditional, because the services or products provided by one mutualist species to another can vary in time and space with changes in physical and biological settings (Addicott 1986, Cushman and Addicott 1991, Setala et al. 1997, van Ommeren and Whitham 2002). Prior study has demonstrated that the level of benefit acquired by mutualists is dependent on many different factors, including partner density (Breton and Addicott 1992, Cushman and Whitham 1989), size or age structure of participants (Cushman and Whitham 1989, Saikonnen et al. 1998) and nutrient levels of the physical environment (Johnson et al. 1997, Setala et al. 1997). For example, the mutualism between plants and their ectomycorrhizal fungi is beneficial for both partners under low nutrient conditions; however, under higher nutrient concentrations the fungi may become parasitic to the plant (Johnson et al. 1997, Setala et al. 1997).

The presence of conditionality in mutualisms has profound implications for the evolution of the interaction. When the outcome or strength of mutualism varies, the direction and/or intensity of selection changes, potentially leading to traits that favour the association in some situations and oppose the association in others (Cushman 1991, Cushman and Addicott 1991). Because of this conditionality, selection should typically favour more generalized beneficial relationships among species rather than obligate co-evolved mutualisms (Cushman and Addicott 1991). Temporal variation in outcome or strength may influence the rate of evolutionary change in the mutualism, whereas spatial variation could lead to variation in the nature of the interaction in different areas of the species ranges (Thompson 1988, Bronstein 1994).

Further, partner species provide solutions for ecological problems faced by mutualists (Cushman and Addicott 1991), and ecological problems vary with environmental condition. Thus the importance of mutualists to their partner species is apt to vary and the mechanisms by which benefit is achieved may change. For example, studies indicate that there are mechanisms in place that allow mutualist partners to adjust or regulate their "behavior" to regulate the degree of benefit acquired (e.g. Addicott 1998, Addicott and Bao 1999, Segraves 2003); thus the internal properties of the interaction may vary in space and time.

Range Edges and Population Dynamics

Range edge populations are geographically peripheral because they are located on the margin of species' ranges (Jonas and Geber 1999), where tolerances for physical conditions abruptly limit the distributions (Brown 1984). Range edge populations can also be ecologically peripheral, where their distributions are limited by biotic and/or abiotic conditions in some areas (Soule 1973, Caughley et al. 1988, Jonas and Geber 1999). Northern range edge populations are both geographically peripheral and ecologically peripheral. Typically northern species' boundaries are determined by climatic conditions and the physiological tolerances of the species in question (Caughley et al. 1988); however, they can also be limited by densities of interacting species (Brown 1984). Populations in locations of high elevation in the core of the range, although they behave to some degree like northern peripheral populations, would be ecologically peripheral rather than geographically peripheral. Populations at high elevations provide some insight on how a species may behave at northern species' boundaries as they too are often at the limit of their physiological tolerances.

Range edge populations tend to be more imperiled relative to more central populations, as they are thought to occur in lower quality habitats, and are often small and isolated (Caughley et al. 1988, Lesica and Allendorf 1995). Peripheral populations that occur at the northern margins of species' ranges are also hampered by harsher environments with shorter growing seasons, lower temperatures and sometimes low nutrient or water availability (Eriksson 1989, Jones and Gliddon 1999, Dorken and Eckert 2001). These factors contribute to increased demographic variability (Curnutt et al. 1996, Jones and Gliddon 1999, Nantel and Gagnon 1999) and increased genetic erosion (Jones and Gliddon 1999, Lönn and Prentice 2002, Cassel and Tammaru 2003), which together lead to increased susceptibility to extinction of peripheral populations relative to core populations. The success of these populations is restricted further by the presence of anthropogenic disturbance and/or habitat destruction (Lönn and Prentice 2002).

Environmental conditions at northern latitudes have been implicated in altering elements of demography, growth and reproduction for numerous species. For example, in the plant *Clarkia unguiculata*, populations at the cooler ends of elevational and latitudinal gradients had slower development rates, smaller sizes and lower gas exchange rates (Jonasa and Geber 1999). A similar response occurs in *Lloydia serotina* where marginal populations produced fewer flowers and seeds (Jones and Gliddon 1999). Woodward and Jones (1984) reported low germination rates, plant survival and seed production in high elevation populations of *Potentilla reptans*. Similar effects occur in insects of northern latitudes where growth and reproduction tend to be constrained by shorter flight periods (Brakefield 1987) and higher mortality rates are heightened by lower temperatures and longer winters (Musolin and Numata 2003, Venette et al. 2004). For example, there was a negative relationship with the number of eggs laid and latitude in the spruce budworm, *Choristoneura fumiferana* (Harvey 1983).

Conditions at the northern edge of range are expected to have important implications for the organisms residing there, leading to altered population demography and dynamics relative to other populations. The impact of living at the edge of range is expected to be greater yet if the species in question is obligately reliant on another species, as is the case between the yucca and the yucca moth, and may have important implications for the evolution of species interactions. This thesis serves to explore the impacts of living at northern edge of range on the yucca and yucca moth and the beneficial interaction between them.

Yuccas and Yucca Moths

Life History

Yuccas (*Yucca* spp., Agavaceae) are perennials of arid to semi-arid regions of North America. The species in this study, *Yucca glauca* Nuttall or Soapweed occurs from Texas north to Alberta, from the Rocky Mountains east to the Mississippi River (Pellmyr 1999). This species of yucca has a sole species of pollinator, the yucca moth (*Tegeticula yuccasella* Riley, Prodoxinae, Incurvariidae).

Tegeticula yuccasella is found from southern Texas to Canada and from the Rocky Mountains east to the Atlantic seaboard (Pellmyr 1999). The moth's range is larger than that of *Y. glauca* because the moth pollinates more than one *Yucca* species. In Alberta and Montana, most adult moths emerge from the soil from the second week in June through to the second week in July. Shortly after emergence, the moths gather and mate in freshly opened soapweed flowers (Riley 1892, Baker 1986, Addicott et al. 1990). Adult female yucca moths actively collect pollen from one plant then usually fly to another inflorescence. Upon finding a fresh flower, a female first inserts her ovipositor through the carpel wall and lays an egg next to the developing ovules (Aker and Udovic 1981, Addicott and Tyre 1995). She then climbs to the tip of the style, and using her maxillary tentacles, appendages unique to yucca moths, she actively transfers pollen into the stylar canal. Moths do not feed as adults and die after 3 to 12 days (Kingsolver 1984, James 1998). Moth eggs hatch after 7 to 10 days and upon hatching, larvae feed on developing seeds. After approximately 50-60 days in the northern portions of their range (D. Hurlburt, personal observation), 4th instar larvae chew their way out of the yucca fruit and drop to the ground via a silken thread (Riley 1892). Larvae burrow 5-20 cm into the soil (Fuller 1990, D. Hurlburt, personal observation), spin a cocoon of silk and sand particles (Davis 1967) and enter a prepupal diapause (Riley 1873, Keeley et al. 1984). After a minimum diapause of approximately 1 year, larvae pupate and emerge from the soil as adults, with the time of emergence usually coinciding with yucca flowering. Most larvae emerge from diapause within 1 to 3 years (Fuller 1990; D. Hurlburt, unpublished data).

The relationship between *Y. glauca* and *T. yuccasella* is obligate; because as in all species of yuccas, the plant cannot sexually reproduce without the moth. The interaction is beneficial to both parties because the yucca is pollinated by the yucca moth and the yucca moth receives oviposition sites and food in the form of seeds for larvae from the plant. The interaction between yuccas and yucca moths is an excellent model for studying the evolution and dissolution of mutualism, given the clear conflicts of interest between partners. Moth larvae feed only on yucca seeds, and in turn, yuccas can only produce seed if the flowers are pollinated by yucca moths. Neither species can survive over the long-term without the other. However, the relationship only remains beneficial if the yucca matures some of the flowers in which the moths laid their eggs and if the moth larvae within mature fruit do not eat all of the yucca's seed. The conflict arises because yuccas and yucca moths act in their own selfish best interests and will attempt to maximize their reproductive output, even at a cost to their partner species.

Species Status

At the very northern edge of the species' distribution, in Alberta and Saskatchewan, the yucca (i.e. Soapweed) is considered "threatened" by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) due to isolation and small population size (Csotonyi and Hurlburt 2000). Although found on prairie flats in the centre of the species' range, they are restricted to eroded south-facing coulee slopes in Canada and much of Montana. Likewise, the yucca moth, *T. yuccasella*, is considered "endangered" due to isolation, small population size and extreme annual variation in recruitment with fewer than 200 larvae emerging from 8499 clones in some years and sites (COSEWIC 2002). Both of these species are currently the subject of a federal joint 'recovery' plan, where the goal is to ensure that both species and the interaction between them persist in Canada (Alberta Soapweed and Yucca Moth Recovery Team, *In prep.*).

Assessing Benefits of Moth - Yucca Mutualism

Critical to the definition and detection of mutualism is an assessment of net benefits. In order for an interaction to be mutualistic, both species must exhibit net benefit, typically as increased fitness. Measuring benefit from the perspectives of all interacting species involved in a mutualism is a difficult task (Cushman et al. 1994) because costs and benefits to different species are not similar in nature, i.e. they are in different currencies (Addicott 1986). The yucca/ moth mutualism is one of the few mutualisms where benefits and costs can be easily compared between mutualists (Addicott 1986, Pellmyr 1989 because most costs and benefits for yuccas and yucca moths are measurable through the same biological unit - seeds (Addicott 1986, Bronstein and Ziv 1997). Yuccas benefit by acquiring viable seeds through the pollinating activities of adult moths and they acquire cost through the seed predation activities of moth larvae. Yucca moths benefit through the consumption of seeds. Associations are mutualistic when benefits exceed costs, or when yuccas gain viable seeds through the pollination activities of yucca moths and moths have yucca seeds to eat. However, seed production is not a complete indication of benefit from the perspective of the yucca moth as it reveals little about individual moth survival, growth or fecundity. Further, seed set may not incorporate all costs incurred due to the association (Bronstein and Ziv 1997), such as the costs to yuccas associated with attracting yucca moths.

Dynamics of Moth-Yucca Populations and Persistence of Mutualism

As with other plants and insects, stochasticity at range edges for yuccas and yucca moths is expected to affect demography and abundance. Further, stochasticity is expected to have major repercussions for the persistence of yucca and yucca moth populations and for the degree and persistence of mutualism between those species at the northern edge of range.

There is an absence of literature specifically concerning northern peripheral populations of yuccas and yucca moths. However there are data available for yucca populations at high elevations, which should behave in similar ways to northern populations. In *Y. glauca*, flowers per inflorescence, fruit set and larval density per fruit were significantly lower at high elevation sites, presumably due to smaller plant size and a decrease in activity of *T. yuccasella* at cooler temperatures (Dodd and Linhart 1994). Powell (1984) and Cruden et al. (1976) found that *Y. schotti* and *Y. glauca* respectively, both exhibited lower fruit set at high elevation sites. Both studies ascribed low fruit set to low levels of adult yucca moths, although it is also plausible that pollination activities may have been reduced by lower temperatures. With longer winters and colder temperatures at high latitudes, fewer moths may survive diapause resulting in low population densities. Although there are data to indicate lower abundances of flowers and moths in harsh environments, it is unknown if peripheral populations of yuccas and yucca moths exhibit higher variation in abundances relative to core populations.

Lower partner abundances and increased variability in population size can change selection pressures and the overall trajectory for the evolution of mutualisms at range edges. The outcome and strength of the moth – yucca relationship is dependent upon the relative density of yucca moths to flowers at any time. High levels of flowers to moths may result in fewer visits per flower, fewer pollinated flowers, and poorer quality pollinations, but would also ensure that fewer seeds are consumed by moths. However, if moth densities were relatively high, pollination may be ensured but higher proportions of viable seeds maybe consumed. In these scenarios, the costs of the interaction disproportionately increase for one partner leading to shifts in benefits obtained by each partner. If one partner consistently acquires more benefit than the other, the interaction may become more parasitic rather than beneficial.

In addition to density, the ability of those partners to align themselves with each other temporally is also critical to the nature of the mutualism. Reproductive failure of moths and yuccas can occur when yucca flowering seasons do not overlap with moth flight seasons (Riley 1892, Wimber 1958, Powell and Mackie 1966, Kingsolver 1984). Disrupted synchrony of flowering and flight leads to variation in moth to flower ratios and influences the outcome and strength of the relationship (Aker 1982). Although there is some evidence to suggest that yucca moths and flowers may have similar environmental cues, like winter precipitation, that serve to synchronize populations (Aker 1982, Fuller 1990), there are numerous examples of relative densities varying considerably among years (e.g. Udovic 1981, Aker 1982, Dodd 1989) which would imply that the abundances of both species fluctuate independently in response to different factors. If northern populations of yuccas and moths are not synchronized and population abundances fluctuate greatly within and among years, there could be profound implications for the degree of benefit obtained by each partner.

Changes in the benefits obtained or in partner abundance had a variety of effects in other mutualistic systems. Species that were limited in access to their mutualists over the long-term were unable to expand their range (Cruden et al.1976), interacted with a different partner species (Bond and Slingsby 1984, Cox 1983) or stopped relying on mutualism altogether (Janzen 1973). The outcomes of interactions with abundances of mutualists are unclear and have been the subject of debate. Some feel that extinction is inevitable (Briand and Yodzis 1982, May 1976), while others believe that dissolution of mutualism between partners will not necessarily lead to extinction for participants (Janzen 1973). Although the extinction of either yuccas or yucca moths is plausible given that they are coevolved and rely on each other for long-term survival, it is possible that the relationship remain tight despite lower availability of moths or flowers. In other species, populations in harsh environments have survived because they are well adapted to their conditions and the unpredictability in the system (Volis et al. 1998, Cassel and Tammaru 2003). Likewise, it is expected that the interaction between northern peripheral populations of yuccas and yucca moths remains tight because of adaptation to their local environment and adaptive changes in regulatory characteristics of yucca and moth populations.

Objectives

The overall purpose of my thesis was to examine how the moth - yucca mutualism persists at its northern edge despite factors such as high demographic variation and low partner density that may lower the benefits acquired by the yucca. In harsh and more variable environments it should be more difficult to acquire pollination services and more difficult to ensure the presence of flowers / fruit for oviposition and larval feeding.

Inability to have access to a mutualist partner may lead to alteration of the relationship through changes in selection pressures, dissolution of the relationship and potentially extinction of either yucca or yucca moth populations at a given location.

More specifically, I examined the success of the moth - yucca mutualism in relation to each of the following factors:

1. Demographic and environmental stochasticity in space and time,
2. Relative densities of moths and flowers within and among years and sites, and
3. Pollen source preference.

Chapter 2 explores the impact of demographic and environmental stochasticity on the survival of yucca populations at the northern periphery of the species' range. First, the effects of reproductive rates from ten natural populations on population growth rates were examined. Second, the impact of flowering and herbivory levels on population persistence and growth was explored using simulated values representing different levels of flower and herbivore intensity. This chapter explored the impact of the factors on population growth to determine which factors could be considered limiting at the northern limits of the species' range.

Chapter 3 investigates the role that spatial and temporal variation in moth and flower densities have on the success of the mutualism. In particular it examines the evolution and functioning of the moth – yucca mutualism at the partner-limited end of the spectrum; whereas previous studies have looked at the evolution of the interaction at high moth densities. This chapter compared the nature of regulation of northern populations relative to other populations of yuccas described in the literature.

Chapter 4 considers how differ selection pressures, like low mutualist densities, isolation and environmental variation, may have led to changes in the breeding systems for yuccas at the northern edge of range. Further, it evaluates what the implications of these changes may be for the persistence of the mutualism.

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CHAPTER 2

Role of demographic and environmental variation in the persistence of an edge of range mutualist, *Yucca glauca*

Summary

Using projection matrix models of *Yucca glauca* population dynamics, insight on future population growth and factors influencing that growth in northern peripheral populations of yuccas in Alberta and Montana was gained. Survivorship and fecundity rates of yuccas were obtained from field measurements of ten populations and incorporated into transition matrices for analysis of potential rates of population increase (λ) and elasticities of northern populations. Since these matrices failed to incorporate patterns of flowering and herbivory intensity on population growth, six additional matrices were generated and "used" in specific combinations in the projection to determine their impacts on lambda.

Field studies showed that all populations exhibited high variation in reproductive rates from year to year and had slightly positive population growth rates. For observed populations, elasticity analysis showed that stasis in adult reproductive stages was more critical to the persistence of populations than was the recruitment of new seedlings. More southern populations exhibited higher rates of growth (λ) relative to the more northerly populations.

Simulations revealed only minor impacts of variation in flowering levels and herbivory on population growth. Populations that had more consecutive years of low flowering naturally had lower lambdas, but surprisingly; herbivory reduced these rates only minimally, if at all. In general, despite high variation in vital rates, peripheral populations of *Y. glauca* are remarkably resilient and should persist into the future under current environmental conditions. Maintenance of mature plants is crucial to population

persistence and management plans should take this into consideration when developing conservation strategies.

Introduction

Compared to core populations, peripheral populations tend to be more isolated and smaller in size (Bengtsson 1993, Lesica and Allendorf 1995). For a given species, the presence of a small marginal population may indicate that the conditions at range margins are less favorable than at the centre of the range (i.e. more harsh) for some elements of the species' life history, such as survival, growth, fecundity, recruitment, asexual propagation and dispersal (Bell and Bliss 1979, Black and Bliss 1980, Woodward and Jones 1984, Carter and Prince 1988). Peripheral populations also exhibit higher temporal variability in abundance and vital rates when compared to core populations (Vucetich and Waite 2003), which in combination with small population size, makes peripheral populations more susceptible to decline and extinction via demographic and catastrophic stochasticity (Lesica and Allendorf 1995). Reduction in or increased variability of fecundity and survival are exacerbated in populations located at the northern periphery of their ranges. As growing seasons become shorter and temperatures become cooler (Jones and Gliddon 1999), organisms become more sensitive to changes in environmental conditions (Lönn and Prentice 2002). An understanding of which demographic parameters make northern peripheral populations more or less susceptible to extinction than others would, therefore, facilitate species preservation and management.

The pollination/ seed predation mutualism between the yucca (*Yucca glauca* Nuttall) and yucca moth (*Tegeticula yuccasella* Riley) at the northern edge of their ranges is an excellent system in which to examine what mechanisms allow the mutualism to persist in the face of large amounts of demographic variation. Yuccas exhibit considerable variation in flowering, fruiting and seed production in all parts of their distribution (Keeley et al. 1984, Addicott 1986, Kingsolver 1986, Chapter 3) and this variation has implications for the regulation and evolution of the mutualism between yuccas and their pollinators (Aker 1982, Addicott 1998). However, northern populations should have even greater variability in reproductive rates and smaller sizes relative to more central populations and the persistence of northern populations of yuccas should be more precarious. As predicted, northern populations of yuccas exhibit high variability in demographic rates (Csotonyi and Hurlburt 2000, Hurlburt 2002); however despite that, most populations of yuccas and moths also continue to exist and engage in a mutualistic relationship with the yucca moth in Alberta and Montana. The long-term persistence of the plant and its apparent good health lead me to ask, how does *Y. glauca* persist at the northern edge of range despite perturbations in vital rates?

The aims of the present study were to identify the critical stages in *Y. glauca*'s life-cycle and to relate population growth to variation in reproductive rates, flowering level patterns and floral herbivory by wild ungulates. To accomplish this I examined demographic parameters in 10 populations of *Yucca glauca* at the northern edge of range in Alberta and Montana. I then examined the consequences of these parameters for population dynamics, using stage-base population matrices. Specifically, I examined the following questions: 1) What are the probable demographic patterns for yuccas at the northern edge of their range and how do those patterns differ in space? To accomplish this, a transition matrix was constructed for each of the 10 populations using parameters measured from each location. The population growth rates and their elasticities were then examined. 2) What role does variation of flowering play in population growth? Do high levels of flowering enhance recruitment more than low levels of flowering? Do populations rely on periodic large scale flowering events to sustain population growth, or are years of low flowering just as critical to population growth? To address these problem, flowering levels were varied in transition matrix models to explore which combinations of flowering levels led to higher population growth rates. 3) Finally, how does florivory and frugivory by wild ungulates interact with variable levels of flowering to influence the population growth of northern yuccas? As complete consumption of inflorescences and/or flowers is common in northern populations (D. Hurlburt, personal observation), is herbivory a limiting factor for the long-term success of yuccas at the northern edge of range?

Materials and Methods

Study Organism and Study Sites

Soapweed (*Yucca glauca* Nuttall) is a polycarpic plant with vegetative propagation. It occurs throughout the Great Plains from southern Texas to southern Alberta, Canada between the Mississippi River and the Rocky Mountains (McKelvey 1947). The seeds germinate in late spring - early summer (D. Hurlburt, personal observation) about 1 year after dispersal from the maternal plant (Kingsolver 1986), producing a small rosette 5-10 cm high (i.e. young juvenile). The number of new seedlings depends upon rain- and snowfall in the previous winter and spring and the overall level of fruit production during the previous flowering season, with the highest numbers being after years of high fruiting and during flowering seasons with relatively high levels of rainfall (D. Hurlburt, personal observation). Age of first reproduction is late at about 15-20 years post-germination (D. Hurlburt, personal observation); each ramet within a genet is capable of producing a single inflorescence about 50-75 cm tall that can produce on average 36 flowers. Seeds are apparently not viable in the soil longer than 1 year, so there is no seedbank (McCleary and Wagner 1973). Plants can also produce numerous, additional rosettes through vegetative reproduction (ramets), each of which is capable of flowering once before dying (Kingsolver 1984).

Soapweed has a mutualistic relationship with a yucca moth (*Tegeticula yuccasella* Riley) where the moth pollinates flowers and the flowers provide oviposition sites for moths (Riley 1892). The developing larvae consume a small proportion of potentially viable seeds per fruit (Addicott 1986). Hence, the plant depends on the yucca moth for sexual reproduction.

Life history parameters for yuccas were estimated from 10 sites at the northern periphery of the yucca's range in southeastern Alberta and central Montana (Table 2.1). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) considers populations of yuccas and yucca moths in Alberta "Threatened" due to isolation, limited distribution and high variation in recruitment and fecundity among years (Csotonyi and Hurlburt 2000, COSEWIC 2002), with few if any fruit or moth larvae produced in some sites and years. Yuccas in northern Montana have a more continuous distribution than those in southern Alberta, but they also experience high variation in fecundity and recruitment.

Field Methods

Life history data for matrix projections were obtained in several ways from 1998 to 2003, although not all populations were examined in every year (see Table 2.1). Climatic normals for each site are provided in Appendix 2.1. For each year and population of observation, data on physical and flowering/fruiting characteristics were collected from approximately 100 clones per population. The number of unflowered rosettes, maximum leaf length, reproductive history and total fruit produced were determined for each clone.

Each clone was then classified according to maximum leaf length, number of rosettes and prior and/or current reproductive history to determine distribution of stages within populations for the development of a stage-based matrix population projection model (Table 2.2). Non-reproductive plants included seeds, young juveniles and old juveniles; young juveniles and old juveniles were differentiated by the number of rosettes the clone contained and maximum leaf length. Young juveniles, which included seedlings, had a considerably higher mortality rate relative to old juveniles. Reproductive individuals were divided into two categories: clones with no previous history of flowering (primiparous) and clones that had flowered before (multiparous). Primiparous clones produced fewer fruit per inflorescence and had fewer inflorescences than multiparous clones. Since reproductive plants do not flower in every given year or successfully produce fruit when they do flower, multiparous plants were divided into flowering, non-flowering and fruiting and non-fruiting.

Aside from the 100 clones per population, the fruit from a minimum of 25 inflorescences and 10 clones from each site were examined to determine total seed production per inflorescence and per clone (Table 2.3). Numbers of seeds (viable, inviable, eaten, and uneaten) and larvae per locule were counted to estimate mean fecundity (number viable, uneaten seeds) per fruit, inflorescence and clone.

Transition probabilities between stages and mortality rates were estimated through data collected from over 500 individual clones within seven 2m x 50m belt transects in Onefour, AB from 1999-2003. In each year, all new seedlings within each transect were individually identified and followed for the duration of study. Germination rates were estimated by planting 1200 viable, uneaten seeds each in twelve 1m x 1m plots in Onefour, AB and Loma, MT. Seeds were placed approximately 1 cm under the soil surface in known locations for monitoring of germination over the following summer.

Matrix Construction

Stage-based matrix models (Lefkovitch 1965, Caswell 2001) were used to analyze population dynamics and population growth rates of *Y. glauca* in the 10 northern edge of range populations. The population projection matrix model is as follows: n(*t*+1) = A * n(*t*), where n(t) is a vector of the number of individuals at each stage and A is the projection matrix. The general matrix used for all populations is shown in Table 2.4. All models were constructed and executed in MS Excel and population growth analyses were also performed in Excel using the algorithms in PopTools.

Few studies of clonal plants use genetic individuals (genets) as the basis of their demographic analyses, but rather focus on ramets, which in *Y. glauca* conveniently die after flowering and are easier to identify (Kingsolver 1984). In this study, the clone was chosen as the unit of study because genetic individuals are the management unit for the Alberta populations and are the basis for natural selection. The dynamics of clones are crucial to the understanding of the development of traits in peripheral populations. Genet identification was not difficult in this study, as considerable variation in floral morphology and coloration existed among adjacent clones allowing them to be readily identified from one another. Ramets can have different origins with some arising from sexual reproduction and others arising vegetatively, which complicates their use in models because the two types of ramets can have different rates of survival and mortality. *Yucca glauca* seedlings (i.e. genets) grow very slowly relative to vegetative ramets and exhibit low survival. Seedlings are particularly poor at competing with grasses or conspecifics (Kingsolver 1986) and often succumb to disturbance by land slides and vehicular traffic (COSEWIC 2002). Additionally, vegetative ramets can flower within 5 years of their establishment but sexually-derived ramets cannot flower for 15-20 years.

The structure of the life cycle used to develop the matrix is represented in Figure 2.1. Solid arrows represent the transition probabilities, the probabilities that a clone will make the transition from one stage to another in a 1-yr period. Dashed arrows note the life stages that contribute seeds to future population growth.

Population Simulations

Three sets of analyses were used in this study to examine the population growth and dynamics of northern populations.

a) Population growth in natural populations - The first set of matrix analyses was used to examine how natural populations of *Y. glauca* at the northern edge of range persisted relative to demographic variation. A separate matrix, that incorporated the real reproductive rates from each population, was generated for each of 10 populations of Soapweed in Alberta and Montana. Demographic stochasticity was added to the model by letting flowering levels per population and fecundity per clone (composed of fruit per clone and viable seeds per clone) to fluctuate randomly within a set range. The range of flowering was predetermined by field observations where the proportion of mature clones flowering over years was assessed.

b) Variation in flowering levels - Although examining population growth through the use of average transition values is a more practical method of examining population growth, it is not completely realistic in nature. Transition probabilities of growth, survivorship and fecundity are not fixed through time, as assumed by using a single population matrix. For example, the proportion of mature flowering clones that become non-flowering in consecutive years changes over time. In this set of simulations, three different matrices with different transition rates for flowering and non-flowering clones were used to counteract this shortcoming.

Northern populations of *Y. glauca* that experience high levels of flowering and subsequent reproduction do not experience high flowering and fruiting in the following year. In general, high years of flowering seem to be followed by lower levels of flowering resulting in a cyclic pattern of flowering. Although the mechanisms are not completely understood, flowering levels seem to be associated with the number of unflowered ramets per clone. If clones have high numbers of unflowered ramets, they have higher probabilities of flowering; however, after high flowering there are fewer unflowered ramets available, leading to lower levels of flowering in subsequent years. Hence consecutive years of high flowering are not likely to occur.

Based on these observations, several flowering scenarios were examined to determine which flowering pattern would be optimal in terms of population growth and whether higher levels of flowering and the increased frequency of years of high flowering were critical to population persistence. To achieve these objectives three new matrices were developed where transition and fecundity values were altered to represent high (H), medium (M) and low (L) flowering levels. Projections were followed through 100 years by staggering the use of the three matrices according to the desired combination of flowering levels. The medium level of flowering (M) was based on the average fecundity levels for all sites over time; high flowering (H) was 30% higher than the baseline level and low flowering (L) was 30% lower than the baseline level of flowering. Six patterns of flowering were examined: 1) L-L-L…, 2) M-M-M… (average), 3) H-L-L…, 4) H-L-L-L…., 5) H-L-L-L-L… and 6) H-H-H…. All patterns, with the exception of H-H-H and M-M-M were observed in at least one population during the study. The other two patterns of flowering were included for comparative purposes.

c) Variation in flowering levels combined with herbivory – Herbivory is common in northern populations of yuccas and its impact varies with flowering levels. It is particularly catastrophic to yuccas during periods of low flowering where few flowers or fruit escape consumption by pronghorn antelope (*Antilocapra americana*) or mule deer (*Odocoileus hemionus*). To assess the impact of herbivory, the flowering simulations from Part B were repeated with modification of fecundity values to reflect herbivory. Fecundity (number of viable seeds per clone) was reduced by 15% during high flowering years, 29% during moderate (average) flowering years, and by 98 % during low flowering years. These values mirrored the reduction in fecundity observed in the field when clones that experienced herbivory were compared to those that had not.

Projection Analyses

In the first set of simulations (natural population comparisons), population growth rates were determined by the dominant eigen-value (λ) of each matrix. Calculations of population growth were different for the second and third set of simulations because three different population matrices with different transition rates were used. Therefore, the populations would never reach stable stage distributions. In these cases, λ was calculated from the population projections, using the equation $N_t = \lambda^t N_0$, $t = 100$ using average values from 1000 Monte Carlo iterations of population growth over 100 years.

Elasticities were determined for each matrix and used to assess the relative importance of each stage to potential population growth (λ) of natural populations. Elasticity is a perturbation measure in matrix projection models that quantifies the proportional change in population growth rate as a function of a proportional change in a demographic class or transition. They indicate the relative "importance" of life history classes for population growth (De Kroon et al. 1986). Further, elasticities are particularly useful because for a given matrix, they are additive and can be summed for any particular stage or groups of stages with similar characteristics (Caswell 2001).

Results

Patterns of fecundity in Yucca glauca

The two elements of fecundity, fruit produced per fruit per clone and viable seeds per fruit, exhibited considerable variation among sites. The number of fruit produced per clone varied significantly among sites (ANOVA: $F_{9,2021} = 14.872$, $P < 0.001$; Table 2.3; Figure 2.2a), with 41% of the variance in number of fruit per clone being explained by site effects. Both Billings and Little Big Horn produced more fruit per clone than other sites, with 5.5 and 7.6 fruit per clone, respectively; the other 8 sites produced similar numbers of fruit, with 3 or 4 per clone (Figure 2.2a). Fruit per clone was higher at lower latitude sites, but there was no effect of elevation, nor an effect of the interaction latitude*elevation on fruit per clone (Forward stepwise regression: $F_{1,9} = 9.812$, $R^2 = 0.522$, $P = 0.012$; Figure 2.3).

The number of viable seeds produced per fruit per clone varied significantly among sites (ANOVA: $F_{9,956} = 16.936$, $P < 0.001$; Table 2.3; Figure 2.2b). Seed production was considerably higher in the Highwood Mountain population at an average of 253 viable seeds per fruit per clone; other sites produced between 119 and 204 seeds per fruit per clone. There were no significant effects of latitude or elevation on viable seeds per fruit per clone.

Matrix analyses – Natural population comparison

For each site, the projection matrices and their dominant eigen values (λ) were calculated using mean reproductive and transition rates from each population for up to 5 years between 1999 and 2003 (Table 2.4). In all sites, the finite rate of population increase (λ) was greater than 1.0, averaging 1.008 ± 0.001 (n = 10), indicating that all populations are expected to increase in size over time, provided environmental conditions remain constant (Figure 2.4). The finite rate of population increase was negatively associated with latitude, with northern yucca populations experiencing lower levels of population growth ($y = -0.002 - 1.121x$; $F_{1,8} = 5.845$, $n = 10$, Adjusted $R^2 = 0.423$, $P = 0.042$; Figure 2.4). There was no effect of elevation, nor an effect of elevation*latitude on lambda.

Elasticities – Natural population comparison

Elasticity analysis indicated an overwhelming importance of multiparous reproductive clones to the potential future growth of the population (Appendix 2.2). When values were averaged for all sites (Table 2.5), stasis in the flowering and fruiting multiparous class accounted for 33.94% of the potential influence for future population growth. Collectively, multiparous individuals accounted for 88.89% of the potential influence on λ, whereas pre-reproductive individuals had only a minor contribution to population growth. A similar pattern existed for each population (Appendix 2.2).

The elasticity value for stasis in the flowering and fruiting multiparous class was positively correlated with latitude. As one moves north, this stage had a larger potential influence on λ ($y = -0.085 + 0.009x$; $F_{1,8} = 5.913$, $n = 10$, $R^2 = 0.425$, $P = 0.041$). There was no effect of elevation, nor the interaction between elevation and latitude on λ, so these variables were removed from the elasticity analysis. Seed survival (i.e. germination rate as measured here) was more critical to potential population growth in southern sites relative to northern sites, but the relationship was not significant ($y = 0.115 - 0.002x$; $F_{1, 8} = 3.603$, $n = 10$, Adjusted $R^2 = 0.331$, $P = 0.094$).

Role of variation in flowering

In all simulations, populations exhibited positive rates of population growth, regardless of the flowering patterns, as even populations with consistently low flowering would grow (Table 2.6). There were only slight differences among simulated populations and populations that consistently had low flowering had a λ that was only 0.01 less than populations that had all high years of flowering. Lambda was lower in simulations with more subsequent low years of flowering.

Role of variation in flowering and herbivory

The addition of herbivory to simulations resulted in little effect on population growth. As with the flowering simulations, all populations exhibited slightly positive rates of population growth, with little variation among simulated flowering patterns (Table 2.6). Variation in herbivory combined with variation in flowering resulted in little change in λ relative to the flowering only simulations.

Discussion

Despite predictions that peripheral populations have a higher probability of extinction (e.g. Menges and Dolan 1998, Nantel and Gagnon 1999), all northern yucca populations studied are stable, despite high variation in reproductive rates. Many disjunct, peripheral and/or small populations have persisted for long periods of time or even have exhibited positive growth rates (e.g. Bengtsson 1993, Nantel and Gagnon 1999, Godinez-Alvarez 2003), especially if they have low reproductive output, long life span and slow growth (Caswell 1982). For example, out of 17 population studies of different species of cacti, all but three populations were stable; two populations exhibited negative population growth and one population exhibited increasing population size (Godinez-Alvarez 2003). Kingsolver (1986) found that populations of Soapweed from central parts of the species' range were also stable.

The remarkable characteristic of these northern yucca populations is their apparent stability despite high levels of demographic and environmental stochasticity. The absence of a negative relationship between variation in vital rates and population growth is widespread among perennials and has been interpreted as an evolutionary adaptation that buffers the large fluctuations in fecundity/survival (Pfister 1998, Pico and Riba 2002).

At the northern periphery of the range of yuccas, recruitment is extremely low (Table 2.4), probably as the result of limited availability of sheltered microhabitats for seed germination. Seeds of the Agavaceae are often found in sheltered areas which reduce exposure to solar radiation which in turn reduces exposure to high temperatures and reduces evaporative water loss from seeds and soil (Jordan and Nobel 1979), but these data are with respect to hotter, desert environments. In my study populations, young juveniles were typically found in areas of disturbance such as around animal dens, slide locations on steep slopes and roadways. However, in relatively harsh environments, such as those at the northern limit of a species' range, the reproduction from seeds is probably less important than the survival of mature plants and vegetative production of ramets (Svensson et al. 1993). This is supported in *Y. glauca* by population growth rates being most sensitive to adult survival, and much less sensitive to fecundity and young juvenile survival (Table 2.5, Appendix 2.2). The yucca's long life span due to low mortality rates makes high recruitment unnecessary for population persistence (Higgins et al. 2000), although some recruitment is still necessary. Kingsolver (1986) found similar results with his stage transition model with ramets as the individual unit of observation, as seeds only played a small role in population growth. Seed production would be more critical to populations that is expanding and must readily invade new habitats. This is not currently likely with northern populations of yuccas, however seed production could become more crucial as northern populations expand northward with global warming. Therefore, low recruitment is not currently a critical issue in northern populations of yuccas as it is only necessary for a yucca to replace itself once during its lifetime for the population to remain stable. This does not mean that recruitment is not important to the survival of northern populations in the long-term, rather it indicates that recruitment contributes little to population growth rate in each time-step of the projection. The survival of adult reproductive plants however contributes most to population growth, especially over the short-term. Although natural mortality of adult yuccas is very low and natural levels of reproductive variability does not appear to negatively influence population persistence, human-caused changes and/or climate change may pose a threat to the survival of northern populations. Northern populations of yuccas are restricted to specific habitat types, which may make yuccas more prone to extinction through habitat destruction and land-use changes. Destruction of mature plants through agricultural activity is common in some locations, as plants are deliberately removed from fields because they jam up agricultural machinery. In the northern regions of the species' range, however, these issues are minimal as most yuccas are found on eroded slopes that are not compatible with crop production.

Perhaps, more common an issue is the conversion of reproductive plants to non-reproductive plants (i.e. retrogression) via herbivory. Small populations and populations with low flowering are particularly susceptible to the complete consumption of stalks by wild herbivores. This results in the entire population becoming functionally non-sexually reproductive plants. Although this study has shown that northern populations are resistant to some levels of herbivory, complete consumption of stalks results in no seed production and does lead to the decline of populations if it occurs repeatedly. This has been observed to be a critical issue in one of two Canadian populations, where the Pinhorn Grazing Reserve population has not produced fruit in more than 7 years due to repeated consumption of flowering stalks. Repeated failure of this population to set fruit has presumably led to the extirpation of yucca moths from the population (COSEWIC 2002). The population of plants continues to persist however via vegetative reproduction and has higher numbers of rosettes per clone relative to the other Canadian population (Csotonyi and Hurlburt 2000) which is thought to buffer yucca populations in times without sexual reproduction (Kingsolver 1986). Ultimately, the long-term survival of the plant at Pinhorn will be dependent upon the relative importance of sexual vs. vegetative reproduction in the persistence of yucca populations and the mortality rate of mature clones.

Although in the short-term population persistence may depend on the survival of adult plants, the long-term survival of these marginal populations may depend to a large extent on future climatic variation. In recent years, the ecological effects of climate change have been increasingly recognized and the effects of climatic variation on the population dynamics of many species has been documented (Stenseth et al. 2002). General Circulation Models predict that North American grasslands will expand northwards as a result of increased summer temperatures, less precipitation and higher rates of evaporation (Shepherd and McGinn 2003, Zavaleta et al. 2003), which should lead to more favorable habitats for yuccas and range expansion northwards. However, it is also predicted that extreme weather events such as droughts, extreme temperatures or occasional heavy rains will become more common (Easterling et al. 2000, Stenseth et al. 2002); all of which can negatively influence flower availability and moth abundance in yuccas (Chapter 3).

Presumably, the future success of the yucca will also depend on the yucca moth for sexual reproduction. Seed production will be more critical to a species that is invading newly opened habitats (Kingsolver 1986). However, future population persistence and range expansion can only occur if yuccas and yucca moths respond in a similar fashion and can evolve rapidly enough to respond to climatic change. Yuccas and yucca moths have different life history patterns and it is plausible that they may react differently to changing conditions and their phenologies become uncoupled (Bond 1995).

Moths spend most of their lives in diapause in the soil (Riley 1892, Fuller 1990) and may not be able to respond as immediately to changing conditions as their host plant. Although yucca moths are short-lived relative to the plant and will undergo many generations during the lifetime of the yucca which allows them to evolve more rapidly, their short lifespan makes them more susceptible to extinction in the face of catastrophic events.

This matrix model based on genets rather than ramets as in Kingsolver (1986) has many advantages for making management decisions being made at the northern edge of *Y. glauca*'s range. Kingsolver chose the ramet as an arbitrary unit of observation because of the difficulty associated with identifying individual genets or clones. Because the current study followed individual clones through time and individual clones exhibited high variation in floral morphology and color, this was not a big problem as individual clones could easily be distinguished from others about 98% of the time. Further, Kingsolver's model assigned seed-derived ramets and vegetatively propagated ramets the same survival probabilities and time to reproduction, even though these two groups clearly have different establishment and survival rates (Campbell and Keller 1932, Jordan and Nobel 1979, Kingsolver 1986).

Most population projection studies use few years and sites due to the difficulty in collecting intensive data at multiple sites over the long term (Damman and Cain 1998, Nantel and Gagnon 1999). These practices may lead to the overestimation of future population growth because they have failed to estimate a full range of variability in vital rates. This study involved the collection of reproductive data at multiple sites over multiple years and I believe that the total range of reproductive variability was captured.

Flowering ranged from 1% to 50% of mature clones in this study and to date, higher flowering has not been observed in other populations not included in this study, nor have higher levels been reported in the literature.

My study, however, had several potential limitations. Transition probabilities were primarily collected from a single site (Onefour, AB). The Onefour population is the most northern used in my study and there was the potential that this site may have been not representative of the other sites. However, the Onefour population did have a similar stage distribution to the other sites and had similar rates of fecundity.

The models assumed that reproductive rates were independent of plant density, but there is generally an absence of information on this topic in the literature. It has been suggested that this is a reasonable assumption for populations of low densities; but density dependence will eventually limit the growth of populations with a lambda greater than one (Caswell 2001). All ten populations examined had lambdas that were slightly above 1 indicating that populations have the potential to grow only very slowly; hence an absence of density dependence in the model likely would likely lead to only a small change in estimated values of lambda.

Several additional analyses should be included in future assessment. First, models of herbivory failed to include years of complete herbivory in the analysis which occurs frequently in northern populations, especially in those of small sizes. Complete reproductive failure is expected to result in decrease population growth rates and possibly decline. This is a common pattern of reproduction in northern populations and is expected to have implications for population persistence. Second, additionally variability could be incorporated in survival and reproductive rates to determine how much variation can be tolerated before populations begin to decline. This would allow one to assess if high variability can lead to population decline as frequently suggested as a reason for poor performance of peripheral populations in the literature. Finally, these analyses did not incorporate the effect of changing moth densities over time. Moth densities are variable in time and space and are positively related to visitation of flowers (Chapter 3). Presumably variation in moth density will have an impact on future population growth.

The persistence of northern peripheral populations is apparently hampered by neither demographic stochasticity nor by low recruitment. More critical to the persistence of these populations is the maintenance of mature reproductive clones in the population. In northern populations, human destruction of these mature individuals is minimal, so environmental conditions may play a larger role in the ability of these populations to persist over the long term. It is plausible that climate change may lead to increased availability of habitat as the environment becomes drier and warmer, however it could also alter the mutualistic interaction between the plant and yucca moth due to differing responses to environmental change leading to altered abundances or phenological shifts in species occurrences.

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Table 2.1 - Location and elevation of populations of *Yucca glauca* used in this study.

	Coordinates	Elevation (m)	Years studied
Onefour Research Substation Cypress Co, AB	49°00'62 N 110°26'70 W	906	1998-2003
Fort Belknap Blaine Co, MT	47°59'35 N 108°42'81 W	951	2000-2003
Loma Choteau Co, MT	47°57'79 N 110°30'03 W	728	1999-2003
Fort Benton Choteau Co, MT	47°50'22 N 110°39'32 N	885	2000-2003
Highwood Mountains Fergus Co., MT	47°23'44 W 110°10'67 N	1055	2000-2003
Judith River Fergus Co, MT	47°16'07 N 109°41'10 W	1090	1999-2003
Wolf Creek Lewis and Clark Co, MT	47°01'22 N 112°02'73 W	1133	1999-2003
Roundup Lewis and Clark Co, MT	46°25'53 N 108°34'19 W	997	2000-2003
Billings Yellowstone Co, MT	45°40'63 N 108°10'49 W	1129	2002-2003
Little Big Horn Big Horn Co, MT	45°31'88 N 107°15'86 W	1056	2000-2003

Table 2.2 - Summary of life history stages for matrix population projections of clones for *Yucca glauca* at the northern edge of range. Individuals reproducing for the first time and mature reproductive plants that flowered were furthered divided into individuals that fruited or did not fruit.

Life stage	Flowering	Maximum leaf length (cm)	Rosettes per clone	Is clone sexually reproductive?
Seeds	No	n/a	n/a	No
Young Juveniles	No	$\leq$ 15 cm	1	No
Old Juveniles	No	> 15 cm	$\geq$ 1	No
Primiparous Adults	Yes	> 15 cm	$\geq$ 1	Yes, no prior reproduction
Multiparous Adults	No	> 15 cm	$\geq$ 1	Yes, did not flower in current year
	Yes	> 15 cm	$\geq$ 1	Yes, flowered in current year

Table 2.3 – Reproductive success of study sites throughout the duration of study demonstrating high variation in fecundity for mature clones among sites. N years indicates the number of years each population reproduced sexually; the number in brackets is the number of years that the population was observed.

Population	Viable seeds / fruit (Mean ± st. dev. (n fruit))	Fruit per clone (Mean ± st. dev. (n clones))	N years
All combined	158.912 ± 89.595 (956)	4.299 ± 2.736 (2021)	N/A
Onefour	146.267 ± 93.916 (221)	3.761 ± 0.627 (589)	6 (6)
Fort Belknap	147.440 ± 88.952 (100)	3.549 ± 2.675 (162)	4 (4)
Loma	142.437 ± 87.857 (112)	3.193 ± 1.932 (353)	4 (5)
Fort Benton	139.567 ± 75.297 (101)	3.562 ± 0.672 (241)	4 (4)
Highwood Mtn	253.209 ± 56.354 (67)	3.682 ± 5.207 (58)	1 (4)
Judith River	172.918 ± 68.674 (73)	4.490 ± 5.197 (138)	3 (5)
Wolf Creek	119.032 ± 76.163 (82)	4.401 ± 2.221 (167)	4 (5)
Roundup	204.964 ± 82.301 (28)	3.952 ± 2.374 (76)	2 (4)
Billings	126.347 ± 93.061 (72)	5.568 ± 1.060 (94)	2 (2)
Little Big Horn	189.490 ± 66.771 (100)	7.658 ± 3.686 (143)	4 (4)

Table 2.4 – Basic transition matrix for all 10 northern populations of *Yucca glauca* demonstrating proportion of individuals moving between stages every year. Definition of stages: 1 = seeds, 2 = young juveniles, 3 = old juveniles, 4 = Non-fruiting primiparous (P) adults, 5 = Fruiting primiparous adults, 6 = Non-flowering multiparous (M) adults, 7 = Flowering and fruiting multiparous adults, 8 = Flowering and non-fruiting multiparous adults. Bold figures indicate fecundity values which differ for each population and are a stochastic multiplicative function of fruit produced per clone (F) by viable seeds per fruit (S).

Stage	1	2	3	4	5	6	7	8
1	0	0	0	0	**$F_{P}S$**	0	**$F_{M}S$**	0
2	0.0006	0.6830	0	0	0	0	0	0
3	0	0.1030	0.8450	0	0	0	0	0
4	0	0	0.0390	0	0	0	0	0
5	0	0	0.1160	0	0	0	0	0
6	0	0	0	0.24300	0.7280	0.6220	0.6220	0.6220
7	0	0	0	0	0	0.1460	0.1460	0.1460
8	0	0	0	0.0150	0.0150	0.2290	0.2290	0.2290

Table 2.5 – Average elasticity matrix for all 10 northern populations of *Yucca glauca* demonstrating potential relative contributions of each stage to future population growth. Definition of stages: 1 = seeds, 2 = young juveniles, 3 = old juveniles, 4 = Non-fruiting primiparous adults, 5 = Fruiting primiparous adults, 6 = Non-flowering multiparous adults, 7 = Flowering and fruiting multiparous adults, 8 = Flowering and non-fruiting multiparous adults. Bold figures indicate fecundity values.

Stage	1	2	3	4	5	6	7	8
1	0	0	0	0	**0.00052**	0	**0.00940**	0
2	0.00992	0.02066	0	0	0	0	0	0
3	0	0.00992	0.05121	0	0	0	0	0
4	0	0	0.00098	0	0	0	0	0
5	0	0	0.00894	0	0	0	0	0
6	0	0	0	0.00092	0.00826	0.33941	0.07847	0.12375
7	0	0	0			0.08569	0.01981	0.03178
8	0	0	0	0.00006	0.00016	0.12571	0.02906	0.04583

Table 2.6 – Simulated population growth rates for populations of *Y. glauca* with varying levels and patterns of flowering and herbivory. Flowering pattern symbols: L = low, M = Medium and H = High. Each flowering pattern was projected for a total of 100 years. Population growth varied little with flowering pattern and with the addition of herbivory to the model, although population growth is predicted to be slightly less in populations with more consecutive years of low flowering.

Flowering pattern	Variation in flowering	Variation in flowering with herbivory
	Lambda	Lambda
LLL	1.0021	1.0021
MMM	1.0076	1.0075
HHH	1.0125	1.0125
HLL	1.0058	1.0060
HLLL	1.0047	1.0049
HLLLL	1.0043	1.0043
HLLML	1.0053	1.0053

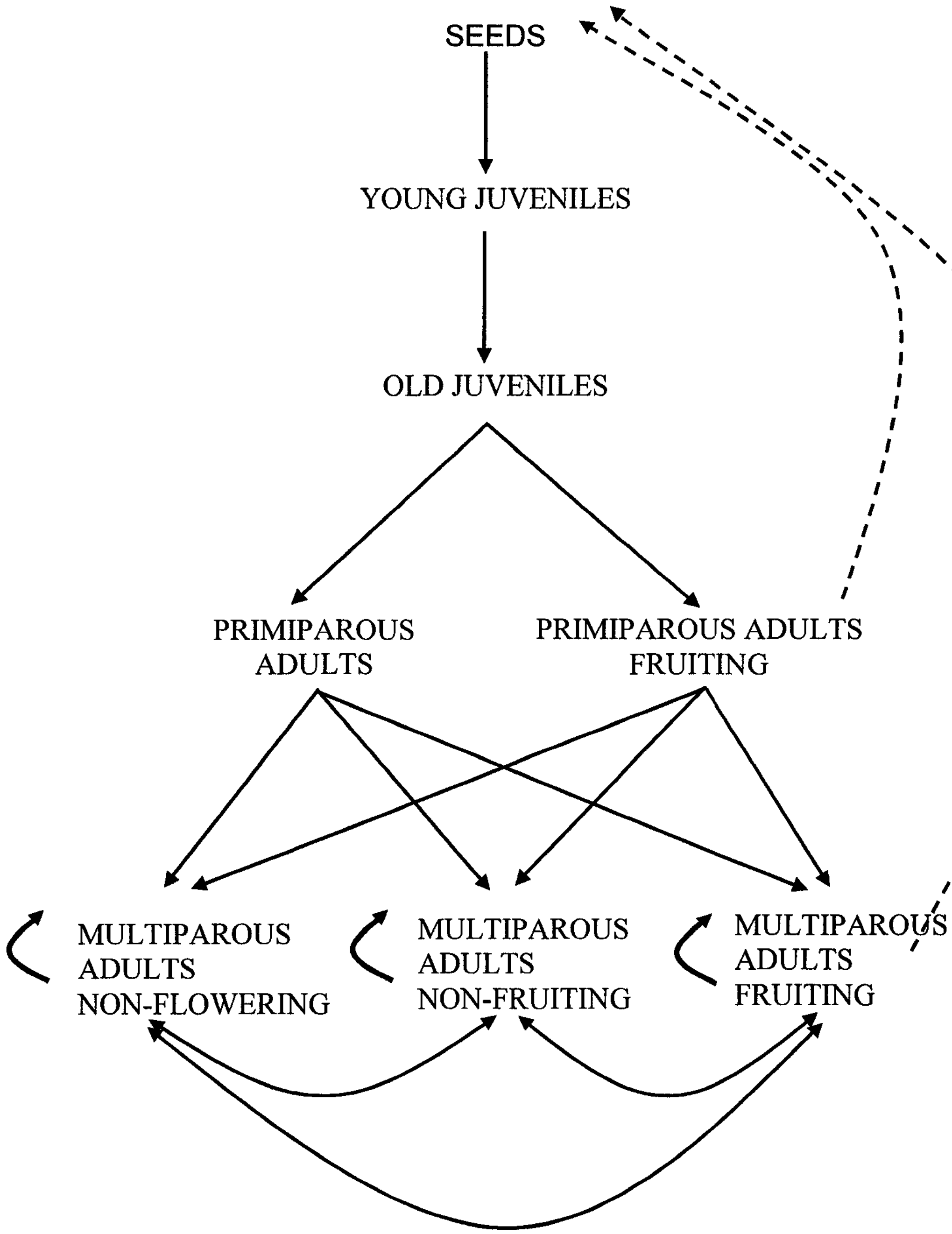

Figure 2.1 - Life history model of *Yucca glauca* reproduction using the clone (genet) as the reproductive unit Circles represent life stages that have unique survival and/or reproductive values. Arrows with solid lines represent transitions between stages. Arrows with dotted lines represent contributions of each stage to the population in the form of seeds.

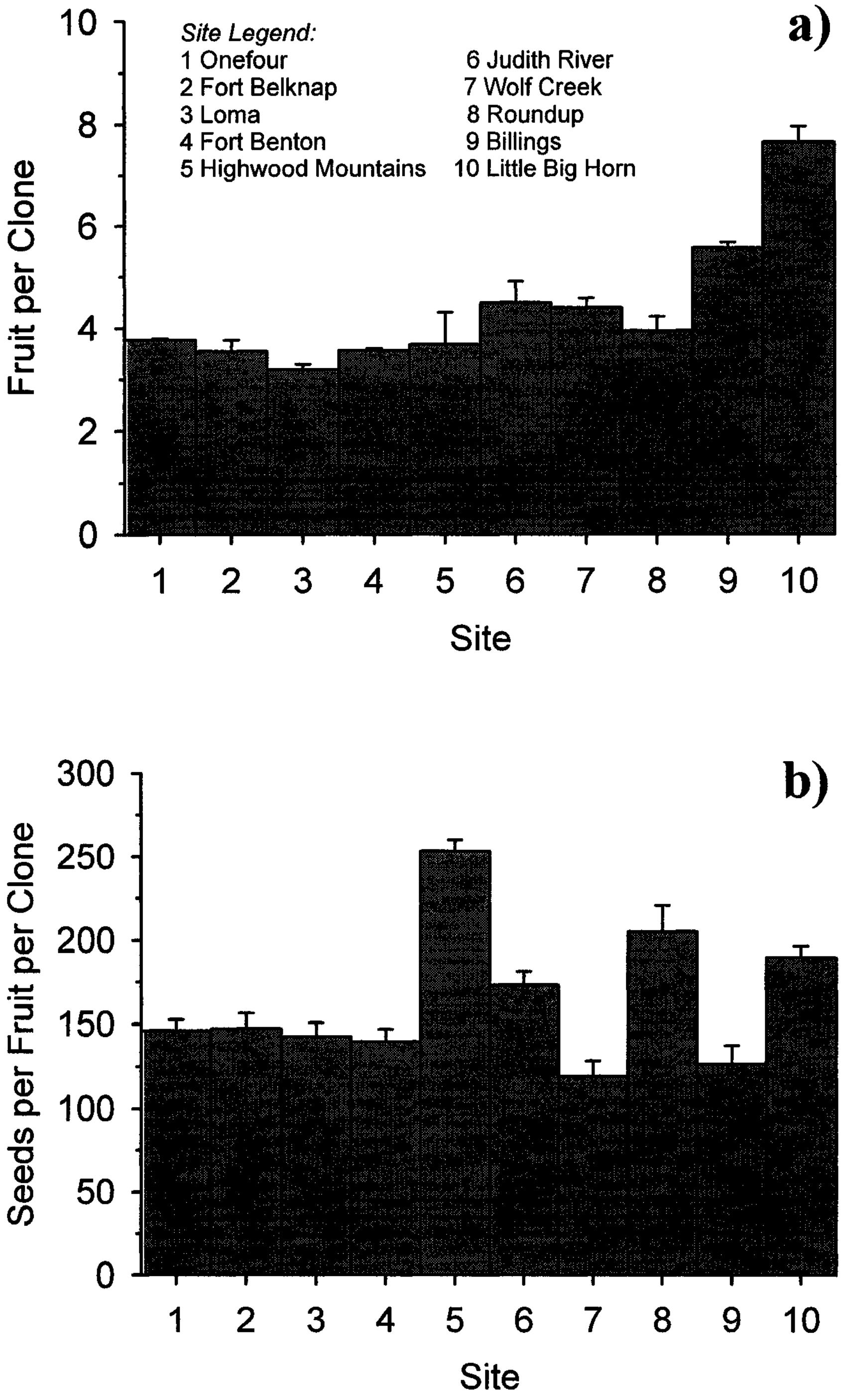

Figure 2.2 – Variation in reproductive rates among study populations for (a) fruit per clone and (b) viable seeds per fruit per clone. Bars are mean values ± standard error.

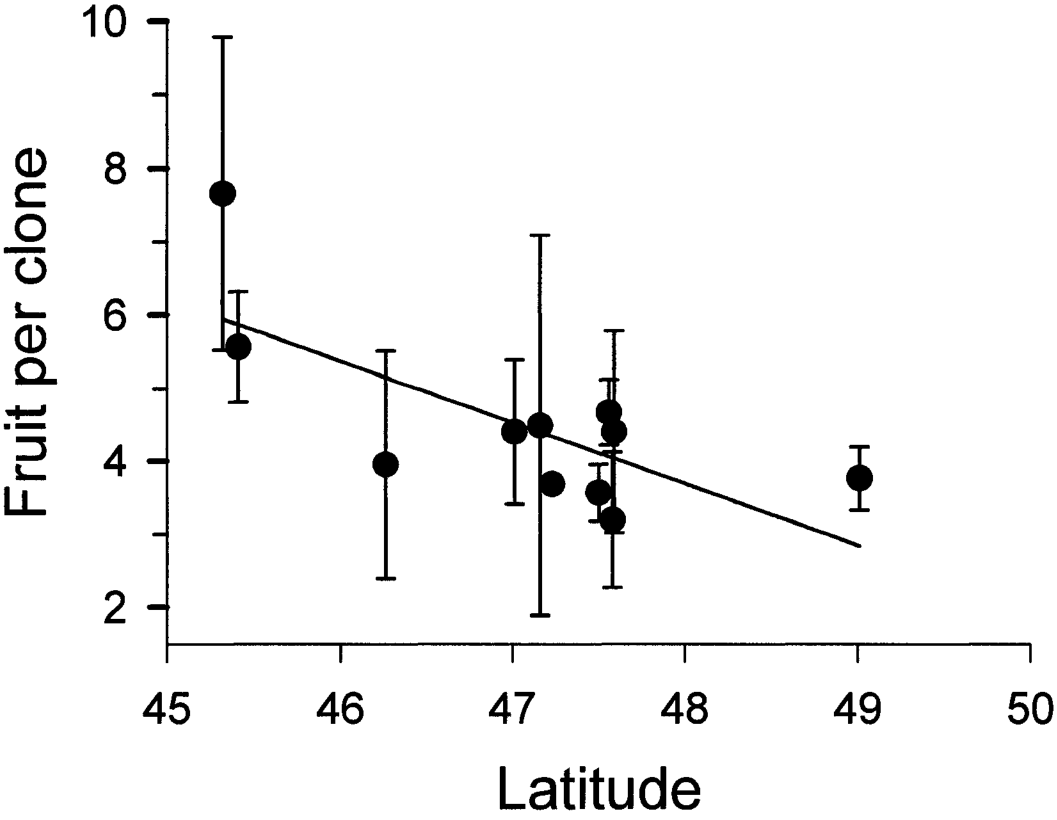

Figure 2.3 – Number of fruit produced per clone as a function of latitude of the population. More northern populations produced fewer fruit than more southern populations in the study region. Points are mean values ± standard errors.

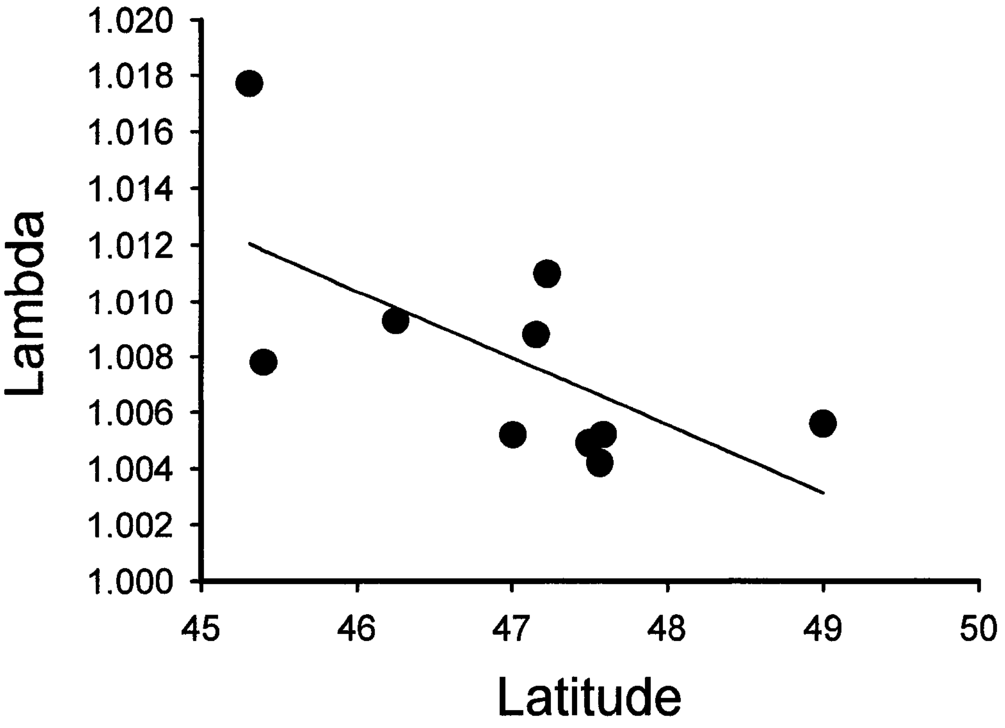

Figure 2.4 – Projected population growth rates (λ) as a function of latitude. More northern populations have lower predicted population growth rates than more southern ones.

Appendix 2.1 – Climatic averages for each study site.

	Max Temp. (°C)	Min Temp. (°C)	Avg Total Prec. (mm)
Onefour Research Substation Cypress Co, AB	11.1	-2.2	330
Fort Belknap Blaine Co, MT	14.3	-1.8	280
Loma Choteau Co, MT	15.5	-1.3	277
Fort Benton Choteau Co, MT	15.5	-0.4	308
Highwood Mountains Fergus Co., MT	15.1	-0.6	327
Judith River Fergus Co, MT	14.3	-0.6	334
Wolf Creek Lewis and Clark Co, MT	13.7	-2.1	321
Roundup Lewis and Clark Co, MT	16.3	0.4	271
Billings Yellowstone Co, MT	16.7	0.5	290
Little Big Horn Big Horn Co, MT	16.6	-0.2	257

Appendix 2.2 – Stage-based elasticity matrices for 10 northern populations of *Yucca glauca,* arranged by latitude (north to south). Definition of stages: 1 = seeds, 2 = young juveniles, 3 = old juveniles, 4 = Non-fruiting primiparous adults, 5 = Fruiting primiparous adults, 6 = Non-flowering multiparous adults, 7 = Flowering and fruiting multiparous adults, 8 = Flowering and non-fruiting multiparous adults. Bold figures indicate fecundity values.

Stage	1	2	3	4	5	6	7	8
Onefour, AB								
1	0	0	0	0	**0.0003**	0	**0.0077**	0
2	0.0080	0.0169	0	0	0	0	0	0
3	0	0.0080	0.0422	0	0	0	0	0
4	0	0	0.0008	0	0	0	0	0
5	0	0	0.0072	0	0	0	0	0
6	0	0	0	0.0008	0.0068	0.3483	0.0808	0.1274
7	0	0	0	0	0	0.0867	0.0201	0.0317
8	0	0	0	<0.0001	0.0001	0.1290	0.0300	0.0472

Fort Belknap, MT								
1	0	0	0	0	**0.0003**	0	**0.0074**	0
2	0.0077	0.0162	0	0	0	0	0	0
3	0	0.0077	0.0405	0	0	0	0	0
4	0	0	0.0008	0	0	0	0	0
5	0	0	0.0069	0	0	0	0	0
6	0	0	0	0.0007	0.0065	0.3500	0.0812	0.1281
7	0	0	0	0	0	0.0869	0.0202	0.0318
8	0	0	0	<0.0001	0.0001	0.1296	0.0301	0.0474

Loma, MT								
1	0	0	0	0	**0.0002**	0	**0.0065**	0
2	0.0068	0.0144	0	0	0	0	0	0
3	0	0.0068	0.0360	0	0	0	0	0
4	0	0	0.0007	0	0	0	0	0
5	0	0	0.0061	0	0	0	0	0
6	0	0	0	0.0006	0.0058	0.3543	0.0824	0.1298
7	0	0	0	0	0	0.0874	0.0203	0.0320
8	0	0	0	<0.0001	0.0001	0.1312	0.0305	0.0481

Fort Benton, MT								
1	0	0	0	0	**0.0003**	0	**0.0071**	0
2	0.0073	0.0155	0	0	0	0	0	0
3	0	0.0073	0.0388	0	0	0	0	0
4	0	0	0.0007	0	0	0	0	0
5	0	0	0.0066	0	0	0	0	0
6	0	0	0	0.0007	0.0062	0.3516	0.0817	0.1287
7	0	0	0	0	0	0.0871	0.0202	0.0319
8	0	0	0	<0.0001	0.0001	0.1302	0.0302	0.0477

Highwood Mountains MT								
1	0	0	0	0	**0.0008**	0	**0.0117**	0
2	0.0125	0.0259	0	0	0	0	0	0
3	0	0.0125	0.0636	0	0	0	0	0
4	0	0	0.0012	0	0	0	0	0
5	0	0	0.0113	0	0	0	0	0
6	0	0	0	0.0011	0.0103	0.3273	0.0753	0.1188
7	0	0	0	0	0	0.0843	0.0194	0.0306
8	0	0	0	<0.0001	0.0002	0.1212	0.0279	0.0440

Judith River, MT								
1	0	0	0	0	**0.0006**	0	**0.0102**	0
2	0.0107	0.0225	0	0	0	0	0	0
3	0	0.0107	0.0555	0	0	0	0	0
4	0	0	0.0011	0	0	0	0	0
5	0	0	0.0097	0	0	0	0	0
6	0	0	0	0.0010	0.0089	0.3354	0.0774	0.1221
7	0	0	0	0	0	0.0852	0.0197	0.0310
8	0	0	0	<0.0001	0.0002	0.1242	0.0287	0.0452

Wolf Creek, MT								
1	0	0	0	0	**0.0003**	0	**0.0074**	0
2	0.0077	0.0162	0	0	0	0	0	0
3	0	0.0077	0.0405	0	0	0	0	0
4	0	0	0.0008	0	0	0	0	0
5	0	0	0.0069	0	0	0	0	0
6	0	0	0	0.0007	0.0065	0.3499	0.0812	0.1281
7	0	0	0	0	0	0.0869	0.0201	0.0318
8	0	0	0	<0.0001	0.0001	0.1296	0.0301	0.0474

Roundup, MT								
1	0	0	0	0	**0.0006**	0	**0.0105**	0
2	0.0111	0.0232	0	0	0	0	0	0
3	0	0.0111	0.0573	0	0	0	0	0
4	0	0	0.0011	0	0	0	0	0
5	0	0	0.0100	0	0	0	0	0
6	0	0	0	0.0010	0.0092	0.3336	0.0769	0.1214
7	0	0	0	0	0	0.0850	0.0196	0.0309
8	0	0	0	<0.0001	0.0002	0.1236	0.0285	0.0449

Billings, MT								
1	0	0	0	0	**0.0005**	0	**0.0094**	0
2	0.0099	0.0207	0	0	0	0	0	0
3	0	0.0099	0.0514	0	0	0	0	0
4	0	0	0.0010	0	0	0	0	0
5	0	0	0.0089	0	0	0	0	0
6	0	0	0	0.0009	0.0083	0.3394	0.0785	0.1237
7	0	0	0	0	0	0.0857	0.0198	0.0312
8	0	0	0	<0.0001	0.0002	0.1257	0.0291	0.0458

Little Big Horn, MT								
1	0	0	0	0	**0.0015**	0	**0.0161**	0
2	0.0176	0.0359	0	0	0	0	0	0
3	0	0.0176	0.0863	0	0	0	0	0
4	0	0	0.0017	0	0	0	0	0
5	0	0	0.0159	0	0	0	0	0
6	0	0	0	0.0016	0.0141	0.3043	0.0693	0.1094
7	0	0	0	0	0	0.0817	0.0186	0.0294
8	0	0	0	<0.0001	0.0003	0.1127	0.0257	0.0451

CHAPTER 3

Regulation of a seed predation - pollination mutualism between yuccas (*Yucca glauca*) and yucca moths (*Tegeticula yuccasella*) under low density conditions

Summary

This study examined the regulation of the mutualism between yuccas (*Yucca glauca*) and yucca moths (*Tegeticula yuccasella*) in Alberta and Montana at the extreme northern edge of the distribution of any yucca species. Partner densities are variable in time in these populations and low partner densities may be experienced more frequently than in other populations, which in turn may have implications for the stability and evolution of the mutualism. I assessed the role that annual and seasonal variation of flowering and moth emergence played on floral visitation and fruit retention in multiple flowering seasons, following individual flowers and inflorescences and their fruiting success.

Northern populations of yuccas and yucca moths exhibited similar levels of reproductive success as other populations despite considerable variation in flowering and lower numbers of ovipositions per flower. This similarity resulted from yuccas selectively abscising flowers with fewer ovipositions and maturing those with more moth visits. Further, northern populations apparently had less mortality of moth eggs, as a higher proportion of ovipositions (eggs) led to moth larvae in mature fruit relative to other populations reported in the literature. Preliminary evidence suggests that this is due to fewer ovules being damaged per oviposition and an overall reduction in damaged ovules, because moths spread their ovipositions throughout the length of the pistil. Therefore the mutualism between yuccas and yucca moths remains strong at the northern periphery of their ranges and populations of yuccas have the ability to regulate moth populations at low densities to ensure reproductive success.

Introduction

One of the most sought after answers in the study of mutualism is how seemingly beneficial relationships persist given the inherently selfish nature of organisms (Axelrod and Hamilton 1981, Connor 1995, Frank 1995). Such conflicts arise between mutualists because the selection pressures on one partner favors its maximized reproduction regardless of the cost to the other partner, at least in the short term. This conflict of interest is especially clear in the interaction between yuccas (*Yucca* spp., Agavaceae) and yucca moths (*Tegeticula* spp., Incurvariidae) (Wilson and Addicott 1998, Addicott and Bao 1999,) where moth larvae feed only on yucca seeds, and in turn, yuccas can only produce seeds if their flowers are pollinated by yucca moths (Riley 1892, but see Dodd and Linhart 1994). Both yuccas and moths are selected to maximize the numbers of their respective progeny and to ensure this they must prevent overexploitation by their mutualist partners (Addicott and Tyre 1995, Addicott and Bao 1999).

For the interaction between yuccas and moths to remain mutualistic, yuccas must mature some of the flowers in which moths have laid their eggs and moth larvae within mature fruit must not eat all of the yucca's seeds (Pellmyr and Huth 1994). The average proportion of seeds destroyed by feeding can be as low as 1% and as high as 45 % per fruit depending upon the species of yucca (Keeley et al. 1984, Addicott 1986). However, some fruit lose all viable seeds to yucca moth larvae (Addicott 1986, D. Hurlburt, personal observation), while other fruit may lose almost no seeds to yucca moth larvae. Further, some individual yucca moths "cheat" yuccas by ovipositing in yucca flowers without transferring pollen (Aker and Udovic 1981, Tyre and Addicott 1993), with 5-

25% of flowers not receiving pollen despite having received ovipositions (Tyre and Addicott 1993).

Yuccas can limit the number of poor quality flowers that they mature through the mechanism of selective abscission. Like many plant species (Stephenson 1981), most yuccas appear to experience resource limitation and can only mature around 10% of their flowers, while abscising the rest whether or not those flowers have been pollinated (Udovic and Aker 1981, Aker 1982, Addicott 1986, Addicott and Tyre 1995, Addicott 1998). Fruit retention is non-random and the plants selectively retain visited flowers of the highest quality (e.g. Pellmyr and Huth 1994, Pellmyr et al. 1997, Wilson and Addicott 1998, Addicott and Bao 1999, Humphries and Addicott 2000), although the mechanisms through which selective abscission occurs are not understood (Marr and Pellmyr 2003). In yuccas, selective abscission of visited flowers occurs in response to high numbers of seeds produced through self-fertilization (Aker and Udovic 1981), high numbers of moth eggs per flower (Pellmyr and Huth 1994, Huth and Pellmyr 1997) and by high numbers of ovules damaged by oviposition (Shapiro and Addicott 2003).

However, the degree of conflict and the need to prevent over exploitation can vary in time and space, especially with variation in mutualist density. The conflict of interest between partners should be reduced under low density conditions and these interactions may be less antagonistic than interactions with mutualists at high densities. Most studies exploring regulation in moth-yucca systems have examined the system at relatively high partner densities. The examinations of moth-yucca mutualisms in sites that are along ecological range edges are particularly amenable to examining regulation because partner densities are highly variable in time and low partner densities may be experienced more frequently than in other populations. Additionally, because of high demographic and environmental stochasticity, yucca flowering and moth emergence may become asynchronous, potentially leading to a dissolution of the mutualism. Opportunities to examine the evolution and functioning of moth-yucca mutualisms at the partner-limited end of the spectrum are rarely presented in the literature and may provide a unique glimpse at how yuccas and yucca moths have evolved in response to low levels of pollination and seed predation.

In this study I address three problems. First, how does the effect of moth density and flowering levels on visitation and fruiting vary between sites and years in populations at the north edge of the range of moth/yucca mutualism? Second, what factors extrinsic to the mutualism, such as weather, drive the systems to low densities in a given year? Third, how do the mechanisms regulating moth mutualism vary between low and high density conditions? Finally, do these populations at the northern edge of range have a different form of regulation than that which occurs in other species and locations?

Materials and Methods

Study System

I studied the interactions between the yuccas (*Yucca glauca* Nuttall) and yucca moths (*Tegeticula yuccasella* Riley) at the northern edge of their ranges in Alberta and Montana. Flowering phenology and moth emergence patterns were determined over a four year period (1999 - 2002) for three populations, including 1) Onefour, Alberta (49° 00. 62' N, 110° 26.70' W, 906 m altitude), 2) Loma, Montana (47° 57.79' N, 110° 30.03' W, 728 m altitude), and 3) Fort Benton, Montana (47° 01.22' N, 110° 39.32' W, 885 m altitude). These populations are among the most northern natural populations of yuccas and yucca moths. Complete data sets were available for Onefour between 1999-2002, Loma between 2000-2002 and Fort Benton between 2001-2002. Additional data were obtained from other sites/years: Wolf Creek, MT (47°01.22' N, 112°02.73' W, 1133 m altitude, 1999), Judith River, MT (47°16.07' N, 109°41.10' W, 1090 m altitude, 2001-2003), and Billings, MT (45°40.63' N, 108°10.49' W, 1129 m altitude, 2002-2003).

Sites were classified as being relatively 'northern' or 'southern' based on biological differences among populations and past geological history. Northern sites included Onefour, AB, Loma, MT, Fort Benton, MT, Fort Belknap, MT and Judith River, MT. These sites exhibited higher variation in flowering, moth densities (D. Hurlburt, unpublished data) and fruit production (Chapter 2) over 5 years of observation (1999 – 2003) and were found on eroded south-facing coulee slopes north of the Missouri River (the Judith River site occurs at the junction of the Missouri River and the Judith River). All other sites were considered southern. Each northern site was covered by ice and experienced disturbance associated with the retreat of glaciers during the last ice age

10,000 years ago (Alt and Hyndman 1986, Beaney and Shaw 2000). In addition to presumably having lower quality habitat than southern sites because of their more northerly location, northern sites may have a relatively recent evolutionary history (Pamilo and Savolainen 1999) and the interaction may have evolved in response to different selection pressures.

Flowering and Moth Emergence Patterns

Individually marked flowers on a subset of inflorescences at each main site were monitored 2-7 times per week to determine the opening date and eventual fate of flowers (e.g. abscised, visited, retained, eaten). To identify individual flowers on inflorescences, a unique identifying number was written on the outer surface of one tepal on each flower using a black Sharpie® marker (see Addicott 1998). Once the petals withered, and if the flower developed into a fruit, the number was re-written on the enlarging ovary. There was no evidence that writing on the flowers/ fruit was detrimental to the plant or that it affects moth behavior, since there was no difference in total numbers of ovipositions per fruit or viable seeds between marked and unmarked fruit (D. Hurlburt, unpublished data).

To determine moth emergence patterns and female moth density per flower, 100 fresh flowers were examined and female yucca moths were counted at each site 2-3 times per week throughout the flowering season. For comparative purposes, moth densities were determined at additional sites in Montana 1-3 times per flowering season using the same technique.

Fruit Dissection

At each of the three main sites, every marked flower was collected as it abscised or at six weeks post-pollination in the case of retained flowers. Collected flowers/fruit were dissected to determine the number of ovipositions per pistil. To dissect for ovipositions, thin sections of the carpel wall were shaved away with a scalpel to reveal ovipositor tracks into the locular cavity. There is a 1:1 correspondence between number of oviposition tracks and the numbers of yucca moth eggs (D. Hurlburt, unpublished data, J. Addicott, personal communication). Additional samples of flowers and fruit from minor sites were also dissected for ovipositions to assess pollinator activity levels. Mature fruit were dissected along each locule wall and the number of yucca moth larvae per locule and fruit were obtained to determine reproductive success of moths. A small sample of 10 day old fruit from Onefour, AB in 2000 was dissected to determine the relationship between oviposition number and number of damaged ovules per fruit. An ovule was considered damaged if it was in the immediate vicinity of an oviposition track and had failed to develop.

Visitation, Fruiting and Retention

Flowers were considered "visited" if they had at least one oviposition mark, because yucca moths only pollinate if they have first laid an egg in a flower (Aker and Udovic 1981, Addicott and Tyre 1995). Visitation rates, the proportion of flowers within inflorescences with at least one oviposition, were determined for each site and year of study. Retention refers to the proportion of visited flowers that were retained as fruit to maturity (approximately 6 weeks after flower opening) and fruiting referred to the overall proportion of flowers that become fruit, whether or not flowers were visited. These measures were calculated within inflorescences and averaged within sites and years. In some cases, multiple inflorescences from the same clone were used and treated as independent samples.

Overall visitation and retention rates for flowers and fruit from each population and year were assessed in response to 1) flowering levels (proportion of clones flowering and proportion of rosettes flowering), 2) the density of female moths per flower, 3) total ovipositions per flower and/or fruit, and 4) seasonal weather conditions including over-winter mean monthly temperature, over-winter total precipitation, spring mean monthly temperature, and spring total precipitation. Daily flowering, visitation, fruiting and retention patterns were plotted to determine correspondence with extreme weather events such as high precipitation, high evening wind speed, high temperatures and high levels of herbivory, and moth density per flower.

Selective Abscission

I examined the effect of the number of ovipositions on the retention of flowers to determine if yuccas selectively retain flowers with fewer ovipositions as observed in most other moth/yucca interactions (Pellmyr and Huth 1994, Huth and Pellmyr 1997). To test for population-level patterns of selective abscission, logistic regressions of flower fate (0=abscised, 1= retained) on numbers of ovipositions per flower were conducted for all flowers over all years at each site. However, this analysis does not necessarily tell us whether individual yuccas are selectively abscising their flowers (Addicott and Bao 1999).

To test for within inflorescence selective abscission I partitioned the mortality of eggs from oviposition in flowers to eggs in fruit that matured into two additive components using k-values (Varley et al. 1975). One is the mortality due simply to abscission of excess flowers, and is measured as the log(1/Retention). The other is my estimate of selective abscission, which measures the mortality of eggs (ovipositions were used as an indicator of eggs) above or below that which would have occurred if abscission of flowers were independent of numbers of ovipositions. k-values for selective abscission were computed as $k_{sa} = \log_{10} (N_x/N_{x+1})$, where N_x and N_{x+1} are the average number of yucca moth eggs in flowers and in fruit, respectively. Calculation and interpretation of k-values are further described in Addicott and Bao (1999) and Shapiro and Addicott (2004).

Results

Annual and Site Variation

Intensity of flowering and moth density - Population levels of flowering were measured in two ways, 1) proportion of clones that flowered and 2) proportion of rosettes that flowered. Proportions of clones that flowered ranged from 0.083 to 0.700 among years and sites and proportion of rosettes flowering ranged from 0.031 to 0.449 (Table 3.1). Both year and site*year had a significant impact on clone-level flowering (Clones flowered: $X^2 = 234.772$, n = 2353, d.f. = 11, $P < 0.001$, Appendix 3.1). The odds of a clone flowering for either Fort Benton or Loma were not different from Onefour (= reference site). Flowering was low in 2000, and high in both 1999 and 2001 relative to 2002 (= reference year). The odds of flowering in 1999 were three times higher than in 2001. The odds of a clone flowering in Fort Benton (1999) was two time less than Onefour (2002). For proportion of rosettes flowered, site, year and the interaction term, site*year were significant predictors of flowering (Rosettes flowered: $X^2 = 542.527$, n = 6870, d.f. = 11, $P < 0.001$, Appendix 3.1). Rosettes in Fort Benton had a lower probability of flowering and those in Loma had a high probability of flowering relative to Onefour. In 1999, the probability of a rosette flowering was almost four times higher than 2002; where as in 2000 and 2001 the probability of flowering was lower. In 2000, the odds of a rosette flowering were only one-tenth of that of 2002. The probability of flowering was highest in Fort Benton (2001), Loma (1999) and Loma (2000) relative to Onefour (2002). On average, Fort Benton had the lowest levels of both clone– and rosette-level flowering. The highest levels of flowering occurred in 1999 and the lowest levels of flowering occurred in 2000 for both flowering measures.

In addition to clone- and rosette-level flowering, the number of flowers produced per inflorescence can also contribute to the total abundance of flowers available for pollination. The mean number of flowers per inflorescence differed significantly among years and sites (Full factorial ANOVA: $F_{6,976} = 12.437$, $P < 0.0001$; Figure 3.1). However, significance arose because of the high number of degrees of freedom. As the model only however accounted for 7.103% of the total variation and differences between populations were small, flowers per inflorescence was not used in subsequent analyses.

Although there were significant differences in flowering levels among sites and years (see above), these differences must be attributed to factor(s) other than temperature or precipitation. Neither winter monthly temperatures, total winter precipitation, spring monthly temperatures, nor total spring precipitation were significant predictors of either clone- or rosette-level flowering (Table 3.2).

Fruiting rates per clone and per rosette were analyzed using full factorial logit models and revealed different patterns than that observed with rates of flowering (Fruiting per Clone: $X^2 = 299.173$, n = 762, d.f. = 11, $P < 0.001$; Fruiting per Rosette: $X^2 = 333.515$, n = 1088, d.f. = 11, $P < 0.001$; Appendix 3.1). In general, high levels of flowering did not lead to high levels of fruiting in any given year or site. For both measures, Loma expressed considerably higher odds of fruiting than Onefour; Fort Benton did not differ from Onefour. For fruiting per clone, the odds of fruiting in 1999 was four times higher than 2002 and in 2000 and 2001, 98% and 50% lower respectively. For fruiting per rosette, 1999 was 2.5 higher and 2000 was 96 % lower than in 2002. In 1999 and 2000, Fort Benton had much lower probabilities of fruiting per clone relative to Onefour 2002, but in 2001, the odds of fruit set was 226 times higher than the reference site and year. For fruiting per rosette, Fort Benton also had lower probabilities in 1999 and 2000, and higher probability (by 28 times) in 2001 relative to Onefour 2002. The odds of fruiting in Loma (1999) however were only 5% of that of the reference site and year.

Moth densities per site per year ranged from 0.043 to 1.087 female moths per freshly opened flower. In most years, a single female moth was found in every 2 or 3 flowers. Non-parametric Kruskal-Wallis tests showed that female moth density varied among sites ($X^2 = 4.900$, n = 9, $P = 0.086$) but not years ($X^2 = 4.089$, n = 9, $P = 0.252$). The relative abundance of moths to flowers within sites and years was positively related to mean spring temperatures and negatively associated with proportion of clones flowering, but was not related to winter precipitation, spring precipitation or winter temperature. There was no relationship between relative moth density and proportion of rosettes flowering (Table 3.3).

Visitation, fruiting and retention - Flowering intensity and moth emergence varied considerably among sites and years, leading to variation in the success of the mutualism in time and space. The nature of the moth – yucca mutualism was assessed using measures of visitation, fruiting and retention.

At the population level the proportions of flowers receiving at least one oviposition (= visitation) were high at all sites and in all years, ranging from 0.751 to 0.950 (Table 3.4). Moth density per flower was the only significant predictor of visitation, as flowering levels and weather conditions were not significant (Table 3.5).

Within inflorescences, there was a positive relationship between the mean number of ovipositions received per flower and visitation per inflorescence (Figure 3.2).

At the population level, the proportion of all flowers that mature as fruit (=fruiting), ranged from .003 to .122 (Table 3.4). Using multiple forward regression, fruiting was positively associated with the proportion of flowering clones, but there was no relationship between fruiting and visitation, mean number of ovipositions per flower, proportion of flowering rosettes, temperature, or precipitation (Table 3.5).

In most cases, retention of visited flowers was high, ranging from .076 to .211 (Table 3.4). None of the variables measures were significant predictors of retention rates (Table 3.5).

Ovipositions and larval survival - Ovipositions received per flower were compared between samples of all flowers (abscised and retained) and retained mature fruit at the population level as an indication of moth success and to assess the presence of patterns of abscission. At all sites and years, except Onefour in 1999, retained fruit had significantly more ovipositions than abscised flowers (Figure 3.3). Similarly, combining the data for all years at each site (except Onefour 1999), abscission of flowers was more likely for flowers with low numbers of yucca moth ovipositions than for flowers with high numbers of ovipositions (Figure 3.4).

Patterns of abscission within inflorescences were also examined in 2001 and 2002 since population level patterns of selective abscission do not necessarily imply that individual inflorescences are selectively retaining some flowers over other. Population-level patterns of selective abscission may arise if visitation varies among plants, is positively correlated with number of eggs per visited flower and is negatively correlated with retention (Addicott and Bao 1999). Values for within inflorescence selective abscission were consistently negative (Figure 3.5), which indicated that inflorescences preferentially retained flowers with higher numbers of ovipositions (Table 3.6). In 2002, plants with more ovipositions had greater k-values. This supports the idea that at very low oviposition levels, flowers with fewer ovipositions will be discriminated against (Figure 3.5). The consistent negative k-values indicate that within inflorescences flowers with more ovipositions are more likely to be retained, but that the degree to which this occurs does not differ among inflorescences with different average numbers of ovipositions per flower.

Flowers in northern populations of yuccas received fewer ovipositions (10.060 ± 0.809, n = 12) than other more southern populations (20.380 ± 4.974, n = 7) (t = 2.048, d.f. = 6, *P* = 0.043, Table 3.7). However there were no significant differences in total ovipositions per retained fruit (northern populations: 13.064 ± 1.206, n = 13, southern populations: 15.120 ± 2.617, n = 5, t = 0.714, d.f. = 6, *P* = 0.281, Table 3.7), nor were there significant differences in numbers of larvae per fruit with population location (northern populations: 5.215 ± 0.547, n = 11, southern populations: 5.765 ± 1.385, n = 13, t = 0.163, d.f. = 14, *P* = 0.436, Table 3.8).

Dissection of a small sample of young fruit from the Onefour, AB site in 2000 showed that with increasing numbers of ovipositions per fruit more ovules were damaged, although the amount of damage acquired per oviposition decreased with additional ovipositions (Figure 3.6).

Within Season Variation

Patterns of flowering and moth emergence - Daily flowering and moth emergence patterns varied among years and sites and may change the nature of the interaction occurring between yuccas and yucca moths on a day to day basis. The distributions of flowering and moth emergence tended to have similar shapes across sites and years. Flowering tended to peak about 2 weeks after first flower and then gradually tapered off in a very long tail (Figures 3.7-3.9). Number of moths per flower varied throughout individual flight seasons with higher numbers per flower earlier and later in the flowering season when flower density was the lowest (Figures 3.10-3.12). The commencement of flowering varied up to 3 weeks among years (Table 3.9), but the first emergence of moths is unknown since it is difficult to count yucca moths in the absence of flowers; however it is obvious from the high numbers of moths per flower on the first day of flowering that moths were out before flowers. The Onefour site started to flower about 7 days after the Montana sites in any given year. Onset of flowering for all sites and years was later in years when total spring precipitation was higher ($F_{2,6} = 15.537$, $n = 9$, Adjusted $R^2 = 0.784$, $P = 0.004$). But the onset of flowering was not related to winter precipitation, mean spring temperature, nor mean winter temperature.

Patterns of visitation, fruiting and retention phenologies - Temporal correspondence between levels of a) flowering and fruiting and b) visitation and fruiting was examined using non-parametric runs tests. Runs tests were used to examine if temporal patterns of flowering and fruiting directly corresponded and were used to determine if days with high flower availability and high visitation, also produced high proportions of fruit. The temporal pattern of flowering did not differ significantly from that of fruiting in any site or year (Table 3.10). Fruiting tended to be proportional to flowers available at any given time, with few unaccountable deviations from the pattern. Most fruit were produced during peak flowering (Figures 3.7-3.9). Likewise, the temporal pattern of visitation did not significantly differ from that of fruiting (Table 3.10). There were also some interesting daily occurrences where extreme declines in flower or moth availability occurred on days exhibiting high temperature (T; daytime temperature > 35 °C), high precipitation (P; > 2.5 cm per day) or high evening wind speed (W; wind speed between 8 PM - 12 AM > 50 km/hr) as noted on Figures 3.7-3.9.

Discussion

In this study, I examined the reproductive output of yuccas and yucca moths at the northern edge of their ranges in relation to annual and seasonal variation in moth emergence / visitation and levels of flowering. I will now compare and contrast the observed patterns in northern populations to other populations of yuccas and provide explanations for the development of different patterns of reproduction among populations.

Variation in Flowering Intensity

The intensity of flowering and moth emergence both varied independently among years and sites, potentially changing the nature of the mutualism over space and time. The mechanisms driving mutualist levels in a given year and/ or site remains unclear, although it is likely that levels in a given year are a result of larval recruitment and intensity of flowering in previous years. Observations suggest that the level of flowering is determined by the number of unflowered rosettes remaining from previous flowering seasons; for example, in Onefour (1999) approximately 39% of rosettes flowered, but in 2000 approximately 3% of rosettes flowered (Table 3.1). A longer term dataset however is necessary to examine this statistically. The more unflowered rosettes available, the higher the probability of flowering (D. Hurlburt, personal observation), if high flowering occurred in one year it is unlikely that it will also occur in the following year. Likewise Smith and Ludwig (1978) found that *Y. elata* in New Mexico exhibited cyclic patterns of flowering and suggested that plants may require several years between reproductive episodes to store sufficient energy for inflorescence and fruit production. Similarly, most moths emerge within 2 years of the onset of diapause (Fuller 1990, D. Hurlburt, unpublished data), so current moth levels are likely a result of fruiting events in recent years. Studies of other yuccas have indicated that high fall, winter and spring moisture levels are positively correlated with high rosette-level flowering (Campbell and Keeler 1932, Smith and Ludwig 1976, Fuller 1990); yet others have found no relationship between weather conditions and flowering in yuccas (Addicott 1998).

Flowering intensity varied among years and sites; however there were many low flowering years relative to high over the duration of the study. High variation in flowering/fruiting is common in yuccas (Riley 1892, McKelvey 1938, Aker 1982, Kingsolver 1984, Powell 1984, Fuller 1990, Addicott 1998). Fuller (1990) followed rosette-level flowering in a population of *Y. glauca* in New Mexico over a five year period. The population exhibited a cyclic pattern alternating between low flowering at approximately 2% and high flowering at 20-22%. Kingsolver (1986) reported that rosette-level flowering varied within years from 13 to 37% across eight populations in the centre of *Y. glauca*'s range. Addicott (1998) indicated that flowering levels varied from 5 to 28% for a single population of *Y. kanabensis* over seven years. Years of low flowering were quite common in this population with less than 7% flowering occurring in 3 of 7 years. Northern populations of *Y. glauca* certainly exhibited a similar range of flowering in comparison to other yuccas; but at this time there are insufficient data to determine whether northern populations experience more years of complete reproductive failure than southern populations, given the short-term nature of most studies. Overall fruit production was also low in most years at our northern sites with every third year or so being highly productive (Table 3.1), corresponding with seasons of high flowering. In

Onefour, for example, flowering levels have ranged from 1% to 50% of clones over a 6 year period (1998-2003) with less than 100 fruit produced from 8499 clones in 4 of those years (1998, 2000, 2001, 2003) and 10s of thousands of fruit produced in the other two years (1999, 2002).

In low flowering years, low levels of reproductive potential are reinforced because of high florivory or fructivory from pronghorn antelope (*Antilocapra americana*) or mule deer (*Odocoileus hemionus*). For example, in Onefour in 2000, herbivory reduced available flowers by over 40% (Hurlburt, unpublished data). A second Alberta population (Pinhorn Grazing Reserve) with just 404 clones (Csotonyi and Hurlburt 2000), has experienced high levels of herbivory (> 95% of inflorescences) for the last 7 years (1997 to 2003) and has failed to produced any fruit during this period, although clone-level flowering has been roughly 30% on average. Patterns suggest that herbivores may experience satiation during years of high flowering or in large populations and have a greatly reduced effect on plant fecundity during those periods or locations (Fuller 1990). It is possible that the frequency of low flowering seasons and/or episodes of low reproduction is higher in marginal populations than in other locations which would make these populations more susceptible to herbivory. Alternately, populations may have evolved this alternating high – low flowering pattern in response to herbivory to ensure that plants successfully reproduce some of the time (Fuller 1990), but longer term studies are needed to assess the role of herbivory in all populations regardless of location. Numerous studies have reported occurrences of high herbivory in yuccas (Campbell and Keeler 1932, Benson and Darrow 1944, Smith and Ludwig 1976, Fuller 1990, Moravec 1994, Kerley and Whitford 1993, Shapiro 2002), but there is little quantification of the effect of herbivory on yucca reproduction within yucca populations over time in the literature.

The distribution of flowering within years was similar to other reported populations in that there was a point of peak flowering from which most fruit originated (Aker 1982); however the duration of flowering was greatly extended at northern sites leading to a skewed distribution like that reported by Rau (1945). The study populations had extremely long flowering periods relative to other reported species, even those such as *Y. whipplei* that have 100 fold more flowers per inflorescence (Table 3.9). It was not uncommon to have several inflorescences with unopened flowers in the first week in September, when most fruit had begun to dehisce. Augspurger (1981) suggests that longer flowering periods within populations are selected for in environments where access to pollinators is limited and unpredictable. This is a way in which populations can spread the risk and ensure that at least some individuals encounter pollinators and set seed. Others have suggested that extended flowering has evolved as a way to mitigate loss of flowers from herbivory or to avoid non-pollinating seed predators (Beattie et al. 1973, Cariveau et al. 2004) such as *Tegeticula corruptrix* which occurs in all of my study sites. This is unlikely in northern populations since the impacts of herbivory are greatest during periods of low flowering such as that which occurs at the tail end of the flowering season.

T. corruptrix oviposits in early stage fruit, consumes developing seeds and does not pollinate (Addicott et al. 1990; James 1998, Pellmyr 1999). By evolving more synchronous flowering and shortening the duration of the flowering season, yuccas may also inadvertently select for earlier emergence times in *T. corruptrix* and increase their presence during peak flowering. At present, *T. corruptrix* emerges about 10 days after peak flowering and only a small proportion of non-pollinating moths oviposit in developing fruit that result from peak flowering; flowers opening after peak flowering get hit more heavily (D. Hurlburt, unpublished data). I speculate that a more condensed flowering period will increase the prevalence of non-pollinating yucca moths during peak flowering and that overall more seeds could be consumed, reducing recruitment in yuccas.

Variation in Moth Density

Throughout the course of this study, female moth numbers per flower varied over 400-fold and ranged from 0.009 to 3.800 for all sites combined (Figures 3.10 – 3.12). This range is greater than that provided by Addicott (1998) for *T. altiplanella* on *Y. kanabensis*, where female moth numbers per flower ranged from 0.04 to 0.31. Other studies described moth densities for males and females combined which makes for a difficult comparison given that relative densities of males to females may change throughout a given flowering season (Pellmyr 1995). Kingsolver (1994) reported *T. yuccasella* numbers ranging from 0.1 to 8.0 per inflorescence per day on *Y. glauca*. Rau (1945) and Aker (1982) found 0.1 to 6 moths per flower per day and 5.4 to 32.5 moths per flower in *Y. elata* and *Y. whipplei* respectively. Although moth densities were variable in other yucca populations, they were much less variable than those reported in this study.

Daily fluctuations in moth abundance and fresh flowers can influence the interactions between yuccas and yucca moths (Aker 1982, Addicott 1998), although in northern sites visitation (a surrogate of moth density) closely tracked flowering resulting in a relatively consistent density of moths throughout the central part of the season where most fruit are produced. Other factors however must be influencing the effectiveness of pollen transfer during peak flowering since not all flowers received equal visits across days. Extreme weather events, such as high temperatures, high/low levels of rainfall or high winds, are not uncommon in southern Alberta or northern Montana and appeared to have had differential effects on flowering and moth activity causing deviations from the pattern described above. The proportion of new flowers opening daily drops considerably on cold or rainy days and at least temporarily, increases moth numbers per flower (Figures 3.7 – 3.12). Although more moths are available per flower when flower abundance declines, oviposition and pollination activity slows considerably as well. Eventually with prolonged cold or rain, moth numbers decline as well. High winds are usually not associated with cold or rain and typically do not influence the proportion of flowers opening. Moths, however, tend not to fly during these periods and stay inside of flowers (Cruden et al. 1976, Aker 1982) which appears to reduce overall visitation rates on those days. The relative frequency of cold temperatures, rain or high winds among years or sites may limit interactions between yuccas and moths, decreasing overall reproductive success. It is unknown if these harsh weather events occur more frequently at the northern edge of range relative to other locations.

Synchrony between yuccas and yucca moths

In addition to the relative abundance of flowers to moths, the success of the mutualism is also dependent upon the degree of overlap between flowering and moth emergence. In most populations, the emergence of moths and the initiation of flowering is relatively asynchronous (Powell and Mackie 1966, Kingsolver 1984, Powell 1984, but see Rau 1945) with moths emerging earlier than the commencement of flowering and with flowering lasting longer than moth emergence (Kingsolver 1984). In this study, flowering was slightly extended relative to moths, but only slightly so (Figures 3.7 – 3.12). It is unclear if moths were present before flowering started since it is not possible to count moths outside of flowers. However, in most years and sites, the first moth census revealed higher numbers of moths per flower than in subsequent samplings which likely indicates that moths had emerged before flowering had started. Additionally, some moths must be present at the tails of flowering since fruiting was not significantly less than predicted by daily flowering levels (Table 3.10).

Visitation, Fruiting and Retention

Relative abundance of flowers and moths varied annually across sites, leading to fluctuations in moth densities per flower over time and space and a potential shift in reproductive success of the plant and moth. Holland and DeAngelis (2001) demonstrated through a functional response model that higher reproductive success in yuccas could be a function of higher pollinator abundance relative to flower abundance, in the absence of selective abscission. In the current study, moth density but not level of flowering was a significant positive predictor of visitation (Table 3.4). Further, there was a significant positive relationship between visitation and number of ovipositions per flower at the inflorescence-level (Figure 3.2). This is not a surprising result as it is expected that visitation will be higher and each flower will receive more ovipositions during periods when there are more moths per flower. However, it was expected that flowering levels would also be directly associated with visitation if moth numbers were fixed over time. If this were the case, one would expect low moth densities during periods of high flowering and vice versa. This indicates that moth abundance is not fixed in time and space, and that both moths and flowering levels vary independently.

Mutualism is typically not limited by visitation (Udovic 1981, Udovic and Aker 1981, Aker 1982, Addicott 1985, James et al. 1994, Addicott 1998). Visitation rates were high in northern populations (68.8 to 95.0 %) relative to those reported for *Y. kanabensis*, even though there were fewer ovipositions per flower in northern populations relative to southern populations (Table 3.7). Addicott (1998) found that visitation ranged from 55 to 80%. Visitation rates can be much lower during adverse weather conditions, for example, Pautenaude (2001) reported visitation rates of 6.4 to 9.1% in *Y. baileyi* and 9 to 14 % in *Y. kanabensis* as a result of a late frost.

High visitation, however, does not imply that flowers have acquired a sufficient amount of pollen, or pollen of sufficient quantity (Humphries 1998), which may be an issue in northern populations as they received fewer ovipositions per flower relative to other yuccas (Table 3.7). Moths do not always pollinate following oviposition (Tyre and Addicott 1993, Addicott and Tyre 1995) and may transfer varying amounts of pollen per pollination event (Pellmyr et al. 1997, Humphries and Addicott 2000). Self-fertilization is undesirable in most yuccas (Aker and Udovic 1981, Fuller 1990, James et al. 1993, Huth and Pellmyr 1997, Richter and Weis 1998, Huth and Pellmyr 2000, Marr et al. 2000) and low levels of flowering combined with decreased movement due to high wind in northern populations may exacerbate this problem. Inter-clone distances are greater in low flowering seasons (D. Hurlburt, unpublished data) and moths may be forced to pollinate flowers using pollen collected from the same flower (autogamy) or from another flower on the same plant (geitonogamy). Populations that experience increased frequencies of low flowering are expected to be more prone to self-fertilization than other populations. Although northern yuccas are tolerant of self pollination and appear to be adapted to some degree to self-fertilization (Chapter 4), they will preferentially retain outcrossed flowers if given a choice.

Higher levels of visitation should lead to higher levels of pollination and subsequent fruit set (Holland and DeAngelis 2001). In the current study, fruiting was positively related to level of flowering, but not to visitation rates (Table 3.4). It is possible that visitation is not a good indicator of fruit set, especially when the rates are consistently high with most flowers receiving ovipositions and presumably pollen. This was supported by Addicott (1998) who found that fruiting was independent of visitation in *Y. kanabensis*. The positive relationship between fruiting and flowering indicated that fruit production is higher during periods of high flowering. Addicott (1998) reported that visitation was inversely related to retention; however, in the present study, none of the measured variables was a significant predictor of retention. Although visitation is likely critical to fruit retention, its value in determining retention is not apparent statistically because visitation is consistently high across all sites and years.

In most yuccas, the proportion of flowers producing mature fruit (i.e. fruiting) is less than 10%. The following rates of fruit production have been reported: 1 – 11% in *Y. glauca* (Cruden et al. 1976, Kingsolver 1984, Dodd 1989, Fuller 1990), 0 - 9% in *Y. whipplei* (Udovic 1981, Udovic and Aker 1981), 4 – 9 % in *Y. kanabensis* (Addicott

1998, Pautenaude 2000), 4 – 6 % in *Y. baileyi* (Pautenaude 2000), and 4 – 7 % in *Y. elata* (Schaffer and Schaffer 1979). Retention, the proportion of visited flowers that matured as fruit, ranged from 7 – 13 % in *Y. kanabensis* (Addicott 1998). In northern populations of *Y. glauca*, fruiting (0.3 to 12.2 %) was similar to other reported rates, but retention rates (7.6 to 21.1 %) were much higher and more variable than those previously recorded for any species. I hypothesize that these higher retention rates have occurred because northern populations reproduce less frequently than more southern yuccas and have more energy available for fruit production during reproductive bouts. However, these populations have fewer flowers than other yuccas and also experience a further reduction in flowers due to herbivory. Plants with fewer flowers may have limited choices when it comes to retaining better quality flowers, so even though retention may be higher, the fruit may be of lower quality than elsewhere.

Presence of "Reverse" Selective Abscission

Yucca glauca at the northern edge of range selectively abscised flowers, but unlike other studies in which yuccas retained flowers with lower egg loads (e.g. Tyre and Addicott 1993, Pellmyr and Huth 1994, Pellmyr et al. 1997, Addicott and Bao 1999, Humphries and Addicott 2000), Alberta and Montana populations retained flowers with higher numbers of ovipositions (Figure 3.3). In other studies, retention peaked at 8-10 ovipositions per fruit and then dropped off markedly (Pellmyr and Huth 1994; Addicott 1998); in northern populations, retention did not decrease at all, and if anything these populations exhibited even higher rates of retention when flowers had in excess of 20 ovipositions (Figure 3.4).

"Reverse" selective abscission may be a response to low moth density, as indicated by fewer ovipositions received per flower (Table 3.7) and a reduced frequency of constrictions in fruit (D. Hurlburt, unpublished data), and be a strategy to increase moth density in future seasons. It is thought that the constriction is generated by a concentration of ovipositions, in a central area of the pistil, that damage ovules and prevent seed development (Riley 1892, Addicott 1986, Marr and Pellmyr 2003, Shapiro and Addicott 2003). Similar observations of "reverse" selective abscission have only been reported in three cases where oviposition resulted in no damage to developing ovules (Wilson and Addicott 1998, Addicott and Bao 1999, Csotonyi and Addicott 2001) than with "deep" ovipositing yucca moths like *T. yuccasella* or *T. altiplanella*. The first two cases are with "shallow" ovipositing moths (*T. superficiella* and *T. cassandra*) which lay their eggs in the outer surface of carpel wall rather than in the locule (Addicott 1996, Segraves 2003) and as a result does not lead to the destruction of developing ovules (Csotonyi and Addicott 2001). The third case occurs in *Yucca baccata* where pollinating moths lay eggs in the apical portion of the ovary where seeds remain inviable despite any level of pollination (Bao and Addicott 1998, Addicott and Bao 1999).

Fewer ovipositions per retained fruit in northern populations (Figure 3.3; Table 3.7) did not translate into fewer larvae per fruit relative to other studies (Table 3.8), thus northern yuccas may regulate low density moth populations by increasing egg survival. However what is the mechanism behind lower egg mortality in northern populations relative to other populations? Observations indicate that *T. yuccasella*, at the northern edge of their range, lay their eggs in different locations of the pistil relative to other populations (D. Hurlburt, unpublished data). Most yucca moths lay eggs in the centre of the pistil creating a zone of damaged ovules (Riley 1892). This stereotypic behaviour reduces the overall number of ovules damaged, since many of the same seeds are repeatedly damaged through subsequent ovipositions (Shapiro and Addicott 2003). Although ovule damage increased with the number of ovipositions, the level of damage per additional oviposition decreased as ovipositions increased in *Y. kanabensis*. Further, it is speculated that only the last eggs inserted in the damage zone survive (J. F. Addicott, personal communication), presumably because eggs are damaged by subsequent ovipositions, or eggs can not survive in locations with damaged ovules.

Northern populations likely suffer from having too few moths and the strategy exhibited in *Y. kanabensis* probably would not work in northern populations. The strategy of laying all eggs in a single location of the pistil could further reduce moth survival and recruitment in northern populations of *Y. glauca*. Edge of range moths often lay eggs throughout the ovary which I believe leads to increased larval survival in fruit, because oviposition does not repeatedly occur in the same location and does not destroy eggs. It is plausible that this activity may lead to increased damage because additional ovules are destroyed as the moths oviposit in different locations. However this may not be the case, preliminary experiments indicate that moths cause less damage per oviposition than in *Y. kanabensis* (Figure 3.6; Shapiro and Addicott 2003). These data however are from a single site and year and this idea warrants further investigation.

Conflict of Interest at Range Peripheries

The density of moths to flowers varies in time and space and the relative levels at any point in time determines the level of conflict between species that is experienced, and the nature of the solution needed to mitigate that problem. When moth numbers are high relative to flower availability, conflicts are particularly high as moths can overexploit flowers and consume higher proportions of seeds. At the northern edge of range, moth emergence and flowering levels fluctuate greatly between sites and years, resulting in fluctuating moth densities that are consistently lower than moth densities reported in other studies. When relative densities of moths to flowers are low, conflicts between mutualists should be low and the benefits of association higher (Bronstein 1994) and yuccas may employ mechanisms to increase moth numbers, as plant reproductive success is dependent upon pollinator density within flowering seasons. In this study, yuccas encourage higher abundances of moths through reverse selective abscission and will even retain flowers with higher numbers of ovipositions or larvae than other populations do. Additionally, there is preliminary evidence that moths damage fewer seeds through oviposition activities which could result in more viable seeds per fruit for the plant, but further study is required to assess whether moths could enhance yucca populations in this way.

Conclusion

Although high variation in mutualist density and low abundances of moths characterizes northern populations, yuccas and yucca moths and the beneficial interaction between them can persist in at the northern periphery of the species' ranges. In general, flowers experience a high level of visitation, with most flowers receiving at least one oviposition. However, each flower receives fewer visits than in other yucca populations. Despite fewer ovipositions per flower, northern populations of yuccas produce similar levels of fruit per inflorescence and larvae per fruit. Through higher fruit retention rates, reverse selective abscission, and possibly higher larval survival via a unique oviposition strategy, yuccas can regulate moth densities to ensure plant reproductive success and maintain the stability of the mutualism.

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Table 3.1 - Flowering and fruiting in northern edge of range populations of *Yucca glauca* (1999-2003). Values are proportions of clones and rosettes that flowered and proportions of flowering clones and rosettes that produced fruit; sample sizes are in brackets.

Site	Year	Proportion flowering		Proportion fruiting	
		Clone	Rosette	Clone	Rosette
Onefour					
	1999	0.514 (344)	0.394 (766)	0.918 (184)	0.844 (301)
	2000	0.083 (337)	0.031 (998)	0.078 (90)	0.092 (87)
	2001	0.344 (413)	0.110 (1331)	0.560 (116)	0.594 (202)
	2002	0.267 (438)	0.147 (1108)	0.771 (109)	0.690 (155)
Loma					
	1999	0.700 (40)	0.449 (98)	0.575 (40)	0.625 (40)
	2000	0.130 (100)	0.087 (229)	0.094 (32)	0.075 (40)
	2001	0.360 (89)	0.151 (332)	0.933 (30)	0.957 (47)
	2002	0.327 (101)	0.214 (495)	0.878 (41)	0.967 (60)
Fort Benton					
	1999	0.185 (200)	0.154 (374)	0.659 (41)	0.408 (49)
	2000	0.169 (65)	0.071 (168)	0.950 (20)	0.880 (25)
	2001	0.347 (124)	0.150 (461)	0.837 (43)	0.839 (62)
	2002	0.293 (102)	0.088 (510)	0.750 (16)	0.850 (20)

Table 3.2 - Multiple forward regression analysis of weather conditions on flowering levels. Neither winter or spring precipitation, nor mean winter of spring temperatures were significant predictors of flowering levels. Proportion of clones flowering: Adjusted $R^2 = 0.323$, ANOVA $F_{4,4} = 1.956$, $P = 0.266$. Proportion of rosettes flowering: Adjusted $R^2 = 0.012$, ANOVA $F_{4,4} = 1.024$, $P = 0.491$. Mean number of flowers per inflorescence: Adjusted $R^2 = 0.000$, ANOVA $F_{4,4} = 0.849$, $P = 0.561$.

Model	Factor	Beta	t	*P*
Proportion Clones Flowering	Constant	0.050	0.207	0.846
	Total Winter precipitation	0.057	2.333	0.080
	Total Spring precipitation	0.004	0.356	0.740
	Mean Winter temperature	-0.065	-2.648	0.057
	Mean Spring temperature	-0.019	-1.097	0.334
Proportion Rosettes Flowering	Constant	-0.175	-0.670	0.540
	Total Winter precipitation	0.052	1.960	0.122
	Total Spring precipitation	0.004	0.329	0.759
	Mean Winter temperature	-0.035	-1.321	0.257
	Mean Spring temperature	-0.002	-0.105	0.951
Flowers per Inflorescence	Constant	15.923	1.516	0.204
	Total Winter precipitation	-0.619	-0.581	0.593
	Total Spring precipitation	0.702	1.348	0.249
	Mean Winter temperature	0.593	0.562	0.604
	Mean Spring temperature	1.358	1.811	0.144

Table 3.3 – Multiple forward regression analysis of the contribution of flowering level and weather conditions on female moths density. Adjusted $R^2 = 0.657$, ANOVA $F_{2,6} = 8.656$, $P = 0.017$.

Model Parameters	Coefficient	t	*P*
<u>Model</u>:			
Constant	0.275	1.253	0.257
Proportion of Clones Flowering	-1.049	-2.136	0.077
Mean Spring Temperature	0.059	3.269	0.017
<u>Excluded variables</u>:			
Proportion Rosettes Flowering	0.228		0.622
Total Winter precipitation	0.551		0.200
Total Spring precipitation	0.110		0.815
Mean Winter temperature	0.470		0.281

Table 3.4 - Summary of flower fate per inflorescence in northern edge of range populations from 1999 to 2002. Visitation is the proportion of flowers visited by yucca moths and exhibit at least one oviposition mark. Fruiting is the proportion of all flowers that were set into fruit. Retention is the proportion of visited flowers that were retained as fruit.

Site	Year	Inflorescences (n)	Flowers (n)	Visitation	Fruiting	Retention
Onefour	1999	300	9184	0.756	0.107	0.097
	2000	87	2854	0.828	0.008	0.076
	2001	204	5277	0.780	0.056	0.100
	2002	156	4943	0.751	0.072	0.150
Loma	1999	43	1376	----	0.056	----
	2000	40	1274	0.950	0.003	0.123
	2001	37	699	0.850	0.122	0.145
	2002	19	641	0.688	0.120	0.207
Fort Benton	2000	31	837	----	0.117	----
	2001	28	723	0.836	0.094	0.211
	2002	32	1072	0.809	0.092	0.144

Table 3.5 - Multiple forward regression analysis of flowering, moth density and weather conditions on visitation, fruiting and retention. A) Moth density was positively correlated with the visitation rate of flowers. Adjusted $R^2 = 0.469$, ANOVA $F_{1,7} = 6.192$, $P = 0.042$. B) Flowering level was a positive predictor of fruiting. Adjusted $R^2 = 0.678$, ANOVA $F_{1,7} = 14.710$, $P = 0.006$. C) None of the variable measured were predictors of retention.

Model	Factor	Coefficient	F	*P*
A) Visitation	Model variables:			
	Constant	0.736		
	Moth density per flower	0.685	6.192	0.042
	Excluded variables:			
	Proportion of rosettes flowering	-0.210	0.521	0.497
	Proportion of clones flowering	-0.195	0.326	0.589
	Total winter precipitation	0.381	2.132	0.195
	Total spring precipitation	-0.121	0.120	0.741
	Mean winter temperature	0.401	2.592	0.159
	Mean spring temperature	-0.479	1.461	0.272
B) Fruiting	Model variables:			
	Constant	-0.010		
	Proportion of clones flowering	0.823	14.710	0.006
	Excluded variables:			
	Proportion of rosettes flowering	-0.231	0.068	0.557
	Visitation	-0.221	0.043	0.416
	Mean ovipositions per flower	-0.126	0.679	0.598
	Total winter precipitation	-0.091	0.116	0.711
	Total spring precipitation	0.362	3.906	0.096
	Mean winter temperature	0.108	0.020	0.672
	Mean spring temperature	-0.148	0.764	0.535
C) Retention	Excluded variables:			
	Proportion of clones flowering	0.327	0.836	0.391
	Proportion of rosettes flowering	0.112	0.090	0.773
	Visitation	-0.423	1.523	0.257
	Mean ovipositions per flower	-0.126	0.113	0.746
	Total winter precipitation	-0.090	0.058	0.817
	Total spring precipitation	0.466	1.947	0.206
	Mean winter temperature	0.042	0.013	0.914
	Mean spring temperature	-0.281	0.599	0.464

Table 3.6 - Mortality of yucca moth eggs caused by within inflorescence selective abscission of flowers at Onefour, AB, Loma, MT and Fort Benton, MT in 2001 and 2002. k-values are means ± standard error.

Site	Year	k-value
Onefour, AB		
	2001	-1.115±0.0742
	2002	-1.160±0.0935
Loma, MT		
	2001	-0.487±0.0821
	2002	-1.063±0.0620
Fort Benton, MT		
	2001	-0.869±0.0917
	2002	-1.299±0.0879

Table 3.7 - Numbers of ovipositions per fruit from this study, as reported in literature, and from surveys conducted in southern Montana. Measures of ovipositions per fruit are means, means + standard error or ranges; ranges are provided in brackets. Species are coded as follows: *Yg* = *Y. glauca*, *Yk* = *Y. kanabensis*, *Ty* = *T. yuccasella*, *Ta* = *T. altiplanella*

Location	Site	Year	Species	Ovipositions All flowers		Ovipositions Retained flowers		Source
Northern	Onefour, AB	1999	*Yg/Ty*	12.647±0.169	(1 – 65)	9.692±1.285	(1 – 34)	this study
		2000	*Yg/Ty*	11.033±0.423	(1 – 72)	18.700±2.091	(2 – 72)	this study
		2001	*Yg/Ty*	8.168±0.319	(1 – 44)	14.389±1.475	(1 – 44)	this study
		2002	*Yg/Ty*	12.399±0.305	(1 – 58)	17.122±0.892	(3 – 41)	this study
		2003	*Yg/Ty*	5.852±0.509	(1 – 17)	6.617±0.309	(1 – 18)	this study
	Loma, MT	1999	*Yg/Ty*	–		13.053±1.442	(1 – 28)	this study
		2000	*Yg/Ty*	13.498±0.611	(1 – 106)	21.452±2.362	(6 – 106)	this study
		2001	*Yg/Ty*	10.063±0.494	(1 – 42)	11.600±0.827	(1 – 26)	this study
		2002	*Yg/Ty*	7.648±0.257	(1 – 38)	8.682±0.236	(1 – 38)	this study
		2003	*Yg/Ty*	8.669±0.429	(1 – 24)	14.298±0.804	(4 – 29)	this study
	Fort Benton, MT	2001	*Yg/Ty*	13.529±0.403	(1 – 44)	14.519±0.551	(4 – 29)	this study
		2002	*Yg/Ty*	5.761±0.195	(1 – 25)	7.870±0.654	(1 – 25)	this study
		2003	*Yg/Ty*	11.450±0.318	(1 – 30)	11.833±0.468	(4 – 26)	this study
Southern	Billings, MT	2002	*Yg/Ty*	–		21.906±1.647	(1 – 50)	Hurlburt, unpubl
		2003	*Yg/Ty*	8.565±0.323	(1 – 24)	14.684±1.564	(3 – 27)	Hurlburt, unpubl
	Judith River, MT	2001	*Yg/Ty*	–		15.895±2.277	(1 – 31)	Hurlburt, unpubl
		2002	*Yg/Ty*	–		17.245±1.109	(3 – 44)	Hurlburt, unpubl
		2003	*Yg/Ty*	7.062±0.228	(1 – 17)	5.868±0.329	(1 – 14)	Hurlburt, unpubl
	Kanab, UT	1991[a]	*Yk/Ta*	17.891±0.544	(1 – 81)			Addicott, unpubl
		1999[a]	*Yk/Ta*	43.343±1.268	(1 – 198)			Shapiro (2002)
		2000[a]	*Yk/Ta*	31.478±0.614	(1 – 85)			Shapiro (2002)
		2000[b]	*Yk/Ta*	21.887±0.391	(1 – 86)			Shapiro (2002)
		2000[c]	*Yk/Ta*	12.435±0.454	(1 – 45)			Shapiro (2002)

Populations: [a]Yellowjacket, [b] Hancock 1, [c] Hancock 2

Table 3.8 - Numbers of *T. yuccasella* larvae per fruit in *Y. glauca* as reported in literature and from this study (study populations are bolded). Measures of larvae per fruit are means ± standard error.

Site	Year	Larvae / fruit	Source
Onefour, AB	**1999**	**3.56 ± 0.47**	**this study**
	2000	**5.92 ± 0.51**	**this study**
	2001	**4.40 ± 0.58**	**this study**
	2002	**8.11 ± 0.60**	**this study**
	2003	**2.90 ± 0.69**	**this study**
Loma, MT	**1999**	**4.64 ± 0.77**	**this study**
	2000	**7.91 ± 1.85**	**this study**
	2001	**4.55 ± 0.72**	**this study**
Fort Benton, MT	**2000**	**4.85 ± 0.89**	**this study**
	2001	**7.15 ± 0.76**	**this study**
Fort Belknap, MT	2003	3.37 ± 0.51	this study
Wolf Creek, MT	1980	5.6	Addicott 1986
	1999	6.29 ± 0.94	this study
Billings, MT	2003	3.26 ± 0.53	this study
Boulder Valley, CO	1986	8.17 ± 0.84	Dodd 1989
	1987	19.73 ± 2.87	Dodd 1989
Buckingham Pk, CO	1986	7.70 ± 1.52	Dodd 1989
County Rd 87, CO	1987	1.40 ± 0.31	Dodd 1989
Gunnison, CO	1980	4.10	Addicott 1986
Jamestown, CO	1986	3.50 ± 1.32	Dodd 1989
Poncha Springs, CO	1980	3.8	Addicott 1986
Clinton, OK	1979	1.40 ± 1.90	Keeley 1984
Clines Corners, NM	1980	0.70	Addicott 1986

Table 3.9 - Duration of flowering in yucca populations. *Yucca glauca*, the focus of this study, represents a small species of yucca with less than 100 flowers per stalk, *Y. kanabensis* is a medium sized yucca with up to several hundred flowers, and *Y. whipplei* is a large-sized yucca with potentially thousands of flowers. Northern populations of *Y. glauca* have greatly extended flowering seasons which are comparable to large-sized yuccas that have 25-30 times as many flowers.

Species	Population	Year	Duration of Flowering (d)	Source
<u>Northern Sites</u>				
Y. glauca	Onefour, AB	1999	83	This study
	Onefour, AB	2000	85	This study
	Onefour, AB	2001	73	This study
	Onefour, AB	2002	64	This study
	Loma, MT	2000	62	This study
	Loma, MT	2001	58	This study
	Loma, MT	2002	18	This study
	Fort Benton, MT	2001	39	This study
	Fort Benton, MT	2002	35	This study
<u>Southern Sites</u>				
Y. glauca	Arapaho Prairie, NB	1993	41	Moravec 1994
	Boulder Co., CO	1987	35	Dodd 1989
	Lake Scott, KS	1982	18	Kingsolver 1984
	Socorro Co., NM	1984	55	Fuller 1990
Y. kanabensis	Kanab, UT	1995	26	Humphries 1997
	Kanab, UT	1990	30	Addicott 1998
	Kanab, UT	1991	22	Addicott 1998
	Kanab, UT	1992	34	Addicott 1998
Y. whipplei	Trabuco Co., CA	1993	43	Richter 1995
	Riverside Co., CA	1979	52	Aker 1981
	Claremont, CA	1957	47	Wimber 1958
	Claremont, CA	1957	59	Wimber 1958
	Claremont, CA	1957	43	Wimber 1958
	El Cajon, CA	1964	75	Powell & Mackie 1983

Table 3.10 - Runs test statistics demonstrating that the temporal pattern of a) flowering and fruiting and b) visitation and fruiting directly correspond in three northern peripheral populations over a four year period.

		a) Flowering vs. Fruiting				b) Visitation vs. Fruiting			
		Runs	*Z*	*n*	*P*	Runs	*Z*	*n*	*P*
Onefour									
	1999	11	-1.165	33	0.244	12	-0.626	27	0.532
	2000	11	-0.554	39	0.579	11	0.000	34	1.000
	2001	9	-0.528	20	0.598	11	1.109	16	0.267
	2002	3	-1.237	15	0.216	3	-0.791	10	0.429
Loma									
	2000	3	-0.788	7	0.431	2	-1.369	6	0.171
	2001	3	-0.380	7	0.704	4	0.000	6	1.000
	2002	4	0.109	5	0.913	2	-0.982	5	0.326
Ft Benton									
	2001	4	0.000	7	1.000	4	0.000	7	1.000
	2002	4	0.000	6	1.000	4	0.000	6	1.000

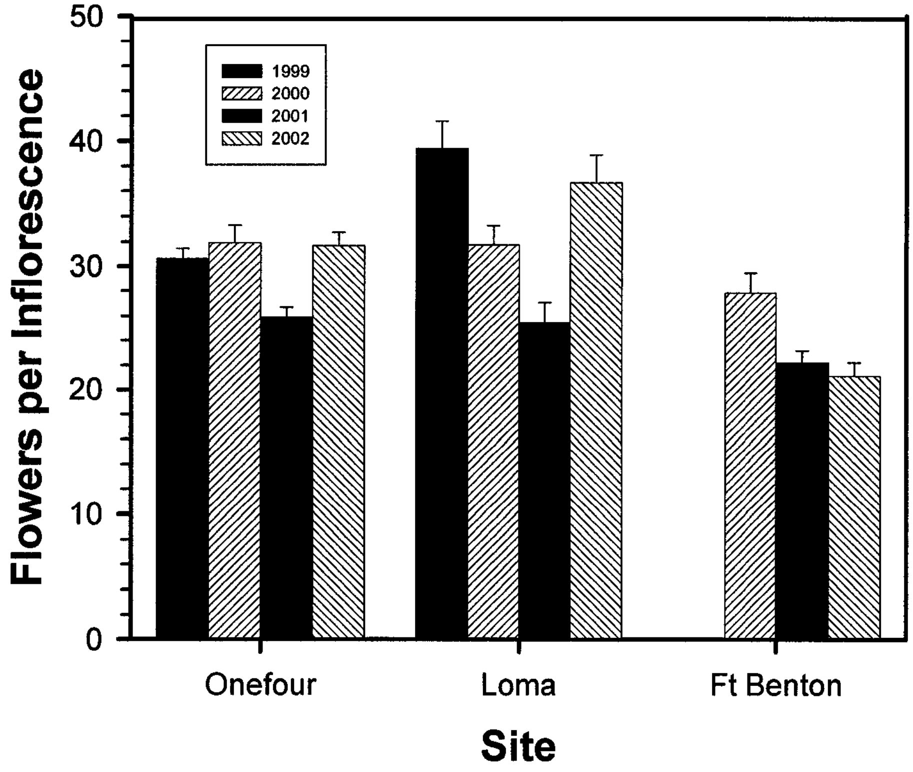

Figure 3.1 – Mean numbers of flowers per inflorescence at three sites from 1999 to 2002.

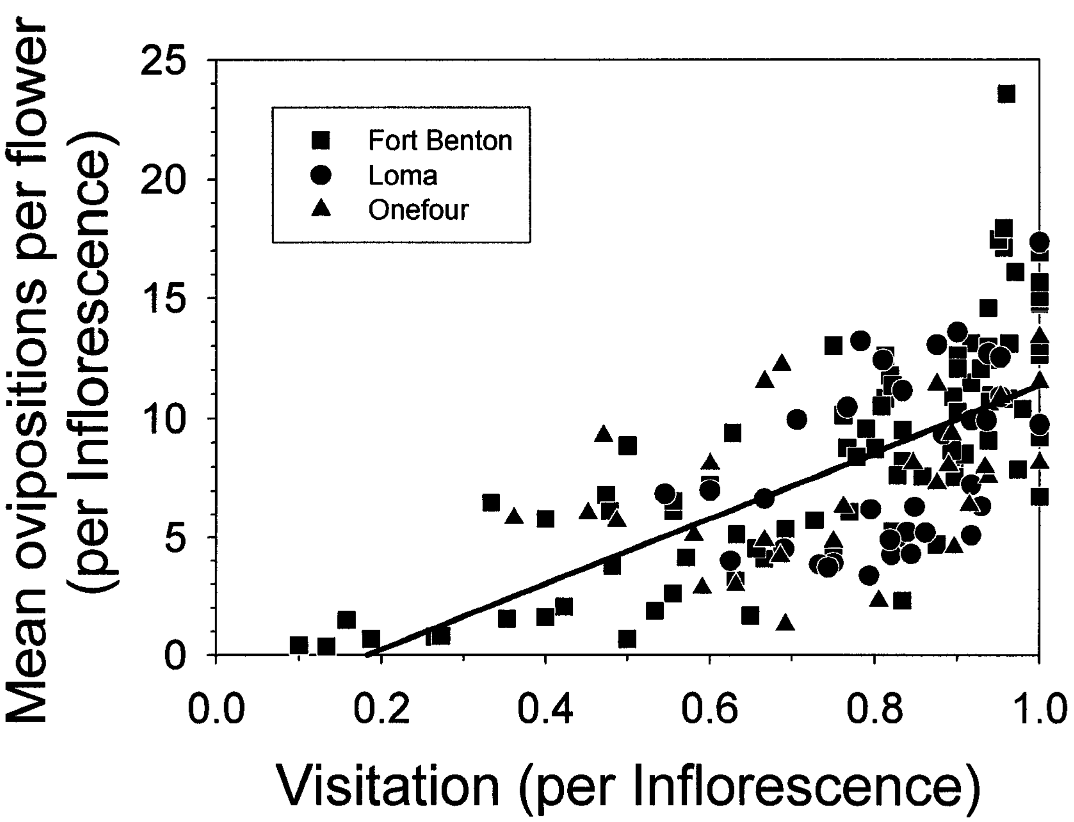

Figure 3.2 – Relationship between visitation per inflorescence and the mean number of ovipositions per flower per inflorescence. Visitation rates were higher on inflorescences with more ovipositions per flower (Ovipositions = 13.927*Visitation - 2.544, $r^2 = 0.444$, F = 120.464, P < 0.0001, d.f. = 152).

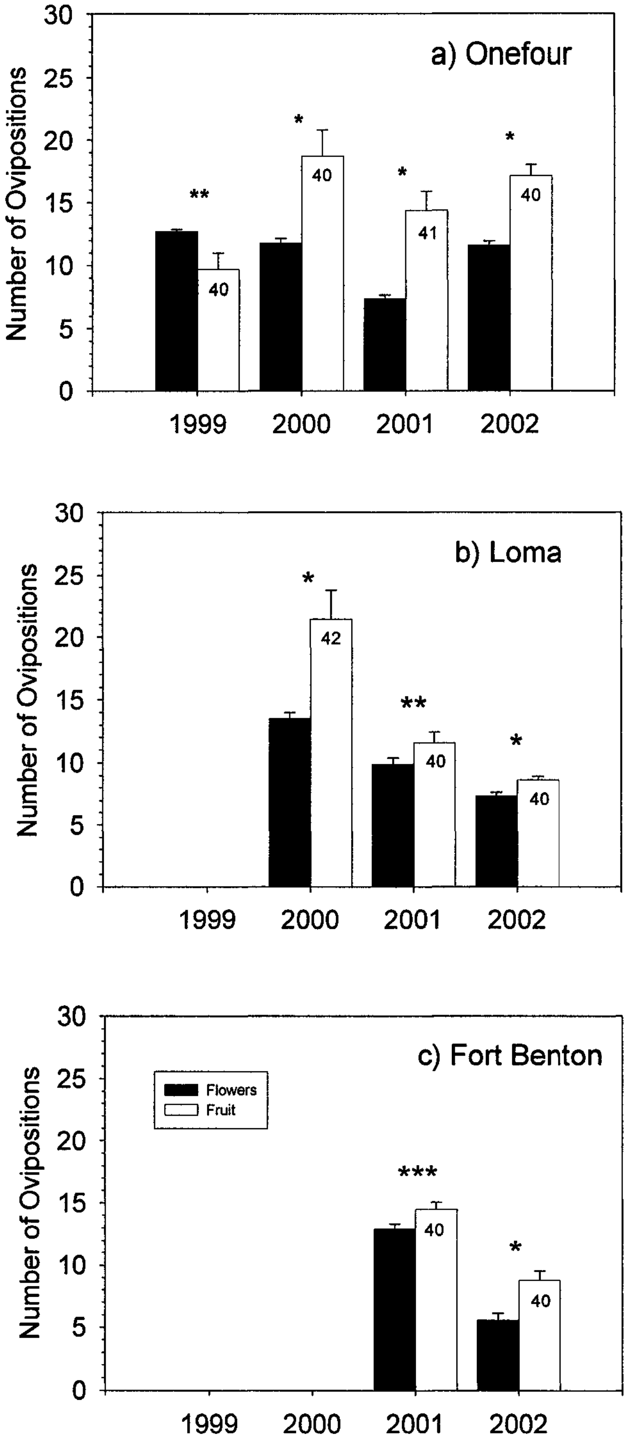

Figure 3.3 – Mean ovipositions per flower (includes abscised and retained flowers) and retained fruit from 3 sites from 1999 to 2002. Overall, flowers the received more ovipositions were retained and matured as fruit. Significant differences in mean ovipositions between abscised flowers and retained fruit within sites and years are denoted with asterisks (* $P < 0.001$; ** $P < 0.05$, *** $P < 0.100$)

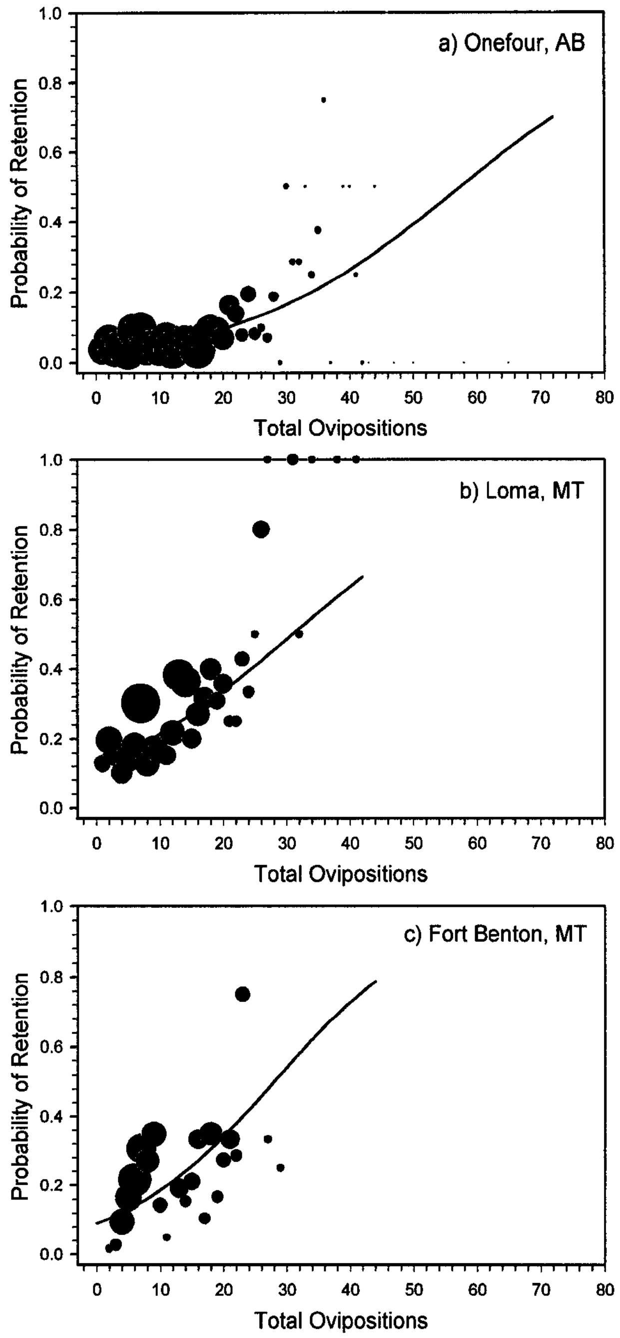

Figure 3.4 - Probability of flower retention (R) as a function of the number of ovipositions (O) a flower received. (a) For Onefour, AB the logistic regression equation obtained was $R = 1/(1 + e^{-(-0.021 + 0.005\,O)})$ (Overall Model $X^2 = 77.291$, df = 1, $P < 0.0001$), where R is the probability of retention and O is the number of ovipositions. Sample sizes are proportional to symbol size (n = 3110 flowers from 1999-2002). (b) For Loma, MT the logistic regression equation obtained was $R = 1/(1 + e^{-(-1.915 + 0.062\,O)})$ (Overall Model $X^2 = 25.212$, df = 1, $P < 0.0001$; n = 745 flowers from 2000-2002). (c) For Fort Benton, MT the logistic regression equation was $R = 1/(1 + e^{-(-2.293 + 0.082\,O)})$ (Overall Model $X^2 = 45.478$, df = 1, $P < 0.0001$; n = 359 flowers from 2001-2002).

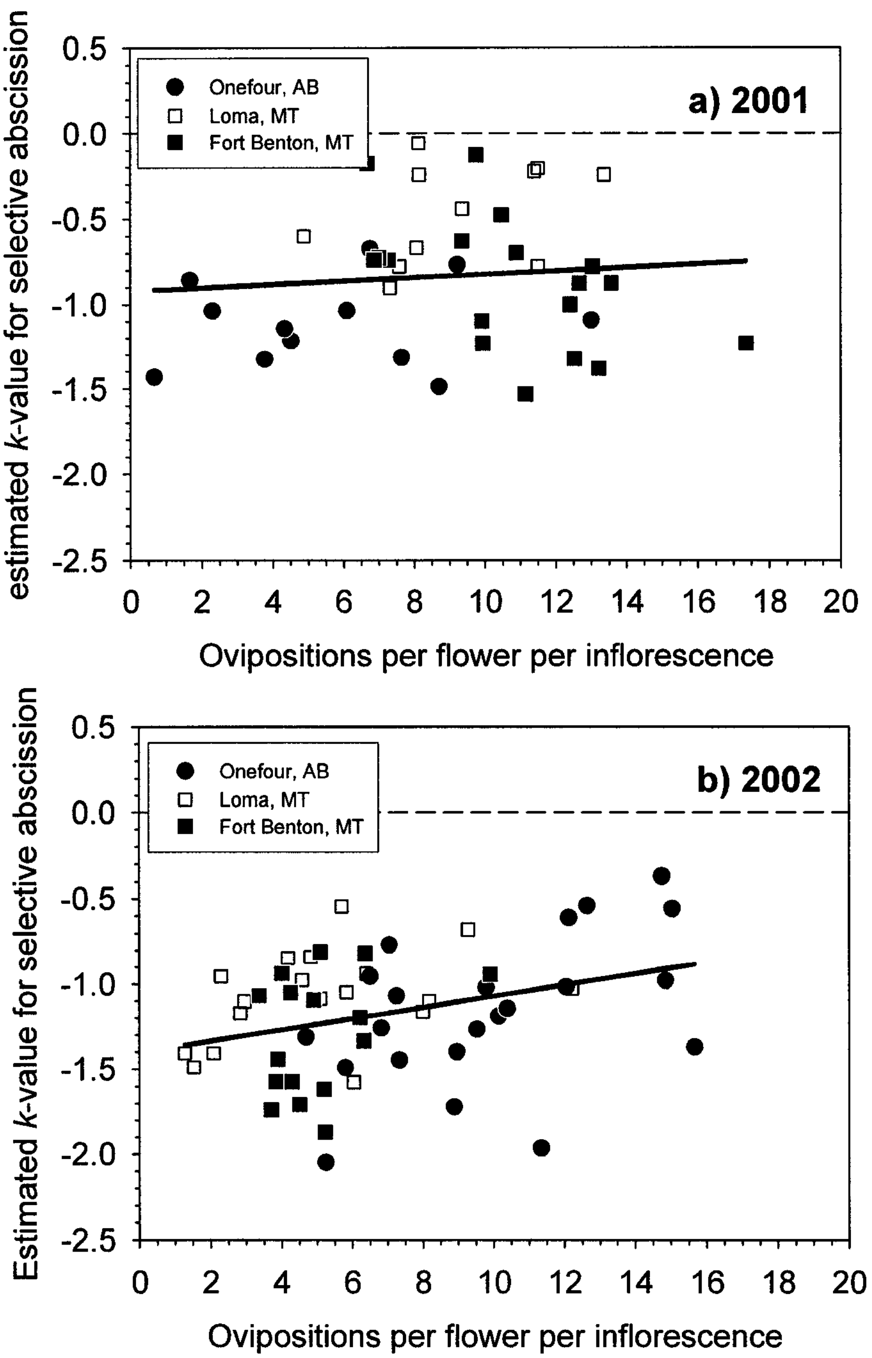

Figure 3.5 - Estimated *k* values for selective abscission per inflorescence as a function of mean numbers of ovipositions per moth per flower for *Tegeticula yuccasella* on *Yucca glauca* for Onefour, AB, Loma, MT and Fort Benton, MT in 2001 and 2002. Relationship for all sites combined in 2001 can be described as Selective k = 0.010 * Ovipositions – 0.920 (Overall model: $R^2 = 0.008$, $P = 0.568$, n = 42). Relationship for all sites combined in 2002 can be described as Selective k = 0.033 * Ovipositions – 1.399 (Overall model: $R^2 = 0.104$, $P = 0.015$, n = 57).

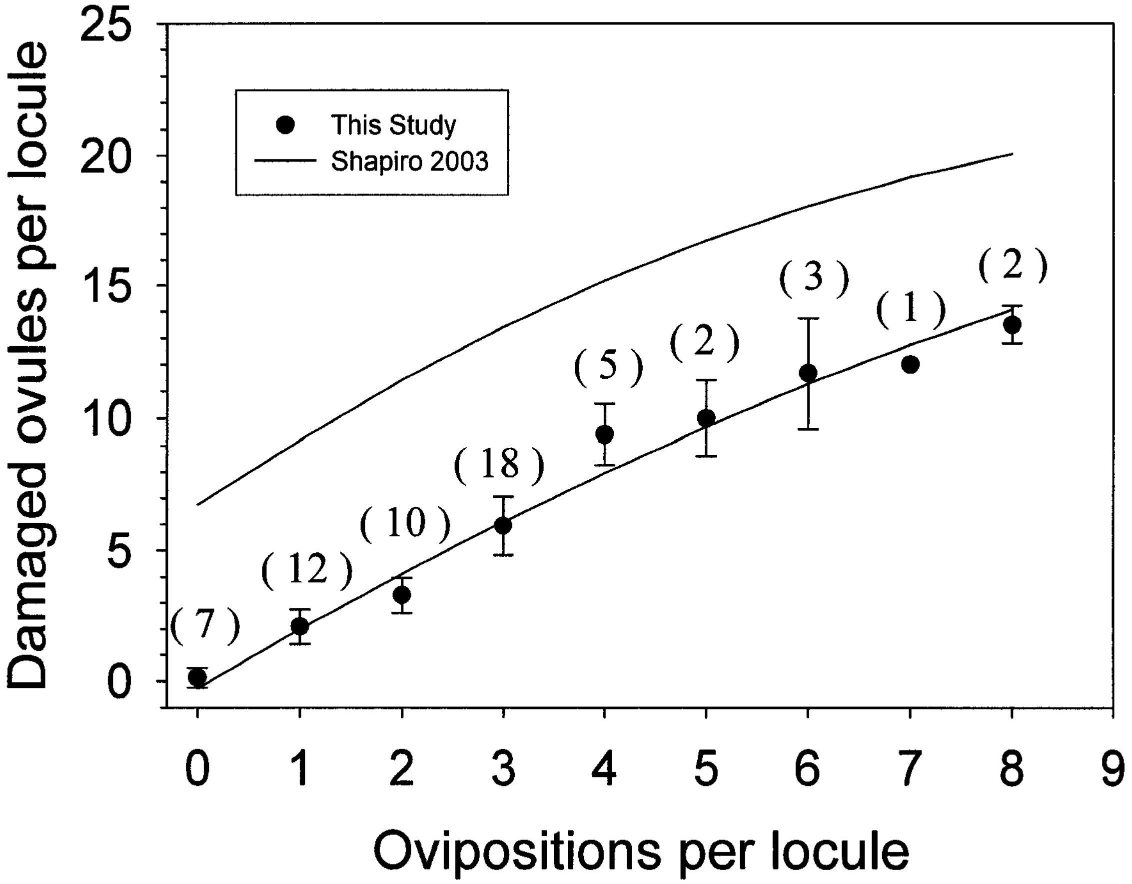

Figure 3.6 - Relationship between ovipositions by *Tegeticula yuccasella* and level of ovule damage per flower (*Yucca glauca*) for Onefour, AB in 2000 and for a similar study by Shapiro (2003) in *Yucca kanabensis*. Linear regression equation is Damage = 1.864 * Ovipositions + 0.239 (Overall model: $r^2 = 0.911$, $P = 0.0001$, d.f. = 58). Sample sizes are shown in brackets.

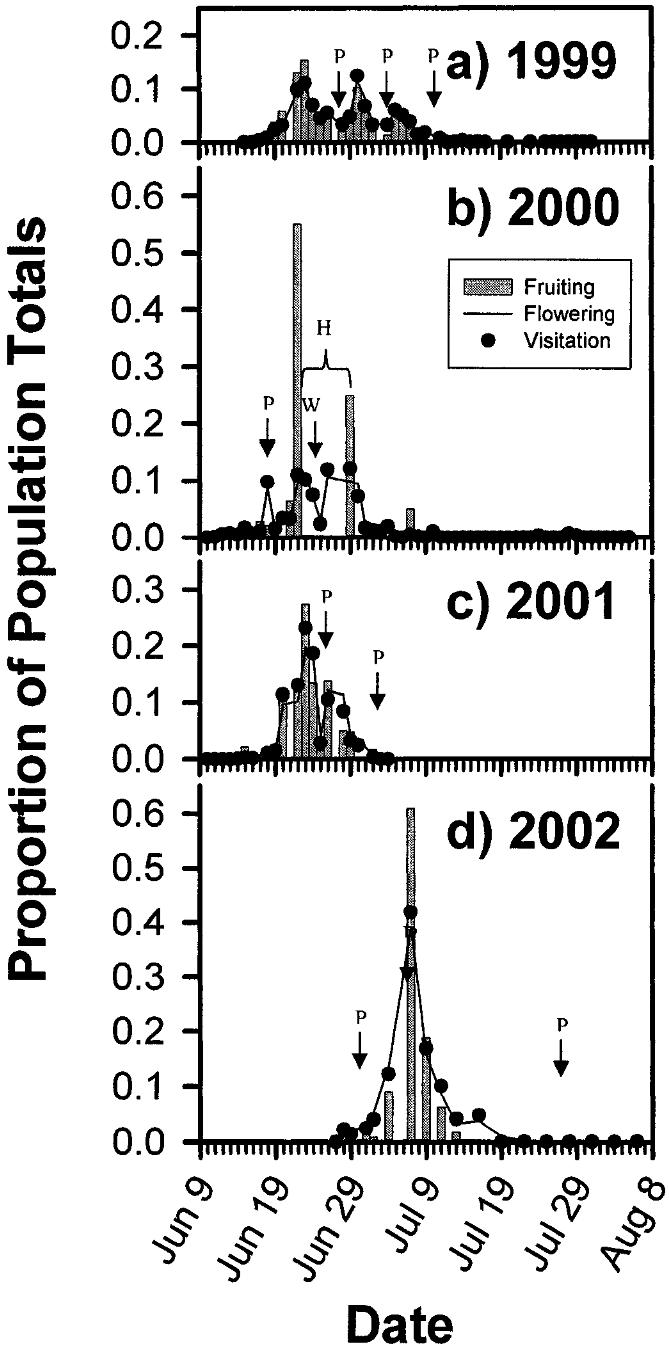

Figure 3.7 - Phenology of fruiting (bars), flowering (solid line) and visitation (circles) for Onefour, AB for a) 1999, b) 2000, c) 2001, and d) 2002. Values are the proportions of all fruit, flowers, or visited flowers that were produced on a given day. Extreme weather events are noted on the figure (P = high precipitation, T = high temperature, and W = high evening winds). H denotes a period of high herbivory levels in 2000.

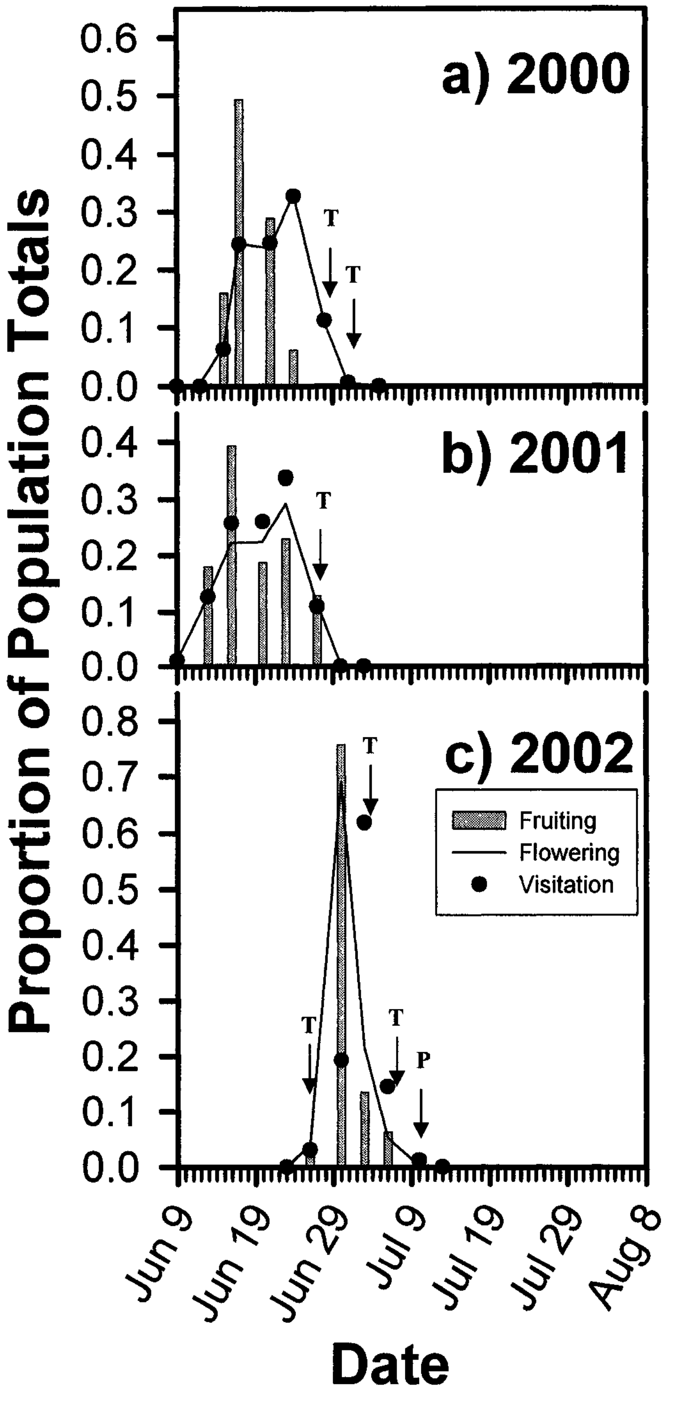

Figure 3.8 - Phenology of fruiting (bars), flowering (solid line) and visitation (circles) for Loma, MT for a) 2000, b) 2001, and c) 2002. Values are the proportions of all fruit, flowers, or visited flowers that were produced on a given day. Extreme weather events are noted on the figure (P = high precipitation, T = high temperature, and W = high evening winds).

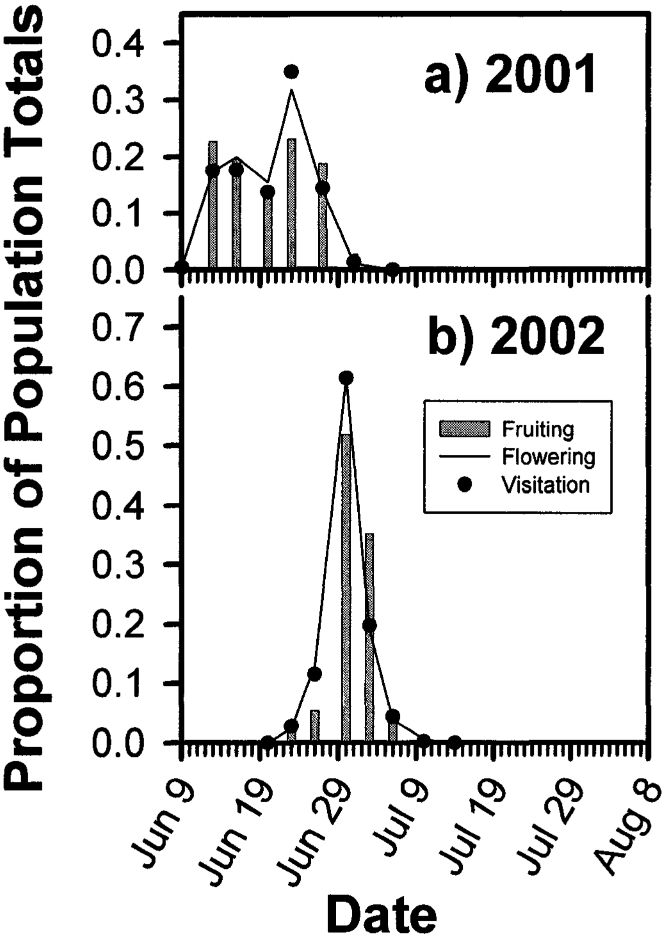

Figure 3.9 - Phenology of fruiting (bars), flowering (solid line) and visitation (circles) for Fort Benton, MT for a) 2001 and b) 2002. Values are the proportions of all fruit, flowers, or visited flowers that were produced on a given day. Extreme weather events are noted on the figure (P = high precipitation, T = high temperature, and W = high evening wind speed).

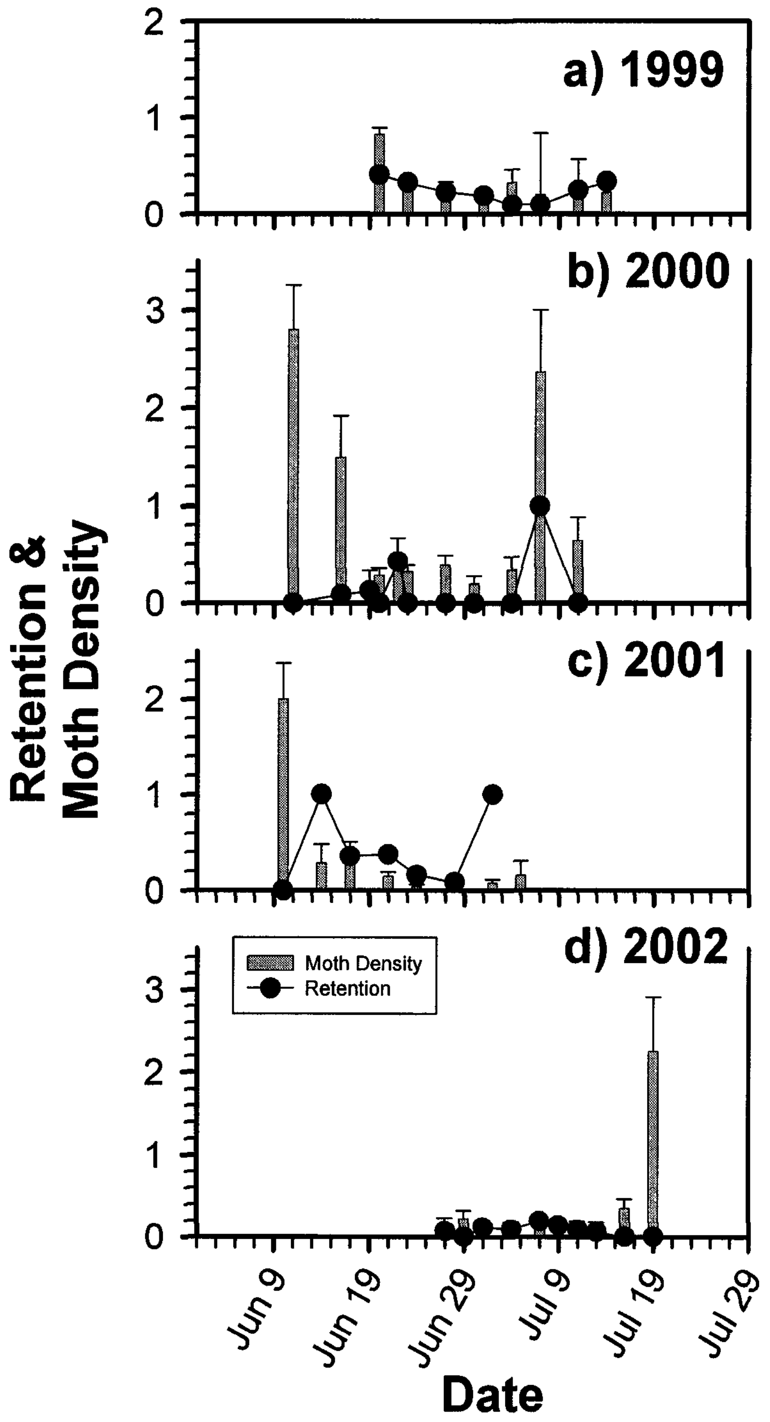

Figure 3.10 - Daily retention (circles) in relation to female moth density per flower (bars) for Onefour, AB from 1999 – 2002.

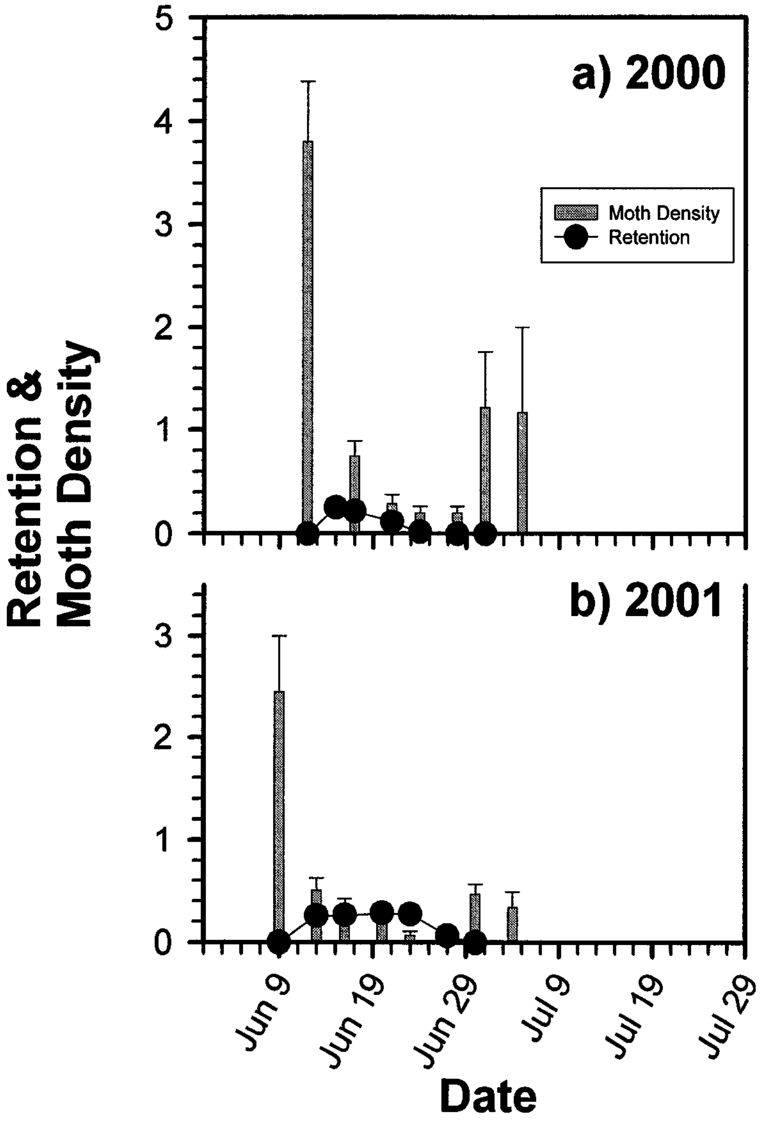

Figure 3.11 - Daily retention (circles) in relation to female moth density per flower (bars) for Loma, MT from 2000 – 2001.

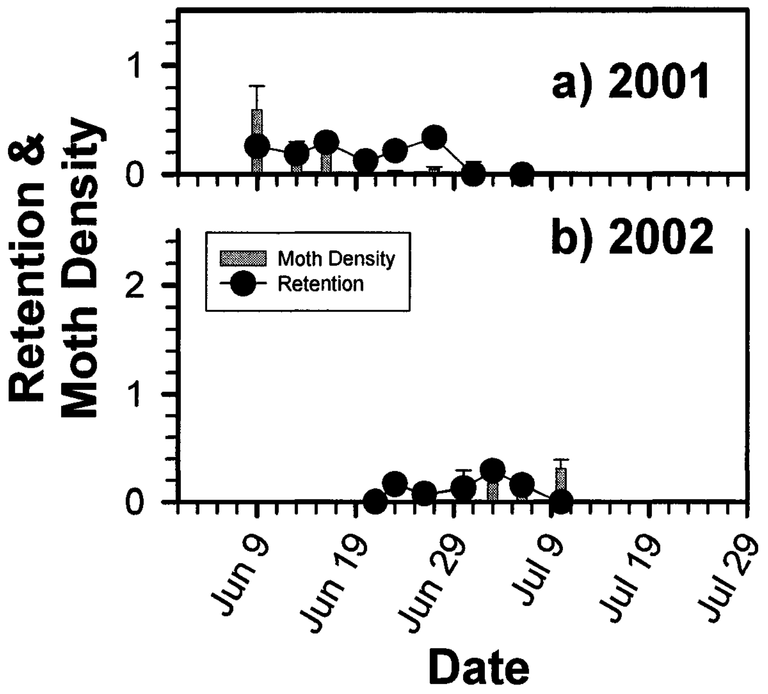

Figure 3.12 - Daily retention (circles) in relation to female moth numbers per flower (bars) for Fort Benton, MT from 2001 – 2002.

Appendix 3.1 - Logit model of the effects of site, year and site*year on a) clone-level flowering, b) rosette-level flowering, c) clone-level fruiting, and d) rosette-level fruiting. Note: Site = Onefour and Year = 2002 were used as a reference and the coefficient for these factors are therefore not expressed below.

Analysis	**Factor**	**Coefficient**	**Odds ratio**	**95% C. I.**	***P*-value**
a) Clone-level flowering					
	Intercept	-0.988	----	----	0.000
	Site				
	Fort Benton	0.112	1.119	0.699-1.791	0.640
	Loma	0.179	1.196	0.856-1.671	0.294
	Year				
	1999	1.046	2.846	2.127-3.806	<0.001
	2000	-1.414	0.243	0.157-0.376	<0.001
	2001	0.321	1.379	1.059-1.794	0.017
	Interaction				
	Fort Benton * 1999	-1.653	0.191	0.102-0.358	<0.001
	Fort Benton * 2000	0.610	1.840	0.841-4.029	0.127
	Fort Benton * 2001	0.698	2.010	0.826-4.892	0.124
	Loma * 1999	0.321	1.379	0.635-2.993	0.427
	Loma * 2000	-0.079	0.924	0.496-1.722	0.804
	Loma * 2001	0.000	1.000	----	----
b) Rosette-level flowering					
	Intercept	-1.744	----	----	<0.001
	Site				
	Fort Benton	-0.591	0.554	0.393-0.780	0.001
	Loma	0.422	1.524	1.232-1.887	<0.001
	Year				
	1999	1.315	3.724	3.016-4.598	<0.001
	2000	-1.696	0.183	0.124-0.271	<0.001
	2001	-0.364	0.695	0.569-0.849	<0.001
	Interaction				
	Fort Benton * 1999	-0.675	0.509	0.320-0.811	0.004
	Fort Benton * 2000	-0.197	0.821	0.511-1.320	0.416
	Fort Benton * 2001	1.467	4.334	2.011-9.341	<0.001

	Loma * 1999	0.672	1.958	1.054-3.639	0.033
	Loma * 2000	0.962	2.618	1.675-4.091	<0.001
	Loma * 2001	0.000	1.000	----	----
c) Clone-level fruiting					
	Intercept	1.102	----	----	<0.001
	Site				
	Fort Benton	-0.004	0.996	0.298-3.331	0.995
	Loma	1.502	4.493	1.950-10.348	<0.001
	Year				
	1999	1.320	3.742	1.907-7.345	<0.001
	2000	-3.575	0.028	0.012-0.067	<0.001
	2001	-0.783	0.457	0.267-0.781	0.004
	Interaction				
	Fort Benton * 1999	-1.762	0.172	0.040-0.745	0.019
	Fort Benton * 2000	-3.622	0.027	0.008-0.086	<0.001
	Fort Benton * 2001	5.421	226.088	19.146-2669.756	<0.001
	Loma * 1999	-1.298	0.273	0.053-1.414	0.122
	Loma * 2000	1.322	3.751	0.844-16.661	0.082
	Loma * 2001	0.000	1.000	----	----
d) Rosette-level fruiting					
	Intercept	0.797	----	----	<0.001
	Site				
	Fort Benton	0.938	2.554	0.715-9.116	0.149
	Loma	2.652	14.189	5.101-39.473	<0.001
	Year				
	1999	0.890	2.436	1.541-3.849	<0.001
	2000	-3.087	0.046	0.020-0.102	<0.001
	2001	-0.413	0.662	0.430-1.018	0.060
	Interaction				
	Fort Benton * 1999	-2.996	0.050	0.012-0.208	<0.001
	Fort Benton * 2000	-3.829	0.022	0.006-0.076	<0.001
	Fort Benton * 2001	3.345	28.356	4.249-189.234	0.001
	Loma * 1999	-2.875	0.056	0.010-0.315	0.001
	Loma * 2000	0.327	1.387	0.320-6.010	0.662
	Loma * 2001	0.000	1.000	----	----

CHAPTER 4

Reproductive biology of an edge of range perennial, *Yucca glauca*: The implications of pollen limitation and self-fertilization for a mutualistic species at risk

Summary

The breeding systems of plant populations at the edges of their ranges are thought to be particularly susceptible to changes in levels of self-compatibility because of low densities, isolation and high levels of environmental variation. I examined northern populations of *Yucca glauca* in Alberta and Montana to determine how differences in selection pressures may have led to changes in the breeding system relative to other areas. Northern populations were pollen limited and were slightly autogamous provided that yucca moths (*Tegeticula yuccasella*) were present. Inflorescences selectively retained cross-pollinated flowers when given a choice between crossed- and self-fertilized flowers. However, inflorescences retained equal proportions of selfed and crossed fruit if no choice was provided. The most isolated populations in Alberta did not exhibit inbreeding depression, while those in Montana, which were more continuously distributed, exhibited very high effects of inbreeding depression in selfed fruit. This study suggests that *Y. glauca* has a mixed mating system and populations can exhibit varying levels of self-compatibility. Pollen limitation and low flowering densities in populations at the northern edge of range have potentially led to higher tolerances to self-fertilization as a form of reproductive assurance and in Alberta populations may have led to the "purging" of deleterious alleles which in effect eliminated the negative effects of frequent self-fertilization (i.e. inbreeding depression). Assessments of unique situations, adaptations and reproductive health in northern edge of range populations are critical in the justification of the conservation of peripheral populations and to demonstrate that edge populations are not necessarily bound for extinction.

Introduction

Animal-mediated pollination for seed set could be a risky pollination method for rare plants in marginal habitats at the ecological or geographic edges of their range. Edge of range plants and pollinators tend to occur at low densities, are relatively isolated and exist in highly variable abiotic and biotic environments (e.g. Lesica and Allendorf 1995, Jones and Gliddon 1999, Nantel and Gagnon 1999, Dorken and Eckert 2001). Plants at the northern edge of their ranges should experience a reduction in pollinator availability and in the availability of conspecifics with which to mate (Sipes and Tepedino 1995). These factors may expose these populations to unique selective pressures that may lead to an alteration of breeding system. Knowledge of the reproductive biology of rare plants and how this may differ from more central populations is necessary to develop effective conservation and management practices for these species.

Populations of *Yucca glauca* Nuttall at the northern edge of its range offer an excellent opportunity to examine how breeding systems may change at range edges. The species is pollinated by a single species of pollinator, a yucca moth (*Tegeticula yuccasella* Riley), which consistently occurs at lower densities in the north relative to other populations. Reliance on a single species for pollination may therefore put *Y. glauca* at great risk should the yucca moth be extirpated (Spira 2001).

The yucca and the yucca moth are considered "Threatened" and "Endangered", respectively, by the Committee on the Status of Endangered Wildlife in Canada (Csotonyi and Hurlburt 2000, COSEWIC 2002) and wildlife managers are currently developing management goals and plans to ensure that both species and the mutualism between them persists in Canada (Alberta Soapweed and Yucca Moth Recovery Team, *In prep.*).

Opportunities for self-fertilization, through within-flower autogamy or via between flower pollen transfers within inflorescences or between flowers among inflorescences with clones (geitonogamy) are high in yuccas since anthers within a flower dehisce the same evening that the stigma becomes receptive and many flowers within an inflorescence are open at the same time. Despite high proportions of cross-pollinated fruit in populations with abundant yucca moths, the incidence of self-pollination behaviour by moths is extremely high and moths frequently transfer pollen among flowers within an inflorescence and among inflorescences within a clone (Marr et al. 2000). In addition, pollen transfer is more likely to occur among near neighbours than among more widely separated individuals that are less likely to be genetically related (Marr et al. 2000). Most yuccas exhibit some level of self-compatibility (Webber 1953, Wimber 1958, Aker and Udovic 1981, James et al. 1993, Dodd and Linhart 1994, Pellmyr et al. 1997, Richter and Weis 1998). However, self-fertilized flowers have a much lower probability of fruit retention relative to cross-pollinated flowers in many species, including *Yucca whipplei* (Aker and Udovic 1981, Richter and Weis 1998), *Y. elata* (James et al. 1993), *Y. filamentosa* (Huth and Pellmyr 1997, Huth and Pellmyr 2000, Marr et al. 2000) and *Y. glauca* (Fuller 1990). Further, self-pollinated progeny in *Y. filamentosa* (Pellmyr et al. 1997) and *Y. whipplei* (Richter 1995) have lighter seed masses, lower germination frequency, lower growth rates and higher seedling mortality than outcrossed seeds. In most populations, yucca plants can selectively abscise lower quality flowers, such as those that arise from self-fertilization, and only allow higher quality fruit to mature (Huth and Pellmyr 2000) to avoid the costs of poor progeny performance.

Pollination levels must be sufficiently large for selective abscission of selfed progeny to be a viable strategy. At the northern edge of range, neither the presence of moths, nor the presence of other individual plants in flower is reliable and the maintenance of a mating system restricted to outcrossing could be costly. Populations of *Y. glauca* in Alberta and much of northern Montana have fewer female moths per flower and fewer ovipositions per fruit than more southern populations (Alberta Sustainable Resource Development 2002, Chapter 3). Self-fertilization, even if pollinator-mediated, would have an advantage in maintaining high and reliable seed production when pollinators are scarce or unpredictable, as long as the performance of progeny is not highly degraded (Herlihy and Eckert 2002); however in northern population, population persistence is not reliant on seed production (Chapter 2). In situations where moth numbers are low (Aker and Udovic 1981, Dodd 1988, Fuller 1990) or where overall fruit set is low (Richter and Weis 1998), yuccas show higher levels of self-compatibility and retention of selfed fruit. At the northern edge of its distribution, I predict that *Y. glauca* will be more tolerant of self-fertilization and will retain self- and cross-pollinated flowers without preference, with no apparent signs of inbreeding depression in selfed fruit.

Materials and Methods

Study organisms

Soapweed (*Yucca glauca;* Agavaceae) is obligately dependent on the yucca moth, (*Tegeticula yuccasella*; Incurvariidae) for sexual reproduction, with active pollen collection and transfer being performed by female yucca moths. Likewise, yucca moths are obligately dependent on yuccas, as yucca moth larvae consume seeds in developing yucca fruit (Riley 1892). Adult female yucca moths actively collect pollen using their maxillary tentacles and typically fly to another fresh flower on another inflorescence (Riley 1892). The moth inserts her ovipositor through the carpel wall and lays an egg next to the developing ovules. She then climbs to the tip of the style, and using her maxillary tentacles, actively inserts pollen into the stylar canal. Upon hatching, the yucca moth larvae feed on developing seeds. Mature larvae emerge from the yucca fruit, burrow into the soil and enter a state of prepupal diapause (Riley 1892; Keeley et al. 1984).

Study sites

Hand pollination experiments were conducted in four populations of *Y. glauca* at the northern edge of the species' range in southern Alberta and northern Montana. All locations were in the Missouri River drainage. Experiments were conducted in Onefour, AB (49° 00" 62' N, 110° 26" 70' W, 906 m altitude), Loma, MT (47° 57" 79' N, 110° 30" 03' W, 728 m altitude), Fort Benton, MT (47° 01" 22', 110° 39" 32', 885 m altitude) and Decision Point, MT (47° 55" 54' N, 110° 29" 63' W, 929 m altitude) from 1999 to 2002.

The Onefour population was subdivided into Onefour-East and Onefour-W to control for density effects.

Hand pollination

All experiments using hand pollination employed the following methods. Inflorescences to be hand pollinated were covered by moth exclusion bags made of window screening during the bud stage to prevent pollination by yucca moths. Each flower on a recipient inflorescence was identified individually by writing a unique number on one tepal of each flower using a permanent marker. For each experiment, approximately 25% of flowers per recipient inflorescence were randomly selected and hand pollinated; all other unused flowers were removed. Yuccas typically mature less than 10% of their flowers into fruit, presumably allowing plants to retain the highest quality fruit (Addicott 1998, Humphries and Addicott 2000). In other experiments fruit retention rates were similar between inflorescences with hand pollinated flowers and inflorescences with moth pollinated flowers (Humphries and Addicott 2000). Removal of non-treated flowers ensures that the flowers retained are a result of treatment and that plants will not abscise poorer quality flowers in anticipation of flowers yet to open receiving higher quality pollen in the future.

Pollen donor plants were haphazardly chosen from at least 100 m away from the recipient plant within the same population to ensure outcross pollen. Fresh flowers with undisturbed pollen were collected shortly before pollination. All the pollen from a single anther was collected and transferred to the stigma of a fresh flower needing pollination using a fresh yucca leaf blade for each transfer. The pollen was packed gently into the stylar canal using the tip of the leaf blade.

Hand pollinated flowers were scored for retention or abscission after 1 week, 2 weeks and 8 weeks. Abscission prior to 1 week is indicative of self-incompatability; whereas, abscission at later stages would indicate that a plant is selectively retaining certain quality flowers over others.

In two experiments (#3 and #4 below) fruit were harvested at 8 weeks post-fertilization. Each fruit was dissected and the number of inviable and viable seeds per fruit was recorded. Ten seeds were randomly selected per fruit, oven dried for 24 hr and dry weight determined to the nearest mg.

Experimental and Observational Procedures

I performed four separate experiments and sets of observations to evaluate the reproductive system of *Y. glauca* at the northern edge of range. The first two descriptive studies focused on factors that may have acted as selective pressures on the breeding system at the northern edge of range. The other two experiments focused on the ability of *Y. glauca* to accept and retain self-fertilized flowers and examined the individual preference of inflorescences when presented with a choice between flowers with outcross and self pollen.

<u>Experiment 1 - Pollen Limitation</u> – The presence of pollen limitation, where plants produce fewer fruit or seeds than their resources could support (Burd 1994), is indicative of inadequate pollen receipt. Pollen limitation may be a result of poor pollen quality or poor pollen quantity which may occur when pollinator abundance is low.

Twenty inflorescences in each site were covered with mesh exclosure bags to prevent natural pollination. Twenty flowers on each covered inflorescence (total n = 400 flowers per site) at Onefour-East, AB (low plant density), Onefour-West, AB (high plant density), Loma, MT and Fort Benton, MT were hand pollinated with outcross pollen to assess whether northern edge of range populations are pollen limited. Twenty flowers per inflorescence were shown in preliminary experiments to be more than enough to initiate resource limitation in *Y. glauca.* Fruit retention of hand pollinated inflorescences was compared to open-pollinated inflorescences of approximately the same size (i.e., numbers of axils and flowers) located within 25m of each covered inflorescence. The closest inflorescence with fresh open flowers to the covered inflorescence was selected for comparison. Paired t-tests were used to analyze differences between numbers of enlarged axils and total numbers of fruit matured between open- and hand-pollinated inflorescences. Enlarged axils indicate that a flower had been visited, pollinated and that the plant had made a preliminary choice to begin to mature that flower into fruit.

Experiment 2 - Autogamous unfacilitated and facilitated self-fertilization - One hundred flowers were covered with small mesh bags in Onefour-West, AB, Loma, MT and Fort Benton, MT to test for autogamous self-fertilization of *Y. glauca* where flowers could become fertilized without a pollinator vector. An additional 100 flowers in each site were covered to exclude pollinators and hand-pollinated using pollen from the same flower to determine if *Y. glauca* was capable of being fertilized autonomously if a pollinator were present.

Flowers in the Onefour population were observed for a total of 18 hours during the peak pollination period in 1999 to determine if insects other than yucca moths could potentially pollinate yucca flowers. Fifty flowers in this population were also hand-pollinated where pollen was wiped gently onto the style, instead of forcibly stuffed down the style as with yucca moths, to determine if yuccas could be passively pollinated, potentially by another species of insect.

Experiment 3 - Plant Donor Choice (PDC) Experiment - At Onefour, AB, Loma, MT and Decision Point, MT, 12 inflorescences from 12 single-inflorescence clones were chosen for hand pollination. Ten flowers per stalk were randomly selected to control for potential differences in retention due to axil position and each of the chosen flowers was assigned to either the outcross (n = 60 per site) or self (n = 60 per site) pollination treatment and hand pollinated accordingly. The number of flowers retained per inflorescence at 8 weeks post-fertilization was compared between sites and pollen source treatments using two-factor ANOVA. Likewise, two-factor ANOVA was used to measure the effects of study site and pollen treatment on seed mass per fruit and proportion of viable seeds per fruit.

Experiment 4 - Plant Donor No Choice (PDNC) Experiment - At Onefour-East, AB, Onefour-W, AB, Loma, MT and Fort Benton, AB, 12 clones containing 2 inflorescences each were chosen for hand pollination. Clones with two stalks were necessary for a paired blocked design to control for differences in fruit retention within and among clones. Stalks within clones were randomly assigned to either the all outcross or all self pollination treatment. Ten flowers per stalk were hand pollinated for a total of 120 crossed flowers and 120 selfed flowers per site. The purpose of this experiment was to test if yuccas would retain selfed flowers and mature them as fruit if given no choice about pollen source.

The effects of study site and pollen treatment were assessed on flower and fruit retention per inflorescence using a two factor ANOVA. The proportion of viable seeds and seed mass *per fruit* were assessed using two factor ANOVA using study site and pollen treatment as factors.

To measure inbreeding depression, I estimated the fitness of selfed fruit relative to crossed fruit using 4 components of fitness from Experiment #4 (Pollen Donor No Choice). I determined the retention rate of selfed and crossed flowers at 1 week and 8 weeks post-fertilization *per inflorescence*. Each matured fruit was dissected and the proportion of viable seeds per fruit was estimated, and then a subset of matured seeds were dried and weighed to the nearest mg. Finally, 20 seeds per fruit were placed in Petri dishes and enclosed in a growth chamber for 15 days to determine germination rate. Inbreeding depression was calculated as per Stevens and Bougourd (1988) in *Allium schoenoprasum* (wild chives). For each variable, the relative fitness of selfs (RFS) was calculated as the ratio of self / cross performance. Inbreeding depression was calculated a 1 - RFS, while total inbreeding depression was calculated by multiplying the independent estimates of RFS from each variable and subtracting the product from 1.

Results

Experiment 1 - Pollen limitation

Edge of range populations were pollen limited, rather than resource limited. Supplementing flowers with pollen increased fruit retention relative to inflorescences with open-pollinated flowers (Figure 4.1). Hand pollination increased the number of fruit initiated by 17% and the number of fruit matured by 56% over open pollination.

Experiment 2 - Autogamous self-pollination

Y. glauca is not capable of unfacilitated autonomous self-pollination at the northern edge of range; none of the covered flowers in any of the sites (n=300 flowers total over 3 sites) enlarged, and all of them abscised. However 5%, 3% and 3% of autogamous flowers were retained as fruit in Onefour, AB, Loma, MT and Fort Benton, MT respectively.

In Onefour, *Y. glauca* did not have alternative pollinators to the yucca moth. No other species of insects were observed in a location within the flower so as to be even remotely capable of accomplishing pollen transfer, should they have been carrying pollen. Further, none of the flowers passively pollinated by hand resulted in pistil enlargement or fruit production. There is, however, some anecdotal evidence that the high winds experienced in these sites may occasionally act as a pollen vector in flowers where the pistil and anthers are of similar length (Hurlburt, unpublished data). Approximately 3 of 3000 inflorescences in Onefour and 1 of 200 inflorescences in Fort Benton produced fruit that were likely pollinated by “wind-whipping”, resulting in severely pollen-limited (pear-shaped) fruit with no moth ovipositions and less than 30 viable seeds per fruit.

Experiment 3 - Pollen Donor Choice Experiment (PDC)

At maturity (8 weeks post-fertilization), crossed flowers were selectively retained over selfed flowers at all 3 sites (Table 4.1). Mature fruit retention per inflorescence was significantly higher in the cross treatment relative to self, but did not vary among sites (Table 4.2); no relationship existed between treatment, site and initial fruit maturation (Table 4.2). Seed mass per fruit and proportion of viable seeds per fruit did not vary between treatments or sites (Table 4.3, Table 4.4).

Experiment 4 - Pollen Donor No Choice Experiment (PDNC)

Yucca inflorescences readily set selfed flowers if they were not given a "choice" between self- and cross pollinated flowers. Initial fruit set was significantly lower in selfed flowers relative to crossed (Table 4.1, Table 4.2), but this effect was not present at fruit maturity. At maturity there was no difference in flower retention due to treatment, but retention was significantly higher in Onefour-W relative to Fort Benton (Table 4.1, Table 4.2, Figure 4.2). Seed mass was higher in Loma relative to Onefour-E (Table 4.3, Table 4.4), and selfed seeds were significantly lighter than crossed seeds (Table 4.4). The proportion of viable seeds was significantly lower in Onefour-W than in the 3 other sites, but did not vary with treatment (Table 4.3; Table 4.4).

Data from Experiment 4 (Pollen Donor No Choice) were used to compute cumulative inbreeding depression for each site, using retention at 1 week, retention at 8 weeks, proportion of viable seeds per fruit, seed mass and germination frequency as fitness components (Table 4.3, Table 4.5). There were no trends suggesting that some fitness components were more critical than others. Loma, MT and Fort Benton, MT exhibited high inbreeding depression, where selfed fruit performed at only 12-22 % as well as crossed fruit (Table 4.5). Neither Onefour-W, nor Onefour-E exhibited inbreeding depression since cumulative inbreeding depression for both sites was less than 0.5.

Discussion

This study supports prior evidence that *Y. glauca* has a mixed breeding system that ranges from mostly cross-pollination to mostly self-pollination among populations. In New Mexico, *Y. glauca* was primarily self-incompatible (Fuller 1990); however, levels of self-compatibility were much higher in high altitude populations where moths were limiting. Additionally, high levels of self-compatibility occurred at four high elevation sites in Colorado (Dodd and Linhart 1994). Not surprisingly, yuccas at the northern edge of range in Alberta and northern Montana behaved similarly to those at other ecologically marginal sites. As with populations at high elevations, high environmental variability and cooler temperatures at high latitudes may have reduced moth densities (Chapter 3). Moth densities at these northern sites are significantly lower than those in more central parts of the species' range (Alberta Sustainable Resource Development 2002). Partial self-compatibility, where some individuals and populations are self-compatible in a predominantly cross-fertilizing species, is common and was reported to occur in 62-84% of temperate plants (mostly herbaceous) and 35-78% of tropical plants (including shrubs, trees, vines and herbs) (Arroyo and Uslar 1993). Overall, almost one-third of taxa studied showed a mixture of selfing and outcrossing among populations (Barrett et al. 1996).

Despite the ability of *Y. glauca* to retain self-pollinated flowers, plants were incapable of reproduction without a pollen vector. No flowers in the unfacilitated autogamy trial produced fruit. Although Dodd and Linhart (1994) suggested that insects other than yucca moths could pollinate flowers, no other potential pollinators were observed in this study. Fertilization through passive pollination is unlikely in these populations, as simply placing pollen on the stigma did not result in fruit production. This implies that passive pollination, as with most bees and flies, would not result in fruit.

The Onefour-E and Onefour-W populations are isolated from other known populations to the south by over 100 km and experienced low levels of flowering in 4 of 6 years (1998-2003). At low floral densities, it is probable that yuccas have increased rates of self-fertilization, with yucca moths "choosing" to self-fertilize another flower on the same plant instead of the risky behaviour of flying to a distant plant. Distances between flowering individuals greatly increased in seasons with low levels of flowering relative to those with higher levels of flowering (D. Hurlburt, personal observation). Using fluorescent powder as a pollen analog, Marr et al. (2000) found that 80% of dye transfers occurred within 8 m of the source plant and 55% of transfers were within plants in *Y. filamentosa*. They also speculated that the benefits of long-distance travel in more central populations maybe negated by the increased risk of predation to moths during long interplant movements (Huth and Pellmyr 2000, Marr et al. 2000). It is also plausible that the risk of predation would be considerably higher in less dense plant populations because of increased interplant distances such as those occurring at the northern edge of range. The Loma and Fort Benton populations are not isolated from other populations of *Y. glauca*, as the species is continuously distributed along the Missouri River.

When given a choice between either self- and cross pollen (PDC experiment), plants at the northern edge of range retained higher proportions of cross-pollinated relative to selfed flowers. However, if the entire inflorescence were either selfed or outcrossed (Experiment 4 – Pollen Donor No Choice), they retained equal proportions of selfed and crossed flowers. This suggests that there is some degree of reproductive assurance associated with retaining potentially lower quality flowers when low levels of cross-pollination occur. Reproductive assurance, where self-fertilization ensures the production of seeds in the face of pollen limitation, is the most widely accepted explanation for the evolution of self-fertilization in angiosperms (Eckert 2000; Herlihy and Eckert 2002; Lloyd 1992), and may be especially important in explaining the widespread occurrence of partial selfing in some species (Eckert and Schaefer 1998).

According to the reproductive assurance hypothesis, selection favors increased self-fertilization in situations in which pollinator service strongly limits reproduction due to scarcity of mates or pollinators (Fausto et al. 2001) because selfing should always be favoured when ovules would otherwise remain unfertilized. The ecological conditions under which pollinators were limiting and reproductive assurance was suggested as the mechanism behind the evolution of self-fertilization include: extreme temperatures (reviewed in Lloyd 1980), high altitudes (Ramsey et al. 1994), small population size (Barrett et al. 1992), low population density (Holsinger 1991), early season plants (primarily annuals) in temperate climates (Lloyd 1965), interspecific competition for pollinators (Lloyd and Schoen 1992), and at the edges of species ranges or at high latitudes (Wyatt 1986, Ramsey et al. 1993, Ramsey et al. 1994).

In general, the relative fitness of selfed to outcrossed progeny determines the evolution of the mating system. Studies have suggested that self-fertilization has evolved to assure reproduction when pollinator activity is reduced (Herlihy and Eckert 2002), implying that selection is sufficient to counteract the effects of inbreeding depression. In cases of pollen limitation, the level below which self-fertilization is preferred ($\delta = 0.50$) is shifted upwards and self-fertilization is favoured despite inbreeding depression.

Increased self-fertilization may also weaken the deleterious effects of inbreeding by exposing recessive mutations to selection (Eckert and Barrett 1994), i.e., self-fertilizing populations may "purge" deleterious effects (Lande and Schemske 1985) and over time a selfing population is expected to reduce inbreeding depression (Goodwillie 2000). Further, selfing allows the rapid spread of favourable recessive alleles (Jain 1976). Populations of *Y. glauca* in Alberta have been isolated from the Missouri populations presumably for about 10,000 years since the end of the hypsithermal. More recently, these populations have been subjected to low moth and flower densities and have likely experienced high levels of self-fertilization over time. Thus, inbreeding depression would be expected to be reduced over time in northern populations of *Y. glauca* because of constant, high levels of self-fertilization. There is some disagreement with regard to the differential effects of purging in very small versus large populations. Purging has little impact in very small populations with regular selfing, but has clear effects in moderate to large populations (Frankham et al. 2001) with regular selfing. The populations at Onefour are large (8499 clones) relative to any other populations studied in Alberta or Montana and according to Frankham et al. (2001) should be a candidate for purging.

It is possible that my estimate of inbreeding depression for the Onefour populations was low, as I did not assess seedling survival of self- and crossed progeny. Huth and Pellmyr (2000) found that seedling survival was a major contributor to overall inbreeding depression. However, if I use the average RFS values for seedling survival (0.72 and 0.92 from Richter (1995) and Pellmyr et al. (1997) respectively) from primarily outcrossing populations of yuccas, overall inbreeding depression for the Onefour populations is still considerably lower than in Loma and Fort Benton. Using these values, the fitness of selfed fruit at Onefour-W was 46.1 % of the outcrossed fruit for Onefour-W and 72.6 % for Onefour-E, which are still only half that of Loma, MT and Fort Benton, MT. I expect that the values for RFS for seedling survival would be lower in Onefour populations than in reported values for other populations/species given other less negative responses to self-pollination. However, estimates of seedling survival are critical to this assessment of inbreeding depression.

Implications for conservation

Geographically peripheral populations are more likely to be imperiled because they are often small in size, are isolated, experience environmental stochasticity and may accumulate deleterious mutations and an increased expression of those mutations upon inbreeding (inbreeding depression). However, peripheral populations are not necessarily doomed for extinction and may be critical to the evolutionary future of the species.

Isolation and extreme environments may introduce selective pressures to the population that are unique or more severe than core populations (Lesica and Allendorf 1995), leading to more rapid divergence than those populations in the centre of their ranges. Thus, peripheral populations may be pre-adapted to future environmental change that might threaten populations across the remainder of the species range. *Yucca glauca* already exhibits signs of unique adaptation to its environment in Alberta and Montana relative to other more central populations. The species tolerates self-fertilized flowers and has greatly extended flowering and moth emergence times within seasons (Alberta Sustainable Resource Development 2002). These northern, peripheral populations of yuccas are particularly important, because they are expected to be on the leading edge of range expansion in the face of climate change as the climate on the prairies becomes increasingly warmer and drier northwards.

Inbreeding depression is often of concern in the conservation of rare species because it reduces fitness and potentially affects persistence of peripheral populations in the future. However this may not be the case in all small, isolated populations as it depends on the evolutionary history of the population. Long-term exposure to self-fertilization may lead to the purging of deleterious recessive genes, which may counterbalance the effects of inbreeding depression. Small populations with relatively sudden increases in self-fertilization levels are expected to experience higher levels of inbreeding depression.

Despite the potential abilities of these populations of yuccas to adapt to a changing environment, their persistence will also depend on the ability of the yucca moth to persist and adapt as well. Although the most northern populations of *Y. glauca* can be self-fertilized with no apparent reduction in fitness through reduced seed set, they are still obligately pollinated by yucca moths. If flowering and moth emergence are controlled by different climatic cues, it is plausible that the mutualistic interaction will become disrupted as climate change continues to occur. Yucca moths would succumb to the loss of their partner relatively quickly, as adults oviposit in yucca flowers and larvae require yucca seeds for food. The plant will likely persist for a greater length of time because of its long life span and ability to survive through vegetative propagation; the species may be able to persist in to the future if it evolves an autogamous breeding system or attracts an alternative pollinator.

Knowledge of the reproductive biology of rare plants is essential to develop effective conservation and management practices. One must have a clear understanding of the mating system and pollination levels of the populations of concern, and how the evolutionary past of those populations may have influenced the breeding strategies of the plant. Comprehension of these factors will clarify the relative importance of various management strategies; for example, in northern populations of *Y. glauca,* introduction of individuals from other populations to increase genetic diversity is apparently not critical as there is no effect of inbreeding. However, *Y. glauca* still relies on a single pollinator, the yucca moth, to transfer pollen among flowers, and intervention may be necessary to prevent pollinator loss through habitat preservation. Species that are pollinated by a variety of species may be less susceptible to pollinator loss. Preservation of plant populations that are reliant on pollinator populations often involve multiple species assemblages which occupy different habitats, hence, a community or ecosystem-approach may be necessary to ensure such populations persist.

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Table 4.1 - Summary of fruit retention for cross- and self-pollination treatments for Pollen Donor Choice (PDC) and Pollen Donor No Choice (PDNC) experiment.

Experiment	Site	Treatment	Flowers (n)	Retention
Pollen Donor Choice	Onefour	Cross	60	0.267
		Self	60	0.133
	Loma	Cross	60	0.517
		Self	60	0.183
	Decision Point	Cross	60	0.533
		Self	60	0.267
Pollen Donor No Choice	Onefour - West	Cross	110	0.428
		Self	110	0.336
	Onefour - East	Cross	110	0.318
		Self	110	0.327
	Loma	Cross	100	0.430
		Self	100	0.170
	Decision Point	Cross	100	0.310
		Self	100	0.220

Table 4.2 - Logistic regression analysis of flower fate (retained or not retained) on site and pollen treatment for PDC and PDNC experiment. Analyses for initial fruit set and mature fruit retention are shown.

Experiment	Stage	Term	Coefficient	d.f.	*P*
Pollen Donor Choice	Initial Fruit Set	Site	-0.802	1	0.091
		Treatment	-0.678	1	0.070
		Constant	1.811	1	0.161
	Mature Fruit Set	Site	1.175	1	0.269
		Treatment	-0.533	1	0.009
		Constant	-0.637	1	0.334
Pollen Donor No Choice	Initial Fruit Set	Site	1.022	1	0.737
		Treatment	-0.469	1	0.001
		Constant	2.661	1	0.001
	Mature Fruit Set	Site	-0.791	1	0.001
		Treatment	0.602	1	0.111
		Constant	1.648	1	0.082

Table 4.3 – Summary statistics for Pollen Donor Choice and Pollen Donor No Choice experiments. Number of fruit per sample is shown in parentheses.

		Crossed		Selfed	
Experiment	Study Site	Seed Mass (g)	Proportion of Seeds Viable	Seed Mass (g)	Proportion of Seeds Viable
Pollen Donor Choice	Onefour	0.141 ± 0.008 (11)	0.738 ± 0.026 (11)	0.135±0.008 (12)	0.674±0.067 (12)
	Loma	0.147±0.007 (25)	0.688±0.059 (25)	0.121±0.011(10)	0.769±0.049 (9)
	Decision Pt	0.144±0.007 (18)	0.767±0.035 (18)	0.156±0.007 (15)	0.667±0.057 (15)
Pollen Donor No Choice	Onefour – West	0.103±0.004 (35)	0.580±0.034 (43)	0.089±0.005 (35)	0.596±0.037 (36)
	Onefour – East	0.107 ± 0.006 (33)	0.694±0.031 (40)	0.112±0.008 (32)	0.719± 0.033 (32)
	Loma	0.140 ± 0.007 (42)	0.780±0.027 (41)	0.118±0.006 (16)	0.620±0.060 (18)
	Fort Benton	0.133 ± 0.012 (13)	0.727±0.052 (13)	0.070±0.205 (21)	0.686±0.030 (21)

Table 4.4 - Summary results of multifactor ANOVAs for the effects of pollen source for PDC and PDNC experiment on proportion of viable seeds and seed mass per fruit in *Yucca glauca*. Significance levels are denoted with asterisks: * P<0.05; ** P<0.01; *** P<0.001

Pollen Donor Choice – Experiment #3

	Seed mass			Proportion viable seeds		
Factor	d.f.	SS	F	d.f.	SS	F
Treatment	1	9.337 X 10^{-9}	0.000	1	0.079	1.705
Site	2	0.007	3.736	2	0.081	0.880
Interaction	2	0.007	3.630	2	0.217	2.341
Error	76	0.080		236	4.073	

Pollen Donor No Choice – Experiment #4

	Seed mass			Proportion viable seeds		
Factor	d.f.	SS	F	d.f.	SS	F
Treatment	1	24265.757	8.761**	1	0.081	2.002
Site	3	68277.136	8.217***	3	0.699	5.795***
Interaction	3	2810.024	0.338	3	0.307	2.057
Error	236	653674.030		219	9.494	

Table 4.5 – Fitness of crossed and selfed progeny of *Yucca glauca* at the northern edge of range by population. The relative fitness of selfed flowers (RFS) was calculated as the ratio of self / cross performance and inbreeding depression (ID) is 1 – RFS. Overall RFS is the product of individual estimates of RFS for each factor.

Site	Factor	Selfed	Crossed	RFS	ID
Onefour - W	Retention (Initial)	0.464	0.518	0.896	0.104
	Retention (Mature)	0.336	0.418	0.804	0.196
	Proportion viable seeds / fruit	0.596	0.580	1.028	-0.028
	Seed weight	0.089	0.103	0.864	0.136
	Germination frequency	0.029	0.033	0.879	0.121
	Cumulative effect			**0.562**	**0.438**
Onefour - E	Retention (Initial)	0.427	0.409	1.044	-0.044
	Retention (Mature)	0.327	0.318	1.028	-0.028
	Proportion viable seeds / fruit	0.719	0.694	1.036	-0.036
	Seed weight	0.112	0.107	1.047	-0.047
	Germination frequency	0.038	0.050	0.760	0.240
	Cumulative effect			**0.885**	**0.115**
Loma	Retention (Initial)	0.400	0.610	0.610	0.344
	Retention (Mature)	0.170	0.430	0.395	0.605
	Proportion viable seeds / fruit	0.620	0.780	0.795	0.205
	Seed weight	0.118	0.140	0.843	0.157
	Germination frequency	0.217	0.305	0.711	0.289
	Cumulative effect			**0.115**	**0.885**
Fort Benton	Retention (Initial)	0.320	0.680	0.471	0.529
	Retention (Mature)	0.220	0.310	0.710	0.290
	Proportion viable seeds / fruit	0.707	0.717	0.986	0.014
	Seed weight	0.070	0.133	0.526	0.474
	Germination frequency	0.127	0.100	1.270	-0.270
	Cumulative effect			**0.220**	**0.780**

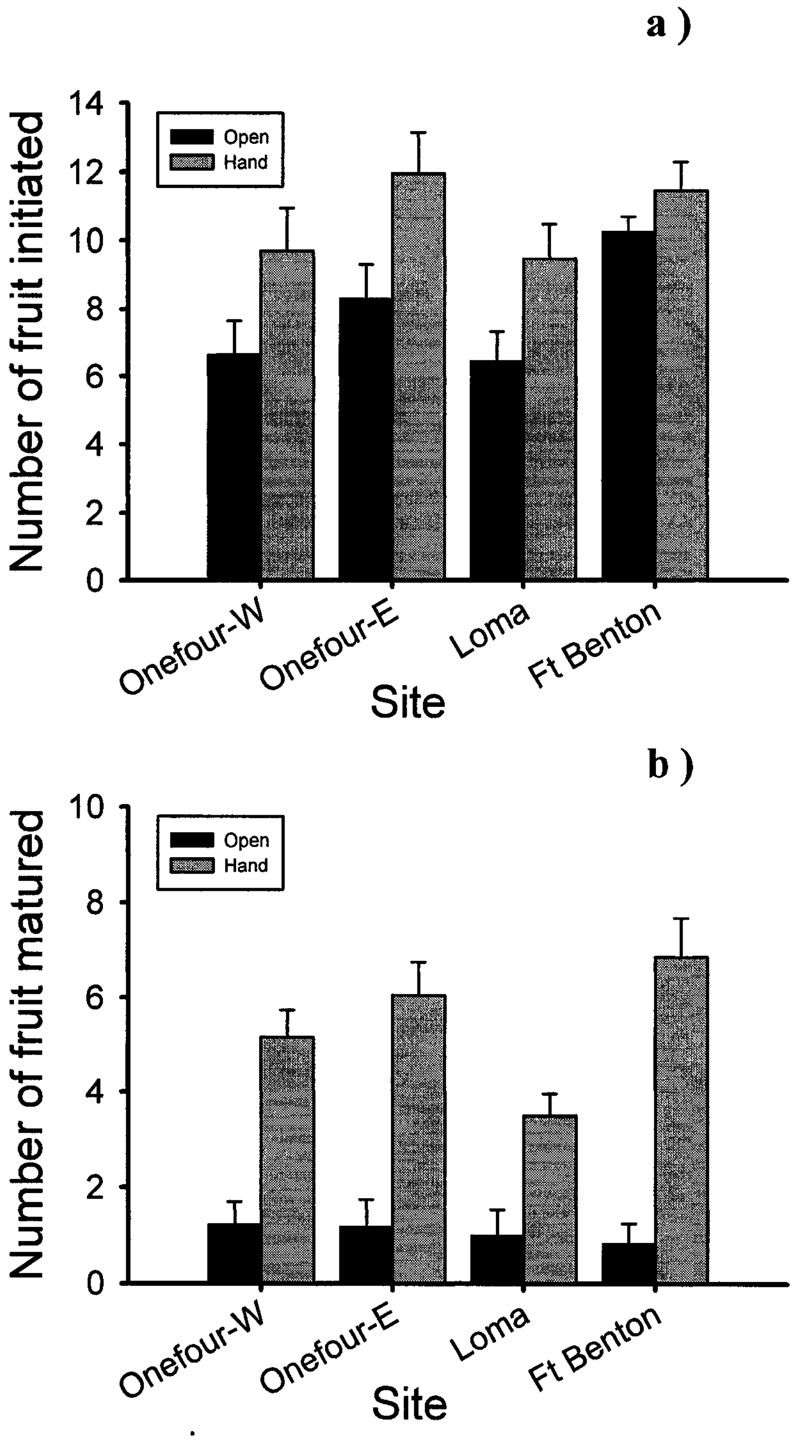

Figure 4.1 - Demonstration of pollen limitation of *Yucca glauca* at the northern edge of range. Initial flower retention and fruit maturation were significantly higher for hand cross-pollinated inflorescences relative to open pollinated inflorescences; analyses of these data are in Table 4.1. A total of 400 flowers among 20 inflorescences were used in each sample.

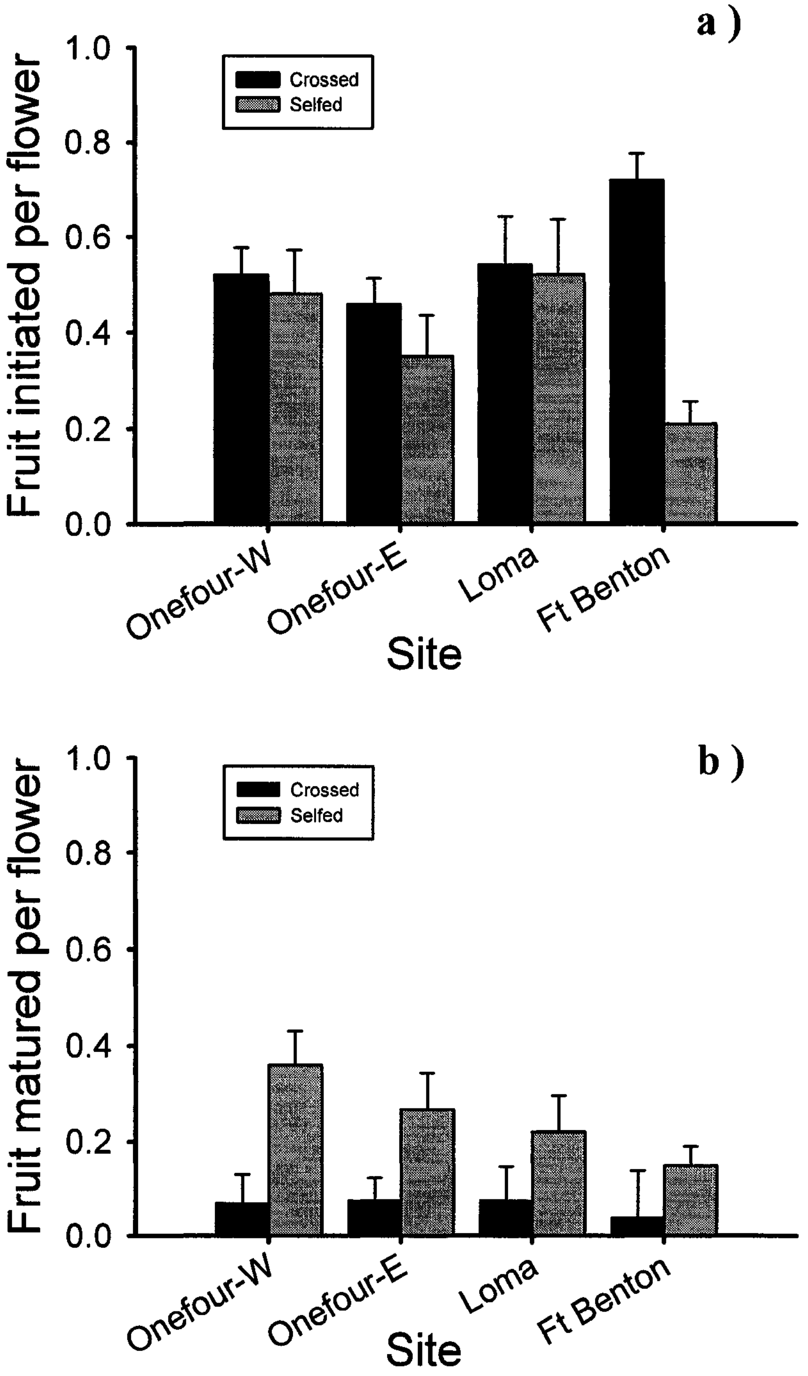

Figure 4.2 - Results of pollination treatment in Pollen Donor No Choice experiment on (a) fruit initiated per flower and (b) mature fruit production per flower at four sites. Values are means (± 1 standard error; n = 10-12 inflorescences per site).

CHAPTER 5

Thesis Discussion

Overview

Numerous studies and simulations have indicated that range – margin populations of species are more sensitive to demographic, environmental and genetic stochasticity than populations from the core of the species' range, which results in low, but variable, population size (Bengtsson 2000). Hence, interacting marginal populations of mutualists should be at lower densities and be demographically more variable relative to those of beneficial interactions elsewhere, especially when they occur at the northern periphery of their ranges. This thesis examined how yuccas and yucca moths living at the northern periphery of their ranges responded to different abiotic and biotic conditions and adapted their life histories to persist in apparently less optimal habitat. Additionally, in many respects this thesis provides a set of counter arguments for the sensitivity of marginal populations.

Literature suggests that most plant and insect populations are suppressed under harsh conditions at ecological range margins (Lesica and Allendorf 1995). This was also observed in yuccas and yucca moths, where yuccas experience lower rates of fruit set in inhospitable environmental conditions because of decreased survival and activity of the yucca moth (Cruden et al. 1976, Powell 1984, Dodd and Linhart 1994). Further, theoretical models suggest that at low mutualist densities, mutualistic interactions will become unstable and the extinction of partners and the dissolution of the mutualism is inevitable (May 1981). Although extinction and the dissolution (via shifts in relative benefits to costs) are evident in some mutualisms (Temple 1977, Stadler and Dixon 1998, Cox 2000), there are examples of mutualisms that are resilient to harsh environments and/or can persist at low partner densities (Breton and Addicott 1992, Bronstein and McKey 1994) and my system seems to support this as well.

Historical data indicated that the mutualism between yuccas and yucca moths was present at the northern edge of range in Alberta (i.e., they produced fruit and larvae) (Milner 1977, Fairbarns 1984), but it was unclear if these populations were in decline and how they dealt with variability in their abiotic and biotic environment. For my Ph.D. thesis, I explored how yuccas and yucca moths deal with several problems that likely occur and influence the mutualism between the species at the northern edge of range.

Chapter 2 explored the role that demographic variation and variation in herbivory plays in the persistence and growth of mutualist populations. I found that, despite high variation in flowering and seed production, yucca populations are stable at the northern periphery. I also explored the implications of variable levels of flowering on population growth, and although the more frequent the occurrence of high flowering the higher the population growth rate, the effects were small. Finally, herbivory in yuccas is common and the effects of herbivory in combination with variable flowering were evaluated. As with variation in flowering, herbivory only reduced population growth rate minimally. Sensitivity analysis revealed that reproduction and recruitment have only minor contributions to population growth in northern populations. The survival of mature reproducing individuals is much more critical to population persistence.

Chapter 3 examined the regulation of the mutualism and how the yucca and yucca moth deal with low partner densities in time and space. I found that abiotic biotic differences between northern peripheral populations and those in the core of the distribution, northern yuccas and yucca moths exhibited similar levels of reproductive success. To accomplish this, northern yuccas engage in "reverse" selective abscission and selectively abscise flowers with fewer ovipositions which presumably increases moth recruitment in future years. Additionally, although data are preliminary, it appears that moths also enhance their survival by laying their eggs in throughout the pistil of the flower rather than in just the centre. This may also benefit the plant, as fewer ovules are damaged through oviposition.

Chapter 4 determined how northern yuccas have dealt with low pollinator availability and years of low flowering given that most yuccas are strictly outcrossing and selectively abscise flowers with pollen of low quantity or quality. This was critical in northern populations since it was expected that low levels of pollinators would reduce the quantity of pollen received by each flower and that low flowering levels would encourage selfing behaviour by moths. The study revealed that northern yuccas will set fruit from selfed flowers and show no preference between selfed and crossed flowers. Further, there appears to be little cost to the plant if it retains selfed flowers as they have similar numbers of viable seeds and seed masses. Relative fitness of selfs analysis showed that the most northern and isolated population had no inbreeding depression.

Overall, northern populations of yuccas and yucca moths are well adapted to reduced partner densities and fluctuations in abundance and can readily engage in a coevolved mutualistic interaction.

Depth vs. Breadth of Study

Perhaps the biggest limitation of this study is the inability to isolate the effects of physiological intolerance, small population size, and / or isolation on the patterns observed. This is a common problem in studies concerning peripheral populations as they are by nature rare, and the motives for research are conservation oriented rather than for the purposes of generating hypotheses. Ideally, it would have been appropriate to include additional study sites from the core of the range and from other peripheral populations in my analyses which would have assisted in deciphering effects, but this was not logistically feasible. There is some indication however that the patterns observed are not a result of small population size. The Onefour population is relatively large population relative to other study sites and it exhibited very different patterns that those observed in other northern populations of a similar size. Further, these patterns have not been reported in any other populations from the core of the range, regardless of size. Additionally, isolation is not thought to be responsible for the patterns of retention in this study, as the reverse selective abscission pattern was also observed in the Loma and Fort Benton populations which are not highly isolated from other populations, as is the case with Onefour. It is my impression that the results obtained in Chapter 2 and 3 are a function of the northern location of my study sites. The case is somewhat different in Chapter 4 however where differences in the breeding system at Onefour relative to Loma and Fort Benton are thought to be a result of the highly isolated nature of the Onefour population.

There are tradeoffs associated with focusing intensively on few populations (i.e. depth) rather than superficially observing patterns in many (i.e. breadth). It is difficult to understand the mechanisms behind patterns if one is focusing on general, large-scale biogeographical patterns of mutualism. Additionally, because this study also has applied implications, the detailed understanding of Alberta populations was necessary. Fortunately, relative to most studies of peripheral populations, there are considerable data available on yuccas and yucca moths for comparisons from the core of their ranges – including the species of concern. The problem with this approach however is that methods are not standardized across studies. Despite this limitation however, this study does provide insight on the functioning of mutualisms at low partner densities (for whatever reason) which has rarely been explored.

Conditionality, Stability and Regulation of Mutualisms

The outcome and success of mutualisms is conditional upon the abiotic and biotic environment in which it occurs and may vary in space and time depending upon costs relative to benefits. Models suggest that mutualisms should be unstable when a mutualist partner is at low density, however, field studies indicate that mutualism strength is actually greatest at low densities because the benefits of association are higher when one occurs in low numbers (e.g. Breton and Addicott 1992). In low density populations, where extrinsic factors, like climate, limit populations, densities may never reach that threshold where costs outweigh benefits.

Mutualists can regulate their partners in response to spatiotemporal variation in density, whether it be high or low, which stabilizes the interaction and ensures its persistence (Cushman and Addicott 1991, Pellmyr and Huth 1994, Addicott 1998, Addicott and Bao 1999). For example, in moth-yucca mutualisms moth abundances are reduced through mechanisms such as selective fruit abscission (e.g. Pellmyr and Huth 1997, Addicott 1998, Segraves 2003) and altered fruit morphology (Addicott and Bao 1999). Similar patterns exist in other obligate mutualisms such as that between figs and fig wasps, where fig trees avoid overexploitation by fig wasps by producing flowers into which wasps cannot oviposit or by limiting access to the specialized inflorescence (West and Herre 1994, Nefdt and Compton 1996). However, most studies have concentrated on intrinsic and extrinsic regulatory mechanisms that prevent overexploitation by mutualists, rather than underexploitation. This is one of the few studies that concentrations on the regulation of mutualisms under low partner conditions and emphasizes the resilience or stability of mutualisms under these rare set of conditions.

Value of Northern Peripheral Populations and Interactions

This study supports the idea that peripheral populations and species interactions are not necessarily dead-end entities that are doomed for extinction (Lomolino and Channell 1995, Lomolino and Channell 1998), despite decreased and variable demographics and less optimal environmental conditions relative to core populations (Volis et al. 1998, Vucetich and Waite 2003). There have been discussions among conservation biologists and managers about the wisdom of investing resources on the preservation of peripheral populations, especially when the species are widespread and secure in most of their range (Lesica and Allendorf 1995). This study emphasizes the value that edge of range populations, especially those at the northern periphery can have from an ecological and evolutionary perspective.

Peripheral populations are of interest because they may reveal information about the factors that delimit species distributions and speciation in general (Bengtsson 1993). When isolated, these populations are often found without predators or competitors (Short et al. 1992), which allow one to examine population dynamics more closely without the presence of confounding factors found in other populations. Peripheral populations are often a source of future speciation events (Lesica and Allendorf 1995) and they may be critical to the survival of species in the face of climate change (Nantel and Gagnon 1999). Peripheral populations may readily adapt to a wide range of environmental conditions relative to core populations as they are typically faced with varying environmental selective pressures which serve to prevent genetic erosion and a variety of genotypes are maintained in the population (Volis et al. 1998, but see Cassel and Tammaru 2003). This inherent adaptability of peripheral populations will be crucial to the persistence of many species as northern peripheral populations will be on the leading edge of range expansion during global warming. Finally, rare species may have higher a higher change of persistence in peripheral populations. Lomolino and Channell (1995, 1998) found that 23 of 31 species of endangered mammals persisted at the periphery of their ranges rather than in the core due to increased anthropogenic disturbance, despite higher population densities and reduced variability in the core part of the range. These features makes northern peripheral populations particularly valuable.

The examination of mutualism at range edges is also of particular value because the dynamics of beneficial interactions are primarily studied in core populations of mutualists. Studies at the periphery provide insight on the stability of interactions when mutualists are dynamic and/or low in abundance, and emphasize the importance of mutualisms to the maintenance of biodiversity in harsh environments. Geographical comparisons of mutualisms in general, show how a wide range of selective pressures can alter the degree of benefit acquired through species interactions (Thompson 1997).

Endangered Canadian Populations of Yuccas and Yucca Moths

Canadian populations of yuccas and yucca moths are considered to be Threatened and Endangered respectively, by the Committee on the Status of Endangered Wildlife in

Canada (Csotonyi and Hurlburt 2000, COSEWIC 2002) because of limited distribution within Canada, low, variable population abundances and recruitment. Both species are currently the subject of a joint recovery plan to maintain the yucca, yucca moth and the interaction between them in Canada (Alberta Soapweed and Yucca Moth Recovery Team *in prep.*). Data collected throughout the course of this thesis has been used to evaluate the status and develop a management plan for both species.

In addition to understanding mutualisms at low mutualist densities, I hoped that this study would shed light on why the mutualism between moths and yuccas abruptly stops in southeast Alberta. There is a tremendous amount of apparently suitable and undisturbed habitat north of the Onefour and Pinhorn population, but it remains unoccupied by yuccas. It does not seem reasonable to suggest that yuccas and yucca moths are at the limit of physiological tolerance as there are several small patches of transplanted yuccas up to 150 km north of our Alberta study sites near Medicine Hat, AB and Fox Valley, SK. These small groups of plants are capable of producing fruit if sufficient amounts of soil containing prepupal larvae were transported with the plants. However, there are also nearby transplanted plants that have never set fruit, apparently because moths are not present. These observations suggest that dispersal of moths is limiting the distribution of the mutualism within the current range of the plant. It is plausible that moths are unable to disperse to suitable sites because they must fly through an inhospitable matrix or that their flying abilities are reduced in the north. North of Medicine Hat however, it is expected that the moth, will be physiologically limited and will be unable to survive possibly due to the inability of larvae to survive soil freezing.

The yucca is expected to have a broader tolerance of climatic conditions as commercial varieties are know to survive in gardens north of the area.

There are only two native populations of yuccas and yucca moths in Canada. Only the Onefour population has been intensively studied throughout this research since the Pinhorn population has failed to reproduce in recent years, although flowering levels, visitation and moth density were reassessed annually. In general, the Onefour population appears to be stable and well adapted to deal with the unique problems that the population faces at the northern edge of range. The mutualism between yuccas and yucca moths persists in this population and results in reproductive output similar to other populations further south, although the mechanics of the interaction are different than that of other yuccas. The Pinhorn population appears to be in decline as the plants have failed to set fruit for at least 7 years and the yucca moths are nearly extinct (no female yucca moths have been observed during the course of this study). In essence, a mutualist partner has become extinct and the mutualism has dissolved. Both populations experience similar climates, frequencies of adverse weather conditions, threats, management practices and likely share a common history as they are both on the same watershed and are less than 20 km apart. The question is why have similar conditions resulted in two different fates for the mutualistic interaction.

The primarily difference between sites is population size of yuccas and the differential effect that herbivory has on populations of different sizes (Pinhorn: 404 clones, Onefour: 8499 clones). Both populations experience wild ungulate herbivory, but the Onefour population is apparently buffered in some years from its effects by high levels of flowering. The available biomass of inflorescences in Pinhorn is always low relative to Onefour and is apparently not large enough to satiate the herbivores. Even when flowering is extremely low in Onefour, some inflorescences escape predation. In essence, herbivory is more catastrophic for Pinhorn than for Onefour, and due to Pinhorn's small population size herbivory is catastrophic every year and eliminates sexual reproduction and recruitment. Repeated catastrophes, in combination with environmental and demographic stochasticity, is particularly detrimental to small populations frequently leading to population extinction (e.g. Mangel and Tier 1994). The ability of the Pinhorn population to naturally recover is extremely low given that moths would have to recolonize the site during a period when deer are low and would only be able to survive if deer densities remained low.

The Onefour population is of particular conservation value because of its large population size, its apparent adaptations to the environment at the northern edge of range and it undisturbed location. This population exhibits considerable phenotypic variation in the timing of flowering and floral morphology / coloration, and is predicted to have high genetic diversity relative to other populations, although this is as of yet unstudied. Although at this time this population is apparently secure, its persistence is reliant on the stability of current conditions. Population growth rates are only slightly above 1 and could begin to decline if the population is not well adapted to deal with increased intensity or variability in factors extrinsic to the mutualism such as herbivory. Although, recruitment of young individuals contributes little to population growth at this time, some seed production is necessary which means that the system must be preserved in tact to ensure the survival of both species and the mutualism between them. Further, in the face of global change, the relative importance of reproductive success will likely increase as yucca populations expand northward and rely on seed production to invade new habitats. Overall, current conditions at Onefour are appropriate for the persistence of the mutualism; however, one must keep in mind that these conditions are not necessarily appropriate for future survival in the face of changing conditions.

Although the yucca continue to exist in the Pinhorn population, moths have been extirpated and the mutualism has dissolved. Eventually, the plant population will decline (although at a slow rate) as individual clones die off and are not replaced by new clones via seedlings. Asexual reproduction via the production of new rosettes slows the process of decline for the plant, but will not prevent the inevitable demise of the population. The population projection model used in Chapter 2 suggests that yucca populations at Pinhorn will have a half-life of 50 years when clones fail to reproduce sexually, provided the no major catastrophic events take place that increase the mortality of adults. The mutualism in Pinhorn will only be reestablished if 1) herbivory is reduced so that flowers can survive and 2) moths recolonize the site after the presence of flowers has been secured. It is unlikely that moths will naturally recolonize the population as they are particularly weak flyers and large numbers of moths will have to colonize the site before the current generation of plants dies off. It is my belief that to conserve the moth-yucca mutualism in Pinhorn, efforts to reduce floral herbivory (via fencing) and reintroduce the moth will need to be conducted in the very near future. Although efforts to reintroduce pollinators to obligate mutualisms are unheard of, moths could be quite easily transported from the Onefour or Montana populations via mature, undehised fruit.

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