

Not Easy Being Mead's: Comparative Herbivory on Three Milkweeds,
Including Threatened Mead's Milkweed (*Asclepias meadii*),
and Seedling Ecology of Mead's Milkweed

BY

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Abstract for Chapter 1: Comparative Herbivory and Herbivore Effects on Reproduction for Three Milkweeds (*Asclepias*) in Two Landscape Contexts.

The ability of herbivores to regulate plant populations depends on many factors including herbivore species, abundance, and phenology. These factors can be dynamic across the landscape and between plant species. Where plant species share common herbivores, they may also interact with each other through apparent competition, a form of associational susceptibility. Milkweeds (*Asclepias*) are an excellent system for studying the relationships between herbivores, plants, and plant reproduction in a complex community. Milkweeds are well-known for their plant defenses and the guild of specialist insects that feed on them, but generalist herbivores also feed on some milkweeds. I compared herbivore activity and its effect on plant reproduction for three milkweed species (*Asclepias syriaca*, *Asclepias viridis*, *Asclepias meadii*) in eastern Kansas. I selected plants of each species at two field sites in distinct prairie landscapes and monitored them for herbivory throughout the growing season, from before bud formation to seed pod maturation. Milkweed herbivores showed major differences in abundance and phenology between plant species and study site. Damage from herbivores was implicated in the reproductive failures approximately half of all *A. meadii* and *A. viridis* ramets but only a small portion of *A. syriaca* ramets. One of the milkweed species, *A. meadii*, is federally threatened, so a better understanding of this milkweed-herbivore community has conservation management implications. High rates of herbivory on *A. meadii* suggest that herbivore control measures, especially for deer, would be beneficial to some populations of this rare plant.

Abstract for Chapter 2: Influence of seed characteristics and site conditions on establishment of a threatened prairie milkweed, *Asclepias meadii*, in Kansas.

Population restoration and reintroduction are critical aspects of many plant conservation efforts but factors affecting the earliest life history stages, critical to the establishment of new individuals, are often poorly understood. I investigated the influence of seed characteristics and manipulations of the field environment on seedling emergence and seedling growth in *Asclepias meadii* (Mead's milkweed), a federally threatened tallgrass prairie species. Seeds of known mass and maternal plant were reared in a greenhouse and also in experimental restoration plots with combinations of burning and soil disturbance treatments. Seed mass was positively correlated with seedling emergence but not seedling growth. There was no detectable effect of burning on seedling emergence, but it had a negative effect on seedling growth. The effects of soil disturbance on both seedling emergence and growth were non-significant. Mass may be a useful metric for evaluating seed stocks for reintroduction and the quality of seeds produced in restored populations. Pre-emergence manipulations of a restoration site do not facilitate emergence or growth and may even be detrimental to restoration efforts. High survivorship of seedlings during their first year and overwinter suggests that direct sowing of seeds into the field is an effective restoration technique for *A. meadii*.

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On the cover: Mead's milkweed (*Asclepias meadii*) experiences high rates of damage from a variety of herbivores, including the familiar monarch caterpillar (*Danaus plexippus*). © Steve Roels 2009.

Introduction

Mead's milkweed (*Asclepias meadii*) is a federally threatened tallgrass prairie species in need of recovery. Populations are small and highly fragmented and only a handful of sites have formal protection. In addition, many populations on private land are under management regimes poorly suited for the plant and the long term existence of these populations is in doubt (USFWS 2003). Individual plants take several years to reach maturity and must run a gauntlet of vertebrate and invertebrate herbivores including deer, rodents, and a suite of specialized insects in order to successfully reproduce.

Understanding the challenges that Mead's milkweed faces throughout its life cycle can inform conservation management decisions critical to the protection of existing populations and the success of future restoration efforts. My research focused on two areas of Mead's milkweed natural history, herbivory and seedling ecology.

Herbivory - The U.S. Fish & Wildlife Service Mead's milkweed recovery plan (2003) specifies herbivory as a key research area stating, "Future research should focus on identifying and determining how to manage critical external factors, such as insect herbivores or pathogens, that can significantly reduce reproductive effort in Mead's milkweed" (pg. 38). Prior Mead's milkweed researchers have also speculated on the importance of herbivores, particularly specialist insects (Betz, 1989; Bowles et al. 1998). High rates of herbivory can not only limit the number of seeds entering natural populations, but also limit the availability of seeds that can be collected for restoration purposes.

Meadow milkweed is only one of several species in the diverse milkweed community in eastern Kansas and prairies with Meadow milkweed commonly have other milkweeds present as well. These congeners are likely to share herbivores with Meadow milkweed, due to their ecological and phylogenetic similarities, which allows for the possibility of herbivore-mediated indirect interactions between milkweed species. Of particular interest are plausible scenarios of "apparent competition" between Meadow milkweed and more common milkweeds. In apparent competition, one plant can reduce the equilibrium abundance of another by supporting an herbivore that feeds on both plants (Holt 1977). An intimate knowledge of the natural history of milkweed-herbivore communities, including the distribution, abundance, and phenology of many organisms, is required to understand the relative importance of the myriad interactions observed between milkweeds and herbivores and recognize possible cases of indirect interactions between milkweeds.

Seedling ecology - The USFWS recovery plan also identifies seedling ecology and establishment as important research topics. The plan references work by Bowles et al. (2001), which demonstrated that the success of Meadow milkweed restorations can be influenced by land management decisions. The USFWS further recommends that "restoration projects should coincide with replication of this [Bowles et al.'s] research and identification of other possible factors influencing recovery" (pg. 36). There is good empirical evidence that certain environmental conditions, like high rainfall and early-season burning, are beneficial for mature plants (Kettle et al. 2000; Grman & Alexander

2005), but management decisions would ideally be based on more comprehensive knowledge of the entire life history of the plant. Seedling establishment in natural populations of Mead's milkweed is largely a "black box." Although some general principles of seedling establishment in grasslands have been proposed (Edwards & Crawley 1999; Isselstein et al. 2002; Jensen & Gutekunst 2003), species-specific conditions for establishment are poorly understood for many grassland plants. Observational greenhouse and field studies of germination and establishment have been important first steps (Betz 1989; Bowles et al. 1998; Row et al. 1999; Bowles et al. 2001), but our understanding of the conditions that influence natural recruitment would benefit from a more experimental approach.

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Chapter 1: Comparative Herbivory and Herbivore Effects on Reproduction for Three Milkweeds (*Asclepias*) in Two Landscape Contexts

Introduction

The role of herbivory in regulating plant populations is increasingly appreciated by plant ecologists (del-Val & Crawley 2005; Halpern & Underwood 2006; Maron & Crone 2006). Even in cases where herbivory does not lead to mortality in mature plants, herbivores can influence population trajectories through damage to reproductive tissues and seeds or by forcing plants to reallocate resources away from reproduction to regrowth or defense. Understanding herbivore identity, abundance, and phenology is critical for insight into the effect of herbivory on a plant population (Russell & Louda 2004; Russell et al. 2007). Additionally, plant-herbivore interactions are not necessarily static across the landscape (Kruess 2003; Tschardtke & Brandl 2004; Barbosa et al. 2009). Plants may grow in areas of varying suitability to a particular herbivore (Miller et al. 2009); patch dynamics may allow some host populations to be unoccupied by an herbivore (Östergård & Ehrlén 2005); plant genotypes may have varying degrees of herbivore resistance (Strauss & Agrawal 1999); and populations of multiple host species may intermingle (Russell & Louda 2005; Barbosa et al. 2009). Where the last situation is true, an emergent pattern of herbivore-mediated indirect interactions between host plants can arise, often called *apparent competition* when the interaction is negative (Holt 1977). By collectively supporting an herbivore that feeds on both plants, two host species can negatively affect each other's equilibrium abundance without directly competing for

resources (Holt 1977). More generally, increased herbivory on one plant due to the presence of another, regardless of the mechanism, is termed *associational susceptibility* (Barbosa et al. 2009). Herbivore-mediated indirect interactions have been observed between native plants and herbivores (Rand 2003), as well as among various combinations of native and exotic species (Russell & Louda 2005; Lau & Strauss 2005). Anthropogenic changes to the landscape, such as agriculture, disturbance, or suppression of natural disturbance, can also influence plant-herbivore interactions (Tschardt & Brandl 2004).

The detrimental effects of herbivores on plant reproductive output are particularly significant for species that are seed limited or rare. Although herbivory is not generally considered a primary reason for species endangerment, high rates of herbivory can hinder species recovery efforts. Consideration of herbivores has become an important part of restoration or reintroduction plans for some rare plants (e.g. Phillips & Maun 1996; Beville et al. 1999). Where plants with conservation needs are growing in proximity to common or invasive species with which they share herbivores, harmful apparent competition may also have conservation management implications (Orrock & Witter 2010).

Milkweeds (Aponcynaceae: *Asclepias*) make an excellent system to explore questions about complex plant-herbivore interactions in a diverse community. Milkweeds are well known for their multifaceted defenses against herbivory, including production of latex and toxic secondary compounds, and the specialist insect herbivores that have evolved to

circumvent those defenses (Agrawal et al. 2008). Despite their defenses, some milkweeds are also damaged by generalist herbivores, including mammals (personal observation). The most familiar species of *Asclepias* are abundant, weedy plants that grow in frequently disturbed areas, but nineteen species of *Asclepias* in the United States are listed as state threatened or endangered and two more are federally threatened (USDA 2011). In eastern Kansas, for example, two weedy species, *Asclepias syriaca* Linnaeus (common milkweed) and *Asclepias viridis* Walter (green antelopehorn milkweed), do well in human-dominated landscapes. In contrast, federally threatened *Asclepias meadii* Torrey ex A. Gray (Meadø milkweed) is found almost exclusively in native prairies with a limited history of human impacts. Populations of the weedy species often occur in close proximity to *A. meadii*, growing both in disturbed areas adjacent to prairies and in the prairies themselves. I investigated the diversity of the milkweed herbivore community and the probable effect of herbivores on reproductive output for these three species of *Asclepias* at two eastern Kansas field sites in distinct prairie landscapes. Key questions include: 1) Which herbivores attack each plant species?; 2) How frequent is herbivory?; 3) How does herbivory differ through time and by landscape?; 4) How does herbivory impact reproduction?; and 5) What herbivore-host relationships are most likely to give rise to herbivore-mediated indirect interactions between host plants?

Methods

Study Species ó Fifteen *Asclepias* species occur in eastern Kansas, USA (Barkley, 1986), and, despite ecological differentiation, many species can often be found in the same

location. In this work, I focus on three species. *Asclepias syriaca* is widespread in open habitats, especially frequently disturbed areas such as roadsides. *Asclepias viridis* is also found in open areas, including mowed grasslands and heavily grazed pastures. These two species are easily the most abundant *Asclepias* species in eastern Kansas (personal observation). In contrast, *A. meadii* is found in high-quality, unplowed tallgrass prairies, which today exist only as isolated fragments in a highly modified landscape (USFWS 2003). *A. meadii* was formerly widespread throughout the tallgrass prairie region of the central Midwest, but land use changes nearly eliminated the plant by the beginning of the 20th century (Betz 1989; USFWS 2003). The majority of known *A. meadii* populations are found in eastern Kansas and the long term existence of many of these populations is in doubt due to stochastic extinction, inbreeding depression, and land use changes by private landowners (USFWS 2003; Kindscher et al. 2009).

Study Sites ó I performed plant/herbivore surveys at two sites in distinct landscape regions in eastern Kansas: the Rockefeller Native Prairie and adjacent areas (RNP) and the Anderson County Prairie Preserve (ACPP), a protected area owned by the Nature Conservancy. These sites were chosen because they each have a diverse milkweed community, including unusually large populations of *A. meadii*. The sites are approximately 95 km apart (Appendix 1-1) and both are units of the University of Kansas Field Station.

The RNP (Leavenworth Co., KS, 39° 2'44.01"N, 95°12'18.41"W) includes a 4.0 ha virgin prairie that has never been plowed and an additional 0.5 ha area restored to native

vegetation in 1957 (Kettle et al. 2000). In recent decades, the RNP has been managed through biennial burning; occasional mowing has also been incorporated into the management regime in recent years. Much of the adjacent land is heavily wooded and the prairie is embedded in a landscape matrix of secondary forest, old fields, and non-native pasture (see Appendix 1-2a for site aerial). Remarkably, ten *Asclepias* species are present on the RNP (pers. comm. Caleb Morse). The *A. meadii* population on the RNP has been studied for over 20 years and all known plants are permanently tagged (Alexander et al. 2009). The study population of *A. syriaca* was growing in the virgin prairie and along a former fencerow on the prairie margin. The study population of *A. viridis* was in an annually mowed field of native vegetation adjacent to the virgin prairie; *A. viridis* does occur on the virgin prairie itself but population density was more suitable for this study in the adjacent field.

The ACPP (Anderson Co., KS, 38°10'53"N 95°16'2"W) includes 554 ha of native prairie, restored prairie, and native pasture and is situated within the largest native grassland complex in eastern Kansas (Nature Conservancy 2011) (see Appendix 1-2b for site aerial). The ACPP study populations of *A. meadii* and *A. viridis* were growing intermingled in a historically hayed native prairie management unit; in the last decade, a varied management regime of haying, burning, and resting has been employed. The ACPP study population of *A. syriaca* was growing along a roadside at the margin of a prairie restoration approximately 500 m away from the *A. meadii* and *A. viridis* populations.

Plant Selection 6 50 flowering individuals of each species were flagged at each field site in early May, 2010. The definitions of the unit studied, however, depend on the species. *A. syriaca* and *A. meadii* often have multiple ramets (above-ground stems) growing from the same genet (individual rhizome), but ramets are typically spaced out, making identifying individuals a challenge; 50 individual ramets were monitored for these species. *A. viridis* ramets grow in tight clumps that likely represent an individual plant, so 50 clumps of ramets were monitored for this species. All selected plants were a minimum of 3 m apart to reduce the non-independence of herbivore damage on neighboring plants and also reduce the likelihood of selecting two ramets from the same genet in *A. syriaca* and *A. meadii*. In *A. meadii*, it appears reasonable to assume ramets more than 1.25 m apart are from different genets (Kettle et al. 2000). Vegetative ramets were not included since plant reproduction was a focus of this study, although herbivory also occurs on these ramets. Heavy losses of RNP *A. meadii* ramets to herbivory early in the study prompted the selection of 23 additional ramets at the RNP and 28 additional ramets on the ACP in mid-June. At the ACP, 14 of the original *A. syriaca* ramets failed to develop buds (reproductive status could not be ascertained at the time of initial selection) and each ramet was replaced with the nearest neighbor with buds or in flower once the non-reproductive status of the original ramets became apparent. Herbivore data for replaced ramets in the ACP *A. syriaca* population is a combination of the original ramet up to the time it was replaced, and the replacement ramet thereafter. I did not notice any difference in herbivore abundance between ramets with and without buds up to the time the non-flowering ramets were replaced. A single ACP *A. syriaca* was destroyed by an ATV mid-study and was removed from the data set.

Data Collection ó I visited each field site approximately every 4 days from early May 2010 through the end of July and approximately every 7 days after, until the last seed pods (follicles) of any species dehisced (mid-September at the ACPP and mid-October at the RNP). During each visit, I inspected plants for herbivore presence or damage. When damage was discovered, I assigned it to a likely herbivore based on the pattern of damage (see results below). I did not assess subterranean herbivory since it would have required destructive sampling of the plants. Once a plant completed flowering, I recorded the number of developing pods on each stem. I infrequently checked individual plants once they no longer had active pods and I stopped monitoring each species once all pods of that species were collected at a site. I coded seed production as a binary character for each plant.

For *A. syriaca* and *A. meadii*, herbivory data refers to a single ramet. In the case of *A. viridis*, data were collected for each ramet within the clump. The 50 clumps selected at the RNP produced a total of 120 flowering ramets. The number of ramets in each clump ranged from 1 to 6 with a mean of 2.4 (s.d. = 1.4). The 50 clumps selected at the ACPP produced a total of 140 flowering ramets. The number of ramets in each clump ranged from 1 to 10 with a mean of 2.8 (s.d. = 2.1). Due to the non-independence of ramets in the same clump, *A. viridis* data are analyzed on both the scale of individual ramets and clumps.

Statistics ó Statistical analyses were done in Minitab 14 (Minitab Inc. 2003) with $\alpha = 0.05$. When sample size was sufficient, I used binary logistic regression models to evaluate the effects of plant species and study site on herbivore presence. I used non-parametric methods to compare plant and herbivore phenologies between species and sites.

Results

Herbivores Identified and Herbivore Damage Patterns ó In almost all cases, herbivore damage could be assigned to a likely species or group of species (Table 1). Herbivory by mammals could be recognized by large scale damage or destruction of a ramet. Two types of mammals visited the milkweeds: white-tailed deer (*Odocoileus virginianus* Zimmerman) and small gnawing mammals. Deer damage was characterized by complete removal of the top portion of the stem, with the bottom portion remaining. Small mammal damage was characterized by stems cut diagonally near the ground and the presence of gnawed stem sections. Insect damage was generally focused on particular tissues of the plant, such as the stem, leaves, buds, or developing pods. I found four main types of insect herbivores: a weevil, *Rhyssomatus lineaticollis* Say; two caterpillars, *Danaus plexippus* Linnaeus and *Cycnia inopinatus* (Hy. Edwards); two bugs, *Lygaeus kalmii* Stål and *Oncopeltus fasciatus* (Dallas); and a longhorn beetle, *Tetraopes tetropthalmus* Förster. *Rhyssomatus lineaticollis* adults feed on leaves, rasping holes in the leaf surface. Females oviposit in the stems and pods, leaving behind distinctive bore holes, and grubs feed on pith tissue or developing seeds (Fordyce & Malcolm 2000).

Danaus plexippus feeds on leaves and buds and *C. inopinatus* feeds on leaves and occasionally pods. Caterpillar damage could not be distinguished between species, so I only assigned damage to one species or the other when the caterpillar was seen on the plant. *Lygaeus kalmii* and *O. fasciatus* attack developing pods, using their long mouthparts to feed on the seeds inside. Feeding by the bugs does not necessarily leave behind obvious external damage, so damage by each species was recorded only when the insects were present. The nymphs of these two species are difficult to tell apart, so they were recorded simply as lygaeid nymphs. *Tetraopes tetrophthalmus* adults feed on leaves and buds; grubs feed on rhizomes but belowground damage could not be assessed (Farrell & Mitter 1998). Other herbivorous insects observed infrequently included aphids (*Aphis* sp.), the milkweed tiger moth (*Euchaetes egle* (Drury)), the delicate cynthia (*Cycnia tenera* Hübner), a stink bug (*Euchistus* sp.), and an unidentified planthopper (Fulgoroidea). One milkweed specialist not observed on any plant in the study was the swamp milkweed leaf beetle (*Labidomera clivicollis* Kirby), which I have observed rarely on *A. viridis* and not at all on *A. syriaca* or *A. meadii*.

Herbivore Frequency ó Herbivores showed a wide range of occurrence rates across the plant populations, which were not uniform between all plant species and only occasionally similar between study sites (Table 2). Occurrence rates appear most similar when the herbivore is very common (e.g. *R. lineaticollis* on *A. viridis* at both sites) or rather uncommon (e.g. *D. plexippus* on *A. syriaca* at both sites). Binary regression models for herbivore presence on *R. lineaticollis*, *O. fasciatus*, and nymphs showed significant effects of plant species and site, as well as interactions between plant species

and site (Appendix 1-3). Although other herbivores did not have sufficient sample sizes for effective regression modeling, there are other notable patterns in the data where an herbivore is common in some populations while absent or rare in others. For example, *D. plexippus* was common on ACPP *A. meadii* but absent from RNP *A. meadii*, and rare on *A. syriaca* and *A. viridis* at both sites (Table 2). Another herbivore, deer, was common on RNP *A. meadii* but absent from all other milkweed populations, except for a single RNP *A. viridis* ramet (Table 2).

Plant Phenologies ó Plant phenology (Tables 3a-f) differed significantly between species, with successive 11-day shifts in flowering time between the earliest, middle, and latest species to flower (Kruskal-Wallis test on day of first flowering, $p < 0.001$; data was pooled between sites, Appendix 1-4). *A. viridis* flowered earliest (median Julian day of first flower = 144), *A. meadii* second (median = 155), and *A. syriaca* last (median = 166). Mann-Whitney U tests showed that the effect of site on plant phenology was not the same for each species (Appendix 1-4). *A. syriaca* flowering was not significantly different between the RNP and ACPP (median RNP = 167, median ACPP = 166, $p = 0.113$). *A. viridis* flowered significantly earlier on the RNP (median RNP = 140, median ACPP = 148, $p < 0.001$). *A. meadii* flowered significantly later on the RNP (median RNP = 159, median ACPP = 152, $p < 0.001$).

Herbivore Phenologies ó Herbivores also had distinct phenologies throughout the season (Tables 3a-f). Some herbivores showed relatively steady rates of occurrence season-long (e.g. *L. kalmii*), while others had sharp peaks in abundance in a narrow time frame (e.g.

R. lineaticollis on *A. viridis*). Herbivore phenologies are presumably driven in large part by intrinsic attributes of the herbivores, but in some cases may be influenced by the phenologies of the plants. For example, there is a significant effect of plant species on the first records of *R. lineaticollis* for a given ramet; the weevil appeared much earlier on *A. viridis* (median day of first record = 160) than on *A. syriaca* (median = 208) (Mann-Whitney, $p < 0.001$, Appendix 1-4). Investigating this example further, *R. lineaticollis* appearance on *A. viridis* was shifted significantly earlier on the RNP (median = 160) than on the ACPP (median = 166) (Mann-Whitney, $p < 0.001$, Appendix 1-4), similar to the shift in *A. viridis* flowering. *R. lineaticollis* appearance was not significantly different on *A. syriaca* on the RNP (median = 208) and the ACPP (median = 209) (Mann-Whitney, $p = 0.934$, Appendix 1-4), as would be expected given the lack of difference in flowering times for *A. syriaca* between the two sites. In this case, the weevil is likely not tracking the flowering phenology per se, but rather the development of the pods, into which females oviposit, following flowering. Although the weevil also oviposits into stems and feeds on leaves and buds, damage to pods comprised the majority of the records for this species, so differences in plant reproductive timing are likely of primary importance for understanding the phenology of *R. lineaticollis*.

Herbivore Effects on Reproductive Output of Ramets ó Mature pods were successfully produced by 69.7% (69/99) of *A. syriaca* ramets, 29.2% (76/260) of *A. viridis*, 13.0% (13/100) of the original *A. meadii*, and 35.3% (18/51) of additional *A. meadii* (Figure 1). Herbivores were likely responsible for complete reproductive failure in 5.1% (5/99) of *A. syriaca*, 50.0% (130/260) of *A. viridis* (individual ramets), 49.0% (49/100) of the original

A. meadii, and 43.1% (22/51) of additional *A. meadii* (Figure 1 and Table 4). Mammals (deer and small mammals) were responsible for only a small proportion of the likely herbivore-caused reproductive failures in *A. syriaca* and *A. viridis* (only 6 total occurrences) but a large proportion of failures in *A. meadii*, especially at the RNP (25 of original 50 stems). Most insects caused only occasional failures, but *R. lineaticollis* caused a remarkable amount of damage to the reproductive efforts of *A. viridis* at both sites (121 failures out of 260 total ramets or 46.5%).

Discussion

Herbivory was common on each milkweed species, but the identity and frequency of each herbivore depended on population studied. Species also differed in reproductive output and the likely importance of herbivory for reproductive output. Isolating the occurrence or effect on reproduction for a single herbivore species is difficult because the full potential of one herbivore to damage plants is confounded by the presence of other herbivores. Herbivores cannot damage a ramet that is already gone or destroy the reproductive effort of a ramet that has already failed; for example, a plant eaten by a deer in May cannot have its buds eaten by a caterpillar in June. It is also important to keep in mind that this study looked at herbivory on individual ramets and ramet death does not equate to genet death in these perennial plants. Genets that send up multiple ramets may still reproduce, even if some portion of their ramets failed to do so. This was often the case in the larger clumps of *A. viridis*, which could suffer fatal damage on several ramets but still produce mature pods on others.

Patterns of Herbivory ó Mammal herbivory was more common overall on the RNP, where there is more surrounding cover for deer. Also, the prairie vegetation grows much denser on the RNP than on the ACPP, which may encourage small mammal activity. Damage to *A. meadii* by deer was confined to a brief, but intense, period on the RNP, while small mammal damage occurred intermittently throughout the season at both sites. In a previous study of the RNP *A. meadii* population by Grman and Alexander (2005), 63.3% of ramets were lost to herbivory, and they speculated that deer and small mammals were responsible for the majority of the damage. The high rate of damage by mammals to *A. meadii* relative to *A. syriaca* and *A. viridis* (Table 2) suggests that, despite its status as a milkweed, *A. meadii* may in fact be an õice creamõ plantõ a plant so appealing that it is always eaten when herbivores come upon it (Crawley 1997). The deer browsing patterns I observed are consistent with this idea; where *A. meadii* was damaged by deer, the neighboring vegetation was usually not mowed down to a height even with the cut *A. meadii* stem, as it would be if deer were browsing indiscriminately. *A. meadii* is a relatively early emerging forb on tallgrass prairies, which could allow deer to find it more easily early in the season. Deer herbivory did not occur after June 3 (Julian Day 154, Table 3f), so perhaps deer had a difficult time finding *A. meadii* once the surrounding vegetation became denser. The small mammal damage pattern seen in *A. meadii* (stems cut into sections with diagonal cuts) is consistent with the foraging behavior of the hispid cotton rat (*Sigmodon hispidus*), a common species in eastern Kansas grasslands (Robert Timm, pers. comm.). Traits that influence the palatability of *A. meadii*, such as lignin content and concentrations of toxic compounds, have not yet been compared to other

milkweeds. The small mammal damage on *A. syriaca* was probably from rabbits; cuts in the stem were coarser than in *A. meadii* and rabbit pellets were found at the base of one damaged plant. This damage occurred late in the season when *A. syriaca* ramets were drying out and the amount of latex exuded from damaged tissues seemed to be lower than earlier in the year.

Overall, damage from specialist insects was common on each milkweed species and at both sites, although not all insects were found on each species or in each population. The weevil, *R. lineaticollis*, was fairly common on *A. syriaca*, although more frequent on the ACPP, nearly ubiquitous on *A. viridis* at both sites, and infrequent on *A. meadii* at both sites. While surveying for this weevil at other locations in eastern Kansas, I found it much less frequently on *A. syriaca* than on *A. viridis* (unpublished data). The caterpillars, *D. plexippus* and *C. inopinatus*, were far more common on the ACPP, but it is possible that the lack of *C. inopinatus* records from *A. viridis* on the RNP reflects the end of data collection in that population just prior to the most active time for *C. inopinatus* (late July/early August or Julian Days 200-220, Tables 3c, e, f). Although it did not occur during this study, I have occasionally observed *D. plexippus* on *A. meadii* on the RNP. The comparative abundance of adult *O. fasciatus* to adult *L. kalmii* and the close temporal alignment of adult *O. fasciatus* and lygaeid nymphs suggest that the majority of the nymphs recorded were young *O. fasciatus* and not *L. kalmii* (ex. Table 3a). *Tetraopes tetrophthalmus* appeared more frequently on all plant species on the ACPP but reasons for this are unclear. Following a broad survey of specialist insects on several milkweed species at multiple sites, Price and Willson (1979) proposed that microenvironmental

variables, especially edaphic conditions and available moisture, were major factors in determining patch occupancy and abundance of herbivorous insects. It is important to remember that insect abundances can vary dramatically between years and locations; any firm conclusions regarding patterns of occurrence should be based on several years of survey data.

Due to the strong seasonal patterns in herbivore activity, data for *A. meadii* ramets added partway through the study should not be directly compared to the original set of *A. meadii*. Most notably, the additional plants were selected after the last occurrence of deer herbivory on the RNP and *D. plexippus* herbivory on the ACPP, explaining why none of the additional RNP stems had records of these herbivores. Also, *A. meadii* pods had begun to develop at both sites when the extra plants were selected, which is why no additional ACPP stems fell in the ðflowered but no podsö category.

Effects of Herbivory on Reproductive Output of Ramets ó Herbivores were likely responsible for the reproductive failure of approximately half of all *A. viridis* and *A. meadii* stems but only a small proportion of *A. syriaca* stems. While study was observational, making definitive links between cause and effect challenging, there is reason to believe that the designated herbivore is the true cause of reproductive failure in these cases. Although resprouting can sometimes occur in *A. meadii* following mammal damage, I have only ever seen one ramet flower after resprouting. The new shoot flowered well after the rest of the population, meaning that it could not be cross-pollinated, which is critical for this self-incompatible species (USFWS 2003). Insects on

all plant species frequently attacked the reproductive structures themselves or caused otherwise healthy ramets with buds or flowers to wither. High herbivore frequency does not necessarily correlate with high culpability for reproductive failure (compare Tables 2 and 4); the impact of each herbivore depended on phenology and feeding behavior. For example, *C. inopinatus* appeared on *A. meadii* well after it flowered and *A. meadii* was able to continue developing pods even when there was heavy damage to the foliage. Reproductive failure only occurred in cases where the caterpillar chewed into the pod itself and destroyed the seeds.

It is likely that the full effects of herbivory exceed what is shown in Table 3 for several reasons. First, ramets were credited with seed production if any pod on the ramet appeared to mature and dehisce naturally. However, the seeds themselves were not evaluated for viability, so reproductive output may be overestimated, especially on ramets with infestations of lygaeid bugs, which can feed directly on developing seeds while causing minimal damage to pod tissues. Second, underground herbivory could not be assessed and it is possible that some of the plant withering I observed was due to rhizome damage, especially by *T. tetraphthalmus* larvae. Finally, seed production was coded as a binary character for each ramet, but all three *Asclepias* can have multiple pods on a single ramet and there were cases where insect herbivory destroyed some, but not all, pods on a ramet. In this study, the number of pods matured per ramet ranged from 1-8 for *A. syriaca*, 1-2 for *A. viridis*, and 1-2 for *A. meadii*. There is also some possibility that herbivory could have lasting effects; a study of *R. lineaticollis* and *D. plexippus* damage on another milkweed, *A. quadrifolia*, showed significantly reduced ramet and

inflorescence size relative to undamaged plants the year after the attack (Chaplin & Walker 1982).

Although damage by *R. lineaticollis* to *A. syriaca* was frequently recorded, the lack of serious harm to reproductive output was surprising, especially in contrast to the drastic reduction in pod production the weevil caused to *A. viridis*. The relationship between *R. lineaticollis* and *A. syriaca* has been frequently studied in other regions of the country (Price & Willson 1979; Fordyce & Malcolm 2000; Agrawal & van Zandt 2003; St. Pierre & Hendrix 2003). In my study, *A. syriaca* pods with weevil larvae present were found only four times at the RNP and two times at the ACPP. This outcome was even more unexpected at the ACPP because *R. lineaticollis* adults and larvae were abundant on a population of *A. sullivantii* (prairie milkweed) directly adjacent to the study population of *A. syriaca*, although this fact may explain the higher frequency of observed weevil damage in the ACPP population. Whether these results represent shifts in host preference by the weevil, the ability of regional populations of *A. syriaca* to resist weevil infestation, or some other mechanism is unclear. One possible explanation relates to the spinose processes on the exterior of *A. syriaca* pods, which vary in length between clones. Price and Willson (1979) observed that pods with longer processes had lower rates of weevil damage; I did not attempt to assess relative spine length in my study populations. A poorly known parasitoid wasp may also play a role in *R. lineaticollis* behavior (Webster 1895).

The large proportion of ACP *A. meadii* that flowered but failed to initiate pods (36% of the initial 50 plants) suggests that pod production in this population may be limited more by problems in the reproductive cycle than by herbivory. Possible reasons for the low fruit set I observed include low genetic diversity, lack of pollinators, and limited maternal resources. *A. meadii* is self-incompatible and populations of *A. meadii* that have a history of mid-summer haying, thereby inhibiting sexual reproduction, have lower genetic diversity than populations on prairies managed with fire (Tecil et al. 1998). Future work on the pollinators and genetics of this globally important population is needed to understand challenges to reproduction other than herbivory.

Possibilities for Herbivore-mediated Indirect Interactions ó While the observational nature of this study does not allow for an explicit test of apparent competition, it does illustrate natural patterns of herbivory for these milkweeds. Biologically relevant indirect interactions between the plants can only occur where they share a common herbivore, which is also present in sufficient numbers and capable of causing significant damage to the plant. It is also important to distinguish between herbivory occurring independently on two plant species by the same herbivore, and herbivory that would not occur were it not for the presence of another plant species in the community. I believe that much of damage to *A. meadii* by specialist insects falls in the latter category. Specifically, I hypothesize that herbivory on *A. meadii* is influenced by the presence of other milkweed species. *Asclepias meadii* is considered rare across its entire extant range and all known populations are small; the sites used in this study are considered some of the largest populations, with a few hundred individuals. This suggests that there would be

insufficient plant material to maintain insect herbivore populations in the absence of other host plants.

In addition to the paucity of individuals, the small stature of *A. meadii* suggests that its use by specialist insects may represent a less than ideal choice of host. Well developed *R. lineaticollis* grubs found in other host species met or exceeded the typical diameter of *A. meadii* stem, making normal grub growth inside stems of this species unlikely, although grubs inside *A. meadii* pods may have sufficient space to grow. The thin stems of *A. meadii* also make the plant more susceptible to weevils; I have never observed a ramet survive following a weevil attack to the stem. This contrasts with *A. syriaca*, where mortality from weevil stem attacks is low (Agrawal & van Zandt 2003). *R. lineaticollis* appears to have a preference for attacking thicker stems in *A. syriaca* (Agrawal & van Zandt 2003), making its presence on slender *A. meadii* even more unexpected. *A. meadii* stems attacked by the lepidopterans were often completely stripped of their leaves; when *C. inopinatus* and *D. plexippus* were observed on other *Asclepias* species, they appeared to consume much more biomass than is present on the average *A. meadii* ramet (personal observation). *T. tetrophthalmus* was rare on *A. meadii*; it is possible that only adults feed on plants because *Tetraopes* species are thought to have strong fidelity to a single host species (Farrell & Mitter 1998). The physical characteristics that make *A. meadii* a poor host for many milkweed specialists, along with its rarity, suggest that it does not independently support populations of the insects that attack it. It is more likely that the insects are maintained in the landscape by other species of *Asclepias*, and that *A. meadii* is, in a sense, collateral damage.

Of the specialist insects, I believe *R. lineaticollis* and *C. inopinatus* have the most potential to be mediators of apparent competition between *A. meadii* and more common *Asclepias* species. Both insects exclusively utilize milkweeds and are residential species, unlike the monarch, which is a highly mobile migrant probably capable of locating *A. meadii* even without an association with other milkweeds. When they do occur, both species have the ability to inflict heavy damage on vegetative tissue and developing pods. Although *R. lineaticollis* was not common on *A. meadii* at either site during this study, it was more frequent during the previous year on the RNP, possibly accounting for an almost 25% reduction in ramets producing pods (unpublished data). *R. lineaticollis* has been previously noted as a possibly important herbivore of *A. meadii* by others (Betz 1989; Bowles et al. 1998). Associational effects influencing weevil damage have been previously suspected between *A. syriaca* and *A. amplexicaulis* (Price & Willson, 1979). Of the alternative host plants, both insects were far more common on *A. viridis* than *A. syriaca*, suggesting that *A. viridis* may be the more important source of insects attacking *A. meadii* in eastern Kansas. Surveys of additional *A. meadii* populations would be useful to determine which of these congeners it is most commonly associated with and the typical density and proximity of congeners to *A. meadii* individuals. An explicit test of the apparent competition hypothesis could be done by surrounding *A. meadii* ramets with varying densities of transplanted or potted congeners, similar to the experimental design by Rand (2003).

Implications for conservation management ó Prior to European settlement of the tallgrass prairie region, *A. meadii* was apparently widespread in upland areas, although probably still uncommon (Betz 1989; USFWS 2003). In contrast, *A. syriaca* and *A. viridis* likely occupied ephemeral disturbances caused by bison, burrowing mammals, and Native Americans (Platt 1975). This historical pattern has been reversed by decades of anthropogenic disturbance, and weedy species now dominate the landscape. The shift in species distributions and abundances may have also influenced the population structure of specialist milkweed insects. For example, in a mark-recapture study of *R. lineaticollis* in Iowa, St. Pierre & Hendrix (2003) demonstrated that despite its sedentary habits, the weevil is rarely absent from patches of *A. syriaca* and probably functions as a patchy population, due to the abundance and short distances between suitable patches. However, the authors believe that in pre-settlement times the population structure was likely more similar to a metapopulation. The near ubiquity of *A. syriaca* and *A. viridis* in modern eastern Kansas may amplify the effects of milkweed insects on *A. meadii*, which cannot escape to enemy-free space (Holt & Lawton 1994), even though it is probably not the preferred host.

Regarding the generalist mammal herbivores, the most striking contrast in herbivory between species and landscapes is the high frequency of deer herbivory on RNP *A. meadii*. Multiple authors in the 1930s and 1940s considered white-tailed deer extirpated in Kansas and in 1963 an estimate of less than 0.5 deer/km² was made for Douglas County, KS, which includes part of the RNP (Hall 1964). Since that time, the encroachment of woody vegetation, the eventual development of secondary forest, and

ample food in agricultural fields have led to explosive growth in the deer population of eastern Kansas. Deer density surveys around the RNP from 2008-2010 estimated approximately 15 deer/km² (pers. comm. Robert Hagen). Anecdotally, I regularly flushed deer from the RNP during the study but never saw a deer at the ACPP, which has almost no suitable cover for deer in the area where *A. meadii* is present. Efforts to reduce deer numbers through culling or elimination of favorable habitat would likely be beneficial to the *A. meadii* at the RNP, as well as a number of other species that suffer from heavy deer browsing. I have also observed deer damage on two other milkweeds occurring on the RNP, *A. purpurascens* and *A. amplexicaulis*, which are considered vulnerable species in Kansas (USDA 2011).

A. meadii can be protected from herbivore damage through the use of exclusion cages (unpublished data), but this is a time intensive method of protection, especially when considering that any enclosure must be partially opened during the blooming period to allow for pollinator access. Another potential method of protection from insect herbivory is careful use of insecticide, which has already been suggested for another threatened plant species (Bevill et al. 1999). Insecticide use would only be advisable in extreme cases of herbivore damage and indirect methods of managing insects, such as restoration of larger areas, thereby reducing local abundance of weedy *Asclepias*, may be a better long-term solution.

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Tables and Figures

Table 1. Frequently observed milkweed herbivores in eastern Kansas. Herbivores were considered generalists if milkweeds do not form a major portion of their diet, specialists if milkweeds are the predominate host plant, and exclusive if they feed solely on milkweeds during some stage of their life cycle.

<i>Species</i>	<i>Common Name</i>	<i>Category (Family)</i>	<i>Selectivity</i>
<i>Odoicoileus virginianus</i>	White-tailed deer	Mammal (Cervidae)	Generalist
-	Small mammals	Mammal	Generalist
<i>Rhyssomatus lineaticollis</i>	Milkweed stem weevil	Beetle (Curculionidae)	Exclusive ¹
<i>Danaus plexippus</i>	Monarch butterfly	Butterfly (Nymphalidae)	Specialist ²
<i>Cycnia inopinatus</i>	Unexpected cycnia	Moth (Arctiidae)	Exclusive ³
<i>Lygaeus kalmii</i>	Small milkweed bug	Bug (Lygaeidae)	Specialist ⁴
<i>Oncopeltus fasciatus</i>	Large milkweed bug	Bug (Lygaeidae)	Exclusive ⁵
<i>Tetraopes tetrophthalmus</i>	Milkweed longhorn beetle	Beetle (Cerambycidae)	Exclusive ⁶

1) St. Pierre & Hendrix 2003; 2) Mattila & Otis 2003; 3) Bess 2005; 4) Wheeler 1983; 5) Farrell & Mitter 1998.

Table 2. Herbivore presence on plants. Each count is the number of unique plants in the survey population that had evidence for the presence of a given herbivore during at least one day of the study. Since herbivore presence on multiple ramets within the same clump of *A. viridis* is non-independent, *A. viridis* data are presented at two scales: entire clumps and individual ramets. "Caterpillar" refers to species other than *D. plexippus* and *C. inopinatus* or cases where caterpillar damage was evident but species was unknown.

Herbivore	<i>A. sylvatica</i>		<i>A. viridis</i> by clump		<i>A. viridis</i> by ramet		<i>A. meadii</i>		Additional <i>A. meadii</i>	
	RNP	ACPP	RNP	ACPP	RNP	ACPP	RNP	ACPP	RNP	ACPP
N	50	49	50	50	120	140	50	50	23	28
Deer	0	0	1	0	1	0	17	0	0	0
Small mammal	9	0	0	0	0	0	9	5	7	3
<i>R. lineaticollis</i>	17	33	47	46	102	105	3	3	1	2
<i>D. plexippus</i>	4	3	1	4	1	4	0	19	0	0
<i>C. inopinatus</i>	3	0	0	27	0	31	4	2	4	15
Caterpillar	0	4	0	2	0	2	5	4	4	3
<i>L. kalmii</i>	9	7	5	5	5	6	1	0	2	0
<i>O. fasciatus</i>	42	28	2	4	2	4	8	0	2	1
Lygaeid nymphs	44	30	4	5	4	5	7	1	5	1
<i>T. tetraphthalmus</i>	12	26	0	11	0	15	0	3	0	2

Tables 3a-f – Herbivore and plant phenologies: Each table shows the activity patterns of herbivores for a given *Asclepias* population. Values in each cell are the number of plants with evidence of new herbivore activity averaged for all survey days within a 10 day period, starting with the column header date (e.g. column “140” there were many cases where multiple ramets within a clump showed herbivore evidence. Herbivores with no records for a particular population are not included as a line in the table. For the flowering phenology line, numbers are the average percentage of ramets (*A. sylvatica* and *A. meadii*) or clumps (*A. viridis*) actively flowering in the study population within a 10 day period. The thick vertical line in the ACPFP *A. sylvatica* table represents the replacement of non-flowering ramets with neighbors. In the *A. meadii* tables, the thick vertical line represents the selection of additional ramets. The additional *A. meadii* ramets selected partway through the study are included in the herbivore phenologies but not the flowering phenology.

Table 3a – RNP *A. syriaca*.

Herbivore	Julian Date															
	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290
Small Mammal	0	0	0	0	0	0	0	0	0	0	0	0	2	1.5	1	3
<i>R. lineaticollis</i>	0	0	0	0.3	0.5	0.7	4.5	4	0	0	0	0	0	0	0	0
<i>C. inopinatus</i>	0	0	0	0	0	0	0	0	0	2	0.5	1	0	0.5	0	0
<i>D. plexippus</i>	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0
<i>O. fasciatus</i>	0	0	0	0	0.5	6	7	8	11	9	12	4	1	4.5	1	1
<i>L. kalmii</i>	0	0	0.5	0	0	0.7	0.5	0.5	0	1	1	3	0	0.5	0	0
Lygaeid nymphs	0	0		0	0	0	7.5	14	22	16	6	2	3	2	2	1
<i>T. tetraphthalmus</i>	0	0.3	2	2	0.5	0.7	0	0	0	0	0	0	0	0	0	0
Flowering %	0	6.7	70	83	8	0	0	0	0	0	0	0	0	0	0	0

Table 3b – ACP *A. syriaca*.

Herbivore	Julian Date											
	140	150	160	170	180	190	200	210	220	230	240	250
<i>R. lineaticollis</i>	0.3	0	0.3	0	0.7	4	7	6	1	0	0	0.5
<i>D. plexippus</i>	0	0	0	0	0	0.5	0	0.5	0.5	0	0	0
<i>O. fasciatus</i>	0	0	0	0	1.3	10	4	4.5	6	12	5	1.5
<i>L. kalmii</i>	0	0	0	0	0	0	0.7	0	0.5	1.5	4	0.5
Lygaeid nymphs	0	0	0	0	0	2.5	12	14	11	10	9	4.5
<i>T. tetraphthalmus</i>	0	0.5	7.3	3.5	2.7	4	0	0	0	0	0	0
Flowering %	0	1.4	74	76	18	0	0	0	0	0	0	0

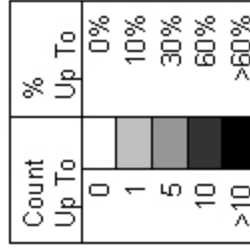


Table 3c – RNP *A. viridis*.

Herbivore	Julian Date						
	130	140	150	160	170	180	190
Deer	0	1	0	0	0	0	0
<i>R. lineaticollis</i>	1	3.3	11	19	0.7	0	0.7
<i>D. plexippus</i>	0	0	0.3	0	0	0	0
<i>O. fasciatus</i>	0	0	0	1	0	0	0
<i>L. kalmi</i>	0	0	0	1.5	0.7	0.5	0
Lygaeid nymphs	0	0	0	0	1.7	0	0
Flowering %	0	80	36	0.5	0	0	0

Table 3d – ACP *A. viridis*.

Herbivore	Julian Date						
	140	150	160	170	180	190	200
<i>R. lineaticollis</i>	2.7	4.5	19	4	1	0.5	0.5
<i>C. inopinatus</i>	0	0	2	0	0	0	14
<i>D. plexippus</i>	0	3.5	0	0	0	0	0
<i>O. fasciatus</i>	0	0	0.3	0	0.3	0	1
<i>L. kalmi</i>	0	0	0	2	1.3	0	0
Lygaeid nymphs	0	0	0.3	0.5	1	1	0.5
<i>T. tetraphthalmus</i>	0	0	3.3	0.5	0.7	1.5	0.5
Flowering %	31	89	24	0	0	0	0

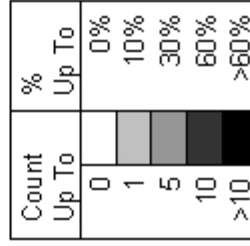


Table 3e – RNP *A. meadii*.

Herbivore	Julian Date															
	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280
Deer	1	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Small Mammal	0	0	0.3	0	0.7	0	0	0	0.5	1	5	1.5	0	0	0.5	1
<i>R. lineaticollis</i>	0	0	1	0	0.3	0	0	0	0	0	0	0	0	0	0	0
<i>C. inopinatus</i>	0	0	0	0	0.3	0	0.3	0.5	2	0.5	0	0	0	0	0	0
<i>O. fasciatus</i>	0	0	0	0	0	0	1	1.5	1.5	0.5	0	0.5	0	0	0	0
<i>L. kaltrii</i>	0	0	0	0	0	0	0	0.5	1	0	0	0	0	0	0	0
Lygæid nymphs	0	0	0	0	0	0	0	1	3	2	0	0.5	1	0	0.5	0
Flowering %	0	0	43	59	2.4	0	0	0	0	0	0	0	0	0	0	0

Table 3f – ACP *A. meadii*.

Herbivore	Julian Date												Count Up To	% Up To	
	140	150	160	170	180	190	200	210	220	230	240	250			
Small Mammal	0	0	0.3	0.5	0.7	0	0.3	0.5	0.5	0	0	0	0	0	0.5
<i>R. lineaticollis</i>	0.3	0.5	0	0.5	0	0.5	0	0	0.5	0	0	0	0	0	0
<i>C. inopinatus</i>	0	0	0	0	0	0.5	4	3	1	0	0	0	0	0	0
<i>D. plexippus</i>	4.7	2	0.3	0	0	0	0	0	0	0	0	0	0	0	0
<i>O. fasciatus</i>	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0
Lygæid nymphs	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
<i>T. tetraphthalmus</i>	0	0	0	0	0.3	1.5	0	0.5	0	0	0	0	0	0	0
Flowering %	0.8	72	38	0	0	0	0	0	0	0	0	0	0	0	0

Figure 1. Reproductive fates of milkweed ramets. Stems either produced mature seed pods or failed to reproduce. Stems that failed could be placed into three categories: those that likely failed to reproduce because of herbivore damage; those that flowered but failed to initiate pods for reasons other than herbivory; and those that failed to reproduce for other reasons, including cases where the stem or buds withered without evidence of herbivory, undamaged pods failed to develop or were aborted, or the plant was attacked by possible pathogens. Sample size is below site name for each population. The identity of the herbivores is illustrated in Table 4.

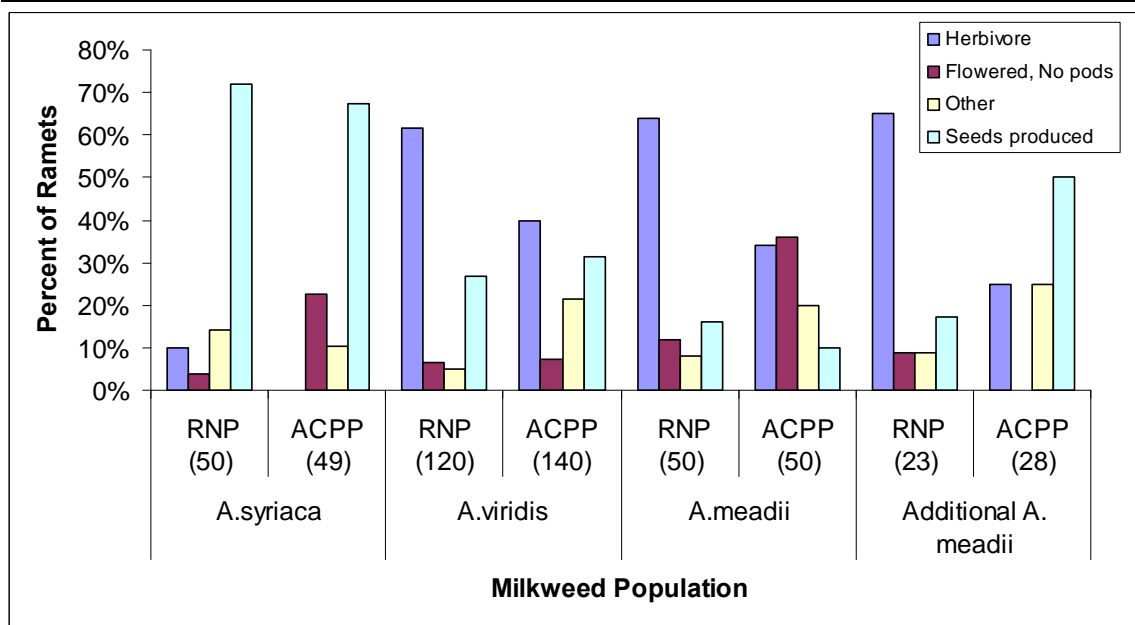


Table 4. Herbivores responsible for reproductive failures of ramets. Each count is the number of plants in the survey population that did not produce mature pods likely due to damage from a given herbivore.

Herbivore	<i>A. sylvatica</i>		<i>A. viridis</i> by ramet		<i>A. meadii</i>		Additional <i>A. meadii</i>	
	RNP	ACPP	RNP	ACPP	RNP	ACPP	RNP	ACPP
N	50	49	120	140	50	50	23	28
Deer	0	0	1	0	17	0	0	0
Small mammal	5	0	0	0	8	3	6	3
<i>R. lineaticollis</i>	0	0	69	52	2	3	1	1
<i>D. plexippus</i>	0	0	0	0	0	6	0	0
<i>C. inopinatus</i>	0	0	0	0	1	0	3	3
Caterpillar	0	0	0	1	1	1	4	0
Unknown Herbivore	0	0	4	3	3	4	1	0

Chapter 2: Influence of seed characteristics and site conditions on establishment of a threatened prairie milkweed, *Asclepias meadii*, in Kansas.

Introduction

The reestablishment of self-sustaining populations is often an important component of recovery plans for threatened and endangered plants (Pavlik et al. 1993, Lofflin & Kephart 2005). A study of species recovery plans for federally listed plants found that 87% proposed reintroduction or population augmentation as part of the recovery effort (Kennedy 2004). However, the conditions necessary for the establishment of many target species are poorly understood. Propagules of rare species are especially valuable and restoration techniques need to be carefully selected to ensure successful population establishment. Factors influencing the outcome of a reintroduction include propagule source and quality (Broadhurst et al. 2008; Vander Mijnsbrugge et al. 2010), as well as conditions and management of the restoration site (Wendelberger & Maschinski 2009). Lessons learned from experimental reintroductions can also be used to encourage recruitment in natural populations and mature restorations (Pavlik et al. 1993).

The physical quality of the propagules, in part reflected by mass, can affect germination rates and seedling growth (Morse & Schmitt 1985; Prinzie & Chmielewski 1994) and thus influence restoration outcomes. Restorers can attempt to facilitate plant establishment by selecting high quality propagules and directly manipulating environmental conditions at the restoration site. Species in a variety of ecosystems

require fire for their seeds to germinate, and natural or prescribed fires can be necessary to maintain native plant communities (Hulbert 1986; Bell et al. 1993; Brockway & Lewis 1997). Soil disturbance has also been shown to influence recruitment in a number of grassland systems, by providing microsites suitable for young seedlings and reducing competition (Hobbs & Huenneke 1992).

Restoration and reintroduction programs are particularly important for plants like *Asclepias meadii* Torrey ex A. Gray (Mead's milkweed), a federally threatened prairie species. Populations are typically small and isolated, limiting gene flow and leaving them vulnerable to inbreeding depression and stochastic extinction (Tecic et al. 1998; Hayworth et al. 2001; USFWS 2003). Prior research has provided insight about suitable restoration techniques for this species but also raised unanswered questions regarding seed ecology and seedling establishment. Germination studies by Betz (1989) and Row et al. (1999) allude to distinctions made between viable and non-viable seed but do not provide quantitative criteria for seed evaluation. Betz (1989) mentions seeds "appearing non-viable;" however, the seedling emergence rate reported pooled both apparently viable and apparently non-viable seed together. Row et al. (1999) discarded "shriveled" seeds before beginning their efforts to propagate *A. meadii*.

The effect of common ecological disturbances on *A. meadii* establishment is also uncertain. Fire is a frequent management tool on grasslands in the midwest United States (Hobbs & Huenneke 1992), including some of the prairie remnants where *A. meadii* is typically found (Tecic et al. 1998). Small scale soil disturbance, such as that caused by

burrowing and grazing mammals, has been demonstrated to facilitate the establishment of other tallgrass prairie species (Platt 1975; Gibson 1989), although its function in actually maintaining or enhancing community diversity has been questioned (Rogers et al. 2001). A better understanding of the early life stages of *A. meadii* would aid development of more effective restoration techniques for this species and possibly other rare plants with similar characteristics.

Through a combined observational greenhouse and experimental field study, I addressed two key questions:

1) *To what degree does seed mass affect emergence and seedling size?* Simple quantitative methods to evaluate *A. meadii* seed quality would ensure that valuable time is not lost working with inviable seed. A seed evaluation technique would also help conservationists distinguish between populations that are successfully reproducing and those that have difficulty creating viable seeds, despite the possible presence of flowering stems and developing fruit.

2) *Do burn or soil disturbance treatments affect emergence and seedling size?* On tallgrass prairies, prescribed burning is usually conducted in the spring, prior to the emergence of new vegetation from the soil. Spring burning appears to increase flowering *A. meadii* stems (Kettle et al. 2000) and also may have a positive effect on seedling and juvenile survivorship (Bowles et al. 1998), but the effect of fire on seed germination and

seedling growth is unclear. The influence of soil disturbance on *A. meadii* establishment has not been previously investigated.

Methods

Study Species

Asclepias meadii (Aponcynaceae) is a long-lived perennial found in tallgrass prairies. It was formerly widespread throughout the central Midwest, but land conversion for agriculture nearly eliminated the plant, which persists in unplowed prairie fragments (Betz 1989; USFWS 2003). The future of many populations on private land is in doubt, making restoration of self-sustaining populations on protected land a priority (USFWS 2003; Missouri Department of Conservation 2009; Nature Conservancy 2011).

Restoration activities have begun in every state with a historical record (IL, IN, IA, KS, MO, and WI) (USFWS 2003). Seed pods (follicles) typically mature between early September and mid-October and pod maturity can be recognized by the dehiscence of the pod wall, allowing the wind dispersed seeds to leave the pod. However, some pods dehisce prematurely, often due to herbivore damage (personal observation), while others show stunted growth, possibly due to lack of maternal resources or selective fruit abortion (Willson & Price 1977). Seeds can often still be collected from these pods, but they may not be viable, despite appearing fully formed by visual inspection. *A. meadii* has previously been used as a model system for population viability analysis (Bell et al.

2003), plant mark-recapture studies (Alexander et al. 1997; Slade et al. 2003; Alexander et al. 2009), and adaptive management (Moore et al., in review).

Study Site

The Rockefeller Native Prairie (RNP) is a unit of the University of Kansas Field Station (KUFS), Leavenworth Co., KS (39° 2'44"N, 95° 12'18"W). A 4.0-ha portion of site has never been plowed and an additional 0.5-ha was restored to native vegetation in 1957 (Kettle et al. 2000). The *A. meadii* population on the RNP has been studied for over 20 years and all known patches are permanently tagged (Alexander et al. 2009). Individual plants can produce multiple above-ground stems (ramets) and stems within 1.25 m of each other are considered to belong to the same patch (Kettle et al. 2000). Thus, each patch is putatively an individual plant, although germination of multiple seeds in the same location can sometimes result in multiple genotypes within a patch (Kettle et al. 2000). The prairie has historically been managed through biennial burns (Alexander et al. 2009). In recent years, occasional mowing has also been incorporated into the management regime, although the prairie was not mowed during this study. The RNP is both the source for seeds included in this study and the location of the experimental restoration.

Experimental Design

Seed Collection and Selection ó In 2008, a total of 18 pods were collected from 13 stems in 5 distinct patches. Pods were monitored during the late summer for signs of maturity, either a drying out of the pod wall and stalk or dehiscence of the pod wall. 9 pods were collected from Patches 2, 4, and 5 in mid-August and 9 pods were collected from Patches 1, 2, and 3 in mid-September. The pods collected in August dehiscenced earlier than expected and some seeds were lost from pods in Patches 4 and 5. For each pod, I recorded the number of seeds and identity of the maternal patch. Individual seeds were separated from their coma and weighed. I randomly selected an equal number of seeds from each pod for greenhouse and field studies, with the exception of two pods from Patch 4, which did not have enough seeds for equal representation in both studies. Patches did not all produce the same number of pods, so they are represented by unequal numbers of seeds in each study. A minimum seed mass of 1.000 mg was required for a seed to be planted in either study, which eliminated 3.7% of seeds, mostly from Patches 4 and 5. Seeds below 1.000 mg were little more than an empty seed coat and clearly underdeveloped.

Greenhouse Study ó 468 seeds from 18 pods (27 seeds each, except two Patch 4 pods with 20 and 16 seeds) were planted 1.5 cm deep into moist 50 mm peat pellets (Jiffy Products of America; Norwalk, OH). The 468 seeds consisted of 135, 54, 108, 63, and 108 seeds from Patches 1-5, respectively. The peat pellets were placed in a cold room (4°C) for 10 weeks, with occasional watering to prevent drying out. In late May, the pellets were moved to a greenhouse and monitored for seedling emergence.

Field Experiment 6 408 round plots 0.5 m in diameter were created in the restored portion of the RNP. I selected 408 seeds from 17 pods (24 seeds each), consisting of 120, 48, 96, 48, and 96 seeds from Patches 1-5, respectively. Plots were arranged in a grid with plots placed in 3 m intervals. Plot treatments were in a factorial design with the following treatment groups: 1) soil disturbance, burn; 2) soil disturbance, no burn; 3) no soil disturbance, burn; and 4) no soil disturbance, no burn. Six seeds from each pod were placed in each treatment type. Plots were randomized within two experimental blocks (A and B) because of a gentle slope to the site. In December 2008, a single seed was planted in each plot at a depth of 1.5 cm and covered with soil. The disturbance treatment consisted of tilling the top layer of soil and litter with a garden claw, to an approximate depth of 7 cm, prior to seed placement in the plot. The fire treatment consisted of patch burns conducted in April 2009, prior to the emergence of new prairie vegetation or *A. meadii* seedlings. A ring of aluminum flashing was placed around each plot to be burned and a propane torch was used to ignite the litter in the plot. The flashing prevented the fire from spreading outside of the plot, such that all plots were embedded in a matrix of unburned vegetation remaining from the previous year. In April 2010, the entire site was burned as part of a larger prescribed burn on the RNP.

Data Collection

In the greenhouse study, peat pellets were checked for seedling emergence every 2 days following removal from cold storage. Measurements of seedling height were taken 16 times over a 78 day period. The first 8 sets of measurements were taken in 2 day

intervals; later measurements were taken at longer intervals as seedling growth slowed. In the field study, all plots were checked for emerging seedlings on May 9, 13, 17, and 24. Measurements of seedling height were taken 14 times over a 60 day period between May 18 and July 16. The first 5 sets of measurements were taken in 2 day intervals, with longer intervals between later measurements. A final check of all field plots for late emerging seedlings on July 16 did not locate any new plants. I revisited all field plots the following year (June 21-24, 2010) to check for surviving juvenile plants or any possible new seedlings resulting from a seed bank effect. Measurements of juvenile height were taken on each plant for comparison to seedling growth the previous year. In both field and greenhouse studies, the cotyledons of some seedlings emerged but a stem never broke the surface of the soil. These seedlings were counted as "emerged" but no growth measurements could be taken on them.

Statistical Analyses

Statistical analyses were done in Minitab 14 (Minitab Inc. 2003) with $\alpha = 0.05$. Means are presented with ± 1 S.E. I created regression models for greenhouse and field seedling emergence (binary logistic regression), field seedling survival during the first year (binary logistic regression), and greenhouse and field final seedling height (general linear model) for both greenhouse and field data. Greenhouse models included seed mass as a covariate and source patches as factors. I initially evaluated field models with seed mass as a covariate, source patches, plot treatments (burning and soil disturbance), and experiment block as factors, and all possible interaction terms. For field emergence, I

used the corrected Akaike Information Criterion (AICc) to select the best model for presentation (Burnham & Anderson 2002). For field growth, I removed non-significant interaction terms from the final model.

Results

Seedling Emergence and Seed Mass ó In the greenhouse study, 123 seedlings emerged out of 468 total seeds (26.3%). In the field study, 74 seedlings emerged out of 408 total seeds (18.1%). However, in both studies, no Patch 4 or 5 seeds produced seedlings. All pods collected from those patches dehiscid approximately one month earlier than the other patches. Some Patch 4 and 5 pods had evidence of insect damage, which may have stunted seed growth and caused them to split open prematurely. Since all seeds from Patches 4 and 5 were likely underdeveloped, they were excluded from the following analyses. Seeds from Patch 2 pods collected at the earlier date had a much higher mean mass than seeds from Patch 4 or 5 pods and did produce some seedlings, so they were included in all analyses. Once Patches 4 and 5 were removed from the data set, overall greenhouse emergence was 41.4% (123/297) and field emergence was 28.0% (74/264).

The remaining 11 pods from Patches 1-3 produced a total of 924 seeds; total seed production was 482, 169, and 273 for Patch 1-3, respectively. Mean number of seeds per pod was 84.0 ± 5.39 , with a minimum of 60 and a maximum of 111. Seeds ranged in mass from less than 1.000 mg to a maximum of 6.841 mg. A histogram of seed mass for Patches 1-3 shows a bimodal distribution (Figure 1); in part, this reflects distributions

within the individual pods, which were often bimodal. Kolmogorov-Smirnov Goodness-of-Fit tests showed that seed mass was not normally distributed within any pod (K-S values ranging from 0.093 to 0.295, p-values all ≤ 0.026 , see Appendix 2-1). An ANOVA showed a significant effect of patch on seed mass ($F = 8.80$, $p < 0.001$, d.f. = 2; Patch 1 mean = 4.327 ± 0.0631 mg; Patch 2 mean = 4.198 ± 0.105 mg; Patch 3 mean = 4.689 ± 0.0803 mg). There was no significant correlation between number of seeds in a pod and average seed mass ($r = 0.22$, $p = 0.516$, $n = 11$).

Factors Affecting Emergence, Survival, and Growth Regression analyses of seedling emergence in both the greenhouse and the field showed a significant effect of seed mass (Tables 1a and 1b); there was an overall trend of increasing emergence rates with seed mass (Table 2). The lowest mass of any successfully emerged seed was similar in the greenhouse and the field, at 3.183 mg and 3.201 mg, respectively. In Patches 1-3, 83.1% of seeds produced were ≥ 3.183 mg, but no seeds from Patches 4 or 5 met this minimum mass threshold, so seeds from those patches had essentially no chance of emergence. There were also significant effects of patch identity on emergence (Tables 1a and 1b); these differences were more evident in the greenhouse than in the field (Table 3). There was no significant effect of burning on field emergence; the p-value for the soil disturbance treatment was 0.051 (Table 1b). Fewer seedlings emerged in field plots with either burning or soil disturbance treatments (Table 4). Field manipulations did not have a significant effect on seedling survival once emerged (Table 4); an analysis of field survival to July 16 (the last day of 2009 measurements) showed no significance of patch, treatment, or any interaction term (not displayed).

I generated seedling growth curves for greenhouse and field seedlings (Figure 2). 8 plants each in the greenhouse and field emerged but never produced a shoot; those plants were omitted from the growth curves and models. The slight decline in field seedling height toward the end of the study was due to herbivory, probably by small mammals, which nipped the tops off some plants. Seedlings did demonstrate some ability to generate new shoots after apical meristem damage. Greenhouse seedlings were significantly taller than field seedlings by their respective seventeenth day (mean difference = 0.88 ± 0.33 cm, $t = 2.64$, $p = 0.009$, d.f. = 141) and by the final day of field measurements, greenhouse seedlings were over twice as tall (mean field on Day 68 = 8.32 ± 0.25 cm; mean greenhouse on Day 61 = 16.53 ± 0.41 cm).

The effects of seed mass, patch identity, and field experimental treatments on final seedling height were evaluated with general linear models. I used the last day of measurements for the greenhouse model (Day 82) but the second to last day for the field model (Day 58) due the drastic height reduction of several plants by herbivores on the last day of measurements. In the greenhouse study, final height differed among seeds from different patches (Table 5a). The final height of Patch 2 seedlings in the greenhouse (mean = 14.70 ± 0.83 cm) was significantly shorter than either Patch 1 or Patch 3 (means = 18.48 ± 0.85 cm and 17.17 ± 0.64 cm, respectively). In the field experiment, the only significant factor was the burn treatment (Table 5b); seedlings in burned plots were shorter (mean = 8.00 ± 0.40 cm) than in unburned plots (mean = 9.24 ± 0.24 cm). Preliminary analyses of field growth showed no significance of any interaction term, so

they were removed from the model. Seed mass was not a significant covariate in either the field or the greenhouse.

Field Juvenile Survival and Growth 6 54 of the original 74 seedlings (73%) reappeared as juvenile plants in 2010 (Table 4). A single plant was also discovered in a plot without a 2009 seedling record; while it is possible that this individual was the result of a seed bank effect, it was probably simply overlooked in 2009. 16 of the 20 seedlings that failed to become 2010 juveniles were already known to have withered, been damaged by herbivores, or failed to produce a shoot during the 2009 field season. There was no significant difference in seed mass between plants surviving to 2010 and those that did not (mean of juveniles = 5.177 ± 0.097 mg; mean of failed seedlings = 5.022 ± 0.20 mg; $t = -0.71$, $p = .485$, d.f. = 28). Individual plants were significantly taller in their second year than they were at the same time the first year (mean height on June 21, 2009 = 8.56 cm, mean height on June 21-24, 2010 = 9.98 cm, mean difference = 1.43 ± 0.29 cm, paired $t = 4.95$, $p < 0.001$, d.f. = 53).

Discussion

Propagule Selection for Restoration

Seed mass and source 6 Understanding the roles of mass and source in the success of each seed helps restorers select quality seed stocks. For *A. meadii*, there is clearly a lower mass limit, below which seeds have little or no chance of emerging, and the probability

of seedling emergence shows a positive correlation with seed mass. Table 2 suggests that seeds with mass lower than 3.0 mg should not be used. However, this study used seed from a single population and the degree of seed mass variation between populations is unknown. Once seedlings emerge, data from both greenhouse and field studies suggest that seed mass has little influence on seedling survival or final height. It has been observed in other species that seeds with low mass compensate for low initial growth with a higher relative growth rate than seeds with high mass, and differences in seedling size are erased over time (Meyer & Carlson 2001). The bimodal distribution of seed masses within pods appears to be the result of the manner in which seeds develop. Relatively low mass seeds are often clustered near the top, implying that seeds are developing unequally and perhaps sequentially.

It is increasingly recognized that the suitability of propagules from different sources to a restoration site can vary widely because of locally adapted genotypes (Gustafson et al. 2004; Sanders & McGraw 2005; Vander Mijnsbrugge et al. 2010). Studies typically focus on population level variation (Hamzé & Jolls 2000; Gustafson et al. 2004; Sanders & McGraw 2005), but using bulk collected seed from each source may mask variation among individuals within a population. The seed collection method I used in this study made it possible to examine propagule quality at the level of source patch. I found significant effects of maternal patch on emergence and seedling growth in the greenhouse, but these effects were mostly lost in the field study. The loss of source patch effects could be due to the greater overall variability caused by field conditions. Alternatively, since all seeds in the field experiment were planted in nearly the exact

location they were sourced from, in theory they were already locally adapted genotypes, regardless of their maternal patch. If genotypes were not equally adapted to novel greenhouse conditions, this could explain why performance differences were evident in the greenhouse but not in the field. Source patch effects are likely a minor concern for large scale field restorations, where high levels of genetic diversity are usually desired, but the presence of these effects under greenhouse conditions may alter genotype representation in cohorts of greenhouse-reared seedlings intended for field restoration.

Choice of propagule type Although direct planting of seeds into restoration areas is the most straightforward, transplanting greenhouse-reared seedling or juvenile plants into the field is sometimes recommended due to lower mortality or increased growth. Previous studies of *A. meadii* favored juveniles over seeds as the restoration unit because the observed germination and seedling growth rates were higher in the greenhouse than in the field (Bowles et al. 1998; Bowles et al. 2001), patterns that were also seen in this study. *A. meadii* appears to need to reach a threshold size before it begins to flower (Bowles et al. 2001), so plants started in the greenhouse may have a quicker path to maturity than those in the field. However, the time invested in rearing juveniles and transplanting them is much greater than introducing seeds directly to the restoration site. The preferred strategy may also differ depending on restoration site. For example, seedlings in this study showed higher survivorship (54 juveniles/74 seedlings = 73.0%) than those in restorations done in Illinois (35%) (Bowles et al. 2001). In cases where the seed supply is ample, but staff time and facilities are not, conservationists may prefer to tradeoff greater

growth and higher germination with the opportunity to introduce more propagules over a greater area.

Although methodologies vary between studies, overall emergence rates in this study were comparable to previous *A. meadii* propagation efforts. Previously reported germination rates included 47.6% (greenhouse) (Betz 1989), 74.3% (greenhouse) (Bowles et al. 1998), and 34.9% (field) (Bowles et al. 2001). Emergence rates could be slightly lower than germination rates, because of the possibility that cotyledons of germinated seeds fail to break the soil surface, although the exact definition of "germination" in older studies is uncertain.

Effects of Burning and Soil Disturbance Treatments

It has been suggested that *A. meadii* has characteristics typical of a late successional species—slow to colonize and reach maturity, but able to persist in a competitive environment (Bowles et al. 1998). Although the competitive ability of *A. meadii* was not directly evaluated in this study, the lack of response, or even negative response, by seeds and seedlings to field manipulations is consistent with this view. Specifically, emergence was reduced in the soil disturbance treatment (at the $p = 0.051$ level); this is opposite the pattern observed in some native annuals where soil disturbance enhances seedling emergence (Moody-Weis & Alexander 2007). There are at least two possible explanations for this result. First, as a late successional prairie species, *A. meadii* may be adapted to establishing in interstitial spaces, and thus not require soil disturbance to play

a role in creating a safe site for seedling establishment. Alternatively, reduced seedling emergence in the soil disturbance treatment could be because seeds were more exposed to seed predators when placed in loose, disturbed soil than when inserted into uniformly packed soil. Burning had no significant effect on seedling emergence, although both burn treatments showed reduced emergence relative to the control plots (Table 4). The patch burns were brief and increases in subsurface soil temperature during low intensity prairie burns is generally minor (e.g. Riechert & Reeder 1972), so damage to subterranean seeds from burning is unlikely. The shorter stature of seedlings in burned plots was an unexpected result. It is possible that burning stimulated the growth of the neighboring vegetation and burned plots were subject to more light competition than unburned plots. Consistent with this idea, mean seedling height in burned treatments was already lower than in unburned treatments early in the study and remained lower throughout the experiment (Figure 2). The visible effects of the burning and soil disturbance treatments were essentially erased by the end of growing season, so it is improbable that these treatments have any long term influence on plant growth or survival.

Restoration Management for Multiple Life Stages

Ideal conditions for young plants and reproductive individuals may not completely overlap and restoration management should account for the needs of all life stages present. Also, meeting long term goals for age structure may require shifting management as a reintroduced population matures. In the case of *A. meadii*, this study shows that burning may have a detrimental effect on seedling growth and possibly seedling

emergence, which may partially offset the benefits spring burns appear to have for flower and fruit production by mature plants (Kettle et al. 2000) in existing populations. While active management through burning or mowing is essential for long term prairie maintenance, managers may want to adjust the management regime and temporarily forgo disturbance treatments during periods when the plant is being reintroduced through seed. However, in populations that already have many reproductive individuals, enhanced seed production may be more important to population growth than increased seedling emergence and seedling growth.

Implications for Practice

1. *A. meadii* seed quality can be easily evaluated through mass. A simple measure of quality is useful when selecting seeds for restoration purposes or when determining the ability of a population to successfully reproduce.
2. Active manipulation of reintroduction areas through soil disturbance or fire is not necessary, and may even be detrimental for *A. meadii* recruitment and seedling growth.
3. Direct planting of *A. meadii* seeds into restoration sites is a reasonable alternative to transplanting juveniles if resources are limited, although lower emergence and growth rates should be expected.

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Tables and Figures

Figure 1. Distribution of seed mass for all seeds from Patches 1-3. Seed mass is binned in 0.33 mg intervals. Distributions of seed mass within individual pods typically follow a similar pattern.

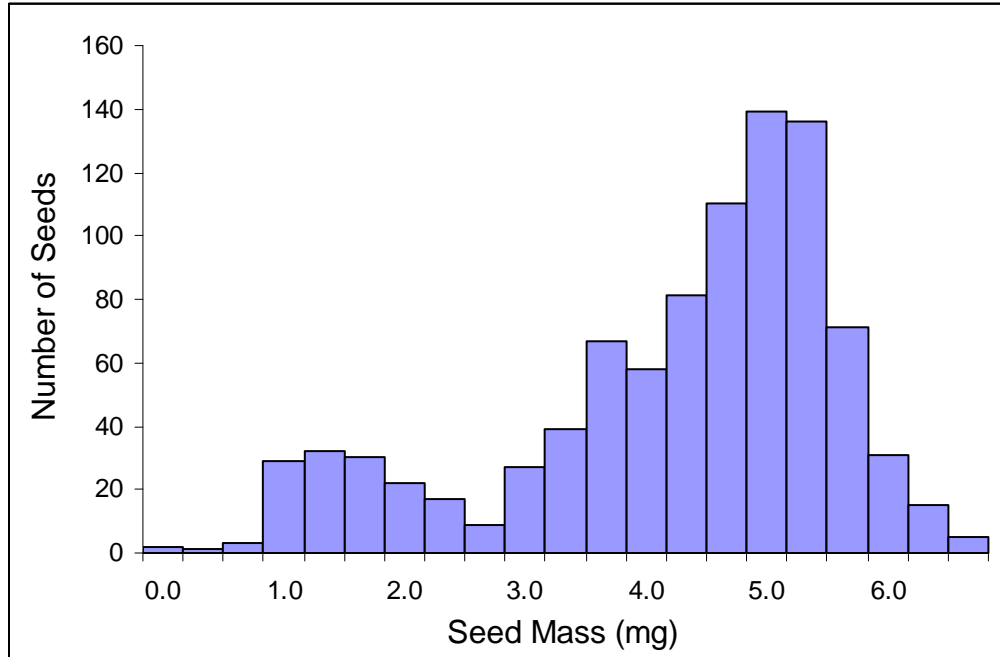


Table 1a. Binary logistic regression model for greenhouse seedling emergence. Patch 1 seeds are treated as a constant to which other predictors can be compared.

<i>Predictor</i>	<i>Coefficient</i>	<i>Coefficient SE</i>	<i>Z</i>	<i>P</i>
Constant	-4.939	0.707	-6.98	<0.001
Mass (mg)	0.826	0.139	5.94	<0.001
Patch 2	1.176	0.371	3.17	0.002
Patch 3	1.553	0.309	5.03	<0.001

Table 1b. Binary logistic regression model for field seedling emergence. The model presented includes all factors plus the set of interactions which provides the lowest possible AICc score. Patch 1 seeds in block ðAö plots that did not have a burn or soil disturbance treatment are treated as a constant to which other predictors can be compared.

<i>Predictor</i>	<i>Coefficient</i>	<i>Coefficient SE</i>	<i>Z</i>	<i>P</i>
Constant	-4.750	0.932	-5.10	<0.001
Mass (mg)	0.773	0.167	4.63	<0.001
Patch 2	0.203	0.633	0.32	0.749
Patch 3	0.978	0.471	2.08	0.038
Burn (Yes)	0.139	0.431	0.32	0.747
Soil (Yes)	-0.603	0.308	-1.96	0.051
Block (B)	0.567	0.551	1.03	0.303
Patch 2 x Block (B)	1.044	0.846	1.23	0.217
Patch 3 x Block (B)	-1.197	0.684	-1.75	0.080
Burn (Yes) x Block (B)	-1.010	0.615	-1.64	0.101

Table 2. Seedling emergence by seed mass. Emergence rates for seeds from Patches 1-3, binned into 1 mg mass categories.

<i>Mass (mg)</i>	<i>Greenhouse emergence</i>			<i>Field emergence</i>		
	<i>N</i>	<i>Emerged</i>	<i>%</i>	<i>N</i>	<i>Emerged</i>	<i>%</i>
1.000-1.999	29	0	0.0%	23	0	0.0%
2.000-2.999	20	0	0.0%	10	0	0.0%
3.000-3.999	43	9	20.9%	47	7	14.9%
4.000-4.999	92	53	57.6%	75	23	30.7%
5.000-5.999	95	49	51.6%	91	34	37.4%
6.000-6.999	18	12	66.7%	18	10	55.6%
Total	297	123	41.4%	264	74	28.0%

Table 3. Seedling emergence by seed source patch.

<i>Patch</i>	<i>Greenhouse emergence</i>			<i>Field emergence</i>		
	<i>N</i>	<i>Emerged</i>	<i>%</i>	<i>N</i>	<i>Emerged</i>	<i>%</i>
1	135	32	23.7%	120	26	21.7%
2	54	25	46.3%	48	16	33.3%
3	108	66	61.1%	96	32	33.3%

Table 4. Field seedling emergence and survivorship by plot treatment.

<i>Soil Disturbance</i>	<i>Burn</i>	<i>N</i>	<i>Emerged</i>	<i>Emerged %</i>	<i>Survived to end of 2009</i>	<i>2010 Juveniles</i>	<i>Overwinter Survival %</i>
Yes	Yes	66	12	18.2%	7	7	100.0%
Yes	No	66	17	25.8%	14	14	100.0%
No	Yes	66	20	30.3%	18	16	88.9%
No	No	66	25	37.9%	21	17	81.0%
Total		264	74	28.0%	60	54	90.0%

Figure 2 6 Growth curves for greenhouse and field seedlings. Mean stem height was calculated for all plants in the treatment group that produced a shoot. Seedlings that withered or were completely destroyed by herbivores were not included in means following the incident. Seedlings that were damaged but continued to grow following herbivory were included in the means for the entire study. Day 0 is the first day cotyledons emerged from any plant in the greenhouse or field. Error bars are ± 1 S.E.

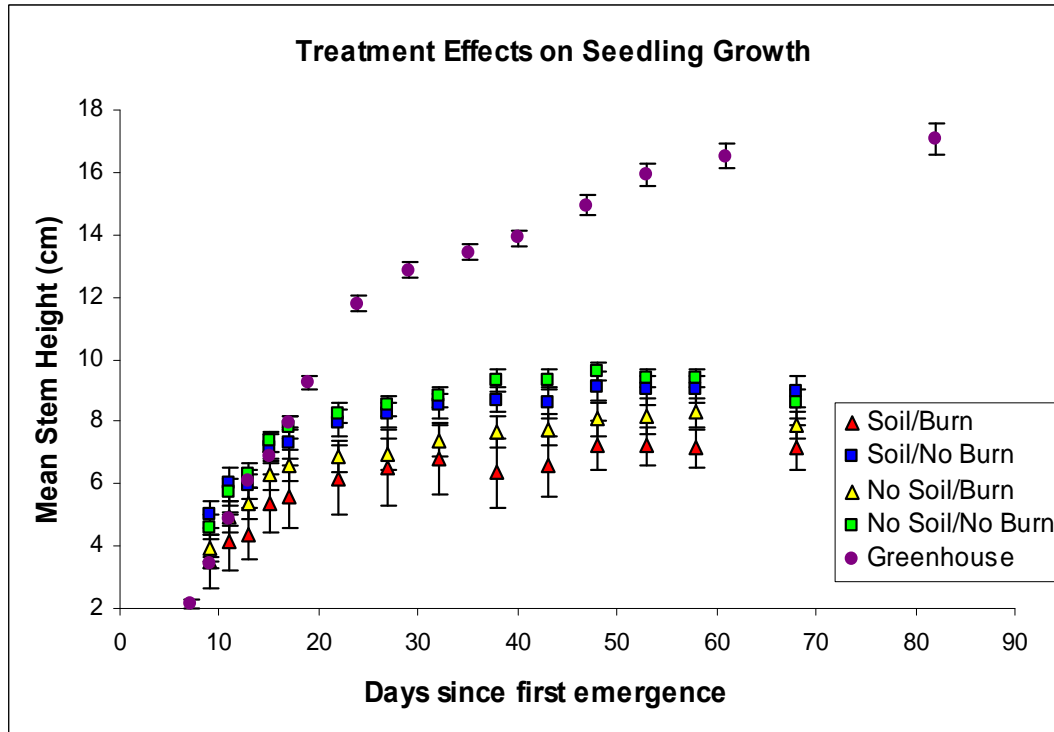


Table 5a. General linear model for greenhouse seedling growth. Patch 1 seeds are treated as a constant to which other predictors can be compared.

<i>Predictor</i>	<i>Coefficient</i>	<i>Coefficient SE</i>	<i>T</i>	<i>P</i>
Constant	13.669	3.457	3.95	<0.001
Mass (mg)	0.625	0.685	0.91	0.364
Patch 2	-2.122	0.854	-2.48	0.015
Patch 3	0.237	0.656	0.36	0.719

Table 5b. General linear model for field seedling growth. Patch 1 seeds in block 1 plots that did not have a burn or soil disturbance treatment are treated as a constant to which other predictors can be compared.

<i>Predictor</i>	<i>Coefficient</i>	<i>Coefficient SE</i>	<i>T</i>	<i>P</i>
Constant	6.663	1.573	4.24	<0.001
Mass (mg)	0.328	0.303	1.08	0.283
Patch 2	-0.607	0.384	-1.58	0.119
Patch 3	0.362	0.319	1.13	0.261
Burn (Yes)	-0.617	0.233	-2.65	0.011
Soil (Yes)	-0.382	0.238	-1.60	0.115
Block	-0.009	0.233	-0.04	0.968

Conclusion

My *Asclepias meadii* is reduced very low. If possible will send at some other time with other plants which you may want. It grows 10-15 inches high, on high, rolling prairies, or did years ago. I have seen it in Missouri, and it has been found near Davenport, Iowa but perhaps the plough has destroyed it.

- Dr. Samuel Barnum Mead in a letter to fellow plant collector,
December 15, 1871 (from Betz, 1989).

The life history of Mead's milkweed can be both a hindrance and a benefit for conservation efforts. Individual plants grow from seed to maturity slowly but are long lived once established. Mead's milkweed fruit have a long development period, which exposes them to seed predators even prior to dispersal. Once seeds germinate, seedling survival is high during the first season and very high for young overwintering plants. I have observed herbivore damage at all life stages from seed to seedling to flowering adult. The extent to which herbivory reduces equilibrium population sizes depends in large part on the degree to which Mead's milkweed is seed limited, an unknown property. A more subtle interplay also exists between herbivory and Mead's milkweed reproduction. My research studying the influence of seed mass on seedling emergence demonstrates a positive correlation between mass and probability of emergence. I have also observed that damage from insect herbivores can stunt developing pods or cause them to open prematurely. In this way, insects can reduce seed mass and seedling emergence, even if the seeds themselves are not directly damaged by insect activity.

Management Recommendations ó Herbivore damage assessments should be completed for significant Meadø milkweed populations. Where herbivory is found to be severe, such as on the Rockefeller Native Prairie, measures can be taken to mitigate herbivore damage. In the case of deer, local herd culling could relieve browsing pressure on Meadø milkweed and other native forbs. Expansion and restoration of Meadø milkweed habitat may also reduce deer damage, by eliminating nearby woody cover. It is possible that habitat enhancement could also lessen insect herbivory by reducing weedy congeners that act as reservoirs for insects. However, the relevant spatial scale for milkweed-herbivore relationships is unknown and doubtless very different for herbivores as diverse as deer, rodents, beetles, butterflies, and bugs.

Reintroduction and restoration efforts through direct planting of seed can successfully establish Meadø milkweed in suitable habitat patches, although waiting for these efforts to literally bear fruit may take extraordinary patience. Active manipulation of the restoration site through burning and soil disturbance does not appear beneficial to seedling emergence, so restorers should focus their efforts on planting large numbers of seeds in broad restoration arrays. Evaluating seeds by individually weighing random samples of seed will screen out poor quality seed and ensure that limited time and resources are not wasted.

The Future of Mead's Milkweed ó Although wholesale habitat destruction in the 19th and 20th centuries is to blame for the endangerment of Meadø milkweed (USFWS 1988), subtler anthropogenic changes to the environment continue to place pressure on existing

populations, even those with protected status. Habitat fragmentation reduces gene flow between populations, potentially leading to inbreeding depression in this self-incompatible species (Tecic et al. 1998; Hayworth et al. 2001). Population isolation also increases the risk of stochastic extinction in the many small populations of Meadø milkweed and limits the possibility that suitable habitat patches will be recolonized naturally. Widespread anthropogenic disturbance including road maintenance, mowing, and livestock grazing may increase weedy milkweeds, which could act as sources for the insect herbivores that attack Meadø. The replacement of natural disturbance regime with artificial regimes including fire suppression and annual mid-summer mowing also place a strain on Meadø milkweed. Fire suppression allows for the encroachment of woody vegetation into prairies, creating direct competition between Meadø milkweed and woody species, while also facilitating the movement of deer into prairie communities through increased cover. Annual mowing that occurs prior to pod maturation in early fall stops plants from reproducing sexually and appears to have led to genetic bottlenecks in populations in long-term hay meadows (Tecic et al. 1998). The host of environmental changes imposed by humans on grasslands may also lead to declines in native pollinators, which Meadø milkweed depends on for fertilization. Despite myriad challenges, there is hope that Meadø milkweed will continue to be part of the prairie landscape. It is a hardy plant which can live for several decades and it has been able to persist in less than ideal conditions in railroad right-of-ways and pioneer cemeteries (Betz 1989). The future of Meadø milkweed depends on the dedicated efforts of conservationists to protect and enhance existing populations and on species specific research that can ensure efficient management of protected populations and restoration of new, self-sustaining populations.

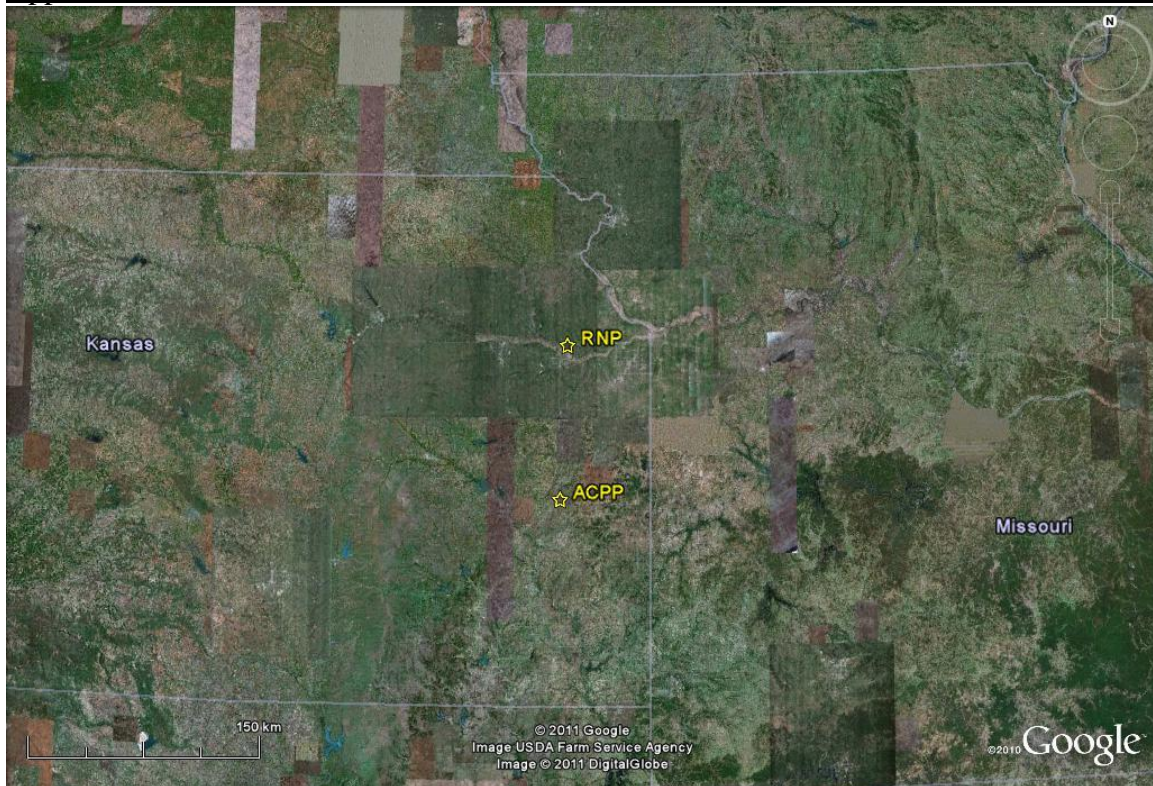
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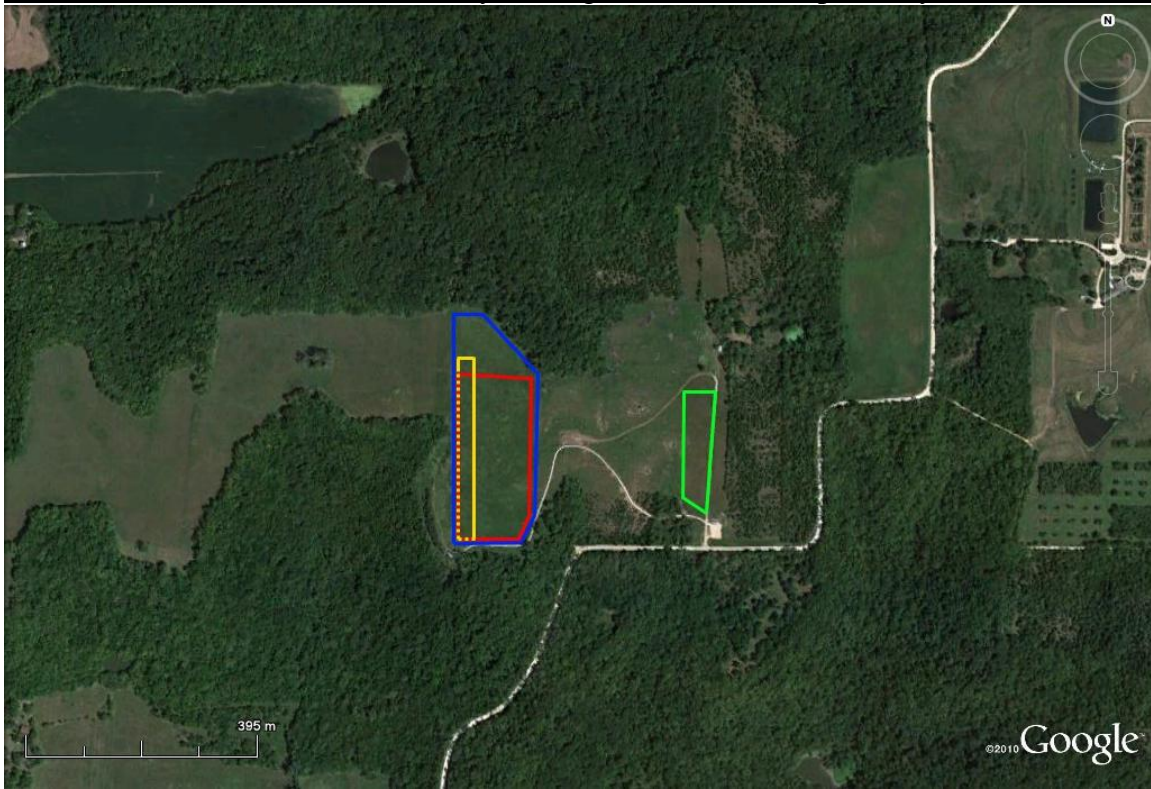
Appendices

Chapter 1

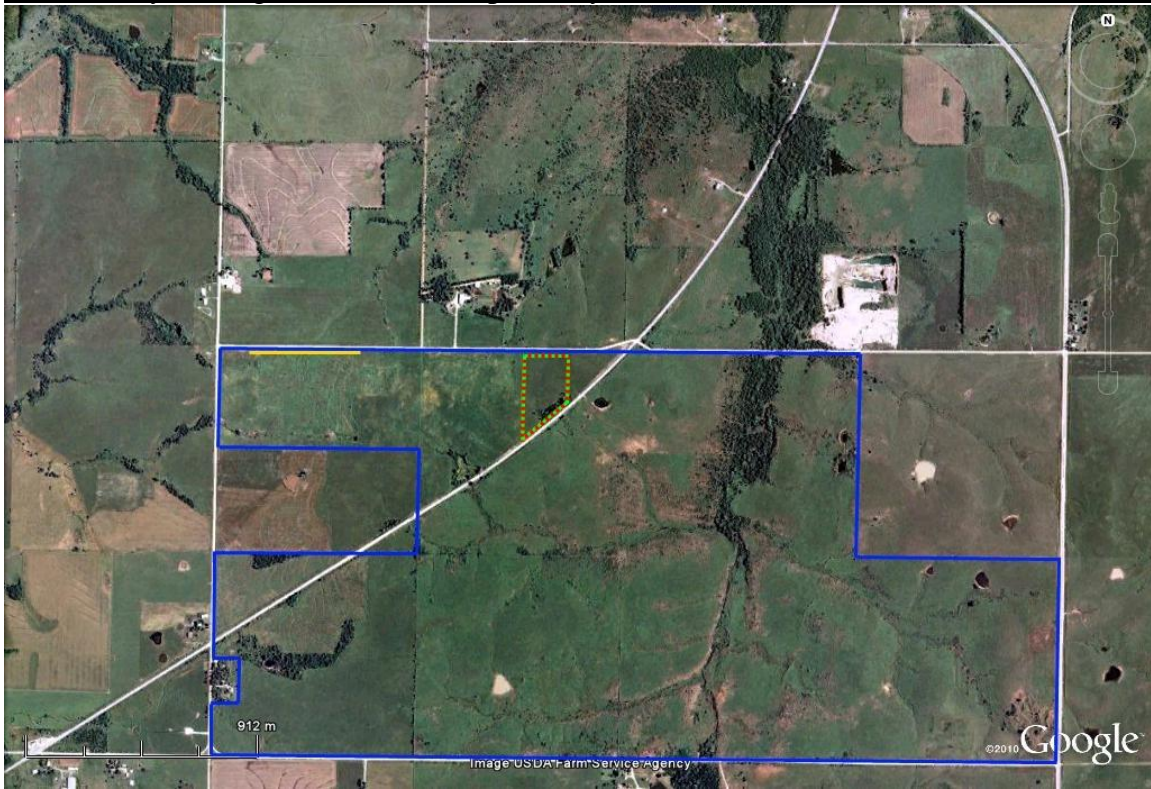
Appendix 1-1. Location of field sites in eastern Kansas.



Appendix 1-2a. Aerial of the Rockefeller Native Prairie and surrounding areas. The native and restored prairie is outlined in blue. The study populations of *A. syriaca*, *A. viridis*, and *A. meadii* are outlined in yellow, green, and red, respectively.



Appendix 1-2b. Aerial of the Anderson County Prairie Preserve. The preserve boundaries are outlined in blue. The study populations of *A. syriaca*, *A. viridis*, and *A. meadii* are outlined in yellow, green, and red, respectively.



Appendix 1-3. Binary Regression Models for Herbivore Presence. For each model, RNP *A. syriaca* is set as a constant to which other populations can be compared. For example, there is a significant difference between the presence of *R. lineaticollis* on RNP *A. syriaca* and RNP *A. viridis* ($p < 0.001$) and between RNP *A. syriaca* and ACPP *A. syriaca* ($p = 0.001$).

<i>R. lineaticollis</i>				
	Coef	SE Coeff	Z	P
Constant	-0.663	0.299	-2.22	0.026
Viridis	3.415	0.666	5.13	<0.001
Meadii	-2.088	0.666	-3.13	0.002
ACPP	1.387	0.427	3.25	0.001
viridis*ACPP	-1.942	0.871	-2.23	0.026
meadii*ACPP	-1.387	0.944	-1.47	0.142

<i>O. fasciatus</i>				
	Coef	SE Coeff	Z	P
Constant	1.658	0.386	4.30	<0.001
Viridis	-4.836	0.818	-5.91	<0.001
Meadii	-3.316	0.546	-6.08	<0.001
ACPP	-1.371	0.482	-2.84	0.004
viridis*ACPP	2.106	1.012	2.08	0.037
meadii*ACPP	-18.450	3956.830	0.00	0.996

Lygaeid Nymph				
	Coef	SE Coeff	Z	P
Constant	1.992	0.435	4.58	<0.001
Viridis	-4.435	0.679	-6.53	<0.001
Meadii	-3.808	0.596	-6.39	<0.001
ACPP	-1.536	0.525	-2.93	0.003
viridis*ACPP	1.781	0.877	2.03	0.042
meadii*ACPP	-0.541	1.209	-0.45	0.655

Appendix 1-4. Phenology Tests

Plant Phenology Comparison by Species

Kruskal-Wallis Test: First Day of Flowering versus Species (Both Sites)

Species	N	Median	Ave Rank	Z
A. meadii	71	155.0	132.9	0.66
A. syriaca	86	166.0	209.7	12.63
A. viridis	98	144.0	52.7	-12.88
Overall	255		128.0	

H = 208.04 DF = 2 P = 0.000

H = 210.45 DF = 2 P = 0.000 (adjusted for ties)

Plant Phenology Comparison by Site within Species

Mann-Whitney Test and CI: First Day of Flowering ACPP A. viridis v. RNP A. viridis

	N	Median
Flow ACPP Av	48	148.00
Flow RNP Av	50	140.00

Point estimate for ETA1-ETA2 is 8.00

95.1 Percent CI for ETA1-ETA2 is (5.00,8.00)

W = 3511.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000

The test is significant at 0.0000 (adjusted for ties)

Mann-Whitney Test and CI: First Day of Flowering ACPP A. syriaca v. RNP A. syriaca

	N	Median
Flow ACPP As	36	166.00
Flow RNP As	50	167.00

Point estimate for ETA1-ETA2 is -1.00

95.1 Percent CI for ETA1-ETA2 is (-1.00,2.00)

W = 1389.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1223

The test is significant at 0.1131 (adjusted for ties)

Mann-Whitney Test and CI: First Day of Flowering ACPP A. meadii v. RNP A. meadii

	N	Median
Flow ACPP Am	43	152.00
Flow RNP Am	28	159.00

Point estimate for ETA1-ETA2 is -4.00

95.1 Percent CI for ETA1-ETA2 is (-7.00,-4.00)

W = 1171.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000

The test is significant at 0.0000 (adjusted for ties)

Weevil Phenology Comparison by Species

Mann-Whitney Test and CI: R. lineaticollis on A. viridis v. A. syriaca (Both sites)

	N	Median
RL Av	93	160.00
RL As	50	208.00

Point estimate for ETA1-ETA2 is -46.00
95.0 Percent CI for ETA1-ETA2 is (-48.00,-42.00)
W = 4495.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)

Weevil Phenology Comparison by Site within Species

Mann-Whitney Test and CI: R. lineaticollis on A. viridis, ACPP v. RNP

	N	Median
ACPP Av	46	166.00
RNP Av	47	160.00

Point estimate for ETA1-ETA2 is 6.00
95.0 Percent CI for ETA1-ETA2 is (4.00,8.00)
W = 2737.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)

Mann-Whitney Test and CI: R. lineaticollis on A. syriaca, ACPP v. RNP

	N	Median
RL ACPP As	33	209.00
RL RNP As	17	208.00

Point estimate for ETA1-ETA2 is -1.00
95.1 Percent CI for ETA1-ETA2 is (-7.00,6.00)
W = 837.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.9347
The test is significant at 0.9342 (adjusted for ties)

Chapter 2

Appendix 2-1. Kolmogorov-Smirnov Tests on Mass Distributions in Individual Pods

Patch	Stem	Pod	Seed	Avg	Norm	KS	p-value
210	2	2	79	3.249	No	0.216	less than 0.01
210	4	1	110	4.053	No	0.237	less than 0.01
210	4	2	108	5.261	No	0.169	less than 0.01
210	5	1	111	5.185	No	0.093	0.026
212	2	1	86	4.178	No	0.190	less than 0.01
212	2	2	83	4.201	No	0.188	less than 0.01
269	1	1	73	4.255	No	0.197	less than 0.01
269	1	2	70	4.283	No	0.220	less than 0.01
269	2	1	60	4.760	No	0.236	less than 0.01
269	2	2	70	5.487	No	0.295	less than 0.01