

FIRST FLOWERING DATE TRENDS IN CLAY COUNTY, MINNESOTA AND
POLLINATION AND LIFE HISTORY CHARACTERISTICS OF HOARY PUCCOON

(LITHOSPERMUM CANESCENS)

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First flowering date trends in clay county, minnesota and pollination and life
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ABSTRACT

Plant species in Clay County, Minnesota have been changing their first flowering dates (FFDs) in response to climate changes. To document those shifts, in 2011 and 2012 I recorded phenological data for Clay County, Minnesota. I added that data to data which had been collected since 1910 for two locations in Minnesota and found that, on average, plants flowered 1 day later than their historical averages in 2011 and 16.1 days earlier in 2012.

I also performed experiments upon *Lithospermum canescens*, a native prairie forb which has shifted its first flowering date (FFD) significantly earlier than in the past century and which is underrepresented in tallgrass prairie restorations. I found that this species does not appear to be pollen limited, that the concurrently blooming plant species have changed noticeably since the early 1900s, and that this species is able to be grown by hand from seed (the first known attempt).

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I dedicate this master's thesis to my husband, Dale Maxson, and my mother, Gail Boehm.

Without their unfailing support throughout the years I would not have been able to complete this goal, and without their wise perspectives I would not have made it to the end sane.

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CHAPTER 1. LITERATURE REVIEW-THE GENUS *LITHOSPERMUM*

Introduction

The genus *Lithospermum* is a taxon with a surprisingly large impact on human life. The plants of this genus are found in some of the earliest human cultural sites and may have played a role in the saving of innumerable human lives through the compounds found in its root fibers (Papageorgiou et al. 2008). Various *Lithospermum* species are valuable to native North American ecosystems as first colonizers of disturbed areas and/or to land managers and ecologists as indicators of the vegetative quality of native plant communities (Weller & Keeler 2000, cited in Molano-Flores 2001). The genus *Lithospermum* exhibits a broad range of morphological and genealogical characteristics and could therefore be useful for taxonomists researching character evolution (Cohen 2011). In addition, various *Lithospermum* species have unusual life history traits (including plasticity in first flowering date in the face of climactic changes) which, if researched more fully, could lead to a greater understanding of plant adaptive traits leading to better management of wild and domesticated plants in the decades to come. My goal in this literature review is to compile the scientific literature published on the genus *Lithospermum* in order to consolidate it and to shed light on areas that could benefit from future research.

Systematics

The genus *Lithospermum* includes approximately 40 species (taxonomists disagree on the exact number). The phylogenetic relationships of *Lithospermum* species have been characterized with molecular tools including ten chloroplast DNA regions in one study (Cohen 2011) and two chloroplast DNA regions and the nuclear ribosomal internal transcribed spacer in another

(Weigend et al. 2009). *Lithospermum* species are clustered around Mexico and the southwest United States, but can be found on every continent except Australia and Antarctica (Cohen & Davis 2009). *Lithospermum* is a member of the tribe Lithospermeae, in the family Boraginaceae. As members of Boraginaceae, these plants have flowers which are generally perfect, in scorpioid inflorescences. The plants tend to be covered in hairs and have four ovules per ovary that can produce a maximum of four nutlets (Levin 1972). Plants in the tribe Lithospermeae are notoriously difficult to divide into genera and species (Cohen & Davis 2009, Govoni 1975) and the tribe Lithospermeae is considered the most primitive tribe in the family Boraginaceae, due to the diversity in its members' pollen characteristics (Liu et al. 2010, Gabel 1987).

Growth Form and Demographics

Many *Lithospermum* species are perennial (Weller 1985a, Ganders 1979) and at least one species (*L. caroliniense*) may live several hundred years (Weller 1985a) (the lifespans of other *Lithospermum* species are unrecorded). In general these species set small numbers of seed compared to their potential seed number (four) and germination of those seeds and seedling survival tends to be low (Weller 1985a, Weller & Keeler 2000, Westelaken & Maun 1985). In some species (e.g., *L. caroliniense*) ovule abortion is common; differences in pollen load do not change seed set significantly nor did removing ovules (Weller 1985a, Levin 1972). These findings are not surprising as fixed abortion rates and low seed production are common in Boraginaceae (cited in Weller 1985a, cited in Weller & Keeler 2000, Levin 1972). Interestingly, some *Lithospermum* studies found that changes in nutlet mass did not noticeably affect germination rates or seedling vigor, even in species where nutlet size varied widely (Weller 1985a, Salisbury & Preston 1949). Possibly because of that variability in reproductive output, large changes in *L. caroliniense* recruitment are common from year to year (Weller 1985a).

Additionally, one study has found that smaller populations (less than one hundred individuals) have lower fecundity levels than larger populations (more than one hundred individuals) (cited in Molano-Flores 2001). Despite low rates of fecundity, in at least one remnant prairie a fragmented *Lithospermum canescens* population retained high levels of genetic diversity decades after it was first disturbed (Kittelson & Handler 2006), possibly because these plants can be long lived (see above).

Members of *L. caroliniense* are able to flower their first year, although a very slow rate of first flowering was more commonly observed in two studies (Weller 1985a, Weller 1985b). *Lithospermum canescens* has been observed to flower in its second year (Kittelson & Handler 2006). In its first year of growth, *L. canescens* sends up one or more vegetative shoots from its below ground apical bud (personal observation). The duration of an open flower has been estimated at approximately four days (Parrish & Bazzaz 1979).

The genus *Lithospermum* is heterostylous; pollen and sexual organs are often noticeably dimorphic and occasionally trimorphic (Ganders 1979, Halsted 1889, Weller & Keeler 2000, Levin 1968). Unequal pollen flow between morphs and unequal pollen production has been measured repeatedly in this genus, with more total pollen coming from the pin morph, but more legitimate pollen (pollen capable of fertilizing ova) coming from thrum anthers (Weller & Keeler 2000, Levine 1968, Ganders 1979). Populations may have unequal ratios of plants per morph, and that inequality can vary from population to population (Westelaken & Maun 1985, Molano-Flores 2001, Levin 1968).

Stratification and scarification are required for germination in some species of *Lithospermum* (cited in Weller 1985a, Parkinson & DeBolt 2005, Westelaken & Maun 1985,

Blake 1935). No doubt, the pericarp of *Lithospermaea* renders scarification helpful, if not essential for *Lithospermum*. The pericarp of that tribe has four layers and is embedded with calcium carbonate and silicon dioxide (Pustovoytov et al. 2004). Depth of burial and supplemental watering in dry years also affected seedling emergence (Weller 1985b, Weller 1989, Chantre & Orioli et al 2009). Dormancy can last for more than two cold periods (Weller 1985b). Germination may be favored by hot, dry autumn weather, but long periods of drought affect *L. canescens*, *L. incisum* and *L. caroliniense* negatively (Blake 1935, cited in Blake 1935). *L. arvense* requires high summer temperatures to germinate (cited in Chantre & Orioli et al 2009).

Some species of *Lithospermum* exhibit early successional characteristics, such as colonization of burned sites and beach dunes, but those characteristics may vary from site to site (Weller 1985a, Humphrey 1984). *Lithospermum ruderales* requires disturbance or it is lost from the landscape (Humphrey 1984).

Lithospermum canescens and *L. caroliniense* have brittle, woody root structures (personal observation, Weller & Keeler 2000). At Palouse Prairie, in Idaho and Washington State, *Lithospermum incisum* has deeply penetrating taproots with few off-branches, and *L. ruderales* has widely spreading roots which penetrate five to six feet deep (Weaver 1958).

Reproduction

All species of *Lithospermum* exhibit heterostyly, distyly or tristly and populations may exhibit cleistogamy as well as chasmogamy (Weller & Keeler 2000, Ganders 1979, Halsted 1889, Levin 1968, Smith 1879, Bessey 1880, Kittleson 2006; but see *L. incisum* notes in Halsted 1889). In addition, at least one species is capable of clonal reproduction, with clonal plants

growing up to one meter in width (Weller 1985a, Weller & Keeler 2000). At least one *Lithospermum* species may be semi-parasitic and can be propagated via cuttings (*L. canescens*, cited in Molano-Flores 2001). Self incompatibility is common in this genus, but some species are weakly self-compatible (Ganders 1979, Parrish & Bazzaz 1979, Levin 1972). In fact, in *L. caroliniense* populations as much as 27% of a cohort's seeds may come from cleistogamous flowers, and cleistogamous reproduction may increase when chasmogamic reproduction is low (Levin 1968, Levin 1972). However, in chasmogamic *L. caroliniense* flowers, pollen from the same morph is inhibited in the style, to ensuring outcrossing (Levin 1968).

Seeds of *Lithospermum caroliniense* do not disperse more than a few meters from the parent plant unless they are carried away by small mammals (Weller 1985a, Weller & Keeler 2000), and that lack of dispersal is probably common throughout *Lithospermum* due to the genus' large, heavy nutlets. In at least in one species large nutlet size is with higher germination rates than those associated with smaller nutlets; *Lithospermum caroliniense* establishes itself upon unstable sand dunes, and its large nutlet may result in nutlet burial by weather events as well as providing a larger taproot which could facilitate seedling survival in drought-prone areas (Weller 1985a). It is plausible that this characteristic could extend to other species growing in dry ecotypes, such as *L. canescens* and *L. incisum*, which grow on tallgrass prairies on well-drained soils (personal observation, Kittelson & Handler 2006). According to Weller (1985a) these heavy nutlets may play a role in the low seed set of this genus; diverting resources to a few nutlets is a potential adaptive value of abortion, however the specific reason and mechanism for ovule abortion is currently unknown.

The genus *Lithospermum* encompasses species with a wide variety of floral characteristics, from small (<10 mm) to large (>30 mm) corollas, which may be blue, yellow,

orange, white or any color in between. At least some species contain nectar (personal observation). Corolla lobes may be entire or lacinate, and corolla tubes also vary in depth (Weigend et al. 2009). Because of this variety, the pollination syndromes of these species vary between melittophily (bee pollination), psychophily (butterfly pollination), phalaenophily (moth pollination), ornithophily (bird pollination), or a combination of those four types. Reported pollinators of *L. caroliniense* are bumblebees, butterflies, sphinx moths and Ruby-throated hummingbirds (Weller 1985a, Weller & Keeler 2000). *Lithospermum canescens* and *L. caroliniense* attract “bees and butterflies” (Kittelsohn & Handler 2006, Levin 1968), long-tongued bees and *Vanessa cardui* (the painted lady, a butterfly; Molano-Flores 2001). Potential pollinators of *L. canescens* are insects including bees and moths (members of Anthophoridae, Apidae, Halictidae, and Lepidoptera; Parrish & Bazzaz 1979). Weller has recorded large annual variations in seed production and pollen load in *L. caroliniense*, which may have been due to the different responses of the species’ main pollinators (bumblebees and butterflies) to the weather (Weller 1985a). In one study, potential pollinators visited *L. canescens* flowers most often from nine a.m. to after five p.m. when temperatures were between 21 to 24 C° (Parrish & Bazzaz 1979).

Osmia illinoensis (a solitary bee) has been seen collecting pollen from *L. canescens* and nectar on *Lithospermum* species, and Weller has observed solitary bees removing pollen from *L. caroliniense* stigmas (Robertson 1925, Weller 1985a, Crosswhite & Crosswhite 1966). Crosswhite and Crosswhite list *Osmia atriventris* as a pollinator of *Lithospermum* species. (Crosswhite & Crosswhite 1966). The larvae of *Ethmia longimaculla* (a moth) have been found eating *L. caroliniense* plants, and the larvae of the moth *Haploa reversa* have been found

predating *L. canescens* plants (Westelaken & Maun 1985, Molano-Flores 2001, personal observation).

Anthropological and Medical Aspects

Human beings have long been fascinated with themselves, specifically with their ancient history and their bodies. *Lithospermum* plays a role in both of those arenas. The seeds of *Lithospermum* species have been found in fossil layers from the Miocene period (5 to 23 million years before present) in South Dakota (specifically *Lithospermum dakotense*), a packrat midden in Texas from the Wisconsin Glacial Episode (10 to 110 thousand years before present) and anthropological sites as early as the Neolithic time period (Baczyńska & Lityńska-Zajac 2005, Pustovoytov et al. et al. 2010, Gabel 1987, Van Devender et al. 1978). *Lithospermum* seeds have been found in about one third of all Mediterranean and Near Eastern anthropologic sites (cited in Pustovoytov et al. 2010). In addition, *Lithospermum officinale* seeds have been found in 13 Polish archaeological sites during routine archaeological investigations – two in Neolithic, four in Bronze Age, two in Roman period and two in Middle Age sites (Baczyńska & Lityńska-Zajac 2005).

The carbon-14 found in biogenic carbonate in *Lithospermum* seeds can potentially be used for dating fossil and archeological sites, especially since the carbonate and silicon dioxide found in the seed's pericarp protect it from microbe chemicals (Pustovoytov et al. 2004, Pustovoytov et al. 2010). In addition, *Lithospermum* nutlets often persist intact in ancient sites because of the silica content in their seed coats (cited in Baczynsak & Lityńska-Zajac 2005).

The seeds found in archeological sites are likely both naturally occurring (from weeds) and intentionally placed there by human inhabitants of the sites (Pustovoytov et al. 2004,

Baczyńska & Lityńska-Zajac 2005). For example, at least two burial sites in Poland have been found in which *Lithospermum* nutlets were deliberately applied to corpses, presumably for perceived medicinal or magical purposes (Baczyńska & Lityńska-Zajac 2005).

If ancient humans were using *Lithospermum* species for medicine, their intellectual offspring followed suit. Ancient herbalists from Pliny the Elder to people of the Ming Dynasty included two Boraginaceae species, *Anchusa tinctoria* and *Lithospermum erythrorhizon*, in their writings and in their medicinal preparations (Papageorgiou et al. 2008, Baczyńska & Lityńska-Zajac 2005). *Lithospermum erythrorhizon* contains shikonin which is a “wound-healing, anti-inflammatory, antimicrobial, antioxidant, antithrombotic and antitumor” chemical (Papageorgiou 2008, Huang 2010). Shikonin and its chiral partner alkannin are found in about 150 species, but are primarily obtained from *L. erythrorhizon* and *Alkanna tinctoria* (another member of Boraginaceae) (Papageorgiou 2008). This chemical pair has been used for centuries for its medicinal purposes, and they continue to be used in the medical community today (Papageorgiou 2008). *Lithospermum radix* contains a chemical which causes apoptosis in human tumor cells, *L. ruderale* and *L. officinale* produce chemicals used for thyroid diseases, and *L. officinale* chemicals regulate hormone secretion in the pituitary gland and strengthen capillary vessels (cited in Baczyńska & Lityńska-Zajac 2005).

Conclusion

Lithospermum is a genus that is found on every continent except Australia and Antarctica and contains approximately 40 species. Some of those species, such as *Lithospermum caroliniense*, have life history characteristics that are well documented, but most species have little or no information published about them; therefore, nothing conclusive can be said about

them other than to assume that characteristics found in the entire Boraginaceae family or Lithospermaea tribe, such as ovule number and inflorescence type, are found in each *Lithospermum* species.

From the life history characteristics that have been recognized, we can say that most *Lithospermum* species are perennial with low germination and seedling survival rates. Heterostyly is common in this genus, and plants set seeds enclosed in tough pericarps which are composed partially of calcium carbonate and silicon dioxide, which impact seed dispersal (Weller 1985a, Weller & Keeler 2000) and allow the seeds to persist for hundreds of years (Baczyńska & Lityńska-Zajac 2005, Pustovoytov et al. et al. 2010, Gabel 1987, Van Devender et al. 1978). At least some *Lithospermum* species have root systems that penetrate deeply into the soil (Weller & Keeler 2000, Weaver 1958). This genus has exhibited cleistogamy (Weller & Keeler 2000, Ganders 1979, Halsted 1889, Levin 1968, Smith 879, Bessey 1880, Kittleson 2006) and clonal reproduction (Weller 1985a, Weller & Keeler 2000) and may be able to be propagated via cuttings (cited in Molano-Flores 2001). Flowers may be blue, yellow, white or any combination of those colors, and corolla tube length is variable (Weigend et al. 2009). At least one species, *L. erythrorhizon* has a compound in it that has been used medically for centuries (Papageorgiou et al. 2008, Baczyńska & Lityńska-Zajac 2005).

Lithospermum canescens, the species included on in some of the studies below (see chapters 3 and 4), has not been the focus of many studies. Some studies on tallgrass prairie plants include one or two mentions of it in vegetative surveys, but only three other studies have been published based on research performed specifically on *L. canescens* (Kittleson & Handler 2006, Molano-Flores 2001, Parrish & Bazzaz 1979). These studies concentrated on the genetic makeup and fecundity of *L. canescens* and found the following: the populations maintained high

levels of genetic diversity generations after habitat fragmenting events (Kittleson & Handler 2006); populations of *L. canescens* may have skewed flower morphology ratios (Molano-Flores 2001); and that potential pollinators visited the *L. canescens* flowers most frequently from nine a.m. to after five p.m. (Parrish & Bazzaz 1979). Some life history characteristics, such as the fact that the species can bloom as early as two years old and that the flowers last approximately four days have also been reported (Kittleson & Handler 2006, Parrish & Bazzaz). It is pollinated by “bees and butterflies” (Kittleson & Handler, Levin 1968, Molano-Flores 2001) and possibly moths (Parrish & Bazzaz 1979). *Haploa reversa* (the reversed haploa, a moth) larvae predate *L. canescens* plants (Molano-Flores 2001). Because of the paucity of studies, and the importance of this species on the landscape (Molano-Flores 2001), it is important to study *Lithospermum canescens* further in order to characterize life history details as well as to determine the research possibilities of the species.

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CHAPTER 2. FIRST FLOWERING DATE TRENDS IN CLAY COUNTY, MINNESOTA

Introduction

For many decades studies have shown that humans are causing widespread changes to occur in global and local climate patterns and these changes have noticeably affected many thousands of species worldwide, including insects, mammals, birds and plants (Parmesan 2006). Specifically, these climactic changes have caused species to change the timing of their life cycle events. Of these, plants may be the easiest to study phenologically (Parmesan 2006), due to their sedentary tendencies, long-time association with humans and the obviousness of certain parts of their life cycles. Throughout the years, researchers have found that phenological changes vary over time primarily because of local climate changes. Phenological responses also vary greatly from species to species, although there is evidence that phenological changes may be similar within higher order taxonomic groups (Mazer et al. 2013). It is important to determine which plant species change their phenology and how so that the effects of future climate changes can be monitored comparatively and, hopefully, anticipated in order to provide guidance for land managers and for future research.

The goals of this study are to 1) monitor current flowering phenology patterns of plant in Clay County Minnesota and 2) compare current phenological patterns to historical patterns for the same species in the same location.

Materials and Methods

In 2011, field observations were made at two locations in Clay County, Minnesota (Figure 2.1): 1) The Nature Conservancy's Bluestem Prairie Scientific and Natural Area

(Bluestem Prairie) and 2) the Jarvis parcel in the Fish and Wildlife Service's Detroit Lakes Wetland Management District (Jarvis).

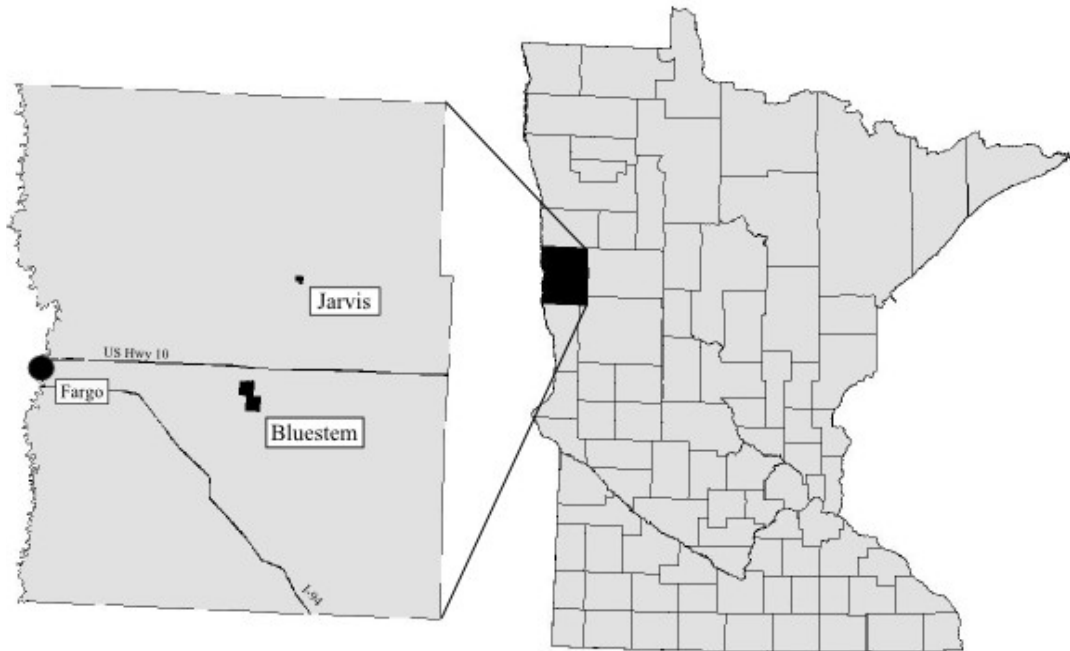


Figure 2.1. Bluestem Prairie Scientific and Natural Area and the Jarvis parcel, both of which are in Clay County, Minnesota. GIS data from the Minnesota DNR MIS Bureau, the Minnesota Department of Transportation, Survey and Mapping and the Minnesota DNR Division of Ecological Services Scientific and Natural Areas Program.

Bluestem Prairie Scientific and Natural Area (Bluestem Prairie) is located six and a half kilometers southeast of Glyndon, Minnesota (Lat/Lon: 46.87°N 96.48°W) (Figure 2.2). My study site at Bluestem Prairie was about 10.5 hectares in size. The predominant soil types are fine sands, with occasional loamy sands, sandy loams and clay loams. My study sites are on or directly adjacent to one of the Lake Agassiz beach ridges; therefore plant communities present include dry-mesic and mesic prairies and wetlands. Woodland species are also present in certain areas on Bluestem Prairie. Both Bluestem Prairie and Jarvis (see below) are classified as Northern Dry Prairies according to the Minnesota Department of Natural Resources' Native Plant

Communities classification system. Between 1977 and 2012 my study site at Bluestem was burned eleven times (an average of once per 3.2 years), had no herbicide applications and was not seeded with native plants.

The Jarvis parcel (Jarvis) is located 45 kilometers east of Hitterdal, Minnesota (Lat/Lon: 46.95°N 96.39°W) (Figure 2.3). I censused an area that was approximately 5 hectares in size. The main soil types are loams. Like Bluestem Prairie, Jarvis is in the tallgrass prairie ecosystem, and the communities that I sampled ranged from dry prairie to mesic prairie. Unlike Bluestem Prairie, Jarvis is not located on a beach ridge and has numerous prairie potholes. Jarvis was acquired in 1970 from a farmer who had used the area as pasture land. Since then it has not been grazed or seeded. The Jarvis parcel has been burned three times since 1995, (in 1995, 2000 and 2010), and had tree removal treatments applied in 2008 and 2012. No herbicide has been applied to the study site since the FWS obtained it in 1970.

In 2011, I initiated censuses of flowering plants at the Jarvis parcel of the Detroit Lakes Wetland Management District and The Nature Conservancy's Bluestem Prairie during the week of April 24th, which was the week herbaceous plants began blooming for that growing season. A census of each site consisted of walking the same route each time and recording the identity of all prairie and woodland plant species in flower. The route surveyed at Bluestem was approximately 3 kilometers long, and the route surveyed at Jarvis was also approximately 3 kilometers long.

These censuses were performed weekly between April and September on Bluestem Prairie, and intermittently between April and August on the Jarvis parcel. In total, I censused Bluestem Prairie approximately twenty times and the Jarvis parcel approximately five times over

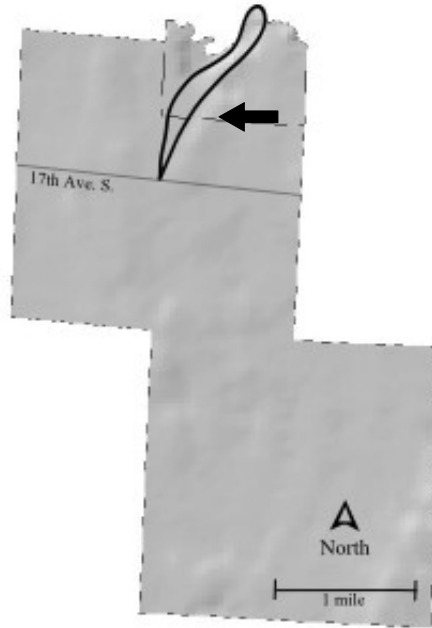


Figure 2.2. Survey route taken on Bluestem Prairie in 2011 and 2012. GIS data from the Minnesota DNR MIS Bureau, the Minnesota Department of Transportation, Survey and Mapping and the Minnesota DNR Division of Ecological Services Scientific and Natural Areas Program.

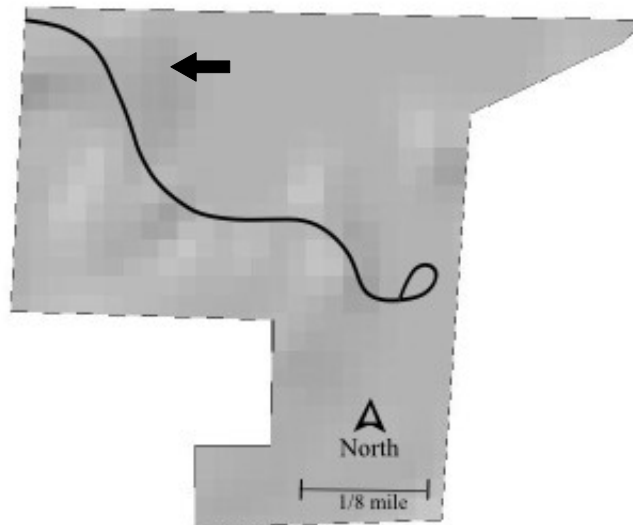


Figure 2.3. Survey route taken on the Jarvis parcel in 2011. GIS data from the Minnesota DNR MIS Bureau, the Minnesota Department of Transportation, Survey and Mapping and the Minnesota DNR Division of Ecological Services Scientific and Natural Areas Program.

the length of the growing season. In addition, as I performed a pollen limitation experiment on Bluestem Prairie and the Jarvis parcel (chapter 3) I recorded newly blooming species when I saw them. This tripled the plant observations I made while censusing each site. Flowering species were first identified from memory, if possible, and then were checked against wildflower field guides. If the species was not recognized initially, field guides were used to determine its identity.

In 2012, I surveyed flowering prairie and woodland plants at Bluestem Prairie using the same methods as in 2011. I began this census March 25th (the beginning of flowering in 2012) and ended the first week of August. In total, I surveyed the Bluestem Prairie plants 18 times. I also recorded newly blooming species as I found them opportunistically while performing a pollen limitation study on Bluestem Prairie (chapter 3). I visited the Jarvis parcel once in the spring, but did not visit it again in 2012 due to time constraints. I did not record any flowering data for the Jarvis parcel in 2012. In addition to regular censuses I made additional observations occasionally between the censuses.

Climate information for this study was collected by Steven Travers (personal communication) from the NOAA Weather Station near Ada Minnesota. Cumulative Annual Growing Degree Units were calculated by summing the degrees above 0° C and below 32.2° C for each day of the year.

The first flowering dates (FFD) for applicable species in 2011 and 2012 were compared to the mean FFD for the same species observed by O. A. Stevens between 1910 and 1960 (Travers & Dunnell 2009). In order to standardize the comparisons among years and varying sample sizes I calculated the deviation from the mean by calculating a z-score for each species in

2011 and 2012. The formula for the z-score was the following: $(z = (\chi - \mu) / \sigma)$ where χ = the FFD in either 2011 or 2012, μ = the mean FFD between 1910 and 1960, and σ = the standard deviation of the mean FFD between 1910 and 1960 (Zar 2010). Comparisons were only made if there were a minimum of 2 values for the 1910 to 1960 mean. In order to compare the mean FFD values for the recent five years compared to the period between 1910 and 1960 I combined observations made by Dunnell and Travers (2010) with my observations from 2011 and 2012. In 2011 and 2012 I observed species flowering that were not included in the comparisons because they were not reported by Stevens. There were a total of eleven species that I observed but did not compare in 2011 and 2012.

In order to examine possible trends across the growing season in tendency to shift flowering time, each species' z-score was plotted against the FFD of that species surveyed in 2011 and 2012 from the same year (Table 2.2).

The mean z-scores for 2011 and 2012 and the Δ FFD (from 1910-1961 to 2007-2012) were compared among the lifeforms of the plants. The lifeforms included graminoid, forb, shrub and tree. Lifeform type was assigned based upon the vegetative characteristics of each species. For example, if the species had one main, persistent, woody stem and was capable of growing taller than three meters it was considered a tree. Mean FFD for each lifeform in 2011 and 2012 was compared with one-way ANOVAs, as was the Δ FFD (current FFD – average FFD from 1910 to 1961). A Tukey-Kramer HSD test was run comparing the Δ FFDs among lifeforms and mean FFDs also. These tests were performed with the statistical program JMP (SAS Institute Inc. 1989-2007).

Mean z-scores and Δ FFD values were calculated for eighteen families in 2011 and seventeen families in 2012. Only families with more than one surveyed species were included. Means among families were compared with a one-way ANOVA using the statistical program JMP (SAS Institute Inc. 1989-2007), and the Δ FFDs were compared among families in the same manner. The mean FFD and Δ FFD values among families were compared with a Tukey-Kramer HSD test with JMP (SAS Institute Inc. 1989-2007).

Results

Long term weather data from the Fargo area (see Dunnell and Travers 2010) indicate that in general the climate at the study sites has shifted to warmer temperatures and more Annual Growing Degree Units (AGDUs) than in the past century. In particular, 2011 was 1.2°C warmer, had 4.6 inches more precipitation, had prior winter snow accumulation of 52.7 more inches than average historically, accumulated 20.9 more inches of snow from January to May, froze 12.0 days earlier and had 446 more Annual Growing Degree Units in comparison to the average values for 1910-1961. In contrast, compared with the data from 1910-1961, 2012 was warmer by 4.9°C, accumulated comparable precipitation (0.8 inches more in 2012), had a prior winter snowfall of 10.9 inches less than in the early 20th century, had 1.5 inches less snowfall from January to March, froze 12.0 days earlier and accumulated 896 more AGDUs. Compared to 2007-2012's average climate indicators, 2011 was slightly cooler than average (by 0.6°C), slightly drier (1.0 inches less precipitation), was following a winter that was snowier than average (by 39.9 inches), had more spring snow (by 8.6 inches), had its first freeze sooner (by 18.8 days) and had 79 more AGDUs in it. In comparison to 2007-2012's averages, 2012 was 3.1°C warmer and had 5.8 fewer inches of precipitation drier, followed a winter with 23.8 fewer inches of snow, had 3.7 fewer inches of snow from January through May, the first freeze of fall

came 16.8 days earlier and it had 529 more AGDUs. In sum, 2011 was warmer than historical and had about the same mean temperature as the average for the last five years. It was also a lot snowier than both periods, had an earlier freeze than usual and had many more AGDUs than the first half of the 20th century and the average for recent years. The year 2012 was much warmer than both the beginning of the last century and this century, had precipitation levels similar to historical levels but much less than the average for this century, had less snowfall than both measures, froze earlier than the early 20th and 21st centuries and accumulated more AGDUs than historical and recent averages.

Table 2.1. Climate information for Ada, in Clay County, Minnesota from 1910 to 2012.

Year(s)	Average Annual Temperature (Celcius)	Average Annual Precipitation (in)	Prior Winter Snowfall (in)	Snowfall January – May (in)	First Freeze of Fall (DOY)	Annual Growing Degree Units
1910-1961 (mean)	5.02	19.37	35.78	22.45	267.98	5287
2007-2012 (mean)	6.81	25.95	48.65	34.7	274.83	5654
2010	6.36	29.5	46.6	20.8	274	5862
2011	6.17	24	88.5	43.3	256	5733
2012	7.96	20.2	24.9	21	257	6183

In 2011, 65 plant species were observed that had been observed by Stevens prior to 1962. In 2012, 67 species were observed that had also been observed by Stevens. Thirty-six of the 96 species in Table 2.2 were observed in both 2011 and 2012. The earliest blooming species in 2011 was *Capsella bursapastoris* with an FFD of 98 (April 8th), and the last blooming flower of that season was *Allium stellatum* which bloomed on the 245th day of the year (September 2nd). In

2012 the first blooming species was *Populus tremuloides* which bloomed on the 75th day of the year (March 15th), and *Liatris aspera* was the last blooming plant with an FFD of 227 (August 14th) (neither *P. tremuloides* or *L. aspera* were recorded in 2011).

In 2011, the z-scores ranged from -7.8 to 7.8 standard deviations and the average was 0.6. In 2012, the z-scores ranged from -12.0 to 7.1 standard deviations and the average was -1.8. A negative z-score indicates a shift earlier in phenology. The by-species shift in mean FFD (Δ FFD) between recent (2007-2012) and historical (1910-1961) records ranged from -33.9 to 78.7 days with a mean of -2.4 days. The Δ FFD shift from historical records vs. 2011 was 1.0 day and the shift from historical records vs. 2012 was -16.1 days.

Regression analysis indicates that the z-score per species was significantly positively related to FFD in both 2011 and 2012 (Figures 2.4 and 2.5). The equation of the fitted line for the relationship between z-score and FFD for 2011 is $z\text{-score} = -3.913 + 0.29\text{FFD}$ and the equation for 2012 is $z\text{-score} = -7.665 + 0.042\text{FFD}$. The r-squared values are 0.108 and 0.402, respectively, with f-ratios of 7.475 and 44.765 and p-values of 0.0008 and <0.0001. The sample sizes for 2011 and 2012 were 64 and 70 species respectively. Observations were unusable for calculations if the species had had too few observations prior to 2011 or 2012 for an accurate standard error to be calculated. The most notable distinctions between the two years are the y-intercepts in each data set's best fit line (-3.913 and -7.665) and the differences in the f-ratios (7.475 and 44.765). The positive f-ratios indicate that the variance in the means of the sample are not due to random chance, and that is especially true for the 2012 data set. The fact that the y-intercept is lower in 2012 versus 2011 indicates that in 2012 species bloomed earlier overall than in 2011.

Table 2.2. Characteristics of native plant species found in Clay County, including family name, scientific and common names, lifeform and first flowering date (FFD) information from 1910 to 2012, including z-scores and mean shift in first flowering date from the 1910-1961 period to the 2007-2012 period.

Family	Scientific Name	Common Name	Lifeform	2011		2012		1910-1961			2007-2012			Δ FFD (# Days)
				FFD (DOY)	z-score (Sd)	FFD (DOY)	z-score (Sd)	mean FFD (DOY)	N	SE	mean FFD (DOY)	N	SE	
Aceraceae	<i>Acer saccharinum</i>	silver maple	tree	103	3.30	78	-18.30	99.2	51	1.2	96.4	5	5.4	-2.8
Aceraceae	<i>Acer negundo</i>	boxelder	tree			83	-24.45	115.4	47	1.3	104.5	4	8.4	-10.9
Apiaceae	<i>Cicuta maculata</i>	spotted water hemlock	herb	192	6.32	191	5.87	177.8	6	2.2	187.0	4	3.4	9.2
Apiaceae	<i>Osmorhiza longistylis</i>	sweet cicily	herb	143	-2.73			150.9	9	2.9	152.0	2	9.0	1.1
Apiaceae	<i>Pastinaca sativa</i>	wild parsnip	herb			158	-7.11	168.3	3	1.5	158.0	1	n/a	-10.3
Apiaceae	<i>Zizia aptera</i>	meadow zizia	herb			118	-8.77	142.6	8	2.8	137.7	3	9.8	-5.0
Apiaceae	<i>Zizia aurea</i>	meadow parsnip	herb			122	-15.13	146.8	22	1.6	140.8	4	6.5	-6.0
Apocynaceae	<i>Apocynum hypericifolium</i>	Indian hemp	herb			162	-5.50	173.9	14	2.2	162.0	1	n/a	-11.9
Asclepiadaceae	<i>Asclepias incarnata</i>	swamp milkweed	herb	192				187.0	2	1.0	192.0	1	n/a	5.0

Table 2.2. Characteristics of native plant species found in Clay Country (continued).

Family	Scientific Name	Common Name	Lifeform	2011		2012		1910-1961			2007-2012			Δ FFD (# Days)
				FFD (DOY)	z-score (Sd)	FFD (DOY)	z-score (Sd)	mean FFD (DOY)	N	SE	mean FFD (DOY)	N	SE	
Asclepiadaceae	<i>Asclepias ovalifolia</i>	oval-leaf milkweed	herb	165	-0.87			167.8	5	3.2	175.0	3	5.8	7.2
Asclepiadaceae	<i>Asclepias speciosa</i>	showy milkweed	herb	192	2.34			181.7	3	4.4	192.0	1	n/a	10.3
Asclepiadaceae	<i>Asclepias syriaca</i>	common milkweed	herb			165	-6.73	176.4	9	1.7	178.3	3	6.7	1.9
Asteraceae	<i>Achillea millefolium</i>	common yarrow	herb	165	-0.69	138	-13.08	166.5	8	2.2	158.8	5	5.7	-7.7
Asteraceae	<i>Ratibida columnifera</i>	upright prairie coneflower	herb	192	1.73	172	-3.80	185.8	8	3.6	184.4	5	5.2	-1.3
Asteraceae	<i>Taraxacum officinale</i>	common dandelion	herb	103	-23.86	86	-40.11	128.0	49	1.0	166.8	5	71.6	38.8
Asteraceae	<i>Antennaria aprica</i>	small-leaf pussytoes	herb			116	-0.97	120.7	6	4.8	116.0	1	n/a	-4.7
Asteraceae	<i>Aster novae-angliae</i>	New England aster	herb			191	-15.58	224.9	17	2.2	191.0	1	n/a	-33.9
Asteraceae	<i>Cirsium altissimum</i>	tall thistle	herb	192	-3.83			215.0	2	6.0	192.0	1	n/a	-23.0
Asteraceae	<i>Gaillardia aristata</i>	common gaillardia	herb	171	-0.15			171.8	5	5.2	168.5	4	3.7	-3.3

Table 2.2. Characteristics of native plant species found in Clay Country (continued).

Family	Scientific Name	Common Name	Lifeform	2011		2012		1910-1961			2007-2012			Δ FFD (# Days)
				FFD (DOY)	z-score (Sd)	FFD (DOY)	z-score (Sd)	mean FFD (DOY)	N	SE	mean FFD (DOY)	N	SE	
Asteraceae	<i>Helianthus maximiliani</i>	Maximilian sunflower	herb			192	-8.12	210.5	16	2.3	194.5	2	2.5	-16.0
Asteraceae	<i>Liatriis aspera</i>	tall blazing star	herb			227	4.18	217.6	5	2.2	214.3	3	11.7	-3.3
Asteraceae	<i>Liatriis punctata</i>	dotted blazing star	herb			215	-0.67	217.0	2	3.0	220.0	2	5.0	3.0
Asteraceae	<i>Liatriis pycnostachya</i>	prairie blazing star	herb			226	13.77	202.8	5	1.7	218.0	2	8.0	15.2
Asteraceae	<i>Solidago canadensis</i>	Canada goldenrod	herb			216	-1.12	218.5	13	2.2	219.5	2	3.5	1.0
Asteraceae	<i>Tragopogon dubius</i>	yellow salsify	herb	161	1.87			158.5	17	1.4	161.3	4	3.1	2.8
Betulaceae	<i>Corylus americana</i>	American hazelnut	tree	103	0.07	78	-9.16	102.8	11	2.7	96.4	5	5.4	-6.4
Betulaceae	<i>Betula papyrifera</i>	paper birch	tree			79	-35.61	125.6	33	1.3	97.5	2	18.5	-28.1
Boraginaceae	<i>Lithospermum canescens</i>	hoary puccoon	herb	138	-0.47	116	-7.67	139.4	7	3.1	129.0	6	3.5	-10.4
Boraginaceae	<i>Lithospermum incisum</i>	narrow-leaved puccoon	herb	153	4.49	158	6.34	140.9	7	2.7	148.6	5	3.5	7.7

Table 2.2. Characteristics of native plant species found in Clay Country (continued).

Family	Scientific Name	Common Name	Lifeform	2011		2012		1910-1961			2007-2012			Δ FFD (# Days)
				FFD (DOY)	z-score (Sd)	FFD (DOY)	z-score (Sd)	mean FFD (DOY)	N	SE	mean FFD (DOY)	N	SE	
Brassicaceae	<i>Capsella bursapastoris</i>	shepherd's purse	herb	98	-7.28	80	-13.40	119.4	15	2.9	91.3	4	6.8	-28.2
Brassicaceae	<i>Erysimum chieranthoides</i>	wormseed wallflower	herb			227	3.18	148.3	3	24.7	227.0	1	n/a	78.7
Campanulaceae	<i>Campanula rotundifolia</i>	bluebell bellflower	herb	167	-1.83	216	5.24	179.7	3	6.9	179.3	4	12.7	-0.4
Campanulaceae	<i>Lobelia spicata</i>	palespike lobelia	herb	192	2.08	191	1.80	184.7	3	3.5	193.3	3	1.9	8.7
Caprifoliaceae	<i>Symphoricarpos occidentalis</i>	western snowberry	shrub			173	-1.99	179.4	9	3.2	174.0	2	1.0	-5.4
Caryophyllaceae	<i>Cerastium arvense</i>	field chickweed	herb	134	-0.25	116	-8.09	134.6	12	2.3	126.4	5	4.3	-8.2
Commelinaceae	<i>Tradescantia bracteata</i>	longbract spiderwort	herb	167	6.26	157	2.64	149.7	10	2.8	162.0	2	5.0	12.3
Cyperaceae	<i>Carex pennsylvanica</i>	Pennsylvania sedge	grasslike	127	2.75	98	-9.98	120.7	15	2.3	112.5	2	14.5	-8.2
Euphorbiaceae	<i>Euphorbia esula</i>	leafy spurge	herb	140	-13.42			163.7	3	1.8	140.0	1	n/a	-23.7

Table 2.2. Characteristics of native plant species found in Clay Country (continued).

Family	Scientific Name	Common Name	Lifeform	2011		2012		1910-1961			2007-2012			Δ FFD (# Days)
				FFD (DOY)	z-score (Sd)	FFD (DOY)	z-score (Sd)	mean FFD (DOY)	N	SE	mean FFD (DOY)	N	SE	
Fabaceae	<i>Medicago sativa</i>	alfalfa	herb	165	2.40	125	-15.17	159.5	15	2.3	145.0	2	20.0	-14.5
Fabaceae	<i>Melilotus alba</i>	white sweetclover	herb	178	3.84	126	-30.31	172.2	13	1.5	152.0	2	26.0	-20.2
Fabaceae	<i>Amorpha canescens</i>	leadplant	shrub			178	-3.22	185.7	7	2.4	184.3	4	4.1	-1.5
Fabaceae	<i>Amorpha fruticosa</i>	desert false indigo	shrub	173	3.41			154.8	6	5.3	173.0	1	n/a	18.2
Fabaceae	<i>Melilotus officinalis</i>	yellow sweetclover	herb	161	1.39			159.0	23	1.4	164.5	2	3.5	5.5
Fabaceae	<i>Trifolium pratense</i>	red clover	herb	165	4.01			159.2	9	1.4	165.0	1	n/a	5.8
Fabaceae	<i>Trifolium repens</i>	white clover	herb	167	9.98			153.0	12	1.4	167.0	1	n/a	14.0
Fabaceae	<i>Vicia americana</i>	American vetch	herb	139	-7.26			150.0	22	1.5	145.7	3	7.7	-4.4
Hydrophyllaceae	<i>Hydrophyllum virginianum</i>	eastern waterleaf	herb	148	0.96			147.0	33	1.1	145.8	4	2.3	-1.2

Table 2.2. Characteristics of native plant species found in Clay Country (continued).

Family	Scientific Name	Common Name	Lifeform	2011		2012		1910-1961			2007-2012			Δ FFD (# Days)
				FFD (DOY)	z-score (Sd)	FFD (DOY)	z-score (Sd)	mean FFD (DOY)	N	SE	mean FFD (DOY)	N	SE	
Iridaceae	<i>Sisyrinchium angustifolium</i>	narrowleaf blue-eyed grass	herb			126	-5.62	141.5	11	2.8	139.0	5	4.5	-2.5
Lamiaceae	<i>Leonurus cardiaca</i>	common motherwort	herb			165	-3.38	185.7	3	6.1	165.0	1	n/a	-20.7
Lamiaceae	<i>Monarda fistulosa</i>	wild bergamot	herb	195	n/a	192	n/a	202.0	1	n/a	193.5	2	1.5	-8.5
Lamiaceae	<i>Prunella vulgaris</i>	common selfheal	herb	178	5.67			169.5	2	1.5	178.0	1	n/a	8.5
Liliaceae	<i>Allium stellatum</i>	autumn onion	herb	245	9.06	216	0.74	213.4	7	3.5	221.4	5	6.7	8.0
Liliaceae	<i>Maianthemum canadense</i>	Canada mayflower	herb	143	-1.00	95	-17.00	146.0	2	3.0	119.0	2	24.0	-27.0
Liliaceae	<i>Trillium cernuum</i>	nodding wake robin	herb	143	1.34	124	-4.39	138.6	7	3.3	137.6	5	4.0	-1.0
Liliaceae	<i>Zigadenus elegans</i>	mountain death camas	herb	178	2.79	162	-8.84	174.2	6	1.4	172.0	5	2.8	-2.2
Liliaceae	<i>Lilium philadelphicum</i>	wood lily	herb	192	9.04			174.5	4	1.9	181.3	4	4.3	6.8

Table 2.2. Characteristics of native plant species found in Clay Country (continued).

Family	Scientific Name	Common Name	Lifeform	2011		2012		1910-1961			2007-2012			Δ FFD (# Days)
				FFD (DOY)	z-score (Sd)	FFD (DOY)	z-score (Sd)	mean FFD (DOY)	N	SE	mean FFD (DOY)	N	SE	
Liliaceae	<i>Uvularia grandiflora</i>	largeflower bellwort	herb			124	-2.69	130.1	7	2.3	130.0	3	3.1	-0.1
Liliaceae	<i>Convallaria majalis</i>	European lily of the valley	herb			122	-7.04	140.8	5	2.7	122.0	1	n/a	-18.8
Linaceae	<i>Linum sulcatum</i>	grooved flax	shrub			191	4.00	179.0	2	3.0	191.0	1	n/a	12.0
Oleaceae	<i>Syringa vulgaris</i>	common lilac	herb	139	0.08	124	-13.29	138.9	44	1.1	128.3	3	5.4	-10.6
Onagraceae	<i>Oenothera biennis</i>	common evening primrose	herb	201	2.93			193.6	13	2.5	199.0	2	2.0	5.4
Onagraceae	<i>Oenothera nuttallii</i>	Nuttall's evening primrose	herb			191	2.69	177.0	6	5.2	195.5	4	1.8	18.5
Orchidaceae	<i>Cypripedium candidum</i>	white lady's slipper	herb	165	2.82			152.7	3	4.4	152.5	4	4.3	-0.2
Oxalidaceae	<i>Oxalis violacea</i>	violet wood sorrel	herb	140	-0.92	121	-13.61	141.4	16	1.5	134.2	6	3.2	-7.2
Oxalidaceae	<i>Oxalis stricta</i>	common yellow oxalis	herb	160	1.31			155.3	6	3.6	148.0	4	11.0	-7.3

Table 2.2. Characteristics of native plant species found in Clay Country (continued).

Family	Scientific Name	Common Name	Lifeform	2011		2012		1910-1961			2007-2012			Δ FFD (# Days)
				FFD (DOY)	z-score (Sd)	FFD (DOY)	z-score (Sd)	mean FFD (DOY)	N	SE	mean FFD (DOY)	N	SE	
Papaveraceae	<i>Sanguinaria canadensis</i>	bloodroot	herb			94	-9.91	116.7	16	2.3	102.7	3	6.3	-14.0
Polygalaceae	<i>Polygala senega</i>	Seneca snakeroot	herb	160	2.41			152.3	3	3.2	160.0	1	n/a	7.7
Ranunculaceae	<i>Anemone canadensis</i>	Canada anemone	herb	160	1.34	139	-10.47	157.6	13	1.8	152.8	4	5.1	-4.9
Ranunculaceae	<i>Anemone cylindrica</i>	candle anemone	herb	171	-0.74	165	-2.74	173.2	5	3.0	171.3	4	2.3	-1.9
Ranunculaceae	<i>Anemone patens</i>	pasque flower	herb	108	2.75	83	-2.98	96.0	3	4.4	100.7	6	4.1	4.7
Ranunculaceae	<i>Caltha palustris</i>	yellow marsh marigold	herb	140	4.29	109	-4.88	125.5	4	3.4	123.5	6	5.1	-2.0
Ranunculaceae	<i>Ranunculus abortivus</i>	littleleaf buttercup	herb	134	2.54	116	-1.99	123.9	10	4.0	128.2	5	4.4	4.3
Ranunculaceae	<i>Ranunculus rhomboideus</i>	prairie buttercup	herb	127	2.58	94	-7.03	118.1	14	3.4	112.3	6	5.6	-5.8
Ranunculaceae	<i>Actaea rubra</i>	baneberry	herb			122	-17.01	141.3	25	1.1	135.0	2	13.0	-6.3
Ranunculaceae	<i>Aquilegia canadensis</i>	wild columbine	herb	148	2.09			144.7	14	1.6	145.5	2	2.5	0.8

Table 2.2. Characteristics of native plant species found in Clay Country (continued).

Family	Scientific Name	Common Name	Lifeform	2011		2012		1910-1961			2007-2012			Δ FFD (# Days)
				FFD (DOY)	z-score (Sd)	FFD (DOY)	z-score (Sd)	mean FFD (DOY)	N	SE	mean FFD (DOY)	N	SE	
Rosaceae	<i>Potentilla arguta</i>	tall cinquefoil	tree	171	-2.13	156	-7.45	177.0	9	2.8	171.3	4	5.5	-5.8
Rosaceae	<i>Prunus americana</i>	American plum	shrub	139	8.34	99	-28.23	129.9	50	1.1	125.8	5	8.1	-4.1
Rosaceae	<i>Rosa arkansana</i>	prairie wild rose	tree	167	0.95	152	-10.84	165.8	14	1.3	163.0	5	4.5	-2.8
Rosaceae	<i>Prunus armeniaca</i>	apricot	shrub			96	-13.64	124.7	16	2.1	96.0	1	n/a	-28.7
Rosaceae	<i>Prunus pumila</i>	sandcherry	shrub			116	-4.27	139.5	2	5.5	116.0	1	n/a	-23.5
Rosaceae	<i>Spiraea alba</i>	white meadowsweet	herb	192	2.46			180.8	5	4.6	192.0	1	n/a	11.2
Rosaceae	<i>Fragaria virginiana</i>	wild strawberry	herb	139	6.72	122	-3.90	128.3	4	1.6	126.8	4	5.5	-1.5
Rubiaceae	<i>Galium boreale</i>	northern bedstraw	tree	165	3.34	152	-5.34	160.0	13	1.5	161.7	3	4.9	1.7
Salicaceae	<i>Populus deltoides</i>	cottonwood	tree	126	7.50	88	-20.86	116.0	42	1.3	108.0	5	6.5	-8.0
Salicaceae	<i>Populus tremuloides</i>	quaking aspen	herb			75	-12.98	110.8	17	2.8	94.3	3	9.7	-16.4

Table 2.2. Characteristics of native plant species found in Clay Country (continued).

Family	Scientific Name	Common Name	Lifeform	2011		2012		1910-1961			2007-2012			Δ FFD (# Days)
				FFD (DOY)	z-score (Sd)	FFD (DOY)	z-score (Sd)	mean FFD (DOY)	N	SE	mean FFD (DOY)	N	SE	
Saxifragaceae	<i>Heuchera richardsonii</i>	Richardson's alumroot	herb	158	-0.54	143	-3.71	160.6	9	4.7	155.3	4	5.3	-5.3
Scrophulariaceae	<i>Castilleja coccinea</i>	scarlet Indian paintbrush	herb	140	-10.00			160.0	2	2.0	140.0	1	n/a	-20.0
Scrophulariaceae	<i>Castilleja sessiliflora</i>	downy painted cup	herb	167	3.42			151.7	3	4.5	157.0	4	3.7	5.3
Scrophulariaceae	<i>Pedicularis canadensis</i>	Canadian lousewort	herb	139	-2.29			146.0	3	3.1	136.2	5	3.3	-9.8
Scrophulariaceae	<i>Penstemon albidus</i>	white penstemon	herb	160	0.81			157.0	4	3.7	158.5	4	4.4	1.5
Scrophulariaceae	<i>Penstemon gracilis</i>	lilac penstemon	herb	167	-2.42			174.5	12	3.1	164.5	4	4.6	-10.0
Scrophulariaceae	<i>Penstemon grandiflorus</i>	large beardtongue	grasslike	167	0.48			165.3	4	3.7	163.5	4	4.2	-1.8
Sparganiaceae	<i>Sparganium eurycarpum</i>	broadfruit burreed	tree			162	-2.85	171.0	4	3.2	162.0	1	n/a	-9.0
Ulmaceae	<i>Ulmus americana</i>	American elm	herb			79	-23.59	110.0	49	1.3	100.3	4	7.6	-9.8
Verbenaceae	<i>Verbena hastata</i>	swamp vervain	herb	192	11.00	191	10.00	181.0	2	1.0	191.5	2	0.5	10.5

Table 2.2. Characteristics of native plant species found in Clay Country (continued).

Family	Scientific Name	Common Name	Lifeform	2011		2012		1910-1961			2007-2012			Δ FFD (# Days)
				FFD (DOY)	z-score (Sd)	FFD (DOY)	z-score (Sd)	mean FFD (DOY)	N	SE	mean FFD (DOY)	N	SE	
Violaceae	<i>Viola pedatifida</i>	prairie violet	herb			127	-2.45	137.0	7	4.1	131.8	5	3.3	-5.2
Violaceae	<i>Viola sororia</i>	common blue violet	tree	138	10.00			128.0	2	1.0	138.0	1	n/a	10.0

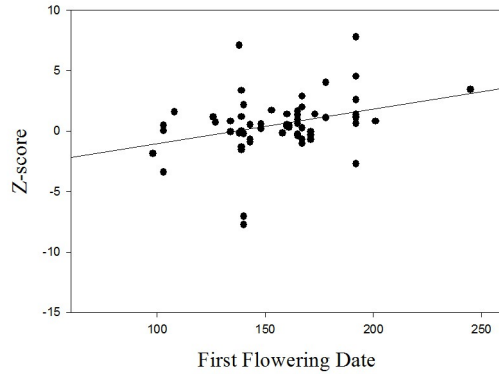


Figure 2.4. First flowering dates (FFDs) of individual species plotted against their z-score for 2011 ($z = (X-\mu)/\sigma$). (The line graphed is a line of best fit.)

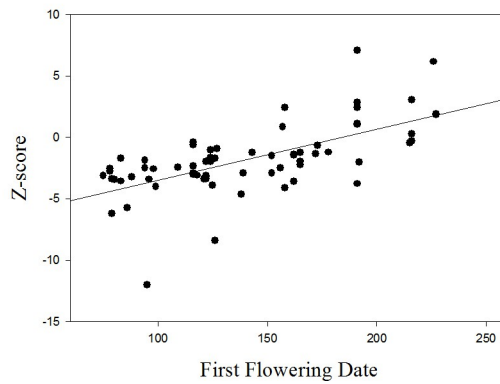


Figure 2.5. First flowering dates (FFDs) of individual species plotted against their z-score for 2012 ($z = (X-\mu)/\sigma$). (The line graphed is a line of best fit.)

The number of species which represented each lifeform varied from 2 to 78, (Table 2.3) with the herb lifeform as the most commonly observed and the grasslike lifeform as the least common. For the grasslike lifeform, there was no mean z-score in 2011, in 2012 the score was -2.0 and the average Δ FFD was -8.6. Herbs had a mean z-score of 0.6 in 2011, an average z-score of -1.5 in 2012, and the mean Δ FFD was -1.1. The 2011 average z-score for shrubs was 0.7, the 2012 score was -2.0 and the average Δ FFD was -2.1. Trees had a mean z-score of 0.7 in 2011 and -3.6 in 2012, with a mean Δ FFD of -12.8. Among the lifeforms, the mean z-scores

were similar for 2011 and 2012 (0.6 to 0.7 and -1.5 to -3.6 respectively) but varied quite a bit in mean Δ FFD, from -1.1 for herbs to -12.8 for trees.

A single factor ANOVA test indicated that there were significant differences among lifeforms in FFD. The ANOVA results from comparing 2011 FFDs among the different lifeforms resulted in an f-ratio of 4.27, a p-value of 0.008 and the degrees of freedom were 3 and 64. From the ANOVA comparing the FFDs from 2012 based upon lifeform, the f-ratio was 7.77, the p-value was 0.0002 and the degrees of freedom were 3 and 66. The Δ FFD ANOVA had 3 and 95 as the degrees of freedom, an f-ratio of 1.939 and a p-value of 0.128. The f-ratios of the 2011 and 2012 ANOVAs tell us that there are differences between the mean FFDs of the lifeforms that cannot be explained by chance, and the p-values support the significance of the tests. However, the ANOVA run on the Δ FFDs did not show significant differences between the lifeform means.

According to the Tukey-Kramer HSD test run on the data, in 2011 the tree lifeform had a significantly different FFD compared to the herb and shrub lifeforms, which were not significantly different from each other. The grasslike lifeform was not significantly different from any of the other lifeforms. The results were the same for 2012 (Figure 2.7). In addition, looking at the graphs of the results of the ANOVA tests (Figure 2.6), we can see that in 2011 the tree lifeform was significantly different from both the shrub and the herb lifeforms. It was not significantly different from the grasslike lifeform, but the grasslike lifeform had an N of 2, which made its SE values quite high. It is possible that if there were more data points for the grasslike lifeform, the tree lifeform would be significantly different from it, but there is currently no way to tell. Regarding the ANOVA test run on the Δ FFDs (Figure 2.8), while the p-value was not significant at an alpha value of 0.05, results of the Tukey-Kramer HSD test comparing the

Δ FFDs between lifeforms show us that the means of herb lifeform and tree lifeform are significantly different.

Table 2.3. Mean z-scores and their standard error values from 2011 and 2012 and average Δ FFD between (1910-1961) and (2007-2012) for plants found in Clay County, grouped by lifeform.

Lifeform	2011 z-score			2012 z-score			Δ FFD		
	N	\bar{x}	SE	N	\bar{x}	SE	N	\bar{x}	SE
grasslike	1	n/a	n/a	2	-2.00	0.58	2	-8.62	0.38
herb	56	0.56	0.34	51	-1.50	0.44	78	-1.09	1.67
shrub	4	0.69	0.33	5	-1.96	0.46	7	-2.06	5.19
tree	4	0.70	0.28	9	-3.58	0.36	9	-12.80	3.24

For plant families, mean z-scores for 2011 varied from -1.1 (Scrophulariaceae) to 1.8 (Liliaceae). 2012's average z-scores ranged from -4.5 (Fabaceae) to 2.0 (Campanulaceae). The mean Δ FFD from 1910 to 2012 was lowest in Betulaceae (-17.3) and Salicaceae (-12.2) and highest in Brassicaceae (25.3) and Onagraceae (11.9). The families that have shifted earlier include (from largest change to smallest change): Betulaceae, Salicaceae, Rosaceae, Oxalidaceae, Aceraceae, Lamiaceae, Scrophulariaceae, Liliaceae, Asteraceae, Apiaceae, Ranunculaceae and Boraginaceae. The families that have shifted later are (from largest change to smallest change): Brassicaceae, Onagraceae, Asclepiadaceae, Campanulaceae, Violaceae and Fabaceae.

The f-ratio and p-value from the ANOVA comparing the FFDs among plant families for 2011 were 2.16 and 0.03, respectively (Figure 2.9). The results of the Tukey-Kramer HSD test for 2011 indicate that Onagraceae, Asclepiadaceae, Liliaceae, Campanulaceae, Lamiaceae, Apiaceae, Fabaceae, Asteraceae, Rosaceae, and Scrophulariaceae had significantly different means from Aceraceae, Betulaceae and Brassicaceae. Ranunculaceae and Salicaceae had means that were significantly different from Onagraceae, Asclepiadaceae and Liliaceae. The f-ratio and

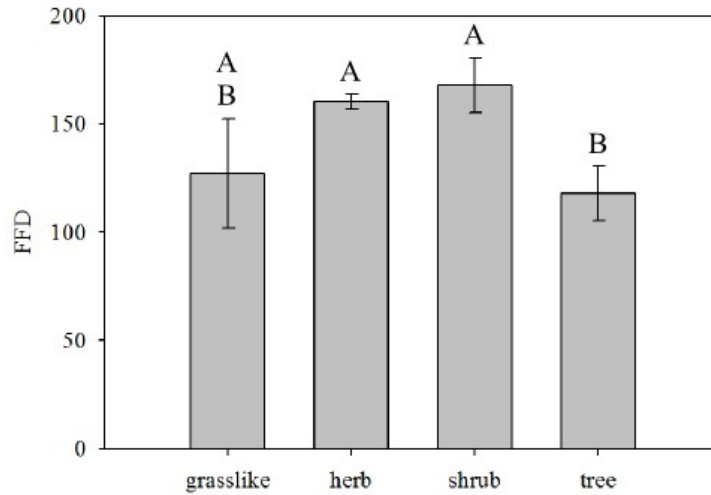


Figure 2.6. Least square mean First Flowering Day (1SE) for plants at Bluestem Prairie in 2011 by lifeform. Single factor ANOVA: $F = 4.27$, $P = 0.01$, $DF = 3, 64$.

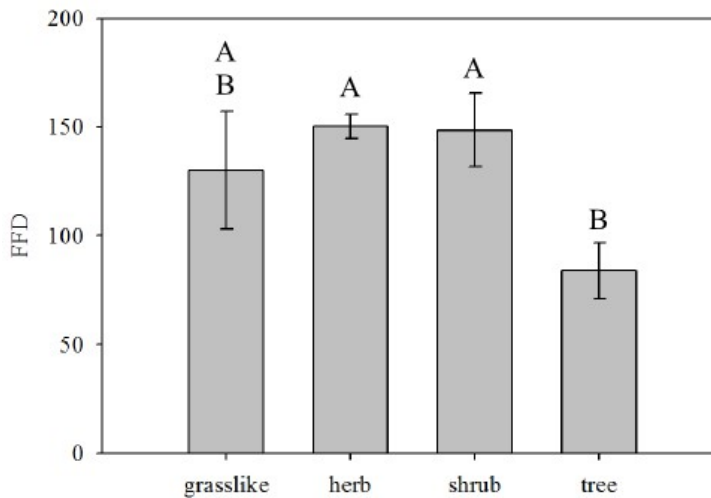


Figure 2.7. Least square mean First Flowering Day (1SE) for plants at Bluestem Prairie in 2012 by lifeform. Single factor ANOVA: $F = 7.77$, $P = 0.0002$, $DF = 3, 66$.

p-value statistics from the ANOVA comparing 2012 FFDs among plant families were 2.15 and 0.03, respectively. The results of the Tukey-Kramer HSD test for 2012 show that Campanulaceae, Onagraceae and Asteraceae had significantly different means from Salicaceae,

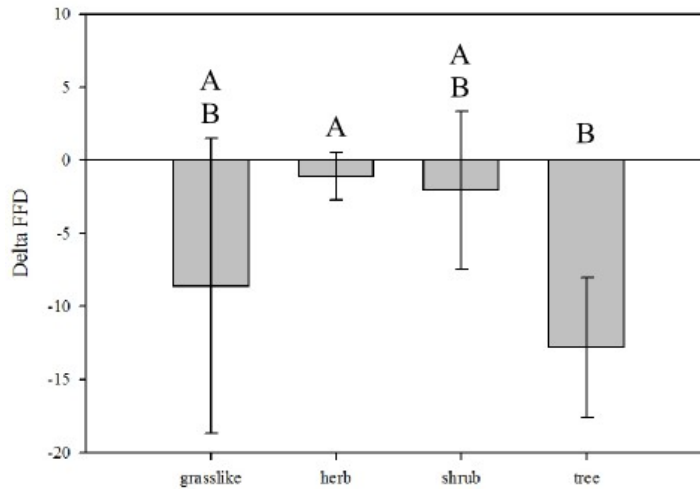


Figure 2.8. Least square mean Δ First Flowering Day (1SE) for plants surveyed in 2011 and 2012 by lifeform. Single factor ANOVA: $F = 1.94$, $P = 0.13$, $DF = 3, 95$.

Aceraceae and Betulaceae (Figure 2.10). Also, Rosaceae and Ranunculaceae had means that are significantly different from the means of Campanulaceae and Asteraceae. The results of the oneway ANOVA that was run on the Δ FFDs from 1910-1961 and 2007-2012 were an f-ratio of 0.84, and a p-value of 0.67 and the degrees of freedom were 17 and 77 (Figure 2.11). According to the Tukey-Kramer HSD test run on the means of the Δ FFD for those families, Brassicaceae was the only family significantly different from any of the rest.

Table 2.4. Mean z-scores, Δ FFD and associated standard error values and confidence intervals for plants growing in Clay County, grouped by plant family.

Family	2011 mean z score				2012 mean z score				mean Δ FFD			
	N	\bar{x}	SE	CI	N	\bar{x}	SE	CI	N	\bar{x}	SE	CI
Betulaceae	1	n/a	0.00	n/a	2	-4.48	1.72	3.37	2	-17.28	10.86	21.28
Salicaceae	1	n/a	n/a	n/a	2	-3.18	0.04	0.07	2	-12.19	4.24	8.31
Rosaceae	5	1.04	0.67	1.32	6	-2.96	0.29	0.57	7	-7.87	5.18	10.15
Oxalidaceae	2	0.15	0.38	0.75	1	n/a	n/a	n/a	2	-7.27	0.06	0.12
Aceraceae	1	n/a	n/a	n/a	2	-3.06	0.50	0.98	2	-6.84	4.06	7.97
Lamiaceae	1	n/a	n/a	n/a	1	n/a	n/a	n/a	2	-6.08	14.58	28.58
Scrophulariaceae	6	-1.08	1.28	2.51	0	n/a	n/a	n/a	6	-5.79	3.78	7.41
Liliaceae	5	1.78	0.96	1.88	6	-3.53	1.79	3.52	7	-4.91	4.95	9.70
Asteraceae	6	-0.89	0.70	1.38	10	-3.17	1.08	2.12	13	-2.49	4.88	9.56
Apiaceae	2	0.83	1.74	3.42	4	-2.01	1.49	2.91	5	-2.21	3.38	6.63
Ranunculaceae	7	0.83	0.31	0.60	7	-2.03	0.36	0.71	8	-1.39	1.52	2.99
Boraginaceae	2	0.76	0.94	1.84	2	-0.25	2.65	5.19	2	-1.34	9.09	17.81
Fabaceae	7	0.86	0.51	0.99	3	-4.51	2.10	4.11	8	0.36	4.68	9.17
Violaceae	1	n/a	n/a	n/a	1	n/a	n/a	n/a	2	2.40	7.60	14.90
Campanulaceae	2	0.07	1.13	2.21	2	2.03	0.99	1.95	2	4.13	4.54	8.90
Asclepiadaceae	3	0.48	1.14	2.23	1	n/a	n/a	n/a	4	6.11	1.78	3.49
Onagraceae	1	n/a	n/a	n/a	1	n/a	n/a	n/a	2	11.94	6.56	12.85
Brassicaceae	1	n/a	0.00	n/a	2	-0.81	2.65	5.19	2	25.26	53.41	104.68

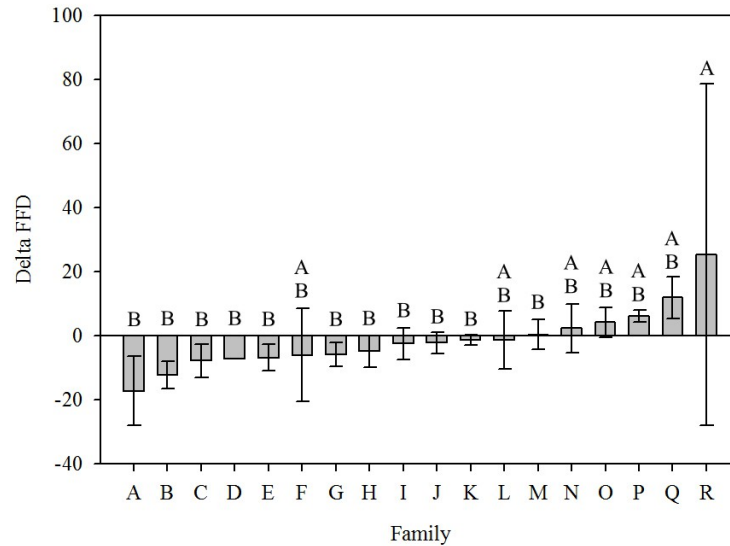


Figure 2.9. Δ FFD from 1910 – 1961 to 2007 – 2012 (1 SE) by plant family. A=Betulaceae, B=Salicaceae, C=Rosaceae, D=Oxalidaceae, E=Aceraceae, F=Lamiaceae, G=Scrophulariaceae, H=Liliaceae, I=Asteraceae, J=Apiaceae, K=Ranunculaceae, L=Boraginaceae, M=Fabaceae, N=Violaceae, O=Campanulaceae, P=Asclepiadaceae, Q=Onagraceae, R=Brassicaceae. (Oxalidaceae has no SE bar because the SE value was too small to be visible when graphed.)

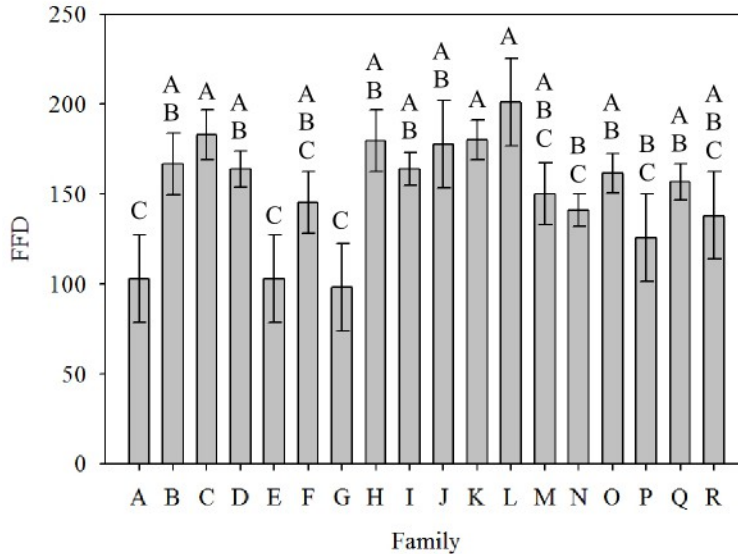


Figure 2.10. Least square means of First Flowering Day (1SE) for 2011 by plant family. Single factor ANOVA: $F = 2.159$, $P = 0.026$, $DF = 17, 53$. A=Aceraceae, B=Apiaceae, C=Asclepiadaceae, D=Asteraceae, E=Betulaceae, F=Boraginaceae, G=Brassicaceae, H=Campanulaceae, I=Fabaceae, J=Lamiaceae, K=Liliaceae, L=Onagraceae, M=Oxalidaceae, N=Ranunculaceae, O=Rosaceae, P=Salicaceae, Q=Scrophulariaceae, R=Violaceae.

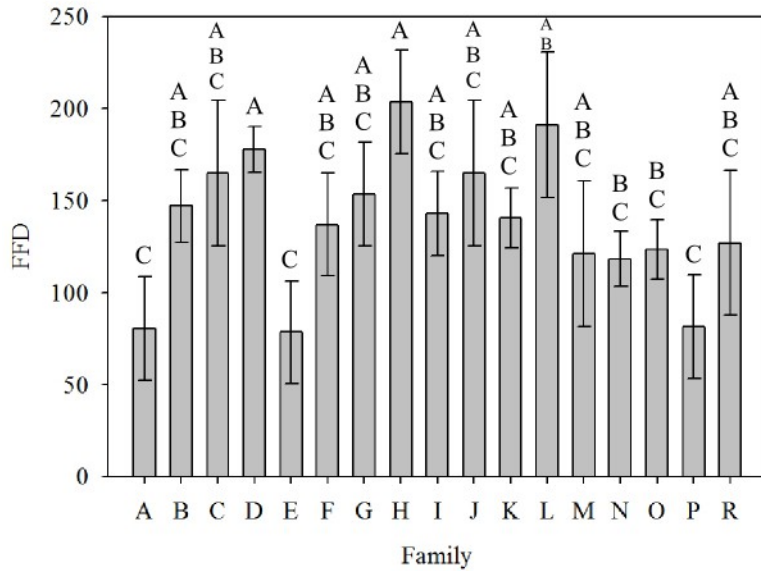


Figure 2.11. Least square means of First Flowering Day (1SE) for 2012 by plant family. Single factor ANOVA: $F = 2.15$, $P = 0.03$, $DF = 17, 52$. A=Aceraceae, B=Apiaceae, C=Asclepiadaceae, D=Asteraceae, E=Betulaceae, F=Boraginaceae, G=Brassicaceae, H=Campanulaceae, I=Fabaceae, J=Lamiaceae, K=Liliaceae, L=Onagraceae, M=Oxalidaceae, N=Ranunculaceae, O=Rosaceae, P=Salicaceae, R=Violaceae.

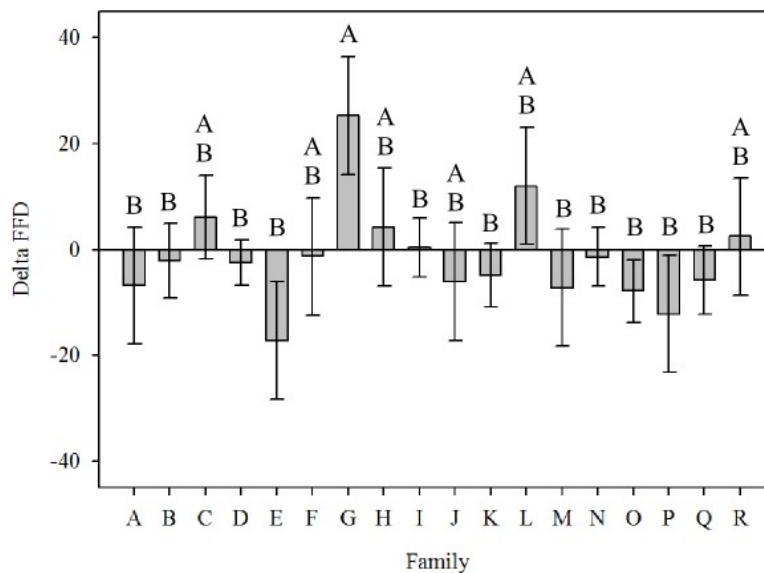


Figure 2.12. Least square mean Δ First Flowering Day (1SE) for plant families surveyed in 2011 and 2012. Single factor ANOVA: $F = 0.84$, $P = 0.65$, $DF = 17, 77$. A=Aceraceae, B=Apiaceae, C=Asclepiadaceae, D=Asteraceae, E=Betulaceae, F=Boraginaceae, G=Brassicaceae, H=Campanulaceae, I=Fabaceae, J=Lamiaceae, K=Liliaceae, L=Onagraceae, M=Oxalidaceae, N=Ranunculaceae, O=Rosaceae, P=Salicaceae, Q=Scrophulariaceae, R=Violaceae.

Discussion

The combination of climactic data and plant flowering data from 1910 to 2012 indicates that recent years have been warmer and, correspondingly, flowering has been earlier. Overall in Clay County, 2011 was warmer and wetter than historical measures and 2012 was much warmer than historical norms. These local climatic changes are consistent with global patterns of climate change. In particular, the average global temperature has increased by 0.13 degrees Celsius per decade since 1955 in response to anthropogenic forcings and greenhouse gas accumulation (IPCC 2007). It appears that the effect of these climactic changes are longer growing seasons and earlier flowering dates for many plant species. For example, 2011 had 446 more Annual Growing Degree Units (AGDUs) than historical, and 2012 had 896 more. In response, plants flowered an average of 1 day later than the historical average in 2011 and 16.1 days earlier in 2012. This is a bit surprising, since both 2011 and 2012 were warmer than the historical mean and had more AGDUs, even though 2011 had 450 AGDUs less than 2012. However, it is possible that the wetness of 2011 and the winter before delayed blooming (but see Mazer et al. 2013). Also, it appears from the regression analysis of 2011 FFDs vs. z-score (Figure 2.4) that late-blooming plants may have pushed the average FFD back. Delayed blooming in late summer/early fall species (Figures 2.4 and 2.5) because of climate change has been found in other studies (Sherry 2007, Menzel 2000, Cook et al. 2012). One explanation of these observations is the late responses of species that require vernalization in order to flower; warmer winters may not sufficiently vernalize these species. These findings, specifically that the shifts in FFD rely upon temperature and the fact that those shifts are more dramatic in warmer years, are supported by the findings of numerous studies regarding the effects of climate change upon plant species (Cook et al. 2012, Menzel 2000, Beaubien & Freeland 2000, Miller-Rushing &

Primack 2008), as well as the significance of the data in this study. For example, when linear regressions of the data were calculated, the y-intercept for 2012 was 3.75 standard deviations less than that for 2011, which was -3.91. The p-values and f-ratios for these regressions are highly significant, indicating strong relationships for both years. Alternatively, late season species may be more dependent on vegetative growth for high reproductive output and therefore increase fitness by growing longer before flowering (cite Matt and Barrett). Because of the results of this study and others, it is to be expected that if climactic trends continue towards warmer years, most plants in Clay County will continue to bloom earlier and earlier while plants that bloom in the late summer and early fall will continue to bloom later and later, as freezing temperatures allow.

Of the lifeforms represented in this study, trees have had the most plastic response to changes in climactic conditions. This is consistent with the findings of other studies which have followed the flowering responses of tree species in relation to climactic changes (Beaubien & Freeland 2000, Chmielewski & Rötzer 2001). We found that from 2011 to 2012, trees had mean z-scores varying from 0.7 to -3.6. They are also the lifeform with the largest shift from historical vs. recent FFDs with a mean change of -12.8 days., a shift that was significantly different from the the shifts of the shrub and herb lifeforms (Figure 2.8.). A possible reason for the highly plastic nature of the trees' response to climatic variables is the fact that they are often the first species to bloom on the landscape and therefore may have phenological cues which respond more to temperature versus day length compared to other lifeforms. In addition, trees have an incentive to bloom early in the year , before leaf-out, in order to facilitate airborne pollen dispersal (Clambey, personal communication) . Whatever the reasons behind the proportionally higher response trees exhibit, it can be expected that they will continue to respond to changes in

temperature more than the other lifeforms present in the study if climactic conditions continue to prove as changeable as they have recently.

Just as there were definite trends in the responses of lifeforms to changes in the local climate, there were also differences in the means of plant family responses to climactic changes. However, according to the results of the ANOVA tests run, those means are not significantly different based upon the f-values (2.16 and 2.15, respectively). However, those tests were significant based upon p-values (0.026 and 0.027, respectively), and the Tukey-Kramer HSD test run on each of the ANOVA tests, including the Δ FFD (from historical to the present), for the families showed significant differences between some of the families (Figure 2.12). Most significant changes in mean family FFD were for families which have shifted earlier; the exception is Brassicaceae, but that family is only represented by two species in the data set from 1910 to 2012 and it is therefore difficult to draw meaningful conclusions from that family's data. In addition, in 2011 and 2012 the families which were most likely to have significantly different means from most of the other families were Aceraceae, Betulaceae, Brassicaceae (in 2011) and Salicaceae (in 2012). Aside from Brassicaceae, those families contain early-blooming trees which probably impacted their mean FFDs to a large degree (see above). Also, Mazer et al. (2013) have found that families differ significantly in FFD within and among numerous study sites. It is worth noting that in general families had more positive z-scores in 2011 compared to 2012. Also, certain families had a much larger shift in FFD when comparing historical data to data from this century.

It is important to keep in mind that these data represent a small (two-year) expanse of time and relationships are probably not fully discernible from that short length of time. Since this is the case, we can assume that the data gathered in this portion of Clay County are accurate,

if not statistically significant, and those changes documented will continue to be progress as climate change continues along its course.

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CHAPTER 3. POLLEN LIMITATION AND POLLINATOR VISITATION IN *LITHOSPERMUM CANESCENS*

Introduction

Phenological studies in recent years have indicated that some plant species are shifting their phenological patterns in response to climate change (citations). An important implication of these patterns is that changing phenology may have fitness consequences by disrupting evolved mutualisms with pollinators and therefore reproductive success. I conducted an experiment to examine reproductive patterns in a prairie species known to have shifted its flowering earlier in the past century.

Lithospermum canescens (hoary puccoon) is a member of the Boraginaceae family, and is found in the open areas of west central and east central United States and into southern Canada and northern Mexico (USDA 2013) (Figure 3.1). It can grow to eighteen inches tall, but has a tendency to sprawl. It has a distylous, five petaled yellow/orange flower with a floral tube of ~1 cm and is pollinated by Lepidopterans (Bishop, personal communication). It may exhibit cleistogamy (personal observation), although that has not been verified. *Lithospermum canescens* can set up to four nutlets per flower and its nutlets have a hard seed coat, from which it derives its genus name. Its species name and the term 'hoary' come from the whitish appearance it receives from the small hairs which cover the plant. 'Puccoon' is a reference to the plants' use as dyestuff (Freckmann Herbarium).

Lithospermum canescens has shifted its First Flowering Date (FFD) significantly in the last century (Travers & Dunnell 2009). Other studies have shown that such shifts in phenology could result in asynchrony between plants and their traditional pollinators (Parmesan 2007, cited

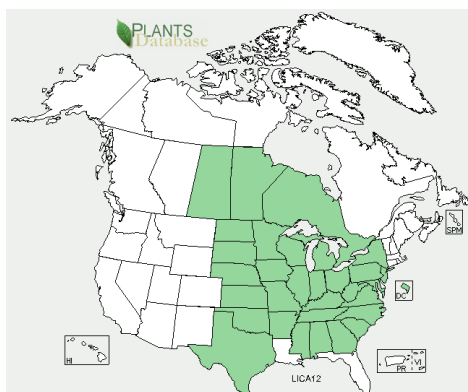


Figure 3.1. The species distribution of *Lithospermum canescens* (USDA 2013).

in Miller-Rushing 2008). It is important to establish whether or not these asynchronies are occurring in Clay County in order to, if possible, take steps to mitigate their effects.

Lithospermum canescens, as a plant with a specialist pollinator syndrome, has the potential to be an excellent indicator species to determine if asynchronies are occurring. This species is also under represented in the prairie restorations in the surrounding area, and can therefore be used as an example of what is happening in plant species that are similarly underrepresented. In order to determine whether or not there is a plant/pollinator mismatch, we ran a pollen-limitation study upon *L. canescens*.

Materials and Methods

In 2011 and 2012 I conducted manipulative experiments in populations of *Lithospermum canescens* at two sites to directly measure natural levels of seed production and to indirectly measure pollen deposition and its complement, pollen limitation on reproduction.

Pollen Limitation Experiment

In spring 2011, I flagged 90 plants each at Bluestem Prairie and Jarvis (see Chapter 2) once the plants began to flower. At both sites the plant community in which the study plants

were found was tallgrass prairie. I chose plants to include in the study at each site as they bloomed and assigned them to a treatment based on the order in which I found them. Each marked plant received one of three experimental treatments for all of the flowers on each plant: open-pollinated (O), extra pollen (X) or bagged (B) (see Figure 3.2).

The bagged treatment was expected to prevent pollination by any animal or insect vectors and was designed to test for possible seed production in the absence of pollen. On bagged plants, one to nineteen flowers per plant were bagged at the beginning of the flowering season using 6 by 6 inch bags made out of bridal veil (white tulle). Wire markers were twisted around the stem of bagged plants to indicate the date they were bagged, with different colored wire indicating different dates. The bags were secured to the plant with plastic coated wire.

The extra pollen treatment (X) was designed to determine how many seeds are produced per flower if there is no limit to the amount of pollen received. Flowers on the plants in this treatment were pollinated by hand with mixed pollen from adjacent *L. canescens* individuals. I attempted to pollinate as many flowers per plant as possible at any one time by hand. Between 1 and 3 flowers per plant were pollinated each day and I pollinated plants a total of seven days. Pollen was collected in the morning from non-study plants by removing mature anthers and mixing them in a vial. This mixed pollen was then applied to the stigma of the focal *L. canescens* flower with a fine paintbrush. If the flower morphology was pin, the pollen was brushed onto the stigma without removing the corolla. If the flower morphology was thrum, the corolla and attached stamens were carefully removed to prevent selfing and outcross pollen was then brushed on to the stigma. Flowers that were hand-pollinated were marked on the flower pedicel with a Sharpee marker to distinguish them from the untreated flowers on the same inflorescence and were left open to further insect pollination.

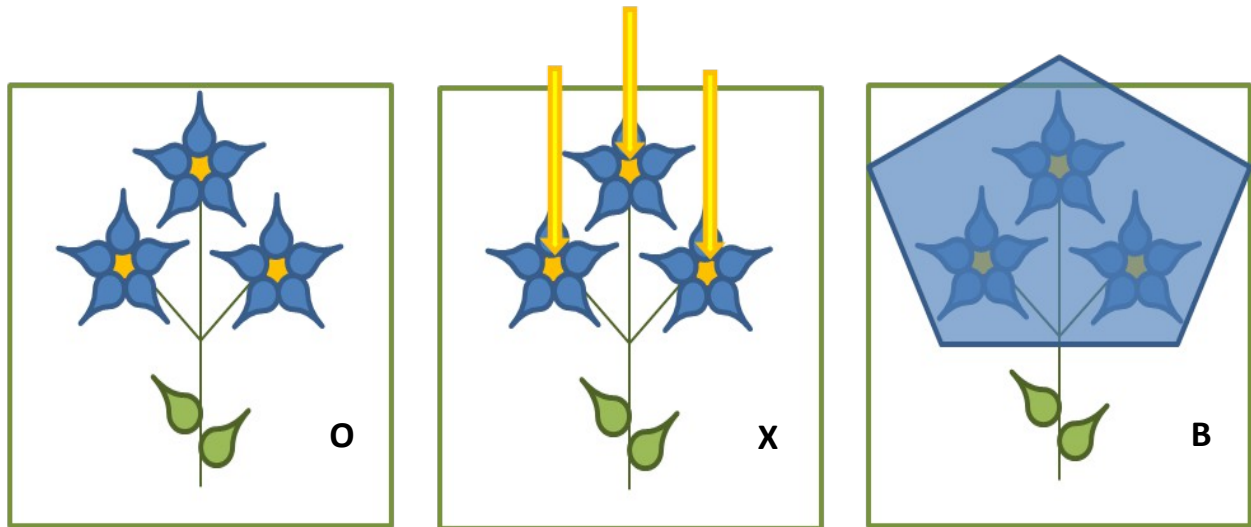


Figure 3.2. The pollen limitation treatments for *Lithospermum canescens*. Treatment O was open-pollinated, treatment X had pollen hand-administered and treatment B was bagged to exclude pollinators. The arrows in the second square represent the hand-administered pollen, and the blue polygon in the third square symbolizes the bag which excludes pollinators.

Plants in the open treatment were left undisturbed and served as a control to assess natural levels of seed production in this species. In this treatment, I monitored the flowers opening during a given week by placing colored wire around the stem of the inflorescence between the last unopened and the first opened flowers once a week. Different colors were used to indicate the different dates that those flowers opened to indicate the timing of maturity of flowers on open treatment plants. In 2011 my experiment lasted for six weeks, from first bloom to last bloom. I treated approximately 5 to 40 flowers per plant, depending upon treatment type.

After all study plants were finished setting seed (in August) I collected the plants and refrigerated them until they could be analyzed. *L. canescens* flowers each have four ovules and can produce up to four seeds. I counted the number of viable seeds produced by examining the nutlet scars left behind after the nutlets had fallen (approximately two to three weeks after the flower was pollinated). Seed set can be determined effectively with this technique (Forrest &

Thomson 2009, and see below). These scars are visible to the naked eye and were considered to represent a viable seed if the nutlet had reached full size before falling off. If the nutlet remained on the plant, it was considered viable if it resisted crushing and had reached a size of 1.5 to 2 mm in length.

In 2012, I repeated the experiment at Bluestem Prairie but not at Jarvis. Only the open-pollinated (O) and bagged (B) treatments were administered at the beginning of the season; the hand-pollination treatment (X) was initiated near the end of the blooming season (June 15th) after a pilot experiment to determine the signals of stigma maturation. After pollinators were observed accessing flowers that had been bagged with tulle, flowers in the bagged treatment were subsequently (after May 9th) covered with cages made of wire and tulle which were firmly staked to the ground. The wire cages were pyramidal with a base of one square foot.

I conducted the last part of the experiment on plants fifty meters to the west of my first experiments on a parcel at Bluestem that had been burned by the Nature Conservancy earlier in the spring (April 2012), which caused a delay in blooming. These plants (at the burned site) were marked after plants in the first community (at the unburned site) had finished blooming. Fifty three additional plants were marked at the burned site for the open (O) treatment.

The hand-pollinated plants (X) were located in the burned unit. Plants were bagged when they had at least three buds remaining. Once two or three flowers (three flowers open on one inflorescence at the same time was unusual) were open, pollen was administered to them in the same manner as in 2011. Red wire was placed below the oldest open flower and blue wire was placed above the youngest open flower to indicate where the pollinated treatment began and ended. After the flowers were hand pollinated, the wire and tulle cages were replaced over the

plants to ensure that there were no other pollination events. I conducted hand pollination of each plant once.

Plants were collected after *Lithospermum canescens* stopped blooming (beginning the week of July 8th) to evaluate seed set. The unburned site pollen limitation experiment took place from April 25th to June 11th and the burned site experiments took place from June 6th to June 25th.

After the seed set data was collected, one-way ANOVA tests were run to compare seed set from site to site and from year to year based upon treatment type. Four ANOVAs were performed using JMP statistical software (SAS Institute Inc. 1989-2007); one comparing seed set among treatments for the *L. canescens* population at Bluestem and Jarvis in 2011, one comparing seed set among the treatment types on Bluestem in 2012, one comparing seed set on Bluestem in 2011 and 2012 and one test comparing the seed set on Bluestem in the burned and unburned areas in 2012.

Background Community Flowering

In order to assess the environmental context in which *L. canescens* was blooming, I monitored the flowering patterns of other species within the flowering period for the experimental *Lithospermum canescens* population at the unburned site in 2012. A single transect was used that was 100 meters long and located within the *L. canescens* population. Once a week from April 30th to June 21st I walked the length of the transect and recorded the species and number of individual flowers within 3 meters on each side of the transect line. If the blooms were too small to distinguish individual flowers from one another I recorded the entire inflorescence. The transect data were recorded weekly during the entire *L. canescens* blooming period at the unburned site. Species surveyed along the transect included hoary puccoon

(*Lithospermum canescens*), pasque flower (*Pulsatilla patens*), pussy toes (*Antennaria aprica*), field chickweed (*Cerastium arvense*), prairie buttercup (*Ranunculus rhomboideus*), prairie smoke (*Geum triflorum*), fringed puccoon (*Lithospermum incisum*), ragwort (*Senecio plattensis*), gaura (*Gaura coccinea*), and thimbleweed (*Anemone virginiana*). Other species were recorded, but these were the most abundant and therefore they are the species that were included in the analysis.

Flower Persistence, Anther Dehiscence, and Nectar Production in *L. canescens*

I conducted an experiment to determine the lifespan of individual *Lithospermum canescens* flowers by observing how long they remained open after being treated with one of two treatments. (This experiment was performed in the burned area at Bluestem Prairie.) One treatment was an unpollinated treatment, in which the flowers were caged for the duration of their open period. The other treatment was a hand-pollinated treatment in which the plants were caged except when I administered outcross pollen with a paintbrush (I pollinated each flower once on the day that it opened). At the beginning of the experiment all open flowers were pinched off, leaving the oldest bud. If subsequent flowers opened on the same inflorescence as the flower being observed they were pinched off as well, leaving one open flower on the inflorescence. The flowers were observed each day to determine if they were open or closed, and those data were analyzed to find the average lifespan of a flower under each treatment. In total I processed eighty *L. canescens* plants for this experiment, forty per treatment (however, there were three mortalities: one in the open treatment and two in the caged treatment). After data on flower length were collected, a Student's t-test was run on the data with JMP statistical software (SAS Institute Inc. 1989-2007).

To determine another *L. canescens* flower characteristic, I ran a short experiment in the burned area of Bluestem Prairie to determine when this species' anthers dehisced. On June 13th and 25th, sequential flowers from ten inflorescences were examined to ascertain when anther dehiscence occurred. First the flower corolla was separated from its sepals and the floral tube was split with a sharpened piece of wire. The inside of the tube was then examined with a hand lens, and pollen abundance was rated on a scale of 0 (no pollen present) to 3 (abundant pollen present). Two values were collected, one for pollen abundance on the anthers and the other for pollen abundance on the corolla tube. From these values I was able to determine when the anthers dehisced, which was when pollen was first visible on the flower parts, and when pollen was most abundant.

The amount of nectar produced and the time of day of nectar production were determined over the course of two days (June 26th and 27th) in the burned area of Bluestem Prairie. To collect nectar, flower corollas were carefully plucked from the sepals, and a 20 μ l capillary tube was used to collect the nectar which was squeezed out of the flower, as well as any nectar left on the sepals. The length of the nectar in the capillary tube was then measured with digital calipers. There were three treatments: plants that had never been bagged (A), plants that had been bagged for all of their blooming period but whose cages were removed for twelve hours to determine if there was a difference in when pollinators visited the plants (B) and bagged plants which did not have their cages removed until nectar was gathered (C). On the evening of the first day of the study, nectar was gathered from ten plants from treatment A and nine plants from treatment C. The cages were removed from eight plants (treatment B) for the next day's measurements. The next morning, nectar levels were taken from the eight plants from treatment B, ten plants from treatment A and five plants from treatment C. Eight more plants were uncovered for the

evening's measurements (treatment B). Nectar levels were measured in those flowers that evening. Data from this experiment were analyzed with an ANOVA test which compared nectar production by treatment and time using JMP statistical software (SAS Institute Inc. 1989-2007).

Verification of the Nutlet Scar Technique

In order to ensure that counting the seed scars left by nutlets was an accurate indication of viable nutlet production I conducted one final experiment on ten separate plants. Each branch of the plants were marked with wire to track each specific branch's nutlet production throughout the season. The plants were visited on a weekly basis, and viable nutlets per flower were counted. On a weekly basis from May 31st to June 26th I revisited the experimental plants and counted the viable nutlets present at each developing flower. The viability of nutlets was determined by nutlet size and color; viable nutlets are stony-looking and greyish and are greater than 1.5mm in length (Figure 3.3). Nutlet scars were counted immediately after all of the nutlets had dropped from each *L. canescens* stem. It was obvious when a nutlet had stopped developing, and when a fully sized nutlet was not viable (nutlets were a papery white and could be crushed with little effort, versus the viable nutlets which were durable and grey in color). A regression analysis was run on this data using JMP statistical software (SAS Institute Inc. 1989-2007).



Figure 3.3. A *Lithospermum canescens* seed without its pericarp (left) and another *L. canescens* seed enclosed in its pericarp (right); the latter, enclosed seed is called a nutlet.

Results

Pollen Limitation Experiment

In 2011, average seed set did not vary significantly between Bluestem and Jarvis with one exception: seed set was significantly higher at Bluestem (1.1 nutlets/flower) than at Jarvis (0.8 nutlets/flower) in the open treatment (Figure 3.4). Average seed set in 2011 varied significantly among pollination treatments; specifically, the bagged (B) treatment resulted in mean seed sets of 0.2 seeds per flower for Bluestem and 0.2 for Jarvis. The hand pollinated (X) treatment yielded average values of 0.5 for Bluestem and 0.6 for Jarvis. In the open (O) treatment, Bluestem had an average of 1.1 seeds set per flower and Jarvis had an average of 0.8. The lowest seed set for all treatments and sites was 0.2 seeds/flower (B treatment, Jarvis) and the highest was 1.1 seeds/flower (O treatment, Bluestem). The ANOVA test comparing the seed set among the treatments on Bluestem and Jarvis in 2011 resulted in an f-ratio of 170.2 and a p-value of <0.0001 , with degrees of freedom of 5 and 8219. The results of the effect test on the difference between the sites were an f-ratio of 1.4 and a p-value of 0.24 with 1 degree of freedom. The results of the effects test for treatment were an f-ratio of 206.9 and a p-value of <0.0001 with 4 degrees of freedom. (Bluestem Prairie had higher seed set than Jarvis, which explains why the interaction term is significant.) When looking at the graph that resulted from the 2011 Bluestem ANOVA, we can see that all three treatments are significantly different from each other. The same is true of the Jarvis results from 2011.

In 2012, only Bluestem was used as a study site. Average seed set differed significantly from treatment to treatment in every case except one; the hand pollinated and bagged treatments were not significantly different. The bagged treatment averaged 0.5 seeds per flower, the hand

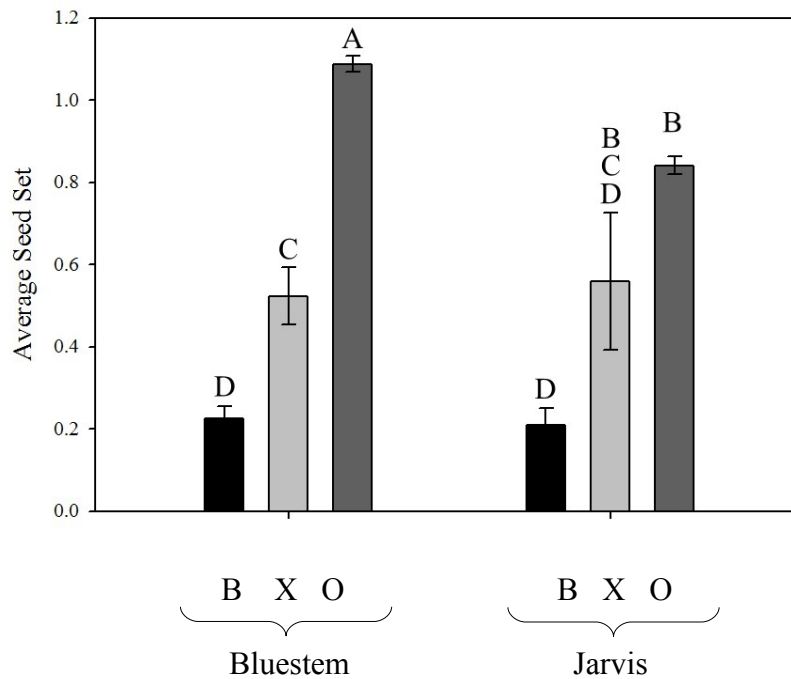


Figure 3.4. Least square means of average seed set in 2011 at Jarvis and Bluestem Prairie (1SE) by treatment. Nested ANOVA: $F = 170.2$, $P < 0.0001$, $DF = 5$, 8219. Effects test for treatment: $F = 206.3$, $P < 0.0001$, $DF = 4$. Effects test for site: $F = 1.4$, $P = 0.24$, $DF = 1$. B=bagged treatment, C=caged treatment, X=hand-pollinated treatment, O=open treatment.

pollinated treatment had 0.7 seeds/flower and the open treatment yielded a seed set of 1.1 (Figure 3.5). The caged and bagged seed sets were also significantly different. The lowest seed set recorded for 2012 was 0.1 in the caged treatment, and the highest was 1.1 in the open treatment. The ANOVA test results from the comparison of seed set among treatments from Bluestem in 2012 were an f-ratio of 45.61 and a p-value of < 0.0001 . On Bluestem in 2012, the bagged (B) and hand-pollinated (X) treatments were not significantly different, but the caged treatment (C) was significantly lower than all of the other treatments with a mean seed set of 0.07 seeds/flower and the open treatment (O) was significantly higher than the other treatments with a mean seed set of 1.07.

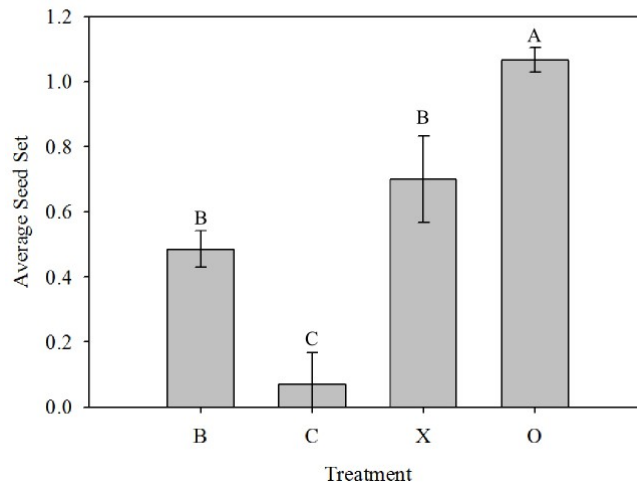


Figure 3.5. Least square means for seed set (1SE) on Bluestem Prairie by treatment for 2012. Single factor ANOVA: $F = 45.61$, $P = <0.0001$, $DF = 3, 1456$. B=bagged treatment, C=caged treatment, X=hand-pollinated treatment, O=open treatment.

The main differences between Bluestem's seed set in 2011 and 2012 were in the bagged treatment; seed set for this treatment in 2011 was an average of 0.2 seeds/flower and in 2012 it was 0.5 seeds per flower, a difference of 0.3 seeds/flower. The open treatment in 2011 and 2012 resulted in an average seed set of 1.1 seeds/flower. The hand pollinated treatment in 2011 yielded 0.5 seeds per flower and in 2012 seed set for this treatment was 0.7 seeds per flower, a difference of 0.2. The results from the nested ANOVA performed on this data were an f-value of 119.43, and a p-value of <0.0001 and the degrees of freedom were 6 and 6518. The results of the effects test for treatment were an f-ratio of 143.3 and a p-value of <0.0001 , with 5 degrees of freedom. The results of the effects test on year were an f-ratio of 0.03 and a p-value of 0.57 with 1 degree of freedom.

Seed set in the burned area versus the unburned area was not significantly different (Figure 3.7); therefore the plants from each area were treated as one population. An ANOVA

test was run comparing the burned and unburned areas; the resulting f-ratio was 0.08, the p-value was 0.79 and the degrees of freedom were 1 and 868.

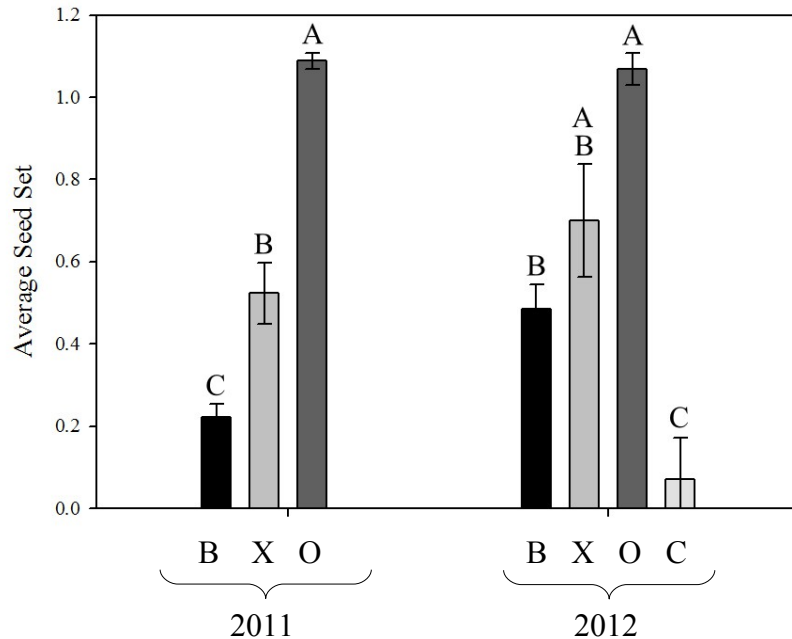


Figure 3.6. Least square means of average seed set in 2011 and 2012 at Bluestem Prairie (1SE) by treatment. Nested ANOVA: $F = 119.43$, $P < 0.0001$, $DF = 6, 6518$. Effects test for treatment: $F = 143.3$, $P < 0.0001$, $DF = 5$. Effects test for year: $F = 0.3$, $P = 0.57$, $DF = 1$. B=bagged treatment, C=caged treatment, X=hand-pollinated treatment, O=open treatment.

Background Community Flowering

Figures 3.8. and 3.9. include the ten most common species along the survey transect. Maximum bloom number varied based upon species, as did blooming duration and bloom distribution. Compared to the other species, *L. canescens* is the third most prolific producer of blooms with a bloom number comparable to ragwort (*Senecio plattensis*), gaura (*Gaura coccinea*) and fringed puccoon (*L. incisum*). The species with the highest bloom number, field chickweed (*Cerastium arvense*), had many times more blooms than the other species found along

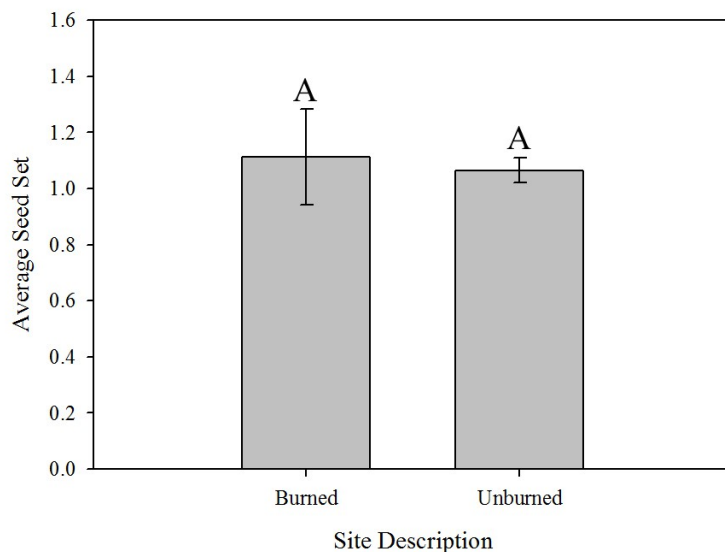


Figure 3.7. Least square means for seed set (1SE) on Bluestem Prairie by burned vs. unburned sites. Single factor ANOVA; $F = 0.08$, $P = 0.79$, $DF = 1, 868$.

the transect. Field chickweed is a generalist pollinated species, as are four other graphed species. Of the other five species, 20% are pollinated by moths (including *L. canescens*), 20% are pollinated by bees and 10% are pollinated by bees and moths. Only one species besides *L. canescens* appears to have had a bimodal distribution of blooms, which is pussy toes (*Antennaria aprica*). There appears to be a spike in bloom abundance at the beginning of *L. canescens*' bloom period which ends around day 135, that spike is followed by a paucity of blooms which lasts until about day 150. Because *L. canescens* is moth pollinated, it may be competing with other moth pollinated species and generalist pollinated species during those periods of bloom abundance. Of the surveyed species, the most abundant moth and generalist pollinated species include pasque flower, pussy toes, fringed puccoon, ragwort, gaura and thimbleweed. In short, all of the species which were the most common in the survey period have the capability to compete with *L. canescens* except one species, and that species (prairie buttercup) is butterfly pollinated and therefore is also a potential competitor.

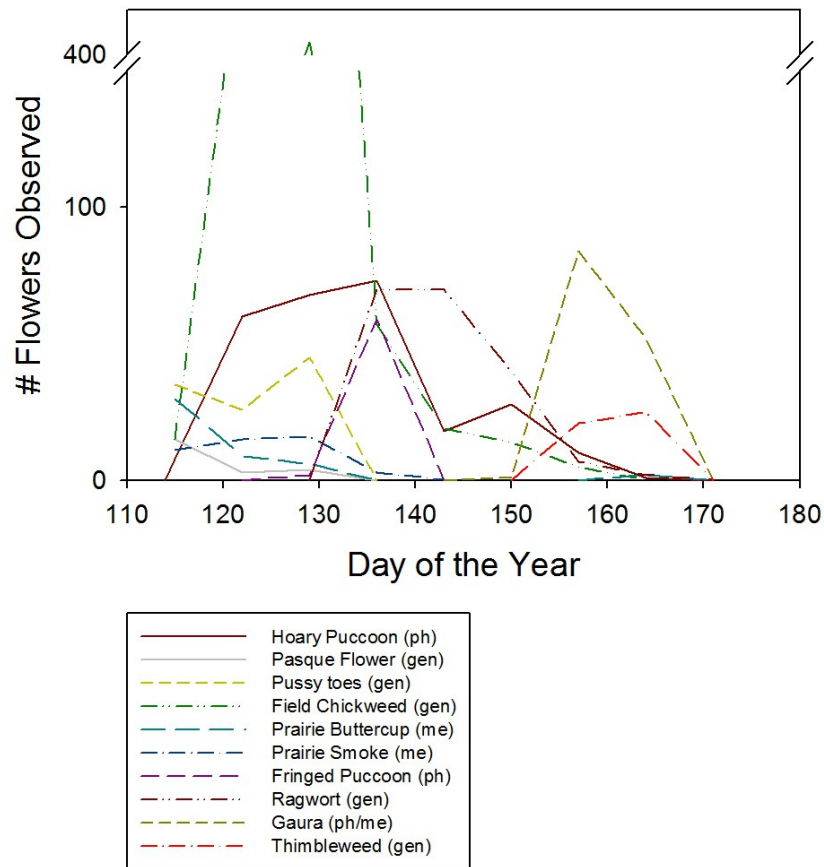


Figure 3.8. Flower abundance for species sampled at Bluestem Prairie during the duration of *L. canescens*' blooming period. Letters in parenthesis indicate pollination syndrome of the species surveyed. Gen=pollinated by a number of animal/insect types, me=melittophily (bee pollination), ph=phalaenophily (moth pollination). The break in the y axis is between 150 and 400 flowers observed.

Following the first plant community graph is a graph of a theoretical historical scenario (Figure 3.10). The bloom distribution of nine of the plants which shared *L. canescens*' bloom period were placed at their historical FFD. The result is a series of species bloom periods that stretches not from day 115 to day 171, as they did in 2012, but from day 97 to day 180. Out of the eight species found blooming alongside of *L. canescens* (there is no historical data for Prairie Smoke), only three historically bloomed concurrently. Three species bloomed before it and two species bloomed after. Of the three species which (theoretically) bloomed at the same time as *L.*

canescens, two are generalists and one is pollinated by moths. Two of the species which flower before have a generalist pollinator syndrome, and one is bee pollinated. One of the two remaining species is pollinated by generalists, and the other is pollinated by bees and moths. There are three gaps in the estimated historical plant community graph in which none of the focal species are blooming.

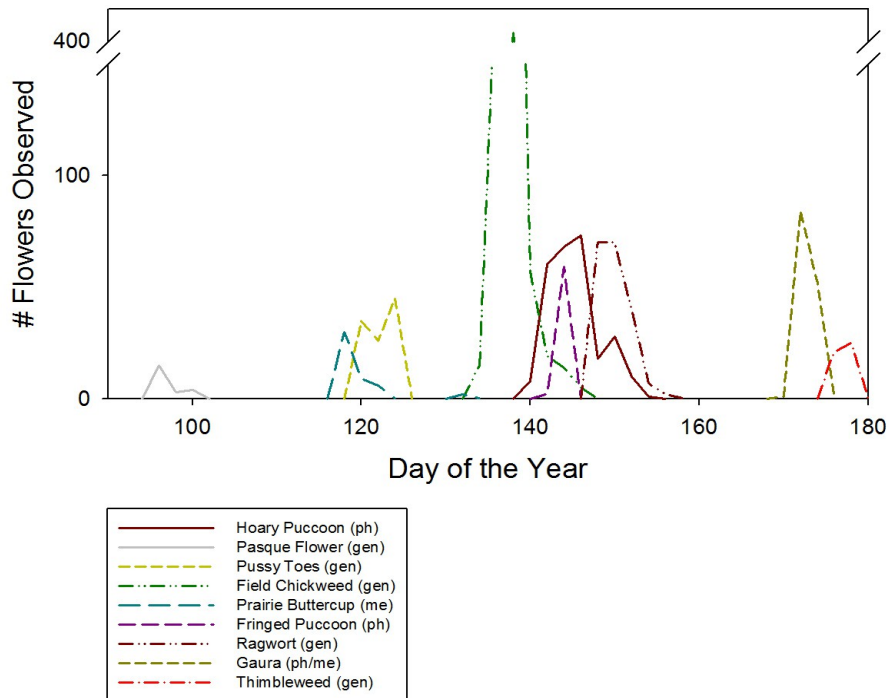


Figure 3.9. Flower abundance curves for species surveyed during *L. canescens*' blooming time spaced according to their historical FFD. (Prairie smoke was not included, due to its absence in the historical data set.) Letters in parenthesis indicate pollination syndrome of the species surveyed. Gen=pollinated by a number of animal/insect types, me=melittophily (bee pollination), ph=phalaenophily (moth pollination).

Flower Maturation

Results from 2012's hand pollinated plants show that seed set is significantly (p-value: 0.01) lower for the oldest flowers versus the middle and youngest flowers (Figure 3.11). The f-

ratio for that test was 4.8 and the degrees of freedom were 2 and 69. There is no significant difference between the seed set of the middle and youngest flowers. This indicates that stigmas are most receptive in the middle and youngest flowers, and receptivity declines once the flowers reach a certain age (approximately 12 days old – see results of flower duration experiment below).

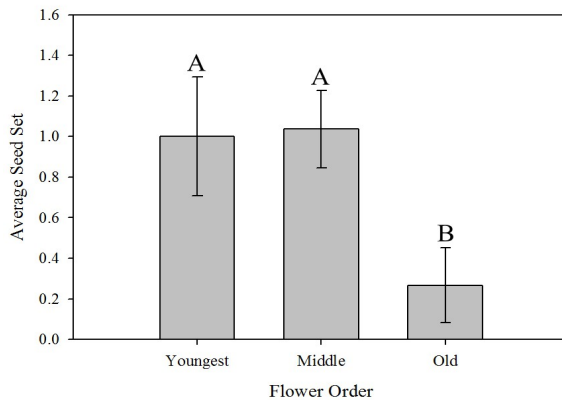


Figure 3.10. Least square means for average seed set (1SE) on Bluestem Prairie for hand pollinated flowers of differing ages. Single ANOVA: $F = 4.8$, $P = 0.01$, $DF = 2, 69$.

Flower Duration

In the flower duration experiment, unpollinated (treatment 1) flowers were open for significantly less time than hand pollinated (treatment 2) flowers. The t test ran on flower duration resulted in a p-value of <0.0001 , a standard error difference of 0.3 and the degrees of freedom were 54 and 73. The flowers were open for a maximum of 9 days in treatment 1 and 5 days for treatment 2, and for a minimum of 1.5 days for both treatments. The average flower duration for treatment 1 was 4.8 days and 3.3 days for treatment 2.

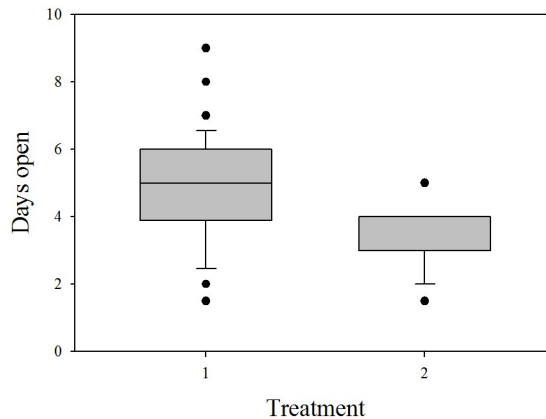


Figure 3.11. Box plot showing flower duration based upon treatment type. The middle line in the box equals the mean and the upper and lower limits to the box are the quantiles. Error bars indicate the 95% confidence limit, and dots represent outliers. Treatment 1: flowers that were caged to exclude pollinators. Treatment 2: flowers that were hand pollinated. Treatment 1's confidence interval is 2.98-3.553 and treatment 2's confidence interval is 4.27-5.36. Thirty nine flowers were tested for treatment 1, and thirty eight flowers were tested for treatment 2. Error bars represent +/- 1 SE from the mean.

Anther Dehiscence

Regarding pollen availability, pollen abundance was lowest for buds (0) and highest for flowers that had just opened (1) (Figure 3.12). Both buds and newly opened flowers had pollen abundances that differed significantly from all of the other flower stages. The 2nd and 3rd flowers did not significantly differ in pollen abundance. This pattern is typical of flowers that are protandrous.

Nectar Production

Nectar production for both the afternoon and the morning was significantly higher for the bagged treatment versus the open treatment. Also, nectar production was significantly higher for the morning versus the afternoon. Results did not vary significantly based on treatment type.

The nested ANOVA test run on the data resulted in a p-value of 0.01, an f-ratio of 3.93 and degrees of freedom of 3 and 51. The effects test for time resulted in an f-ratio of 6.95 and a p-value of 0.01 with 2 degrees of freedom. The results of the effects test for treatment were an f-ratio of 1.96 and a p-value of 0.15 with 1 degree of freedom. Average nectar production for the afternoon bagged treatment was 0.03 μl , and the average produced for the afternoon open treatment was 0.00 μl . Mean nectar production in the morning bagged treatment was 0.12 μl and the average for the morning open treatment was 0.05 μl . The minimum for each time period and treatment was 0.00 μl .



Figure 3.12. Box plot of pollen abundance measured by flower order. 1 indicates that the flower was still a bud, 2 is a newly opened flower, 3 is the second oldest flower and 4 is the oldest. The pollen abundance scale goes from 0 (no pollen visible) to 6 (the highest amount possible of pollen visible). Twenty seven plants were surveyed.

Verification of the Nutlet Scar Technique

There was a strong positive relationship between the number of nutlets which matured and the number of nutlet scars counted for the same flower. Regression analysis produced an r square value of 0.91, indicating that there is a strong relationship between nutlet scars and

number of nutlets, so predictability is high ($\text{Visible Scars} = -0.933 + 0.964\text{Nutlets}$, $F = 128.95$, $P = <0.0001$, $SE = 0.08$). All of these parameters lead us to believe that judging seed set based upon the scars left after the nutlets fall is valid.

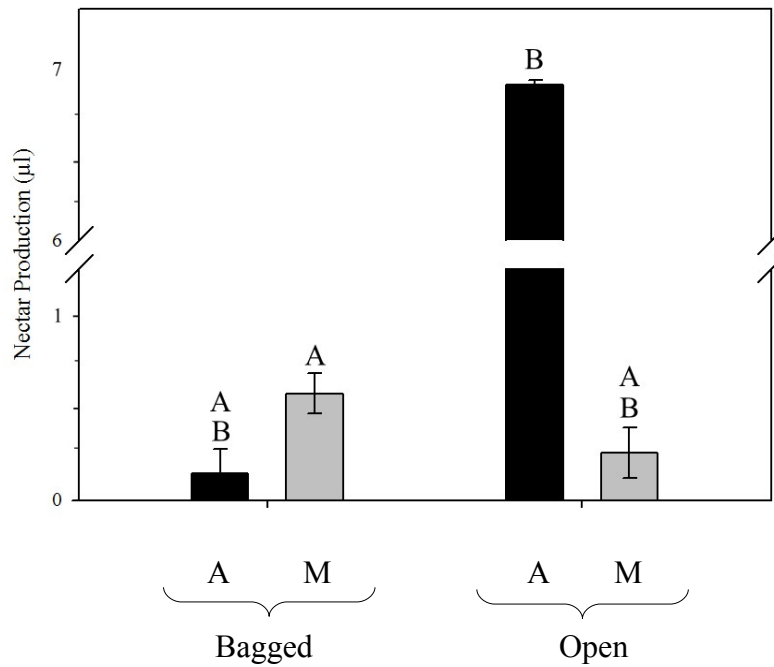


Figure 3.13. Least square means of nectar production (1SE) by treatment and time. Nested ANOVA: $F = 3.9$, $P = 0.01$, $DF = 3$, 51. Effects test for time: $F = 6.95$, $P = 0.0$, $DF = 2$. Effects test for treatment: $F = 1.96$, $P = 0.15$, $DF = 1$. A = Afternoon, M = Morning.

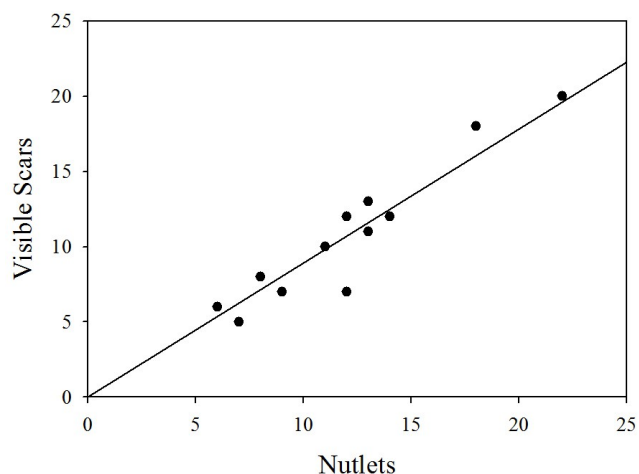


Figure 3.14. Number of nutlets produced per plant compared to the scars visible per plant. $x = -0.933 + 0.964y$, $F = 128.95$, $P = <0.0001$.

Discussion

The results of the experimental manipulation of pollen availability suggest several things about pollen limitation in this species and about the influence of growing season and plant community on reproductive success. For example, it appears that *Lithospermum canescens* is not pollen limited. Each of the treatments resulted in significantly different seed set at both Jarvis and Bluestem in 2011, and the open treatment (1.1) and caged (0.1) treatment were significantly different from each other and from the hand-pollinated and bagged treatments (these treatments were not significantly different from each other, with seed sets of 0.7 and 0.5, respectively) at Bluestem in 2012. The results of 2011's seed set on Jarvis were: O = 0.8, X = 0.6 and B = 0.2. The results for the 2011 Bluestem treatments were: O = 1.1, X = 0.5 and B = 0.2. The fact that in all cases the open-pollinated treatment resulted in a significantly higher seed set than the hand-pollinated treatment supports the hypothesis that *Lithospermum canescens* plants in these populations are not pollen limited.

Seed set for *Lithospermum canescens* was higher in 2012 versus 2011, even though this species had an earlier FFD in 2012 (116 vs. 138). Higher seed set in the hand pollinated treatment (X) (from 0.5 to 0.7 seeds/flower) may be explained by an improvement in pollination methods from 2011 to 2012, but that does not explain the increase in seed set in the bagged (B) treatment (from 0.2 seeds set/flower to 0.5 seeds set/flower). A potential explanation for that increase is the fact that bags were only used during part of the season for this treatment and bagged seed set could have been higher during the first half of the season, but that is not supported by seed set results from the open treatment. Seed set for the open (O) treatment from each year was quite comparable (1.00 in 2011 vs. 1.06 in 2012). Whatever the reasons for the increases in seed set in the X and B treatments, the fact remains that in all treatments seed set stayed at the same levels or increased from 2011 to 2012. This leads me to conclude, at least from this study, that there is no pollen limitation occurring in the *Lithospermum canescens* population I studied. Of course, it is possible that pollen limitation is occurring due to the absence of a plant or pollinator that is no longer on the landscape (Parrish & Bazzaz 1979), but that is impossible to ascertain that from this study. Also, judging from the projected historical plant community, plant species are blooming differently in relationship to each other (i.e. species are now blooming together with different species than they were historically), which may have resulted in changes in the way pollinators behave on the landscape (Sherry et al. 2007). Whether or not that is the case, no pollen-limitation was discernible on the bases of local climactic variables during this study. As an aside, part of the 2012 population of *L. canescens* had a delayed blooming period due to a prescription fire that burned half the site. Despite this delay, the seed set for this half of the population was statistically the same as the unburned half, which supports the hypothesis that *L. canescens* is not suffering from pollen limitation. Thus, this

species appears able to withstand the potential climate uncertainties facing it in the near future, both because of that lack of pollen limitation and because it has shown that it has the ability to adapt its blooming period from one year to another, and studies have shown that that plasticity can result in higher fitness in species (Cleland 2012, Mazer et. al 2013).

It is probable that the conclusions drawn above for the Bluestem *L. canescens* population hold true for Jarvis, even though that site was not included in the 2012 study. This inference is based upon the fact that two of the three treatments (B and X) performed in 2011 were not significantly different from Bluestem to Jarvis. The O treatment was significantly different, but I believe that the lower seed set at Jarvis for this treatment can be explained by the overwhelming presence of white sweet clover (*Melilotus alba*) on half of the study site, which surpassed *Lithospermum canescens* in growth during its blooming period and eventually reached a height surpassing two meters, effectively cutting off sunlight for over half of the *L. canescens* plants sampled (*M. alba* was found to negatively affect the growth of other plant species by Spellman & Wurtz in 2011).

Regarding the bagged treatment, it is important to point out a valuable fact which came to light in 2012, namely, that Lepidopteran pollinators were able to access *L. canescens* blooms through the plastic tulle (bridal veil) that the plants were bagged with. Not only was a moth observed accessing a *L. canescens* flower through the bag (Bishop, personal communication), but the seed set for bagged flowers was significantly different when compared to the seed set of flowers that had had cages built of wire and tulle placed around them (0.49 seeds/flower vs. 0.07 seeds/flower). As bagging flowers with bags made of plastic tulle is a common practice in pollen-limitation studies (Németh & Smith-Huerta 2003, Scott 2007, McCall 1996), I hope that

this information will be useful for studies involving pollinators with long proboscises in the future.

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CHAPTER 4. GERMINATION OF *LITHOSPERMUM CANESCENS*

Introduction

Lithospermum canescens is a species that is underrepresented in local prairie restorations, mainly because of its seed characteristics (they shatter readily in the middle of the summer, when few companies and agencies are out collecting), and the fact that it is a difficult species to propagate (which is not an unusual trait in prairie forbs, see Blake 1935). However, *L. canescens* is a valuable part of the tallgrass prairie ecosystems where it is found. Tallgrass prairies are highly diverse systems comprised of hundreds of plant species which have undergone centuries of coevolution. Because of this coexistence, tallgrass prairie species have become adapted to specific pollinator niches, and the loss of one species on the prairie landscape affects the other species in its community (Parrish & Bazzaz, 1979). Sadly, even in remnant prairies, forbs such as *L. canescens* are especially at risk (McLachlan & Knispel 2005).

In addition to its value to native plant communities, *L. canescens* has the potential to be an excellent research species. It has a scirpoid inflorescence in which flowers mature sequentially. Also, it is perennial and individual plants are able to be easily monitored from year to year. Lastly, its reproductive strategy with distylous morphology and obligate outcrossing make this species valuable for pollination studies. In order to increase *L. canescens*' value as a research species by artificially extending its bloom season and in the hopes of finding a method of propagation which could be used by others, I attempted to propagate *L. canescens* in the winter of 2011-2012.

Materials and Methods

There are no records of *Lithospermum canescens* being grown from seed or cuttings in an artificial setting. However, development of propagation techniques would be informative and

promote the use of *Lithospermum* as a research tool and beneficial to prairie restoration efforts in the upper Midwest. Our objectives were to: 1) develop propagation methods to facilitate reintroduction efforts, and 2) produce an experimental population for a pollen limitation study. I developed propagation methods based on *L. ruderal* methods of propagation (Green 1950).

I began by stratifying the seeds I had collected the summer of 2011 from the Jarvis parcel of the Fish and Wildlife Service's Detroit Lakes Wetland Management District. The seeds (~800) were mixed with dampened, sterilized sand and placed in a freezer at ~4°C on October 10th, 2011 in a paper bag. I established four cohorts from these seeds by removing collections of seeds and planting them at four different times. To prepare for germination, seeds were soaked for three to six hours in tap water at room temperature. The seed coats were removed via scarification with sand paper. After the scarification, the seeds were soaked in tap water for another twenty four hours, then taken up to the greenhouse and planted in Sunshine Mix 1 growing medium (Sun Gro Horticulture) which had been thoroughly saturated with water. The seeds were sprinkled over seed trays, then covered with ~1/8" of growing medium. Seeds were watered at least once a week with a water/chamomile tea solution (one teabag soaked in 4 liters of water for one day) to inhibit fungus formation. The seedlings were initially fed with Peters Professional Water Soluble Mix 20-20-20 fertilizer (The Scotts Company LLC) mixed in water once every two weeks, then as the seedlings began to show symptoms of nutrient deficiencies they were watered with a solution of Happy Frog Jump Start Organic Fertilizer (FoxFarm Soil and Fertilizer Company), which contained the micronutrients the plants were lacking, as well as mycorrhizal organisms.

Seedling cohorts were begun December 1st, 2011, December 14th, 2011, January 13th, 2012 and January 30th 2012. Cohorts were staggered in an attempt to spread out their blooming season, with a month between cohorts two and three to allow for the natural *L. canescens*

blooming period to take place. Sample sizes in each cohort were: 211, 255, 196 and 146 seeds for cohorts 1 through 4 respectively. Each cohort was started in its own seed tray. Once a seedling had germinated and had one set of true leaves, that individual was transplanted to its own 4" or 6" diameter plastic pot, which had been filled with Sunshine Mix 1 potting soil (Sun Gro Horticulture). Each pot was labeled with the cohort the seedling belonged to. A seed was considered germinated if it produced a seedling at the cotyledon stage.

Initially this study was designed solely to see if *Lithospermum canescens* could be propagated by hand from seed. Because of that, no specific data were collected during the first two months of the study until February 1. Beginning in February, I counted and recorded the number of seedlings in each cohort once a week until the third week of March when greenhouse equipment malfunctioned and high temperatures killed all of the seedlings. Because the cohorts were started at different times each cohort was observed for a different total number of weeks (Cohort 1= 7 weeks, cohort 2= 6 weeks, cohort 3 = 4 weeks and cohort 4 = 3 weeks).

Germinated plants were placed into two categories: plants with cotyledons present and plants with true leaves present. Each week, the total number of plants with cotyledons present and plants with true leaves present were counted for each cohort. New plants with true leaves present were not distinguished from previously germinated plants with true leaves present, mainly because growth rate was quite slow for most individuals.

The mean number of plants in each category of development each week was calculated for each cohort at the end of the study. I also calculated the mean number of plants in each category across cohorts (n=4) that were present each week. I used oneway ANOVA to test for differences among weeks in the mean number of plants in each development category. I used a

Tukey-Kramer HSD test to compare means among seedlings with cotyledons by cohort, seedlings with true leaves by cohort, seedlings with cotyledons by week and seedlings with true leaves by day of the experiment. All analyses were done with JMP statistical software (SAS Institute Inc. 1989-2007).

Results

Consistent with findings in other closely related species, germination was low. Germination rate (percentage of seeds planted per cohort that sprouted cotyledons) for each of the four cohorts were 11.8%, 7.1%, 4.6 and 2.1%, respectively from cohorts 1 through 4. According to the Tukey-Kramer HSD test, the mean number of plants with true leaves in the 1st and 2nd cohort did not differ significantly, nor did the mean number of plants with true leaves in the 3rd and 4th cohorts (Figure 4.1). However, the mean number of plants with true leaves was significantly different between the 1st and 2nd cohorts and the 3rd and 4th cohorts. None of the cohorts with sufficient numbers to be tested had means which varied significantly among plants with cotyledons.

Throughout the study, the mean number of plants with true leaves did not vary significantly based upon day of the experiment according to the Tukey-Kramer HSD test, and neither did the means of the seedlings with cotyledons (Figure 4.2). However, the oneway ANOVA for the plants with true leaves by day of the experiment resulted in a p-value of 0.94 (and an f-value of 0.23 with 5 and 21 degrees of freedom), which means that those findings are not significant. The ANOVA test on plants with cotyledons yielded another non-significant p-value of 0.332, and an f-value of 0.29, with 5 and 16 degrees of freedom. The mean number of plants with true leaves varied from 6 to 14.7 and the average number of plants with cotyledons present ranged from 1 to 6 throughout the duration of the experiment. These results support

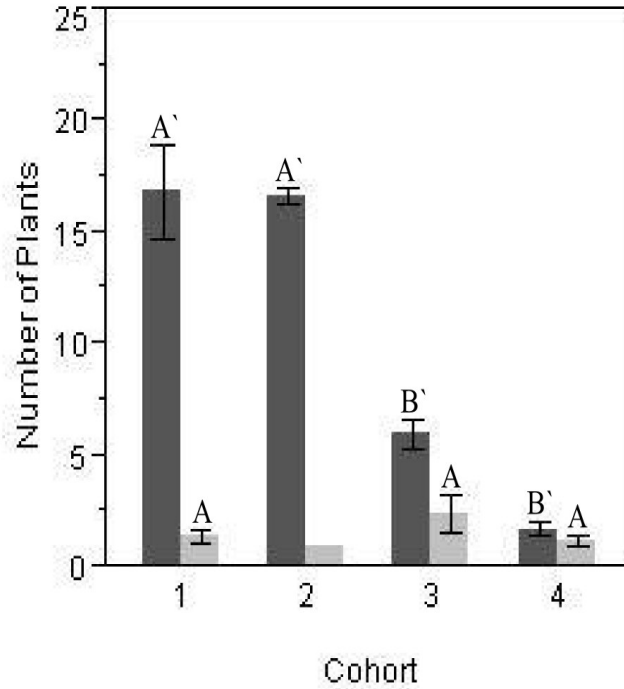


Figure 4.1. The average number of *L. canescens* seedlings with true leaves present (dark grey) and seedlings with cotyledons present (light grey) produced in each cohort (1SE) throughout the entire study. (Due to insufficient data for standard error calculation, error bars are missing from the cotyledons present bar in the 2nd cohort.).

observations made in the greenhouse that, despite the periodic germination of new plants, some seedlings with cotyledons never grew true leaves and both categories of seedlings inexplicably died throughout the length of the experiment, which meant that numbers of plants with true leaves present stayed roughly the same throughout the experiment.

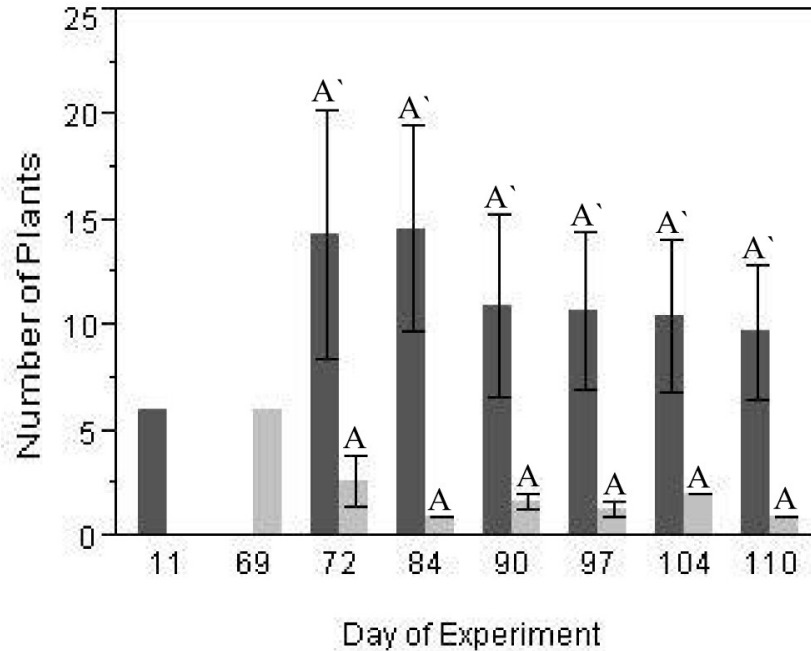


Figure 4.2. The average number of all *L. canescens* seedling with true leaves (dark grey) and seedlings with cotyledons (light grey) throughout the experiment (averaged across cohorts). Error bars represent +/- 1 standard error from the mean. Single factor ANOVA on seedlings true leaves present: $F = 0.23$, $P = 0.94$, $DF = 5, 21$. Single factor ANOVA on cotyledons presents: $F = 1.35$, $P = 0.32$, $DF = 5, 16$. (Due to insufficient data for standard error calculation, error bars are missing from the first two bars.)

Discussion

Both germination and seedling survival were very low for this study. The overall low germination levels may be explained by greenhouse conditions, which were very different from the conditions under which *L. canescens* usually germinates. According to a comprehensive prairie seedling study undertaken by A. K. Blake (1935), *Lithospermum* germination was quite high in hot, dry summer conditions. Blake also found that plants of various species which set seed in certain abiotic conditions (e.g, hot and dry) had lower germination rates in dissimilar conditions (e.g, cool and humid). The greenhouse that my plants were grown in was humid and subject to cold drafts due to its placement at the top of a campus building in a region that has

quite cold winters. It is also possible that, despite efforts to the contrary, a nutrient or microorganism was missing in the soil medium that *L. canescens* requires for survival. It has also been suggested that *L. canescens* is at least partially parasitic on other plants (cited in Molano-Flores 2001) or may exhibit germination polymorphism (Clambey, personal communication). Lastly, it is possible that as a perennial *L. canescens* has evolved a low rate of annual seedling germination. Its nutlets are quite durable, and it's possible that one of *L. canescens*' reproduction strategies is a bet-hedging one, and more viable nutlets are produced than are needed to germinate in the next year. (As an example of this strategy, see Tamm 1972 and Bierzychudek 1982.) This has been found to be the case with most non-weedy prairie plant species, which produce large amounts of seeds with low percentages of viability (Blake 1935) and low seedling production (Blake 1935, Weaver 1950).

Sadly, *Lithospermum canescens*' low germination rate in an artificial setting may be detrimental to its future. Often species that are relatively easy to propagate are the species chosen to be used in prairie restorations (personal observation). Because of this, none of the land management agencies or native seed suppliers in Clay County collect or propagate *L. canescens* seeds (personal observation). This absence of propagation could quickly result in *Lithospermum canescens*' absence on the landscape as more remnant prairies are plowed for agriculture and others areas are restored to native vegetation in Clay County.

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APPENDIX A. PLANT PHENOLOGIES FROM CLAY COUNTY MINNESOTA 1910-1938

First flowering dates of plant species found in Clay County Minnesota, including family name, scientific and common names, life-form and first flowering date (FFD) information from 1910 to 1938.

Scientific Name	Family	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938
<i>Acer ginnala</i>	Aceraceae	149
<i>Acer negundo</i>	Aceraceae	92	114	112	.	121	103	125	127	103	120	126	110	117	120	122	100	112	115	122	114	104	105	113	112	118	125	124	121	104
<i>Acer saccharinum</i>	Aceraceae	79	110	98	102	104	92	107	102	84	103	107	92	96	108	96	88	105	100	91	92	93	95	104	104	102	100	108	105	84
<i>Acer saccharum</i>	Aceraceae	126	.	137
<i>Acerates viridiflora</i>	Asclepiadaceae
<i>Achillea millefolium</i>	Asteraceae	165	166	.	165	.	166	.	175	154	.	171
<i>Acnida altissima</i>	Amaranthaceae	214	226
<i>Actaea rubra</i>	Ranunculaceae	.	.	.	145	144	143	138
<i>Actinella acaulis</i>	Asteraceae
<i>Aesculus glabra</i>	Hippocastanaceae
<i>Agastache anethiodora</i>	Lamiaceae	195	206	201	185
<i>Agoseris glauca</i>	Asteraceae	158	.	.	158	.	168
<i>Agrimonia striata</i>	Rosaceae	176
<i>Agropyron repens</i>	Poaceae	173	166	.	176	.	185	.	176	.	.	.	167

Scientific Name	Family	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938
<i>Cerastium arvense</i>	Caryophyllaceae	130	132	.	.	.	133	.	.	.	137	150	128	.	.	148	142	.	.	131
<i>Cerastium nutans</i>	Caryophyllaceae
<i>Cerastium vulgatum</i>	Caryophyllaceae
<i>Chamaerhodos erecta</i>	Rosaceae	154
<i>Chenopodium album</i>	Chenopodiaceae	169	156	.	181	177	174	.	175	180	.	172
<i>Chenopodium gigantospermum</i>	Chenopodiaceae	195
<i>Chenopodium glaucum</i>	Chenopodiaceae	215	237	229	.	.	.	239
<i>Chenopodium leptophyllum</i>	Chenopodiaceae
<i>Chenopodium rubrum</i>	Chenopodiaceae	235	228
<i>Chenopodium strictum</i>	Chenopodiaceae	231	229	.	237	226	.	.
<i>Chrysanthemum coccineum</i>	Asteraceae
<i>Chrysanthemum leucanthemum</i>	Asteraceae
<i>Chrysanthemum uliginosum</i>	Asteraceae	252	252	.	260	255	253	.	251	251	252	263	255	256	260
<i>Chrysopsis villosa</i>	Asteraceae	.	168	191	171

Scientific Name	Family	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	
<i>Chrysothamnus graveolens</i>	Asteraceae	
<i>Cichorium intybus</i>	Asteraceae	183	
<i>Cicuta maculata</i>	Apiaceae	173	.	.	181	.	185	178	180	170	
<i>Cinna latifolia</i>	Poaceae
<i>Circaea latifolia</i>	Onagraceae	202	
<i>Cirsium altissimum</i>	Asteraceae	221	209	
<i>Cirsium arvense</i>	Asteraceae	187	.	.	181	.	.	.	195	177	205
<i>Cirsium undulatum</i>	Asteraceae	180	.	.	181	.	192	191	187	.	.	181
<i>Cirsium vulgare</i>	Asteraceae	199
<i>Clematis virginiana</i>	Ranunculaceae	221	.	.	203	192
<i>Cleome serrulata</i>	Capparaceae	186
<i>Collomia linearis</i>	Polemoniaceae	163
<i>Comandra pallida</i>	Santalaceae	139
<i>Conringia orientalis</i>	Brassicaceae	156	154	.	154	.	148	.	.	154	.	.	141	133	.	131	151
<i>Convallaria majalis</i>	Liliaceae
<i>Convolvulus arvensis</i>	Convolvulaceae	174	186	172	167	173	.	.	.
<i>Convolvulus repens</i>	Convolvulaceae	169	158	173	.	.	173	164	.	168	.	163	163	173

Scientific Name	Family	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938
<i>Eupatorium maculatum</i>	Asteraceae
<i>Eupatorium rugosum</i>	Asteraceae	250
<i>Euphorbia esula</i>	Euphorbiaceae	161	167
<i>Euphorbia glyptosperma</i>	Euphorbiaceae
<i>Euphorbia serpyllifolia</i>	Euphorbiaceae	167	156	.	.	153	.	.	170	171	.	.	.	163	.	.	151	.	.
<i>Festuca elatior</i>	Poaceae	166	.	.	165
<i>Festuca obtusa</i>	Poaceae	171
<i>Forsythia ovata</i>	Oleaceae	103	.	.	.
<i>Fragaria americana</i>	Rosaceae	.	.	.	126
<i>Fragaria virginiana</i>	Rosaceae	.	126	127	.	.	133	127
<i>Fraxinus lanceolata</i>	Oleaceae	.	133	127	136	.	137	133	127	124	125	140	110	119	131	128	.	.	.	130	.	125	138	134	126	119
<i>Fraxinus nigra</i>	Oleaceae
<i>Fritillaria atropurpurea</i>	Liliaceae
<i>Fumaria officinalis</i>	Fumariaceae
<i>Gaillardia aristata</i>	Asteraceae	.	168	165	.	170	192
<i>Galinsoga ciliata</i>	Asteraceae
<i>Galium aparine</i>	Rubiaceae	141	147	146	151	149	146	.	149	.	144	164	.	.	.	154	.	.	.	147	149	.	.	.	146	.	152	.	.	.

Scientific Name	Family	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938
<i>Impatiens capensis</i>	Balsaminaceae	201	184
<i>Iva axillaris</i>	Asteraceae
<i>Iva xanthifolia</i>	Asteraceae	231	.	227	224	236	.	.	230	228	.	236	230	.	229	229	.	.	.	227	.	.	233	226	.
<i>Juglans cinerea</i>	Juglandaceae	138	145	.	146	140	145	140
<i>Juglans nigra</i>	Juglandaceae	145	144	159	159	155	.	151	.	.	147	150	155
<i>Juncus balticus</i>	Juncaceae	167	.	.	151	154
<i>Juncus interior</i>	Juncaceae	167
<i>Juncus nodosus</i>	Juncaceae
<i>Juncus torreyi</i>	Juncaceae
<i>Juniperus scopulorum</i>	Cupressaceae	128	120	118	130	130	116	.	.	127	132	.	131	130	133	126	137	135	133	121
<i>Juniperus virginiana</i>	Cupressaceae	112	.	.	125	115	.	118	122	125	122	125	125	.	104	
<i>Kochia scoparia</i>	Chenopodiaceae
<i>Koeleria cristata</i>	Poaceae	.	166	175	.	.	173
<i>Kuhnia eupatorioides</i>	Juncaceae	.	.	.	206
<i>Lactuca biennis</i>	Asteraceae
<i>Lactuca canadensis</i>	Asteraceae	181
<i>Lactuca ludoviciana</i>	Asteraceae	179	196	.	.	.	186	.	185	.	199	180
<i>Lactuca pulchella</i>	Asteraceae	181	184	.	181	.	.	.	195	173	.	185	184	.	180

Scientific Name	Family	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	
<i>Populus balsamifera</i>	Salicaceae	126	110	118	121	
<i>Populus deltoides</i>	Salicaceae	92	117	.	.	121	108	125	129	105	120	.	110	118	121	124	100	112	117	123	114	104	106	114	.	120	.	127	122	107	
<i>Populus tremuloides</i>	Salicaceae	85	107	.	108	.	.	.	116	99	111	120	.	.	116	
<i>Portulaca oleracea</i>	Portulacaceae	171	183	.	213	.	.	.	173	
<i>Potentilla anserina</i>	Rosaceae	131	147	.	.	
<i>Potentilla arguta</i>	Rosaceae	171	.	.	.	174	193	.	186	.	.	180	167	.	177	
<i>Potentilla concinna</i>	Rosaceae	.	123	.	135	
<i>Potentilla fruticosa</i>	Rosaceae	161	
<i>Potentilla norvegica</i>	Rosaceae	162	166	.	170	166	168	.	167	.	.	173	.	.	166	.	167
<i>Potentilla paradoxa</i>	Rosaceae	
<i>Potentilla pennsylvanica</i>	Rosaceae	175	166	186	
<i>Potentilla pentandra</i>	Rosaceae	168	
<i>Prenanthes alba</i>	Asteraceae	228	
<i>Prenanthes racemosa</i>	Asteraceae	239	
<i>Prinsepia sinensis</i>	Rosaceae	130	129	126	

Scientific Name	Family	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938
<i>Zigadenus elegans</i>	Liliaceae	174	180	171
<i>Zizania aquatica</i>	Poaceae	226	210
<i>Zizia aptera</i>	Apiaceae	141	141	.	.	148	137	149
<i>Zizia aurea</i>	Apiaceae	127	140	146	144	149	142	.	145	.	146	143	.	.	.	148	143	.	.	147

APPENDIX B. PLANT PHENOLOGIES FROM CLAY COUNTY MINNESOTA 1939-2012

First flowering dates of plant species found in Clay County Minnesota, including family name, scientific and common names, life-form and first flowering date (FFD) information from 1940 to 2012.

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012	
<i>Acer ginnala</i>	Aceraceae	144	140	.	.	140	.	158	135	157	146	141	149	156	.	.	.	152	.	.	.	
<i>Acer negundo</i>	Aceraceae	124	115	110	113	119	97	105	122	114	.	134	119	113	123	118	105	131	116	.	.	128	.	.	121	114	100	.	83	
<i>Acer saccharinum</i>	Aceraceae	108	99	92	97	103	80	85	109	106	100	111	112	106	95	99	97	110	106	92	92	105	.	.	107	104	90	103	78	
<i>Acer saccharum</i>	Aceraceae	137	120	142	.	.	.	139	135	
<i>Acerates viridiflora</i>	Asclepiadaceae
<i>Achillea millefolium</i>	Asteraceae	170	155	168	168	.	165	138
<i>Acidanthera ?</i>	Iridaceae	261
<i>Acnida altissima</i>	Amaranthaceae
<i>Actaea rubra</i>	Ranunculaceae	143	130	.	146	143	149	146	143	138	131	153	137	143	147	143	132	147	137	137	140	141	.	.	148	.	.	.	122	
<i>Actinella acaulis</i>	Asteraceae	148	145
<i>Aesculus glabra</i>	Hippocastanaceae	142	149
<i>Agastache anethiodora</i>	Lamiaceae
<i>Agoseris glauca</i>	Asteraceae	144	159	161
<i>Agrimonia striata</i>	Rosaceae
<i>Agropyron repens</i>	Poaceae	163	177	176	.	169	175	.	165	169

128 128

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012
<i>Antennaria aprica</i>	Asteraceae	114	133	116
<i>Antennaria microphylla</i>	Asteraceae
<i>Anthemis cotula</i>	Asteraceae
<i>Antirrhinum majus</i>	Scrophulariaceae	173
<i>Aplopappus lanceolatus</i>	Asteraceae	214
<i>Aplopappus spinulosus</i>	Asteraceae
<i>Apocynum androsaemifolium</i>	Apocynaceae	165	162
<i>Apocynum hypericifolium</i>	Apocynaceae	182	.	.	.	177	169	170	184	.	.	.	177	162
<i>Aquilegia canadensis</i>	Ranunculaceae	.	135	143	143	153	.	.	.	137	147	.	149	.	.	143	.	148	.
<i>Arabis divaricarpa</i>	Brassicaceae	134	.	.	.	131
<i>Arabis hirsuta</i>	Brassicaceae
<i>Aralia nudicaulis</i>	Araliaceae	161	143	149	161	139	.	160	.	140	153
<i>Arctium minus</i>	Asteraceae	208	206
<i>Arenaria laterifolia</i>	Caryophyllaceae	134	156
<i>Aretium tomentosum</i>	Caryophyllaceae	210
<i>Arisaema atrorubens</i>	Araceae	134	.	.

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012
<i>Aster laevis</i>	Asteraceae	225	234
<i>Aster nova.angliae</i>	Asteraceae	.	203	219	229	227	223	.	.	222	.	232	.	.	229	231	212	239	.	217	224	.	221	191
<i>Aster paniculatus</i>	Asteraceae	.	.	.	231	247	.	.	.	210	.	234
<i>Aster punicens</i>	Asteraceae	225	214
<i>Astragalus bisulcatus</i>	Fabaceae
<i>Astragalus canadensis</i>	Fabaceae	201	193
<i>Astragalus caryocarpus</i>	Fabaceae	.	.	119	129	127	132
<i>Astragalus flexuosus</i>	Fabaceae
<i>Astragalus hypoglottis</i>	Fabaceae	163	134	.	.	.	134
<i>Astragalus missouriensis</i>	Fabaceae	138
<i>Astragalus pectinatus</i>	Fabaceae
<i>Astragalus plattensis</i>	Fabaceae
<i>Astragalus racemosus</i>	Fabaceae	152
<i>Astragalus striatus</i>	Fabaceae	163	159
<i>Astragalus triphyllus</i>	Fabaceae
<i>Atriplex confert</i>	Chenopodiaceae	171

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012
<i>Cardaria draba</i>	Brassicaceae	175	147
<i>Cardaria pubescens</i>	Brassicaceae	163	144	159
<i>Carduus crispus</i>	Asteraceae	191
<i>Carex aquatilis</i>	Cyperaceae
<i>Carex assiniboinensis</i>	Cyperaceae
<i>Carex blanda</i>	Cyperaceae	140	.	.	.	153
<i>Carex brevior</i>	Cyperaceae	156
<i>Carex deweyana</i>	Cyperaceae	140
<i>Carex eleocharis</i>	Cyperaceae	.	.	119
<i>Carex filifolia</i>	Cyperaceae
<i>Carex gravida</i>	Cyperaceae
<i>Carex laeviconica</i>	Cyperaceae
<i>Carex lanuginosa</i>	Cyperaceae
<i>Carex pennsylvanica</i>	Cyperaceae	.	116	119	114	127	98
<i>Carex praegracilis</i>	Cyperaceae
<i>Carex rosea</i>	Cyperaceae
<i>Carex spregelii</i>	Cyperaceae	134
<i>Carex vulpinoidea</i>	Cyperaceae
<i>Castilleja coccinea</i>	Scrophulariaceae	162	158	140

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012
<i>Clematis virginiana</i>	Ranunculaceae	199	.	.	210	190	182	197	185	202	563	221
<i>Cleome serrulata</i>	Capparaceae	181	186	188
<i>Collomia linearis</i>	Polemoniaceae
<i>Comandra pallida</i>	Santalaceae	148	134	156	137	.	.
<i>Conringia orientalis</i>	Brassicaceae	153
<i>Convallaria majalis</i>	Liliaceae	143	146	136	.	.	.	133	.	.	.	146	122
<i>Convolvulus arvensis</i>	Convolvulaceae	177	188	.	.	.
<i>Convolvulus repens</i>	Convolvulaceae	159	157	.	170	169	175	.	179
<i>Convolvulus sepium</i>	Convolvulaceae
<i>Coreopsis tinctoria</i>	Asteraceae
<i>Cornus alba</i>	Cornaceae	133	151	151	134	154	150	.	149	147	150
<i>Cornus baileyi</i>	Cornaceae	135	142
<i>Cornus racemosa</i>	Cornaceae	165
<i>Cornus stolonifera</i>	Cornaceae	154
<i>Corydalis aurea</i>	Fumariaceae
<i>Corylus americana</i>	Betulaceae	111	.	104	108	106	105	90	103	78

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012
<i>Dactylis glomerata</i>	Poaceae	149
<i>Dalea alopecurioides</i>	Fabaceae
<i>Daphne cneorum</i>	Thymelaeaceae	119	.	132	125	.	131	.	137	135	118	141	129
<i>Daucus carota</i>	Apiaceae
<i>Delphinium bicolor</i>	Ranunculaceae
<i>Delphinium virescens</i>	Ranunculaceae	168	.	.	182	172
<i>Descurainia pinnata</i>	Brassicaceae	159	144	.	.	137	.	151	.	.	140
<i>Descurainia richardsonii</i>	Brassicaceae
<i>Descurainia sophia</i>	Brassicaceae	137	.	153	149	132	148	.	136
<i>Desmodium acuminatum</i>	Fabaceae	195
<i>Desmodium canadense</i>	Fabaceae	203
<i>Dictamnus albus</i>	Rutaceae	159	135	158	.	144	151	150	155
<i>Digitaria ischaemum</i>	Poaceae	213	216	.	.	211
<i>Digitaria sanguinalis</i>	Poaceae	210	.	.	226
<i>Dirca palustris</i>	Thymelaeaceae	98
<i>Disporum trachycarpa</i>	Liliaceae	144

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012
<i>Epilobium angustifolium</i>	Onagraceae	191
<i>Equisetum arvense</i>	Equisetaceae
<i>Eragrostis cilianensis</i>	Poaceae	186	187	188
<i>Eragrostis hypnoides</i>	Poaceae
<i>Eragrostis pectinacea</i>	Poaceae	192	188
<i>Erigeron caespitosus</i>	Asteraceae
<i>Erigeron canadensis</i>	Asteraceae	201	199	210	.	200
<i>Erigeron glabellus</i>	Asteraceae	156	.	145	.	162
<i>Erigeron philadelphicus</i>	Asteraceae	134	161	.	145	.	162	.	.	161	.	108	.	.
<i>Erigeron strigosus</i>	Asteraceae
<i>Eriogonum flavum</i>	Polygonaceae
<i>Eriogonum multiceps</i>	Polygonaceae
<i>Eriophorum angustifolium</i>	Cyperaceae
<i>Erucastrum gallicum</i>	Brassicaceae
<i>Erysimum asperum</i>	Brassicaceae	139	144	148

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012	
<i>Erysimum chieranthoides</i>	Brassicaceae	227	
<i>Erysimum parviflorum</i>	Brassicaceae	162		
<i>Erythronium albidum</i>	Liliaceae	130		
<i>Euonymus alatus</i>	Celastraceae	165	158	150		
<i>Euonymus atropurpurea</i>	Celastraceae	163	.	176		
<i>Euonymus nanus</i>	Celastraceae	.	.	145	145	141	.	143	137	.	.	134	158	150	150		
<i>Eupatorium maculatum</i>	Asteraceae	196		
<i>Eupatorium rugosum</i>	Asteraceae		
<i>Euphorbia esula</i>	Euphorbiaceae	163	140	
<i>Euphorbia glyptosperma</i>	Euphorbiaceae		
<i>Euphorbia serpyllifolia</i>	Euphorbiaceae	165	.	.	.	166	.	.	153		
<i>Festuca elatior</i>	Poaceae	161	162		
<i>Festuca obtusa</i>	Poaceae		
<i>Forsythia ovata</i>	Oleaceae	83	
<i>Fragaria americana</i>	Rosaceae		
<i>Fragaria virginiana</i>	Rosaceae	132	.	114	139	122

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012
<i>Helianthus maximiliani</i>	Asteraceae	205	.	217	233	209	220	.	.	197	192
<i>Helianthus petiolaris</i>	Asteraceae	195	.	.	184	197	173
<i>Helianthus rigidus</i>	Asteraceae
<i>Helianthus tuberosus</i>	Asteraceae
<i>Heliopsis helianthoides</i>	Asteraceae	182	.	.	.	177	.	.	.	179
<i>Hemerocallis fulva</i>	Liliaceae	175
<i>Heracleum lanatum</i>	Apiaceae	167
<i>Hesperis matronalis</i>	Brassicaceae	147	141	.	.	136	153	159	136	156	152	.	146	150	150	.	150	148	.	.	.
<i>Heuchera richardsonii</i>	Saxifragaceae	.	.	152	145	168	152	.	158	143
<i>Hibiscus trionum</i>	Malvaceae	191	178
<i>Hieracium canadense</i>	Asteraceae
<i>Hierochloa odorata</i>	Poaceae	120
<i>Hippophae rhamnoides</i>	Elaeagnaceae	132
<i>Hordeum jubatum</i>	Poaceae	170	.	175
<i>Hosta lancifolia</i>	Liliaceae	249

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012	
<i>Leonurus cardiaca</i>	Lamiaceae	165	
<i>Lepidium densiflorum</i>	Brassicaceae	152	.	145		
<i>Lepidium ramosissimum</i>	Brassicaceae	161		
<i>Lesquerella ?</i>	Brassicaceae		
<i>Leucocrinum montanum</i>	Liliaceae	137	.	.	148	140	128	150	136		
<i>Liatris aspera</i>	Asteraceae	191	225	.	.	.	227	
<i>Liatris punctata</i>	Asteraceae	225	.	.	.	215	
<i>Liatris pycnostachya</i>	Asteraceae	205	197	201	.	.	.	210	.	.	226	
<i>Lilium philadelphicum</i>	Liliaceae	171	181	181	.	192		
<i>Lilium pumilum</i>	Liliaceae	165		
<i>Limonium ?</i>	Plumbaginaceae	206		
<i>Limosella aquatica</i>	Scrophulariaceae		
<i>Linaria vulgaris</i>	Scrophulariaceae		
<i>Linum lewisii</i>	Linaceae	148	.	164		
<i>Linum rigidum</i>	Linaceae		
<i>Linum sulcatum</i>	Linaceae	191	
<i>Linum usitatissimum</i>	Linaceae		
<i>Lithospermum canescens</i>	Boraginaceae	143	127	136	134	123	138	116

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012	
<i>Lithospermum incisum</i>	Boraginaceae	140	.	148	147	137	153	158	
<i>Lobelia siphilitica</i>	Campanulaceae	
<i>Lobelia spicata</i>	Campanulaceae	186	.	190	.	.	197	.	192	191	
<i>Lolium perenne</i>	Poaceae	168	.	.	.	193	
<i>Lolium persicum</i>	Poaceae	182	
<i>Lomatium foeniculaceum</i>	Apiaceae	
<i>Lomatium orientale</i>	Apiaceae	124	124	
<i>Lonicera dioica</i>	Caprifoliaceae	150	.	141	151	148	.	144	.	144	140	157	141	130	149	151	134	154	144	.	.	148
<i>Lonicera maacki</i>	Caprifoliaceae
<i>Lonicera tatarica</i>	Caprifoliaceae	151	132	.	146	149	149	136	.	141	134	.	136	125	145	147	125	151	139	.	139	144	147	.	.	148	.	.	.	
<i>Lotus americanus</i>	Fabaceae
<i>Lupinus argenteus</i>	Fabaceae
<i>Lupinus pusillus</i>	Fabaceae
<i>Lycopus americanus</i>	Lamiaceae	192	
<i>Lycopus asper</i>	Lamiaceae
<i>Lycoris squamigera</i>	Liliaceae	266	247	234	.	224	222	241	233	226	232	232	225	237	232	240	230	233	227
<i>Lygodesmia juncea</i>	Asteraceae	195	194

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012	
<i>Lysimachia ciliata</i>	Primulaceae	182	.	189	182	.	190	188	184	196	.	191	195	189	185	
<i>Lysimachia longifolia</i>	Primulaceae	196	
<i>Lysimachia thyrsiflora</i>	Primulaceae	163	
<i>Lythrum alatum</i>	Lythraceae	181	
<i>Lythrum salicaria</i>	Lythraceae	178	.	180	186	.	183	
<i>Lythrum salicaria</i>	Lythraceae	175	.	178	.	.	177	
<i>Maianthemum canadense</i>	Liliaceae	143	143	95	
<i>Malus baccata</i>	Rosaceae	138	125	125	138	134	121	.	140	121	.	129	129	.	139	142	
<i>Malus sylvestris</i>	Rosaceae	.	125	125	138	142	138	120	141	136	127	150	134	121	138	138	120	146	129	128	.	136	141	.	139	
<i>Malva rotundifolia</i>	Malvaceae	159	171	.	.	.	177
<i>Mamillaria vivipara</i>	Cactaceae	
<i>Matricaria matricarioides</i>	Asteraceae	163	149	
<i>Medicago falcata</i>	Fabaceae	169	
<i>Medicago lupulina</i>	Fabaceae	160	163	167	163	160	161	169	164	
<i>Medicago sativa</i>	Fabaceae	165	151	165	125	
<i>Melilotus alba</i>	Fabaceae	170	.	167	177	178	126	
<i>Melilotus officinalis</i>	Fabaceae	.	147	154	.	159	149	161	157	155	.	155	162	.	.	168	.	161	.	

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012
<i>Oxalis stricta</i>	Oxalidaceae	165	147	164	152	116	160	
<i>Oxalis violacea</i>	Oxalidaceae	139	.	.	.	140	146	130	145	.	.	.	130	140	141	133	140	121
<i>Oxytropis campestris</i>	Fabaceae	138	
<i>Oxytropis lambertii</i>	Fabaceae	152	.	.	
<i>Oxytropis splendens</i>	Fabaceae	
<i>Panicum capillare</i>	Poaceae	
<i>Panicum leibergii</i>	Poaceae	172	
<i>Panicum virgatum</i>	Poaceae	175	.	.	.	
<i>Papaver orientale</i>	Papaveraceae	160	.	.	159	159	159	148	161	.	151	.	159	
<i>Parietaria pennsylvanica</i>	Urticaceae	
<i>Parnassia glauca</i>	Saxifragaceae	198	
<i>Parnassia palustris</i>	Saxifragaceae	198	213	
<i>Paronychia sessiliflora</i>	Caryophyllaceae	
<i>Parthenocissus vitacea</i>	Vitaceae	186	196	.	183	
<i>Pastinaca sativa</i>	Apiaceae	171	158
<i>Pedicularis canadensis</i>	Scrophulariaceae	.	.	152	.	.	142	130	144	141	127	139	

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012	
<i>Plantago rugelii</i>	Plantaginaceae	186	
<i>Poa annua</i>	Poaceae	144	170	134	158	
<i>Poa compressa</i>	Poaceae	170	
<i>Poa palustris</i>	Poaceae	
<i>Poa pratensis</i>	Poaceae	163	.	.	158	.	134	158	.	147	.	.	151	
<i>Polanisia graveolens</i>	Capparaceae	
<i>Polemonium reptans</i>	Polemoniaceae	125	.	134	.	.	140	124	144	131	.	.	139	141	
<i>Polygala alba</i>	Polygalaceae	
<i>Polygala senega</i>	Polygalaceae	.	.	152	158	160	.	
<i>Polygonatum commutatum</i>	Liliaceae	176	160	163	167	174	160	169	168	165
<i>Polygonum achoreum</i>	Polygonaceae	180	176	149	181	
<i>Polygonum aviculare</i>	Polygonaceae	160	.	159	.	180	.	160
<i>Polygonum coccineum</i>	Polygonaceae	203
<i>Polygonum convolvulus</i>	Polygonaceae
<i>Polygonum lapathifolium</i>	Polygonaceae	205
<i>Polygonum pennsylvanicum</i>	Polygonaceae
<i>Polygonum persicaria</i>	Polygonaceae
<i>Polygonum ramosissimum</i>	Polygonaceae	205	203

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012
<i>Prunella vulgaris</i>	Lamiaceae	168	178	
<i>Prunus americana</i>	Rosaceae	133	122	116	132	135	129	117	136	132	122	146	130	119	132	137	118	142	125	.	.	134	139	.	138	138	115	139	99
<i>Prunus armeniaca</i>	Rosaceae	130	118	.	.	.	117	111	124	128	119	141	127	117	126	132	115	137	.	.	.	132	96
<i>Prunus pennsylvanica</i>	Rosaceae	.	122	118	139	.	137	118	137	.	119	.	130	119	134	138	119	145	.	127	
<i>Prunus pumila</i>	Rosaceae	134	116
<i>Prunus tomentosa</i>	Rosaceae	141	127	117	126	132	114	137	118	.	.	131	130	111	
<i>Prunus triloba</i>	Rosaceae	140	130	.	146	131	120	134	138	119	145	125	.	.	135	140	.	.	140	.		
<i>Prunus virginiana</i>	Rosaceae	144	130	135	146	.	147	127	.	139	131	153	137	124	145	147	125	149	134	133	140	142	144		
<i>Psoralea argophylla</i>	Fabaceae	174	.	.	195	.	.	176	.	203	188	.		
<i>Psoralea esculenta</i>	Fabaceae	
<i>Psoralea lanceolata</i>	Fabaceae	
<i>Puccinellia airoides</i>	Poaceae	163	
<i>Pycnanthemum virginianum</i>	Lamiaceae	195	192
<i>Pyrethrum coccineum</i>	Asteraceae	152	.	154	
<i>Pyrola elliptica</i>	Pyrolaceae	195	
<i>Pyrus communis</i>	Rosaceae	134	

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012
<i>Rorippa sinuata</i>	Brassicaceae	149
<i>Rosa arkansana</i>	Rosaceae	170	.	.	165	.	.	162	.	153	168	175	.	167	152	
<i>Rosa blanda</i>	Rosaceae	155	.	.	162	
<i>Rosa hugonis</i>	Rosaceae	158	.	.	.	169	158	146	163	164	140	162	154	154	156	157	157	
<i>Rubus strigosus</i>	Rosaceae	
<i>Rubus occidentalis</i>	Rosaceae	159	159	135	.	.	151	148	153	
<i>Rubus pubescens</i>	Rosaceae	160	.	131	
<i>Rubus strigosus</i>	Rosaceae	
<i>Rudbeckia laciniata</i>	Asteraceae	208	.	213	
<i>Rudbeckia serotina</i>	Asteraceae	168	
<i>Rumex acetosella</i>	Polygonaceae	
<i>Rumex crispus</i>	Polygonaceae	163	.	.	165	
<i>Rumex mexicanus</i>	Polygonaceae	148	.	.	163	.	.	165	.	.	153	
<i>Rumex occidentalis</i>	Polygonaceae	151	
<i>Rumex persicarioides</i>	Polygonaceae	
<i>Rumex venosus</i>	Polygonaceae	
<i>Sagittaria cuneata</i>	Alismataceae	189	174	183	.	179	
<i>Salix interior</i>	Salicaceae	155	

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012
<i>Scirpus atrovirens</i>	Cyperaceae
<i>Scirpus fluviatilis</i>	Cyperaceae	163
<i>Scirpus heterochaetus</i>	Cyperaceae	184
<i>Scirpus paludosus</i>	Cyperaceae	179
<i>Scirpus validus</i>	Cyperaceae	168	156
<i>Scolochloa festucacea</i>	Poaceae
<i>Scolochloa festucacea</i>	Poaceae
<i>Scrophularia leporella</i>	Scrophulariaceae	152	157
<i>Scutellaria galericulata</i>	Lamiaceae
<i>Scutellaria lateriflora</i>	Lamiaceae
<i>Scutellaria parvula</i>	Lamiaceae
<i>Secale cereale</i>	Poaceae	165	.	.	.	163	149	155
<i>Senecio aureus</i>	Asteraceae	153	.	156
<i>Senecio canus</i>	Asteraceae
<i>Senecio congestus</i>	Asteraceae	134
<i>Senecio integerrimus</i>	Asteraceae	138

Scientific Name	Family	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	2007	2008	2009	2010	2011	2012	
<i>Viola adunca</i>	Violaceae	
<i>Viola conspersa</i>	Violaceae	
<i>Viola eriocarpa</i>	Violaceae	124	115	.	
<i>Viola nuttallii</i>	Violaceae	134	
<i>Viola papilionacea</i>	Violaceae	134	115	.	127	129	.	.	.	
<i>Viola pedatifida</i>	Violaceae	120	127	144	134	127	.	127	
<i>Viola rugulosa</i>	Violaceae	.	121	110	134	117	.	117	.	.	128	
<i>Viola sororia</i>	Violaceae	138	.	
<i>Vitis riparia</i>	Vitaceae	156	167	165	.	.	.	151	.	157	161	
<i>Xanthium echinatum</i>	Asteraceae	223	
<i>Xanthium italicum</i>	Asteraceae	
<i>Yucca glauca</i>	Agavaceae	
<i>Zanthoxylum americanum</i>	Rutaceae	.	.	129	136	.	.	148	138	.	.	
<i>Zephyranthes</i>	Liliaceae	190	
<i>Zigadenus elegans</i>	Liliaceae	176	172	.	.	.	172	171	177	172	.	178	162
<i>Zizania aquatica</i>	Poaceae	
<i>Zizia aptera</i>	Apiaceae	131	156	.	148	147	.	.	118	
<i>Zizia aurea</i>	Apiaceae	150	138	146	161	.	.	153	164	.	.	149	142	153	153	.	151	143	147	.	.	122	