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Survival Strategies Of The Endangered Physaria Ludoviciana (Silvery Bladderpod; Brassicaceae)

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Survival Strategies of the Endangered *Physaria ludoviciana*

(Silvery Bladderpod; Brassicaceae)

BY

Marissa Catherine Jernegan Grant

THESIS

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Abstract

Physaria ludoviciana (Nuttal) O'Kane and Al-Shehbaz is an endangered species in Illinois and Minnesota sand prairies, but has many individual populations in western states. Areas where *P. ludoviciana* occurs have high summer temperatures, a sandy soil with low water holding capacity and high drainage, the frequent disturbance of blowing sand and little canopy vegetation, giving it a full sun environment. This habitat is where few other plants are able to establish, providing *P. ludoviciana* with little competition. *Physaria ludoviciana* exists in a niche where it is adapted and able to establish and persist. The focus of this study was to investigate survival strategies of *P. ludoviciana.* The objectives were to understand *P. ludoviciana* with regards to: 1) seed biology, 2) growth and development with different light conditions and with root competition, 3) structural and physiological adaptations, and 4) a survey of soils and associated species that occur with *P. ludoviciana.* Seed biology was studied at the Henry Allan Gleason Nature Preserve (HAGNP) in Mason County, Illinois from 1999-2008. Beneficial aspects of seed biology include high seed production averaging 192 to 555 seeds per plant, an afterripening response that staggers seed germination, high germination percentages (around 70%), and maintained viability of seeds at least for 6.5 years when collected mature and stored at 4°C with less than 50% relative humidity. Detrimental aspects of seed biology include no apparent mechanism for seed dispersal as evidenced by seeds not being dispersed farther than 1 meter from mother plant and limited persistence of seeds in the seed bank with only 4% of seeds in June still being found in November of same year. These seed conditions create variable establishment of seedlings from year to year at HAGNP with 0 to 11 seedlings per m^2. Growth and

development with different light intensities or photoperiods and with root competition were studied to simulate effects of encroaching vegetation that was observed in areas with *P. ludoviciana* at HAGNP. Flowers developed when plants were transferred to both long (16 hr light/8 hr dark) and short (8 hr light/16 hr dark) day photoperiods after being grown in long days for 4 months. Flowers likely were initiated in long days. Plants grown at higher light intensity (584 μmol/m^2/sec) had significantly greater leaf areas, leaf numbers, fresh and dry masses, and root development than those grown at lower light (174 μmol/m^2/sec). Container size affected both vegetative and reproductive development of plants when 4 months old plants were transferred to larger containers (control 20 cm X 4 cm, 2 depths: 22 cm=short and 36 cm=tall and 2 widths: 12 cm=narrow and 23 cm=wide) and then harvested at 4 and 5 weeks. Both light and root competition affected the development of *P. ludoviciana* stressing the importance of little or minimal competition of other species for optimal growth. *Physaria ludoviciana* possesses many structural and physiological adaptations for sand prairie conditions. A long taproot extending to 46 cm can help anchor the plant and help attain ground water. Early in development, plants put more energy into roots than shoots corresponding to a larger root to shoot ratio for younger plants (0.5) than for older plants (0.3). This root to shoot ratio also corresponds to more secondary growth present in roots than in stems. *Physaria ludoviciana* is an evergreen herbaceous perennial so it does not need to regenerate all of its leaves each spring in its water and nutrient limited environment. Plants have C_3 photosynthesis based on anatomy and an isotope analysis, allowing it to photosynthesize during cooler times of the year. A palisade layer occurs on both the top and bottom of the leaf to maximize light collection. Plants also have dense stomates (329

to 698/mm^2) and trichomes (31 to 48/mm^2) which allow for transpirational cooling while still minimizing water loss. Plants have a lower water potential in June and September compared to March and May, allowing them to absorb water from the soil even in dry conditions. No water storage tissues were present in plants. Surveys of soil and associated species were compared in areas where *P. ludoviciana* was present or absent in three states including Illinois, Minnesota and Nebraska. Associated plant species were consistent throughout the range of *P. ludoviciana* being typical grassland and dry sand prairie forbs and grasses with no apparent differences between areas were *P. ludoviciana* was present or absent. Substantial differences in soil characteristics were not found between sites with *P. ludoviciana* present or absent. Seed production per plant for *P. ludoviciana* was high in all sites, 234 to 305; however seedling establishment was low. This study suggests that the ability of *P. ludoviciana* to survive depends more on its ability to arrive and establish in an area than it does on the associated plant species and soil characteristics. Disjunct populations could be a result of poor seed dispersal and limited persistence in the seed bank. So rather than soil characteristics being the limiting factor, establishment and seed dispersal could be the limiting factors. Our understanding of adaptations and requirements for *P. ludoviciana* can aid management decisions for sand prairie species, especially for *P. ludoviciana*.

Acknowledgments

My time with *Physaria ludoviciana* has been enjoyable and rewarding. I will forever be grateful to Dr. Janice Coons for her guidance and the unwavering support and encouragement she has shown me. Her eternal optimism and organizational skills made her an excellent mentor and I appreciate all the time and care she took in my development as a professional.

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I am also indebted to Ms. Nancy Coutant, who, although not an official member of my committee, was always willing to help with anything, whether it was practicing a talk with me, or helping with field work. She was always there.

I would like to thank my parents, who have always valued education and have shown how proud they are of me. Lastly, I owe my husband, Gabe, a big thank you for his support and his ability to keep me calm when this botanical masterpiece got out of control.

I had many collaborators on individual portions of this project and those people are acknowledged at the end of each respective chapter.

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Chapter 1

Introduction

Adaptations in plants are developed as a survival strategy to increase their chances of reproduction for the next generation. The adaptations used by plants vary widely by species and environment. Plants living in harsh environments are modified in different ways for survival. *Physaria ludoviciana* (Nuttal) O'Kane & Al-Shehbaz (silvery bladderpod; Brassicaceae) is an endangered species that relies upon adaptations for existence in a harsh sand prairie habitat that is on the edge of its natural range in Illinois.

Physaria ludoviciana was formerly known as *Lesquerella ludoviciana* until 2002 when Al-Shehbaz and O'Kane (2002) renamed the species from the genus *Lesquerella* into the genus *Physaria* based on DNA sequencing. The genera *Physaria* and *Lesquerella* are indistinguishable morphologically except for one feature of fruit morphology. *Physaria* and *Lesquerella* have the same leaf morphology, trichome type, inflorescence, flower color, fruiting pedicels, and all aspects of seed-coat sculpture and embryo type (Al-Shehbaz and O'Kane, 2002). Historically they were two genera based on fruit. Rollins (1993) separated the two genera because *Physaria* had didymous fruits with deep sinuses between the valves distally (apically), and often proximally (basally), whereas *Lesquerella* had non-didymous fruits with no, or shallow, distal sinuses. A Bayesian analysis of DNA sequences of the internal transcribed spacers of ribosomal DNA was used to compare the two species. Molecular work showed that *Physaria* is nested within and evolved more than once from *Lesquerella.* Based on ITS spacers, *Physaria* and *Lesquerella* should be one genus (Al-Shehbaz and O'Kane, 2002). The

International Botanical Code states that the first established name takes precedence, therefore *Lesquerella* (established 1948) species were transferred in *Physaria* (established in 1888).

Physaria ludoviciana is a perennial forb that forms a rosette of linear, basal leaves which are covered in thick trichomes, which gives the plant a silvery appearance (Beach *et al.,* 2001a,b) (Figure 1.1). Indeterminate racemes bear yellow flowers (Figure 1.2) with four distinct sepals and four distinct petals, as observed in many members of the Mustard Family (Brassicaceae) (Judd *et al*., 2008). Flower stalks elongate as flower buds form and flowers open. From April into August, *P. ludoviciana* flowers on sandy soils (Rollins, 1939; Rollins and Shaw, 1973). Fruits begin to mature from the bottom while flowers are still present on top of the flower stalk (Figure 1.3). Mature seed is set in June.

Physaria ludoviciana is distributed from Illinois north to Wisconsin, west to Montana, south to Nevada, and east to Illinois (Figure 1.4). It is listed as an endangered species in Illinois (Herkert and Ebinger, 2002; Illinois Department of Natural Resources, 2006), Minnesota (Minnesota Department of Natural Resources, 2007) and Wisconsin (Wisconsin Department of Natural Resources, 2004). In each of these states, *P. ludoviciana* is reported in only one county although Claerbout (2003) reported that it may no longer be found in Wisconsin. In these locations, it occurs on the easternmost edge of its geographic range (Rollins and Shaw, 1973). In western states (Arizona, Colorado, Kansas, Montana, Nebraska, Nevada, New Mexico, North Dakota, South Dakota, Utah and Wyoming), it is found scattered throughout many different counties in individual populations (United States Department of Agriculture, Natural Resources Conservation

Service, 2007). Populations often are disjunct, occurring in very adverse conditions. Plants frequently are found on steep slopes in highly disturbed areas and often within a few miles of large rivers (Claerbout, 2003). Commonly *P. ludoviciana* is found in well-drained, mobile sand in blowouts of sand prairies (i.e. vegetated sand dunes) (Coons *et al.*, 2004) (Figure 1.5 and Figure 1.6). With constant disturbance and low soil moisture, these areas may create the ideal niche for *P. ludoviciana*. Many other species lack the adaptations to establish in such environments, which allows *P. ludoviciana* to remain with minimal competition for light, water, nutrients or physical space (Over *et al.*, 2005).

In Illinois, sand deposits can be found in three areas, one of them is Mason County (Figure 1.7). One of the most studied populations is in Illinois at the Henry Allan Gleason Nature Preserve (HAGNP) in Mason County (Herkert and Ebinger, 2002). This area has a unique habitat that was formed after glaciers receded at the end of the Wisconsinan glaciation about 12,000 years ago. The glacial meltwaters suspended large amounts of gravel, sand and clay. Also, meltwaters were collected in glacial lakes that eventually exceeded capacity and busted moraines. The surging of the water has become known as the Kankakee Torrent (Gleason, 1910; Willman and Frye, 1970). The water of the Kankakee Torrent carried tremendous volumes of sand and gravel downstream of the "Big Bend" at Hennepin where the river channel narrowed. The river valley widens below Hennepin so the water lost its velocity causing the sand and gravel to deposit. The heavier sediments such as sand and gravel were deposited most frequently in areas where the river floodplains naturally widened (Lamar and Willman, 1958). As these sand deposits dried, they were exposed to wind action, resulting in large sand dunes (McClain, 1997). These sand deposits developed a truly unique ecosystem at HAGNP. *Physaria*

ludoviciana at the preserve grows on remnant sand dunes that are fully exposed and have been eroded by the wind into three distinct bowl-shaped depressions (Claerbout, 2003). The blowouts where *P. ludoviciana* is found are higher on the dunes, suggesting a more exposed environment. Three distinct colonies are present: the North Colony Lower Bowl (NCLB), the North Colony Upper Bowl (NCUB) and the South Colony (SC) (Beach *et al.*, 2001b) (Figure 1.8).

Features of seed biology for *Physaria ludoviciana* may affect its survival. In Illinois, flowering occurs in April-May, and mature seed is set in May-June (Beach *et al.*, 2002a,b) when temperatures are usually lower and water is more plentiful than later in summer months of July and August. Fruits begin to mature from the bottom while flowers are still present on top of the flower stalk. *Physaria ludoviciana* plants are successful at producing fruits with viable seeds at HAGNP (Claerbout *et al.*, 2007). Estimated seed production per plant in 2002 was 500 (Claerbout, 2003), but it is not known if this number varies from year to year. Plants do not appear to have any mechanisms for seed dispersal. Since plants occur near rivers, it is possible that rivers played a role in dispersal of seeds (Coons *et al.*, 2004). Seeds do not require any stratification or scarification to germinate (Coons *et al.*, 2000), but it is unclear if they need afterripening. It is unknown if seed remains viable after several years in controlled storage or in the soil bank. Claerbout (2003) did not find any *P. ludoviciana* seeds in the seed bank when sifting and planting soil cores, which was not expected given that numerous seeds were produced and that seedlings are present at HAGNP. Other techniques to quantify seeds in the seed bank need to be investigated. The large quantity of seeds produced that lack dormancy presumably creates a greater chance of germination

and establishment of seedlings immediately during the early summer when more soil moisture is available from snow melt or spring rains. However seedling establishment may not occur every year. Therefore little seed in the bank may limit the spread of *P. ludoviciana* populations (Claerbout, 2003; Over *et al.*, 2005). A better understanding of the seed biology (production, afterripening, longevity, dispersal and seedling establishment) of *Physaria ludoviciana* is important to manage for its survival.

Harsh conditions such as disturbance from blowing sand dunes and exposure to sunlight due to little over story vegetation may create the ideal niche for *Physaria ludoviciana* while helping prevent establishment of many other species that might compete for light, water, nutrients and physical space. This niche allows *P. ludoviciana* to be successful (Over *et al.*, 2005). *Physaria ludoviciana* blooms early in the spring when competition is less for pollinators and other resources. Knowing what initiates floral development in *P. ludoviciana* would be beneficial. Microclimates are found above-and-below ground in areas where *P. ludoviciana* resides. Places where *P. ludoviciana* is absent may have thick vegetation, not allowing for *P. ludoviciana* growth because of above-ground microclimates interfering with the amount of light needed for growth and development. Observations made of below-ground microclimates suggested that *P. ludoviciana* does not do well with competition. Greenhouse studies showed that although roots do not become root-bound, plant growth and vigor is revitalized when transferred to larger containers (Coons, personal observation). Since *P. ludoviciana* is listed as endangered in two states, some of these factors may be inhibiting its growth and development. Light (both duration and intensity) and competition (both above ground and below ground) could be affecting the development of *P. ludoviciana*.

Physaria ludoviciana has adapted to growing in very adverse environmental conditions (Figure 1.9). The soil, consisting of mostly sand, is well-drained and therefore water is a limiting factor. Soil temperatures at HAGNP were on average 2-5°C higher compared to those at a local environmental monitoring station 25 km south southwest of HAGNP (Over *et al.*, 2005). These areas have little competition from other species as evidenced by 62% of the area being open sand (see chapter 5). Little to no shade occurs for plants with light intensity during the summer months averaging around 1900 $\mu mol/m^2/sec$, so plants receive full sunlight (Over *et al.*, 2005). Wind also is a constant disturbance. Many sand prairie plants share a number of common morphological adaptations that help reduce stress from low soil and air moisture levels (Moore, 1999). For example *P. ludoviciana* has trichomes that create a boundary layer by creating a humid layer of air near the leaf surface; a short stature that allows leaves to warm quickly in spring, since sandy soils typically warm quicker than loam soils, which may allow for the plant to begin photosynthesis earlier (Coons *et al.*, 2000); stomatal densities also may give an indication of potential for transpirational cooling; and tolerance for low water potentials may allow for water uptake by the roots even if soils are drier (Over *et al.*, 2005). Descriptions of structural and physiological adaptations of *P. ludoviciana* could help to understand how it is able to flourish in these areas where few other species can.

The population of *Physaria ludoviciana* at HAGNP occurs in a very unique habitat. Three separate colonies are found at this preserve. Seemingly similar habitat connects the North Colonies to the South Colony, but *P. ludoviciana* has never established between the two. The scattered and disjunct nature of the *P. ludoviciana* observed at HAGNP and in western states (Claerbout, 2003) brings many questions. In

Illinois, other areas with apparently similar habitat and environmental conditions to HAGNP are found where *P. ludoviciana* is absent as well as similar areas in other states where it is absent. Due to this interesting habitat in Illinois, we decided to expand the survey beyond HAGNP in Illinois and look at sites in different states where *P. ludoviciana* is present and absent to identify possible parameters that might relate to its disjunct distribution. Associated species and soil traits often are indicative of differences in habitat. Seed and seedling traits often are used as indicators of population viability because aspects of the seed production may affect seedling establishment for *P. ludoviciana*. Associated species and soil traits in areas where *P. ludoviciana* is present and absent in different states would provide more comparisons to help explain its disjunct distribution as well as to compare development of reproductive structures and plant densities of *P. ludoviciana* at different locations.

The goal of this thesis was to investigate survival strategies of *Physaria ludoviciana* to its unique ecological niche. It is important to learn more about the survival strategies of this endangered species in order to make more informed management decisions. The objectives of the study were to investigate seed biology, effects of light and root competition on development, and structural and physiological adaptations, as well as to survey areas where *P. ludoviciana* is present and absent to help interpret how these plants survive in their adverse environments.

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Figure 1.1. Habit of *Physaria ludoviciana* with buds forming and stem arising from a basal rosette covered in thick trichomes giving it a silvery appearance.

Picture courtesy of Janice Coons.

Figure 1.2. Elongating flower stalk of *Physaria ludoviciana* with an indeterminate raceme bearing yellow flowers (Claerbout, 2003).

Figure 1.3. Fruits of *Physaria ludoviciana* mature from the bottom towards the top.

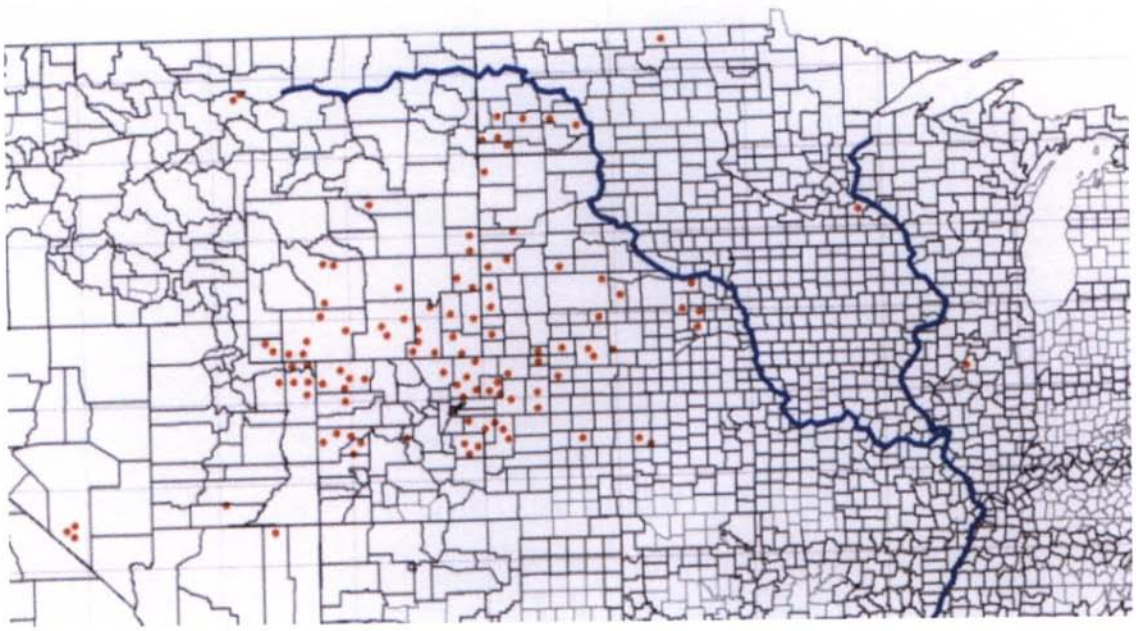

Figure 1.4. Distribution of *Physaria ludoviciana* throughout the United States (Modified from Rollins and Shaw, 1973).

Picture courtesy of Ann Claerbout.

Figure 1.5. Commonly *Physaria ludoviciana* is found in well-drained, mobile sand in blowouts of sand prairies (i.e. vegetated sand dunes).

Picture courtesy of Janice Coons.

Figure 1.6. *Physaria ludoviciana* growing in typical sand prairie habitat at the Henry Allan Gleason Nature Preserve, Mason County, Illinois (Claerbout, 2003).

Figure 1.7. Sand deposits of Illinois (Modified from McClain, 1997) (Claerbout, 2003).

Figure 1.8. Colonies of *Physaria ludoviciana* at the Henry Allan Gleason Nature Preserve in Mason County, Illinois (Modified from Claerbout, 2003).

Figure 1.9. *Physaria ludoviciana* in its habitat at the Henry Allan Gleason Nature Preserve, Mason County, Illinois, with little moisture and blowing sand resulting in little competition.

Chapter 2

Seed Biology of the Endangered *Physaria ludoviciana* (Silvery Bladderpod; Brassicaceae)

Abstract

Physaria ludoviciana (Nuttal) O'Kane & Al-Shehbaz (silvery bladderpod) is an endangered species of the Illinois sand prairies. The only naturally occurring population in Illinois is at the Henry Allan Gleason Nature Preserve in Mason County. An overview of how seed biology affects its ability to persist is lacking. Our goal was to evaluate how seed biology (production, dispersal, longevity in seed bank, afterripening, longevity in storage, emergence of seedlings from seed, and seedling establishment in the field) affects the persistence of *P. ludoviciana* in the seed bank. Inflorescence structures were counted to estimate seed production on a per plant basis. In June 2008, 20 soil scrapes (10 cm X 10 cm) were collected at 0, 1 and 2 m from reproductive plants to estimate seeds dispersal. Soil was separated from seed by JFNew Native Plant Nursery using a two-step screen cleaning machine based on seed weight, size and shape, including a high air flow with various screen sizes. For seed longevity in the seed banks, soil scrapes were taken immediately adjacent to a reproductive plant in June 2008 and November 2008. The same procedure for separating soil was followed as in seed dispersal. Afterripening studies were performed using seeds collected in June 2007, which were imbibed at two-month intervals for ten months after harvest. Three replicates of 50 seeds per trial were germinated in Petri dishes at 25°C. Seeds were considered germinated when the radicle emerged. To consider longevity of seeds in storage, fruits were collected including nine seed lots from the upper and lower stalks with different harvests in June of 1999-2002.

Germination was tested from 2000-2006 using four to five replications of 10-50 seeds per seed lot with the same conditions for afterripening. Seed vigor of stored seeds was tested by planting seeds that had the highest germination in 2006, using one seed lot from each year. Numbers of emerged plants, leaves, width of rosettes and masses were measured. For seedling establishment and plant density, quadrats (0.25 m^2) were placed on alternating sides along a 45 m transect. Plant densities were counted from 2000-2008. Seedlings ($\leq$ 6 leaves), vegetative (> 6 leaves) and reproductive (flower stalks present) plants were counted. Seed production in May and June was high between 71-744 seeds per plant. Most seeds did not disperse farther than from the mother plant and only 4% of *P. ludoviciana* seeds in June were found in November. In afterripening trials, germination rates increased greatly when seeds were stored at room temperature for six months compared to seeds without any storage. For seed longevity, seeds stored at 4°C with 40-50% relative humidity remained viable for at least six and a half years. Seed vigor was affected more by maturity of seed than date or year of collection. Seedling establishment densities ranged from 0-11 seedlings/m^2 during surveys in June 2000-2002, 2007 and 2008. Many factors can affect the seed biology of *P. ludoviciana*. Seed production, afterripening and the longevity of seeds in storage were good survival strategies for *P. ludoviciana,* but no mechanisms for dispersal and few seeds in the seed bank were poor survival strategies for *P. ludoviciana*. These seed conditions create variable establishment of seedlings from year to year. Studies in seed biology allow us to predict the recruitment of *P. ludoviciana.* A better understanding of the seed biology of this species is important for its survival as well as for making informed restoration and management decisions.

Introduction

Physaria ludoviciana (Nuttal) O'Kane & Al-Shehbaz (silvery bladderpod; Brassicaceae) was formerly known as *Lesquerella ludoviciana* until Al-Shehbaz and O'Kane (2002) transferred it to the genus *Physaria* based on molecular evidence. *Physaria ludoviciana* is a perennial forb that forms a rosette of linear, basal leaves covered in thick trichomes, which gives a silvery appearance (Beach *et al.,* 2001a,b). Flower stalks elongate as flower buds form and yellow flowers are produced. From April into August, *P. ludoviciana* flowers on sandy soils (Rollins, 1939; Rollins and Shaw, 1973). Fruits begin to mature from the bottom while flowers are still on top of the flower stalk.

Physaria ludoviciana is distributed from Illinois north to Wisconsin, west to Montana, south to Nevada, and east to Illinois. It is listed as an endangered species in Illinois (Herkert and Ebinger, 2002; Illinois Department of Natural Resources, 2006), Minnesota (Minnesota Department of Natural Resources, 2007) and Wisconsin (Wisconsin Department of Natural Resources, 2004). However Claerbout (2003) reported it may no longer be in Wisconsin. In each of these states, *P. ludoviciana* is reported in only one county. In these locations, it occurs on the eastern-most edge of its geographic range (Rollins and Shaw, 1973). In other more western it is found scattered in individual populations (United States Department of Agriculture, Natural Resources Conservation Service, 2007). Populations are frequent and disjunct, occurring in adverse conditions. Commonly, it is found in well-drained, mobile sand in blowouts of sand prairies (i.e. vegetated sand dunes) (Coons *et al.*, 2004).

One of the most studied populations is in Illinois at the Henry Allan Gleason Nature Preserve (HAGNP) in Mason County (Herkert and Ebinger, 2002). The most significant geological feature at HAGNP is the large sand dune known as "Devil's Tower," which comprises about half of the preserve and is more than 25 m tall (McClain *et al.*, 2005). At the preserve *Physaria ludoviciana* grows on remnant sand dunes that are fully exposed and have been eroded by the wind into three distinct bowl-shaped depressions (Claerbout, 2003). The blowouts where *P. ludoviciana* is found are higher on the dunes, creating a more exposed environment. The three distinct colonies are the North Colony Lower Bowl (NCLB), the North Colony Upper Bowl (NCUB), and the South Colony (SC) (Beach *et al.*, 2001b). Of these three colonies, the North Colony Lower Bowl is a stabilized blowout immediately west of and below the highest peak of Devil's Tower and is the largest colony of *P. ludoviciana* (Coons *et al.,* 2004). This colony is located in the presumably younger, less recovered blowout. In 1999, its area was 2,050 m^2 with approximately 10,300 silvery bladderpod plants (Coons *et al.*, 2000). In 1999, the NCUB area was 660 m^2 with approximately 220 silvery bladderpod plants (Coons *et al.*, 2000), being the smallest colony based on plant numbers with the heaviest plant cover from other species, and relatively sparse distribution of *P. ludoviciana* (Beach *et al.*, 2001a,b). The SC was 272 m^2 with approximately 900 silvery bladderpod plants in 1999 (Coons *et al.*, 2000).

Potential adaptations to this open sand environment that *Physaria ludoviciana* has made include features of seed production and dispersal. In Illinois, flowering occurs April to May, and mature seed is set from May to June (Beach *et al.*, 2002a, b). Setting seed during this time is beneficial because temperatures are lower and water is more

plentiful than in the later summer months. Plants produced large numbers of seeds, around 500 per plant in 2002 (Claerbout, 2003), but it is not known if this number varies from year to year. No structures were apparent to facilitate widespread seed dispersal, which might limit its range (Claerbout, 2003; Over *et al.*, 2005). Since plants occur near rivers, it is possible that rivers played a role in dispersal of seeds. *Physaria. ludoviciana* plants are successful at producing fruits with seeds at HAGNP (Claerbout *et al.*, 2007). High fruit production is typical in this genus and its relatives. The related *Physaria* (as *Lesquerella*) *fendleri*, a desert perennial species, produces one to several hundred fruits per plant with 1-30 seeds per fruit (Cabin and Marshall, 2000). But in *Physaria* (as *Lesquerella*) *arctica,* a typical arctic perennial herb, flowers per inflorescence are 3-8 and seeds per fruit are 6-8 (Aiken *et al.,* 1999). In *Physaria* (as *Lesquerella*) *arctica*, seeds often are dispersed only a short distance due to the lack of adaptations for wind dispersal, and seedlings often occur in the vicinity of the parent plant (Elberling, 2000; Marchand and Roach, 1980).

Whether *Physaria ludoviciana* is able to maintain a seed bank is unknown. Other related species, like *Physaria* (as *Lesquerella*) *fendleri,* are able to maintain a seed bank (Hyatt *et al.*, 1999). According to Parker *et al.* (1989), persistent seed banks usually are found where environmental and/or disturbance regimes are unpredictable, and thus the probability of seedling success is low or variable. While *P. ludoviciana* lives in such disturbed regimes, Claerbout (2003) did not find any seeds in the seed bank when sifting and collecting soil cores, which was not expected given the presence of seedlings. These findings indicate that other techniques to quantify seeds in the seed bank need to be investigated.

Seeds of *Physaria ludoviciana* do not require any stratification or scarification to germinate (Coons, *et al.*, 2000), but it is unclear if they experience dormancy after they are shed and need an afterripening period. If no afterripening is required, the large quantity of seeds produced presumably creates a greater chance of germination and establishment of seeds during the early summer rains. *Physaria* (as *Lesquerella*) *fendleri* has a cyclic dormancy-nondormancy cycle, where seed collected had a maximum germination of 30% in year one and 95% in year two (Hyatt *et al.*, 1999). The dormancy cycle of *P. ludoviciana* needs to be investigated.

Several factors affect the germination and emergence of *Physaria ludoviciana,* including maturity of seed and its age or longevity in storage (Claerbout, 2003). In the early part of the growing season, seeds from the lower portion of the flower stalk are mature, while seeds on the upper portion are not. Later in the growing season, seeds from the upper stalk are mature while those on the lower portion of the stalk are shed (Claerbout, 2003). In 2002, Claerbout (2003) germinated *P. ludoviciana* seeds collected from HAGNP in 1999-2002. Seasonal differences were observed from the different years. Since several factors including seed collection dates, position of seed on the flower stalk, maturity of seed when collected and storage time affect seed vigor, multiple years and collection dates are needed to test how longevity affects seed vigor of *P. ludoviciana* (Beach *et al.*, 2001b; Claerbout, 2003).

If proper environmental conditions are not present, seedling establishment may not occur every year. Since no seed was found in the seed bank, the spread of *Physaria ludoviciana* populations could be limited (Coons *et al.*, 2000; Claerbout, 2003) from year to year. Seedlings are present at HAGNP (Beach *et al.*, 2001a) from year to year, but it is

unknown over successive years whether seedling establishment is increasing or decreasing. For plants, a short time frame often occurs when conditions are favorable enough to allow germination and establishment. Thus favorable environmental conditions can be a limiting factor (Elberling, 2000). An environment with unstabilized blowing sand, such as the sand prairie, could bury seeds and not allow them to germinate (Martinez and Moreno-Casasola, 1993). The optimal depth for seedling emergence could be strongly influenced by seed mass (Li *et al.*, 2006). Larger seeds are generally superior to smaller seeds by having a higher probability of emergence, and by developing into seedlings with better competitive ability, higher survival, and better performance in later life stages (Li *et al.*, 2006).

The goal of this study was to investigate seed biology of *Physaria ludoviciana* including production, dispersal, longevity in seed bank, afterripening, longevity in storage, emergence of seedlings from seed, and seedling establishment in the field in *P. ludoviciana*. In this study, aspects of seed biology were compared in all three colonies of *P. ludoviciana* at HAGNP in Illinois with more in-depth comparisons at the largest colony (NCLB).

Materials and Methods

Site Description

Henry Allan Gleason Nature Preserve (HAGNP) is located in extreme northwestern Mason County, southwest of Goofy Ridge, and about 15 km northeast of Havana, Illinois (SE 1/4 S6, NE 1/4, Section 7 Township 22N Range 7W) (McClain *et al.*, 2005). The site lies within the Illinois River Section of the Mississippi River and the

Illinois River Sand Area Natural Division, and is within Sand Ridge State Forest (Schwegman, 1973).

Seed Production

Seed production was surveyed at the Henry Allan Gleason Nature Preserve in Mason County, Illinois in three colonies (North Colony Lower Bowl (NCLB), North Colony Upper Bowl (NCUB), and South Colony (SC)) in 1999 (8 and 22 June), 2000 (1 June), 2001 (14 May, 6 June, and 24 July), 2002 (3 June), 2007 (4 June) and 2008 (26 June) although not every colony on every date. On each date, 30 to 50 plants in the reproductive stage were selected randomly to count numbers of flower stalks per plant, pedicels per stalk, flowers per stalk, fruits per stalk and fruits with holes per stalk (fruit apparently damaged from herbivory), and to measure height of the tallest stalk. Twenty-five fruits were collected from each of four plants. In the lab, numbers of seeds in each fruit were counted. Means and standard errors were calculated for all parameters. A high estimate of number of seeds per plant was determined based upon the assumption that each pedicel would have a fruit. Calculations were made by multiplying average number of seeds per fruit, by pedicels per stalk, by number of flower stalks per plant. A low seed estimate based on number of fruit also was calculated by multiplying average number of seeds per fruit, by number of fruits per stalk, by number of flower stalks per plant. Means and standard errors using Microsoft® Office Excel 2003 (11.8211.8202) SP3 were found for height of tallest flower stalk, number of flowers per stalk, and number of fruits with holes.

Seed Dispersal and Longevity in Seed Bank

To estimate seed dispersion, soil was collected directly adjacent to ten different reproductive *Physaria ludoviciana* plants, and at 1 and 2 m away from the plant. Samples were collected on 26 June 2008 from HAGNP in the NCLB, while *P. ludoviciana* was shedding its seeds. For each plant, a set of samples at each distance was taken. Soil scrapes (10 X 10 cm as measured with cut-out of card stock was used along with an ice scraper to scrape the top 2 cm of sand into a dust pan) which were placed into Ziploc® bags. Soil scrapes were air dried individually in plastic trays (10 X 20 X 6 cm) for at least seven days. Two soil samples were combined together to make one replicate, for a total of five replicates at each distance. These soil samples then were sent to JFNew Native Plant Nursery (Walkerton, IN) where seeds were separated from sand using two steps on screen seed cleaning machines, where one uses seed mass, size and shape while the other uses a higher air flow setting in combination with screen sizes. A Clipper 2 Seed Cleaner was used which forced air from below to separate seeds through a number of screens. The top screen was ASTM (American Society of Testing Materials) 1/14, materials not passing over this screen included larger debris including *P. ludoviciana* seed pods. The bottom screen was ASTM 1/18, materials passing through 1/18 (bottom) screen were small sand particles, dust, etc. Materials collected from the bottom base tray and sieves were screened for final separation. All other materials were collected in the air-blow-dust collection port; these materials were inspected and added to bulk sample remnants. Material from the bottom base tray was further separated using brass sieve trays, (ASTM 1/10, 1/12, 1/14, 1/18, 1/25, and 1/35). *Physaria ludoviciana* seed was separated at the 1/14 and 1/18 sieve tray levels and placed in marked plastic Ziploc®

bags. Material from all other sieve screens was inspected and placed with other bulk sample remnants. Numbers of seeds per square meter were determined along with mean and standard errors.

Seed longevity in the seed bank is defined as how long seeds persist in the soil. Seed longevity was measured at HAGNP in the NCLB. Twenty soil scrapes were collected in 2008 (26 June and 4 November) near 20 reproductive plants and placed in Ziploc® bags. These samples were dried for at least seven days, and four samples were combined together to make five replications for each sampling date, which were sent to JFNew where they were separated using the same technique as outlined for dispersal. Numbers of seeds per square meter were determined along with means and standard errors using Microsoft® Office Excel 2003 (11.8211.8202) SP3.

Afterripening

Seeds collected from HAGNP in Mason County, Illinois from the NCLB were used in this study. Seeds collected on 4 June 2007 were sorted by upper and lower stalk. Seeds also were collected on 18 June 2007, when most seed from the lower stalk had matured and was shed, therefore only seed from the upper stalk was used. The first germination tests were started after 18 June 2007, with additional tests initiated every two months until May 2008. Petri dishes (100 X 15 mm plastic, Fisherbrand®, Pittsburg, Pennsylvania) contained two sheets of 90 mm filter paper (Whatman #1, Fisherbrand®, Pittsburg, Pennsylvania) that was moistened with 5 mL distilled water. Each trial had three dishes with 50 seeds each from each collection. Seeds were dusted with thiram powder (50% tetramethylthiuram disulfide from Loveland Industries, Cambridge, Cambridgeshire, UK) to reduce fungal growth. Petri dishes were sealed with Parafilm®

(Parafilm® "M" Laboratory film, Pechiney Plastic Packaging, Chicago, Illinois) and randomly placed in a Rubbermaid® plastic tub (33 cm X 24 cm X 10 cm). These containers were placed in a Percival Scientific® (Perry, Iowa) seed germinator at 24.9 ± 0.0°C as measured by a readout on chamber with constant light provided with fluorescent bulbs at 18.8 ± 1.0 μmol/m^2/sec as measured with Apogee® Quantum meter (Logan, Utah). Additional water was added as needed. Number of seeds germinated or contaminated was recorded every three days for the first six months and then once a week for an additional one to eleven months depending when imbibition was started. If contamination was observed, contaminated seeds were dusted with thiram powder, which eliminated contamination. Seeds were considered germinated when the radicle was evident.

All ungerminated seeds were tested with 0.5% of 2,3,5-triphenyl tetrazolium chloride (tetrazolium) (Fisherbrand®, Pittsburg, Pennsylvania) to determine if they were viable. Seeds were placed in 100 X 15 mm plastic Petri dishes with one sheet of 90 mm filter paper (Whatman #1) that was moistened with 3 ml distilled water. Seeds were cut in half using a dissecting needle and a razor blade. When seeds were cut, the seed coat was broken so that the embryo and cotyledon were removed or at least exposed. It was noted whether a liquid or solid emerged from the seed as well as the color of the embryo and cotyledon. Tetrazolium was dripped over seeds. Petri dishes were placed in a Rubbermaid® tub that was covered with aluminum foil and a black garbage bag to keep light exposure to a minimum. The bag then was placed in the seed germinator at 25.0°C. Seeds were observed for a color change at six and 24 hours after tetrazolium application. Seeds were viewed under a dissecting microscope. If seeds were alive/viable, the

tetrazolium would stain the seeds pink, and if the seeds were dead/not viable, no color change occurred.

Means and standard errors for percentages of seeds that germinated were calculated at 48 days, at six months, and at the end of this study. Means and standard errors of percentages also were calculated for seeds that were alive and dead when tested with tetrazolium. Percentages were arcsin transformed to stabilize the variances. Arcsin transformed data also were analyzed by analysis of variance followed by a Duncan's multiple range test at the 5% level using SPSS (Version 16 for Windows) done at 48 days, six months, end of study and for seeds that were alive or dead when tested with tetrazolium.

Longevity in Storage-Seed Germination

Germination trials included nine seed lots from upper and lower seed stalks from harvests made in 1999-2002. Seeds were collected at HAGNP from the NCLB in 1999 (8 and 22 June), 2000 (1 and 16 June), 2001 (6 June), and 2002 (3 June). Seeds from the first collection dates in 1999 and 2000 and from the collection date in 2001were separated into upper and lower stalk seed. Seed collected in 2002 was a mix of mature lower stalk seed and immature upper stalk seed. Seeds were stored in coin envelopes in a refrigerator (Fisher Scientific, Isotemp, Laboratory Refrigerator, Pittsburg, Pennsylvania) at 4°C with 40-50% relative humidity until used in study. Four to five repetitions of each seed lot were used with 10-50 seeds for each repetition. Earlier trials used fewer seeds when seed collections were more limited before seed production had been estimated. Later trials used more seeds. Germination was tested in 2000 (11 April and 7 November), 2001 (11 November), 2002 (19 February), 2003 (20 February) and 2006

(January 19). Plastic Petri dishes were 100 X 15 mm that contained two sheets of 90 mm filter paper (Whatman #1), which was moistened with 5 ml of distilled water. Seeds were dusted with thiram powder (50% tetramethylthiuram disulfide) to reduce fungal growth. Petri plates were sealed with Parafilm® and randomly placed in a Rubbermaid® plastic tub (33 cm X 24 cm X 10 cm) which was placed in a Percival Scientific seed germinator at 25.0 ± 0.1°C as measured by readout on chamber with constant light provided with fluorescent bulbs at 17.4 ± 1.2 μmol/m^2/sec as measured with Apogee® Quantum meter. Additional water was added as needed. Number of seeds germinated or contaminated was recorded for two weeks. Seeds were considered germinated when the radicle was evident.

For germination data analyses, Microsoft® Office Excel 2003 (11.8211.8202) SP3 and SPSS (Version 16 for Windows) were used. Means and standard errors were calculated for percentage germination for all seeds. Percentages were arcsin transformed to stabilize the variances. Different seed lots were compared using analysis of variance on arcsin transformed data followed by a Duncan's multiple range test at the 5% level.

Emergence of Seedlings from Stored Seed

Seed was collected at HAGNP from the NCLB. One seed lot collected from each year 1999-2002 (22 June 1999, upper stalk seed; 16 June 2000, upper stalk seed; 6 June 2001, upper stalk seed; and 3 June 2002 mixed stalk seed) was tested. These seed lots were chosen because germination trials in January 2006 showed that these seeds were the most viable. Seeds were stored in coin envelopes in a refrigerator (Fisher Scientific, Isotemp, Laboratory Refrigerator, Pittsburg, Pennsylvania) at 4°C with 40-50% relative humidity until used in study. Three repetitions were used for each seed lot with 25 seeds

each. On 9 February 2006, seeds were planted in trays (10 X 20 X 6 cm) filled with soilless mix (peat, vermiculite and perlite) (Fafard Growing Mix #2, Conrad Fafard, Inc., Agawam, Massachusetts) and moistened with distilled water. Twenty-five seeds were placed in each tray. Seeds were planted about one centimeter below the surface of the mix. Trays were placed in a growth chamber at 25.9 ± 0.1°C with a photoperiod of 16 hours of light from fluorescent bulbs at 309 ± 15 μmol/m^2/sec as measured with Apogee® Quantum meter. Number of emerged seedlings were counted daily for four weeks. Number of leaves and width of the plants were taken weekly. Plants were watered as needed with distilled water. Fresh masses of shoots were taken when plants were harvested. Plants were dried in an oven for 24 hours at 111°C and then weighed again.

Means and standard errors were calculated for percentage emergence, number of leaves, width of plants, fresh masses and dry masses using Microsoft® Office Excel 2003 (11.8211.8202) SP3. Then all parameters were analyzed by analysis of variance followed by a Duncan's multiple range test at the 5% level using SPSS (Version 16 for Windows).

Seedling Establishment and Plant Densities

Plant densities were counted of *Physaria ludoviciana* seedling, vegetative and reproductive plants for the three different colonies at the HAGNP in 2000 (1 June), 2001 (14 May, 2 June and 24 July), 2002 (6 May and 3 June), 2007 (4 June) and 2008 (30 April and 26 June). A forty-five meter transect was extended through the colonies, with a quadrat (0.25 m^2) directly adjacent on alternating sides of the transect. In the NCLB a forty-five meter transect was extended running south to north, in the NCUB two transects were extended; a thirty meter transect running west to east and a fifteen meter transect running south to north. In the SC three fifteen meter transects were extended parallel to

each other west to east. In each of the quadrats, number of seedlings (≤ 6 leaves), vegetative plants (> 6 leaves, but no flower stalk) and reproductive plants (flower stalks) were counted. For data analyses, the number of plants at each growth stage per square meter was calculated. Means and standard errors were calculated using Microsoft® Office Excel 2003(11.8211.8202) SP3. Percentage of plants at each growth stage also was calculated.

Results

Seed Production

Seed production does not appear to be a limiting factor for *Physaria ludoviciana* survival. Between 22 and 744 seeds were produced per plant. The estimate based on fruit numbers is a low estimate because it only counts the number of fruits that were on the tallest stalk at the time of the survey. The estimate based on the number of flower pedicels is a high estimate because the number of pedicels were counted on the tallest stalk and assumed that every pedicel would produce a fruit. No standard error was calculated since it was an estimate. On a given date when seed estimates were taken comparing all three colonies, the North Colony Lower Bowl had the highest number of seeds (Table 2.1). Flowers were only present in June of 2002, whereas in other years flowers had already gone to fruit or no data were collected. Fruits with holes were noticed, which could be due to herbivory of the fruits (Table 2.2). A Pearson's Correlation was used to correlate flower stalk height to pedicels/stalk for years 2007-2008. The correlation was found to be significant at the 0.01 level with N=135 and the Pearson Correlation = 0.658.

Seed Dispersal and Longevity in Seed Bank

Most seeds do not disperse far from the mother plant. When plants were shedding seeds, 380 seeds/m^2 were found in soil samples taken from scrapes right next to a reproductive plant compared to 10 and 20 seeds/m^2 found at 1 and 2 meters, respectively, from reproductive plants. There was a significant difference between soil cores taken right next to reproductive plants compared to those soil cores taken at 1 and 2 meters away from reproductive plants. When soil scrapes were taken, reproductive plants within close proximity of each other (2 meters) were not used. However it is possible that other reproductive plants were not noticed and were closer then suspected, thus giving a higher number of seeds. Only 4% of *Physaria ludoviciana* seeds in June were still found in November. There was also a significant difference found in seed numbers between those found in June compared to the seeds found in November The longevity of *P. ludoviciana* seed in the seed bank is limited for the next season (Table 2.3).

Afterripening

Physaria ludoviciana seeds demonstrated a need for an afterripening period. For the lower stalk seed germination rates at 0-4 months after seeds harvest were slow at the beginning whereas at 6-10 months rates increased and total percentages were higher (Figure 2.1). Upper stalk seed had slower rates of germination in 0-2 months after harvest, whereas rates for 4-10 months after harvest increased. Months 2, 6 and 8 had higher germination than 0, 4, and 10 months after harvest (Figure 2.2). In the mixed stalk seed we saw a similar pattern to the lower stalk seed. Zero, 2, and 4 months after harvest have a slower rate of germination than 6 and 10 months after harvest, but 8 months was more similar to 0, 2, and 4 months. Lower germination percentages were found for 0, 2,

4, and 8 months (Figure 2.3). For the lower stalk seed germination percentages after 48 days, months after harvest 6, 8 and 10 were significantly higher than months after harvest 0, 2, and 4 (Table 2.4). Germination percentages at 6 months after imbibition were higher in plants started 10 months after harvest (Table 2.4). At final counts for the lower stalk seed eventually germination percentages became more similar and most viable seed did germinate. At final counts all seeds were the same age and maturity.

Tetrazolium tests from the lower stalk seed showed that a very low percentage of seed were not viable. Seed that had been imbibed right after harvest and had been in Petri dishes for 17 months had 20% of the seeds non-viable (Table 2.4). Tetrazolium tests for the lower stalk seed showed that seeds started at 4 and 8 months after harvest had the highest percentage of viable seeds that had not geminated (Table 2.4). For upper stalk seeds, at 6 months germination percentages for seeds started 10 and 0 months after harvest were significantly lower than other months. From the upper stalk seed, tetrazolium tests showed that non-viable seeds were significantly similar for all trials (Table 2.5). In the mixed stalk seed at 48 days germination was higher in 6 and 10 months than in 0, 2, 4 and 8 months (Table 2.6). At 6 months after imbibiton begins, germination was significantly lower for 0 and 2 months. Tetrazolium tests showed no significant difference in non-viable seeds (Table 2.6). Seed contamination was not a problem for *Physaria ludoviciana*. If contamination was seen, those seeds were dusted with thiram.

Longevity in Storage-Seed Germination

Germination is high in seed even after several years in storage as long as seeds are mature when collected. Some of the seed lots had significant increases in germination

after being stored (Table 2.7). Those seeds lots included the second upper stalk harvest in 2000 and the mixed stalk in 2002. The only significant decrease in germination after being stored was the first harvest lower stalk seed in 1999. Seed from 2002 contained both mature and immature seed as both upper and lower stalks were mixed, thus accounting for the low germination percentage. Seed remained viable in storage for at least 6.5 years.

Emergence of Seedlings from Stored Seed

Emergence was first seen in seeds from 1999-2001 on day five, whereas seeds from 2002 did not emerge until day 6 (Figure 2.4). Four weeks after planting, seed from the 1999 upper stalk had the highest percent of emergence with 85.5%, and seed from 2002 had the lowest emergence with 18.7%. Seed collected from earlier years had higher emergence than seed collected from later years (Table 2.8). Rosette widths varied between the seed years, as plants from seeds with higher emergence percentages had a greater width of the rosettes than those from seed years with low emergence percentages (Figure 2.5). Figure 2.6 shows that the average number of leaves was consistent among the years no matter how many seedlings emerged. Fresh and dry masses of plants from 1999 had the highest mass. Fresh and dry masses of plants from 2002 were lowest (Table 2.8). A Pearson's Correlation was used to correlate width of rosette to leaf number and fresh and dry masses for years 1999-2002. The correlation between width of rosette and leaf number was found to be significant at the 0.01 level with N=165 and the Pearson Correlation = 0.400.

Seedling Establishment and Plant Densities

Seedling densities fluctuated in different years. Table 2.9 shows that the density of seedlings was higher at the NCLB than other colonies in every year and sampling time. Seedling were the most numerous at the NCLB in June 2007 with 10.8 seedlings /m^2, at the NCUB in June 2008 with 1.3 seedlings /m^2, and at the South Colony in June of 2000 with 1.2 seedling /m^2. The NCLB had the greatest number of vegetative plants at all sampling times (Table 2.10). For all three colonies, vegetative plants were the highest in June except in 2008 when vegetative plants were highest in April at the NCLB and equal in April and June at the NCUB. Reproductive plants were highest at the NCLB except in June 2000 when the SC had the greatest number of reproductive plants. The NCLB overall has the largest population with the greatest number of *Physaria ludoviciana* plants per m^2. Figures 2.7, 2.8 and 2.9 show great variability in numbers of seedling, vegetative and reproductive plants at different sampling times for the three colonies. Seedlings often were found in close proximity of a reproductive plant (Figure 2.10).

Discussion

Seed Production

Seed production was variable from year to year, with plants producing from 22 to 744 seeds per plant. In July 2001, when *Physaria ludoviciana* plants were surveyed, most of the fruits had already fallen from the plant, giving a lower number of fruits/stalk and seeds/plant compared to estimates taken in June. The variability in seed production in different years could be due to environmental conditions. Production of seeds was high, which is not uncommon in this genus. *Physaria* (as *Lesquerella*) *gordonii*, a spring flowering desert annual plant, produces between 14.8 and 28.8 fruits per plant and

averages between 3.35 and 6.99 seeds per fruit (Delph, 1986). *Physaria* (as *Lesquerella*) f*endleri* can produce more seeds than *P. ludoviciana,* with one to several hundred fruits per plant and each fruit containing one to 30 seeds (Cabin *et al.*, 1998). In *Physaria* (as *Lesquerella*) *arctica,* a typical high Arctic perennial herb, flowers per inflorescence are 3-8 and seeds per fruit are 6-8 (Aiken *et al.,* 2009). It was hypothesized by Delph (1986) that high fruit set is an adaptive response to unpredictable, variable resource levels and high herbivory risk. Holes in the fruits due to herbivory were fewer than two fruits per plant in populations of *P. ludoviciana* at the Henry Allan Gleason Nature Preserve. In some of the colonies, a large percentage of pedicels were missing flowers and fruits, indicating they were being removed by either naturally abscising or possibly being eaten (Claerbout *et al.*, 2007). Because seed production is in the range of other species in the genus, it does not appear to be a limiting factor for the survival of *P. ludoviciana.*

Seed Dispersal and Longevity in Seed Bank

Soil scrapes were collected while seeds were being shed from the plant. Scrapes were taken right next to a reproductive plant, as well as at 1 and 2 meters away. Most seeds did not disperse far from the mother plant. According to Cabin *et al.* (1998), the vast majority of uneaten seeds in *Physaria* (as *Lesquerella) fendleri* remain within 1 m of the parent. It is unlikely that *Physaria ludoviciana* is dispersing its seeds farther than 2 m because there are no apparent structures to facilitate widespread seed dispersal (Claerbout, 2003; Coons *et al.*, 2000). Even though the Illinois River is near HAGNP, *P. ludoviciana* seeds do not appear to be using water as their dispersing mechanism, since no populations exist directly along the river. If seeds had mechanisms to disperse further, they would appear in the compatible habitat present around these populations. Not

having the ability to disperse seeds except through wind dispersal along the ground, could be a limiting factor. *Physaria* (as *Lesquerella) arctica* is only able to disperse its seeds a short distance due to lack of dispersal adaptations (Marchand and Roach, 1980). According to Chen and Maun (1999), the large losses of seed that occur during dispersal could be because of herbivory, emigration to unsuitable habitats, and soil factors. Having no way to disperse broadly its seeds could be a limiting factor for the survival of *P. ludoviciana.*

Soil scrapes were collected while seeds were being shed from the plant (June) and after shedding of seeds (November). Scrapes were taken immediately adjacent to a reproductive plant. Only 4% of *Physaria ludoviciana* seeds in June were still found in November. Other studies done on seed bank production that compare the amount of seed production with the density of subsequent seed banks also found the great majority of new seeds fail to persist in the soil (Rabinowitz, 1981). In *Physaria* (as *Lesquerella*) *fendleri,* desert granivores such as ants and rodents can affect the population by eating the seeds (Cabin and Marshall, 2000). In sand desert systems, granivores are highly effective at finding and depleting relatively dense soil seed patches, and predator populations can rapidly increase following periods of high seed production (Cabin and Marshall, 2000).

Very few *Physaria ludoviciana* seeds remained in the seed bank. Seed production was high, but not all of the seeds emerged, and most likely seeds are not dispersing to new areas because of the limited dispersal. Since seeds are not persisting in the seed bank, seeds may fall victim to herbivory by rodents or insects. Seed is produced in early June, when there may not be many other food options, so *P. ludoviciana* seeds may be

the only food source for these predators. Having few seeds remaining in the seed bank is a limiting factor for the long-term survival of *P. ludoviciana.*

Afterripening

Physaria ludoviciana seeds needed an afterripening period, as not all seeds were ready to germinate at the same time. Tetrazolium which tests for metabolic activity, (Hyatt *et al.,* 1999; Lakon, 1948) showed that a high percentage of the seeds are alive, and germination tests from seeds that were started soon after harvest indicated that if seeds are viable they eventually germinate. Physiological dormancies are well documented in annual and perennial species (Baskin and Baskin, 1998). Dormancy is likely to be adaptive because it allows seeds to synchronize their germination with environmental conditions that favor their growth and reproduction, while reducing the chance of germinating at unfavorable times during the year (Hyatt *et al.*, 1999). *Physaria ludoviciana* could potentially benefit from this same dormancy strategy. Timing of germination is important to *P. ludoviciana,* especially because it establishes itself in areas that experience four seasons. If seeds are mature in late summer followed by a mild fall with high temperatures and rainfall, seedlings might start to emerge only to die with the first frost. When *P. ludoviciana* populations were visited in the winter, the plants were evergreen (Over *et al.*, 2005). If seedlings can establish quick enough, they will survive the winter, but otherwise fall establishing seedlings will die. Dormancy cycles like *P. ludoviciana*'s afterripening and *Physaria* (as *Lesquerella*) *fendleri's* dormancy-nondormancy cycles change over time (Hyatt *et al.*, 1999). Germination responses to light, temperature and moisture change over time (Baskin and Baskin, 1977; Hyatt *et al.*, 1999). As dormancy decreases, more germination occurs, but it can occur at an increased

range of temperature and light conditions (Hyatt *et al.*, 1999). It is possible that the quickly maturing seeds are getting environmental cues, but in order to test this theory with *P. ludoviciana* seeds, they would need to be tested in a range of temperature and light conditions over time. Dormancy is common in this genus, especially when they grow in unpredictable environments like deserts or sand prairies (Hyatt *et al.*, 1999). There is a selective advantage for *P. ludoviciana* to have seeds that mature and germinate at different times, so having an afterripening period would be beneficial in this species.

Longevity in Storage-Seed Germination

Seed vigor varied with collection dates and position on the flower stalk. The maturity of the seeds, as indicated by position on the flower stalk and the collection date, affects how well the seeds will germinate after storage. In early June, seeds on the lower portion of the flower stalk mature before seeds on the upper portion of the flower stalk. Later in the seed production season (mid-June), seeds are only left on the upper portion of the stalk (Claerbout, 2003) as seed on the lower part of the stalk has been shed. Effects of seed maturity are evident when looking at seeds from 1999. Seeds collected from the lower portion of the flower stalk had a higher germination rate than seeds collected from the upper portion of the flower stalk in early June. However, high germination occurred in seeds from the upper portion of the stalk when collected in mid-June. This response is probably because seeds from the first collection date were not as mature in the upper portions of the flower stalk (Beach *et al.*, 2001a). A similar pattern was observed in seeds collected in 2000, except in trials in November of 2000 and 2001, when the upper stalk seed germination is either equal to the later harvest seed or better. In 2001, only one collection date occurred in early June. At that time in the growing season, seeds from the

lower portion of the flower stalk were more mature than the upper portion of the flower stalk, which is evident from germination tests. Seeds on the upper flower stalk are closer to the apical meristem and therefore less mature than those seeds further from the apex (on the lower stalk). This response might account for the high germination in lower stalk seed because it was able to mature on the mother plant for a longer period of time. Low maturity in 2002 seed might reflect the maturity of the seed collected and not the seasonal differences since both mature and immature seed were mixed. Differences in germination between seasons were most likely related to collection date and maturity.

Yearly differences in germination might be due to seasonal variations in rainfall, light, and temperature as all are limiting factors in plant development, and could have an effect on fruit and seed development (Claerbout, 2003). Environmental stresses, such as low moisture or high temperature might negatively affect seed development. There is evidence that *Physaria ludoviciana* is most likely cross-pollinated by insects, and insects would be most active on sunny days during peak flowering in the months of May and June (Claerbout, 2003; Claerbout *et al.*, 2007). The activity of herbivorous insects that might feed on the fruit and seeds of *P. ludoviciana* might also be at its peak during these months (Claerbout, 2003).

The longevity of P*hysaria ludoviciana* seeds can be very beneficial to its survival. Seeds harvested in late June remained viable for up to 6.5 years. Several factors can affect germination, including years in storage, harvest date, afterripening and seed maturity. The longevity of *P. ludoviciana* seeds is beneficial to its survival, especially when seed is collected and stored for possible restoration efforts if seeds in the seed bank are lost and seedling establishment is low.

Emergence of Seedlings from Stored Seed

Percent emergence of *Physaria ludoviciana* varied greatly between 1999 and 2002. All seeds were taken from the last collection day of the year and from the upper portion of the flower stalk, with the exception of the 2002 seed, which only had one early collection date containing mixed mature and immature seeds. Seed from the upper stalk would have been able to mature on the mother plant for a longer period of time. Seed maturity on the stalk when collected had an effect on the emergence percentage, as seeds harvested when mature had greater vigor as expressed by emergence.

The collection date affected seedling emergence. In the beginning of the growing season, seed on the lower portion of the flower stalk is mature while seed on the upper portion of the flower stalk is not mature. Later in the growing season only seeds left on the upper portion of the stalk are mature (Claebout, 2003), which is because the lower stalk seed was mature and had already shed from the plant so it is not available to collect. Since seed from 1999 and 2000 were collected in late June (when the seeds were mature) higher emergence rates were observed. In 2001, seeds were collected in early June and were not quite as mature, which can account for the lower emergence percentage. Seeds from 2002 had the lowest emergence percentage, not only because they were collected very early in June, but also because it was a mix of both mature and immature seed.

Mature seeds collected in late June from the upper portion of the stalk showed the highest emergence but it is unknown how long after harvest emergence declines. As long as seed is mature when harvesting, storage does not have an effect on seed longevity at least for 6.5 years, which is potentially advantageous to the survival of *Physaria ludoviciana*.

Seedling Establishment and Plant Densities

Plant densities varied throughout the sampled colonies. The NCLB had the highest number of seedlings as well as vegetative and reproductive plants, which seems to indicate high population viability. However, the establishment of seedlings was variable in these populations. With the high percentage of seed produced, higher seedling densities would be expected, but with no mechanism for dispersal, few seeds in the seed bank, and a necessary afterripening period, seedling establishment was low and unpredictable. It is evident that a number of seeds are not geminating given that if the number of reproductive plants per m^2 is multiplied by the number of seeds produced per plant, there is an estimated 479 to 1340 seeds per m^2. A number of factors can account for the low seedling numbers. *Physaria* (as *Lesquerella*) *fendleri* had seedlings in the field in both the spring and the fall, depending on the amount and timing of rainfall (Cabin *et al.*, 1998; Hyatt *et al.*, 1999). *Physaria ludoviciana* seedlings were observed in the field in the fall at densities of 1.3 ± 0.7 per m^2 (Appendix B). Seedling emergence in the fall may be a problem because plants that are not established probably die during the winter due to limitations in water and low temperature. Although more mature plants are evergreen, so it is not for sure whether plants are establishing and dying off or not establishing at all. If a lot of seeds have broken dormancy in the fall and are germinating and subsequently dying, that could account for a significant number of seeds that have been removed from the seed bank and essentially "wasted." Another possibility is that seeds and seedlings are getting buried and killed in the blowing sand of the sand prairie. According to Li *et al.* (2006), many factors can influence seeds not germinating. The optimal depth for a seed could be affected by its mass, as larger seeds often have higher

germination rates than smaller seeds at deeper depths. If large seeds get buried deep, they have more resources to survive until they emerge. Burial of seeds by blowing sand is critical for the establishment of many species, but blowing sand might bury and kill small seeds like *P. ludoviciana*, which could explain why so few *P. ludoviciana* seeds are found in the seed bank. Claerbout (2003) found no *P. ludoviciana* seeds in soil cores collected at a depth of 10 cm. The time it takes for a seed to emerge often determines whether or not the plant can complete its life cycle, because if it gets buried too deep it will have a shorter growing season. Li *et al.* (2006) also noted that seed burial is very beneficial to the plant because exposure to air is reduced. In addition to wind, seeds could get buried over the winter by the freezing and thawing of the ground (Chen and Maun, 1999). In studies done by Martinez and Moreno-Casasola (1993) on a sand dune legume, one of the limiting factors for seedling establishment was sand movement. Smaller seedlings were the most susceptible to being buried or desiccated by sand. Sand burial can be beneficial because it provides high humidity as well as protection from high and low temperature and predators (Li *et al.*, 2006).

It is likely that a combination of factors is causing low seedling establishment numbers at HAGNP. In a sand dune system, survivorship of seedlings is affected by a number of physical and biotic factors such as predation, disease, desiccation, competition, salt spray, nutrient deficiency, high soil surface temperatures and sand movement (Martinez and Moreno-Casasola, 1993). At HAGNP, if seeds and seedlings were not buried too deep or fall victim to predators, seedling densities might be higher.

Summary

Many factors can affect the seed biology and survival of P*hysaria ludoviciana.* Seed production, afterripening, and variable establishment can be considered beneficial to *P. ludoviciana.* No mechanisms for dispersal and few seeds in the seed bank (possibly because of herbivory), can be considered potential detriments to survival of *P. ludoviciana. Physaria ludoviciana* produces a lot of seeds that seem to be unable to disperse. Afterripening is beneficial, but seeds germinating at times that are not favorable and seeds not persisting are limiting the populations at HAGNP. Seed longevity and vigor in stored seed are good for restoration, as long as seeds are mature when collected. Seedling establishment depends on how many mature seeds are left in the seed bank when conditions are favorable for emergence. Everything seems to depend on persistence in the seed bank. With seeds being removed at a rapid rate, it is hard for *P. ludoviciana* to complete its life cycle, and factors that may benefit the seed biology of *P. ludoviciana* are useless if it cannot maintain a seed bank. For land managers, my study indicates that to increase the density of *P. ludoviciana,* seeds should be collected in late June, stored over the winter, and scattered in the spring. Future studies on *P. ludoviciana* should identify causal agents of seed herbivory and its timing.

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Table 2.1. Estimated seed production using counts of reproductive structures of *Physaria ludoviciana* in 3 colonies at Henry Allan Gleason Nature Preserve, Mason County, Illinois in different months from 1999-2008.

Year	Date	Site	Flower Stalks/Plant	Fruits/Stalk	Pedicels/Stalk	Seeds/Fruit[a]	Estimated Seeds/Plant (Based on Fruit)	Estimated Seeds/Plant (Based on Pedicels)
1999	8 June	NCLB[b]	6.9 ± 0.6 [e]	12.8 ± 1.2	ND[f]	2.5 ± 0.4	221	ND
1999	22 June	NCLB	5.9 ± 0.4	14.6 ± 1.2	ND	2.9 ± 0.2*	249	ND
2000	1 June	NCLB	3.2	14.4	ND	2.9 ± 0.2*	134	ND
2000	**1 June**	**NCUB[c]**	**2.1**	**11.6**	**ND**	**2.9 ± 0.2***	**71**	**ND**
2000	*1 June*	*SC[d]*	*4.0*	*10.6*	*ND*	*2.9 ± 0.2**	*122*	*ND*
2001	14 May	NCLB	4.7 ± 0.4	19.8 ± 1.4	ND	2.9 ± 0.2*	270	ND
2001	**14 May**	**NCUB**	**2.4 ± 0.2**	**16.6 ± 1.0**	**ND**	**2.9 ± 0.2***	**116**	**ND**
2001	*14 May*	*SC*	*4.0 ± 0.3*	*18.3 ± 1.1*	*ND*	*2.9 ± 0.2**	*212*	*ND*
2001	22 June	NCLB	5.1 ± 0.4	11.4 ± 0.7	ND	2.9 ± 0.2*	168	ND
2001	**22 June**	**NCUB**	**2.2 ± 0.2**	**11.5 ± 1.1**	**ND**	**2.9 ± 0.2***	**73**	**ND**
2001	*22 June*	*SC*	*3.7 ± 0.3*	*14.0 ± 1.2*	*ND*	*2.9 ± 0.2**	*150*	*ND*
2001	24 July	NCLB	4.9 ± 0.6	1.5 ± 0.2	ND	2.9 ± 0.2*	22	ND
2002	3 June	NCLB	6.2 ± 0.6	23.7 ± 1.7	30.9 ± 2.1	3.4 ± 0.5	500	651
2007	4 June	NCLB	6.2 ± 0.4	22.8 ± 1.1	30.9 ± 1.4	2.5 ± 0.3	353	479
2008	26 June	NCLB	6.7 ± 0.6	12.4 ± 1.3	34.7 ± 2.3	3.2 ± 0.1	266	744
2008	**26 June**	**NCUB**	**5.3 ± 0.6**	**9.4 ± 1.6**	**29.7 ± 2.8**	**3.1 ± 0.2**	**154**	**488**
2008	*26 June*	*SC*	*4.3 ± 0.5*	*14.0 ± 2.2*	*31.1 ± 3.0*	*3.1 ± 0.2*	*187*	*415*
Averages							192	555

[a] seeds per fruit is the average of 25 fruits; for years with *, fruits were not collected, so average calculated from other years

[b] North Colony Lower Bowl at the Henry Allan Gleason Nature Preserve in Mason County, Illinois

[c] **North Colony Upper Bowl at the Henry Allan Gleason Nature Preserve in Mason County, Illinois**

[d] *South Colony at the Henry Allan Gleason Nature Preserve in Mason County, Illinois*

[e] means ± standard errors

[f] ND=No data

Table 2.2. Height of flower stalks, fruits with holes per stalk and percentage of fruits with holes per stalk for *Physaria ludoviciana* in 3 colonies at Henry Allan Gleason Nature Preserve, Mason County, Illinois from 2002-2008.

Year	Date	Site	Height (cm) [d]	Fruits with Holes/Stalk	% of Fruits with Holes/Stalk
2002	3 June	NCLB[a]	19.6 ± 0.9 [e]	1.8 ± 0.3	8.0
2007	4 June	NCLB	17.5 ± 0.5	0.2 ± 0.1	0.8
2008	26 June	NCLB	22.7 ± 0.8	0.3 ± 0.1	2.4
2008	**26 June**	**NCUB[b]**	**22.7 ± 0.9**	**0.3 ± 0.1**	**3.2**
2008	*26 June*	*SC[c]*	*22.7 ± 1.0*	*0.2 ± .1*	*1.4*

[a] North Colony Lower Bowl at the Henry Allan Gleason Nature Preserve in Mason County, Illinois

[b] **North Colony Upper Bowl at the Henry Allan Gleason Nature Preserve in Mason County, Illinois**

[c] *South Colony at the Henry Allan Gleason Nature Preserve in Mason County, Illinois*

[d] Height of tallest flower stalk per plant

[e] means ± standard errors

Table 2.3. Dispersal and longevity of *Physaria ludoviciana* seeds in the seed bank when soil scrapes collected at the Henry Allan Gleason Nature Preserve, Mason County, Illinois on 26 June and 4 November 2008.

Meters From Plant	Seeds/m^2	
	June	November
0	380 ± 158 a[ab]	15 ± 15 b
1	10 ± 10 b	ND[c]
2	20 ± 12 b	ND

[a] means ± standard errors

[b] means within a column or row followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level using arcsin transformed data

[c] ND=No data

Table 2.4. Percentage of seeds (lower stalk) of *Physaria ludoviciana* at 48 days, 6 months or later that germinated when imbibed 0-10 months after harvest on 4 June 2007 or were not germinated and tested positive or negative for viability with tetrazolium.

Month After Harvest	48 Days[a]	6 Months[b]	Final Count[c]	TZ positive[d]	TZ negative[e]
0	19.3 ± 5.7 c[f, g]	46.0 ± 8.7 d	78.7 ± 4.8 (17) c	1.3 ± 0.7 b	20.0 ± 4.2 a
2	42.0 ± 4.2 b	65.3 ± 7.1 c	97.3 ± 0.7 (15) a	0.0 ± 0.0 b	2.7 ± 0.7 c
4	30.7 ± 2.4 bc	46.7 ± 1.3 d	82.7 ± 5.3 (13) c	12.7 ± 3.7 a	4.6 ± 1.8 bc
6	61.3 ± 2.4 a	84.7 ± 3.5 ab	87.3 ± 3.7 (11) bc	0.0 ± 0.0 b	12.7 ± 3.7 ab
8	58.7 ± 8.7 a	76.0 ± 4.6 bc	88.7 ± 5.5 (9) abc	8.0 ± 6.1 ab	3.3 ± 2.4 c
10	73.3 ± 1.7 a	93.3 ± 0.7 a	94.7 ± 1.3 (7) ab	2.7 ± 1.3 b	2.6 ± 1.3 c

[a] 48 days after imbibition
[b] 6 months after imbibition
[c] final count-number of months after imbibition are in parentheses
[d] cotyledon and/or embryo turned pink after 24 hours in tetrazolium
[e] cotyledon or embryo did not turn pink after 24 hours in tetrazolium
[f] means ± standard errors
[g] means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level using arcsin transformed data

Table 2.5. Percentage of seeds (upper stalk) of *Physaria ludoviciana* that germinated at 48 days, 6 months or later when imbibion began 0-10 months after harvest on 4 June 2007 or were not germinated and tested positive or negative for viability with tetrazolium.

Month After Harvest	48 Days[a]	6 Months[b]	Final Count[c]	TZ positive[d]	TZ negative[e]
0	40.0 ± 9.5 d[f, g]	66.7 ± 4.1 cd	92.0 ± 3.1 (17) a	0.0 ± 0.0 b	8.0 ± 3.1 a
2	77.3 ± 5.9 a	86.7 ± 0.7 a	94.7 ± 1.3 (15) a	0.7 ± 0.7 b	4.6 ± 0.7 a
4	58.7 ± 1.8 bc	76.0 ± 2.3 bc	88.7 ± 3.5 (13) a	2.0 ± 2.0 b	9.3 ± 1.8 a
6	77.3 ± 4.4 a	88.0 ± 4.0 a	95.3 ± 1.8 (11) a	0.0 ± 0.0 b	4.7 ± 1.8 a
8	74.7 ± 2.4 ab	85.3 ± 1.3 ab	88.5 ± 1.2 (9) a	1.8 ± 1.3 b	9.7 ± 2.9 a
10	46.0 ± 1.2 cd	56.7 ± 3.5 d	68.0 ± 5.0 (7) b	18.0 ± 8.7 a	14.0 ± 6.1 a

[a] 48 days after imbibition
[b] 6 months after imbibition
[c] final count-number of months after imbibition are in parentheses
[d] cotyledon and/or embryo turned pink after 24 hours in tetrazolium
[e] cotyledon or embryo did not turn pink after 24 hours in tetrazolium
[f] means ± standard errors
[g] means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level using arcsin transformed data

Table 2.6. Percentage of seeds (mixed stalk) of *Physaria ludoviciana* that germinated at 48 days, 6 months or later when imbibion began 0-10 months after harvest on 18 June 2007 or were not germinated and tested positive or negative for viability with tetrazolium.

Month After Harvest	48 Days[a]	6 Months[b]	Final Count[c]	TZ positive[d]	TZ negative[e]
0	8.6 ± 4.1 c[f, g]	22.0 ± 5.8 c	86.7 ± 3.3 (17) ab	7.3 ± 5.5 abc	6.0 ± 2.3 a
2	17.3 ± 1.3 b	32.0 ± 8.0 c	89.3 ± 5.2 (15) ab	0.7 ± 0.7 c	10.0 ± 5.3 a
4	22.0 ± 2.3 b	54.7 ± 1.3 b	76.7 ± 7.4 (13) b	18.7 ± 9.8 a	4.6 ± 2.4 a
6	53.3 ± 2.9 a	82.7 ± 4.8 a	94.0 ± 1.2 (11) a	4.0 ± 1.2 abc	2.0 ± 1.2 a
8	26.0 ± 2.0 b	52.7 ± 4.7 b	74.5 ± 7.2 (9) b	13.8 ± 4.7 ab	11.7 ± 2.7 a
10	52.0 ± 5.0 a	80.7 ± 1.8 a	87.3 ± 1.8 (7) ab	2.3 ± 2.0 bc	10.4 ± 2.3 a

[a] 48 days after imbibition
[b] 6 months after imbibition
[c] final count-number of months after imbibition are in parentheses
[d] cotyledon and/or embryo turned pink after 24 hours in tetrazolium
[e] cotyledon or embryo did not turn pink after 24 hours in tetrazolium
[f] means ± standard errors
[g] means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level using arcsin transformed data

Table 2.7. Percent germination at 2 weeks after imbibition of *Physaria ludoviciana* seeds of different maturity collected in 1999, 2000, 2001 and 2002 when germinated in 2000, 2001, 2002, 2003 and 2006.

Year	Germinated Date	Harvest 6-8-99 Lower Stalk	Harvest 6-8-99 Upper Stalk	Harvest 6-22-99 Upper Stalk	Harvest 6-1-00 Lower Stalk	Harvest 6-1-00 Upper Stalk	Harvest 6-16-00 Upper Stalk	Harvest 6-6-01 Lower Stalk	Harvest 6-6-01 Upper Stalk	Harvest 6-3-02 Mixed Stalk
2000	11 April	62.0 ± 5.8 a[a, b]	20.0 ± 4.5 a	70.0 ± 6.3 abc	ND[c]	ND	ND	ND	ND	ND
2000	7 November	50.0 ± 5.5 ab	22.0 ± 8.0 a	74.0 ± 8.1 a	50.0 ± 8.9 a	34.0 ± 6.8 a	34.0 ± 9.3 b	ND	ND	ND
2001	11 November	56.0 ± 10.3 a	10.0 ± 4.5 a	72.0 ± 4.9 ab	18.0 ± 4.9 b	36.0 ± 2.4 a	30.0 ± 5.5 b	ND	ND	ND
2002	19 February	46.0 ± 6.8 ab	16.0 ± 5.1 a	50.0 ± 5.5 bc	32.0 ± 5.8 ab	28.0 ± 5.8 a	48.0 ± 8.6 ab	70.0 ± 10.5 a	64.0 ± 6.8 a	16.0 ± 6.8 b
2003	20 February	46.0 ± 6.8 ab	12.0 ± 5.8 a	48.0 ± 5.8 c	30.0 ± 5.5 ab	24.0 ± 4.0 a	40.0 ± 7.7 b	66.0 ± 12.9 a	60.0 ± 4.5 a	16.0 ± 6.8 b
2006	19 January	29.5 ± 1.7 b	21.0 ± 4.7 a	61.5 ± 2.1 abc	31.5 ± 9.0 ab	38.5 ± 2.6 a	70.0 ± 5.2 a	72.0 ± 5.2 a	65.5 ± 1.5 a	42.0 ± 3.4 a

[a] means within a column followed by different letters are significantly different using Duncan's multiple range test at p=0.05 level using arcsin transformed data

[b] means ± standard errors

[c] ND=no data

Table 2.8. Percent emergence, width of rosettes, number of leaves, and fresh and dry masses of *Physaria ludoviciana* plants when harvested after 4 weeks. Seed was collected in 1999, 2000, 2001 and 2002 then planted in 2006.

Seed Lots	Emergence (%)	Width (cm)	# of Leaves	Fresh Mass (g)	Dry Mass (g)
22 June 1999 upper stalk	85.5 ± 3.5 a[a,b]	3.3 ± 0.2 a	5.8 ± 0.2 a	0.86 ± 0.03 a	0.148 ± 0.009 a
16 June 2000 upper stalk	62.7 ± 5.8 b	3.3 ± 0.3 a	5.5 ± 0.3 a	0.52 ± 0.03 b	0.093 ± 0.004 b
6 June 2001 upper stalk	57.3 ± 1.3 b	2.7 ± 0.3 b	5.4 ± 0.3 a	0.43 ± 0.03 b	0.071 ± 0.007 c
3 June 2002 mixed stalk	18.7 ± 2.7 c	2.1 ± 0.3 c	5.5 ± 0.3 a	0.10 ± 0.01 c	0.013 ± 0.002 d

[a] means within a column followed by different letters are significantly different based upon Duncan's multiple range test at p=0.05 level

[b] means ± standard errors

Table 2.9. Number of seedlings/m^2 for *Physaria ludoviciana* in 3 colonies at the Henry Allan Gleason Nature Preserve, Mason County, Illinois.

Year	Date	NCLB[a]	NCUB[b]	SC[c]
2000	1 June	3.1 ± 7.1[d](36%)[e]	0.5 ± 2.5 (28%)	1.2 ± 3.0 (25%)
2001	14 May	1.1 ± 0.5 (18%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)
2001	2 June	1.1 ± 0.4 (16%)	0.5 ± 0.4 (24%)	0.0 ± 0.0 (0%)
2001	24 July	1.2 ± 0.4 (25%)	0.1 ± 0.1 (20%)	0.0 ± 0.0 (0%)
2002	6 May	0.2 ± 0.1 (3%)	ND[f]	ND
2002	3 June	0.5 ± 0.3 (8%)	ND	ND
2007	4 June	10.8 ± 4.7 (79%)	ND	ND
2008	30 April	6.8 ± 1.7 (66%)	0.3 ± 0.2 (28%)	0.0 ± 0.0 (0%)
2008	26 June	2.5 ± 1.5 (45%)	1.3 ± 0.8 (48%)	0.0 ± 0.0 (0%)

[a] North Colony Lower Bowl
[b] North Colony Upper Bowl
[c] South Colony
[d] Means ± standard errors
[e] Numbers in parentheses indicate percent of seedlings relative to total number of plants at all developmental stages
[f] ND=no data

Table 2.10. Number of vegetative plants/m^2 of *Physaria ludoviciana* in 3 colonies at the Henry Allan Gleason Nature Preserve, Mason County, Illinois.

Year	Date	NCLB[a]	NCUB[b]	SC[c]
2000	1 June	4.4 ± 8.0[d] (51%)[e]	1.2 ± 3.7 (67%)	2.2 ± 3.6 (46%)
2001	14 May	3.2 ± 0.7 (53%)	0.1 ± 0.1 (14%)	0.4 ± 0.2 (29%)
2001	2 June	3.8 ± 0.8 (54%)	1.3 ± 0.6 (62%)	1.4 ± 0.4 (56%)
2001	24 July	2.8 ± 0.6 (58%)	0.4 ± 0.4 (80%)	0.6 ± 0.3 (46%)
2002	6 May	1.0 ± 0.5 (17%)	ND[f]	ND
2002	3 June	1.5 ± 0.4 (25%)	ND	ND
2007	4 June	0.9 ± 0.5 (6%)	ND	ND
2008	30 April	1.4 ± 0.5 (14%)	0.4 ± 0.3 (36%)	0.0 ± 0.0 (0%)
2008	26 June	1.3 ± 0.4 (23%)	0.4 ± 0.4 (15%)	0.4 ± 0.2 (44%)

[a] North Colony Lower Bowl
[b] North Colony Upper Bowl
[c] South Colony
[d] Means ± standard errors
[e] Numbers in parentheses indicate percent of vegetative plants relative to total number of plants at all developmental stages
[f] ND=no data

Table 2.11. Number of reproductive plants/m^2 of *Physaria ludoviciana* in 3 colonies at the Henry Allan Gleason Nature Preserve, Mason County, Illinois.

Year	Date	NCLB[a]	NCUB[b]	SC[c]
2000	1 June	1.1 ± 2.3[d] (13%)[e]	0.1 ± 0.6 (5%)	1.4 ± 2.6 (29%)
2001	14 May	1.7 ± 0.5 (29%)	0.6 ± 0.4 (86%)	1.0 ± 0.4 (71%)
2001	2 June	2.1 ± 0.5 (30%)	0.3 ± 0.2 (14%)	1.1 ± 0.3 (44%)
2001	24 July	0.8 ± 0.4 (17%)	0.0 ± 0.0 (0%)	0.7 ± 0.3 (54%)
2002	6 May	4.6 ± 1.4 (80%)	ND[f]	ND
2002	3 June	4.2 ± 0.9 (68%)	ND	ND
2007	4 June	1.9 ± 0.6 (15%)	ND	ND
2008	30 April	2.1 ± 0.7 (20%)	0.4 ± 0.3 (36%)	1.1 ± 0.5 (100%)
2008	26 June	1.8 ± 0.4 (32%)	1.0 ± 0.6 (37%)	0.5 ± 0.2 (56%)

[a] North Colony Lower Bowl
[b] North Colony Upper Bowl
[c] South Colony
[d] Means ± standard errors
[e] Numbers in parentheses indicate percent of reproductive plants relative to total number of plants at all developmental stages
[f] ND=no data

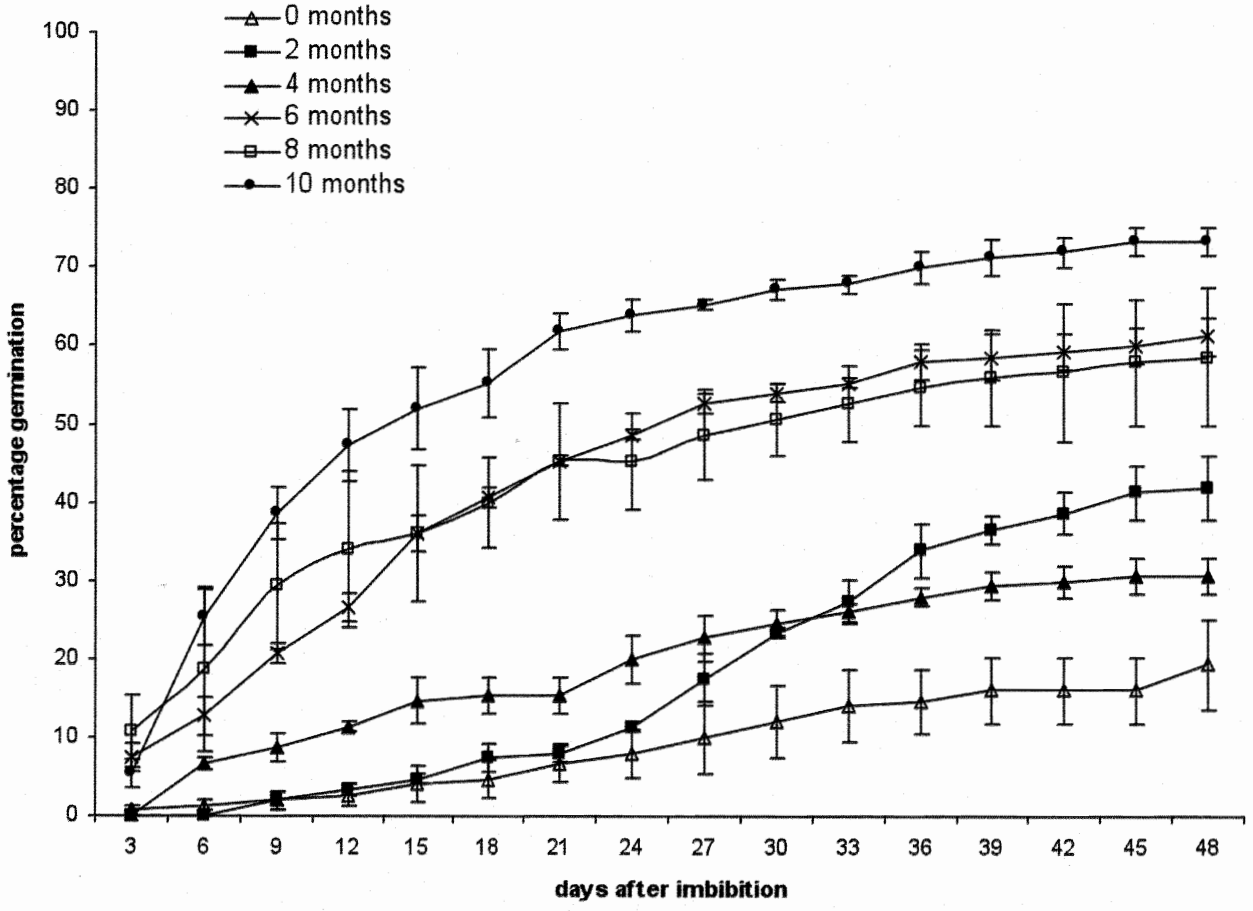

Figure 2.1. Cumulative germination (%) over 48 days for seed of *Physaria ludoviciana* from lower stalk when imbibition started 0-10 months after seed harvested on 4 June 2007. Means ± standard errors.

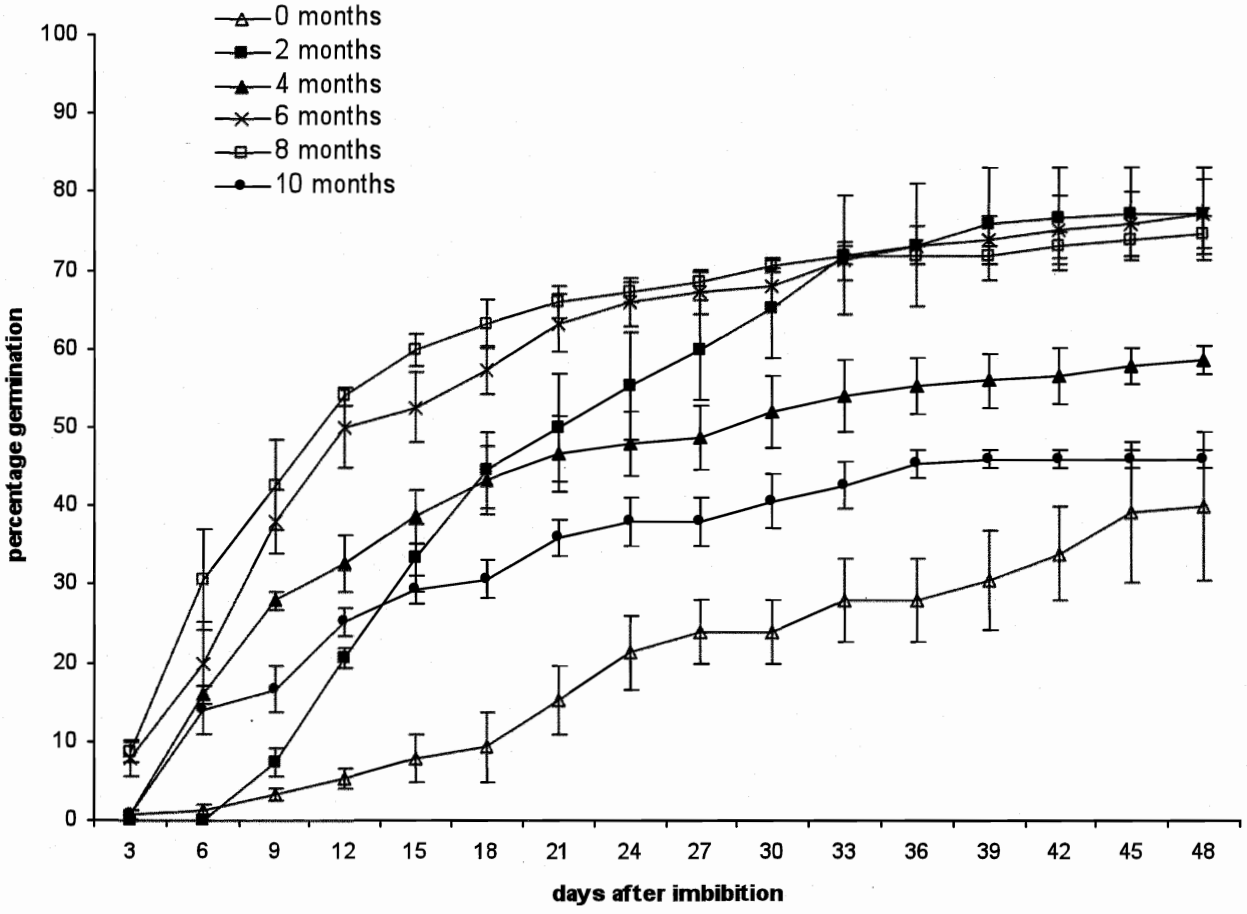

Figure 2.2. Cumulative germination (%) over 48 days for seed of *Physaria ludoviciana* from upper stalk when imbibition started 0-10 months after seed harvested on 4 June 2007. Means ± standard errors.

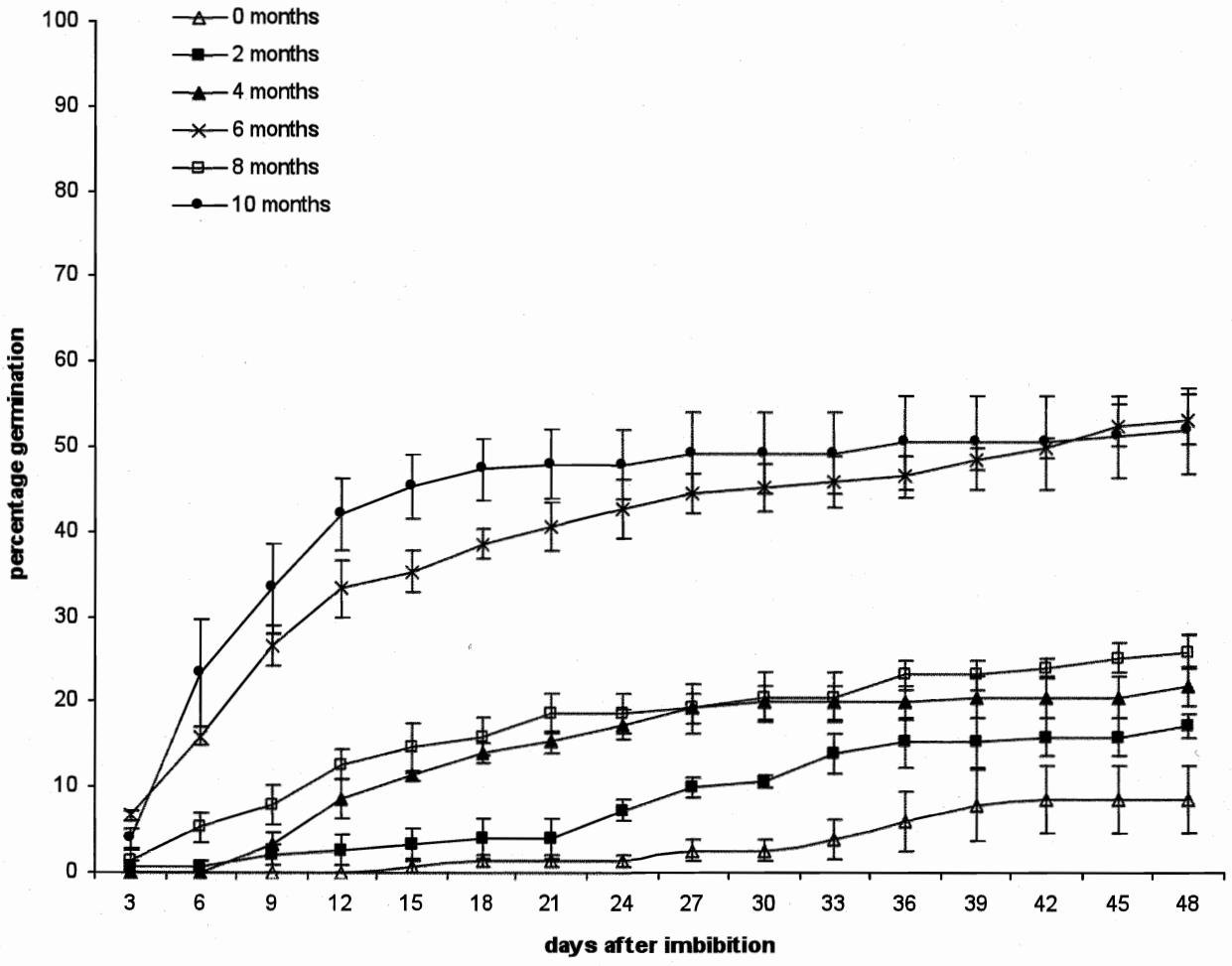

Figure 2.3. Cumulative germination (%) over 48 days for seed of *Physaria ludoviciana* from mixed stalk when imbibition started 0-10 months after seed harvested on 18 June 2007. Means ± standard errors.

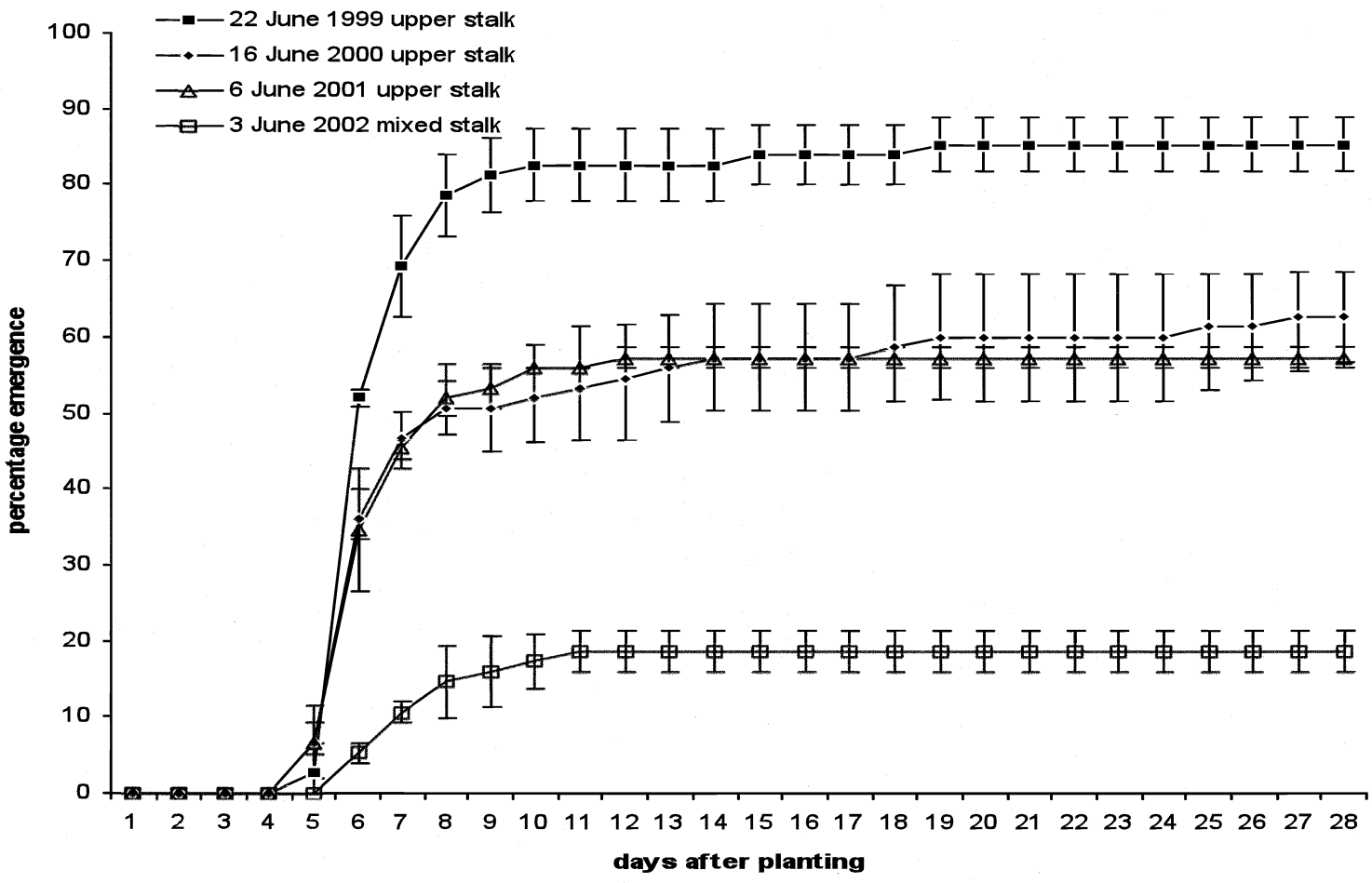

Figure 2.4. Cumulative emergence (%) of seedlings over 28 days for seed lots of *Physaria ludoviciana* collected in 1999-2002 and stored until planting in February 2006. Means ± standard errors.

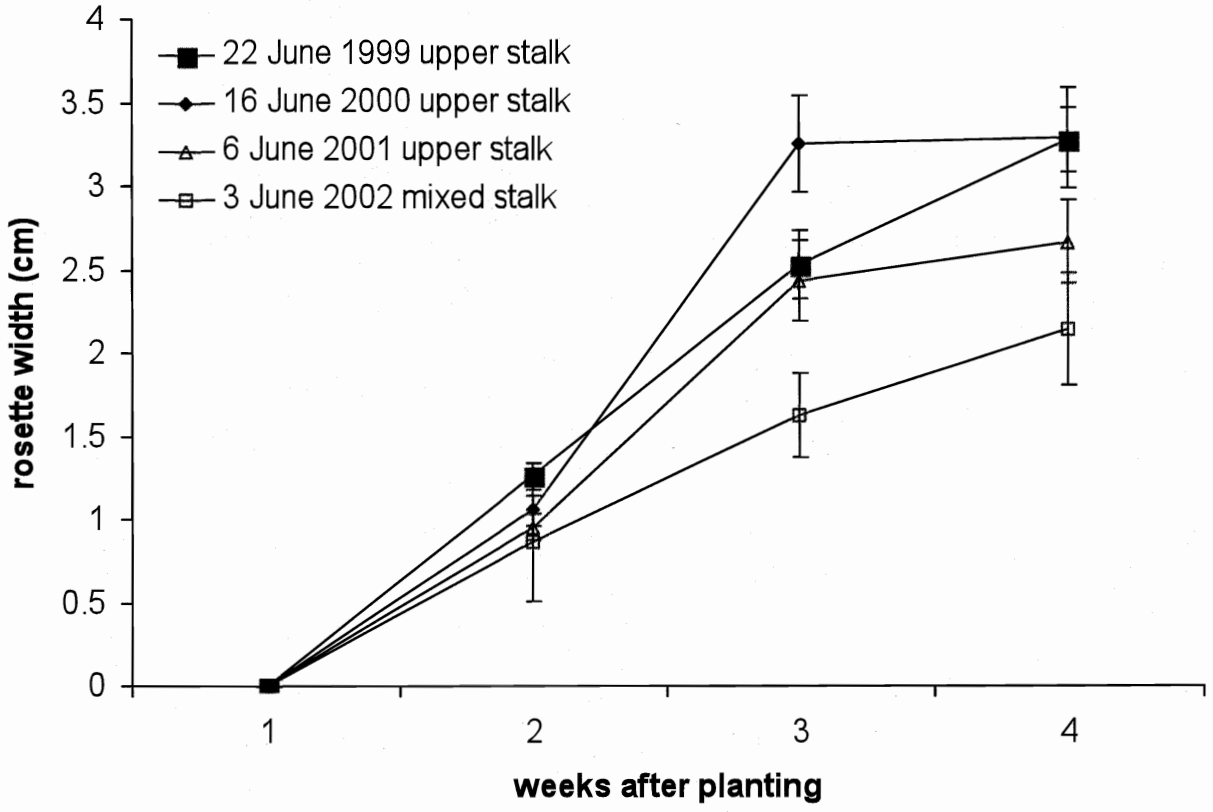

Figure 2.5. Rosette width (cm) for seedlings of *Physaria ludoviciana* over 4 weeks for seed lots collected in 1999-2002 and stored until planting in February 2006. Means ± standard errors.

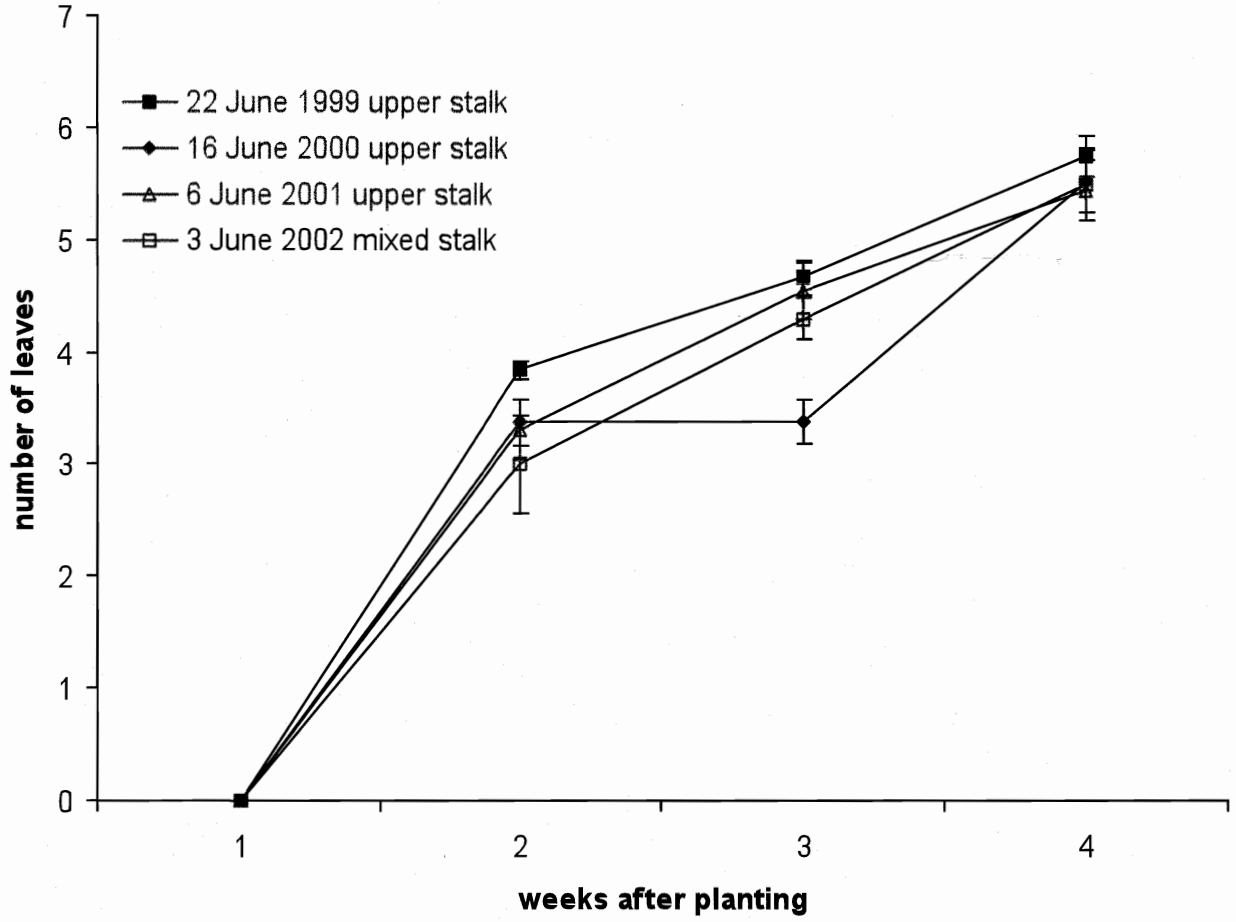

Figure 2.6. Number of leaves for seedlings of *Physaria ludoviciana* over 4 weeks for seed lots collected in 1999-2002 and stored until planting in February 2006. Means ± standard errors.

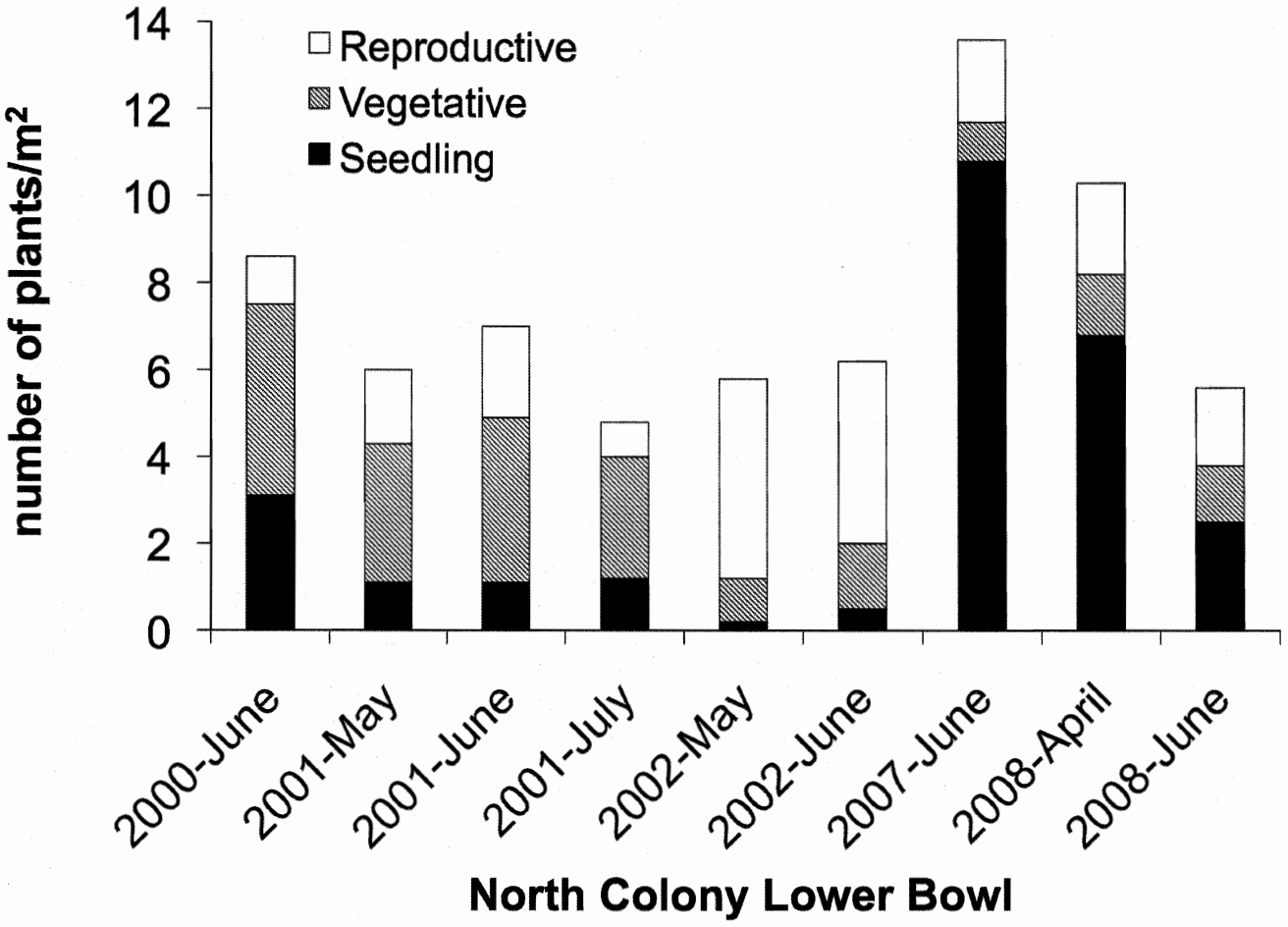

Figure 2.7. Density of *Physaria ludoviciana* plants in the North Colony Lower Bowl at the Henry Allan Gleason Nature Preserve, Mason County, Illinois at different developmental stages (seedling, vegetative, and reproductive) in different months from 2000-2008.

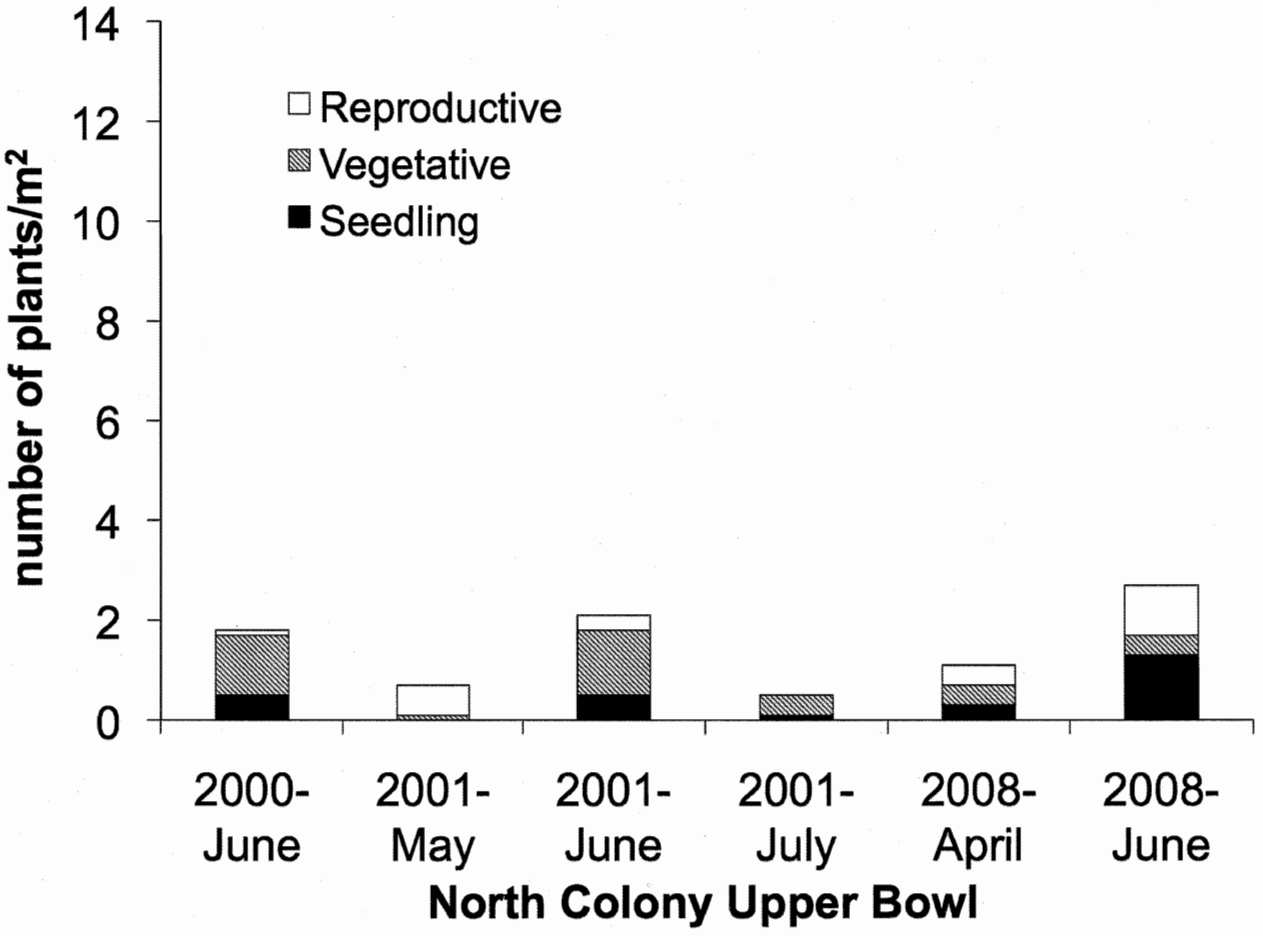

Figure 2.8. Density of *Physaria ludoviciana* plants in the North Colony Upper Bowl at the Henry Allan Gleason Nature Preserve, Mason County, Illinois at different developmental stages (seedling, vegetative, and reproductive) in different months from 2000-2008.

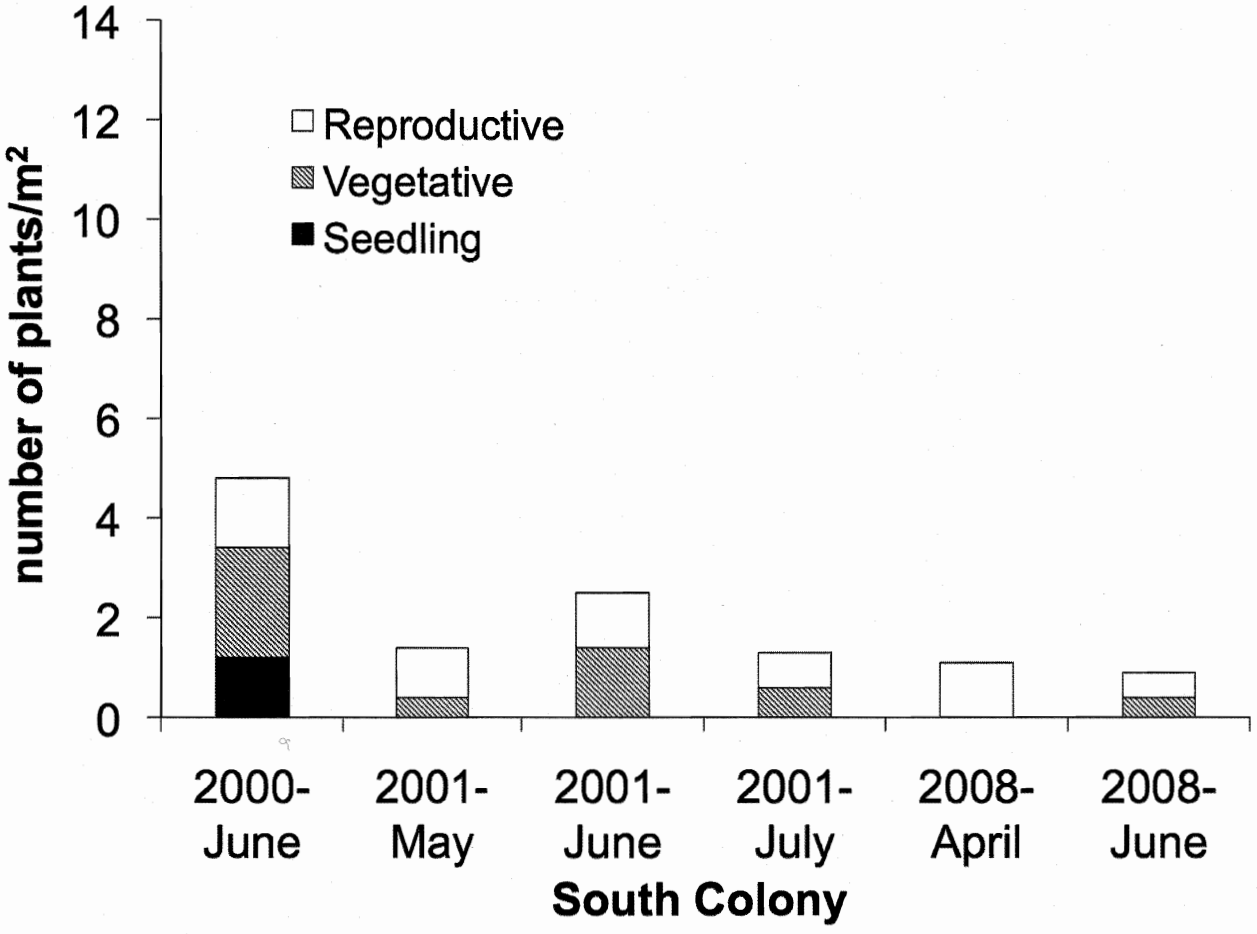

Figure 2.9. Density of *Physaria ludoviciana* plants in the South Colony at the Henry Allan Gleason Nature Preserve, Mason County, Illinois at different developmental stages (seedling, vegetative, and reproductive) in different months from 2000-2008.

Figure 2.10. Reproductive plants of *Physaria ludoviciana* within close proximity to seedling of *Physaria ludoviciana*.

Chapter 3

How Light and Root Competition Affect Development of *Physaria ludoviciana* (Silvery Bladderpod; Brassicaceae)

Abstract

Physaria ludoviciana (Nuttal) O'Kane & Al-Shehbaz (silvery bladderpod; Brassicaceae) is a state endangered species of Illinois, Minnesota, and Wisconsin sand prairies where in each state it is only reported in one county. *Physaria ludoviciana* is located in areas with low water holding capacity, frequent disturbances, full sunlight, and limited competition from other species. This environment, where few other species establish, appears to create an ideal niche for *P. ludoviciana.* If *P. ludoviciana* is an early succession plant, competition for light from increased canopy vegetation as well as competition below ground might hinder its growth, development, and ability to reproduce. Understanding environmental factors and competition that limit or restrict its growth and development will aid in proper management of this endangered species. Our objective was to investigate how light (duration and intensity) and root competition affect development of *P. ludoviciana*. Duration of light was studied using a long day (16 hr/8 hr light/dark) and a short day (8 hr/16 hr light/dark) photoperiod. Plants were started in a long day photoperiod growth chamber at 25°C, and after four months plants were placed in two chambers with either a long or a short day photoperiod. Weekly for 17 weeks, numbers of open flowers, numbers of inflorescences, and heights of inflorescence per plant were recorded. At harvest, numbers of leaves, numbers of leaf clusters (clusters of leaves where the leaf size is less than 4 cm long), leaf areas, and fresh and dry masses were recorded. For comparing intensity of light, plants were started from seed in Cone-tainers™ and placed in two growth chambers with a 16 hr/8 hr (light/dark) photoperiod at

25°C. Light intensity was either 584 ± 21 or 174 ± 2 μmol/m^2/sec. Development was quantified for four weeks by measuring width of plants, leaf area, fresh and dry masses, leaf numbers, and root development. Root competition for *P. ludoviciana* was simulated using five different container sizes. Plants were grown in Cone-tainers™ for five months, and then transplanted to the various container sizes. Plants were harvested at three, four and five weeks after transplanting, and various reproductive parameters (length of tallest stalk, fresh and dry mass of stalks, numbers of stalks, pedicels, flowers and fruits per plant) and vegetative parameters (crown diameter of rosette, fresh and dry mass of leaves, leaf area, number of leaves and root length per plant) were measured. In the photoperiod study, short day plants produced flowers first, but long day plants produced a greater number of flowers peaking at week six with 11.3 flowers per plant, whereas short day plants peaked at weeks two and eight with 6 flowers per plant. Inflorescences per plant increased for both photoperiods throughout the study, but more were produced in the long day plants (5.8 at week 15) compared to the short day plants (4.6 at week 13). Number of leaves, leaf area, and masses for plants harvested at 17 weeks were higher in long day than short day plants. *Physaria ludoviciana* flowered with both photoperiods, so they do not require short days to flower since flowers were likely initiated earlier while in long days. It is also a possibility that plants have no photoperiod requirement, and flowering just depends on the maturity of the plant. Plants grown at higher light intensity had significantly greater leaf areas, fresh and dry masses, leaf numbers and root development than those at lower light intensity. Hence decreased light intensity from competing vegetation would limit growth of *P. ludoviciana*. Container size affected both reproductive and vegetative parameters when plants were harvested at

four and five weeks, with the smallest container having the least growth. This response suggests that root competition in the field could affect plant growth and development. Both light and root competition greatly affected the development of *P. ludoviciana*, stressing the importance of little or minimal competition from other species for optimal growth of *P. ludoviciana*.

Introduction

Physaria ludoviciana formerly was known as *Lesquerella ludoviciana* until 2002 when Al-Shehbaz and O'Kane renamed species in *Lesquerella* into *Physaria* based on DNA sequencing. *Physaria ludoviciana* (Nuttal) O'Kane & Al-Shehbaz (silvery bladderpod; Brassicaceae) is listed as an endangered species in Illinois (Herkert and Ebinger, 2002; Illinois Department of Natural Resources, 2006), Minnesota (Minnesota Department of Natural Resources, 2007) and Wisconsin (Wisconsin Department of Natural Resources, 2004) where in each state *P. ludoviciana* is reported in only one county. However Claerbout (2003) reported it may no longer occur in Wisconsin. These three states represent the eastern most edge of its geographic range, although it occurs in scattered populations in other parts of the western United States (Rollins and Shaw, 1973; Claerbout, 2003; United States Department of Agriculture, Natural Resources Conservation Service, 2007).

Physaria ludoviciana is a perennial forb that forms a rosette of linear, basal leaves covered in thick trichomes, which gives a silvery appearance (Coons *et al.,* 2000; Beach *et al.,* 2001a, b). From April into August, *P. ludoviciana* flowers (Rollins, 1939; Rollins and Shaw, 1973). Fruits begin to mature from the lower portion of the inflorescence

while flowers are still open on the upper portion (Beach *et al.*, 2002). In Illinois, fruit matures early to mid-June, whereas in western states flower and fruit set is later.

Physaria ludoviciana is found commonly in well-drained, mobile sand in blowouts of sand prairies (i.e. vegetated sand dunes) (Coons *et al.*, 2004). With constant disturbance, from blowing sand dunes, and with full sunlight, this type of area may create the ideal niche for *P. ludoviciana*. Failure of other species to establish would allow *P. ludoviciana* to remain with minimal competition for light, water, nutrients or physical space (Over *et al.*, 2005). Plants frequently are found on southwest-facing, steep slopes in highly disturbed areas and often within a few miles of large rivers (Claerbout, 2003). Known populations often are scattered and disjunct, occurring in very adverse conditions. Since *P. ludoviciana* is endangered, some of these factors may be inhibiting its growth and development. Light (both duration and intensity) and competition (both above ground and below ground) can affect development of *P. ludoviciana*.

The species floral biology is a feature that has allowed for adaptation to this harsh environment, as seeds develop early in the growing season (May-June) on flower stalks when temperatures are lower and water is more plentiful than later in the summer. In Illinois, *Physaria ludoviciana* flowers in May (Beach *et al.*, 2002) and sets seed in June, thus avoiding the hottest, driest times of the year to complete its reproductive biology. This timing also may maximize visitations from insects, since few other plants are flowering at this time. Flowers of many species that bloom in the early spring are initiated by vernalization of plants during cold winter months (Hartmann *et al.,* 1988) or by short days of autumn (Garner and Allard, 1920; Lambers *et al.*, 2000). In the short days of autumn, if floral development is initiated, the low temperatures of winter stops

floral development until temperatures become more favorable in spring (Lambers *et al.*, 2000). D'Aloia *et al.* (2008) reported that winter cold (vernalization) and photoperiod are the primary factors controlling flowering time. While short days and vernalization could be floral triggers for *P. ludoviciana*, floral development also could be triggered when plants reach a certain developmental age (Claerbout, 2003).

Effects of photoperiod on the development of flowers have not been extensively investigated in *Physaria ludoviciana*. When floral initiation is triggered by photoperiod, plants can either be categorized as long day or short day plants (Garner and Allard, 1920). For floral initiation, a long day plant requires exposure to light for 12 hours or more, whereas a short day plant requires exposure to light for 12 hours or less. *Physaria ludoviciana* plants grown in the greenhouse for several years with no added light other than natural light, bloomed in December each year (Coons and Claerbout, personal observation). Claerbout *et al.* (2007) studied *P. ludoviciana* and found that long day plants developed inflorescences first, but short day plants produced more inflorescences (10.8/plant) compared to long day plants (7.1/plant). The number of open flowers on their peak flowering day per plant in long days was 4.9 compared to short days, which were 3.5. The influence of photoperiod in other Brassicaceae species had been studied. *Arabidopsis thaliana* was a quantitative long day plant that would eventually flower under any photoperiod, although flowering was accelerated greatly by long days (Napp-Zinn, 1985; Irish and Sussex, 1990). *Sinapis alba* was induced to flower by a single long day (D'Aloia *et al.*, 2008).

At the Henry Allan Gleason Nature Preserve (Mason County, Illinois) where *Physaria ludoviciana* is found in three colonies, *Rhus aromatica*, a woody shrub, has

become more prevalent since 2002 (see chapter 5). Competition from other plants will limit light which may influence growth and development of *P. ludoviciana*. At Red Wing, Minnesota, only a few mature *P. ludoviciana* plants were growing in the shade of an evergreen tree, but most plants, including seedlings, were in the sun (see chapter 5). Competition for light is a factor that could contribute to the disjunct distribution of *P. ludoviciana*. Microclimates are found above ground between *P. ludoviciana* and dominant plant species. Typical flora in areas where *P. ludoviciana* occurs, include sand prairie grasses and forbs (McClain *et al.,* 2005). Some of these species may be outcompeting *P. ludoviciana* for light through increased above ground vegetation. For many endangered species, limited light results in a decrease in population size or even elimination from an area.

In most plant species, variation in light intensity has morphological and physiological effects on stem elongation, root formation, leaf area, fresh mass, relative leaf production rate, photosynthetic rate, and chlorophyll concentration (Rice and Bazzaz, 1989; Smith and Longstreth, 1994; Callan and Kennedy, 1996; Alvarenga *et al.,* 2003). Depending on their growth habitat, plants can be classified as sun plants or shade plants (Packham and Willis, 1977; Smith and Longstreth, 1994). Sun plants grow best in sunny environments because they are able to increase photosynthetic activity when light intensities are high, whereas shade plants grow best in shade because they decrease photosynthetic activity when subjected to high light intensities (Smith and Longstreth, 1994).

With competition for light, other species may crowd *Physaria ludoviciana* above ground, but competition also may occur below ground with interactions of the roots.

When *P. ludoviciana* was grown in greenhouses, it needed to be transplanted to larger containers periodically; otherwise, plants deteriorated in appearance. When transplanted, roots of *P. ludoviciana* did not appear root bound although the roots were distributed throughout the container (Claerbout *et al.*, 2007). After transplanting, plants appeared healthy again and actively grew. Since plants in containers received ample water and fertilizer, it is speculated that physical competition to the roots may have caused the problem. Vegetation in areas where *P. ludoviciana* flourishes is relatively sparse, suggesting that it establishes early in succession. As more species establish in an area, *P. ludoviciana* populations tend to decrease, possibly due to competition for light, water, nutrients and/or space. Perhaps responses of plants grown in containers in the greenhouse suggest a lack of ample growing space for the roots of this species, and may provide insight to field conditions where more species establish in an area causing *P. ludoviciana* roots to experience the same decline in health.

Container size or volume has an impact on the performance of greenhouse grown floral crops (NeSmith and Duval, 1998). Varying the container size alters the volume for the roots which affects overall growth and appearance of plants. Plants undergo many physiological and morphological changes in response to reduced root volume such as root and shoot growth, biomass accumulation and partitioning, photosynthesis, leaf chlorophyll content, plant water relations, nutrient uptake, respiration, flowering and yield (NeSmith and Duval, 1998). According to Latimer (1991), marigold seedling development and performance were reduced by root restriction.

If *Physaria ludoviciana* is an early succession plant, competition both above and below ground from increased vegetation might hinder its development. Understanding

how individual environmental components of this competition affect its growth and development would be useful in preservation efforts. The goal of this study was to investigate how light (duration and intensity) and root competition affect development of *P. ludoviciana*. The specific objectives of this study were: 1) to determine how photoperiod affects floral initiation and development, 2) to evaluate how light intensity affects plant development, and 3) to quantify how container size (various depths and widths) affects vegetative and reproductive development of *P. ludoviciana*. This information will increase our understanding of how environmental components of competition affect the establishment and persistence of *P. ludoviciana* plants.

Materials and Methods

Plant Material and Culture

Physaria ludoviciana seeds collected from the Henry Allan Gleason Nature Preserve in Mason County, Illinois on 22 June 1999 were used in all studies. Seeds were stored in coin envelopes in a refrigerator (Fisher Scientific, Isotemp, Laboratory Refrigerator, Pittsburg, Pennsylvania) at 4°C with 40-50% relative humidity until used in this study. Seeds were planted in Cone-tainers™ (Stuewe & Sons, Inc., Corvallis, Oregon) that were 4 cm wide x 20 cm deep with a removable sleeve. SB500 High Porosity mix (Sun Gro, Seneca, Illinois) was used as the media because of its good drainage. Seeds were planted on 7 October 2007 in a Conviron® (Conviron CMP 4030, Winnipeg, Manitoba, Canada) growth chamber (16 hr light/8 hr dark) at 25.0 ± 0.0°C as measured by readout on chamber provided with fluorescent and incandescent bulbs at 174 ± 2 μmol/m^2/sec as measured with Apogee® Quantum meter (Logan, Utah). Water

was added as needed and fertilizer (Stern's Miracle-Gro®, 15-30-15, granular, 10.0 g in 8 L of water) was added once a week.

Photoperiod

Plants were transplanted into larger containers (12 cm wide x 36 cm deep, 4070 cm^3) (TPOT1, Stuewe & Sons Inc., Corvallis, Oregon) on 15 December 2007 and placed in a Conviron® growth chamber with the same conditions mentioned previously. On 25 January 2008, plants were separated into long day (16 hr/8 hr light/dark) and short day (8 hr/16 hr light/dark) treatments with all other environmental parameters remaining the same except the average light intensity for the long day plants was 229 ± 5 $\mu mol/m^2/sec$ and for the short day plants was 443 ± 10 $\mu mol/m^2/sec$. Thirty plants with no flower buds or flowers were placed in each growth chamber. Number of flower stalks (stalks refer to the stems of the inflorescence), height of stalks and number of open flowers were counted weekly for 17 weeks. Leaves and stalks were not removed as they senesced. Fungus gnats appeared in chambers during week one, so fly paper was placed in chambers, and Gnatrol (a Bt derivative) was applied weekly for three weeks. After 20 weeks, plants were harvested; leaves were divided by greater than 4 cm or less than 4 cm long. For each plant, the numbers of leaves $\geq$ 4 cm per plant were counted. Fresh and dry masses (XE series model 100A, Denver Instruments, Arvada, Colorado) were taken separately for each category of leaf size. For dry masses, plants were dried in an oven for 24 hours at 120°C (Despatch, Minneapolis, Minnesota) and then weighed again. For leaves greater than 4 cm, leaf area (LI 3100 leaf area meter, Li-Cor, Inc., Lincoln, Nebraska) per plant was measured. The numbers of leaf clusters (clusters of leaves where the leaf size was less than 4 cm) also were counted.

Light Intensity

Plants were grown from seed in Cone-tainers™ in two different Conviron® growth chambers, one with high intensity at the maximum setting for the chamber (584 ± 21 μmol/m^2/sec) and one with low light intensity (174 ± 2 μmol/m^2/sec) in the same chamber conditions. Although light intensities in the field were between 1200 -1600 μmol/m^2/sec as recorded at Henry Allan Gleason Nature Preserve (Over *et al.*, 2005), the maximum light intensity of the chambers was less. The low light intensity was about a third of the high light, and was chosen because in Red Wing, Minnesota, a light intensity of 175 μmol/m^2/sec was observed near *P. ludoviciana* plants growing in the shade of an evergreen tree. Two hundred seeds were planted for each light intensity and from that 20 plants were selected randomly to record number of leaves and width of plants weekly. After four weeks, 20 plants were harvested to measure various parameters. Fresh mass and dry mass were measured for shoots. Plants were placed in a drying oven for 24 hours at 120°C, cooled, and then weighed to obtain dry masses. Leaf area was measured using a Li-Cor leaf area meter. Width of plant and number of leaves per plant also were measured. Root comparisons were made using a score based on development of lateral roots and length of roots where 1 = few lateral roots and root length was less than 15 cm, 2 = numerous lateral roots with fairly good branching but root length still less than 15 cm, 3 = few lateral roots but root length was greater than 15 cm, and 4 = numerous lateral roots throughout and root length was greater than 15 cm (Figure 3.1).

Container Size

On 15 December 2007, plants from the growth chamber were moved into a greenhouse at Eastern Illinois University where they remained for the duration of the

study. Temperature and relative humidity were monitored with a hygrothermograph (Cole-Parmer, Vernon Hills, Illinois). In the greenhouse, air temperatures and relative humidity ranged from 13.4°C to 28.4°C and 41.2% to 81.9%, respectively.

Plants other than controls were transplanted into containers of different sizes obtained from Stuewe & Sons, Inc. (Corvallis, Oregon) containing soilless mix (Fafard Growing Mix #2, Conrad Fafard, Inc., Agawam, Massachuseutts). Five different containers sizes were used: TPOT5 (Tall-Wide) = 36 cm x 23 cm (15,000 cm^3); TPOT5 cut short (Short-Wide) = 22 cm x 23 cm tall (4570 cm^3); TPOT1, Tall One (Tall-Narrow) = 36 cm x 12 cm (4070 cm^3); TPOT1/Tall One cut short (Short-Narrow) = 22 cm x 11 cm (2090 cm^3); and Cone-tainer™ (control) = 20 cm x 4 cm (250 cm^3). Uniform sized plants were selected for each replication. Crown diameter of stems (using Digimatic micrometer IP65, Mitutoyo Corporation, Japan), number of leaves and number of flower stalks were measured on the day of transplanting when plants were 4 months old. Nine replications of each container size were used.

Three plants from each container size were harvested after each of three, four, and five weeks. For shoots, the following parameters were measured: number of leaves, fresh mass of all leaves, dry mass of all leaves (75°C for 24 hours), leaf area of all leaves and crown diameter of stem. For roots, root length was quantified after removal of soilless mix via shaking and dipping in water. For reproductive structures, length of flower stalks (total of all stalks on a plant where stalks refer to the stems of the inflorescence), fresh and dry masses of flower stalks, and numbers of stalks, pedicels, flowers and fruits per plant were recorded.

Data Analysis

Means and standard errors were calculated using Microsoft® Office Excel 2003 (11.8211.8202) SP3 and analyzed by SPSS (Version 16 for Windows) univariate one way ANOVA at the 5% level. A Duncan's multiple range test (mean separation at the 5% level) was used when more than two means were compared.

Results

Photoperiod

During the study, not all of the plants in either of the treatments produced inflorescences or flowers stalks (Table 3.1). In the short day photoperiod, 3-20% of the plants produced an inflorescence in any one week, which is 1-6 plants producing reproductive structures compared to the long day plants that had 0-27% of the plants or 0-8 plants producing an inflorescence structure in any one week during the 17 weeks. Hence, at any given time, both photoperiods had less than 30% of plants developing inflorescences. For the figures that follow, instead of dividing by the total number of plants (i.e. 30), calculations were based on the total number of plants that had inflorescences at the time a measurement was taken. During the first week, inflorescences were observed in the short day plants, but not in long day plants. At the end of the study, plants grown in the long day photoperiod had developed more inflorescences than those in short days (Figure 3.2). Number of inflorescences peaked for long days at week 15 with 5.8 stalks per plant and at week 13 for short day plants with 4.6 stalks per plant. In Figure 3.3, flower stalks of the long day photoperiod plants are getting shorter throughout the study. Each week the number of flower stalks and height of stalks were taken. Height of an individual plants were not kept separate each week,

just the average height of all flower stalks. As more frequent smaller flower stalks developed, bringing down the height average, the numbers for heights are getting smaller. Overall, long day plants had higher numbers of open flowers in early weeks during the 17 weeks than short day plants (Figure 3.4). Flowering peaked at week 6 for long day plants with an average of 11.3 open flowers per plant and at weeks 2 and 8 for the short day plants with an average of 6 open flowers per plant. Plants transferred into long day photoperiods had a peak bloom earlier than plants transferred into short days.

Table 3.2 shows the various growth parameters for the two photoperiods when plants were harvested after 20 weeks. Number of leaves per plant (leaf size greater than 4 cm) was significantly greater for the long day plants than the short day plants, as was leaf area, and fresh and dry masses for leaves per plant greater than 4 cm. The fresh and dry masses of the leaves less than or equal to 4 cm and the number of leaf clusters were not significantly different for the two photoperiods.

Light Intensity

Figure 3.5 shows plants grown in high light and low light after 6 weeks. For plants checked weekly for six weeks (Figure 3.6), those grown with high light consistently had a greater number of leaves than those grown with low light, except at week one, when the average number of leaves in both chambers was 2.0 with standard errors of zero. In Figure 3.7, plants grown with low light had a greater width of plants than those at high light.

Table 3.3 shows growth parameters after four weeks when grown in two light intensities. After four weeks, plants grown at high light had significantly greater leaf areas and leaf numbers than those at low light. Width of plants was significantly greater

in low light than high light. After four weeks, plants grown at high light had significantly greater fresh and dry masses than those at low light. Also at four weeks, plants grown at high light had significantly more root development than those at low light based on a scale of 1 to 4.

Container Size

Pre-experiment measurements for numbers of flowers, leaves, lateral rosettes, and crown diameters showed no significant differences for plants transplanted to different container sizes at the beginning of the experiment (Figure 3.8). Hence, whatever differences were present at weeks three, four and five were not pre-existing.

For week three, none of the reproductive parameters (length of flower stalk, fresh and dry mass of flower stalks, numbers of stalks, pedicels, open flowers and fruits per plant) were significantly different between the five different container sizes (Table 3.4). At week three, only one vegetative parameter, leaf area, showed a significant difference due to container size, while other parameters (crown diameter, fresh and dry mass of leaves, number of leaves per plant, and root length per plant) showed no differences. The Short-Wide container had a larger leaf area than the Cone-tainer™ or the Tall-Wide container (Table 3.5).

At week four, more significant differences due to containers were seen for the reproductive parameters (Table 3.6). Flower stalk length, fresh and dry masses of stalks, flower stalks per plant, and number of open flowers per plant had significant differences between the container sizes. For parameters with a significant difference, the Cone-tainer™ consistently had the lowest values with the Short-Narrow and Tall-Narrow frequently having the highest values. For vegetative parameters at 4 week (Table 3.7),

the only parameter with no significant difference was root length per plant. Of the parameters that had a significant difference, the Cone-tainer™ plants had the lowest values. Variable responses were found from the different containers at week four.

For the final harvest at five weeks after transplanting to the various container sizes the only reproductive parameters that were not significantly different were number of flowers per plant and stalk length (Table 3.8). For parameters with significant differences, the Tall-Narrow container consistently had the highest values. The Cone-tainer™, Short-Narrow, and Tall-Wide containers had the lowest values for at least one parameter. Table 3.9 shows the vegetative parameters at the final harvest. For vegetative parameters at week 5, crown diameter, dry mass of leaves, and root length did not have significant differences due to containers. The parameters that were significantly different were fresh mass of leaves, leaf area, and leaf number per plant where the Cone-tainer™ had the lowest values.

Discussion

Photoperiod

This study provides insight relative to how factors including developmental age, vernalization and photoperiod affect floral initiation and development in *Physaria ludoviciana.* Plants in this study were approximately four months old when flowering began and had not flowered or produced reproductive structures prior to this study. By measuring various growth parameters, we can see that more developed and therefore more mature plants are more likely to flower. Since plants flowered after one week in the different photoperiods, floral initiation already occurred while plants were in long days. Thus plants do not require short days to initiate flowers. Hence, flower initiation was

likely a response to the developmental age or maturity of plants or to long days or short days. Vernalization, which occurs when temperatures are between 0-10°C is another factor that can aid in floral initiation (Hartmann *et al.,* 1988). While vernalization could occur during winter where *P. ludoviciana* grows, it was not required for floral initiation as plants in this study flowered in growth chambers where the temperature never dropped below 10°C. These finding agree with Claerbout *et al.* (2007) who reported that *P. ludoviciana* plants kept in warm greenhouses flowered from December to April once they had reached a certain maturity. In our study, extra *P. ludoviciana* plants grown for the photoperiod study, but not used in this study, were brought to the greenhouse in December (when other plants were moved to long or short days), and the greenhouse plants flowered starting in March suggesting plants flower regardless of photoperiod. A third factor that might initiate flowers is photoperiod. Flowering in *P. ludoviciana,* was not triggered by short days as plants began to flower after being only in long days. With a long day photoperiod, plants produced more inflorescences and more flowers. However, photoperiod was not an absolute requirement for development of *P. ludoviciana* flowers, since both long and short day plants developed flowers (when being moved into two different photoperiods at four months of age after possible initiation while growing in long days). *Physaria ludoviciana*'s flowering cycle is very similar to another Brassicaceae species *Arabidopsis thaliana*. *Arabidopsis thaliana* is a quantitatively long day plant that will eventually flower under any photoperiod, although flowering is greatly accelerated by long days (Napp-Zinn, 1985; Irish and Sussex, 1990; Karlsson *et al.*, 1993). For *P. ludoviciana*, if conditions are favorable, plants will flower regardless of photoperiod. The plants are in ideal conditions, where there are well

watered and fertilized with temperature control, so they mature and flower. In the greenhouse, nothing is inhibiting them from flowering at any photoperiod and, unlike in nature, the temperature is not preventing development. The timing in greenhouse plants versus plants in nature differs because in nature around November and December it gets cold, thus slowing development. In Illinois over the winter, plants are evergreen (Coons, personal observation). Then, in the spring with the higher temperatures (Lambers *et al.*, 2000), plants are able to flower quickly since they are evergreen and don't need to develop vegetative structures.

Latitudinal variation often provides environmental cues that signal plants to flower at appropriate times for a given climate (Stinchcombe *et al.*, 2005). Since *Physaria ludoviciana* developed flowers with both photoperiods, perhaps early spring provides other survival advantages. *Physaria ludoviciana* plants might need to be a certain age, and after the cold, the warmth of spring allows them to initiate floral development. With plants being evergreen and able to bloom early in the spring before many other plants bloom, an advantage occurs especially when plants require a pollinator. Claerbout *et al.* (2007) showed cross-pollinated flowers produced fruits while self-pollinated ones did not. While competition for pollinators could be an inhibiting factor, *P. ludoviciana* may overcome this competition by blooming before many other plants to increase its chances for insect pollination. Another advantage of a spring bloom is more moisture in the soil and lower temperatures when *P. ludoviciana* is flowering and fruiting relative to temperatures in summer.

Light Intensity

Light intensity greatly affected the development of *Physaria ludoviciana*. Plants developed faster in higher light than in lower light. Since all of the parameters measured at six weeks and most of the parameters at four weeks were significantly greater for plants grown at higher light than those at lower light, it is possible that photosynthetic rates were greater for plants grown at the higher light. Width of the plant was higher in the lower light intensity compared to the higher light intensity after six weeks. This response is not what was expected. With a higher width of the plant, giving it a higher surface area the plant could be trying to compensate with a greater width of the plant so it can trap more light. To understand this response, it would have been useful to measure length, width and thickness of leaves. These measurements would indicate the entire surface area of the leaf. This response may relate to the way the plant grows, as the new leaves stand upright while the older leaves start to lay flat. Higher photosynthetic rates for plants grown in higher light is a common response as the more light a plant receives, the faster it can develop. In *Gossypium hirsutum* (Malvaceae), Smith and Longstreth (1994) found a greater net rate of carbon dioxide uptake in leaves per unit of leaf area when leaves were exposed to higher light intensity compared to those leaves exposed to lower light, which resulted in greater leaf and mesophyll areas for the leaves exposed to higher light. *Iliamna remota* also grew faster at higher than lower light intensities (McDonnell, 2006). After three weeks at two different light intensities, plants in a higher light chamber had significantly greater numbers of side and main leaves, shoot heights, root lengths, leaf areas, root volumes, fresh and dry masses of shoots and roots, and fresh weight shoot-to-root ratios than those at lower light (McDonnell, 2006). Variations in

light intensity have morphological and physiological effects. Rice and Bazzaz (1989) found when *Abutilon theophrasti* was transferred from lower light to higher light, it caused an immediate stimulation of growth and photosynthetic rates. Many plant species have a positive correlation between light intensity during growth and biomass production (Smith and Longstreth, 1994). This response is evident in *P. ludoviciana* as leaf area, leaf number, fresh and dry masses and root branching were all greater in plants grown at higher than lower light intensities. Width of plants taken at harvest was the only parameter that was greater in lower light than higher light. According to Garner and Allard (1920), a reduction in light intensity causes the thickness of the leaf lamina to be reduced causing the plant to be less compact, which could cause a greater plant width with reduced light.

Physaria ludoviciana can be classified as a sun plant because it develops more quickly in higher than lower light intensities (Smith and Longstreth, 1994). Also personal observations show that plants are found in open areas with full sun, whereas fewer plants are found where dense vegetation competes. For many native sand prairie species, reduced light is a problem which results in decreased population sizes or even elimination from an area when woody species start to establish. In the habitat near the *P. ludoviciana* population in Illinois over the past years, fragrant sumac (*Rhus aromatica*) has increased which may threaten the *P. ludoviciana* population. A lower light intensity may be an adverse effect of above ground competition from other plants for light. The minimum amount of light necessary for survival and the amount of light necessary to maximize growth can vary dramatically for different species. Findings from this study

stress the importance of management to control woody species to reduce competition for light, and hence to preserve *P. ludoviciana* populations.

Container Size

Root competition below ground, especially for *Physaria ludoviciana,* can be an inhibiting factor for reproductive and vegetative plant growth. In this container size study, root crowding affected reproductive and vegetative parameters of *P. ludoviciana* after four or five weeks, as at three weeks no significant differences were seen in any of the parameters. Plants harvested at three weeks might not have had time to develop enough to show effects of different container sizes. At weeks four and five, significant differences were seen between the plants grown in different containers. According to Cantliffe (1993) and NeSmith and Duval (1998), as container size increases, plant leaf area, shoot biomass and root biomass increase. Roots rely upon plant aerial portions for photosynthesis and various hormones, while plant aerial portions rely on the roots for water, nutrients, support and hormones (Tonutti and Giulivo, 1990). The delicate balance between roots and shoots can be upset when the root system is restricted in a small rooting volume. This restriction would explain why a decrease was seen in root length as well as in vegetative and reproductive parameters with the smaller container. Reproductive development of plants can be influenced by container size which can increase or prolong root restriction. Peterson *et al.* (1991) found that as root restriction increased in tomatoes, the flowering time was reduced. In our study, the number of reproductive structures tended to be the lowest in the Cone-tainer™. The Cone-tainer™, which was the smallest container, had the lowest numbers for fresh and dry mass of stalks and stalk length in weeks four and five. The root restriction on these plants reduces the

number of stalks. In natural habitats, if the plant is exposed to root restriction, seed production may be reduced, reducing chances for maintaining a viable population.

Physical space and possible inhibition mechanisms are other factors that could affect growth of *Physaria ludoviciana*. Growth and development of *P. ludoviciana* was affected by space in the containers. Smaller containers had less developed plants. Container size and growth of plants can relate to how plants grow in nature. If competition is great below ground in the roots, *P. ludoviciana* may not be able to grow as well. According to Schenk (2006), root competition may reduce the availability of a soil resource to roots that is caused by other roots. This reduction can become a problem for competitor plants because resources are less available. If habitat productivity is not increased, plants can become deficient in nutrients and space. However, even in ideal conditions for resources to *P. ludoviciana*, plants began to deteriorate in appearance when the container was too small. When plants were removed from containers during transplanting, no signs were seen that they were root bound. Mounting evidence has emerged over the last couple of years showing that roots can detect and react to the presence and even identity other roots in the soil, as well as the presence of inert objects, such as the wall of a soil container before they make contact (Coomes and Grubb, 2000). Therefore plants may detect the difference between "self" and "non-self" roots and may inhibit growth of self roots once they start to intermingle (Mahall and Callaway, 1996; Coomes and Grubb, 2000; de Kroon *et. al.*, 2003; Falik *et al.*, 2003; Holzapfel and Alpert 2003; Gruntman and Novoplansky, 2004; Schenk, 2006). Studies on a variety of herbaceous and woody plants species show that roots behave differently when encountering "self" vs. "non-self" roots. It is possible that some chemical signal occurs

in *P. ludoviciana*, where it is able to sense the presence of other plant roots or inert objects like the side of a container, and thus it stops growing even when it does not appear to be in competition with other plants or to be root bound.

Knowing that *Physaria ludoviciana* is not able to compete very well may be useful to management efforts as an incentive to control non-native species. The increasing number of non-native species encroaching many native habitats has given rise to many studies on the effects of competition on native and/or endangered species. When established ryegrass competed with invading grass seedlings, the competition was mainly below ground, probably for nitrogen (Snaydon and Howe, 1986). The invading grass seedlings would decrease when in root competition with ryegrass in the absence of nitrogen. In the field, *P. ludoviciana* might not only be competing with other plants for space but also for nutrients and water. Given *P. ludoviciana*'s hot and dry environment, root competition for water may be more important for structuring the plant community than it would be in moist soils because the lack of water tends to kill species whereas the reduction of nutrients merely stunts growth (Coomes and Grubb, 2000; Schenk, 2006)

Summary

Many environmental factors affected the success of *Physaria ludoviciana*, including light duration (photoperiod) as well as competition both above ground (light intensity) and below ground (root competition). From this photoperiod study, flowers developed with either a long day or short day photoperiod, with favorable conditions such as temperature, water and fertilizer, which aided in reproduction of the plant. Another aspect of light that was important to this species was the intensity. Plants developed faster with higher light than lower light, suggesting that increased woody vegetation

encroaching near them would inhibit their growth. Competition both above ground for light and below ground between roots affected *P. ludoviciana.* The container size study showed that even if plants were not root bound, the amount of plant mass decreased in smaller containers. These studies on light and root competition showed the influence of environment on the establishment, growth and development of *P. ludoviciana.*

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Table 3.1. Percentage of *Physaria ludoviciana* plants that produced inflorescences for each week after start of short day (8 hr) and long day (16 hr) photoperiods.

Week	Short Day	Long Day
1	3[a]	0
2	3	3
3	3	3
4	3	3
5	3	3
6	3	10
7	3	20
8	3	20
9	3	27
10	7	27
11	3	27
12	7	27
13	20	23
14	13	20
15	7	17
16	13	23
17	10	27

[a] data are shown as percentage of *Physaria ludoviciana* on a given week and not a cumulative total

Table 3.2. Growth parameters of *Physaria ludoviciana* after 20 weeks in short day (8 hr) and long day (16 hr) photoperiods.

	Short Day	Long Day
Leaves/Rosette > 4 cm	32.1 ± 1.9 b[a, b]	38.0 ± 2.0 a
Leaf Area (cm^2)	170.9 ± 11.6 b	208.6 ± 13.7 a
Fresh Mass of Leaves > 4 cm (g)	14.1 ± 1.1 b	20.1 ± 1.4 a
Fresh Mass of Leaves ≤ 4 cm (g)[c]	5.2 ± 0.5 a	5.9 ± 0.8 a
# of Leaf Clusters[d]	15.3 ± 1.3 a	14.6 ± 2.2 a
Dry Mass of Leaves > 4 cm (g)	2.7 ± 0.2 b	4.2 ± 0.3 a
Dry Mass of Leaves ≤ 4 cm (g)	1.2 ± 0.1 a	1.5 ± 0.2 a

[a] means ± standard errors

[b] means within a row followed by different letters are significantly different based upon Duncan's multiple range test at p=0.05 level

[c] leaf length ≤ 4 cm

[d] length of leaves in rosette ≤ 4 cm

Table 3.3. Growth parameters per plant of *Physaria ludoviciana* after 4 weeks when grown in high (584 µmol/m^2/sec) and low (174 µmol/m^2/sec) light intensities.

Light Intensity	Width of Plant (cm)	Leaf Area (cm^2)	Leaf Number
Shoots			
High Light	11.4 ± 0.3 b[a,b]	12.5 ± 0.7 a	10.2 ± 0.2 a
Low Light	12.7 ± 0.3 a	8.1 ± 0.5 b	8.7 ± 0.1 b
Masses of Shoots			
	Fresh (g)	Dry (g)	
High Light	0.86 ± 0.04 a	0.15 ± 0.01 a	
Low Light	0.45 ± 0.03 b	0.06 ± 0.01 b	
Root Development			
High Light	3.2 ± 0.3 a[c]		
Low Light	2.3 ± 0.3 b		

[a] means ± standard errors

[b] means within a column followed by different letters are significantly different based upon Duncan's multiple range test at p=0.05 level

[c] Root comparisons were made using a score based on development of lateral roots and length of roots. 1 = few lateral roots and root length was less than 15 cm. 2 = numerous lateral roots with fairly good branching but root length still less than 15 cm. 3 = few lateral roots but root length was greater than 15 cm. 4 = numerous lateral roots throughout and root length was greater than 15 cm

Table 3.4. Reproductive parameters of *Physaria ludoviciana* per plant harvested after 3 weeks when grown in 5 different containers after being transplanted from Cone-tainers™ when 4 months old.

	Flower Stalk Length (cm)	Fresh Mass Flower Stalks (g)	Dry Mass Flower Stalk (g)	Flower Stalks/Plant	Pedicels/Plant	Open Flowers/Plant	Fruits/Plant
Cone-tainer™[a]	2.3 ± 1.2 a[f, g]	0.21 ± 0.13 a	0.07 ± 0.04 a	1.7 ± 1.2 a	12.0 ± 6.2 a	0.0 ± 0.0 a	1.0 ± 0.6 a
Short-Wide[b]	1.7 ± 1.7 a	0.29 ± 0.29 a	0.06 ± 0.06 a	1.7 ± 1.7 a	7.0 ± 7.0 a	0.0 ± 0.0 a	1.7 ± 1.7 a
Short-Narrow[c]	1.8 ± 1.8 a	0.22 ± 0.22 a	0.06 ± 0.06 a	1.3 ± 1.3 a	6.7 ± 5.7 a	0.3 ± 0.3 a	1.7 ± 1.7 a
Tall-Wide[d]	4.2 ± 0.8 a	1.08 ± 0.46 a	0.13 ± 0.04 a	3.3 ± 0.7 a	19.3 ± 7.8 a	0.0 ± 0.0 a	1.7 ± 0.9 a
Tall-Narrow[e]	3.1 ± 0.3 a	0.77 ± 0.40 a	0.09 ± 0.03 a	3.7 ± 0.9 a	8.7 ± 4.1 a	1.3 ± 0.9 a	1.0 ± 1.0 a

[a] 20 cm x 4 cm (250cm^3)
[b] 22 cm x 23 cm (4570 cm^3)
[c] 22 cm x 11 cm (2090 cm^3)
[d] 36 cm x 23 cm (15,000 cm^3)
[e] 36 cm x 12 cm (4070 cm^3)
[f] means ± standard errors
[g] means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level

Table 3.5. Vegetative parameters of *Physaria ludoviciana* per plant harvested after 3 weeks when grown in 5 different containers after being transplanted from Cone-tainers™ when 4 months old.

	Crown Diameter (mm)	Fresh Mass Leaves (g)	Dry Mass Leaves (g)	Leaf Area (cm^2)	Leaf #/Plant	Root Length (cm)
Cone-tainer™[a]	5.25 ± 0.66 a[f, g]	2.55 ± 0.30 a	0.59 ± 1.47 a	30.3 ± 6.3 b	20.7 ± 4.2 a	25.5 ± 1.7 a
Short-Wide[b]	4.20 ± 0.16 a	3.69 ± 0.24 a	0.70 ± 0.50 a	52.0 ± 4.6 a	27.0 ± 4.7 a	24.3 ± 2.4 a
Short-Narrow[c]	3.97 ± 0.39 a	2.70 ± 0.58 a	0.52 ± 0.14 a	43.9 ± 9.0 ab	19.0 ± 5.2 a	23.0 ± 4.6 a
Tall-Wide[d]	3.90 ± 0.54 a	2.67 ± 0.22 a	0.54 ± 0.03 a	31.2 ± 0.6 b	20.0 ± 0.6 a	24.8 ± 3.1 a
Tall-Narrow[e]	4.62 ± 0.24 a	3.00 ± 0.28 a	0.66 ± 0.09 a	37.8 ± 3.3 ab	22.3 ± 0.9 a	22.0 ± 1.9 a

[a] 20 cm x 4 cm ($250cm^3$)
[b] 22 cm x 23 cm ($4570 cm^3$)
[c] 22 cm x 11 cm ($2090 cm^3$)
[d] 36 cm x 23 cm ($15{,}000 cm^3$)
[e] 36 cm x 12 cm ($4070 cm^3$)
[f] means ± standard errors
[g] means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level

Table 3.6. Reproductive parameters of *Physaria ludoviciana* per plant harvested after 4 weeks when grown in 5 different containers after being transplanted from Cone-tainers™ when 4 months old.

	Flower Stalk Length (cm)	Fresh Mass Flower Stalks (g)	Dry Mass Flower Stalk (g)	Flower Stalks/Plant	Pedicels/Plant	Open Flowers/Plant	Fruits/Plant
Cone-tainer™[a]	2.1 ± 2.1 b[f, g]	0.09 ± 0.09 b	0.02 ± 0.02 b	1.0 ± 1.0 b	6.7 ± 1.2 a	1.0 ± 1.0 b	1.3 ± 0.3 a
Short-Wide[b]	8.0 ± 4.6 ab	0.26 ± 0.16 ab	0.06 ± 0.04 ab	4.0 ± 1.7 ab	19.7 ± 15.0 a	0.0 ± 0.0 b	6.0 ± 6.0 a
Short-Narrow[c]	12.6 ± 2.7 ab	0.69 ± 0.17 a	0.17 ± 0.04 a	3.3 ± 0.7 ab	22.3 ± 9.4 a	5.7 ± 3.7 ab	8.0 ± 4.5 a
Tall-Wide[d]	5.7 ± 3.0 ab	0.20 ± 0.13 ab	0.05 ± 0.03 ab	2.3 ± 1.2 b	11.0 ± 6.4 a	0.7 ± 0.7 b	4.7 ± 3.0 a
Tall-Narrow[e]	16.1 ± 4.8 a	0.66 ± 0.18 a	0.15 ± 0.04 a	6.7 ± 0.9 a	32.7 ± 10.7 a	8.0 ± 1.7 a	11.0 ± 3.6 a

[a] 20 cm x 4 cm (250cm^3)
[b] 22 cm x 23 cm (4570 cm^3)
[c] 22 cm x 11 cm (2090 cm^3)
[d] 36 cm x 23 cm (15,000 cm^3)
[e] 36 cm x 12 cm (4070 cm^3)
[f] means ± standard errors
[g] means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level

Table 3.7. Vegetative parameters of *Physaria ludoviciana* per plant harvested after 4 weeks when grown in 5 different containers after being transplanted from Cone-tainers™ when 4 months old.

	Crown Diameter (mm)	Fresh Mass Leaves (g)	Dry Mass Leaves (g)	Leaf Area (cm^2)	Leaf #/Plant	Root Length (cm)
Cone-tainer™[a]	2.25 ± 1.13 b[f, g]	1.15 ± 0.49 b	0.29 ± 0.83 c	14.1 ± 5.9 b	12.0 ± 3.2 b	19.9 ± 4.3 a
Short-Wide[b]	4.29 ± 0.17 ab	3.86 ± 0.35 a	0.74 ± 0.05 b	44.5 ± 6.4 a	22.7 ± 0.9 a	19.2 ± 0.8 a
Short-Narrow[c]	3.48 ± 0.24 ab	3.33 ± 0.11 a	0.67 ± 0.03 bc	43.9 ± 3.8 a	18.0 ± 1.2 ab	20.2 ± 2.0 a
Tall-Wide[d]	4.50 ± 0.61 a	3.20 ± 0.52 a	0.60 ± 0.10 bc	42.3 ± 7.3 a	20.0 ± 3.6 ab	21.3 ± 0.7 a
Tall-Narrow[e]	3.90 ± 0.35 ab	3.61 ± 1.04 a	1.17 ± 0.22 a	47.3 ± 13.7 a	21.0 ± 2.5 a	23.6 ± 1.0 a

[a] 20 cm x 4 cm (250cm^3)
[b] 22 cm x 23 cm (4570 cm^3)
[c] 22 cm x 11 cm (2090 cm^3)
[d] 36 cm x 23 cm (15,000 cm^3)
[e] 36 cm x 12 cm (4070 cm^3)
[f] means ± standard errors
[g] means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level

Table 3.8. Reproductive parameters of *Physaria ludoviciana* per plant harvested after 5 weeks when grown in 5 different containers after being transplanted from Cone-tainers™ when 4 months old.

	Flower Stalk Length (cm)	Fresh Mass Flower Stalks (g)	Dry Mass Flower Stalk (g)	Flower Stalks/Plant	Pedicels/Plant	Open Flowers/Plant	Fruits/Plant
Cone-tainer™[a]	3.0 ± 0.4 a[f, g]	0.36 ± 0.10 c	0.10 ± 0.03 b	2.7 ± 0.3 bc	28.7 ± 6.7 b	0.0 ± 0.0 a	13.0 ± 4.0 ab
Short-Wide[b]	5.2 ± 0.9 a	1.12 ± 0.10 ab	0.23 ± 0.01 ab	4.3 ± 0.3 b	49.0 ± 4.9 b	4.3 ± 2.2 a	9.7 ± 5.0 ab
Short-Narrow[c]	4.8 ± 0.7 a	0.49 ± 0.28 bc	0.13 ± 0.07 b	2.0 ± 1.0 c	24.0 ± 7.0 b	0.7 ± 0.3 a	7.0 ± 3.1 b
Tall-Wide[d]	4.6 ± 1.0 a	0.67 ± 0.02 abc	0.17 ± 0.01 ab	3.7 ± 0.3 bc	21.7 ± 8.7 b	3.3 ± 3.3 a	1.0 ± 1.0 b
Tall-Narrow[e]	4.5 ± 0.6 a	1.26 ± 0.33 a	0.33 ± 0.09 a	6.3 ± 0.3 a	88.0 ± 20.6 a	5.3 ± 2.9 a	22.3 ± 6.3 a

[a] 20 cm x 4 cm (250cm^3)
[b] 22 cm x 23 cm (4570 cm^3)
[c] 22 cm x 11 cm (2090 cm^3)
[d] 36 cm x 23 cm (15,000 cm^3)
[e] 36 cm x 12 cm (4070 cm^3)
[f] means ± standard errors
[g] means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level

Table 3.9. Vegetative parameters of *Physaria ludoviciana* per plant harvested after 5 weeks when grown in 5 different containers after being transplanted from Cone-tainers™ when 4 months old.

	Crown Diameter (mm)	Fresh Mass Leaves (g)	Dry Mass Leaves (g)	Leaf Area (cm^2)	Leaf #/Plant	Root Length (cm)
Cone-tainer™[a]	3.37 ± 0.14 a[f, g]	1.84 ± 0.26 b	0.47 ± 0.90 a	25.9 ± 4.0 b	10.0 ± 0.6 b	22.8 ± 1.3 a
Short-Wide[b]	3.53 ± 0.33 a	3.70 ± 0.18 a	0.70 ± 0.06 a	48.4 ± 4.8 a	18.3 ± 2.0 a	20.6 ± 0.2 a
Short-Narrow[c]	3.36 ± 0.17 a	3.69 ± 0.75 a	0.80 ± 0.16 a	46.2 ± 8.0 a	19.7 ± 4.1 a	23.2 ± 1.7 a
Tall-Wide[d]	3.24 ± 0.08 a	3.69 ± 0.36 a	0.78 ± 0.09 a	51.0 ± 5.4 a	20.3 ± 1.9 a	22.6 ± 1.4 a
Tall-Narrow[e]	3.73 ± 0.22 a	3.53 ± 0.31 a	0.80 ± 0.10 a	46.8 ± 6.1 a	19.7 ± 1.2 a	23.9 ± 0.6 a

[a] 20 cm x 4 cm ($250cm^3$)
[b] 22 cm x 23 cm ($4570\ cm^3$)
[c] 22 cm x 11 cm ($2090\ cm^3$)
[d] 36 cm x 23 cm ($15{,}000\ cm^3$)
[e] 36 cm x 12 cm ($4070\ cm^3$)
[f] means ± standard errors
[g] means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level

1 2

3 4

Figure 3.1. Root comparisons based on development of lateral roots and length of roots (roots are highlighted in green).

Scale of: 1 = few lateral roots and root length was less than 15 cm
2 = numerous lateral roots with fairly good branching but root length still less than 15 cm
3 = few lateral roots but root length was greater than 15 cm
4 = numerous lateral roots throughout and root length was greater than 15 cm

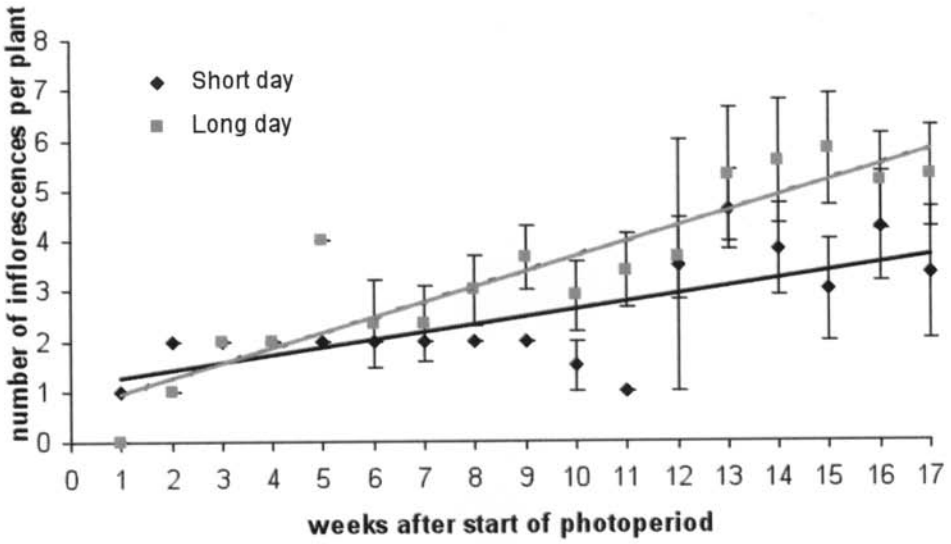

Figure 3.2. Number of inflorescences per plant (of those producing inflorescences) over 17 weeks for *Physaria ludoviciana* when 4 month old plants were transferred into short day (8 hr) and long day (16 hr) photoperiods. Means ± standard errors.

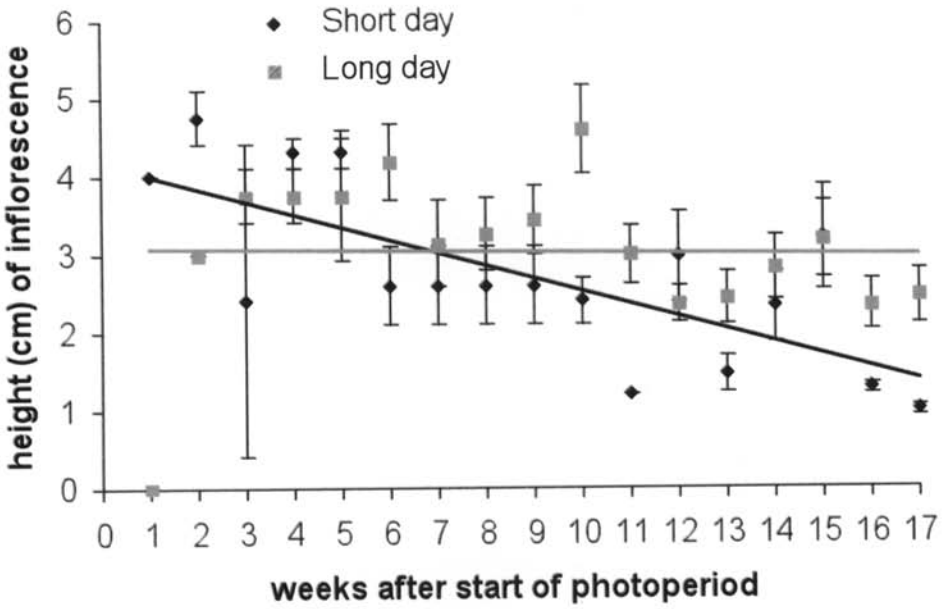

Figure 3.3. Height of inflorescences per plant (of those producing inflorescences) over 17 weeks for *Physaria ludoviciana* when 4 month old plants were transferred into short day (8 hr) and long day (16 hr) photoperiods. Means ± standard errors.

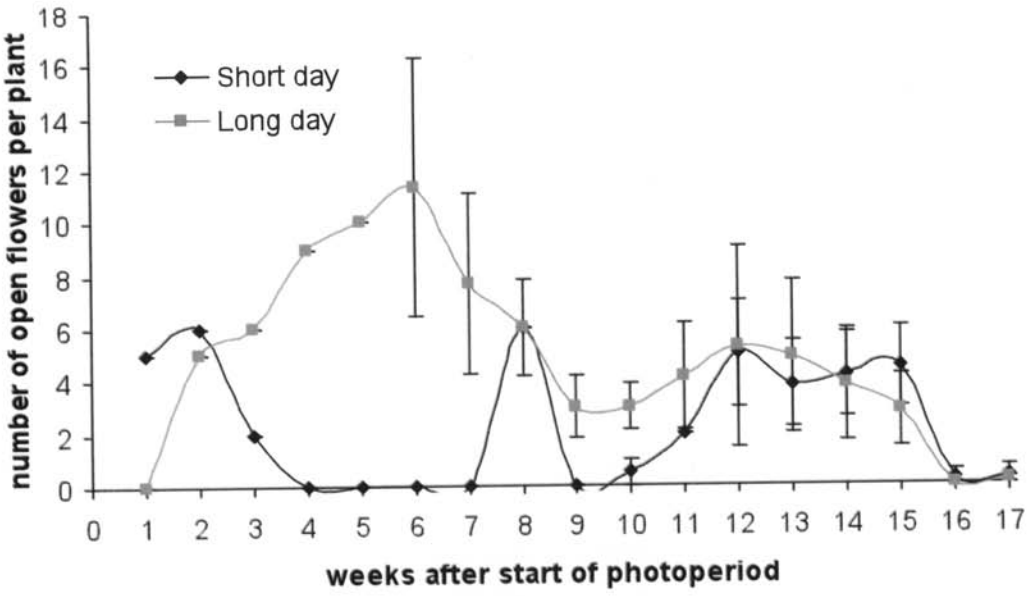

Figure 3.4. Number of open flowers per plant (of those producing inflorescences) over 17 weeks for *Physaria ludoviciana* when 4 month old plants were transferred into short day (8 hr) and long day (16 hr) photoperiods. Means ± standard errors.

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Figure 3.5. *Physaria ludoviciana* plants at 6 weeks when grown with high light (top) (584 μmol/m^2/sec) and low light (bottom) (174 μmol/m^2/sec) intensities.

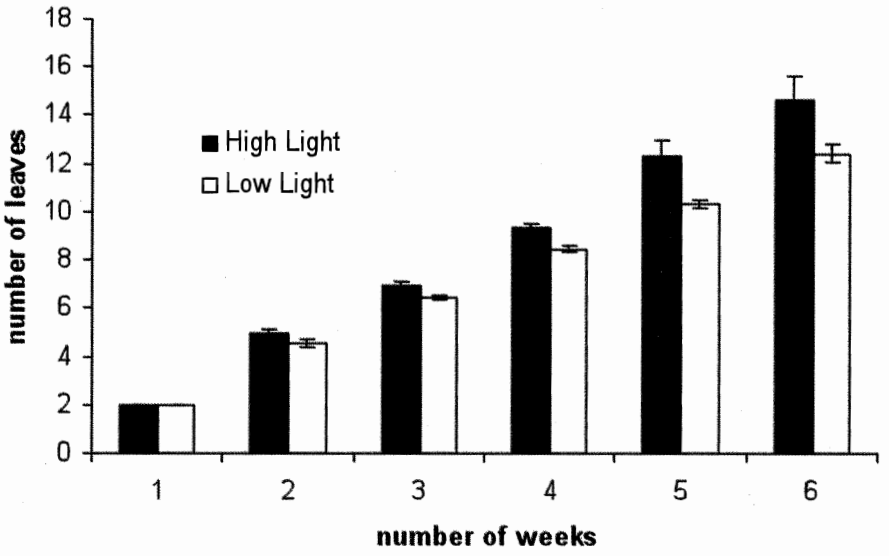

Figure 3.6. Number of leaves on *Physaria ludoviciana* plants over 6 weeks when grown with high (584 μmol/m^2/sec) and low (174 μmol/m^2/sec) light intensities. Means ± standard errors.

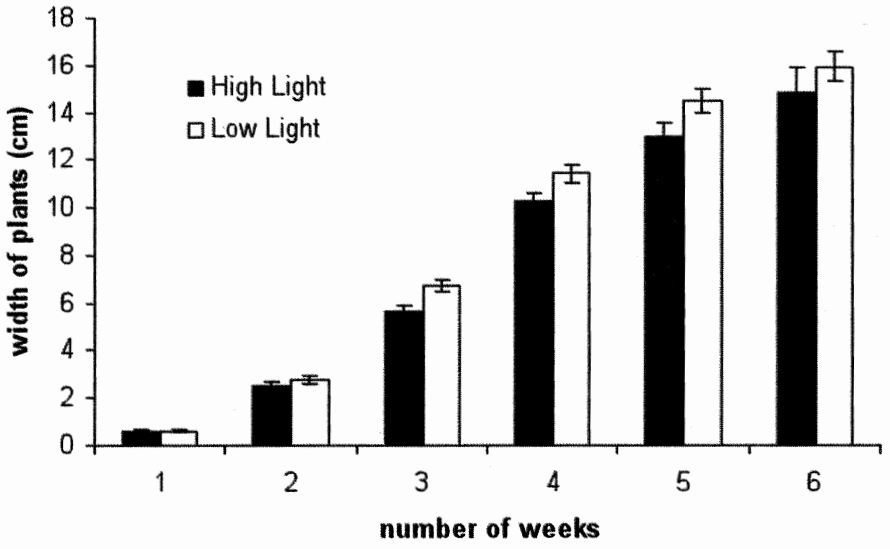

Figure 3.7. Width of plant for *Physaria ludoviciana* plants over 6 weeks when grown with high (584 μmol/m^2/sec) and low (174 μmol/m^2/sec) light intensities. Means ± standard errors.

Figure 3.8. Vegetative rosettes in 5 different container sizes with: control (20 cm X 4 cm), 2 depths (22 cm=short and 36 cm=tall) and 2 widths (12 cm=narrow and 23 cm=wide).

Picture courtesy of Janice Coons.

Chapter 4

Structural and Physiological Adaptations of *Physaria ludoviciana* (Silvery Bladderpod; Brassicaceae) to Sand Prairies

Abstract

Physaria ludoviciana (Nuttal) O'Kane & Al-Shehbaz (silvery bladderpod; Brassicaceae), formerly known as *Lesquerella ludoviciana*, is endangered in Illinois and Minnesota but is widespread in western states. *Physaria ludoviciana* is a perennial forb that flowers in early spring and commonly is found in blowouts on vegetated sand dunes (i.e. sand prairies). Areas where *P. ludoviciana* is found, have high summer temperatures, high light, and low soil moisture retention, which could stress plant growth. Our objective was to describe structural and physiological adaptations of *P. ludoviciana* that may allow it to survive in this environment. Morphological characteristics were measured using three larger and three smaller plants from the Henry Allan Gleason Nature Preserve (HAGNP) in Mason County, Illinois, including root length, area, volume and fresh mass; leaf number and area; shoot fresh mass; and ratio of root:leaf area as well as root:shoot fresh mass. Plants also were evaluated during winter months for evergreen characteristics. Anatomy of the leaves, stems, and roots was examined using permanent stained slides of transverse sections. Leaf clearings also were done to count stomates and trichomes, and to measure length and width of stomates. Anatomy of plants from sites in Illinois, Minnesota and Nebraska was compared to plants grown in controlled environments. Transpirational cooling of leaves with accompanying environmental conditions was measured at the HAGNP. Water potentials also were measured on leaves of *P. ludoviciana* in March, May, June and September. An isotope analysis was done to

determine if plants used C_3 or C_4 photosynthetic pathways. *Physaria ludoviciana* has several structural adaptations for sand prairie conditions including a long taproot, large root:shoot ratio and evergreen shoot characteristics as well as leaves that exhibit C_3 anatomy which also is supported by isotope analysis. Leaves were isobilateral with no evidence of water storage tissues. Stomate and trichome densities on leaves were 328 to 698 and 31 to 48 per mm^2, respectively. Leaves exhibited transpirational cooling, and water potentials ranged from -1.2 to -1.7 MPa. Understanding both structural and physiological adaptations of *P. ludoviciana* may improve our management efforts of sand prairies for survival of plants in harsh conditions.

Introduction

Physaria ludoviciana is a perennial forb that forms a rosette of linear, spatulate, basal leaves covered by thick trichomes, which gives a silvery appearance (Beach *et al.,* 2001). It is commonly found in well-drained, mobile sand in blowouts of sand prairies (i.e. vegetated sand dunes) (Coons *et al.*, 2004). Plants frequently are found on southwest-facing, steep slopes in highly disturbed areas, and often within a few miles of large rivers (Claerbout, 2003). Sand prairies have harsh environmental conditions to which *P. ludoviciana* plants have adapted. Summer temperatures are very high and soil temperatures at the Henry Allan Gleason Nature Preserve (HAGNP), Mason County, Illinois were on average 2-5°C higher compared to those at a local environmental monitoring station 25 km southwest of HAGNP (Over *et al.*, 2005). *Physaria ludoviciana* grows on a sandy soil and has low moisture due to low water holding capacity. Blowing sand due to wind also is a constant disturbance. These areas have little competition from other species, as evidenced by 62% of the area being open sand

(see chapter 5). Because there is little competition, plants receive nearly full sunlight with light intensities during summer months averaging around 1900 μmol/m^2/sec (Over *et al.*, 2005). These harsh conditions seem to create an ideal niche for *P. ludoviciana* to establish itself.

Sand prairies often create drought-like conditions, requiring plants to adapt to a water stressed environment. One of the ways that plants are able to adapt is through their morphological characteristics. Drought-tolerant plant species typically have higher root to shoot ratios, especially if they are perennial (Fitter and Hay, 2002). Plants that grow in a drought-prone environment must develop root systems quickly in order to survive the water deficit. *Boechera holboellii* is a perennial that can adapt itself to mesic, xeric and alpine habitats, and therefore in a dry environment, locally adapted genotypes of *B. holboellii* will have higher root to shoot ratios than populations from more mesic habitats (Knight *et al.*, 2006). These morphological characteristics relate to the plant's physiological ones. Looking at the ratio between photosynthesis and transpiration, water use efficiency (WUE) is useful for determining the plant strategy for dealing with drought (Fitter and Hay, 2002). Drought tolerant species typically have higher WUE if they lack deep roots that can't access water during a dry period. This physiological characteristic is attributed to xerophytes having smaller, thicker leaves, giving a higher ratio of photosynthetic mesophyll to transpiring leaf area, compared to mesophytes (Abrams *et al.*, 1994). Small, thick leaves also alter biophysical properties by tending to reduce the heat load (Fitter and Hay, 2002). Plants that are able to avoid drought typically have lower WUE, grow fast, and set seed before the onset of drought (Knight *et al.,* 2006). *Physaria ludoviciana* has adapted to growing in these very adverse

environmental conditions by developing features which may allow it to persist in these harsh, dry conditions including a basal rosette that allows leaves to warm quickly in spring. Since sandy soils typically warm quickly, this warming may allow the plants to begin photosynthesis earlier than the surrounding competition (Coons *et al.*, 2000). Having a basal rosette of leaves also reduces exposure to wind, which may reduce water loss.

Another structural characteristic that influences water use efficiency in plants is stomatal density (Woodward and Kelly, 1995). Stomata occur on all aerial parts of the plant, but are most abundant on the leaves (Esau, 1977). Stomatal initiation may be a response to light conditions, as plants developing with increased levels of light develop more stomates. Since light intensities are high near *P. ludoviciana,* stomatal densities might correspondingly be high. The variation in stomatal densities is of interest because when stomates are open to collect CO_2 for photosynthesis the plants also are transpiring and losing water. Stomatal density of *P. ludoviciana* may be another predictor for its water usage and transpiration rates.

External appendages that can aid in preventing water loss in plants are trichomes on the epidermis. Rollins and Banerjee (1975) report that trichome diversity is outstanding in *Physaria* (as *Lesquerella*) and that it may be as great or greater than any other genus in Brassicaceae. Trichomes consist of a distinct, primary axis (stalk) and two (forked) or more branches (dendritic) in Brassicaceae (Beilstein *et al.*, 2006). It is likely that these trichomes insulate the leaf mesophyll from excessive heat (Esau, 1977). According to Fahn (1986), the presence of trichomes on the leaves and young stem is commonly regarded as an adaptation associated with arid conditions. Trichomes affect

transpiration rates by influencing the water diffusion boundary layer of the transpiring leaf surface. Dense trichomes would increase boundary layer resistance by creating a humid layer of air near the leaf surface. Trichomes also may reflect some light and help decrease the temperature of *P. ludoviciana.* Therefore trichome densities can give us an idea of water usage in *P. ludoviciana.*

Plants also have physiological adaptations to deal with harsh environments. Transpiration is a mechanism that cools the plant (Taiz and Zeiger, 2002). According to Patino and Grace (2002), it is not clear whether flowering plants in the tropics or other hot environments have adaptations to prevent overheating. However they may control their temperature by evaporative cooling at the expense of water loss. *Physaria ludoviciana* might be cooling itself via transpiration, and because of its low growing stature, it is possible that less water is needed. Differences between temperatures of leaves and their surroundings can indicate if transpirational cooling is occurring.

Another physiological adaptation to deal with limited water conditions is a modification of water potential. Measurements of water potentials of plants at different times during the year, correlated with soil water content and air temperature, can give an estimate of how water stressed plants are (Over *et al.,* 2005). Another parameter that would cause fluctuations in water stress would be the depth of the soil. Moisture fluctuations are most rapid and extreme in shallow soil and comparatively slow and more moderate with greater soil depths (Cable, 1969; Noy-Meir, 1973; Schwinning and Ehleringer, 2001). In shallow soil, every rain event generates a pulse of moisture that, depending on the event size and evaporative demand of the atmosphere, can last from a few hours to many weeks (Sala *et al.,* 1989). Single events usually do not recharge the

soil below 20-30 cm, but when clustered rain events occur, the water is able to penetrate into deeper layers of soil (Sala and Lauenroth, 1982). Sand has a very low water holding capacity, so when it rains, water will not stay in the sand because of its high drainage properties. A possible adaptation of plants in a water stressed environment is the ability to lower its water potential in the root, in response to low water potential in the soil (Taiz and Zeiger, 2002), which would allow water to move into the root. The availability of water in the soil at different times of the year as well as the water potential of *P. ludoviciana* will help to understand water availability and requirements.

Another physiological parameter affected by the environment is photosynthesis which is one of the most temperature sensitive aspects of growth (Jones, 1992). Plants growing in high temperatures or low water often have modified photosynthetic pathways such as C_4 or CAM. C_3 is the dominant photosynthetic pathway of species from cool, temperate or moist habitats, whereas C_4 plants are adapted for hot environments and are common in tropical and semi-arid habitats. Plants that use a CAM pathway are generally succulents adapted to both hot and dry conditions (Jones, 1992). Evergreen leaves extend photosynthesis to cooler times of the year and might also be an adaptation to hot, dry environments (Mooney *et al.*, 1977). Knowing the photosynthetic pathway of *P. ludoviciana* would help to predict how it adapts to water stress.

Water availability is a primary factor limiting distribution and abundance of plants. Understanding the mechanisms of how plants cope with water stress is a central topic in plant physiology (Shrantz & Piemesal, 1927; Stebbins, 1952; Bohnert *et al.*, 1995; Bray 1997; Knight *et al.*, 2006). The objective of this study was to describe structural and physiological adaptations of *Physaria ludoviciana* that allow it to survive

in high summer temperature, high light and low soil moisture. Structural adaptations studied include morphology of shoots and roots; anatomy of leaves, stems, and roots; and evergreen characteristics. Physiological adaptations include transpirational cooling, water potentials, and C_3 versus C_4 photosynthesis. Knowing the biology of *P. ludoviciana* will be useful in the management of *P. ludoviciana* as well as other sand prairie species.

Material and Methods

Site Descriptions

Plant material was collected and physiological characteristics were measured at the Henry Allan Gleason Nature Preserve (HAGNP). At the preserve, *Physaria ludoviciana* grows on remnant sand dunes that are fully exposed and have been eroded by wind into distinct bowl-shaped depressions. Leaves also were collected for anatomical studies at two sites in Minnesota and one in Nebraska. Goodhue County is the only county in Minnesota where *P. ludoviciana* is found. The first location in Minnesota was the Richard J. Dorer Memorial Hardwood State Forest, Hay Creek Management Unit. In this area, *P. ludoviciana* was located on a steep slope with rocky and sandy outcrops. *Physaria ludoviciana* was concentrated in these outcrops where plants were concentrated. The second location in Minnesota of *P. ludoviciana* plants was Red Wing. Here plants were growing along exposed rocky and sandy areas on the slope of a steep bluff where the vegetation consisted of mainly grasses with some hardwoods. The single population sampled in Nebraska was in Dawes County, in Chadron, near the campus of Chadron State College. *Physaria ludoviciana* was found south of campus, on the southeast side of the water tower, where sandy, rocky outcrops were found.

Structural

Morphological Characteristics

Three larger and three smaller adjacent plants were removed near the periphery of the North Colony Lower Bowl at the HAGNP. Each larger plant had one separate, smaller plant in close association with it. Fresh masses of roots and lengths of taproots were measured. Root volumes were determined by water displacement. Root areas were determined using a Li-Cor leaf area meter (LI 3100, Li-Cor, Inc., Lincoln, Nebraska). Leaf areas were estimated using a transparent grid marked with 0.25 cm^2 squares, in order to keep the rosette structures intact. Shoot fresh masses and leaf numbers also were measured. Root:shoot ratios were calculated for root area and for shoot fresh mass. Means and standard errors were calculated for all measured parameters using Microsoft® Office Excel 2003 (11.8211.8202) SP3. Dry masses were not determined, so that voucher specimens could be prepared for deposit in the Eastern Illinois University Stover-Ebinger Herbarium, Charleston, Illinois. Duplicate herbarium specimens were sent to the Illinois Natural History Survey, Champaign, Illinois and to the Missouri Botanical Gardens, St. Louis, Missouri. Evergreen characteristics were documented with digital images taken during the winter months.

Anatomical Characteristics

Plant Material

For comparison, plants were collected from Illinois, Nebraska and two sites in Minnesota. These wild populations also were compared with plants that were grown using seed from HAGNP at Eastern Illinois University (EIU) in a controlled environment. Plants were started from seeds in Cone-tainers™ (Stuewe & Sons Inc.,

Corvallis, OR) with removable sleeves in a Conviron® (Conviron CMP 4030, Winnipeg, Manitoba, Canada) growth chamber on 7 October 2007 at 25°C as measured by readout on chamber. Light was provided with fluorescent and incandescent bulbs at 174 $\mu mol/m^2/sec$ as measured with an Apogee® Quantum meter (Logan, Utah) for 16 hour/8 hour (light/dark). They were fertilized once a week with Stern's Miracle Grow 15-30-15 (10.0 g in 8 L of water) and watered as needed. On 15 December 2007, plants were moved into a greenhouse where they remained until leaves were collected for study on 22 January 2008, when plants were 3.5 months old. One leaf from each of three plants and one whole plant were collected in June 2007 from each of the field sites and in January 2008 from EIU.

Histology

Parts were fixed in FAA (0.5 part 40% formalin, 0.5 part glacial acetic acid, and 9 parts 70% ethyl alcohol) for one week, and then stored in 70% ethyl alcohol. Transverse sections were made of unembedded leaves, roots and stems (when available) using a sliding microtome at a thickness of 30-90 μm. Sections were stained using safranin and Heidenhain's iron-alum hematoxylin, dehydrated in a graduated ethanol series, followed by clearing in limonene (Citrisolv™, Fisherbrand®, Pittsburg, Pennsylvania) as modified from Carlsward *et al.* (1997). Sections then were permanently mounted on microscope slides with Canada balsam. Slides were dried on a slide warmer at 50°C for 1 month. Observations were made using a compound microscope (Zeiss Axioskop 40, Germany) with attention for type of photosynthetic pathway, evidence of water storage tissue, secondary tissue, palisade layers and trichomes. Digital photographs were taken of

leaves, roots and stems (Pixera Pro 150 and software Pixera Viewfinder, West Chester, PA), and image quality was adjusted in Adobe Photoshop CS2 (San Diego, California).

Stomatal and Trichome Densities

Abaxial leaf epidermal preparations were made from three different leaves at each site. An emery board first was used to remove the dense layer of trichomes. Leaves were soaked in bleach for 1 hour, rinsed with de-ionized water, and stained overnight in 50% safranin solution. Leaf sections were dehydrated in a graduated ethyl alcohol series, followed by clearing in limonene. Sections then were permanently mounted on microscope slides with Canada balsam. Slides were placed on a slide warmer at 50°C for 1 month. Permanent slides were viewed using a Zeiss Axioskop 40 microscope, and images were taken using a Pixera Pro 150 digital camera. Stomata and hair bases were counted on 4 April 2009 at 10 random sections on each leaf at 400X. Area of view was measured using ImageJ (Rashband, 2009). Means and standard errors were calculated for areas within a leaf using Microsoft® Office Excel 2003 (11.8211.8202) SP3. An analysis of variance followed by a Duncan's multiple range test at the 5% level and a nested analysis of variance were done using SPSS (Version 16 for Windows). Dimensions of ten pairs of guard cells were measured on one leaf from each location using ImageJ. Means and standard errors were calculated for length and width as well as an analysis of variance followed by a Duncan's multiple range test at the 5% level.

Physiological

Transpirational Cooling

Transpirational cooling was estimated by comparing leaf versus soil surface temperatures on 9 different days at HAGNP and once at a single population in

Minnesota. An infrared thermometer (Mannix™, Model # IRT2, New York, New York) was used to compare temperatures of leaf versus soil surface next to plant at ten random spots. Leaf temperatures were taken in the center of a rosette. Average temperatures were calculated for soil, leaf and the difference between the two. To document the environment when transpirational cooling was estimated, several other parameters were measured. Soil moisture was measured using a scale of 1 to 10, which was calibrated using a calibration curve, with one reading using a soil moisture probe (Lincoln Irrigation, Inc., Lincoln, Nebraska) at a depth of 20 cm. Soil temperature at a depth of 12 cm was measured with one reading using a soil thermometer (Taylor®, Model 9841, Oak Brook, Illinois). Using an Apogee® quantum meter with a strip containing four sensors (Model BQM, Logan, Utah), the light intensity was measured for 1 minute to obtain a range from high to low. A light strip was placed on top of sand where there was minimal vegetation. Relative humidity was measured with a thermo-hygrometer (Mannix™, Model PTH8708, New York, New York) with one reading taken per visit. Wind speed was measured using a mini thermo-anemometer (Extech Instruments, Model 45118, Waltham, Massachusetts) with readings taken for 1 minute to obtain a range from high to low. Air temperature was measured for 1 minute to record a range from high to low with a thermo-hygrometer (Mannix™, Model PTH8708, New York, New York). Differences between soil surface and leaf surface temperatures were plotted against each environmental parameter for indications of how transpirational cooling related to each parameter. A Pearson Correlation using SPSS was calculated showing the relationship between each environmental parameter and differences between soil surface and leaf surface temperature.

Leaf Water Potential

Leaf water potential was measured on six leaves each from a different plant located in the largest colony (North Colony Lower Bowl) at HAGNP using a pressure bomb (Soil Moisture Equipment Corporation, 3005-Series, Santa Barbara, California). Leaves selected were intermediate in age, i.e. not the youngest or the oldest. These measurements were taken between 11:30-13:30 on 31 March 2006, 11 May 2004, 21 June 2006, and 2 September 2004. Soil temperature and moisture were obtained on these same days from a datalogger (HOBO Weather Station by Onset Computer, Bourne, Massachusetts) with soil temperature and moisture sensors (ECH2O by Decagon Devices, Pullman, Washington) at two depths (17 and 45 cm) installed near the center of the same *Physaria ludoviciana* colony. Also on these days, air temperatures were obtained from a weather station at Kilbourne (15 miles southwest of HAGNP). Means and standard errors of water potentials were calculated. A Pearson Correlation was done using SPSS for water potential compared to soil temperature and to soil water content at two depths.

Isotope Analysis

Three leaves from each of five different randomly selected plants were collected at HAGNP in April 2007. Leaves from each plant were placed in coin envelopes and air dried. Elemental analysis-isotope ratio mass spectrometry (EA-IRMS) was performed to obtain isotope ratios of leaves and determine the photosynthetic pathway that plants were utilizing (C_3, C_4, or CAM). Isotope analysis was done in the Stable Isotope Laboratory at the University of Georgia's Savannah River Ecology Laboratory. Samples were loaded in the autosampler of a Carlo Erba Elemental Analyzer (NA 1500) attached to a

continuous flow isotope ratio mass spectrometer (Finnigan Delta C). Stable isotope ratios are reported in per mil units (‰) using standard delta (δ) notation, where X is ^{13}C and R_{sample} and $R_{standard}$ are the corresponding ratios of heavy to light isotopes ($^{13}C/^{12}C$) in the sample.

$$\delta X = [R_{sample}/R_{standard})-1] \times 10^3$$

Measurements were calibrated against reference standards (powdered dogfish muscle) of known ^{13}C. The standard deviation for replicate measurements of standards was < 0.2‰.

Results

Structural

Morphological Characteristics

As plants grew larger, their roots increased in length with larger plants having roots up to 46 cm long (Table 4.1). In comparing smaller plants to larger plants only a 51% percent increase in length of the main root occurred. Whereas comparing leaf number we see an increase of over 200% from smaller plants compared to larger plants. Early in development, plants are putting the majority of their energy into the formation of root. This allocation corresponds to the larger root to shoot ratio in smaller plants (0.5) compared to larger plants (0.3). Root area, volume and fresh masses increased around 300% in larger plants compared to smaller plants. Relative to shoot development, larger plants had an average of 73 leaves per plant compared to smaller plants with an average of 23 leaves per plant. Leaf area increased over 200% and shoot fresh mass increased over 1000% in larger plants compared to smaller ones. *Physaria ludoviciana* plants developed more root growth early and then shoot growth suggesting that more allocations

were put into roots than shoots during early growth relative to later growth. Plants demonstrated an evergreen appearance as evidenced by Figure 4.1.

Anatomical Characteristics

Physaria ludoviciana exhibits a C_3 or Calvin-Benson pathway for photosynthesis and lacks any of the C_4 characteristics such as Kranz anatomy (Esau, 1977) as seen in transverse leaf section (Figure 4.2). In the leaf mesophyll there were few organelles and rather small chloroplasts, making the cells appear empty and clear. There was also a lack of an orderly arrangement of mesophyll cells around the bundle sheath, and the bundle sheath itself was composed of small mesophyll cells with inconspicuous chloroplasts. The spatial association between the mesophyll and the vascular bundles also were not close, being indicative of C_3 plants compared to C_4 plants (Figure 4.2). Distinctly developed palisade layers are present on both the upper and lower surfaces of the leaf. Numerous dendritic trichomes are present on both surfaces of the leaf section. They are in several layers, and have prominent tubercles (wart like projections) that increase in size towards the center of the trichome (Figure 4.3).

No water storage tissues are apparent in either roots or stems (Figures 4.4 and 4.5). Root sections show primary and secondary xylem and phloem and a cork cambium that is neatly organized, as well as large intercellar spaces in the cortex. More secondary growth was present in roots from plants that were grown in the greenhouse compared to plants collected in the field (Figure 4.4). The stem sections showed limited secondary growth with large air spaces in the cortex and lateral branches (Figure 4.5).

Stomatal and Trichome Densities

Range of stomatal densities on the abaxial leaf surface varied between sites and within sites (Table 4.2). Trichome densities did not vary within leaves from the same site, but differences were found between sites (Table 4.2). Plants grown in the controlled environment had the lowest stomatal densities. Trichome densities were similar in all plants grown in the controlled environment, Dawes County (Nebraska), Hay Creek (Minnesota), and Mason County (Illinois). Figures 4.6 and 4.7 are leaf epidermal preparations of *Physaria ludoviciana*. In Figure 4.6, trichomes were removed for stomata counts. Hair buttresses are evident where trichomes were. Figure 4.7 shows a staining where trichomes were not removed.

Table 4.3 shows length and width of stomatal guard cell pairs. Stomates open and close at random and therefore length is a better indicator of the size of the stomates than width if counting both open and closed complexes. Illinois was significantly lower than other sites.

The stomates lacked subsidiary cells and were anomocytic (Figures 4.6 & 4.7). Stomates were found on both the adaxial and abaxial surfaces, but stomatal densities were calculated on the abaxial surface only. Figure 4.8 shows stomata on the upper surface.

Physiological

Transpirational Cooling

Temperature differences between soil surfaces and leaves demonstrated transpirational cooling. The maximum leaf temperature observed was in September at 34.7°C. Leaf temperatures ranged from -4.2 to 34.7°C. Leaf temperatures were usually

cooler than soil temperatures, except in January when the leaf temperature was 4.2°C warmer (Table 4.4). Figure 4.9 shows the fluctuation in temperature differences over different months. With increases in soil moisture and wind speed, less transpirational cooling occurred (Figure 4.10). With higher soil temperature, higher light intensity and higher air temperature, more transpirational cooling occured (Figure 4.10). However relative humidity had little effect on transpirational cooling (Figure 4.10). Although trends were apparent between cooling and environmental parameters, Pearson correlations showed no significant correlation between differences in leaf and soil surface temperature, relative to soil temperature, light intensity and air temperature. Transpirational cooling appears to be effective for *P. ludoviciana* to stay cool in this water stressed environment.

Leaf Water Potential

Leaf water potentials were significantly higher when sampled in March and May than they were in June and September ($p=0.0$). These higher water potentials in March and May were in spring when rainfall is greater. Whereas in June and September, vegetation is drier and rainfall is less. Water potentials were higher when soil water content was higher at 17 cm and when soil or air temperatures were lower (Table 4.5). Figure 4.11 shows that with lower water potentials in June and September, soil water content was lower at 17 cm, but not always at 45 cm, relative to values in March and May. The Pearson correlation did not show a significant correlation between water potential and soil water content. With lower water potentials, soil temperatures are higher (Figure 4.11). Significant differences were not found between water potential and air temperature.

Isotope Analysis

Plants having a C_3 pathway of photosynthesis also was supported by isotope analysis. C_3 and C_4 plants possess distinctly different $^{13}C/^{12}C$ ratios. *Physaria ludoviciana* carbon ratio averages were -29.48 ± 0.35. Typically C_3 plants have a range of -25 to 29% (Lambers *et al.*, 2000).

Discussion

Structural

Morphological Characteristics

Initially, plants put a lot of energy into the development of roots, which is evident from the secondary growth in the roots compared to the stem and from the root to shoot ratios in smaller versus older plants. This response is typical of plants adapted to soils with low water and/or nutrients and high transpirational demands. *Physaria ludoviciana* has a long taproot which is able to penetrate the soil more deeply than branched roots. This long taproot also could help with anchorage in a blowing sand environment. Once roots are developed sufficiently to reach lower regions in the soil profile containing water and nutrients, *P. ludoviciana* plants appear to increase allocations into shoot development to maximize competition for light and to photosynthesize. Overall, roots do not appear to be storing any water, as they are rather slender, very deep and have no water storage tissues. Deep taproots are a good survival strategy because even if enough water is in the shallow part of the soil during pulse rainfall events, it will not compensate for deeper ground water which will not be depleted as quickly as shallow soil moisture (Schwinning and Ehleringer, 2001). Since no evidence of water storage tissues in the roots was found, the taproot may be long enough to reach groundwater when the plant needs water.

Branched roots would not be able to penetrate the soil as deep as a taproot. Having a long taproot that is able to access water also could be a reason why no water storage tissues were seen in the stems or roots.

Growth of the shoots and roots are strictly interconnected as shoots depend on roots for water, minerals and hormones while roots depend on leaves for carbohydrates and hormones (Tonutti and Giulivo, 1990). The larger root to shoot ratio in the smaller plants compared to larger plants is a good strategy for plants establishing themselves when grown in well-drained soil. Drought-tolerant species typically have higher root to shoot ratios because they grow in drought-prone environments and must develop deep root systems quickly to uptake the water needed for survival (Knight *et al.*, 2006). A large root to shoot ratio means that the plant is producing more biomass as roots below ground than shoots above ground. Other observed traits of plants in a water stressed environment that *Physaria ludoviciana* demonstrates included clump growth (to retain moisture) and growing low to the ground.

Evergreen characteristics were observed in January when snow was on the ground. By mid-March, flower buds were observed on plants in the field, suggesting their initiation from the previous year. Evergreen characteristics would be a survival strategy for *Physaria ludoviciana* because it extends photosynthesis into cooler seasons, so that *P. ludoviciana* is able to flower and fruit when more water and lower temperatures are present. Since it is perennial and evergreen, it does not need to spend resources generating all new leaves each year.

Anatomical Characteristics

C_3 or the Calvin-Benson pathway is the most common photosynthetic cycle characterized by the three-carbon compound 3-phosphoglyceric acid as the first stable product of photosynthesis (Taiz and Zeiger, 2002). Based on leaf anatomy and isotope analysis, *Physaria ludoviciana* uses C_3 and not C_4 photosynthesis. Because *P. ludoviciana* has flowers and fruits early in the year when temperatures are lower, C_3 photosynthesis would be more effective at these lower temperatures.

The leaf anatomy of *Physaria ludoviciana* is helpful in understanding its survival strategies in a xeric environment. *Physaria ludoviciana* has a palisade layer on both the top and the bottom sides of the leaf, allowing for excess light to be absorbed on both surfaces (Taiz and Zeiger, 2002).

Stomatal and Trichome Densities

Stomatal density varied both within and between leaves at the same site as well as at different sites. According to Esau (1977), stomatal frequencies vary greatly, even on different parts of the same plant. The variation we observed might be due to developmental age of the leaf, leaf position on the plant, habitat or small sample size. Leaves were selected randomly from plants and we did not record leaf age or leaf position. Plants grown in the controlled environment and at Red Wing, Minnesota had the lowest number of stomates. The controlled environment plants were well watered and temperatures were controlled so *Physaria ludoviciana* may not have needed as much cooling. Whereas in the field, temperatures are not controlled and more transpirational cooling would be needed. High stomatal densities seen at most field sites could be a result of the need for transpirational cooling. Hay Creek, Minnesota had the highest

number of stomates. At Hay Creek, *P. ludoviciana* was found on a very steep slope of a bluff where the soil consisted of mostly sand. Since the site was a steeper hill it probably had low water holding capacity with higher drainage and greater water runoff as compared to other field sites where the inclines were more gradual. Higher numbers of stomates presumably result in increased transpirational cooling. Sample size was another factor that could have played a role in the density variation we observed. Three leaves per site were chosen and ten different sections on each leaf were counted. This sample size might have been too small. In future studies, perhaps more samples should be observed. However, variation between stomatal densities is common in this family. On the lower epidermis, some species of *Brassica* exhibit variation. *B. oleracea* had ranges from 185/mm^2 to 411/mm^2, and *B. rapa* had ranges from 252/mm^2 to 364 mm^2 (Pant and Kidwai, 1967).

Stomatal development and size are affected by environmental conditions (Jones, 1992). The wide variation in stomatal density and dimensions could be attributed to the microclimate conditions on a particular spot of the leaf. Stomatal characters such as density are affected by a number of growth conditions including light intensity (Gay and Hurd, 1975; Poole *et al.*, 2000), water availability (Gindel, 1969), nutrient availability (Poole, *et al.*, 2000), CO_2 concentrations (Woodward and Kelly, 1995), leaf age (Davis *et al.*, 1977), humidity, temperature and pollutants (Jones, 1992). Since water availability affects stomatal densities, we would expect to see the variation between plants from different areas depending on moisture levels. Intrinsic stomatal variation as a response to external differences over a leaf surface could be attributed to uneven guard cell differentiation, uneven expansion of the epidermal cells after differentiation, or a

combination of the two (Poole *et al.*, 1996). Pant and Kidwai (1967) measured length and width of guard cells in five different *Brassica* species, and found a range of sizes from 21-29 μm and 7.5-10 μm, respectively.

Stomatal densities were only counted on the lower surface, however stomates were present on the upper surface making leaves amphistomatic. Other genera in Brassicaceae (as Cruciferae) studied by Pant and Kidwai (1967), particularly *Brassica* are amphistomatic and have many more stomata on the lower surface than on the upper surface. In some *Brassica* species, stomates on the upper surface ranged from 83-220/mm^2 whereas the lower surface densities averaged 163-316/mm^2. In Asteraceae, *Ambrosia cordifolia* had stomatal densities of 133 and 390/mm^2 for the upper and lower surfaces, respectively (Mott *et al.*, 1982). According to Mott *et al.* (1982), amphistomaty in plants serves to increase the maximum leaf conductance to CO_2. Plants that have a high photosynthetic capacity and live in open areas with full sun that experience rapidly fluctuating or continuously available water are identified as deriving an adaptive advantage from high maximum leaf conductance (Mott *et al.*, 1982). Species with the highest known photosynthetic rates (plants with C_4 photosynthesis) all have amphistomatic leaves. Amphistomatic species are successful in full-sun, high light habitats (Mott *et al.*, 1982), which are similar to habitats where *Physaria ludoviciana* is found.

Stomates have an important role in photosynthesis and transpiration. Photosynthesis is dependant on stomatal complexes for its supply of CO_2 which enters the leaf through the stomata. Therefore stomatal size and density play an influential role on photosynthetic rates via stomatal conductance (Poole *et al.*, 2000). When guard cells

are open, collected CO_2 comes with a price as plants also lose water due to transpiration. Transpiration is the evaporation of water from the surface of leaves and stems (Taiz and Zeiger, 2002). Stomates tend to open as the temperature increases (Jones, 1992), as plants need to be kept cool through transpiration and evaportranspiration while at the same time be conservative as to how much water they can lose especially if water availability is limited. To meet the contradictory demands of maximizing CO_2 uptake while limiting water loss, plants have evolved adaptations to control water loss from leaves and to replace water lost to the atmosphere (Taiz and Zeiger, 2002). Plant temperatures were less than sand surface temperatures, and therefore transpirational cooling is occurring. The many stomates on the epidermis of *Physaria ludoviciana* aid in keeping it cool in this water stressed, high temperature and high light environment.

Trichomes are highly variable appendages of the epidermis. They occur on all parts of the plant and may persist through the life of the plant or may fall off early in development (Esau, 1977). Significant differences were found between trichome densities within the site in the nested analysis and between sites with the one way ANOVA. However significant differences were not found in the nested analysis comparing sites. The variation in trichomes could be caused by the same factors as discussed for stomates: developmental age of leaves, leaf position on the plant, habitat and the small sample size.

Trichomes protect the leaf meosphyll from excessive heat, by reflecting light (Wolpert, 1962; Esau, 1977; Fahn, 1986). Finding more trichomes on leaves at the Red Wing, Minnesota site than in the controlled environment (with lower light) was not surprising. Without trichomes, *Physaria ludoviciana* could overheat when light

intensities are high and water is limited, as was seen at the field sites. Having a greater number of trichomes also would impact transpirational cooling. Trichomes are an adaptation associated with arid conditions, as they affect transpiration by influencing the water diffusion boundary layer of the transpiring leaf surface (increasing boundary later resistance).

Physiological

Transpirational Cooling

Deep soil moisture is governed primarily by longer, seasonal weather patterns (Fernandez and Caldwell, 1975; Reynolds *et al.,* 1999). Often maximal recharge of soil water will occur in spring and greatest depletion in late summer (Schwinning and Ehleringer, 2001). On dates with less soil moisture (18 June and 13 Septemeber 2007, Table 4.4), larger differences between leaf and soil surface temperatures were observed relative to dates when soil moisture was higher. In summer months, when sand temperatures are hotter than leaf temperatures, evidence of transpirational cooling was found. When air temperatures are higher in the summer months, a bigger difference is seen in sand versus leaf temperatures than at times when air temperatures are lower. Given the high summer temperatures and the low moisture of the sandy environment, transpiration is evident by cooling of leaves. The cooling of leaves that occurs when a plant is transpiring appears to be a very important factor keeping *Physaria ludoviciana* cool in the hot, dry conditions of the sand prairie. In January 2008, soil surface temperatures were lower than leaf temperatures. This difference may indicate that the plant is generating heat via respiration and is able to stay warmer than the soil, and hence may be actively metabolizing in January.

Leaf Water Potential

With water potentials ranging from -1.15 to -1.67 MPa, plants appear to be in water stress. According to Taiz and Zeiger (2002) plants are considered under mild water stress when water potential is between -0.8 to -2.0 MPa. Water potential often is used as a measure of the water status of a plant. According to Larcher (1995), the minimal water potential values of assimilative organs of plants in periodically dry regions range from -3.5 to -8.0 MPa, and plants that are xerophytes range from -0.8 to -8.0 MPa. Plants are seldom fully hydrated, due to transpirational water loss to the atmosphere. Water transport in plants is a passive process, where plants only absorb water when the plant water potential is less than the soil water potential. Since the water potentials are low, if soil moisture is available, the plant will absorb it. Comparing plant water potential of *Physaria ludoviciana* to soil water content, lower water potentials often correspond to less moisture in the soil. June and September are hotter, drier months than March and May. During the spring (March and May), more rain and so more moisture is available in the soil. This spring season is when *P. ludoviciana* is more hydrated compared to June and September. Cell growth and expansion are the processes most affected by water deficit. March and May are when *P. ludoviciana* is actively growing reproductive structures, and therefore more water is needed during that time. Comparing water potential to soil temperature at two depths, lower water potentials were found with higher soil temperatures. With water potentials ranging from -1.15 to -1.19 MPa, it seems likely that if moisture were in the soil from a rainfall event, *P. ludoviciana* would absorb it.

Summary

Living in a water stressed environment may have drawbacks even with the adaptations that *Physaria ludoviciana* has. In a study on *Physaria* (*Lesquerella*) *fendleri* in which irrigation was reduced to a biweekly application during mid-flowering and during seed formation and ripening, very low seed counts were produced (Hunsaker *et al.*, 1998). Excessive water stress of *P. ludoviciana* may result in a decrease in its survival and population size. Stress-resistance traits frequently increase plant survival, but often result in diminished growth and reproductive output under non-stressed conditions (Maun, 1981).

A mechanism that *Physaria ludoviciana* employs is known as drought escape or drought avoidance (Taiz and Zeiger, 2002). In this mechanism, the plant completes its life cycle during the wet season in order to avoid a shortage of water. By *P. ludoviciana* flowering in the spring and setting seed in the early part of the summer, it avoids the hottest time of the year. *Physaria ludoviciana* has long roots, dense trichomes, and a small stature that help it avoid drought. It is also evergreen, so it is able to photosynthesize for extended times of the year when conditions are not hot and dry.

Plants grown in a variety of habitats show different morphological and anatomical characteristics that can be interpreted as evolutionary adaptations to conditions specific to that habitat. The availability of water is one of the more important factors affecting the survival of plants. The sand prairies where *Physaria ludoviciana* resides have high light, high temperature, and low moisture, so plants in these areas must adapt to these conditions. *Physaria ludoviciana* has developed many adaptations to cope with this environment. Morphological characteristics include a low-growing rosette of leaves, a

long tap root, and an evergreen life history. It also has a large root:shoot ratio, signifying that energy is put into development of the root, before development of the shoots. *Physaria ludoviciana* has several strategies for xeric sand prairie conditions, including very dense stomates (328 to 698/mm^2), dendritic trichomes (31 to 48/mm^2) on the leaves, and an isobilateral mesophyll. However, it is a C_3 plant with no evidence of water storage tissues. In addition to the large root:shoot ratio, the ability of the plant to cool by transpiration is a very important adaptation to a xeric environment. Understanding the adaptations of *P. ludoviciana* to extreme xeric environmental conditions will aid in the understanding of other sand prairie species.

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Table 4.1. Morphological characteristics for larger and smaller plants of *Physaria ludoviciana* from the Henry Allan Gleason Nature Preserve in Mason County, Illinois.

	Smaller Plants	Larger Plants	% Increase
Length of Main Root (cm)	30.4 ± 5.0[a]	45.9 ± 7.3	51
Root Area (cm^2)	3.3 ± 1.2	12.8 ± 3.6	288
Root Volume (cm^3)	1.0 ± 0.5	4.3 ± 0.9	330
Root Fresh Mass (g)	0.8 ± 0.3	3.6 ± 1.2	350
Leaf Number	23.3 ± 4.5	72.7 ± 27.2	212
Leaf Area (cm^2)	19.1 ± 2.6	61.8 ± 25.8	224
Shoot Fresh Mass (g)	1.6 ± 0.6	17.8 ± 9.8	1013
Root:Leaf (Area)	0.2 ± 0.0	0.2 ± 0.1	
Root:Shoot (Fresh Mass)	0.5 ± 0.1	0.2 ± 0.1	

[a] means ± standard errors

Table 4.2. Stomate and trichome densities on the abaxial surface of *Physaria ludoviciana* plants grown in different locations.

	Stomates/mm^2			Trichomes/mm^2		
Site	**Mean**	**Minimum**	**Maximum**	**Mean**	**Minimum**	**Maximum**
Controlled Environment	328.9 ± 11.2 c[a, b, c, d, e]	242.9	485.8	31.0 ± 3.0 b[f, g, h]	0.0	81.0
Dawes County, NE	405.8 ± 26.3 b	242.9	823.2	35.1 ± 3.2 b	0.0	67.5
Hay Creek, MN	698.1 ± 36.6 a	323.9	1052.6	39.5 ± 4.2 ab	13.5	108.0
Mason County, IL	428.2 ± 16.9 b	242.9	647.8	37.4 ± 2.2 b	27.0	67.5
Red Wing, MN	376.1 ± 17.5 bc	188.9	580.3	48.2 ± 2.8 a	27.0	67.5

[a] means ± standard errors
[b] one way ANOVA for site: df=4, F=38.2, p=0.000
[c] nested ANOVA for sites: df=4, F=6.4, p=0.008
[d] nested ANOVA for leaves within site: df=10, F=9.6, p=0.000
[e] means within a row followed by different letters are significantly different based upon Duncan's multiple range test at p=0.05 level
[f] one way ANOVA for site: df=4, F=4.1, p= 0.004
[g] nested ANOVA for sites: df=4, F=1.4, p=0.390
[h] nested ANOVA for leaves within site: df=10, F=4.4, p=0.000

Table 4.3. Dimensions of guard cell pairs on the abaxial surface of *Physaria ludoviciana* plants grown in different locations.

	Length (µm)			Width (µm)		
Site	**Mean**	**Minimum**	**Maximum**	**Mean**	**Minimum**	**Maximum**
Controlled Environment	19.8 ± 0.6 a[a, b]	15.8	21.8	15.5 ± 0.6 b	11.0	18.0
Dawes County, NE	21.1 ± 0.4 a	18.2	22.7	17.7 ± 0.5 a	14.4	19.8
Mason County, IL	17.7 ± 0.4 b	15.0	19.0	14.0 ± 1.1 bc	9.6	20.1
Red Wing, MN	20.4 ± 0.5 a	17.1	22.3	13.0 ± 0.4 c	10.1	15.5

[a] means ± standard errors

[b] means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level

Table 4.4. Estimation of transpirational cooling with various environmental conditions for populations of *Physaria ludoviciana* in Illinois and Minnesota.

Date	Surface Soil Temperature (°C)	Leaf Temperature (°C)	Difference[a] (°C)	Soil Moisture[b] (%)	Soil Temperature (°C)	Light Intensity (µmol/m^2/sec)	Relative Humidity (%)	Wind Speed (m/s)	Air Temperature (°C)
Mason County, IL									
4/27/2007	28.5 ± 0.5	22.5 ± 1.5	6.0	ND[c]	23.6	1709 - 1971	ND	ND	22.5
6/4/2007	31.1 ± 0.4	27.3 ± 0.4	3.8	30	25.1	1335 - 1385	72.5	0.0-2.1	28.0-28.9
6/18/2007	51.1 ± 0.5	33.9 ± 0.9	17.2	10	31.3	1370 - 1542	52.7	0.8 - 1.3	31.0 - 31.6
9/13/2007	44.5 ± 0.8	34.7 ± 0.7	9.8	10	31.1	1492 - 1730	35.5	0.8 – 3.0	ND
10/16/2007	30.1 ± 0.8	24.4 ± 0.7	5.7	25	21.2	662 - 1345	47.6	1.6 - 3.4	20.2-21.8
1/22/2008	- 8.4 ± 0.5	- 4.2 ± 0.5	-4.2	20	0.6	ND	47.7	5.8-7.2	ND
4/30/2008	36.5 ± 1.4	27.9 ± 1.6	8.6	20	18.7	939 - 1110	30.6	0.3-15.4	23.7
6/26/2008	37.6 ± 1.1	31.3 ± 0.7	6.3	55	32.1	1508 - 1581	55.8	1.1-1.6	27.8
11/4/2008	21.2 ± 0.5	21.0 ± 0.9	0.2	35	20.1	504 - 555	30.4	3.6-6.0	24.4
Hay Creek, MN									
6/8/2007	43.2 ± 1.3	30.5 ± 1.3	12.7	15	26.6	1877 - 1885	26.5	0.5 - 2.6	21.7 - 24.3

[a] average soil temperature minus average leaf temperature

[b] soil moisture probe (Lincoln Irrigation, Inc., Linclon, Nebraska)

[c] ND=no data

Table 4.5. Water potential of *Physaria ludoviciana* plants from Henry Allan Gleason Nature Preserve in Mason County, Illinois taken at midday in March, May, June and September.

Date	Water Potential (-MPa)	Soil Water Content[a] (m^3/m^3)		Soil Temperature[a] (°C)		Air Temperature[b] (°C)
		17 cm	45 cm	17 cm	45 cm	
March 2006	1.19 ± 0.03 a [c, d]	0.0995	0.0776	12.8	9.9	15.1
May 2004	1.15 ± 0.03 a	0.0637	0.0525	23.4	21.3	12.5
June 2006	1.67 ± 0.10 b	0.0464	0.0510	29.1	26.9	28.2
September 2004	1.58 ± 0.22 b	0.0616	0.0582	26.7	25.3	21.9

[a] datalogger (HOBO Weather Station by Onset Computer, Bourne, Massachusetts) installed at Henry Allan Gleason Nature Preserve, Mason County, Illinois

[b] Kilbourne Weather Station 25 km southwest of HAGNP

[c] means ± standard errors

[d] means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level

Figure 4.1. *Physaria ludoviciana* at Henry Allan Gleason Nature Preserve, Mason County, Illinois in January 2008 and March 2004.

Pictures Courtesy of Janice Coons.

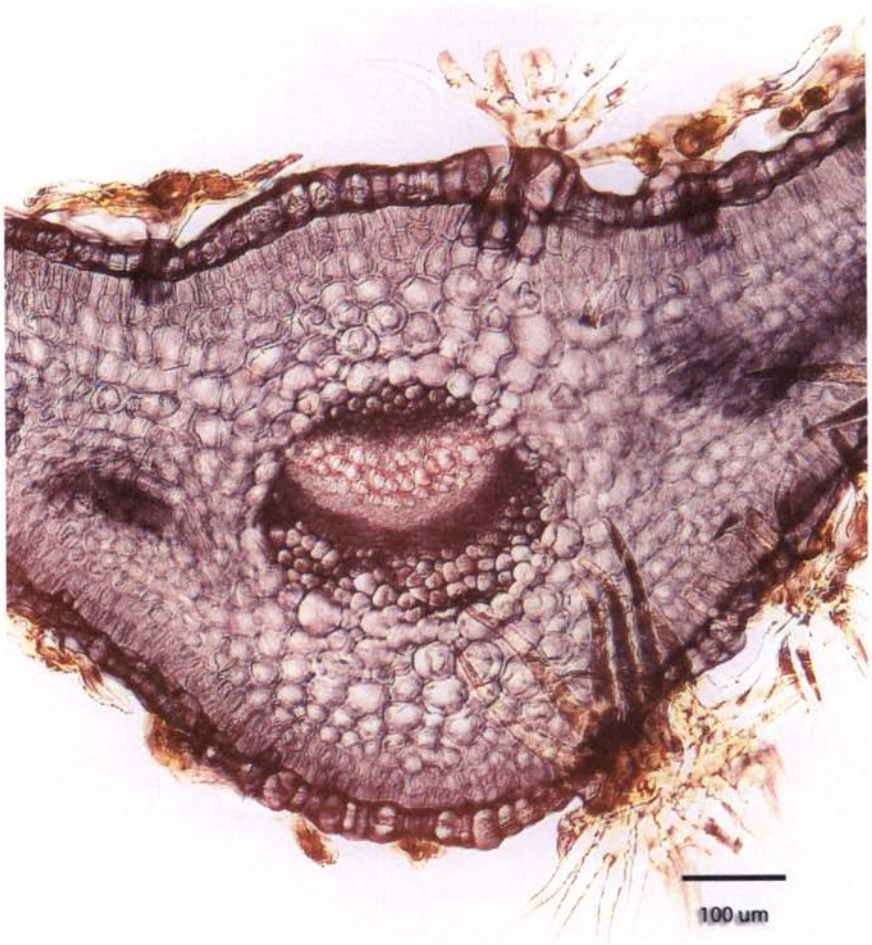

Figure 4.2. Leaf cross section of *Physaria ludoviciana* from Henry Allan Gleason Nature Preserve, Mason County, Illinois showing dendritic trichomes, C_3 anatomy and palisade layers on both surfaces.

Figure 4.3. Trichome of *Physaria ludoviciana* with tubercles present.

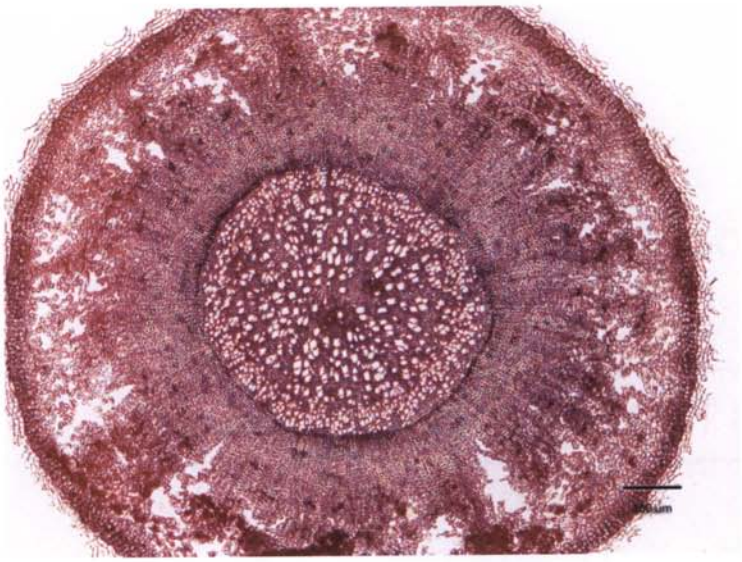

Figure 4.4. Root cross section of *Physaria ludoviciana* (3.5 months old) from controlled environment showing secondary growth.

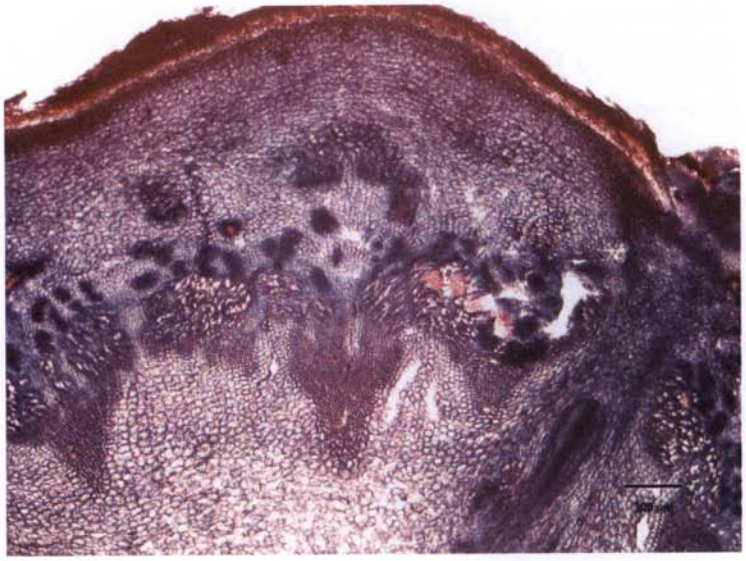

Figure 4.5. Stem cross section of *Physaria ludoviciana* from Hay Creek, Minnesota, showing secondary growth with air spaces and branches forming.

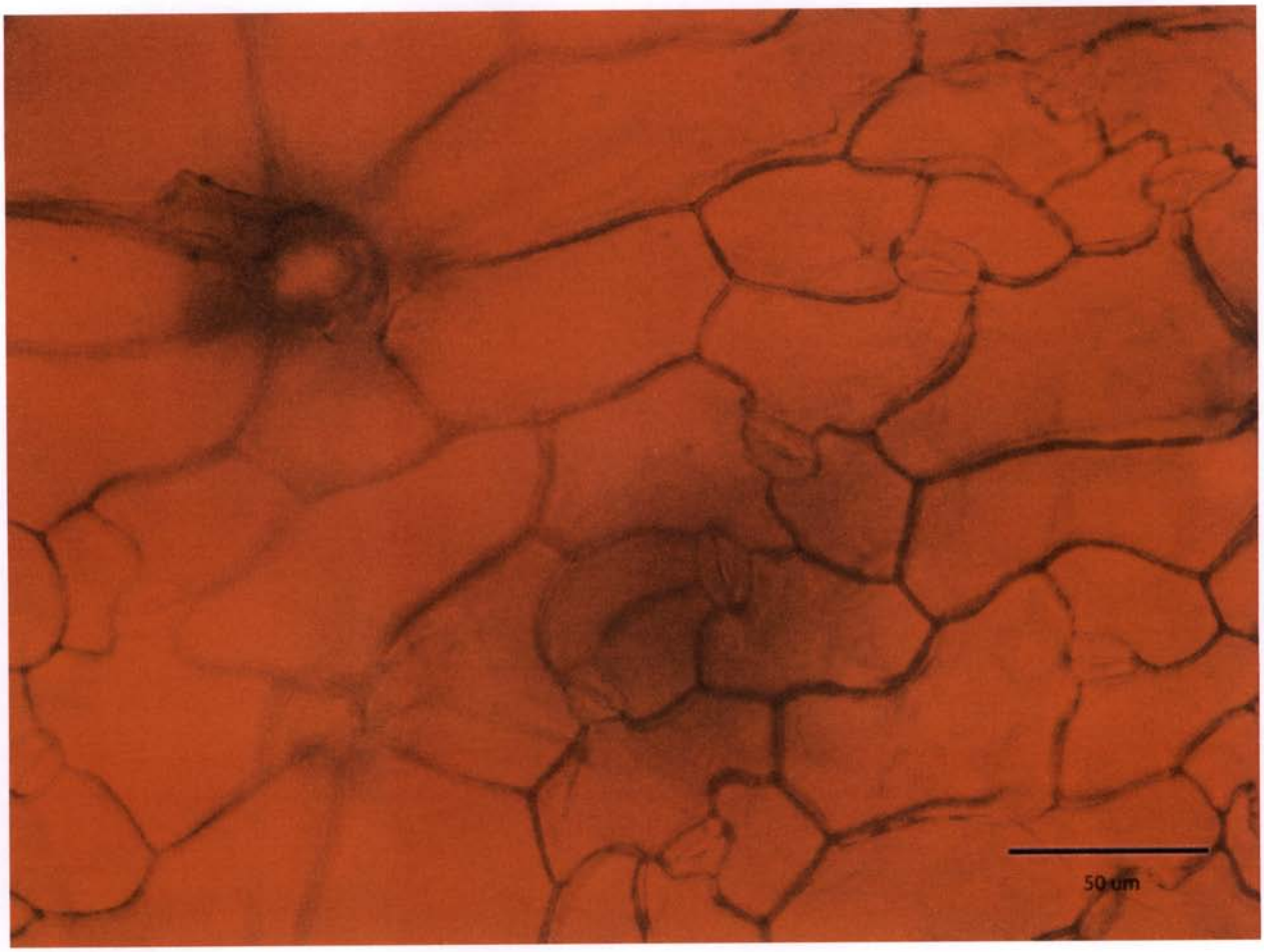

Figure 4.6. Clearing of abaxial leaf surface of *Physaria ludoviciana* from Red Wing, Minnesota showing hair buttresses where trichomes were.

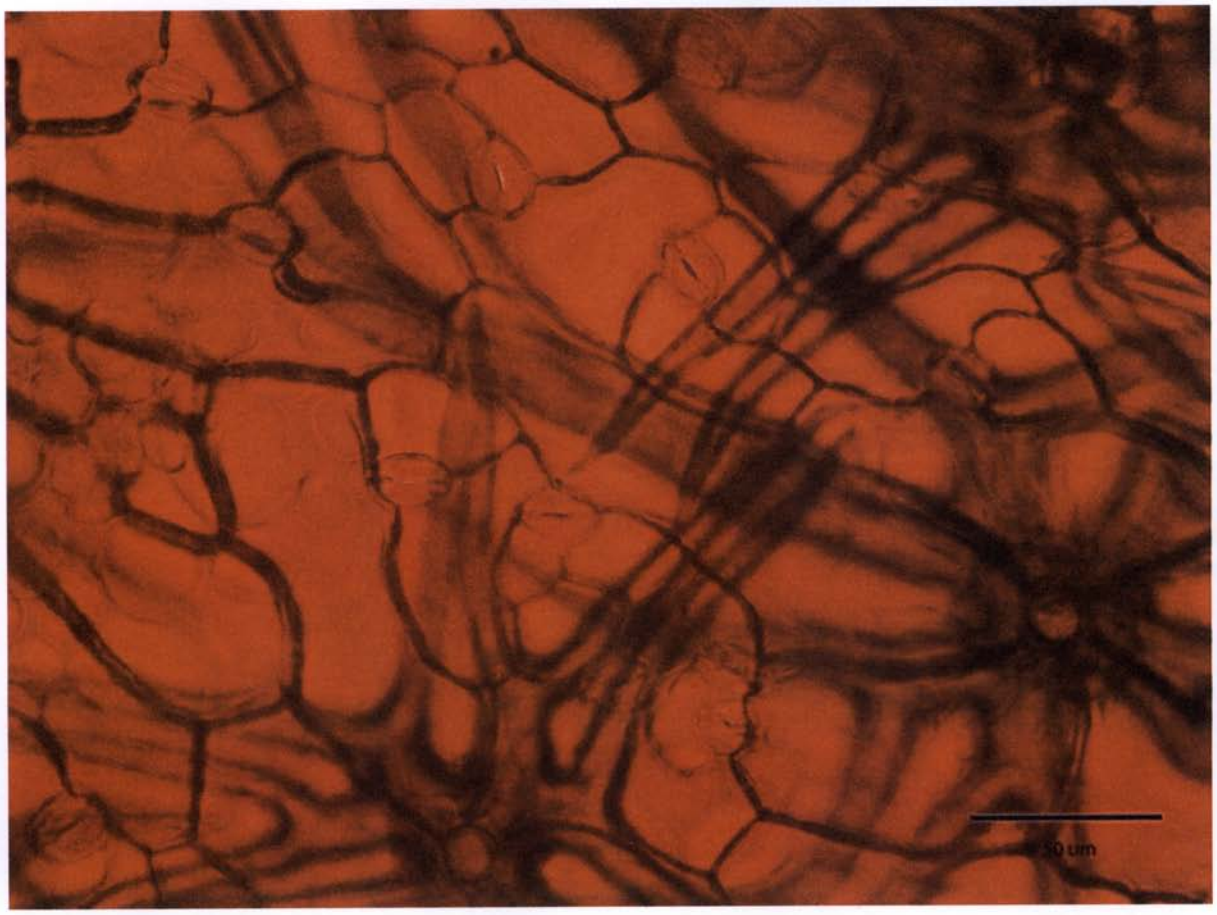

Figure 4.7. Clearing of abaxial leaf surface of *Physaria ludoviciana* from Red Wing, Minnesota showing staining where trichomes were not removed.

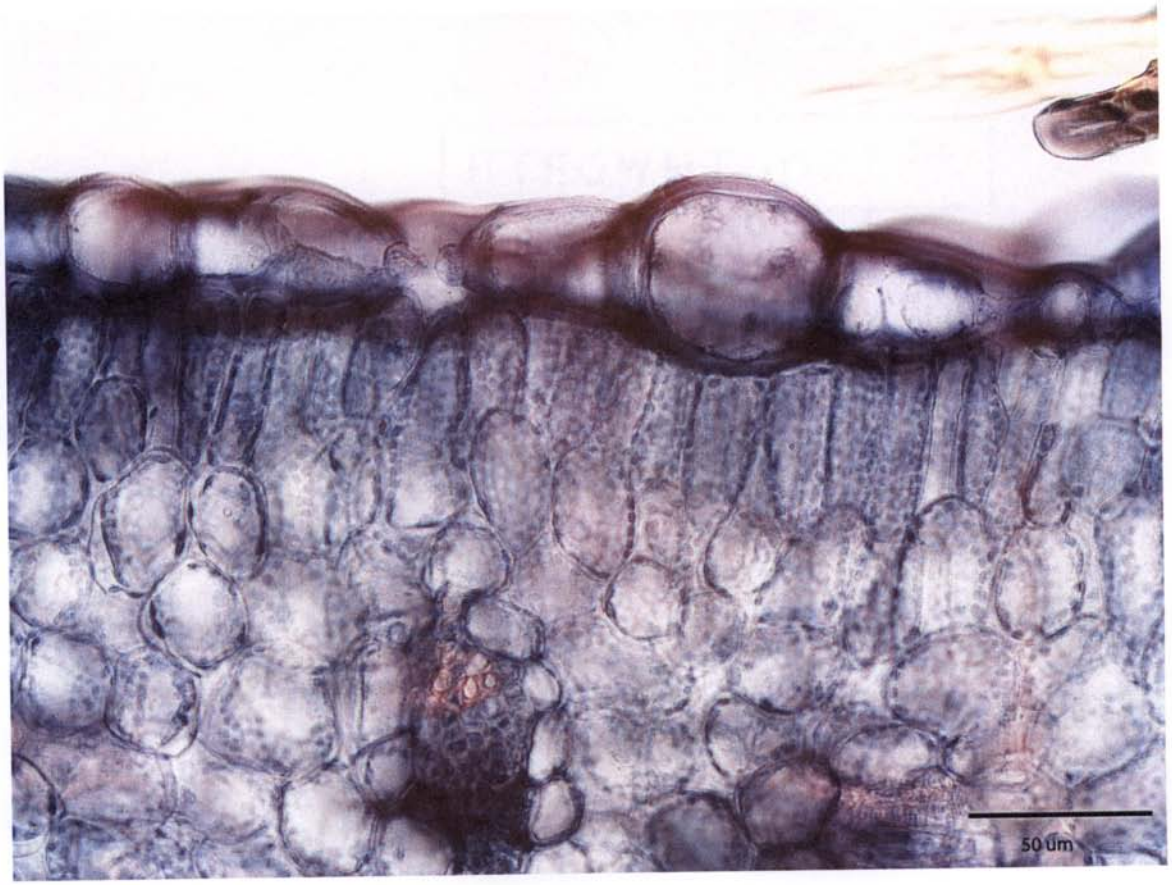

Figure 4.8. Adaxial palisade layer of *Physaria ludoviciana* from Red Wing, Minnesota.

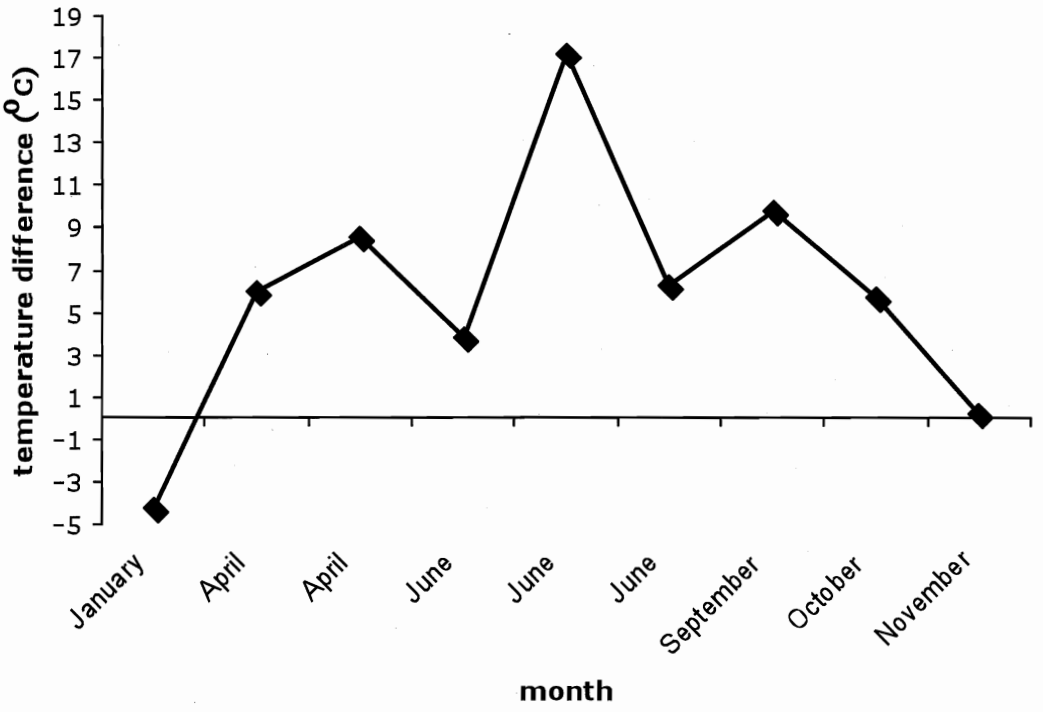

Figure 4.9. Temperature difference (soil surface subtracted from leaf temperature) of *Physaria ludoviciana* in different months.

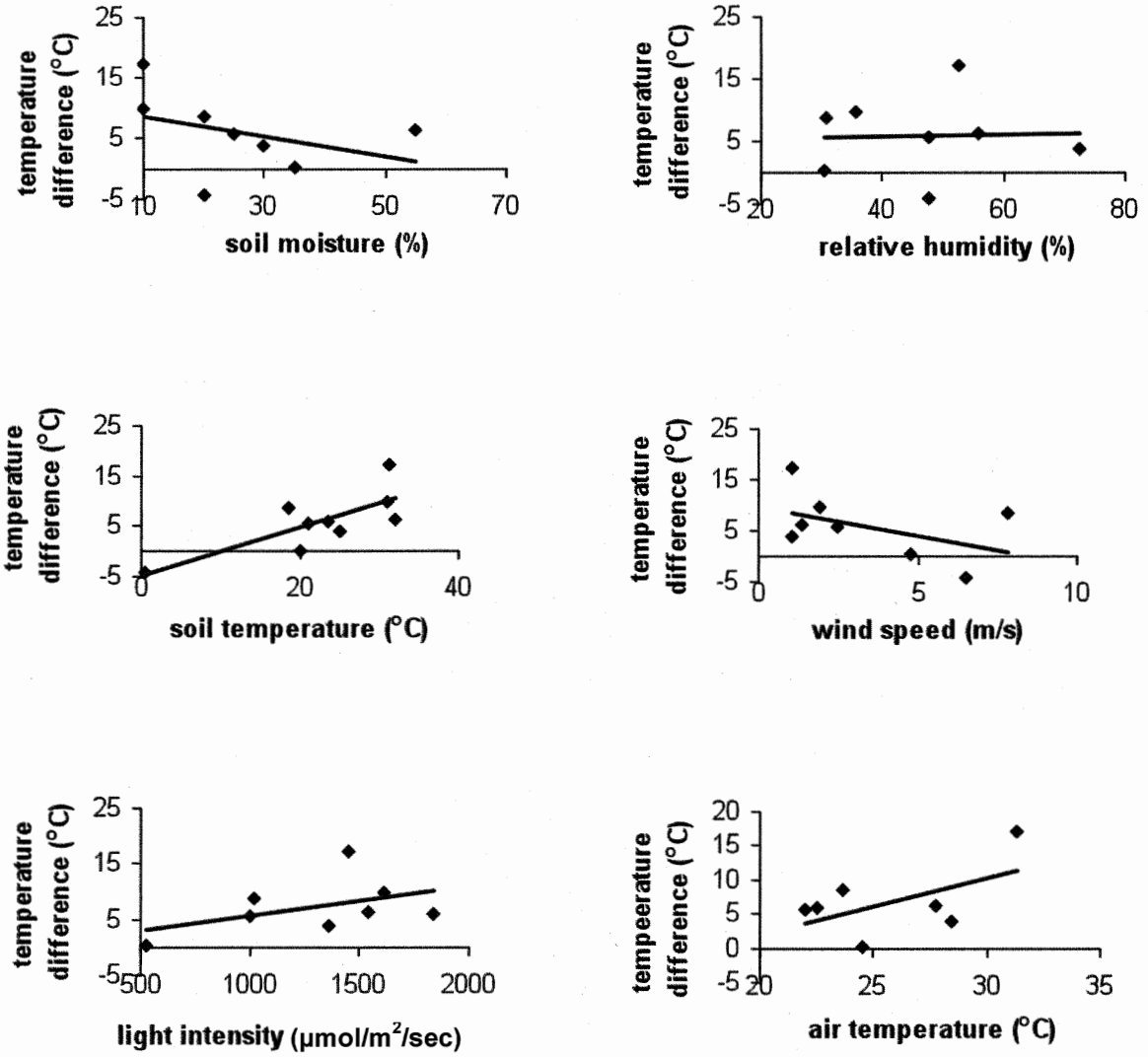

Figure 4.10. Temperature differences between soil surface and leaf of *Physaria ludoviciana* compared to environmental conditions at Henry Allan Gleason Nature Preserve, Mason County, Illinois. Soil moisture: Pearson correlation=-0.372 and p=0.365. Soil temperature: Pearson correlation=0.763 and p=0.017. Light intensity: Pearson correlation=0.461 and p=0.250. Relative humidity: Pearson correlation=0.020 and p=0.963. Wind speed: Pearson correlation=-0.462 and p=0.249. Air temperature: Pearson correlation=0.545 and p=0.206.

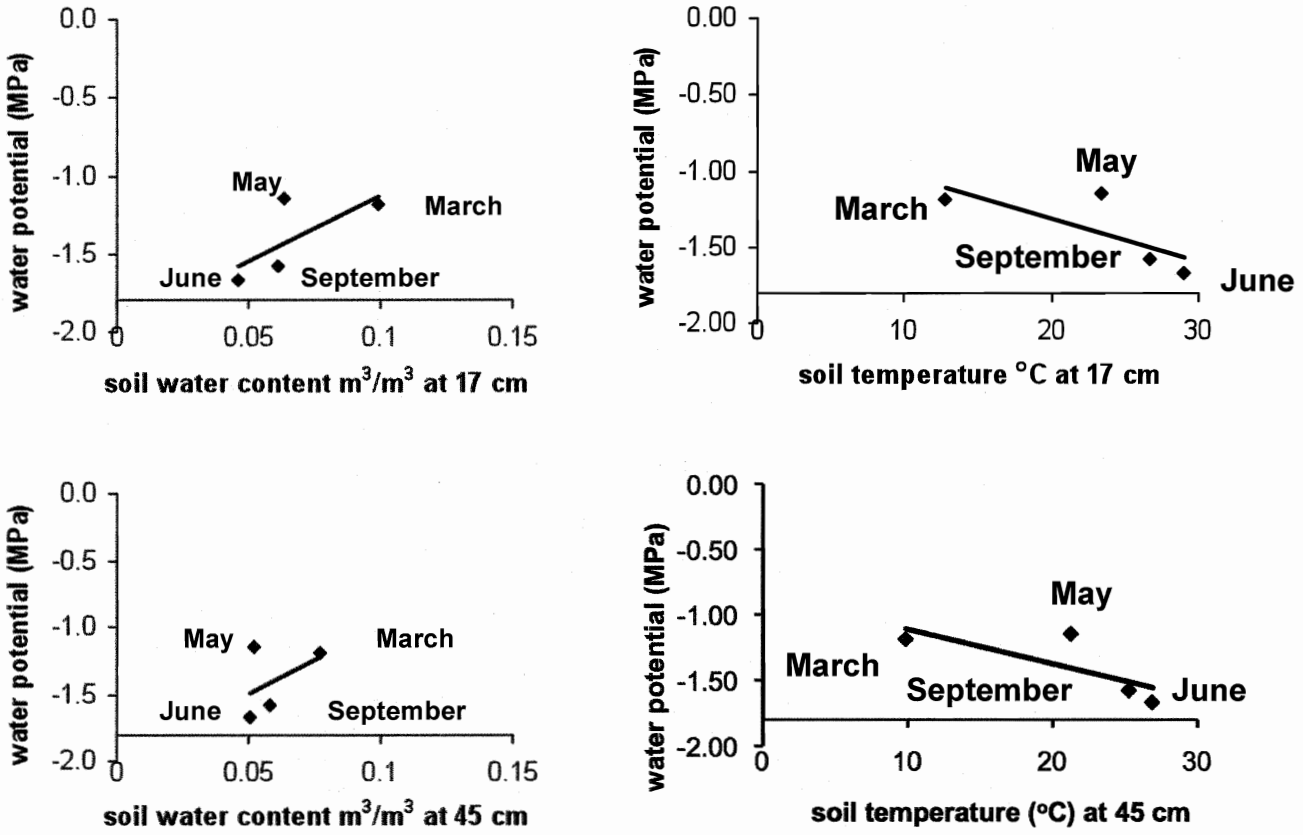

Figure 4.11. Water potential of *Physaria ludoviciana* relative to soil water content and soil temperature at 17 and 45 cm. Soil water content: Pearson correlation=0.698, 0.469 and p=0.302, 0.531 at 17 and 45 cm, respectively. Soil temperature Pearson correlation= -0.760, -0.756 and p=0.240, 0.244 at 17 and 45 cm, respectively.

Chapter 5

Survey of Plant Species and Soil Traits at Sites with *Physaria ludoviciana* Present or Absent (Silvery Bladderpod; Brassicaceae) in Three States

Abstract

Physaria ludoviciana (Nuttal) O'Kane and Al-Shehbaz (silvery bladderpod; Brassicaceae) is an endangered plant of Illinois and Minnesota sand prairies but occurs more commonly in western states in scattered pockets. The populations occur in adverse conditions isolated from each other. It is unclear why these populations are so disjunct. The goal of this study was to investigate parameters which might be associated with locations where *P. ludoviciana* is found with specific objectives: 1) to compare associated plant species in similar areas were *P. ludoviciana* is present or absent, 2) to compare soil traits in similar areas with *P. ludoviciana* present or absent, and 3) to survey reproductive structures and plant densities of *P. ludoviciana*. Similar sites with *P. ludoviciana* present or absent were surveyed in Illinois, Minnesota, and Nebraska in June 2007. At each site, associated plant species were surveyed and soil cores were collected and analyzed for various traits. Seed production was estimated by counting inflorescence structures. Numbers of holes and galls on fruits per stalk also were counted. Plant densities of *P. ludoviciana* at different developmental stages were estimated during surveys. Sites with *P. ludoviciana* present or absent had 85 and 82 species, respectively, totaling 119 plant species overall. Substantial differences in species were not found between the presence and absence of *P. ludoviciana*. Soils at all sites were sand, loamy sand or sandy loam. At sites with *P. ludoviciana* present, sand percentage was 80-100%, and pH was 7.4-8.2. At sites with *P. ludoviciana* absent, sand percentage was 64-96%,

and pH was 5.3-7.8. Substantial soil differences at sites with *P. ludoviciana* present and absent were not found. Illinois had the greatest estimated seeds per plant (353 and 479), and Hay Creek, Minnesota had the lowest (103 and 127) when estimates were based on fruits and pedicels, respectively. Holes were present in 1.1% or less of fruits, and galls were present only in Nebraska on 7.4% of the fruits. Illinois had the greatest density of seedlings (10.8/m^2) compared to the other two states. Knowledge gained provides useful information about this species to aid management of these disjunct populations, especially in states where this species is endangered.

Introduction

Physaria ludoviciana (Nuttal) O'Kane and Al-Shehbaz (silvery bladderpod; Brassicaceae) formerly was known as *Lesquerella ludoviciana* until Al-Shehbaz and O'Kane (2002) renamed the species from the genus *Lesquerella* into the genus *Physaria* based on DNA sequencing. *Physaria ludoviciana* is distributed from Illinois north to Wisconsin, west to Montana, south to Nevada, and east to Illinois. However Claerbout (2003) reported it may no longer be in Wisconsin. It is listed as an endangered species in Illinois (Herkert and Ebinger, 2002; Illinois Department of Natural Resources, 2006), Minnesota (Minnesota Department of Natural Resources, 2007) and Wisconsin (Wisconsin Department of Natural Resources, 2004). In each of these states, *P. ludoviciana* is reported in only one county. In these locations, it occurs on the easternmost edge of its geographic range (Rollins, 1939; Rollins and Shaw, 1973). In other more western states (Arizona, Colorado, Kansas, Montana, Nebraska, Nevada, New Mexico, North Dakota, South Dakota, Utah and Wyoming), it is scattered throughout many different counties (United States Department of Agriculture, Natural Resources

Conservation Service, 2007). Populations are often in very adverse environmental conditions. Commonly it is found in well-drained, mobile sand in blowouts of sand prairies (i.e. vegetated sand dunes) (Coons *et al.*, 2004; Over *et al.,* 2005). Plants frequently are found on southwest facing, steep slopes in highly disturbed areas and often within a few miles of large rivers (Over *et al.,* 2005). With constant disturbance, these areas may create the ideal niche for *P. ludoviciana.* Failure of other species to establish would allow *P. ludoviciana* to remain with minimal competition for light, water, nutrients or physical space (Coons *et al.*, 2004; Over *et al.,* 2005). Populations are scattered and disjunct. Other sites appearing similar to those with *P. ludoviciana* occur nearby, but *P. ludoviciana* is absent.

One of the most studied populations of *Physaria ludoviciana* is in Illinois at the Henry Allan Gleason Nature Preserve (HAGNP) in Mason County (Herkert and Ebinger, 2002). This area has a unique habitat that was formed during the Kankakee Torrent about 12,000 years ago (Gleason, 1910; Willman and Fyre, 1970). The water of the Kankakee Torrent carried tremendous volumes of sand and gravel downstream of the "Big Bend" at Hennepin where the river channel narrowed. The river valley widens below Hennepin and the water lost its velocity causing the sand and gravel to be deposited. As these sand deposits dried, they were exposed to wind action, resulting in large sand dunes (McClain, 1997). These sand deposits developed a truly unique ecosystem at HAGNP. The most significant geological feature at HAGNP is the large sand dune known as "Devil's Tower," which comprises about half of the preserve and is more than 25 m tall (McClain *et al.*, 2005). *Physaria ludoviciana* at the preserve grows on remnant sand dunes that are fully exposed and have been eroded by wind into three distinct bowl-shaped depressions

(Claerbout, 2003). The blowouts where *P. ludoviciana* is found are higher on the dunes, suggesting a more exposed environment. Three distinct colonies are known as the North Colony Lower Bowl (NCLB), the North Colony Upper Bowl (NCUB) and the South Colony (SC) (Beach *et al.*, 2001ab; Beach *et al.,* 2002). Parameters that influence why *P. ludoviciana* is present in some areas and absent in others is not understood.

Associated species are often indicative of differences in habitat. In previous surveys of *Physaria ludoviciana* populations throughout its range, associated plant species frequently found were *Artemisia frigida*, *Bouteloua gracilis*, *Euphorbia corollata*, *Oenothera albicaulis*, *Opuntia humifusa*, *Rhus aromatica*, and *Schizachyrium scoparium* (Claerbout, 2003). These species included a mix of grasses, forbs, cacti, and even a woody species typical of sand prairies. However, these surveys of associated species were not all-inclusive, and focused only on those that were most visible as dominant species in populations of *P. ludoviciana* in Illinois, Minnesota, Colorado, and North Dakota. In a more detailed survey done by McClain *et al.* (2005) in Illinois at the HAGNP, dominant associated species were *Ambrosia psilostachya*, *Aster oblongifolius, Bouteloua hirsuta*, *Eragrostis trichodes*, *Koeleria macrantha*, *Oenothera rhombipetala*, *Opuntia humifusa*, and *Rhus aromatica*. This survey was detailed for the area in Illinois with *P. ludoviciana*, but did not include a survey of an area without *P. ludoviciana* or surveys on other states. Additional surveys are needed to characterize associated species both in areas where *P. ludoviciana* is present and absent from the community structure.

Physaria ludoviciana resides on nutrient poor soils in highly disturbed areas with well drained sand or gravel soils having high pH (~8), low organic matter (~2%), and high calcium (~9%) in Illinois, Colorado, Minnesota and North Dakota (Claerbout,

2003). In Illinois at the HAGNP, soils in areas with *P. ludoviciana* were higher in pH, nitrate-nitrogen, calcium, magnesium and micronutrients than areas without *P. ludoviciana* (Coons *et al.*, 2004). In areas where *P. ludoviciana* was absent, soils were lower in calcium, acidic in pH and higher in sulfur and phosphorus compared to areas with *P. ludoviciana* (Coons *et al.* 2004). None of these studies compared soils for sites with *P. ludoviciana* present and absent in locations beyond HAGNP in Illinois.

Reproductive traits such as seeds and seedlings often are used as an indicator of population status because these features may affect *Physaria ludoviciana* population size. Plants produced around 500 seeds per plant in Illinois in 2002, however in Colorado, Minnesota, and North Dakota estimated seed production ranged from 88-447 (estimates based on fruits per plant) (Claerbout, 2003). High fruit production is typical in this genus. *Physaria* (as *Lesquerella*) *fendleri*, a desert perennial species, produces one to several hundred fruits with 1-30 seeds per fruit (Cabin and Marshall, 2000). In *Physaria* (as *Lesquerella*) *arctica,* a typical high arctic perennial herb, flowers per inflorescence were 3-8, and seeds per fruit were 6-8 (Aiken *et al.,* 2008). The number of *P. ludoviciana* seedlings/m^2 in June 2002 in Illinois, Minnesota, Colorado and North Dakota were 0.5, 0.4, 0.0 and 0.0, respectively (Claerbout, 2003). If proper environmental conditions do not occur, seedling establishment may not occur every year, and no seed was found in the seed bank (Claerbout, 2003), possibly limiting the spread of *P. ludoviciana* populations from year to year. A short time frame may occur when conditions are favorable for germination and establishment, and thus these conditions can be a limiting factor (Marchand and Roach, 1980; Elberling, 2000). An environment with

blowing sand, such as the sand prairie that is not stabilized, could bury seed and not allow for germination (Martinez and Moreno-Casasola, 1993; Li *et al.,* 2006).

The goal of this study was to investigate parameters which might be associated with locations where *Physaria ludoviciana* is found with specific objectives: 1) to compare associated plant species in similar areas with *P. ludoviciana* present or absent, 2) to compare soil traits in similar areas with *P. ludoviciana* present or absent, and 3) to survey reproductive structures and plant densities of *P. ludoviciana*. These comparisons may help to understand factors that explain why *P. ludoviciana* is present in some sand prairies but absent from others. This study will increase our understanding about survival and distribution of *P. ludoviciana*, which will aid long-term management of sand prairies to protect rare species.

Materials and Methods

Site Selection

Illinois, Minnesota, and Nebraska populations of *Physaria ludoviciana* were chosen as study sites. Illinois and Minnesota both have populations that are classified as endangered. Nebraska is a population that is not on the edge of the range, yet is near Illinois and Minnesota. In Illinois, the only population is at the Henry Allan Gleason Nature Preserve in Mason County. In Minnesota, two populations are found in Goodhue County including one in the Hay Creek Management Unit on the southwest side of a bluff and a second one across from 2127 Twin Bluff Road, north of Twin Bluff Middle School in Red Wing. In Nebraska, *P. ludoviciana* is scattered throughout the state. The population surveyed was near the campus of Chadron State College in Dawes County. In each state, one study site was where a population occurs, and another was in what

appeared as a similar area where no *P. ludoviciana* occurs. The sites with *P. ludoviciana* absent were the Sand Prairie-Scrub Oak Nature Preserve in Mason County, Illinois; on the southeast side of a bluff in the Hay Creek Management Unit in Goodhue County, Minnesota; and at the Oglala National Grassland, Hudson Meng Bison Bonehead in Sioux County, Nebraska. All three states were visited in June 2007.

Study Sites

Illinois-Henry Allan Gleason Nature Preserve, Mason County

The Henry Allan Gleason Nature Preserve in Mason County (Figure 5.1), Illinois is where the only natural population of *Physaria ludoviciana* exists in the state. HAGNP is located in extreme northwestern Mason County, just southwest of the town of Goofy Ridge, and about 15 km northeast of Havana, Illinois (SE 1/4 Section 6, NE 1/4, Section 7, Township 22N Range 7W, N40° 22.786`, W89° 55.733`) (McClain *et al.*, 2005). The site lies within the Illinois River Section of the Mississippi River and the Illinois River Sand Area Natural Division, and is within Sand Ridge State Forest (Schwegman, 1973). Three distinct colonies occur, the North Colony Lower Bowl, the North Colony Upper Bowl and the South Colony. Of the three colonies, the North Colony Lower Bowl was surveyed which is in a stabilized blowout immediately west of the largest dune in the preserve (Devil's Tower), and is the largest colony of *P. ludoviciana*. The soil where *P. ludoviciana* colonies are found at HAGNP is the Plainfield sand and mixed mesic. This soil is excessively well-drained, high in permeability and low in available water content and organic matter (Calsyn, 1995). *P. ludoviciana* in the preserve grows on remnant sand dunes that are fully exposed and are eroded by wind into distinct bowl-shaped depressions.

Illinois-Sand Prairie-Scrub Oak Nature Preserve, Mason County

This prairie is located in Mason County (Figure 5.2), (N40° 11. 416`, W90° 04. 463`) and is a mixture of dry sand prairie, savanna and forest (Adelman and Schwartz, 2001). This prairie is mature, and the sand blowouts are stabilized. The soil is Plainfield sand (Calsyn, 1995). The sand blowout surveyed was about 5 meters deep and about 75 meters across. It was oblong shaped with very little blowing sand apparent.

Minnesota-Richard J. Dorer Memorial Hardwood State Forest, Hay Creek Management Unit, Goodhue County

The Richard J. Dorer Memorial Hardwood State Forest (Figure 5.3) is part of the Hay Creek Management Unit located in Hay Creek about two miles south of Red Wing in northern Goodhue County. The location where *Physaria ludoviciana* was surveyed was near a deciduous oak forest. The location surveyed and surrounding area are a dry mesic and mesic forest on loess, glacial till, outwash sands or alluvium. This dry prairie is on loess over bedrock on steep south to west facing bluffs with frequent rock outcrops. *Physaria ludoviciana* was concentrated in these outcrops of sand. Two different outcrops were surveyed (Area 1 and Area 2, N44° 31. 297`, W92° 32. 916`) to remain within the area with *P. ludoviciana* plants. Area 1 and Area 2 were about 30 to 60 meters apart, and were about 6 to 15 meters away from the forest edge on the southwest side of a bluff. Data from areas 1 and 2 were combined.

Minnesota-Red Wing, Goodhue County

In Minnesota, a second site with *Physaria ludoviciana* present also was in the city of Red Wing (Figure 5.4) across from 2127 Twin Bluff Road just north of Twin Bluff Middle School (N44° 32.744`, W92° 32. 568`). Plants were growing along exposed

rocky and sandy areas on the slope of a steep bluff and the vegetation was mainly grasses with some hardwoods. This population also was surveyed in 2002, so it was surveyed again to assess changes.

Minnesota-Richard J. Dorer Memorial Hardwood State Forest, Hay Creek Management Unit, Goodhue County

This area with *Physaria ludoviciana* absent was located near the area with *P. ludoviciana*, but was on the other side of the bluff (N44° 31. 493`, W92° 32. 893`) on the southeast side (Figure 5.5). The survey area was on a very steep slope with no rock or sandy outcrops.

Nebraska-Chadron State College, College Water Tower, Dawes County

The population in Nebraska was in Dawes County in Chadron (Figure 5.6) near the campus of Chadron State College. *Physaria ludoviciana* was found south of campus, on the southeast side of the water tower (N42° 48. 905`, W102° 59. 614`). This area had sandy, rocky outcrops with Kadoka silt loam soil (Ragon *et al.*, 1977). This area had not been burned in quite a while until summer 2006 when a naturally occurring fire swept through the southern most edge of the area. Fire lines and obvious areas of fire effects were avoided when surveying this area.

Nebraska-Oglala National Grassland, Hudson Meng Bison Bonehead, Sioux County

This study site was in Sioux County, Nebraska (Figure 5.7) (N42° 49. 639`, W103° 36. 148). The area surveyed was on top of a hill that was flat and densely covered with vegetation. The soil type is Bridget very fine sandy loam (Willoughby *et al.,* 1998). This area is part of the Mammoth Site where the area surveyed had not been excavated.

Associated Plant Species

Plant species were surveyed in Illinois on 18 June 2007, in Minnesota (Hay Creek) on 8 June 2007 and in Nebraska on 6 June 2007. To survey associated plant species, quadrats (0.25 m^2) were placed at every meter located directly adjacent on alternating sides of a 30 or 45 meter transect. In each quadrat, plant species were identified and estimated for percent cover using the Daubenmire canopy cover class system (Daubenmire, 1959) as modified by Cox (1996) (class 1 = 0 to 1%; class 2 = 1 to 5%; class 3 = 5 to 25%; class 4 = 25 to 50%; class 5 = 50 to 75%; class 6 = 75 to 100%). Nomenclature was according to Brodo *et al.* (2001), Mohlenbrock (2002) or Kaul *et al.* (2006). At the Illinois site with *Physaria ludoviciana* present, a 45 meter transect was established north to south through the North Colony Lower Bowl. At the Illinois site with *P. ludoviciana* absent, a 45 meter transect was established north to south in a sandy bowl-like depression. At the Minnesota site with *P. ludoviciana* present, Area 1 was a 10 meter transect and Area 2 was a 20 meter transect. These transects were oriented parallel to slopes of the hill and in the center of a cluster of *P. ludoviciana* plants. At the Minnesota site with *P. ludoviciana* absent, on the other side of the bluff, a 30 meter transect was established oriented parallel to the slope of the hill. At the Nebraska site with *P. ludoviciana* present, three transects of 15 meters each were established oriented parallel to the slope of the hill. Two of them ran north to south and one ran east to west across the population. At the Nebraska site with *P. ludoviciana* absent, a 45 meter transect was established across the hilltop approximately running north to south. Voucher specimens were collected and prepared for deposit in the Eastern Illinois University Stover-Ebinger Herbarium. Frequency (% of quadrats where each species present),

average cover (% of area covered using midpoint of class as value for each quadrat), relative frequency (% frequency for each species/sum of % frequency for all species X 100), relative cover (% average cover for each species/sum of % average cover for all species X 100) and importance value (sum of relative frequency plus relative cover) were determined for each species at each location. In addition, voucher specimens were collected for some species found in each area, but not occurring in surveyed quadrats.

Soil Traits

Soil was collected in Illinois on 4 June 2007, in Minnesota (Hay Creek) on 8 June 2007 and in Nebraska on 6 June 2007. Using a soil corer, 18 soil cores from the top 5-10 cm of soil were taken randomly throughout each site. Cores were combined for each site, and a 450 g subsample was sent to A & L Analytical Laboratories, Inc. (Memphis TN) for an analysis of fertility (phosphorus, potassium, calcium, magnesium, sulfur, boron, copper, iron, manganese, zinc, sodium, organic matter, cation exchange capacity, soil pH and buffer pH) and texture. Comparisons were made to determine differences and similarities between sites with *Physaria ludoviciana* present or absent. With each site being considered a replication, data also were analyzed by an analysis of variance (ANOVA) followed by a Duncan's multiple range test at the 5% level using SPSS for comparison of sites with *P. ludoviciana* present or absent.

Seed Production

Seed production was estimated in Illinois on 4 June 2007, in Minnesota (Hay Creek) on 8 June 2007, in Minnesota (Red Wing) on 9 June 2007 and in Nebraska on 6 June 2007. Thirty-two to 45 plants in the reproductive stage were selected randomly to count numbers of flower stalks per plant, pedicels per stalk, flowers per stalk, fruits per

stalk, and fruits with holes or galls per stalk. If flowers and flower buds were present, they were counted. Also, height of the tallest stalk was measured. Twenty-five fruits were collected from each of four plants. In the lab, numbers of seeds in each fruit were counted. A high estimate of number of seeds per plant was calculated based upon the assumption that each pedicel would have a fruit. Calculations were made by multiplying average number of seeds per fruit, by pedicels per stalk, by flower stalks per plant. A low seed estimate based on number of fruit present also was calculated by multiplying average number of seeds per fruit, by fruits per stalk, by flower stalks per plant. Means and standard errors were calculated for height of tallest flower stalk, number of fruits with holes (fruit apparently damaged from herbivory), and number of fruits with galls using Microsoft® Office Excel 2003 (11.8211.8202) SP3. Data also were analyzed by analysis of variance (ANOVA) followed by a Duncan's multiple range test at the 5% level using SPSS (Version 16 for Windows) for comparison of sites with *Physaria ludoviciana* in three states.

Seedling Establishment and Plant Densities

Plant densities were determined for *Physaria ludoviciana* seedlings, vegetative and reproductive plants in Illinois on 4 June 2007, in Minnesota (Hay Creek) on 8 June 2007, in Minnesota (Red Wing) on 9 June 2007 and in Nebraska on 6 June 2007. A forty-five meter transect was extended through the colonies, with a quadrat (0.25 m^2) directly adjacent on alternating sides of the transect. The same transects and quadrats were used for surveying the plant density of *P. ludoviciana* as were used for surveying associated plant species with the addition of a 45 meter transect at Red Wing, Minnesota. At Red Wing, a 20 meter transect was oriented parallel to the side of the hill and a 25

meter transect was oriented perpendicular to the hill. In each of the quadrats, numbers of seedlings (≤ 6 leaves), vegetative plants (> 6 leaves, but no flower stalk) and reproductive plants (flower stalks) were counted. For data analyses, the number of plants at each growth stage per square meter was calculated. Means and standard errors were calculated using Microsoft Excel. Percentage of plants at each growth stage also was calculated. Data also were analyzed by analysis of variance followed by a Duncan's multiple range test at the 5% level using SPSS to compare plant densities in three states.

Results

Associated Plant Species

The top three species at each site were identified based on their importance values. The top three plant species at the site in Illinois where *Physaria ludoviciana* was present were *Ambrosia psilostachya*, *Eragrostis trichodes* and *Schizachyrium scoparium* (Table 5.1). At the site in Illinois where *P. ludoviciana* was absent the top three species were *Schizachyrium scoparium*, *Dichanthelium villosissimum* and *Opuntia humifusa* (Table 5.2). The site in Minnesota with *P. ludoviciana* present had *Bouteloua curtipendula*, *Schizachyrium scoparium* and *Andropogon gerardii* as the top three species (Table 5.3). The Minnesota site with *P. ludoviciana* absent had *Schizachyrium scoparium*, *Bouteloua* sp. and *Andropogon gerardii* as the top three species (Table 5.4). In Nebraska at the site with *P. ludoviciana* present, *Carex filifolia*, *Heterostipa comata* and *Vulpia octoflora* were the top three species (Table 5.5). *Heterostipa comata, Carex filifolia* and *Carex eleocharis* were the top three species at the site with *P. ludoviciana* absent in Nebraska (Table 5.6). All of the species of grasses and forbs found were typical of sand prairies (McClain *et al.*, 2005).

Figure 5.8 shows what percentage of species were in different plant families for sites where *Physaria ludoviciana* was present and absent. The Asteraceae and Poaceae were dominant at all sites. In Illinois, 13 plant families were found where *P. ludoviciana* was present and nine where it was absent. Cyperaceae accounts for a larger percentage of species at Illinois absent (18%) than at Illinois present (4%). In Minnesota 12 plant families were found where *P. ludoviciana* was present as compared to 15 plant families where it was absent. The Minnesota present site had more representatives of Brassicaceae (11%) than the absent site which had only 4%. The Nebraska site with *P. ludoviciana* had 17 plant families as compared to 14 plant families in the site with no *P. ludoviciana*. In the Nebraska absent site, Fabaceae accounts for 16% of the plant species, whereas Fabaceae is not represented in the Nebraska present site.

Figure 5.9 shows plant habits for species surveyed. Forbs were the dominant plant habit at all sites followed by grasses. The Illinois absent site was dominated by more grasses and sedges than Illinois present which was dominated by forbs. Proportionally in Illinois, more sedges occurred in the absent site than in the present site. In Minnesota, woody species (trees and shrubs) accounted for a small percentage of the total. In Nebraska, cacti were present in sites with *Physaria ludoviciana*, but not in sites without.

In all sites, native species dominated over non-native or exotic species (Figure 5.10). In Illinois and Minnesota more non-native species were found at sites with *Physaria ludoviciana* present (4% and 3%, respectively) than at absent sites where non-natives were not found. In Nebraska, no non-native species were found at either site.

The biodiversity of plant species was greater where *Physaria ludoviciana* was present than where it was absent (Table 5.7). The greatest total number of species was in Nebraska and the least was in Illinois. In Nebraska, very little bare ground was observed and sandy outcrops were fewer. Table 5.8 lists all the species found and where they were found. No plant species were found in all *P. ludoviciana* present sites or all *P. ludoviciana* absent sites, nor were any species found in all sites. *Schizachyrium scoparium* was found in 5 of the 6 sites, making it the most frequent species at the sites. Species were found in Illinois and Minnesota that were not found in Nebraska, and vice versa. Also, other vascular plant species in Illinois were found at the sites, but not in the quadrats. These species were typically forbs and grasses (Table 5.9). There had already been a comprehensive study done on Illinois flora so plants outside quadrats were not recorded.

Nonvascular plants were found in two of the sites. At the Sand Prairie-Scrub Oak (Illinois site with *P. ludoviciana* absent), *Cladina rangiferina*, *Cladonia cristatella*, and an unknown moss were found. At the Minnesota site with *P. ludoviciana* present, an unknown moss was present in the quadrats.

Soil Traits

Substantial differences in soil traits were not found between sites with *Physaria ludoviciana* present or absent. Soils with *P. ludoviciana* were either sand or loamy sand, whereas soils with *P. ludoviciana* absent were either sand, loamy sand or sandy loam (Table 5.10). Sites with *P. ludoviciana* present had an average pH of 7.9, whereas sites with *P. ludoviciana* absent had an average pH of 6.9. In Minnesota and Nebraska, percent organic matter was lower in sites with *P. ludoviciana*, at 0.6% and 1.3%, respectively,

compared to sites without *P. ludoviciana* at 3.3% and 2.9%, respectively. In Illinois, organic matter was similar (1.2 to 1.1%) at both sites. Trends for macronutrients (phosphorus, potassium, calcium, magnesium and sulfur) and micronutrients (boron, copper, iron, manganese, zinc and sodium) in soils were inconsistent when *P. ludoviciana* was present or absent. All soil parameters were analyzed by analysis of variance followed by a Duncan's multiple range test at the 5% level using SPSS with sites in each state being replications. No significant differences were found between any individual soil parameters in sites with *P. ludoviciana* present or absent.

Seed Production

Physaria ludoviciana plants in Illinois had significantly more flower stalks per plant than any other site. Illinois also had more fruits per stalk than the other sites except Nebraska (Table 5.11). Illinois and Nebraska had significantly greater pedicels per stalk than both Minnesota populations. Red Wing, Minnesota had significantly more seeds per fruit than other sites. This difference could be due to the fact that when fruits per seed were counted, the seeds at Red Wing had not matured and were still green inside the fruit. As seeds mature in the fruit some seeds may be aborted. The population in Illinois produced the most seeds per plant based on calculations between 353 to 479 depending on how the estimates made, whereas Hay Creek, Minnesota produced the least between 103-127 (Table 5.11). None of the four sites were significantly different in height of flower stalks or in numbers of fruits with holes per stalk (Table 5.12). Holes (possibly due to herbivory) affected 1.1% or fewer fruit. Galls found only in Nebraska, affected 7.4% of the fruit. Flowers and flower buds were only present in Nebraska, as in other

states flowers had already developed into fruit. In Nebraska, flowers were 2.3 ± 0.5 per stalk and flower buds were 4.4 ± 1.8 per stalk.

Seedling Establishment and Plant Densities

Seedling densities fluctuated at the different sites (Table 5.13). Illinois had the highest seedling densities with 10.8 seedlings/m^2, while Hay Creek, Minnesota had no seedlings. Vegetative plant densities were greater in Hay Creek, Minnesota than in Red Wing, Minnesota or Nebraska. Reproductive plant densities were greater in Illinois and Hay Creek, Minnesota than in Nebraska.

Discussion

Associated Plant Species

Populations were surveyed in three different states where *Physaria ludoviciana* was present or absent. In Claerbout (2003), four states where *P. ludoviciana* was present were surveyed, but only dominant plant species were noted which limited conclusions that could be drawn because differences in the number of associated plant species in each state could be due to the partial identification. McClain *et al.* (2005) found associated plant species in areas with and without *P. ludoviciana* to be typical sand prairie forbs and grasses of active, partially stabilized and stabilized sand prairie blowouts. In our study of associated plant species, typical dominant species are present such as representatives from Asteraceae, Cyperaceae and Poaceae. More species were found in sites where *P. ludoviciana* was present than where absent. Nebraska had a greater number of species than Illinois or Minnesota. Also, Nebraska had very dense vegetation with little bare ground compared to the other states. Percent bare ground in quadrats was recorded in Illinois and Minnesota, but not in Nebraska because there was little or none. In Illinois

and Minnesota, the top three species consistently included *Schizachyrium scoparium* regardless of whether *P. ludoviciana* was present or absent. In Minnesota, *Andropogon gerardii* was in the top three species at both sites with and without *P. ludoviciana*. In Nebraska, two of the top three species were the same at sites with both the presence or absence of *P. ludoviciana*, i.e. *Carex filifolia* and *Heterostipa comata*. Hence, associated species did not show clear differences at sites with and without *P. ludoviciana*.

According to McClain *et al.* (2005), *Schizachyrium scoparium*, *Opuntia humifusa* and *Dichanthelium villosissimum* are plant species associated with mature sand prairies. *Schizachyrium scoparium* was found in every sand prairie surveyed except for the site in Nebraska where *P. ludoviciana* was absent. *Opuntia humifusa* was found in both sites in Illinois and *Dichanthelium villosissimum* was found at the site in Illinois with *Physaria ludoviciana* absent. With these indicator species present, areas surveyed can be considered as mature sand areas. Though not apparent from this study, woody species are becoming more widespread at the HAGNP in Illinois and more frequent fires will be needed to stop woody encroachment (McClain *et al.,* 2005). While fires will help control woody vegetation, it is uncertain what the effect fire will have on *P. ludoviciana* since it is evergreen. In Illinois, much of the woody encroachment observed is from *Rhus aromatica*, a woody shrub becoming more prevalent in the preserve. This shrub could start to appear in similar sand prairie habitats other than those with *P. ludoviciana*. Given the number of non-native species in Illinois and Minnesota, some could become aggressive, thus limiting the range of *P. ludoviciana*.

Soil Traits

No significant differences were found between any individual soil parameter in the sites with *Physaria ludoviciana* present or absent using ANOVA. No trends were observed for macronutrients or micronutrients between states or between with and without sites. Soils found at all sites were sand, loamy sand or sandy loam. As for soil characteristics, Claerbout (2003) showed that areas with *P. ludoviciana* prefer a sandy habitat and blowing sand with low soil fertility and low water holding capacity as well as high pH (~8), low organic matter (~2%), and high calcium (~9%). Coons *et al.* (2004) showed that areas sampled where *P. ludoviciana* was present had higher calcium, magnesium, manganese, zinc, soluble salts, nitrate-nitrogen and cation exchange capacity but lower phosphorus and sulfur than areas with *P. ludoviciana* absent. No significant differences in soil characteristics between sites with *P. ludoviciana* present or absent indicates, that if seed of *P. ludoviciana* reached alternative areas, it might have a good chance of establishing itself.

Seed Production

Seed production was variable in the different sites, with plants producing from 103 to 479 seeds per plant. Production of seeds is high, which is not uncommon in this genus. *Physaria* (as *Lesquerlla*) *gordonii*, a spring flowering desert annual plant produces between 14.8 to 28.8 fruit per plant, and averages between 3.35 and 6.99 seeds per fruit (Delph, 1986). *Physaria* (as *Lesquerella*) *fendleri* can produce more seeds than *Physaria ludoviciana* with one to several hundred fruits per plant and each fruit containing 1 to 30 seeds (Cabin *et al.*, 1998). In *Physaria* (as *Lesquerella*) *arctica,* a typical high Arctic perennial herb, flowers per inflorescence are 3 to 8 and seeds per fruit

are 6 to 8 (Aiken *et al.,* 2008). Delph (1986) hypothesized that high fruit set is an adaptive response to unpredictable, variable resource levels, and high herbivory risk. Holes in the fruits due to herbivory were fewer than 1.1% of fruit per plant in populations of *P. ludoviciana.* In some colonies, a large percentage of pedicels were missing flowers and fruits indicating that they were being removed, naturally abscising or possibly being eaten (Claerbout *et al.*, 2007). Galls were only observed in Nebraska in this study, although Clarebout (2003) also found galls on a large percentage of plants in North Dakota. With seed production in the range of other species in the genus, seed production does not appear to limit *P. ludoviciana.* Seed production at Red Wing, Minnesota was lower in 2007 than in 2002. When surveyed in 2002, 446 to 515 seeds per plant were produced (Claerbout, 2003) compared to the seed estimate in 2007 of 339 to 383 seeds per plant (both estimates based on the number of fruits and pedicels). This decrease could be due to sampling time, as Claerbout sampled 24 June 2002, but in 2007 sampling was on 9 June. However these numbers are still higher than the Nebraska population (that is not listed as endangered), which produced 141 to 231 seeds per plant and the Hay Creek, Minnesota population which produced 103 to 127 seeds per plant. These differences in fruit production across populations and seasons also could be attributed to sampling at various stages of development. *Physaria ludoviciana* produces a fixed number of flower stalks at the onset of flowering, and those flower stalks continue to elongate and produce pedicels while in the flowering stage. Therefore, height of the flower stalk and number of pedicels is dependent on the plant's developmental stage, while the number of flower stalks is independent (Claerbout, 2003).

Seedling Establishment and Plant Densities

Plant densities varied throughout the sampled sites. Illinois had the highest number of seedlings. Hay Creek, Minnesota had a higher number of vegetative plants than Red Wing, Minnesota or Nebraska. Illinois, and Hay Creek, Minnesota had higher numbers of reproductive plants than Nebraska. It was noted that seedlings were within close proximity of reproductive plants. With the high percentage of seed produced, higher seedling densities might be expected, but with no mechanism for dispersal (Coons *et al.,* 2004), seedling establishment is low and unpredictable. Comparing plant densities at Red Wing in 2002 to 2007, an increase was seen in seedlings/m^2 and vegetative plants/m^2 from 0.0 and 0.2 in 2002 (Claerbout, 2003) to 0.4 and 0.3 in 2007, however reproductive plants/m^2 decreased from 1.9 in 2002 to 1.5 in 2007. This decrease could be due to sampling dates at different times of the year. In 2002 and 2007 the number of plants/m^2 was the same (2.2). There were fewer reproductive plants, but more seedlings and vegetative plants (Claerbout, 2003). A number of factors could account for the low seedling numbers. *Physaria* (as *Lesquerella*) *fendleri* had seedlings in the field in both spring and fall depending on the amount and timing of rainfall (Cabin *et al.*, 1998; Hyatt *et al.*, 1999). *Physaria ludoviciana* seedlings were observed in the field in the fall at densities of 1.3 ± 0.7 in the North Colony Lower Bowl in Illinois (see appendix B). Seedling emergence in fall may be a problem because plants may die during the winter. If significant numbers of seeds have broken dormancy by fall and are germinating and dying, that could account for a significant number of seeds that are "wasted." Another possible explanation for low seedling numbers could be that seeds and seedlings are getting buried in the blowing sand of the sand prairie. According to Li *et al.* (2006),

many factors can influence seeds being buried too deep. The optimal depth for a seed could be influenced by its mass, as often larger seeds have higher germination rates than smaller seeds at deeper depths. If large seeds get buried deeply, they have the resources to survive until they emerge. Burial of seeds by blowing sand is critical for the establishment of many seeds, but blowing sand might bury small seed like *P. ludoviciana* too deep. *Physaria ludoviciana* seeds getting buried below the top two centimeters of soil could explain why so few are found in the seed bank when the plant produces so many seeds. Claerbout (2003) found no *P. ludoviciana* seeds in soil cores collected at a depth of ten centimeters. The time it takes for a seed to emerge often determines whether or not the plant can complete its life cycle because if it gets buried too deep it will have a shorter growing season. Seeds also could get buried too deep in the seed bank over the winter by the freezing and thawing of the ground (Chen and Maun, 1999). In studies done by Martinez and Moreno-Casasola (1993) on a sand dune legume, one of the limiting factors for seedling establishment was sand movement. Smaller seedlings were the most susceptible to being buried or desiccated by sand. Sand burial can be beneficial because it provides mulch that provides high humidity and protection from high and low temperature and predators (Li *et al.*, 2006). Another factor that could be causing low seed numbers are physical and biotic factors. In a sand dune system, survivorship of seedlings is affected by factors such as predation, disease, desiccation, competition, salt spray, nutrient deficiency, high soil surface temperatures and sand movement (Martinez and Moreno-Casasola, 1993). Hence, possibly several factors are influencing the variability seen in the establishment of seedlings at the sites.

Summary

This study and others by Claerbout (2003) and Over *et al.* (2005) suggest that the ability of *Physaria ludoviciana* to survive depends more on its ability to arrive and establish in an area than it does on the associated plant species or soil characteristics. The ability of *P. ludoviciana* to survive in harsh growing conditions was the most striking similarity between the populations throughout the surveyed sites. Associated plant species were consistent throughout the range of *P. ludoviciana* and were identified as typical grassland and dry sand prairie forbs and grasses. Although soil characteristics showed differences in areas with *P. ludoviciana* present or absent, areas did not have a consistent characteristic that defined them. Seed production of *P. ludoviciana* is very high per plant in all sites; however seedling establishment is low and variable. *P. ludoviciana* could be an early succession plant that is able to establish in harsh conditions. The disjunct populations could be a result of poor seed dispersal and therefore instead of associated plant species or soil characteristics being the limiting factor, establishment and seed dispersal could be the limiting factors.

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Table 5.1. Frequency (%), average cover (% of total area), relative frequency, relative cover and importance values for vascular plant species at the Henry Allan Gleason Nature Preserve in Mason County, Illinois where *Physaria ludoviciana* was present.

Plant Species[a]	Frequency	Average Cover	Relative Frequency	Relative Cover	Importance Value
Ambrosia psilostachya DC. (western ragweed)	91	8.70	17.04	22.04	39.08
Asclepias verticillata L. (whorled milkweed)	13	0.12	2.43	0.30	2.73
Asclepias viridiflora Raf. (green milkweed)	4	0.40	0.75	1.01	1.76
Aster oblongifolius Nutt. (aromatic aster)	7	0.03	1.31	0.08	1.39
Bouteloua hirsuta Lag. (hairy grama)	64	3.51	11.99	8.89	20.88
Calamovilfa longifolia (Hook.) Scribn. (prairie sandreed)	18	1.01	3.37	2.56	5.93
Chamaecrista fasciculata (Michx.) Greene (showy partridge pea)	18	0.37	3.37	0.94	4.31
Chamaesyce geyeri (Engelm.) Small. (geyer's spurge)*	4	0.02	0.75	0.05	0.80
Commelina erecta L. (dayflower)	16	0.51	3.00	1.29	4.29
Conyza canadensis (L.) Cronquist (horseweed)	16	0.73	3.00	1.85	4.85
Coreopsis lanceolata L. (lance-leaf tickseed)	4	0.08	0.75	0.20	0.95
Eragrostis trichodes (Nutt.) A.W. Wood (sand lovegrass)	80	9.43	14.98	23.89	38.87
Euphorbia corollata L. (flowering spurge)	9	0.48	1.69	1.22	2.91
Heterotheca camporum (Greene) Shinners	13	1.14	2.43	2.89	5.32
Koeleria macrantha (Ledeb.) Schult (Junegrass)	2	0.01	0.37	0.03	0.40
Lepidium sp. L. (pepper-grass, hoary cress)	2	0.07	0.37	0.18	0.55
Lespedeza capitata Michx. (round-head bush clover)	9	0.16	1.69	0.41	2.10
Liatris aspera Michx. (rough blazing star)	20	1.23	3.75	3.12	6.87
Lithospermum croceum Fern. (hairy puccoon)*	2	0.33	0.37	0.84	1.21
Oenothera rhombipetala Nutt. *ex* Torr. & A. Gray (fourpoint evening-primrose)	7	0.03	1.31	0.08	1.39
Opuntia humifusa (Raf.) Raf. (eastern prickly-pear, bigroot prickly-pear, western prickly-pear)	24	2.17	4.49	5.50	9.99
Phlox bifida Beck. (cleft phlox)*	20	0.32	3.75	0.81	4.56
Physaria ludoviciana (Nutt.) O'Kane & Al-Shehbaz (silvery bladderpod)	27	0.73	5.06	1.85	6.91
Salsola tragus L. (Russian thistle)*	2	0.01	0.37	0.03	0.40
Schizachyrium scoparium (Michx.) Nash (little bluestem)	60	6.49	11.24	16.44	27.68
Strophostyles helvola (L.) Elliott (wild bean)	2	1.39	0.37	3.52	3.89
Totals	534	39.47	100	100	200
Bare ground and litter		64.30			

[a]Nomenclature used for vascular plants was Kaul *et al.,* 2006 unless denoted by * when nomenclature was Mohlenbrock, 2002

Table 5.2. Frequency (%), average cover (% of total area), relative frequency, relative cover and importance values for vascular plant species at the Sand Prairie-Scrub Oak Nature Preserve in Mason County, Illinois where *Physaria ludoviciana* was absent.

Plant Species[a]	Frequency	Average Cover	Relative Frequency	Relative Cover	Importance Value
Ambrosia psilostachya DC. (western ragweed)	29	0.86	5.81	3.08	8.89
Aristida tuberculosa Nutt.(needle grass)*	51	0.26	10.22	0.93	11.15
Bouteloua hirsuta Lag. (hairy grama)	2	0.01	0.40	0.04	0.44
Calamovilfa longifolia (Hook.) Scribn. (prairie sandreed)	16	0.30	3.21	1.08	4.29
Carex muhlenbergii Schk. (Muhlenberg's sedge)	22	0.33	4.41	1.18	5.59
Carex sp. L. unknown B (sedge)	2	0.01	0.40	0.04	0.44
Commelina erecta L. (dayflower)	20	1.88	4.01	6.74	10.75
Conyza canadensis (L.) Cronquist (horseweed)	73	1.73	14.63	6.20	20.83
Crotonopsis linearis Michx. (rushfoil)*	24	0.18	4.81	0.65	5.46
Cyperus schweinitzii Torr. (Schweinitz's flatsedge)	13	0.07	2.61	0.25	2.86
Dichanthelium villosissimum (Nash) Freckm. (hairy panic grass)*	51	4.78	10.22	17.14	27.36
Eragrostis spectabilis (Pursh) Steud. (purple lovegrass)	2	0.07	0.40	0.25	0.65
Eragrostis trichodes (Nutt.) A.W. Wood (sand lovegrass)	11	0.38	2.20	1.36	3.56
Krigia virginica (L.) Willd. (dwarf dandelion)*	31	0.27	6.21	0.97	7.18
Lespedeza capitata Michx. (round-head bush clover)	7	0.41	1.40	1.47	2.87
Liatris aspera Michx. (rough blazing star)	2	0.33	0.40	1.18	1.58
Lithospermum croceum Fern. (hairy puccoon)*	2	0.01	0.40	0.04	0.44
Oenothera rhombipetala Nutt. *ex* Torr. & A. Gray (fourpoint evening-primrose)	4	0.08	0.80	0.29	1.09
Opuntia humifusa (Raf.) Raf. (eastern prickly-pear, bigroot prickly-pear, western prickly-pear)	33	5.27	6.61	18.90	25.51
Paspalum pubiflorum Rupr. (bead grass)	33	0.50	6.61	1.79	8.40
Schizachyrium scoparium (Michx.) Nash (little bluestem)	67	9.49	13.43	34.03	47.46
Tradescantia ohiensis Raf. (spiderwort)	4	0.67	0.80	2.40	3.20
Totals	499	27.89	100	100	200
Bare ground and litter		47.44			

[a]Nomenclature used for vascular plants was Kaul *et al.,* 2006 unless denoted by * when nomenclature was Mohlenbrock, 2002

Table 5.3. Frequency (%), average cover (% of total area), relative frequency, relative cover and importance values for vascular plant species at the Richard J. Dorer Memorial Hardwood State Forest, Hay Creek Management Unit in Goodhue County, Minnesota where *Physaria ludoviciana* was present.

Plant Species[a]	Frequency	Average Cover	Relative Frequency	Relative Cover	Importance Value
Ambrosia psilostachya DC. (western ragweed)	70	1.02	11.67	2.90	14.57
Andropogon gerardii Vitman (big bluestem)	20	5.52	3.33	15.67	19.00
Arabis lyrata L. (sand cress)*	17	0.25	2.83	0.71	3.54
Artemisia campestris L. (field sagewort, Great Plains wormwood)	40	0.70	6.67	1.99	8.66
Aster sericeus Vent. (silky aster)	3	0.10	0.50	0.28	0.78
Bouteloua curtipendula (Michx.) Torr. (sideoats grama)	80	11.45	13.33	32.51	45.84
Bouteloua hirsuta Lag. (hairy grama)	7	0.52	1.17	1.48	2.65
Calamovilfa longifolia (Hook.) Scribn. (prairie sandreed)	27	0.55	4.50	1.56	6.06
Carex tonsa (Fern.) Bicknell	10	0.13	1.67	0.37	2.04
Castilleja coccinea (L.) Spreng (Indian paintbrush)*	3	0.50	0.50	1.42	1.92
Coreopsis palmata Nutt. (finger coreopsis)	7	0.03	1.17	0.09	1.26
Euphorbia corollata L. (flowering spurge)	10	0.05	1.67	0.14	1.81
Hedeoma hispida Pursh (rough false pennyroyal)	20	0.10	3.33	0.28	3.61
Helianthus divaricatus L. (woodland sunflower)*	30	0.65	5.00	1.85	6.85
Lepidium densiflorum Schrader (pepper-grass, hoary grass)	20	0.10	3.33	0.28	3.61
Lithospermum canescens (Michx.) Lehm. (hoary puccoon)	10	0.05	1.67	0.14	1.81
Lithospermum incisum Lehm. (fringed puccoon)	13	0.07	2.17	0.20	2.37
Panicum leibergii (Vasey) Scribn. (leiberg panicum)	20	0.83	3.33	2.36	5.69
Panicum linearifolium Scribn. ex Britton (slimleaf panicum)	27	0.63	4.50	1.79	6.29
Physaria ludoviciana (Nutt.) O'Kane & Al-Shehbaz (silvery bladderpod)	23	0.28	3.83	0.80	4.63
Prunus susquehanae Willd (sand cherry)*	3	0.02	0.50	0.06	0.56
Quercus sp. L. (oak)	3	0.02	0.50	0.06	0.56
Schizachyrium scoparium (Michx.) Nash (little bluestem)	57	7.97	9.50	22.63	32.13
Senecio plattensis Nutt. (prairie ragwort)	10	0.05	1.67	0.14	1.81
Silene antirrhina L. (sleepy catchfly)	3	0.02	0.50	0.06	0.56
Solidago nemoralis Aiton subsp. *decemflora* (DC.) Brammall *ex* Semple (gray goldenrod)	20	1.32	3.33	3.75	7.08
Sporobolus cryptandrus (Torr.) A. Gray (sand dropseed)	10	0.22	1.67	0.62	2.29
Sporobolus heterolepis (A. Gray) A. Gray (prairie dropseed)	7	1.35	1.17	3.83	5.00
Tradescantia ohiensis Raf. (spiderwort)	23	0.60	3.83	1.70	5.53
unknown forb B (possible harebell)	7	0.12	1.17	0.34	1.51

Table 5.3 Continued

Plant Species[a]	Frequency	Average Cover	Relative Frequency	Relative Cover	Importance Value
Totals	600	35.22	100	100	200
Bare ground and litter		57.80			

[a]Nomenclature used for vascular plants was Kaul *et al.*, 2006 unless denoted by * when nomenclature was Mohlenbrock, 2002

Table 5.4. Frequency (%), average cover (% of total area), relative frequency, relative cover and importance values for vascular plant species at the Richard J. Dorer Memorial Hardwood State Forest, Hay Creek Management Unit in Goodhue County, Minnesota where *Physaria ludoviciana* was absent.

Plant Species[a]	Frequency	Average Cover	Relative Frequency	Relative Cover	Importance Value
Amorpha canescens Nutt. *ex* Pursh (leadplant)	23	1.73	3.30	3.59	6.89
Andropogon gerardii Vitman (big bluestem)	50	6.52	7.18	13.53	20.71
Arabis lyrata L. (sand cress)*	3	0.10	0.43	0.21	0.64
Asclepias viridiflora Raf. (green milkweed)	13	0.23	1.87	0.48	2.35
Aster oolentangiensis Riddle (azure aster)	3	0.10	0.43	0.21	0.64
Aster sericeus Vent. (silky aster)	10	0.22	1.44	0.46	1.90
Bouteloua sp. Lag (grama grass)	87	7.60	12.50	15.77	28.27
Carex sp. L. unknown A (sedge)	30	1.53	4.31	3.17	7.48
Carex tonsa (Fern.) Bicknell	57	2.48	8.19	5.15	13.34
Castilleja sessiliflora Pursh (dwarf Indian paintbrush)	17	0.73	2.44	1.51	3.95
Comandra umbellata (L.) Nutt. (false/bastard toadflax)	10	0.22	1.44	0.46	1.90
Dalea purpurea Venten (purple prairie-clover)	10	0.13	1.44	0.27	1.71
Euphorbia corollata L. (flowering spurge)	40	1.82	5.75	3.78	9.53
Liatris sp. Gaertn ex Schreb. (gayfeather, blazing star)	50	2.85	7.18	5.91	13.09
Linum sulcatum Riddell (grooved flax)	7	0.03	1.01	0.06	1.07
Lithospermum canescens (Michx.) Lehm. (hoary puccoon)	3	0.02	0.43	0.04	0.47
Lobelia sp. L. (lobelia)	17	0.33	2.44	0.68	3.12
Panicum linearifolium Scribn. *ex Britton* (slimleaf panicum)	23	1.68	3.30	3.49	6.79
Rhus glabra L. (smooth sumac)	13	0.80	1.87	1.66	3.53
Schizachyrium scoparium (Michx.) Nash (little bluestem)	73	13.02	10.49	27.02	37.51
Senecio plattensis Nutt. (prairie ragwort)	37	0.27	5.32	0.56	5.88
Sisyrinchium atlanticum Bickn (blue-eyed grass)*	33	0.17	4.74	0.35	5.09
Solidago nemoralis Aiton subsp. *decemflora* (DC.) Brammall *ex* Semple (gray goldenrod)	33	0.17	4.74	0.35	5.09
Sporobolus heterolepis (A. Gray) A. Gray (prairie dropseed)	27	4.97	3.88	10.31	14.19
unknown forb B (possibly harebell)	17	0.25	2.44	0.52	2.96
unknown grass A	3	0.10	0.43	0.21	0.64
Viola pedatifida G. Don (prairie violet)	7	0.12	1.01	0.25	1.26
Totals	696	48.19	100	100	200
Bare ground and litter		33.22			

[a]Nomenclature used for vascular plants was Kaul *et al.,* 2006 unless denoted by * when nomenclature was Mohlenbrock, 2002

Table 5.5. Frequency (%), average cover (% of total area), relative frequency, relative cover and importance values for vascular plant species at Chadron State College in Dawes County, Nebraska where *Physaria ludoviciana* was present.

Species[a]	Frequency	Average Cover	Relative Frequency	Relative Cover	Importance Value
Andropogon hallii (Hack.) J. Wipff (sand bluestem)	4	0.40	0.59	0.53	1.12
Artemisia campestris L. (field sagewort, Great Plains wormwood)	7	0.09	1.03	0.12	1.15
Artemisia filifolia Torr. (sandsage)	9	0.42	1.32	0.55	1.87
Artemisia frigida Willd. (fringed sage, prairie sagwort)	4	0.08	0.59	0.11	0.70
Asclepias pumila (A. Gray) Vail (plains milkweed)	9	0.53	1.32	0.70	2.02
Asclepias viridiflora Raf. (green milkweed)	2	0.01	0.29	0.01	0.30
Aster falcatus Lindl. (western heath aster)	9	1.01	1.32	1.33	2.65
Bouteloua gracilis (Wild. *ex* Kunth) Lag. *ex* Griffiths (blue grama)	40	1.99	5.87	2.62	8.49
Bromus japonicus Thunb. *ex* Murr. (Japanese brome, hairy chess)	38	3.26	5.58	4.30	9.88
Bromus squarrosus L. (one-way brome)	2	0.07	0.29	0.09	0.38
Bromus tectorum L. (downy brome, cheat, cheatgrass)	2	0.01	0.29	0.01	0.30
Calamovilfa longifolia (Hook.) Scribn. (prairie sandreed)	16	1.66	2.35	2.19	4.54
Calylophus serrulatus (Nutt.) P.H. Raven (plains evening-primrose)	2	0.01	0.29	0.01	0.30
Carex eleocharis L.H. Bailey (sedge)	2	0.01	0.29	0.01	0.30
Carex filifolia Nutt. (threadleaf sedge)	98	26.99	14.39	35.56	49.95
Carex heliophila Mack. (sedge)	4	0.13	0.59	0.17	0.76
Comandra umbellata (L.) Nutt. (false/bastard toadflax)	11	0.11	1.62	0.14	1.76
Conyza canadensis L. Conquist (horseweed, mare's-tail)	9	0.04	1.32	0.05	1.37
Eriogonum annuum Nutt. (annual wild-buckwheat)	2	0.01	0.29	0.01	0.30
Erysimum asperum (Nutt.) DC. (western wallflower)	2	0.01	0.29	0.01	0.30
Gaura coccinea Pursh (scarlet gaura, scarlet bee-blossom)	22	0.17	3.23	0.22	3.45
Heterostipa comata (Trin. & Rupr.) Barkworth. (needle grass)*	100	21.03	14.68	27.70	42.38
Koeleria macrantha (Ledeb.) Schult (Junegrass)	22	1.73	3.23	2.28	5.51
Lepidium densiflorum Schrader (pepper grass, hoary grass)	16	0.08	2.35	0.11	2.46
Leucocrinum montanum Nutt. *ex* A. Gray (star lily)	7	0.09	1.03	0.12	1.15
Liatris punctata Hook. (gayfeather, blazing star)	9	0.10	1.32	0.13	1.45
Lithospermum sp. L. (puccoon, gromwell)	2	0.01	0.29	0.01	0.30

Table 5.5 Continued

Species[a]	Frequency	Average Cover	Relative Frequency	Relative Cover	Importance Value
Lygodesmia juncea (Pursh) D. Don *ex* Hook (skeletonweed)	13	0.07	1.91	0.09	2.00
Medicago lupulina L. (black medick)	2	0.01	0.29	0.01	0.30
Opuntia fragilis (Nutt.) Haw. (little prickly-pear)	7	0.03	1.03	0.04	1.07
Penstemon angustifolius Nutt. *ex* Pursh (narrowleaf beardtongue)	2	0.01	0.29	0.01	0.30
Phlox andicola E.E. Nelson (plains phlox)	11	0.06	1.62	0.08	1.70
Physaria ludoviciana (Nutt.) O'Kane & Al-Shehbaz (silvery bladderpod)	4	0.13	0.59	0.17	0.76
Plantago patagonica Jacq. (woolly plantain)	4	0.02	0.59	0.03	0.62
Poa secunda J. Presl (candy bluegrass)	20	0.64	2.94	0.84	3.78
Psoralidium lanceolatum (Pursh) Rydb. (lemon scurf pea, lance leaf scruf-pea)	4	0.02	0.59	0.03	0.62
Psoralidium tenuiflorum (Pursh) Rydb. (slender-flowered scruf-pea, wild alfalfa)	4	0.02	0.59	0.03	0.62
Tradescantia occidentalis (Britton) Smyth	20	0.16	2.94	0.21	3.15
Tragopogon dubius Scop (goat's beard, western salsify, Johnny-go-to-sleep-at-noon)	11	0.28	1.62	0.37	1.99
Vulpia octoflora (Walter) Rydb. (six-weeks fescue)	98	9.60	14.39	12.65	27.04
Yucca glauca Nutt. (yucca)	31	4.80	4.55	6.32	10.87
Totals	681	75.90	100	100	200

[a]Nomenclature used for vascular plants was Kaul *et al.,* 2006 unless denoted by * when nomenclature was Mohlenbrock, 2002

Table 5.6. Frequency (%), average cover (% of total area), relative frequency, relative cover and importance values for vascular plant species at the Oglala National Grassland, Hudson Meng Bison Bonehead in Sioux County, Nebraska where *Physaria ludoviciana* was absent.

Plant Species[a]	Frequency	Average Cover	Relative Frequency	Relative Cover	Importance Value
Andropogon hallii (Hack.) J. Wipff (sand bluestem)	4	0.13	0.52	0.12	0.64
Artemisia frigida Willd. (fringed sage, prairie sagewort)	60	5.37	7.81	4.96	12.77
Asclepias viridiflora Raf. (green milkweed)	2	0.01	0.26	0.01	0.27
Aster falcatus Lindl. (western heath aster)	16	0.57	2.08	0.53	2.61
Astragalus gilviflorus E. Sheldon (plains orophaca)	2	0.01	0.26	0.01	0.27
Bouteloua gracilis (Wild. *ex* Kunth) Lag. *ex* Griffiths (blue grama)	33	1.16	4.30	1.07	5.37
Bromus japonicus Thunb. *ex* Murr. (Japanese brome, hairy chess)	69	3.16	8.98	2.92	11.90
Carex eleocharis L.H. Bailey (sedge)	49	11.99	6.38	11.07	17.45
Carex filifolia Nutt. (threadleaf sedge)	76	19.91	9.90	18.38	28.28
Carex heliophila Mack. (sedge)	7	1.79	0.91	1.65	2.56
Cirsium undulatum (Nutt.) Spreng. (wavy-leaf thistle)	4	0.02	0.52	0.02	0.54
Dalea purpurea Venten (purple prairie-clover)	9	0.21	1.17	0.19	1.36
Elymus lanceolatus (Scribn. & J.G. Smith) Gould (wild-rye, wheatgrass)	51	8.41	6.64	7.76	14.40
Elymus smithii (Rydb.) Gould (western wheatgrass)	18	0.37	2.34	0.34	2.68
Erigeron canus A. Gray (hoary fleabane)	2	0.07	0.26	0.06	0.32
Erysimum asperum (Nutt.) DC. (western wallflower)	4	0.13	0.52	0.12	0.64
Fabaceae-unknown in this family	2	0.01	0.26	0.01	0.27
Gaura coccinea Pursh (scarlet gaura, scarlet bee-blossom)	16	0.13	2.08	0.12	2.20
Heterostipa comata (Trin. & Rupr.) Barkworth. (needle grass)*	84	30.74	10.94	28.37	39.31
Koeleria macrantha (Ledeb.) Schult (Junegrass)	47	2.89	6.12	2.67	8.79
Lepidium densiflorum Schrader (pepper-grass, hoary grass)	11	0.06	1.43	0.06	1.49
Leucocrinum montanum Nutt. *ex* A. Gray (star lily)	7	0.09	0.91	0.08	0.99
Liatris punctata Hook. (gayfeather, blazing star)	13	0.61	1.69	0.56	2.25
Linum compactum A. Nelson (bushy flax)	2	0.01	0.26	0.01	0.27
Lithospermum incisum Lehm. (fringed puccoon)	4	0.02	0.52	0.02	0.54
Lupinus plattensis S. Watson (Platte lupine)	29	2.10	3.78	1.94	5.72
Oxytropis lambertii Pursh (purple locoweed)	4	0.34	0.52	0.31	0.83
Penstemon albidus Nutt. (white beardtongue)	2	0.01	0.26	0.01	0.27
Phlox andicola E.E. Nelson (plains phlox)	33	1.42	4.30	1.31	5.61
Plantago patagonica Jacq. (woolly plantain)	9	0.04	1.17	0.04	1.21

Table 5.6 Continued

Plant Species[a]	Frequency	Average Cover	Relative Frequency	Relative Cover	Importance Value
Poa pratensis L. (Kentucky bluegrass)	2	0.01	0.26	0.01	0.27
Psoralidium tenuiflorum (Pursh) Rydb (slender-flowered scurf-pea, wild alfalfa)	7	0.09	0.91	0.08	0.99
Schizachyrium scoparium (Michx.) Nash (little bluestem)	36	9.78	4.69	9.03	13.72
Tragopogon dubius Scop. (goat's beard, western salsify, Johnny-go-to-sleep-at-noon)	4	0.08	0.52	0.07	0.59
Vulpia octoflora (Walter) Rydb. (six-weeks fescue)	24	0.12	3.13	0.11	3.24
Xanthium spinosum L. (spiny cocklebur)	2	0.01	0.26	0.01	0.27
Yucca glauca Nutt. (yucca)	24	6.48	3.13	5.98	9.11
Totals	768	108.35	100	100	200

[a]Nomenclature used for vascular plants was Kaul *et al.,* 2006 unless denoted by * when nomenclature was Mohlenbrock, 2002

Table 5.7. Total number of vascular plant species in sites with *Physaria ludoviciana* present or absent in Illinois, Minnesota and Nebraska when surveyed June 2007.

	Illinois	Minnesota	Nebraska	Total
Present	26[a]	30[b]	41[c]	85
Absent	22[d]	27[e]	37[f]	82
Total	36	45	56	119

[a] number of plant species includes *Lepidium* sp.
[b] number of plant species includes *Quercus* sp. and an unknown forb
[c] number of plant species includes *Lithospermum* sp.
[d] number of plant species includes *Carex* sp.
[e] number of plant species includes *Carex* sp., *Liatris* sp., *Lobelia* sp., an unknown forb and an unknown grass
[f] number of plant species includes Fabaceae-unknown in this family

Table 5.8. Plant species found in areas with *Physaria ludoviciana* present or absent in Illinois, Minnesota and Nebraska when surveyed June 2007.

Plant Species[a]	Illinois Present	Minnesota Present	Nebraska Present	Illinois Absent	Minnesota Absent	Nebraska Absent
Ambrosia psilostachya	√	√		√		
Amorpha canescens					√	
Andropogon gerardii		√			√	
Andropogon hallii			√			√
*Arabis lyrata**		√			√	
*Aristida tuberculosa**				√		
Artemisia campestris		√	√			
Artemisia filifolia			√			
Artemisia frigida			√			√
Asclepias pumila			√			
Asclepias verticillata	√					
Asclepias viridiflora	√		√		√	√
Aster falcatus			√			√
Aster oblongifolius	√					
Aster oolentangiensis					√	
Aster sericeus		√			√	
Astragalus gilviflorus						√
Bouteloua curtipendula		√				
Bouteloua gracilis			√			√
Bouteloua palmate	√	√		√		
Bouteloua sp.					√	
Bromus japonicus			√			√
Bromus squarrosus			√			
Bromus tectorum			√			
Calamovilfa longifolia	√	√	√	√		
Calylophus serrulatus			√			
Carex eleocharis			√			√
Carex filifolia			√			√
Carex heliophila						√
Carex muhlenbergii				√		
Carex sp L. unknown A					√	
Carex sp. L. unknown B				√		
Carex tonsa		√			√	
*Castilleja coccinea**		√				
Castilleja sessiliflora					√	
Chamaecrista fasciculata	√					
*Chamaesyce geyeri**	√					
Cirsium undulatum						√
Comandra umbellata			√		√	
Commelina erecta	√			√		
Conyza canadensis	√		√	√		
Coreopsis lanceolata	√					

Table 5.8 Continued

Plant Species[a]	Illinois Present	Minnesota Present	Nebraska Present	Illinois Absent	Minnesota Absent	Nebraska Absent
Coreopsis palmate		√				
*Crotonopsis linearis**				√		
Cyperus schweinitzii				√		
Dalea purpurea					√	√
*Dichanthelium villosissimum**				√		
Elymus lanceolatus						√
Elymus smithii						√
Eragrostis spectabilis				√		
Eragrostis trichodes	√			√		
Erigeron canus						√
Eriogonum annuum			√			
Erysimum asperum			√			√
Euphorbia corollata	√	√			√	
Fabaceae-unknown in that family						√
Gaura coccinea			√			√
Hedeoma hispida		√				
*Helianthus divaricatus**		√				
*Heterostipa comata**			√			√
Heterotheca camporum	√					
Koeleria macrantha	√		√			√
*Krigia virginica**				√		
Lepidium densiflorum		√	√			√
Lepidium sp.	√					
Lespedeza capitata	√			√		
Leucocrinum montanum			√			√
Liatris aspera	√			√		
Liatris punctata			√			√
Liatris sp.					√	
Linum compactum						√
Linum sulcatum					√	
Lithospermum canescens		√			√	
*Lithospermum croceum**	√			√		
Lithospermum incisum		√				√
Lithospermum sp.			√			
Lobelia sp.					√	
Lupinus plattensis						√
Lygodesmia juncea			√			
Medicago lupulina			√			
Oenothera rhombipetala	√			√		
Opuntia fragilis			√			
Opuntia humifusa	√			√		

Table 5.8 Continued

Plant Species[a]	Illinois Present	Minnesota Present	Nebraska Present	Illinois Absent	Minnesota Absent	Nebraska Absent
Oxytropis lambertii						√
Panicum leibergii		√				
Panicum linearifolium		√			√	
Paspalum pubiflorum				√		
Penstemon albidus						√
Penstemon angustifolius			√			
Phlox andicola			√			√
*Phlox bifida**	√					
Physaria ludoviciana	√	√	√			
Plantago patagonica			√			√
Poa pratensis						√
Poa secunda			√			
*Prunus susquehanae**		√				
Psoralidium lanceolatum			√			
Psoralidium tenuiflorum			√			√
Quercus sp.		√				
Rhus glabra					√	
*Salsola tragus**	√					
Schizachyrium scoparium	√	√		√	√	√
Senecio plattensis		√			√	
Silene antirrhina		√				
*Sisyrinchium atlanticum**					√	
Solidago nemoralis		√			√	
Sporobolus cryptandrus		√				
Sporobolus heterolepis		√			√	
Strophostyles helvola	√					
Tradescantia occidentalis			√			
Tradescantia ohiensis		√		√		
Tragopogon dubius			√			√
unknown forb B		√			√	
unknown grass A					√	
Viola pedatifida					√	
Vulpia octoflora			√			√
Xanthium spinosum						√
Yucca glauca			√			√

[a]Nomenclature used for vascular plants was Kaul *et al.*, 2006 unless denoted by * when nomenclature was Mohlenbrock, 2002

Table 5.9. Associated plant species[a] in surveyed areas but not in quadrats at sites where *Physaria ludoviciana* is present or absent in Minnesota and Nebraska[b].

Minnesota *Physaria ludoviciana* Present	Minnesota *Physaria ludoviciana* Absent
Amorpha canescens Nutt. *ex* Pursh (leadplant)	*Asclepias verticillata* L. (whorled milkweed)
Antennaria neglecta Greene (pussytoes, ladies'-tobacco)	*Campanula rotundifolia* L. (harebell, bluebell, bells of Scotland)
Asclepias viridiflora Raf. (green milkweed)	*Delphinium carolinianum* Walt. (wild blue larkspur)*
Castilleja sessiliflora Pursh.(dwarf Indian paintbrush)	*Dichanthelium linearifolium* (Scribn.) Gould. (panic grass)*
Cornus racemosa Lam. (gray dogwood)*	*Erigeron canus* A. Gray (hoary fleabane)
Dalea purpurea Venten (purple prairie-clover)	*Scutellaria leonardii* Epling (small skullcap)*
Delphinium carolinianum Walt. (wild blue larkspur)*	unknown grass B
Dianthus armeria L. (deptford pink)	
Dichanthelium linearifolium (Scribn.) Gould. (panic grass)*	
Dichanthelium oligosanthes (Schult.) Gould. (panic grass)*	
Erigeron strigosus Muhl. *ex* Willd (daisy fleabane, prairie fleabane, rough fleabane)	
Heterostipa sparta (Trin.) Barkworth (porcupine grass)*	
Koeleria macrantha (Ledeb.) Schult (Junegrass)	
Liatris pycnostachya Michx. (blazing star)	
Mirabilis sp. L. (four-o'clock)	
Prunus americana Marsh (American plum)	
Sorghastrum nutans (L.) Nash (Indian grass)	
unknown forb C (possible harebell)	
unknown grass	
unknown moss	

Nebraska *Physaria ludoviciana* Present	Nebraska *Physaria ludoviciana* Absent
Antennaria neglecta Greene (pussytoes, ladies' tobacco)	*Lilium* sp. L. (lily)
Asclepias verticillata L. (whorled milkweed)	*Opuntia* sp. Mill. (prickly-pear)
Erysimum repandum L. (bushy wallflower)	unknown mosses
Onagraceae unknown species	
Orbexilum sp. Rydb.*	
Oenothera perennis L. (little sundrops)	

[a]Nomenclature used for vascular plants was Kaul *et al.*, 2006 unless denoted by * when nomenclature was Mohlenbrock, 2002.
[b]In Illinois other associated species were not collected.

Table 5.10. Soil pH, fertility and texture analysis for soil samples from colonies with *Physaria ludoviciana* present or absent in Illinois, Minnesota and Nebraska.

Parameter Tested	Illinois Present	Minnesota Present	Nebraska Present	Illinois Absent	Minnesota Absent	Nebraska Absent
pH	8.1	8.2	7.4	5.3	7.8	7.7
phosphorus (kg/h)	26.9	9.0	35.8	67.2	42.6	42.6
potassium (kg/h)	85.1	29.1	557.8	71.7	123.2	719.0
calcium (kg/h)	3608.6	542.1	4540.5	351.7	4381.4	12219.2
magnesium (kg/h)	264.3	143.4	387.5	56.0	766.1	365.1
sulfur (kg/h)	22.4	9.0	13.4	20.2	40.3	13.4
boron (kg/h)	0.9	1.1	0.9	1.1	5.2	1.8
copper (kg/h)	1.8	1.3	1.3	1.3	3.1	1.8
iron (kg/h)	118.7	56.0	56.0	145.6	109.8	40.3
manganese (kg/h)	98.6	504.0	47.0	31.4	389.8	60.5
zinc (kg/h)	7.6	2.5	0.9	3.1	12.5	1.3
sodium (kg/h)	38.1	29.1	44.8	33.6	42.6	51.5
organic matter (%)	1.2	0.6	1.3	1.1	3.3	2.9
OM-ENR (kg/h)[a]	76.2	61.6	78.4	73.9	123.2	114.2
CEC (meq/100g)[b]	7.4	1.5	10.0	1.4	10.6	23.7
potassium (%)	1.2	2.1	6.0	5.5	1.3	3.3
calcium (%)	86.0	63.7	80.1	44.3	72.9	90.9
magnesium (%)	12.2	32.7	13.3	13.7	24.7	5.3
hydrogen (%)	0.0	0.0	0.0	30.6	0.0	0.0
sodium (%)	1.0	3.8	0.9	4.7	0.8	0.4
potassium:magnesium	0.10	0.06	0.44	0.39	0.05	0.61
Texture	sand	sand	loamy sand	sand	loamy sand	sandy loam
Sand (%)	96	100	80	96	76	64
Silt (%)	4	0	18	4	24	32
Clay (%)	0	0	2	0	0	4

[a] organic matter and estimated nitrogen release
[b] cation exchange capacity

Table 5.11. Estimated seed production using counts of reproductive structures for *Physaria ludoviciana* at Illinois, Minnesota and Nebraska in June 2007.

Site	Flower Stalks/Plant	Fruits/Stalk	Pedicels/Stalk	Seeds/ Fruit	Estimated Seeds/Plant (Based on Fruit)	Estimated Seeds/Plant (Based on Pedicels)
Mason County, IL	6.2 ± 0.4 a[a, b]	22.8 ± 1.1 a	30.9 ± 1.4 a	2.5 ± 0.3 b	353	479
Hay Creek, MN	2.7 ± 0.2 c	13.1 ± 0.9 c	16.2 ± 0.9 b	2.9 ± 0.4 b	103	127
Red Wing, MN	4.8 ± 0.4 b	16.8 ± 1.6 bc	19.0 ± 1.5 b	4.2 ± 0.1 a	339	383
Dawes County, NE	2.4 ± 0.3 c	18.9 ± 3.5 ab	31.0 ± 4.4 a	3.1 ± 0.4 b	141	231
					234	305

[a]means ± standard errors

[b]means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level

Table 5.12. Height of flower stalks, fruits with holes/stalk, fruits with galls/stalk and percentage of fruits with holes and galls per stalk of *Physaria ludoviciana* at Illinois, Minnesota and Nebraska in June 2007.

Site	Height (cm)[a]	Fruits with Holes/Stalk	Fruits with Galls/Stalk	% of Fruits with Holes	% of Fruits with Galls
Mason County, IL	17.5 ± 0.5 a[b, c]	0.18 ± 0.07 a	0.0 ± 0.0 b	0.8	0.0
Hay Creek, MN	15.8 ± 0.8 a	0.04 ± 0.03 a	0.0 ± 0.0 b	0.3	0.0
Red Wing, MN	16.4 ± 0.9 a	0.18 ± 0.06 a	0.0 ± 0.0 b	1.1	0.0
Dawes County, NE	16.1 ± 1.3 a	0.13 ± 0.07 a	1.4 ± 0.8 a	0.7	7.4

[a]height of tallest flower stalk per plant
[b]means ± standard errors
[c]means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level

Table 5.13. Plant densities of *Physaria ludoviciana* at different developmental stages in Illinois, Minnesota and Nebraska in June 2007.

Site	Seedlings/m^2	Vegetative/m^2	Reproductive/m^2
Mason County, IL	10.8 ± 4.7 a (79%)[a, b, c]	0.9 ± 0.5 ab (7%)	1.9 ± 0.6 a (14%)
Hay Creek, MN	0.0 ± 0.0 b (0%)	1.9 ± 0.5 a (43%)	2.5 ± 0.5 a (57%)
Red Wing, MN	0.4 ± 0.4 b (18%)	0.3 ± 0.2 b (14%)	1.5 ± 0.4 ab (68%)
Dawes County, NE	1.7 ± 0.7 b (68%)	0.4 ± 0.3 b (16%)	0.4 ± 0.2 b (16%)

[a]means ± standard errors

[b]means within a column followed by different letters are significantly different based on Duncan's multiple range test at p=0.05 level

[c]numbers in parentheses indicate percent of plants at each developmental stage relative to total plants of *Physaria ludoviciana*

Figure 5.1. Henry Allan Gleason Nature Preserve, Mason County, Illinois-site where *Physaria ludoviciana* was present.

Picture courtesy of Janice Coons.

Figure 5.2. Sand Prairie-Scrub Oak Nature Preserve, Mason County, Illinois-site where *Physaria ludoviciana* was absent.

Figure 5.3. Richard J. Dorer Memorial Hardwood State Forest, Hay Creek Management Unit, Goodhue County. Minnesota-site where *Physaria ludoviciana* was present.

Figure 5.4. Red Wing, Goodhue County. Minnesota-site where *Physaria ludoviciana* was present. Taken June 2002.

Picture courtesy of Ann Claerbout.

Figure 5.5. Richard J. Dorer Memorial Hardwood State Forest, Hay Creek Management Unit, Goodhue County, Minnesota-site where *Physaria ludoviciana* was absent.

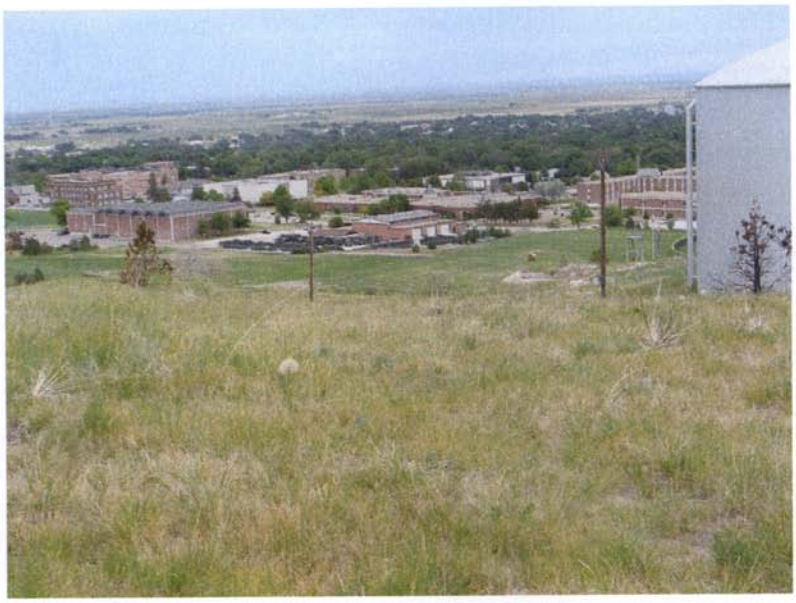

Figure 5.6. Chadron State College, College Water Tower, Dawes County, Nebraska-site where *Physaria ludoviciana* was present.

Picture courtesy of Janice Coons.

Figure 5.7. Oglala National Grassland, Hudson Meng Bison Bonehead, Sioux County, Nebraska-site where *Physaria ludoviciana* was absent.

Picture courtesy of Janice Coons.

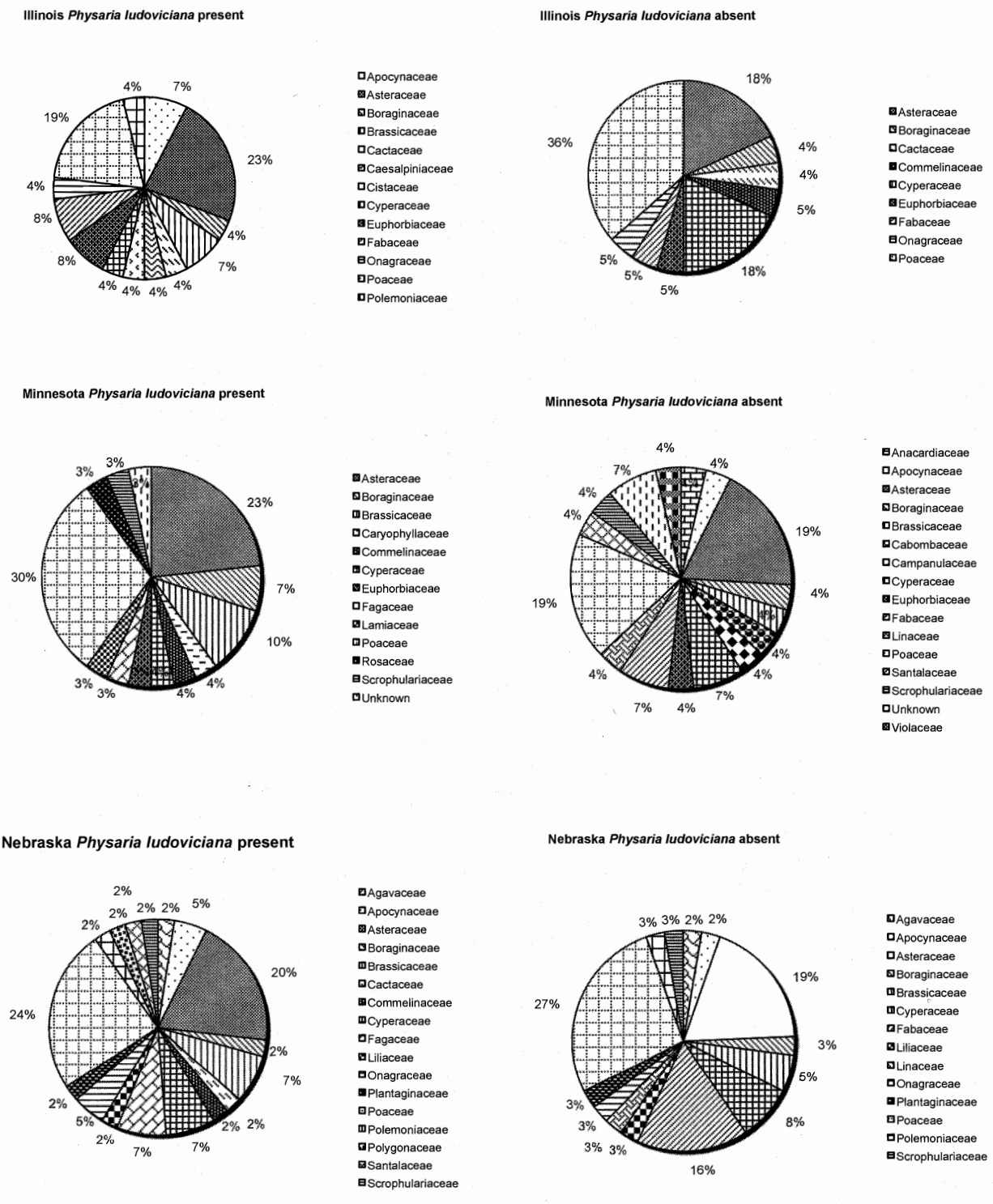

Figure 5.8. Plant families for species at sites where *Physaria ludoviciana* was present and absent in Illinois, Minnesota and Nebraska in June 2007.

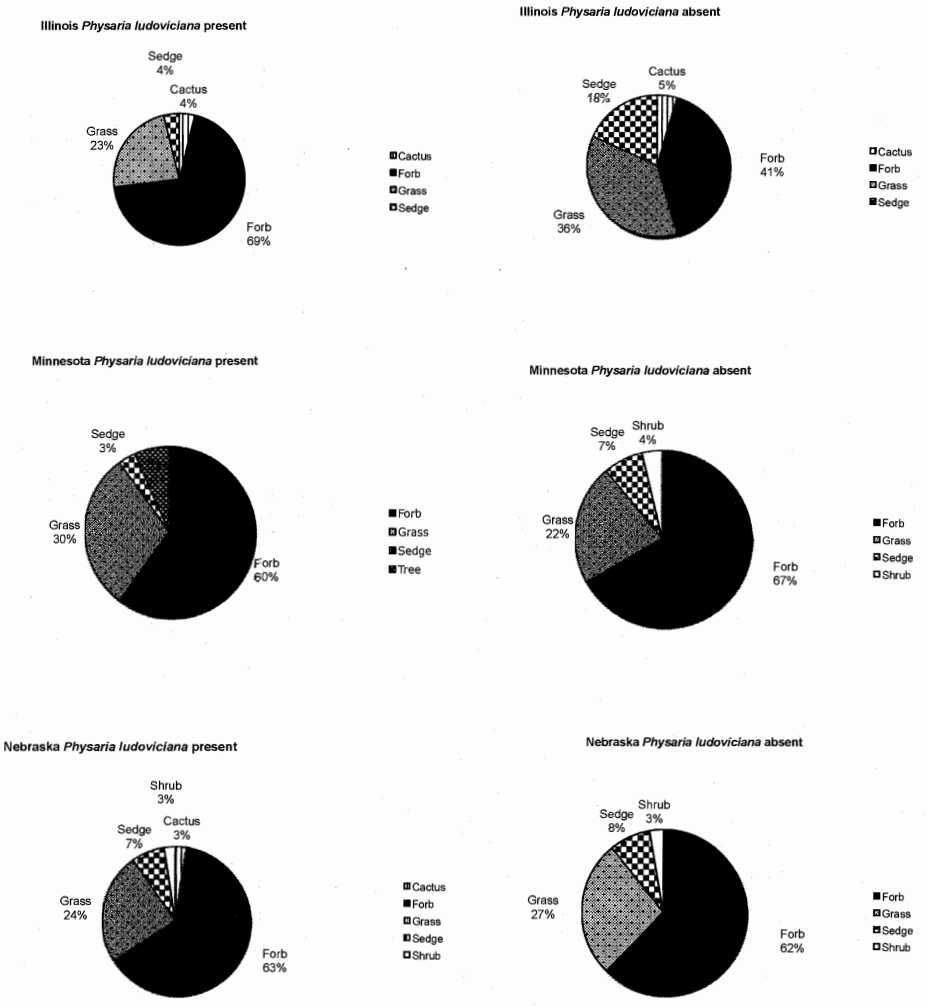

Figure 5.9. Plant habits for species at sites where *Physaria ludoviciana* was present and absent in Illinois, Minnesota and Nebraska in June 2007.

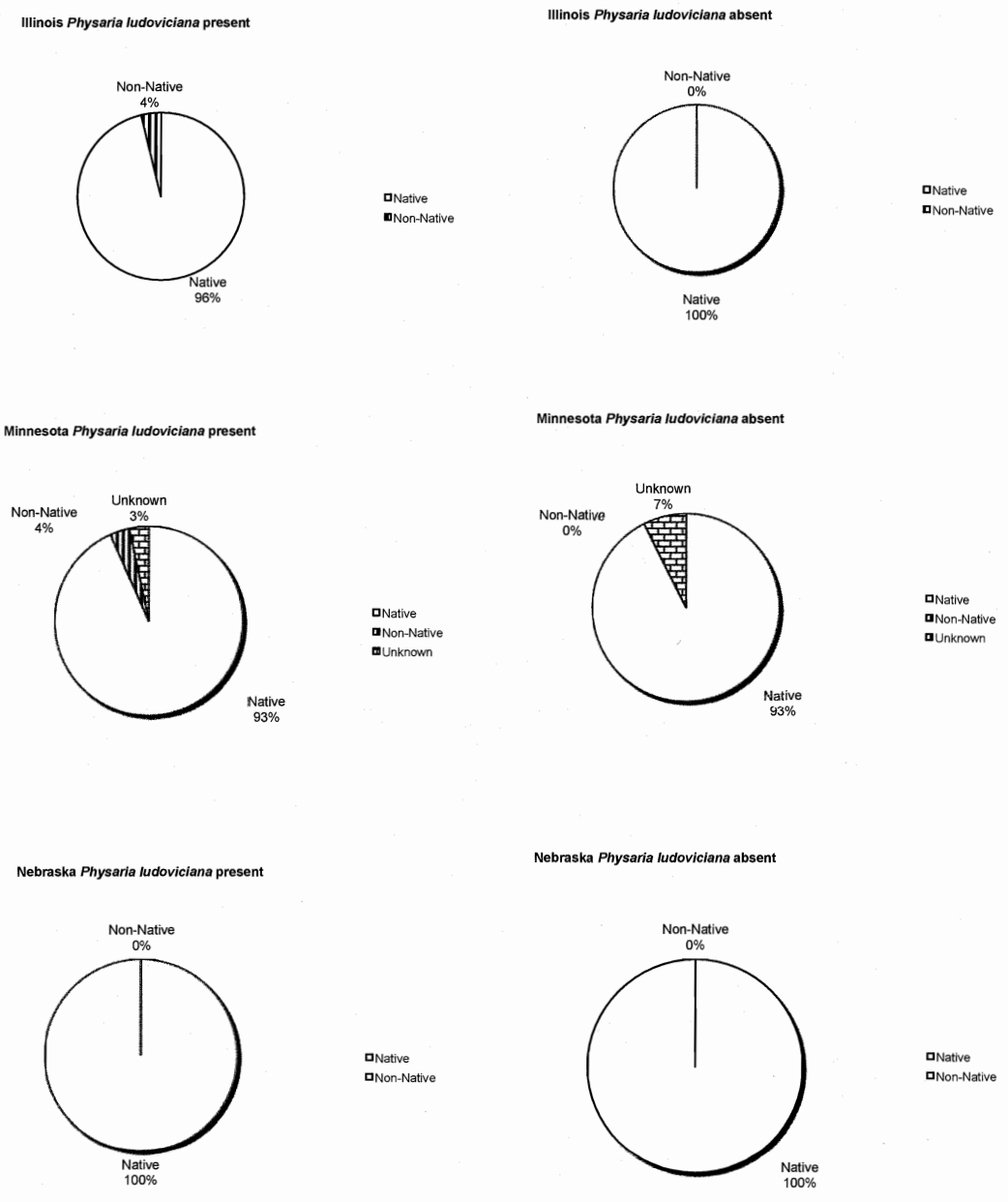

Figure 5.10. Native and non-native plant species at sites where *Physaria ludoviciana* was present and absent in Illinois, Minnesota and Nebraska in June 2007.

Chapter 6

Summary/Importance

This work investigated survival strategies for *Physaria ludoviciana,* an endangered sand prairie species in Illinois. This plant is able to survive in harsh sand habitats that are unfavorable to many plants. The conditions of the sand prairie include low water holding capacity, high summer temperature, frequent disturbance, and full sunlight resulting in limited competition from other species. Survival strategies during all stages of growth and development (seed, seedling, vegetative and reproductive) as well as surveys of areas with *P. ludoviciana* present and absent to evaluate its habitat requirements were studied to help understand its survival in these sand prairies.

Physaria ludoviciana has many factors affecting its seed biology. Seed production was not a limiting factor. Over 9 years, seed production per plant was high with low estimates of 110 to 500 and high estimates of 479 to 744. Not all seeds germinate at once after shed as an afterrippening period was required, which could be beneficial especially if conditions are not ideal when seeds are shed from the mother plant. Seed longevity in the field may be limiting as only 4% of *P. ludoviciana* seeds in soil in June were still found in November, indicating that herbivory is a possible factor. Seed dispersal may be a limiting factor as with no apparent mechanism for dispersal, most seeds were not dispersed further than 1 meter from the mother plant. However, seeds collected when mature and stored at 4°C, remained viable for at least 6.5 years. Studies on seed biology help to understand the recruitment of *P. ludoviciana.*

The ability of seedlings to establish might be limiting population growth. Seedling densities ranged from 0 to 10.8 per m^2, which fluctuated more than vegetative

and reproductive plant densities (0 to 4.4 and 0.5 to 4.6 per m^2, respectively). Low seedling densities indicate relatively few of the many seeds produced were developing into seedlings. Seedlings observed in the field were found within close proximity to reproductive plants. After 4 weeks, plants grown at higher light intensity (585 $\mu mol/m^2/sec$) had significantly greater leaf areas, leaf numbers, fresh and dry masses, and root branching than those at lower light intensity (175 $\mu mol/m^2/sec$). Seedling development from stored seed correlated to maturity at time of collection, indicating the importance of mature seed for the viability of the seedling. Limitations to plant growth with smaller containers suggest that root competition may limit the plant's growth and development. These limitations from restricted root growth occurred even when plants were well watered and fertilized. These studies indicate that encroaching vegetation may hinder the development of *P. ludoviciana* seedlings.

Physaria ludoviciana had several structural and physiological characteristics that allow vegetative plants to adapt to sand prairie conditions including palisade layers on both upper and lower leaf surfaces, which allow for harvesting light from both surfaces. Dense stomates (350 to 510 per mm^2) and trichomes (35 to39 per mm^2) were found. Water loss would be slowed from stomates as dendritic trichomes would trap water and increase humidity in the boundary layer. Early in development, plants put more energy into root than shoot formation giving them a large root:shoot ratio as well as a very long tap root that could access ground water since no water storage tissues were present in the roots or stems. The evergreen characteristic would benefit plants as they would not need water, nutrients and energy for the formation of all new leaves every season. Leaf temperatures compared to soil surface temperatures were higher in the winter showing

that metabolism is possible throughout the year. The anatomy of *P. ludoviciana* is C_3, which aids in its survival, since it has reproductive needs of flowers and fruits in the early spring, and thus has great needs for photosynthates when temperatures are lower compared to summer months. An isotope analysis also supported its C_3 photosynthesis. *Physaria ludoviciana* exhibited greatest transpirational cooling in the summer when soil temperature, light intensity, and air temperature were highest as evidenced by cooler leaf temperatures than soil surface temperatures. Water potentials in March and May were significantly higher than those taken in June and September. A lower water potential in the plant during the drier times of the year helps to ensure that even a little moisture in the soil will be absorbed by the plant. These adaptations are beneficial for *P. ludoviciana* given the harsh environmental conditions of sand prairies.

Floral initiation and fruit set were investigated in reproductive plants of *P. ludoviciana. Physaria ludoviciana* flowered with both photoperiods, long day (16 hr light/8 hr dark) and short day (8 hr light/16 hr dark), so we cannot say for sure if photoperiod is an absolute requirement. Since all plants were started in long days, flowers might have been initiated earlier in long days. However, certainly plants do not require short days. It is also possible that plants do not have a photoperiod requirement, but will flower when mature. *Physaria ludoviciana* plants in Illinois had significantly more flower stalks per plant than any other site corresponding to the higher seed production in Illinois than Minnesota or Nebraska. Holes (possibly due to herbivory) affected around ~1% or fewer fruit. Galls found only in Nebraska affected 7.4% of the fruit. These studies on the reproductive needs of *P. ludoviciana* to flower and produce seed for the next generation could provide useful information to land managers.

In areas where *Physaria ludoviciana* was found, colonies often were within localized areas. The tendency for disjunct and scattered *P. ludoviciana* colonies was found at all areas surveyed. Associated plant species were consistent throughout the areas with *P. ludoviciana* present, and were identified as typical grassland and dry sand prairie forbs and grasses. No differences occurred between the associated species in the areas with *P. ludoviciana* present or absent. Substantial differences in soil traits were not found between sites *P. ludoviciana* present or absent. The disjunct populations could be a result of poor seed dispersal, and therefore instead of associated plant species or soil characteristics being the limiting factor, seed dispersal and establishment could be the limiting factors.

In light of these findings, land managers can better maintain *Physaria ludoviciana* populations. With no mechanism for dispersal and limited seed in the seed bank, it is recommended that seed is collected in late June, since large quantities are produced. This seed could either be stored at 4°C with low humidity or seedlings could be started in large containers, deep enough for tap roots, with high light and temperatures optimal for C_3 photosynthesis to allow for maximum seed growth and development. Once seedlings are established, care needs to be taken to ensure that they are able to develop. Also *P. ludoviciana* could be introduced to other areas since no specific plant species and soil characteristics were associated, so it is likely that most sand prairies would support it. Encroaching vegetation should be kept to a minimum, and aggressive species (*Rhus aromatica*) should be controlled with frequent pulling. As long as the sand prairie habitat remains unchanged, this species should continue to thrive and compete for scarce resources.

Prospectus

While this extensive study on *Physaria ludoviciana* has answered many questions, other unknowns could be answered by further studies. The disappearance of seeds in the seed bank is suspected by herbivory, although future studies should identify agents to lower seed herbivory and investigate herbivory at different times. If herbivory is not the cause of low seedling numbers, it could be that seeds are getting buried too deep. Studies that consider different soil depths for the burial of *P. ludoviciana* would be beneficial. Although the photoperiod study provided good information, it would be useful to conduct a photoperiod study where plants are started in either long or short days from seeds and interrupt the length of the night, as another way to investigate photoperiod effects. *Physaria ludoviciana* plants seemed to stop growing in containers even when other factors such as light, water, and nutrients were constant. Chemical signals could be playing a role in the root identifying self versus non-self but further study is needed. As GIS maps continually improve, over-laying areas with and without *P. ludoviciana* with environmental features such as rivers, elevation, soil type and vegetation could give us knowledgeable insight. The more that is known about *P. ludoviciana,* the better recommendations can be for other species in sand prairie environments, particularly endangered plants.

Appendix A- Seed Dispersal and Longevity in Seed Bank Using Soil Cores

To estimate seed dispersal, soil was collected directly adjacent to ten different reproductive *Physaria ludoviciana* plants, and at 1 and 2 meters from the plant. Samples were collected on 4 June 2007 and 18 June 2007 from the Henry Allan Gleason Nature Preserve (HAGNP) from the North Colony Lower Bowl (NCLB) in Mason County, Illinois, while *P. ludoviciana* plants were shedding seeds. Ten reproductive plants were sampled and a set of samples were taken at each distance. Soil cores were 3 cm diameter at a depth of 20 cm, and were placed in Ziploc® bags. Soil cores were air dried individually in plastic trays (10 X 20 X 6 cm) for at least seven days. Soil cores were weighed and then two cores were combined together to make one replication at each distance, for a total of five replications at each distance. Soil samples then were sent to JFNew Native Plant Nursery (Walkerton, Indiana) where seed was separated from sand using two steps on screen seed cleaning machines, where one uses seed mass, size and shape while the other uses a higher air flow setting in combination with screen sizes. A Clipper 2 Seed Cleaner was used which forced air (from base of screens) to separate seeds through a number of screens. The top screen was (American Society of Testing Materials) ASTM 1/14 where materials not passing over this screen included larger debris such as *P. ludoviciana* seed pods. The bottom screen was ASTM 1/18 where materials passing through 1/18 screen were small sand particles, dust, etc. Materials collected from the bottom base tray and sieves were screened for final separation. All other sized materials were collected in the air blow dust collection port; these materials were inspected and added to bulk sample remnants. Material from the bottom base tray was further separated using brass sieve trays, (ASTM 1/10, 1/12, 1/14, 1/18, 1/25, and

1/35). *Physaria ludoviciana* seed was separated at the 1/14 and 1/18 sieve tray levels and placed in marked plastic Ziploc® bags. Material from all other sieve screens was inspected and placed with other bulk sample remnants. Results using this method showed no seeds in the seed bank.

Seed longevity in the seed bank investigated how long seeds persist in the soil. This study was done at HAGNP in the NCLB. Soil cores were collected 27 April 2007 and in 2008 on 22 January, 30 April, 13 September and 4 June. Samples were taken at a depth of 20 cm with a 3 cm diameter soil corer, and placed in Ziploc® bags. Soil cores were air dried individually in plastic trays (10 X 20 X 6 cm) for at least seven days.

The following procedure was done on the samples collected on 27 April 2007. When dry, samples were sieved with ASTM #35 screen mesh soil sieves to sort seeds and larger particles from soil, since *Physaria ludoviciana* would not pass through that size. After sieving, larger particles that did not go through the ASTM #35 sieve were placed in a coin envelope (6.4 X 10.8 cm) and soil was placed in a 22.0 X 30.5 cm envelope. Envelopes were placed at room temperature. Plastic trays (10 X 20 X 6 cm) were filled with a layer of SB500 High Porosity potting mix (Sun Gro, Seneca, Illinois) and a layer of sand from the recombined soil cores. High Porosity Mix was used because it has good drainage. Each soil core was placed in a separate tray. Trays were placed in greenhouse, watered when needed, and counts were taken of emerged seedlings weekly. No *Physaria ludoviciana* seedlings were found. In the seed bank, the following species were found: *Chenopodium album* L. (lamb's quarters), *Schizachyrium scoparium* (Michx.) Nash. (little bluestem), *Sonchus* sp. L. (sow thistle) and *Sporobolus* sp. R. Br. (dropseed).

Remaining soil samples collected on other dates were sent to JFNew Native Plant Nursery (Walkerton, Indiana). Soil samples were combined and weighed to create five replications for each collection day. The same procedure used for seed dispersal separation was used.

When cores were examined, no seeds were present, which was unbelievable given the number of seeds produced per plant. The next year we switched to soil scrapes, collecting the top 2 cm of soil in a 10 X 10 cm area.

Appendix B-*Physaria ludoviciana* Seedling Establishment and Plant Densities in November 2008

Plant surveys were conducted of *Physaria ludoviciana* seedling, vegetative and reproductive plants in the North Colony Lower Bowl (NCLB) at the Henry Allan Gleason Nature Preserve (HAGNP), Mason County, Illinois on 4 November 2008. A forty-five meter transect was extended through the colony, with a quadrat (0.25 m^2) directly adjacent to transect on alternating sides of the transect with the transect extending south to north through the center of the bowl. In each of the quadrats, number of seedlings ($\leq$ 6 leaves), vegetative plants (> 6 leaves, but no flower stalk) and reproductive plants (flower stalks) were counted. Vegetative and reproductive plants were lumped together since no flower stalks were present. For data analyses, the number of plants at each stage per square meter was calculated. Means and standard errors were calculated. Percentage of plants at each growth stage also was calculated. Findings from this plant density survey are found in Table B1.

Table B1. Number of *Physaria ludoviciana* plants/m^2 sampled in the North Colony Lower Bowl at the Henry Allan Gleason Nature Preserve with quadrats placed at every meter on alternating sides of a 45-meter transect.

Year	Date	Seedling	Vegetative/ Reproductive
2008	4 November	1.3 ± 0.7 (43%)[a,b]	1.7 ± 0.8 (57%)

[a] Means ± standard errors

[b] Numbers in parentheses indicate percent of total plants per population counted as seedling or vegetative/reproductive plants

Appendix C-Vegetation Analysis of *Physaria ludoviciana* Using Geographic Information Systems (GIS)

To see if certain vegetation types occur in areas where *Physaria ludoviciana* was present, a vegetation analysis was completed. Herbaria from across the United States were contacted and asked to send collection information *P. ludoviciana* specimens. Latitude and longitude information was collected for Colorado and Utah because Geographic Information Systems (GIS) maps were very accessible with accurate data. These latitudes and longitudes were put into Universal Transverse Mercator (UTM) coordinates and overlaid on vegetation maps of Colorado and Utah using ArcCatolog and ArcMap (ESRI's ArcGIS suite, St. Paul, Minnesota). Vegetation maps of Colorado were found at http://ndis1.nrel.colostate.edu/cogap/cogaphome.html and of Utah at http://gis.utah.gov/sgid-gap-vegetation/gap-vegetation-land-cover-datasets-2. In Colorado, North American Datum (NAD) 27 and UTM zone 13 was used and in Utah NAD 83 and UTM zone 12 was used. Points around *P. ludoviciana* coordinates were given a 30 meter buffer. Using metadata and an attribute table, vegetation around *P. ludoviciana* was summarized. Percentages of each vegetation type were recorded for each buffered point.

Tables C1 and C2 show vegetation cover in areas where *Physaria ludoviciana* points were. In the vegetation analysis of Colorado, the majority of the points are in the short grass prairie or the sand dune complex. The data received from the herbaria had an exact latitude and longitude point for each plant (plant fully grown have no more than a ¼ meter radius). We gave each data point a large buffer of 30 meters. It is expected that some of the vegetation types in that buffer are areas where *P. ludoviciana* will not occur. For example it is unlikely that it is going to occur in open water, and so when looking at

these datam, the buffer area needs to be taken into consideration. The vegetation data in Utah showed *P. ludoviciana* in a variety of habitats ranging from evergreen such as junipers and pinyon to grassland and shrub areas. While no consistent patterns appeared in the vegetation where *P. ludoviciana* occurred, this finding was not surprising given its disjunct and scattered nature.

It is recommended that for future studies, individual plants are mapped for accurate latitude and longitude, and those points are then over-laid using environmental features.

Table C1. Vegetation types (in percentage) of Colorado where coordinates of *Physaria ludoviciana* were taken for 13 plants.

# of Cover Types	Urban	Dryland Crops	Irrigated Crops	Shortgrass Prairie	Sand Dune Complex (Shrubland)	Open Water Lakes	Forested Wetlands
3	0	0	0	54	0	42	4
2	0	2	0	98	0	0	0
1	0	0	0	100	0	0	0
1	0	0	0	100	0	0	0
2	0	2	0	98	0	0	0
1	0	0	0	0	100	0	0
2	0	19	0	81	0	0	0
4	2	1	0	89	0	0	8
2	0	0	1	0	99	0	0
3	0	0	0	54	0	42	4
2	0	19	0	81	0	0	0
1	0	0	0	0	100	0	0
2	0	0	0	93	0	0	7

Table C2. Vegetation types (in percentage) of Utah where coordinates of *Physaria ludoviciana* were taken for 20 plants.

Plant #	# of Cover Types	Spruce/Fir	Mountain Fir	Juniper	Pinyon	Pinyon/Juniper	Sagebrush	Sagebrush/Perennial Grass
1	3	0	0	1	0	93	6	0
2	3	0	0	27	0	67	6	0
3	2	0	0	0	0	0	0	18
4	4	0	0	0	0	0	0	63
5	2	0	0	0	0	0	0	0
6	2	0	0	0	93	0	0	7
7	2	0	0	0	47	0	53	0
8	3	0	35	0	64	0	1	0
9	4	45	16	0	0	0	0	27
10	4	78	1	0	0	0	0	9
11	4	45	16	0	0	0	0	27
12	2	0	0	0	0	0	0	0
13	3	0	0	2	0	93	5	0
14	3	0	0	24	0	0	25	0
15	3	0	0	27	0	67	6	0
16	2	0	0	0	0	0	0	18
17	4	0	0	19	10	64	0	7
18	4	0	0	0	0	0	0	63
19	2	0	0	0	0	0	84	16
20	3	0	0	0	0	0	53	0

Table C2 Continued

Plant #	Grassland	Dry Meadow	Barren	Ponderosa Pine/Mountain Shrub	Mountain Riparian	Salt Desert Scrub	Blackbrush	No Data
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	82	0	0	0	0	0	0
4	3	0	32	2	0	0	0	0
5	0	0	0	0	0	0	61	39
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	12	0	0	0
11	0	0	0	0	12	0	0	0
12	0	0	0	0	0	0	61	39
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	51	0	0
15	0	0	0	0	0	0	0	0
16	0	82	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0
18	3	0	32	2	0	0	0	0
19	0	0	0	0	0	0	0	0
20	0	0	0	0	5	42	0	0