Reproductive biology and seed germination of the federally endangered *Dalea foliosa* in Illinois

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Introduction

The federally endangered *Dalea foliosa* (A. Gray) Barneby (= *Petalostemum foliosa*, Fabaceae, leafy prairie clover) is a short-lived, herbaceous perennial endemic to the cedar glades of central Tennessee and northern Alabama with disjunct populations in Illinois (USFWS, 1996; USFWS, 1991). A lot of information has been gathered on the subject of seed germination, population demographics, reproductive output, and population genetics for this species (Baskin and Baskin 1989, 1998; Glass et al. 1996; Molano-Flores, 2001, 2004; Wiltshire 1994). Nonetheless, limited information is available regarding the reproductive biology of *Dalea foliosa*, in particular its breeding system. The only piece of information on the subject came from Wemple (1970) who reported that the genus *Dalea* was hermaphroditic and protandrous. In addition, he pointed out that most members of the genus do not self-pollinate. Bowles et al. (1999) indicated that the assessment of the *D. foliosa* breeding system is essential because of the potential effect it might have on population viability.

This study had two main objectives, first to determine if *Dalea foliosa* is a self-compatible or self-incompatible species. Second, to determine if seed germination differences were found between dented smaller seeds vs. well rounded larger seeds (Molano-Flores 2004), since several studies have shown that smaller seeds may have lower seed germination than larger seeds (Hendrix and Trapp 1992; Ress 1997; Venable 1992, see citation within these articles). Gathering this information will allow us to develop a better conservation plan for the species in Illinois.

Methods and Materials

Reproductive biology

In 2003, 20 inflorescences from 20 individuals were covered with bridal veiling before and after hand pollinations to prevent external sources of pollen from reaching the stigma (Fig. 1). Two flower rows per inflorescence, either at the top or bottom of the inflorescence, were used to conduct hand-pollinations with self-pollen to determine if the species was self-compatible or self-incompatible. Hand-pollinations were conducted by removing dehiscent anthers with fine forceps and applying the pollen to the stigmatic surface. The top or bottom two rows from the opposite end of the hand-pollinations were used to determine if the species was autogamous (i.e., could self-pollinate). Different rows from the same inflorescences were used for two main reasons: 1) the USFWS permit to work on this species limits the number of inflorescences that can be studied (i.e., up to 20 inflorescences), and 2) based on the acropetal development of the inflorescence (see below), the same inflorescence can be used. Infructescences were collected during October 2003 and fruit set was determined by classifying fruits as fertilized (i.e., seeds developed) or unfertilized (i.e., no ovular development = flower).

In 2004, due to concerns with potential pollen contamination in 2003, 20 inflorescences from 20 individuals were again covered with bridal veiling to determine if self-pollination (i.e., autogamy) could occur. At the end of the fruiting season (i.e., early September), infructescences were collected and reproductive success was determined by randomly selecting 50 fruits from the middle of the infructescence and classifying them as fertilized (i.e., seeds developed) or unfertilized (i.e., no ovular development). This portion of the study (2003 and 2004) was conducted at the Midewin National Tallgrass Prairie (MNTP) in Will County where most of the plants in the population were caged (Fig. 2). A paired-t test was used to determine if autogamy occurs in *Dalea foliosa* by examining differences between hand-pollinated and not hand-pollinated flowers in 2003 and between fertilized and unfertilized fruits in 2004 (SigmaStat 1997). From the 20 inflorescences that were used in 2003, two were lost and one I was not able to hand-pollinate. In 2004 from the 20 inflorescences that were used, one was lost. In 2003 phenological observation of the flowers and inflorescences were made.

Figure 1: Inflorescence protected with bridal veiling.

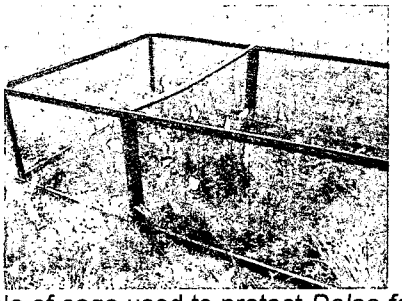


Figure 2: Example of cage used to protect *Dalea foliosa* at MNTP.

Seed germination

Seeds collected from MNTP population from 1998-2001 were used to determine seed germination differences between dented seeds produced by smaller fruits and well rounded seeds produced by plump larger fruits. Fifty plump and 50 dented seeds per year were randomly selected and placed on filter paper within a petri dish. This was replicated four times for a sample size of 200 seeds per year. I followed the seed germination requirements of Baskin and Baskin (1998), 30/15 C and 12light/12dark. Seeds were monitored for germination every other day for a period of 30 days. A seed was considered to have germinated when the radical (root) emerged. Seeds that did not germinate were tested with tetrazolium (TTC) for viability, which stains living tissues red. A two-way Anova was used to determine differences for percent seed germination and percent of seeds that imbibed water (done and reported only for *Dalea foliosa*) among years and type of seed. The percent of seeds that imbibed water was arcsine square root transformed to meet normality. In addition to *Dalea foliosa* seeds, I tested *Dalea purpurea* and *Dalea candida* collected in 2001 from MNTP seedbeds for seed germination. This was done to allow comparisons between *Dalea foliosa* and widespread congeners. Percent seed germination among species (i.e., *Dalea foliosa* and widespread congeners) and type of seed was tested using a two-way Anova. This portion of the study was conducted at the greenhouses growth chambers of the Illinois Natural History Survey in Champaign, IL. All statistical tests were conducted using SigmaStat (1997).

Results

Reproductive biology

The hand pollinations conducted to determine if *Dalea foliosa* was self-compatible or self-incompatible resulted in a total of 60% fruit set suggesting that the species is self-compatible. No significant differences were found for fruit set between hand-pollinated and not hand-pollinated flowers in 2003 ($t = -1.288$, $df = 16$, $P = 0.216$; Fig. 3). In addition, inflorescences covered in 2004 to determine autogamy did not have significant differences between fertilized (mean [± se] = 0.530 ± 04) and unfertilized fruits (mean [± se] = 0.470 ± 04) ($t = -0.663$, $df = 18$, $P = 0.515$). The results (2003 and 2004) suggest that autogamy is a possibility in *Dalea foliosa*.

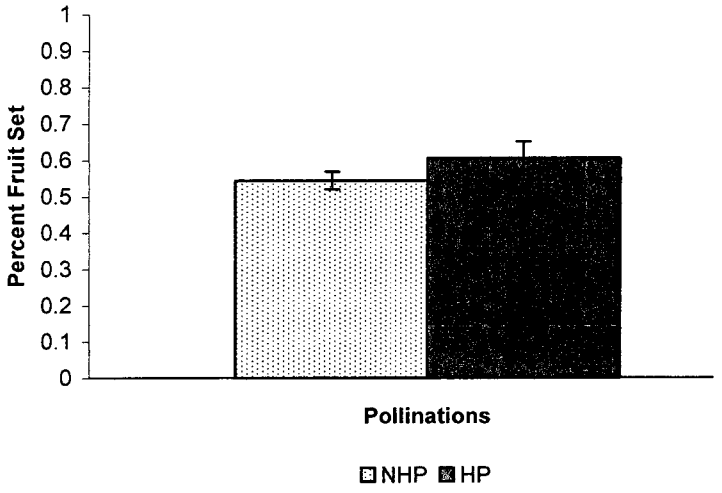


Figure 3: Percent fruit set for pollinations.
(NHP = not hand-pollinated flowers and HP = hand pollinated flowers)

Phenological observations show that the inflorescence of *Dalea foliosa* has an acropetal development (Fig. 4). Usually two rows of flowers open simultaneously and bees can be observed collecting pollen (Fig. 5). For each flower within these rows, the sepals open slightly to allow the petals, stamens, and style to get out. The stamens emerge first and at this point, the two upper anthers are in the process of dehiscing. Later, two more will dehisce until finally the last one dehisces. Anthers change color from yellow (flower closed) to orange (flower open). When a flower is closed, the style is in a hooked shape (Fig. 6a). As the flower opens the style unhooks and becomes linear, later showing a slight downward bend (Fig. 6b). There is no contact between the stamens and style after the flower opens. However, the styles of flowers in an upper row of the same inflorescence occasionally can be observed making contact with the dehisced anthers of the row immediately below. The stigmas become receptive when the style is in the slight downward bending position. Later the style will start bending upward and become hook shaped again. By this point the petals and stamens have started to shrivel up and the stamens will fall off. The hooked style will also shrivel up and recede back into to the sepals (Fig. 6c). Once this happens the next two rows of flowers will start this flowering phenology. This sequential inflorescence development, with its temporal separation between the bottom and top flowers, allows for some inflorescences to have fruits developed at the bottom while flowers are still blooming at the top (Fig. 4).

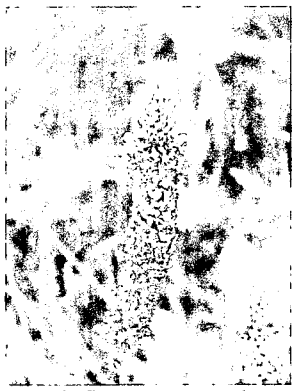
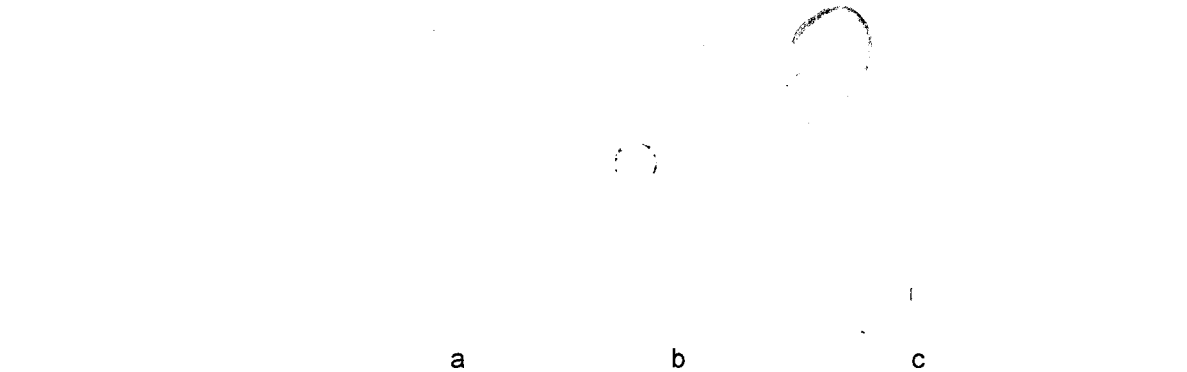


Figure 4: Inflorescence phenology

Figure 5: Bees collecting *Dalea foliosa* pollen



a b c

Figure 6: *Dalea foliosa* pistil: a) style in hook position when inside flower bud; b) style extended after flower opens; c) style in hook position after petals and stamens have shriveled up.

Seed germination

For seed germination, significant differences were found for seed type (plump vs. dented: F= 9.1; df = 1; P = 0.008), but not for year (F= 2.7; df = 3, P = 0.079). Plump seeds had greater percent germination than dented seeds (Fig. 7). However, the interaction was significant (F= 5; df = 3; P = 0.011), meaning that in some years differences between seed types were found. In the case of seeds that imbibed water but did not germinate, significant differences were found for both seed type (plump vs. dented: F= 218; df = 1; P = <0.001) and year (F= 7.1; df = 3; P = 0.003, Fig. 8). A greater percent of dented seeds were able to take up water than plump seeds. Also, the interaction was significant (F= 4.1; df = 3; P = 0.022). The tetrazolium test determined that all *Dalea foliosa* plump seeds were viable, but that none of the dented seeds that did not germinate were viable (Fig. 9a-b).

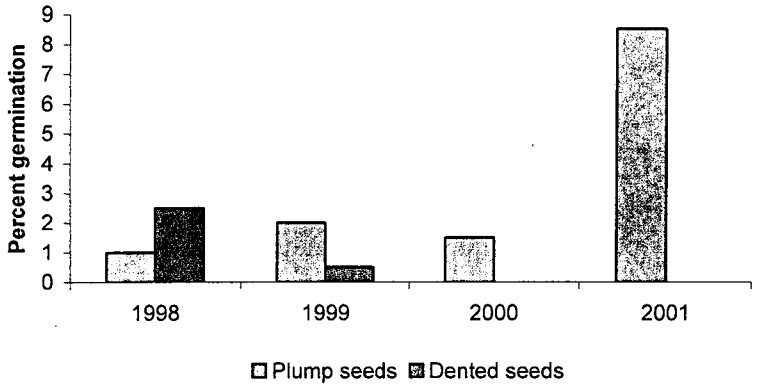


Figure 7: Percent seed germination for *Dalea foliosa* 1998-2001.

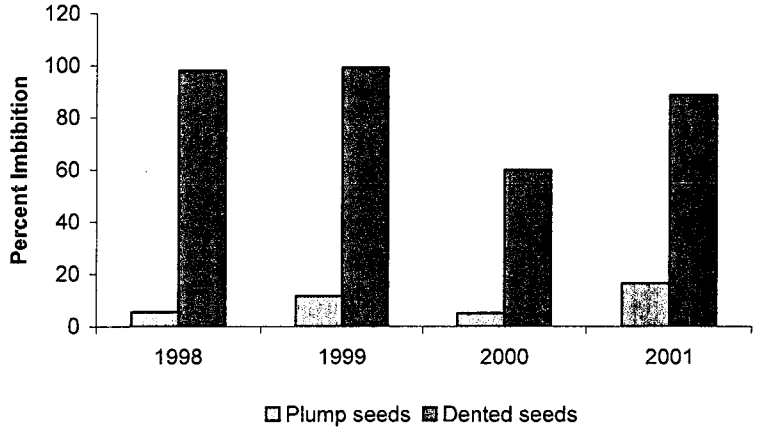


Figure 8: Percent seed imbibition for *Dalea foliosa* 1998-2001.

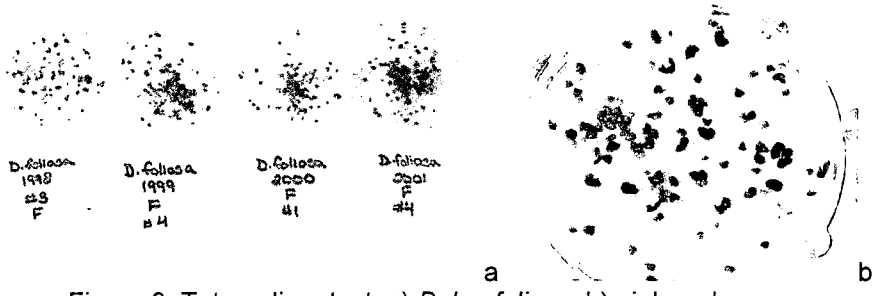


Figure 9: Tetrazolium test: a) *Dalea foliosa*; b) pink embryos

Seed germination differences were also found among species (*Dalea foliosa*, *Dalea purpurea*, and *Dalea candida*; F= 244.2; df = 2; P = <0.001) and type of seeds (F= 416.1; df = 1; P = <0.001) (Fig. 10). Percent seed germination was greatest for *Dalea purpurea* followed by *Dalea candida* and then by *Dalea foliosa*. In addition, regardless of the species, plump seeds had better percent germination than dented seeds. Also, the interaction was significant (F= 83.2; df = 2; P = <0.001).

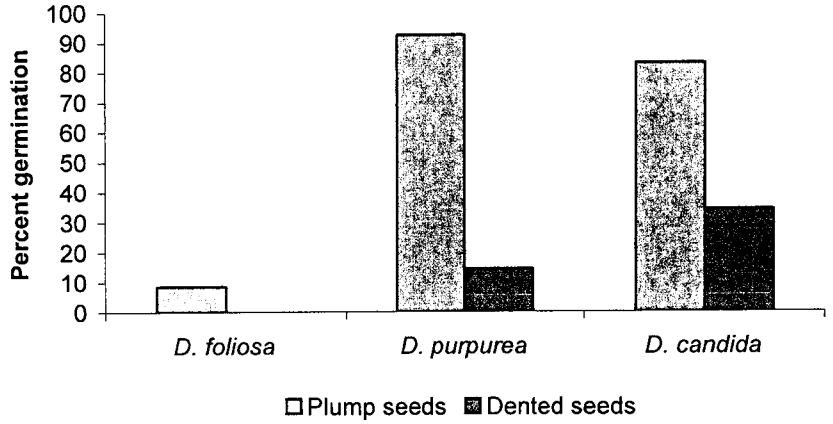


Figure 10: Percent seed germination for *Dalea* spp.

Discussion

Based on fruit set that resulted from hand pollinations using self-pollen, *Dalea foliosa* is a self-compatible species. In addition, phenological observations and fruit set of not hand-pollinated flowers on bagged inflorescences suggest that self-pollination (i.e., autogamy) can occur in *Dalea foliosa*. Wemple (1970) pointed out that self-pollination cannot occur in members of the genus *Dalea* because *Dalea* spp. are protandrous and because of anther position in relation to the stigma (e.g., filaments diverge from the longitudinal axis of the flowers and the style is long with a stigma that extends beyond the anthers). This is true in the case of *Dalea foliosa*, however, it was noticed that the stigmas could touch the anther from the row below potentially resulting in self-pollination (i.e., autogamy). Even though the results of this study suggest that self-pollination can occur in *Dalea foliosa*, this should be taken with caution since although the best efforts were made to exclude pollinators (i.e., inflorescences were covered to prevent pollen contamination), pollinators were observed touching stigmas through the bridal veiling in some inflorescences in both years. Even if autogamous self-pollinations occur, based on phenological observation I do not think that autogamy plays a major role in the reproduction of *Dalea foliosa*. However, geitonogamous pollination, another form of self-pollination, is more common in *Dalea foliosa* based on pollinator observation (data not shown).

In the case of seed germination in *Dalea foliosa*, I was able to determine that plump seeds had greater seed germination than dented seeds, suggesting better fitness in plump seeds. Overall differences between plump vs. dented seeds can be the result of a thinner seed coat or less endosperm associated with dented seeds. This will allow these seeds to absorb more water than plump seeds. However, even though these seeds were able to take up water, the seeds were not viable as seen by a negative tetrazolium test (i.e., the embryos did not turn pink). Also, compared to other congeners *Dalea foliosa* seed germination was very low. In 2001, when congeners were collected and tested against *Dalea foliosa*, seed germination was 8.5 percent compared to over 89 percent for the congeners (Fig. 10).

Two potential reasons for the seed germination differences among *Dalea* species are seed dormancy and seed viability. Seed germination was conducted for a period of 30 days. It possible that this was not enough time to allow *Dalea foliosa* seeds to break dormancy. Seed viability (i.e., lack of it) as a possible explanation for low to no seed germination can be eliminated since the tetrazolium test was positive for the plump seeds that did not germinate (i.e., seeds were viable). Seed scarification, or lack of it, cannot explain the differences among *Dalea* species since all of them were scarified. The low levels of seed germination associated with *Dalea foliosa* may be the

result of inbreeding depression. A study conducted by Edwards et al. (2004, *in press*) found that the Midewin population has no genetic variation.

Summary

Overall the results of this study have provided evidence that *Dalea foliosa* is self-compatible and that self-pollination (i.e., autogamy) is possible. In addition, compared to more widespread *Dalea* species, *Dalea foliosa* has very low seed germination. As pointed out by Molano-Flores (2004), low recruitment associated with this particular population should be addressed since the population size has not increased at this particular study site.

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