

## Does Pollen Supply Limit Seed Set of *Baptisia bracteata*?

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### ABSTRACT

*Baptisia bracteata* is a perennial legume native to tallgrass prairie that flowers early in the growth season and produces a relatively low seed set compared to a taller sympatric congener, *B. alba*. This study tested for evidence that *B. bracteata* is pollen limited. The study site was a reconstructed tallgrass prairie located in northeastern Illinois. Experimental treatments included a control, and two hand-pollination treatments, one where pollen transfer was limited to the same plant and the other where pollen was taken from other plants. Analysis of covariance (ANCOVA) was used to test the effect of treatment on two indicators of pollination success of a plant, i.e., arcsine  $\sqrt{x}$  transformations of pods inflated/flower and seeds matured/flower.  $\text{Log}_{10}(\text{Flower count}/\text{plant} + 1)$  provided a covariate in both ANCOVAs, while likewise transformed counts of a seed predator, *Apion rostrum*, provided a second covariate to seeds matured/plant. Based on ANCOVA, pollination treatment did not affect the number of pods inflated/flower or seeds matured/flower. Flower count/plant showed a significant effect in both comparisons. *A. rostrum*, which synchronizes its life cycle to *B. alba*, did not affect seeds matured/flower of *B. bracteata*. Using Spearman Rank Correlation, flower count/plant was positively related to seeds matured/plant, indicating the importance of inflorescence size to seed set. Count of *A. rostrum*/plant was significantly correlated to pods inflated and seeds matured per plant. Factors not eliminated as affecting seed set of *B. bracteata* were resource limitations and pre-dispersal seed predation by *A. rostrum*.

### INTRODUCTION

Environmental factors linked to low seed set include pollen limitation<sup>pl</sup>, resource scarcities<sup>rs</sup>, and pre-dispersal seed predation<sup>sp</sup> (pl,sp Cariveau et al., 2004; pl Coupland et al., 2005; rs Fulkerson et al., 2012; pl,rs Haig and Westoby, 1988; pl,sp Hainsworth et al., 1984; sp Langer and Rohde, 2005). Pollen supplies may be inadequate due to shortages or unreliability of pollinators particularly in extreme or highly disturbed environments like those in alpine, upper latitude (Fulkerson et al., 2012), fragmented (Holzschuh et al., 2012), or urban locations (Pellissier et al., 2012). Environmental factors causing low pollen supply may select for more apparent optical traits, such as a larger inflorescence, and more attractive floral scents. However, subsequent greater pollination success may result in seeds that a plant cannot support to maturity due to resource limits (Haig and Westoby, 1988) and the attraction of consumers (Adler and Theis, 2012; Ehrlen et al., 2012).

*Baptisia bracteata* Muhl. ex. Ell. (Cream Wild Indigo = *B. leucophaea*) is native to tallgrass prairie of the Midwest (Swink and Wilhelm 1994). The perennial legume produces a low seed set compared to its sympatric congener, *B. alba* (L.) Vent (White Wild Indigo = *B. leucantha*) (Haddock and Chaplin, 1982; Petersen and Wang, 2006). *B. bracteata* blooms during May, a month before *B. alba*, with

little overlap in blooming period. Unlike the taller *B. alba* which can exceed 1m in height, *B. bracteata* rarely exceeds 0.3m. Each *B. bracteata* consists of subterranean rhizomes from which up to several dozen aerial shoots emerge to form a concentric cluster. Racemes bearing yellow flowers, arch outward from the cluster. Flowers last about 4 days, with 2 days spent in a staminate phase, followed by 2 days in a pistillate phase (Haddock and Chaplin, 1982). Self pollination can occur as pollinators, primarily *Bombus* spp., move down an indeterminate raceme. Pods inflate from pollinated flowers. Each pod bears an average of 18 to 19 ovules from which more than half can be expected to initiate seeds (Haddock and Chaplin, 1982; Petersen and Wang, 2006). Pod maturation is complete by August and seeds disperse as pods dehisce.

The cause of low seed output by *B. bracteata* compared to *B. alba* is unknown, but has been hypothesized to be explained by pollen scarcity (Haddock and Chaplin, 1982), limited resources, and avoidance of a seed predator (Haddock and Chaplin, 1982; Petersen and Wang, 2006). The objective of our study was to determine if *B. bracteata* is pollen limited by examining if hand-pollination could increase pollination success.

Complicating factors considered in the experiment were size of inflorescence and pre-dispersal seed predation. Plants

with larger inflorescences are typically presumed to have the resources be able to produce a larger seed set, although this may not always be the case in light of pre-dispersal seed predators (Ohashi and Yahara, 2000). The major pre-dispersal seed predator in our study area located in northeastern Illinois, is *Apion rostrum* Say (Coleoptera: Apionidae). Overwintering weevils oviposit into pods as they inflate. The resulting larvae consume seeds as their only source of nutrition. The adult stage is reached by August, with the new generation of weevils dispersing as pods open.

### METHODS

The study took place during 2012 in the 7.1 ha, reconstructed Russell Kirt Tallgrass Prairie located on the campus of College of DuPage, IL. The prairie plot, reconstructed beginning in 1984, is characterized by the grasses big bluestem (*Andropogon gerardii* Vitman), prairie dropseed (*Sporobolus heterolepis* Gray), and Indian grass (*Sorghastrum nutans* (L.) Nash), plus some 150 species of forbs to include *B. bracteata*. The prairie plot was burned during March of 2012 after a six-year period in which it was not burned.

A concentric cluster of *B. bracteata* was assumed to be one individual based on inspection of excavated *B. bracteata* not used in the experiment. Plants were selected randomly as they flowered during May. Flowers of plants in the control treatment

were not manipulated, while those of the other treatments were hand-pollinated using paint brushes. A “selfing” treatment involved introducing pollen to a stigma where pollen came from racemes of the same cluster, while in a “crossing” treatment, pollen was introduced from racemes of other clusters. These treatments did not limit pollen from contrary sources, but did permit examination of how supplemental pollination from a source could change pollination success. Hand pollination was repeated a week apart as to include flowers as they developed along indeterminate racemes. Due to the availability of individual *B. bracteata*, sample sizes were 18 for both a control and a selfing treatment, and 17 for a crossing treatment.

Counts taken were flowers/plant, pods inflated/plant, pods matured/plant, seeds matured/pod, and *A. rostrum*/pod. Counts of seeds matured/plant and *A. rostrum*/plant were pro-rated from the total number of ripe pods of a plant in the case when some ripe pods were damaged and contents could escape. Pollination success was quantified by pods inflated/flower and seeds matured/flower.

All statistical summarization was done using Statistica (Statsoft, 2001). Analysis of covariance (ANCOVA) was performed on pods inflated/flower (IP/F), with flower count/plant entered as covariate, and also on seeds matured/flower (S/F), with counts of flowers/plant and *A. rostrum*/plant as covariates. In the ANCOVA involving weevils, plants were eliminated from analysis if all ripened pods had holes from which weevils could have escaped prior to sampling. Prior to analyses, pods inflated/flower (IP/F) and seeds matured/flower (S/F) of a plant were arcsine  $\sqrt{x_i}$  transformed, and counts of flowers/plant were  $\log_{10}(x + 1)$  transformed to meet assumptions of parametric analysis (Zar, 1984). Counts of *A. rostrum*/plant also were  $\log_{10}(x + 1)$  transformed, but remained skewed (1.114 among all treatments). Hence, parametric analysis involving weevil counts is possibly spurious because of this violation. Preliminary analyses involving IP/F and S/F indicated that the “pollination treatment X flower” interaction was not significant ( $P = 0.758$ ) and that the “pollination treatment X flower count/plant X *A. rostrum* count/

plant” interaction was not significant ( $P = 0.649$ ), respectively, satisfying homogeneity of slopes.

Due to failure in meeting assumptions of parametric testing with plant counts of weevils and seeds, Spearman Rank Correlation was used to test for relationships between plant counts of flowers and seeds matured, *A. rostrum* and pods inflated, and *A. rostrum* and seeds matured. The first contrast provided insight if a particular size of inflorescence could have advantage in seed set, and the last two how components of reproductive yield may attract the weevil to *B. bracteata* plus the potential effect of weevil abundance on

seed yield.

## RESULTS

Table 1 summarizes data from pollination treatments. Pollination treatment had no effect on pods inflated/flower, and also seeds matured/flower, although in both cases, flower count/plant did (Tables 2 and 3). With the absence of a treatment effect, group data were pooled to illustrate the relationship of flower count/plant to seeds matured/plant (Figure 1). Plants with a larger inflorescence showed a higher seed output ( $r_s = 0.489$ ;  $df = 51$ ;  $P < 0.05$ ). *A. rostrum* count/plant was positively related to inflated pod count/plant ( $r_s = 0.679$ ;  $df = 41$ ;  $P < 0.05$ ) and seeds matured/plant ( $r_s =$

**Table 1.** Summary (sample mean  $\pm$  standard error) of select reproductive parameters and weevil infestation according to treatment. Sample size = 18 unless noted otherwise by subscript.

Treatment Variable	Control	Selfing	Crossing
Flower count/plant	105.1 $\pm$ 32.8	97.9 $\pm$ 25.1	61.9 $\pm$ 21.7 <sub>17</sub>
Pods inflated/plant	36.2 $\pm$ 12.4	47.1 $\pm$ 16.4	34.3 $\pm$ 19.4 <sub>17</sub>
Pods inflated/flower count	0.39 $\pm$ 0.086	0.38 $\pm$ 0.07	0.45 $\pm$ 0.07 <sub>17</sub>
Seeds matured/plant	44.9 $\pm$ 21.4	62.2 $\pm$ 30.0	65.2 $\pm$ 27.8 <sub>17</sub>
<i>Apion rostrum</i> count/plant	15.3 $\pm$ 8.0 <sub>15</sub>	21.0 $\pm$ 10.9 <sub>13</sub>	17.8 $\pm$ 11.7 <sub>15</sub>

**Table 2.** Results of ANCOVA showing the effects of pollination treatment (control, selfing, crossing) and flower count/plant on pods inflated/flower. Symbol: F = flower count.

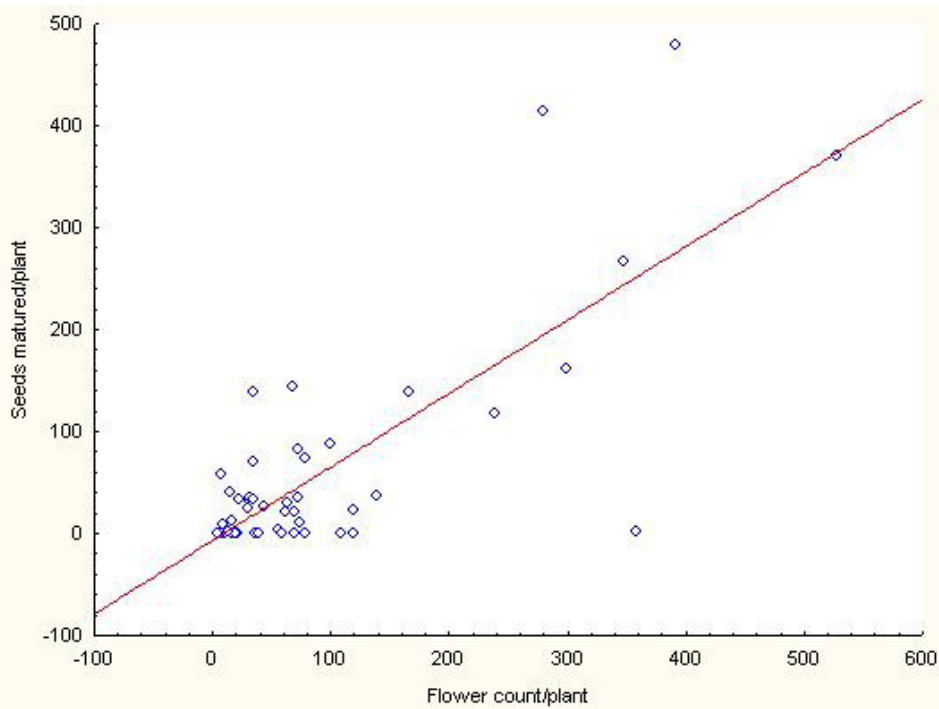
Effect	df	MS	F	P
$\log_{10}(F/\text{plant} + 1)$	1	22.19	129.03	<0.001
Pollination treatment	2	0.215	1.25	0.296
Error	50	0.172		
Model vs. SS Residual $r^2$	0.72			

Note: Univariate Tests of Significance for arcsine  $\sqrt{\text{pods inflated/flower}}$ ; Sigma-restricted parameterization; Type III decomposition.

**Table 3.** Results of ANCOVA showing the effects of pollination treatment (control, selfing, crossing), flower count/plant, and weevil count/plant on seeds matured/flower. Symbols: F = flower count, A = *Apion rostrum* count.

Effect	df	MS	F	P
$\log_{10}(F/\text{plant} + 1)$	1	5.38	21.91	<0.001
$\log_{10}(A/\text{plant} + 1)$	1	0.27	1.08	0.307
Pollination treatment	2	0.43	1.75	0.192
Error	28	0.25		
Model vs. SS Residual $r^2$	0.63			

Note: Univariate Tests of Significance for arcsine  $\sqrt{\text{pods inflated/flower}}$ ; Sigma-restricted parameterization; Type III decomposition.



**Figure 1.** Scatter plot showing the relationship between counts of flowers/plant and seed matured/plant for *Baptisia bracteata*.

0.496;  $df = 41$ ;  $P < 0.05$ ).

## DISCUSSION

We did not find evidence that *B. bracteata* is pollen limited. Hand-pollination did not result in higher pod inflation/flower or seeds matured/flower. In view of the higher ovule to seed initiated ratio of *B. bracteata* found in an earlier study (Petersen and Wang, 2006), it is possible that seed set of the species is resource limited and cannot develop all pollinated ovules. Others have proposed that plants, in effect, bet hedge resource availability, where they produce more ovules during an average year than can be expected to develop into mature seeds (Burd et al., 2009; Fulkerson et al., 2012). Under occasional conditions of higher resource availability, these plants can mature more seeds. As our study only involved one season; any bet hedging could not be assessed.

Pre-dispersal seed predation has also been proposed to be a selective force that influences reproductive characteristics of plants including the timing of flowering (Elzinga et al., 2007; Kolb et al., 2007; Tsvuura et al., 2011). However, the earlier flowering time of *B. bracteata* compared to *B. alba*, unlike-

ly would have deterred *A. rostrum* from synchronizing its lifecycle around the latter. The weevil appears quite adaptive to exploiting species of *Baptisia* around the Midwest and South (Evans et al., 1989; Horn and Hanula, 2004). The additive characteristic of lower seed set, whether explained genetically and/or by resource limitations, may enable *B. bracteata* to escape the brunt of seed predation. Hence, a divergent flowering period and initiating fewer seeds than *B. alba* may actually promote seed set of the cream wild indigo over time.

In our study, *A. rostrum* appeared attracted to *B. bracteata* based on the positive relationship of the weevil count/plant to inflated pod count/plant. More pods have the potential to produce more seeds, explaining the positive correlation of *A. rostrum* count to seeds matured/plant. However, the latter relationship was not negative as would be predicted if seed production was severe. Nonetheless, seed predation has been shown to be highly variable over time (Kolb et al., 2007), to include that by *A. rostrum* (Petersen et al., 2010), necessitating longer-termed study focusing on the relationship between *B. bracteata* and *B. alba*. High error values in the reproductive and

weevil infestation measurements of *B. bracteata* (Table 1) may also reflect the small sample sizes available in our study. Future study should include larger populations of the *Baptisia* congeners to reduce errors that can affect statistical comparisons.

## LITERATURE CITED

- Adler, L. S. and N. Theis. 2012. Advertising to the enemy: enhanced floral fragrance increases beetle attraction and reduces plant reproduction. *Ecology* 93:430-435.
- Burd, M., T. Ashman, D. R. Campbell, M. R. Durdash, M. O. Johnston, R. M. Knight, S. J. Mazer, R. J. Mitchell, J. A. Steets, and J. C. Vamosi. 2009. Ovule number per flower in a world of unpredictable pollination. *Am. J. Bot.* 96:1159-1167.
- Cariveau, D., R. E. Irwin, A. K. Brody, L. S. Garcia-Mayeya, and A. von der Ohe. 2004. Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits. *Oikos* 104: 15-26.
- Coupland, G. T., E. I. Paling, and K. A. McGuinness. 2005. Floral abortion and pollination in four species of tropical mangroves from northern Australia. *Aquat. Bot.* 84:151-157.
- Ehrlen, J., A. Borg-Karlson, and A. Kolb. 2012. Selection on plant optical traits and floral scent: Effects via seed development and antagonistic interactions. *Basic Appl. Ecol.* 13:509-515.
- Elzinga, J. A., A. Atlan, A. Biere, L. Gigord, A. E. Weis, and G. Bernasconi. 2007. Time after time: flowering phenology and biotic interactions. *Trends Ecol. Evol.* 22:432-439.
- Evans, E., C. C. Smith, and R. P. Gendron. 1989. Timing of reproduction in a prairie legume: seasonal impacts of insects consuming flowers and seeds. *Oecologia* 78: 220-230.
- Fulkerson, J. R., J. B. Whittall, and M. L. Carlson. 2012. Reproductive ecology and severe pollen limitation in the polychromic tundra plant, *Parrya nudicaulis* (Brassicaceae). *Plos One* 7:1-8.
- Haddock, R. C. and S.J. Chaplin. 1982. Pollination and seed production in twophenologically divergent prairie legumes (*Baptisia leucophaea* and *B. leucantha*). *Am. Midl. Nat.* 108: 175-186.
- Haig, D. and M. Westoby. 1988. On limits to seed production. *Am. Nat.* 131:757-759.
- Hainsworth, F. R., L.L. Wolf, and T. Mercier. 1984. Pollination and pre-dispersal seed predation: net effects on reproduction and inflorescence characteristics in *Ipomopsis aggregate*. *Oecologia* 63:405-409.
- Holzschuh, A., J. Dudenhöffer, and T. Tschardtke. 2012. Landscapes with wild bee pollination, fruit set and yield of sweet cherry. *Biological Conserv.* 153:101-107.
- Horn S. and J. L. Hanula. 2004. Impact of seed

- predators on the herb *Baptisia lanceolata* (Fabales: Fabaceae). *Fl. Entomol.* 87:398-400.
- Kolb, A., J. Ehrlén, and O. Eriksson. 2007. Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. *Perspect. Plant Ecol. Evol. Syst.* 9:79-100.
- Langer, V. and B. Rohde. 2005. Factors reducing yield of organic white clover seed production in Denmark. *Grass Forage Sci.* 60:168-174.
- Ohashi, K. and T. Yahara. 2000. Effects of flower production and predispersal seed predation on reproduction in *Cirsium purpuratum*. *Can. J. Bot.* 78:230-236.
- Pellissier, V., A. Muratet, F. Verfaillie, and N. Machon. 2012. Pollination success of *Lotus corniculatus* (L.) in an urban context. *Acta Oecol.* 39:94-100.
- Petersen, C. E. and W. Wang. 2006. Congener host selection by the pre-dispersal seed predator, *Apion rostrum* (Coleoptera: Apionidae). *Gt. Lakes Entomol.* 39:68-74.
- Petersen, C. E., A. A. Mohus, B. A. Petersen, and B. A. McQuaid. 2010. Multiyear study of factors related to flowering phenology and reproductive yield of *Baptisia alba* in Northeastern Illinois. *Trans. Ill. State Acad. Sci.* 103:109-117.
- Swink, F., and G. Wilhelm. 1994. *Plants of the Chicago Region*, 4th ed. The Indiana Academy of Science, Indianapolis, IN.
- StatSoft. 2001. *Statistica AX 6.0*. 2001. StatSoft, Tulsa, OK.
- Tsvuura, Z., M. E. Griffith, R. M. Gunton, and M. J. Lawes. 2011. Predator satiation and recruitment in a mast fruiting monocarpic forest herb. *Ann. Botany* 107: 379-387.
- Zar, J.H. 1984. *Biostatistical Analysis*, 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, NJ, USA.