

Environmental Factors Associated with Modular Growth and Flowering Phenology of *Baptisia bracteata* Muhl. ex. Ell.

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ABSTRACT

Baptisia bracteata Muhl. ex. Ell. reproduces sexually and clonally like many perennial plants. The rhizomatous legume forms conspicuous clumps of ramets bearing pale yellow flowering racemes during spring. This study investigates environmental factors governing the modular growth and flowering phenology of the species. The study was conducted in a reconstructed tallgrass prairie located in northeastern Illinois. From 2007 to 2013, excluding 2010, genets were sampled annually for counts of ramets, racemes, flowers, pods inflated, seeds matured, and infestation by a pre-dispersal seed predator, *Apion rostrum* Say (Apionidae). Pods inflated/flower, flowers/ramet, and *A. rostrum* counts/pod provided estimates of pollination success, nutritional allocation to flower production, and weevil infestation, respectively. Sampling also included 32 genets that were followed from 2008-2013. Based on ramet counts, larger genets produced more racemes and flowers, but not more mature seeds. Larger genets were also negatively correlated to flower count/ramet, and to change in genet size over the following season. Coupled with a higher coefficient of variation among larger genets, the declining flower production/ramet and growth of larger genets suggest effects of resource limitation. Counts of pods inflated and weevil infestation of pods were not related to genet size. Findings indicate that interspecific competition selects for larger genet size to the limits where resource limitation restricts further growth. Sexual reproduction may provide enough genetic variation to withstand the heterogeneity of the tallgrass environment through time and space. Seed predation by *A. rostrum* was not so severe as to be negatively related to seed yield.

INTRODUCTION

Baptisia bracteata Muhl. ex. Ell. (Cream Wild Indigo = *B. leucophaea*) and its congener, *B. alba* (L.) Vent (White Wild Indigo = *B. leucantha*) (Fabaceae), coexist in savannas and prairies of Northeastern Illinois (Mohlenbrock, 2002; Swink and Wilhelm, 1994). *B. bracteata* has an earlier flowering period, and is shorter in stature, flowering, and seed yield compared to *B. alba*. This phenology may enable *B. bracteata* to reduce competition with *B. alba* (Haddock and Chaplin, 1982) and also infestation by *Apion rostrum* Say (Apionidae) which is a pre-dispersal seed predator of both perennials (Petersen et al., 2012).

In contrast to *B. alba* which typically bears a main stem that extends over 1m in height, *B. bracteata* grows to a height of about 0.3m and has a spread of over 0.5m in rhizomatous genets that project multiple arching ramets. *B. bracteata* blooms during May when the prairie is coming out of a winter's senescence, forming conspicuous clumps of ramets with pale yellow-flowering racemes among the emerging vegetation. In contrast, *B. alba* flowers during June. *Bombus* spp. are the major pollinators of the congeners although both show a minor degree of autogamy (Haddock and Chaplin, 1982). Pod inflation in both species indicates successful pollination.

Growth patterns and reproductive traits of perennial plants are known to be affected by selective pressures in the forms of herbivores (Ehrlén, 1995; Osada and Sugiura, 2006), pollinators (Fulkerson et al., 2012; Méndez and Díaz, 2001), seed predators (Cariveau et al., 2004; Ehrlén et al., 2012; Mduma et al., 2007; Petterson, 1994), and competition for physical resources (Chen et al., 2010; Dong et al., 2013; Sánchex-Humanes et al., 2011; Szymura and Szymura, 2013). Plants that reproduce by sexual reproduction and clonal growth, like *B. bracteata*, may benefit from both forms of reproduction (Hartnett and Bazzaz, 1985; Schmid et al., 1995). Sexual reproduction can produce phenotypes that promote persistence in a changing environment and seeds can be dispersed away from the parent plant. In contrast, clonal growth does not disrupt an adaptive genetic type and can offer to buffer against environmental risks, e.g., predation of a ramet does not eliminate the genet and joined ramets may share resources.

There is no evidence that *B. bracteata* is pollen-limited (Petersen et al., 2013). Larger genets, based on counts of ramets, produce more flowers, suggesting an importance of a larger floral display. However, for producing so many flowers, the legume yields few seeds. A change in ramet counts/genet over a 3-year period indicated declining

growth with genet size, reflecting possible resource limitation in larger genets (Petersen et al., 2013). Seed predation by *A. rostrum* does not appear to be negatively correlated to the number of seeds matured. In the course of studying *B. bracteata*, the high variances in seeds matured/pod or pods inflated/genet among plants and across years have limited the scope of conclusions that could be drawn from short-term studies. This study involves a multi-year study of the patterns of growth and reproduction of *B. bracteata* with the objectives of identifying environmental factors governing the modular growth and flowering phenology of the legume.

METHODS

The study location was the reconstructed 7.4ha Russell Kirt Tallgrass Prairie located on the main campus of College of DuPage, IL. Reconstruction of the prairie plot began in 1984. Major grasses are big bluestem (*Andropogon gerardii* Vitman), prairie dropseed (*Sporobolus heterolepis* Gray), and Indian grass (*Sorghastrum nutans* (L.) Nash). Data were collected during the years 2007-2013, except for year 2010. During this 6-year period, the prairie was burned once, March of 2012.

A concentric cluster of stems was assumed to be one individual genet of *B. bracteata* based on inspection of excavated plants not

included in the study. Sampling of genets was the same across years and designed to examine advantages of genet size to pollination success and seed yield. Genet size was estimated by counts of ramets/genet, pollination success by genet counts of pods inflated/flower, nutritional allocation to flowering by genet counts of flowers/ramet, and seed yield by seeds matured/genet. Included with this analysis were flowering onset and duration, and infestation of pods by *A. rostrum*.

Genets of *B. bracteata* were randomly selected for study and each were counted for ramets, racemes, flowers, inflated pods, and seeds matured, date of onset of flowering, and flowering duration. Counts of *A. rostrum*/pod were also taken at the time of seed counts to estimate infestation rates. If pods had holes from which weevils could escape, *A. rostrum* were prorated from the total pod count using mean weevil counts of intact pods.

Thirty-two of the genets had continually been sampled from 2008 to 2013, excluding 2010. These genets were used to investigate how genet size was related to future growth.

Statistical analyses were done using Statistica 6 (Statsoft, 2001). Relationships among the reproductive parameters and weevil infestations were examined using Spearman Rank Correlation when assumptions of parametric testing could not be met (Zar, 1984).

Regression analysis was done to examine the effects of ramet count/genet on flower investment for a given year, and using the 32 plants followed from 2008 through 2013, on future genet growth. Ramet and flower counts were first log10(x+1) transformed prior to analyses to meet normality.

Coefficients of variation (CV) were compared between smaller *B. bracteata* and those larger. Based on ramet counts/genet, the 32 genets were divided approximately in half by size for each year prior to analysis. Finding that the distributions followed normal distributions, the grand mean CVs from smaller and larger genets were compared across years using 2-tailed dependent t-tests to investigate the relationship between genet size and CV.

RESULTS

Table 1 shows the variation in growth parameters of *B. bracteata* and infestation of pods by *A. rostrum* across the years of the study. Genets with more ramets produced more racemes and flowers (Table 2). During the majority of the years, more ramets were also linked to more pods inflated, an earlier onset of flowering, and a longer flowering duration. Nevertheless, more ramets did not translate to more seeds matured/genet or to weevil counts/pod. A greater floral display, based on raceme and flower counts per genet, was positively related pods inflated, and during some years, to seeds matured, but showed no relationship to weevil counts (Table 3). Similarly to ramet counts, plants with larger floral displays tended to

flower earlier and for a longer duration. Pods inflated and seed matured per genet were always positively related to one another, and to counts of pods inflated/flower (Table 4). Only during two years of the study were the counts of pods inflated and *A. rostrum*/pod correlated, and positively. Counts of seeds matured/genet were never found to be related to the seed predator.

Larger genets yielded lower counts of flowers/ramet (Figure 1). In addition, the least growth in ramet counts/genet occurred among larger genets during 3 of the 4 sampling intervals and overall from 2008 to 2013 (Figure 2). Grand mean CV's computed across years was significantly greater for larger genets than those smaller (Table 5).

Table 1. Summary (mean ± 95% confidence limits) of *Baptisia bracteata* growth parameters/genet and rates of *Apion rostrum* pod infestations according to year of sampling. Except where noted by subscript, sample sizes were 51 for 2007, 50 for 2008, 2009, and 2011, 53 for 2012, and 65 for 2013.

Parameter	Year					
	2007	2008	2009	2011	2012	2013
Ramets	9.5 ± 1.9	8.0 ± 2.0	9.6 ± 1.9	10.7 ± 2.2	13.6 ± 3.6	13.6 ± 2.6
Racemes	5.55 ± 1.61	6.26 ± 1.91	7.02 ± 1.57	6.86 ± 2.92	6.59 ± 2.24	11.12 ± 2.30
Flowers	136 ± 47	114 ± 32	95 ± 24	105 ± 42	100 ± 37	160 ± 37
Flowering onset (days)	11.4 ± 1.4 ₄₇	9.7 ± 1.5	9.2 ± 1.6	14.0 ± 2.0 ₄₂	9.2 ± 1.1	2.8 ± 0.5
Flowering duration (days)	14.5 ± 1.1 ₄₇	16.8 ± 1.3	17.2 ± 1.1	16.5 ± 2.2 ₄₂	13.4 ± 1.2	18.1 ± 1.0
Pods inflated	41.6 ± 21.4	9.8 ± 6.2	32.3 ± 12.9	23.0 ± 18.9	48.8 ± 24.7	21.6 ± 7.4
Seeds matured	45.1 ± 37.3	28.1 ± 32.7	62.5 ± 41.3	7.2 ± 11.2	30.3 ± 16.1	9.9 ± 9.2
<i>Apion rostrum</i> /pod	0.99 ± 0.27 ₃₉	0.43 ± 0.35 ₂₀	0.28 ± 0.12 ₃₂	0.83 ± 0.33 ₂₄	0.32 ± 0.13 ₄₃	0.55 ± 0.13 ₄₉
Inflated pods/flower count	0.27 ± 0.08 ₄₇	0.07 ± 0.03	0.35 ± 0.12	0.14 ± 0.06 ₄₇	0.82 ± 0.09	0.18 ± 0.06
Flowers/ramets	18.3 ± 8.3	16.1 ± 2.7	11.8 ± 2.7	10.1 ± 3.2	8.1 ± 1.9	12.3 ± 2.2

Table 2. Spearman rank correlation comparing ramet counts to those for growth parameters of *Baptisia bracteata* and *Apion rostrum*/pod according to year. All counts are per genet except for counts of the weevils which were weighted per pod of a genet. Except where noted by subscript, sample sizes were 51 for 2008, 50 for 2007, 2009, and 2011, 53 for 2012, and 65 for 2013. Bold type indicates significant (P<0.05). Symbols for counts are denoted Ra=racemes, Fl=flowers, PI=pods inflated, Se=seed matured, and AR=*A. rostrum*. Fl On=flowering onset and Fl Du=flowering duration.

Parameter	Year	Ra	Fl	PI	Se	AR	PI/Fl	FlOn	FlDu
Ramets	2007	0.50	0.33	0.17	0.02	0.20 ₃₉	0.07 ₄₇	-0.32 ₄₇	0.03 ₄₇
	2008	0.55	0.65	0.15	-0.06	0.00 ₂₀	0.09	-0.20	0.13
	2009	0.45	0.41	0.43	0.10	-0.06 ₃₂	0.20	-0.35	0.36
	2011	0.33	0.35	0.28	0.24	0.02 ₂₄	0.21	-0.07	0.45
	2012	0.57	0.54	0.33	0.24	0.27 ₄₃	-0.07	-0.38	0.49
	2013	0.78	0.33	0.33	0.13	0.18 ₄₈	0.33	-0.28	0.31

DISCUSSION

Expansion by *B. bracteata* through more ramets may enable establishment in a competitive environment. *B. bracteata* is short in stature among prairie vegetation to include among the tall grasses. After flowering, the legume remains green, presumably replacing energy and nutrient reserves

needed for the next flowering season before senescing back to the soil at summer's end. Coverage of more space by a larger genet increases access to light, compensating to a degree for short height. We found little evidence that larger genets have higher pod inflation, yield more seeds, or attract more *A. rostrum* even though they tended to flower over a longer duration. Pod infes-

tation rates by *A. rostrum* also failed to relate to counts of seeds matured/genet. The absence of correlations of weevil counts/pod to inflated pods or seeds matured per genet could indicate that larger plants saturate the seed predator, or that ovipositing females mark pods, limiting the pod number of feeding larvae as has been shown by other weevils including *Apion* spp. (Hod-
 dle, 1991; Kozlowski et al., 1983).

Table 3. Spearman rank correlation comparing counts of racemes and flowers to those for growth parameters of *Baptisia bracteata* and *Apion rostrum*/pod according to year. All counts are per genet except for counts of the weevils which were weighted per pod of a genet. Except where noted by subscript, sample sizes were 51 for 2008, 50 for 2007, 2009, and 2011, 53 for 2012, and 65 for 2013. Bold type indicates significant (P<0.05). Symbols for counts are denoted by Fl=flowers, PI=pods inflated, Se=seed matured, and AR=*A. rostrum*. Fl On=flowering onset and Fl Du=flowering duration.

Parameter	Year	Fl	PI	Se	AR	PI/Fl	FlOn	FlDu
Racemes	2007	0.91	0.61	0.23	0.17 ₃₉	0.12 ₄₇	-0.55₄₇	0.33₄₇
	2008	0.80	0.32	0.18	0.27 ₂₀	0.27	-0.22	0.20
	2009	0.83	0.61	0.27	0.05 ₃₂	0.25	-0.58	0.42
	2011	0.89	0.52	0.38	-0.14 ₂₄	0.32	-0.35	0.50
	2012	0.93	0.56	0.40	0.21 ₄₃	-0.04	-0.63	0.63
	2013	0.92	0.58	0.16	0.13 ₄₉	0.58	-0.39	0.42
Flowers	2007		0.70	0.31	0.18 ₃₉	0.20 ₄₇	-0.42₄₇	0.28 ₄₇
	2008		0.21	0.00	0.23 ₂₀	0.11	-0.36	0.37
	2009		0.59	0.20	0.06 ₃₂	0.15	-0.55	0.43
	2011		0.60	0.49	-0.15 ₂₄	0.37	-0.32	0.54
	2012		0.71	0.43	0.27 ₄₃	0.10	-0.63	0.66
	2013		0.66	0.11	0.20 ₄₉	0.66	-0.27	0.32

Table 4. Spearman rank correlation comparing counts of pods inflated and seeds matured to those for growth parameters of *Baptisia bracteata* and *Apion rostrum* according to year. All counts are per genet except for counts of the weevils which were weighted per pod of a genet. Except where noted by subscript, sample sizes were 51 for 2008, 50 for 2007, 2009, and 2011, 53 for 2012, and 65 for 2013. Bold type indicates significant (P<0.05). Symbols for counts are denoted by Se=seed matured, AR=*A. rostrum*, PI=pods inflated, and Fl=Flowers. Fl On= flowering onset and Fl Du=flowering duration.

Parameter	Year	Se	AR	PI/Fl	FlOn	FlDu
Pods inflated	2007	0.57	0.32₃₉	0.77₄₇	-0.26	0.25
	2008	0.56	0.00 ₂₀	0.98	0.00	-0.06
	2009	0.52	-0.01 ₃₂	0.82	-0.28	0.52
	2011	0.70	0.18 ₂₄	0.93	-0.02	0.38
	2012	0.55	0.55 ₄₃	0.67	-0.49	0.48
	2013	0.48	0.19 ₄₉	1.00	-0.13	0.26
Seed matured	2007		0.02 ₃₉	0.54₄₇	-0.17	0.24
	2008		0.32 ₂₀	0.60	0.23	-0.20
	2009		0.10 ₃₂	0.49	0.02	0.20
	2011		-0.11 ₂₄	0.61	0.17	0.45
	2012		0.12 ₄₃	0.33	-0.24	0.24
	2013		-0.01 ₄₉	0.48	0.22	0.07

Table 5. Grand mean (± 95% confidence limits) coefficients of variation (CV) for ramet counts from small and large genets measured across the years, 2007, 2008, 2009, 2011, 2012, and 2013. Per year, genet size was categorized based on being below or greater than the median count of ramets/genet. Grand means differed significantly (t=2.615; df=10; P<0.05).

Genet size	Mean ± SE
Small	0.390 ± 0.059
Large	0.485 ± 0.116

Stagnant to declining genet growth and counts of flowers/ramet, plus a higher CV in ramet counts among larger genets across years, indicate resources are becoming limited. Ramets of larger genets should be expected to suffer more unevenly from resource limitation than those smaller, resulting in a higher CV's for ramet counts. Although larger genets produced more racemes and flowers than those smaller, greater pod inflation and higher seed yield were occasional events mainly associated with the latter variables.

Trade-offs among components of reproductive yield are central to life history evolution (Roff, 1992). Increasing resource allocations to sexual reproduction or asexual reproduction can be at the expense of the other (Bazzaz et al., 1987; Cheplick, 1995; Ronsheim and Bever, 2000). Contrarily, larger size may enable plants to allocate more energy to both modes of reproduction (Coelho et al., 2005). We did not find clear evidence tradeoffs between sexual and asexual reproduction based on changing genet size. Larger genets despite producing more flowers, did so in decreasing proportion to size. The change in ramet growth also showed a negative relationship to genet size. With respect to growth pattern, *B. bracteata* appears to progress to a size limited by resources, with even the larger genets maturing a small but continuous number of seeds.

In conclusion, our results indicate that interspecific competition appears to be the force in selecting for larger genet size to the limits where resource limitation affects further growth. During some years, the size of inflorescence and flowering duration may function to promote flowers pollinated and seeds matured. Seed yield, which is small relative to flowers produced, may provide enough dispersal capability, plus the genetic variation needed to tolerate

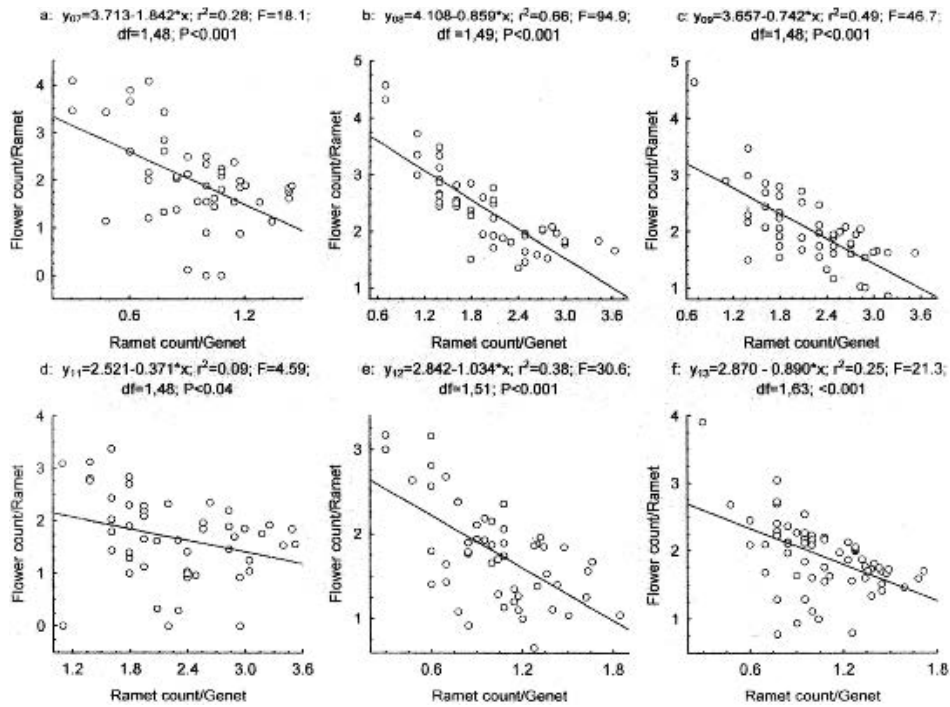


Figure 1. Regression analysis of flowers/ramet as affected by ramet count for genets of *Baptisia bracteata*. Outcomes of testing are provided. All counts have been $\log_{10}(x+1)$ transformed.

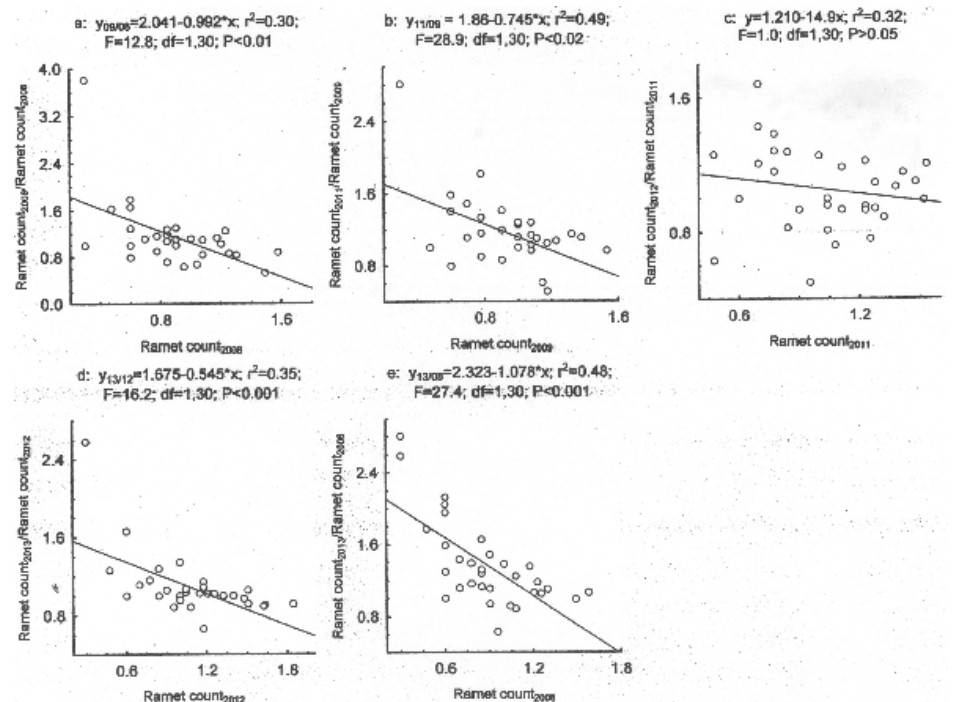


Figure 2. Regression analysis from *Baptisia bracteata* of change in ramet count/genet as affected by ramet count at the beginning of the sampling period. Outcomes of regression testing are provided. All counts have been $\log_{10}(x + 1)$ transformed.

the unpredictable aspects of the tallgrass environment for even the larger genets constrained in expansion by resource limitation. Pre-dispersal seed predation by *A. rostrum* appears not to be so severe as to be related negatively to seed yield. Our findings do not discount the view that *A. rostrum* synchronizes its life cycle more around the prolific *B. alba*.

LITERATURE CITED

Bazzaz, F., N. R. Chiariello, P. D. Coley, and L. F. Pitelka. 1987. Allocating resources to reproduction and defense. *BioScience* 37:58-67.

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.

Cheplick, G. P. 1995. Life history trade-offs in *Amphibromus scabrivalvis* (Poaceae): Allocation to clonal growth storage, and cleistogamous reproduction. *Am. J. Bot.* 82:621-629.

Chen, S. Y., J. L. Zhang, P. Jia, J. Xu, G. Wang, and S. Xiao. 2010. Effects of size variation and spatial structure on plastic response of plant height to light competition. *Chinese Science Bulletin* 55:1135-1141.

Coelho, F. F., L. Deboni, and F. S. Lopes. 2005. Density-dependent reproductive and vegetative allocation in the aquatic plant, *Pistia stratiotes* (Araceae). *Rev. Biol. Trop.* 53:369-376.

Dong, B., J. Wang, R. Liu, M. Zhang, and F. Yu. 2013. Effects of heterogeneous competitor distribution and ramet aggregation on the growth and size structure of a clonal plant. *PLOS ONE* 8:1-12.

Ehrlén, J. 1995. Demography of the perennial herb *Lathyrus vernus*: I. Herbivory and individual performance. *J. Ecol.* 83:287-295.

Ehrlén, 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.

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.

Hartnett, D. C. and F. A. Bazzaz. 1985. Integration of neighborhood effects by clonal genets in *Solidago canadensis*. *J. Ecol.* 73:415-427.

Hodde, M. S. 1991. Gorse pod susceptibility and oviposition preference to the gorse seed weevil, *Apion ulicis* (Forster)(Coleoptera: Apionidae). *New Zeal. J. Zool.* 18:31-35.

- Kozłowski, M. W., S. Lux, and J. Dmoch. 1983. Oviposition behavior and pod marking in the cabbage seed weevil, *Ceutorhynchus assimilis*. *Entomol Exp. Appl.* 34:277-282.
- Mduma, S. A., A. R. E. Sinclair, and R. Turkington. 2007. The role of rainfall and predators in determining synchrony in reproduction of savanna trees in Serengeti National Park, Tanzania. *J. Ecol.* 95:184-196.
- Méndez, M. and A. Díaz. 2001. Flowering dynamics of *Arum italicum* (Araceae): Relative role of inflorescence traits, flowering synchrony, and pollination context on fruit initiation. *Am. J. Bot.* 88:1774-1780.
- Mohlenbrock, R. H. 2002. Vascular Flora of Illinois. Southern Illinois University Press, Carbondale, IL.
- Osada, N. and S. Suguira. 2006. Effects of pollinators and flower bud herbivores on reproductive success of two ericaceous woody species differing in flowering season. *Can. J. Bot.* 84:112-119.
- Pettersson, M. W. 1994. Large plant size counteracts early seed predation during the extended flowering season of a *Silene uniflora* (Caryophyllaceae) population. *Ecography* 17:264-271.
- Petersen, C. E., S. M. Bauzys, F. A. Speranske, and B. A. Petersen. 2012. The varying phenology and growth patterns of *Baptisia bracteata* (Fabaceae) in reconstructed prairie. *Trans. Ill. State Acad. Sci.* 105:79-84.
- Petersen, C. E., S. J. Detloff, S. K. Shukin, and B. A. Petersen. 2013. Does pollen limit seed set of *Baptisia bracteata*? *Trans. Ill. State Acad. Sci.* 106:1-8.
- Roff, D. A. 1992. The Evolution of Life Histories. Chapman and Hall, New York, NY.
- Ronsheim, M. L. and J. D. Bever. 2000. Genetic variation and evolutionary trade-offs for sexual and asexual reproductive modes in *Allium vineale* (Liliaceae). *Amer. J. Bot.* 87:1769-1777.
- Sánchez-Humanes, B., V. L. Sork, and J. M. Espelta. 2011. Trade-offs between vegetative growth and acorn production in *Quercus lobata* during a mast year: the relevance of crop size and hierarchical level within the canopy. *Oecologia* 166:101-110.
- Schmid, B., F. A. Bazzaz, and J. Weiner. 1995. Size dependency of sexual reproduction and of clonal growth in two perennial plants. *Can. J. Bot.* 73:1831-1837.
- Szymura, M. and T. H. Szymura. 2013. Soil preferences and morphological diversity of goldenrods (*Solidago* L.) from south-western Poland. *Acta Soc. Bot. Pol.* 82:107-115.
- StatSoft. 2001. Statistica AX 6.0. 2001. StatSoft, Tulsa, OK.
- Swink, F. and G. Wilhelm. 1994. Plants of the Chicago Region, 4th ed., Indiana Academy of Science, Indianapolis, IN.
- Zar, J.H. 1984. Biostatistical Analysis, 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, NJ, USA.