



Experimental Pollinator Exclusion in Low and High Density Stands of *Chamaecrista fasciculata* (Partridge Pea)

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Abstract - Long-term persistence of native plant species in Iowa's fragmented tallgrass prairies will be dependent upon several interacting factors. For outcrossing species, the lack of pollinators may limit seed set and genetic diversity. Small isolated populations may be most at risk of pollinator limitation if the collective floral display fails to attract adequate numbers of pollinators. This field study addressed the effect of experimental pollinator exclusion on the seed set in high density and low density stands of a "buzz pollinated" native plant, *Chamaecrista fasciculata*. Exclusion was achieved by enclosing flowering stems in mesh bags before the flowers had opened. We manipulated the degree of exclusion by varying mesh size. Following fruit set, legumes were removed from the plants and dry weights of the fruits or seeds were compared. We hypothesized that pollinator exclusion would reduce seed set, predicting an inverse relationship between seed set and degree of exclusion (i.e. high exclusion results in low seed production). In addition, we hypothesized pollinator exclusion to have a greater effect in the high density stand. Our results did not support our hypotheses. Pollinator exclusion did not result in significant differences in seed set in the high density stand. In the low density stand, seed set was significantly lower in the control treatment (no exclusion).

Introduction:

Pollinator availability is a limiting factor for many plants, especially those species that are obligate outcrossers. Even self-compatible species may experience inbreeding depression and reduced fecundity if sufficient pollinators are unavailable. In highly altered, fragmented and/or degraded habitats lacking pollinator refugia, native plant species relying upon specific types of pollinators may be particularly at risk of extirpation. Conversely, fecundity of native species is often positively associated with pollinator abundance and diversity (Slagle and Hendrix 2009).

Population density can also affect plant fecundity. For both wind and animal pollinated plants, studies indicate a positive relationship between the size of the plant population and the fecundity of the plants (Fenster 1991, Agron 1996). Several interacting factors may contribute to these observations. In general, stochastic processes at work in small populations of self-incompatible plants may result in a reduction of genetic variability that limits the number of suitable mates available for outcrossing. For plants dependent upon animal pollinators, high density stands will appear more attractive to pollinators than plants in populations of low density. Finally, even when pollinator availability and suitable mates do not limit a plant from reproducing, inbreeding depression, often occurring in populations of small density, could result in reduced fecundity or in offspring that are of lower quality than those in comparable high density populations (Agron 1996).

Given the significant losses and fragmentation of tallgrass prairie habitat in Iowa, research addressing the relationships between plant population size, pollinator availability, and native plant persistence is of increasing interest.

In our study, we examined the effect of pollinator exclusion on the seed set of an annual legume, *Chamaecrista fasciculata*, in high and low density stands. *C. fasciculata* is self-compatible, but due to its unique anatomy, it rarely self-pollinates (Fenster 1991).

We hypothesized that :

- Overall *C. fasciculata* seed set will be higher in high vs. low density plots.
- Increasing levels of pollinator exclusion will reduce seed set of *C. fasciculata*.
- The overall effect of pollinator exclusion on *C. fasciculata* seed set will be more pronounced in high vs. low density plots.

Materials and Methods:

Study Site – This study was conducted at the Landsmeer Golf Club in Orange City, IA. The greens and fairways of the course are surrounded by variable sized patches of "prairie" plantings (predominantly native grasses and forbs). Initially established in 1994, these patches are managed by occasional burns and selective weed removal. *Chamaecrista fasciculata* was not included in the original seed mix, but occurs as a volunteer in some of the habitat patches. A large population is now established west of the 12th fairway and has spread to the east side of the fairway. These patches were designated as our high and low density plots (Figure 1).

Experimental Design – We used bags of 3 different mesh sizes (4mm, 1mm, and 0.25mm) for our pollinator exclusion treatments. On August 26, 2010, bags were tied to stem tips supporting unopened flower buds. Strings tied under unopened flower buds on stem tips acted as our control treatment. Sample sets consisted of 4 closely growing plants each assigned to one treatment. Initially, we had 23 sample sets (92 plants total) in high density plots and 24 sample sets (96 plants total) in low density plots.

The stem tips were harvested on October 7, 2011. Fruits (legumes) were removed from the bags and allowed to air dry for 3 weeks. All seeds from each legume were weighed and seed weight was used as an indicator of fecundity. In addition, a subset of seeds per legume (all treatments) was regressed against seed weight per legume to assess whether seed weight is a good indicator of seed number per legume (Figure 2). Within plot significance of treatment type was assessed via 1-way ANOVAs and Tukey's HSD (modified for unequal sample sizes). Between plot differences for each treatment were assessed via unpaired TTESTs.



Figure 1. Location of Study Site (googlemaps.com)

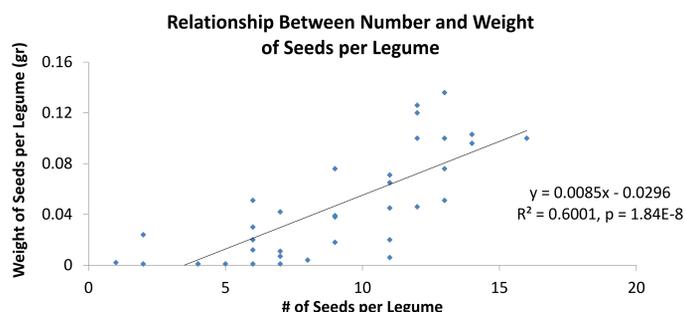


Figure 2. Regression of seed weight per legume against number of seeds per legume is positive and significant.

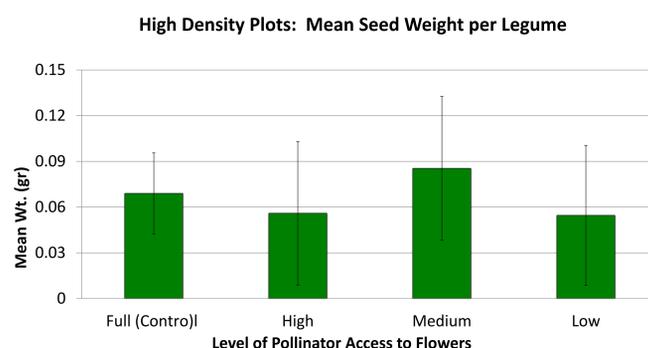


Figure 3. In high density plots, pollinator exclusion did not have a significant effect on fecundity (ANOVA $F_{3,24} = 0.81, P = 0.50$). Standard deviations are indicated by error bars.

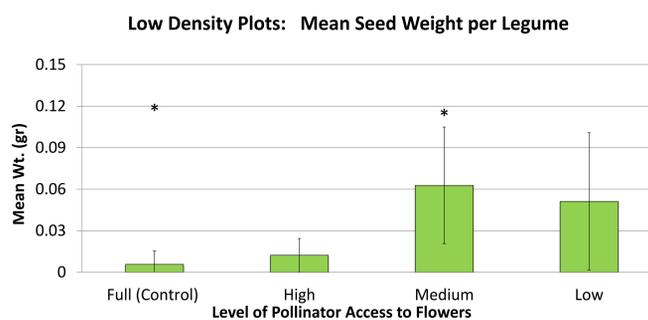


Figure 4. In low density plots, pollinator exclusion had a significant effect on fecundity (ANOVA $F_{3,19} = 4.14, P = 0.02$). The treatments indicated with * differ significantly (Tukey's HSD $q_{4,19} = 4.05, p < 0.05$). Standard deviations are indicated by error bars.

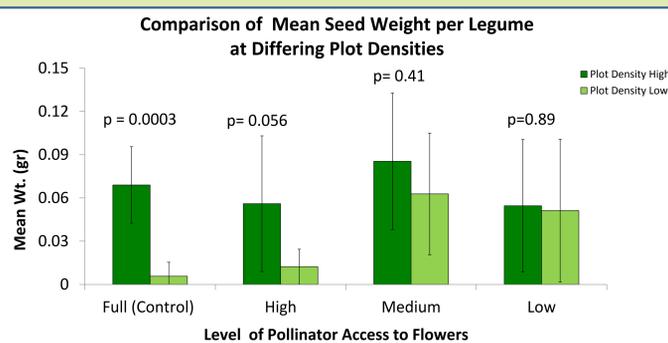


Figure 5. Seed set was consistently lower in low density plots than high density plots in all treatment comparisons. Differences were significant in control treatments (Ttest). Ttest P values given above each treatment type. Standard deviations are indicated by error bars.

Results:

Due to periodic high winds during our experiment, approximately 2/3 of our samples (stem tips) were lost or damaged. The results are based only on stem tips that were undamaged and attached to the plants at the end of the experiment. A comparison of total seed weight with number of seeds per legume indicates a strong positive relationship between these variables ($R^2 = 0.60, p = 1.8 \times 10^{-8}$, Figure 2), therefore, we used seed weight per legume as an indicator of overall fecundity in all further analyses.

Means seed weight per legume was variable in all treatments (Figures 3-5, error bars represent +/- standard deviations). Overall fecundity was not influenced by pollinator access in the high density plots (Figure 3, ANOVA $F_{3,24} = 0.81, P = 0.50$). In low density plots, fecundity was significantly impacted by our manipulations (Figure 4, ANOVA $F_{3,19} = 4.14, P = 0.02$), with the mean control seed weight significantly lower than that of "medium" (1mm mesh) exclusion treatment (Tukey's HSD $q_{4,19} = 4.05, p < 0.05$).

Overall, fecundity was lower in the low density plots regardless of treatment (Figure 5). The effect of plot density on fecundity was most pronounced for the control samples (unpaired Ttest $P = 0.0003$) and decreased with increasing pollinator limitation.

Discussion:

Significantly lower seed set in low density plots was observed in our control treatments. We hypothesized that high density stands would present a more attractive target for potential pollinators and, conversely, low density stands might not attract pollinators in high number. However, we were unsure if this effect would be evident at the small scale of our study. These results are consistent with those of Fenster (1991) in which he found lower seed set at lower floral densities. In addition, he observed that pollinator flight distances were inversely related to flower density, with most pollinator flights averaging 0.3 meters. These distances are well below the scale of our patch sizes and might result in pollinators staying within high density plots.

Pollinator access did not effect seed set in high density plots. We hypothesized that seed set would be most strongly impacted by pollinator access in these plots. *Chamaecrista fasciculata* is a buzz-pollinated plant, relying heavily upon large bees for pollination. Pollen is expressed from the poricidal anthers in response to high frequency "buzzing" by large bees during a visit. In addition, the unique structure of the *Chamaecrista* stigma also requires the "buzz" for successful transfer of pollen to its receptive surface (Arceo-Gómez et al. 2011). Given the need for large bodied buzz pollinators, we were surprised that pollinator exclusion did not effect seed set.

Decreased pollinator access resulted in higher seed set in low density plots. We expected seed set to be lowest in low density plots with low pollinator access. However, contrary to our expectations, seed set was higher in pollinator exclusion treatments than in the controls in low density plots.

The unique anatomy of *Chamaecrista fasciculata* flowers requires buzz pollination for successful seed set in natural settings. With the aid of human "pollinators", this species can self-fertilize and produce viable seeds (e.g. Fenster 1991). As we considered how our bagged plants successfully set seed without buzz pollinators and human intervention, we recalled observations made while observing the flower morphology in our lab. In several flowers, we observed pollen-covered thrips (Order Thysanoptera) crawling into and out of *Chamaecrista* anthers. At the time, we considered this could be a potentially confounding effect, but assumed the effect would be low relative to the importance of buzz pollination.

We propose that thrips may have acted as pollinators in our manipulation. Though poorly studied, it is possible that symbiotic mutualisms could exist between a plant like *C. fasciculata* and pollen-feeding thrips. Studies have shown that thrips can be important pollinators in some plants species (e.g. Baker and Cruden, Williams et al. 2001, García-Fayos and Goldarazena 2008, Ophir 2009). The use of smaller mesh treatments appears to have had a positive effect on the mean seed weight of plants in low density plots, whereas this use had a negligible effect on the mean seed weight of plants in high density plots (Figures 3-5). Thrips are approximately 1.3 mm in length and might, if already present, have been trapped by our medium and small mesh bags. If this is the case, contribution of thrips to pollination would be abnormally high because they were forced to stay in close proximity to the flowers.

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