

An Evaluation of Pollination Mechanisms for Purple Prairie-clover, *Dalea purpurea* (Fabaceae: Amorphaeae)

ABSTRACT.—Purple prairie-clover (*Dalea purpurea* Ventenat) is a common perennial forb that flowers during mid-summer throughout the Great Plains and adjacent biomes. Seed of *D. purpurea* is used for prairie restoration. This study characterizes the reproductive biology of *D. purpurea*. Manual pollination field trials showed that *D. purpurea* has a mixed pollination system. It is primarily xenogamous (45% of flowers manually pollinated with outcross pollen yielded plump seeds), but partially self-compatible (19% of selfed flowers yielded seeds). Flowers neither visited by bees nor manually pollinated rarely yielded mature seed (0–6% of flowers). A review of extensive bee community surveys from sites with prairie wildflower communities revealed that *D. purpurea* attracts a rich and diverse native bee fauna. Managed agricultural pollinators (*Apis mellifera*, *Megachile rotundata*) also work its flowers for pollen and nectar, yielding >20,000 seeds per plant. These observations bode well both for pollinating *D. purpurea* in small commercial seed fields as well as for its value for sustaining native pollinator faunas in prairie restorations.

INTRODUCTION

The 161 described species of *Dalea* Lucanus (prairie-clovers) plus the smaller genus *Marina* Liebmann (38 species) together form the largest monophyletic clade within the tribe Amorphaeae Borissova emend. Barneby (Papilionidae: Fabaceae) (Barneby, 1977; McMahon and Hufford, 2004). Reflecting tribal distribution, species of *Dalea* are also found throughout much of the Americas (exclusive of the lowland tropics); they are especially diverse and abundant in Mexico. In North America, species of *Dalea* are found from the Appalachian Mountains west to the Sierra Nevada and Cascade Mountains (Barneby, 1977). They are perennial forbs or small shrubs. As a consequence of its beauty, *D. purpurea* Ventenat has been cultivated in gardens for several decades. More recently, it has been farmed to produce large quantities of seed for prairie restoration and roadside beautification.

Farming seed crops often requires pollinator supplementation. To evaluate pollination needs, the reproductive biology of a plant species must be understood first. However, there are no published accounts of reproductive biologies for any species of *Dalea*, or any other species of the tribe Amorphaeae. The objective of this study was to characterize the reproductive biology of *D. purpurea* and evaluate the necessity of pollinators for producing seed.

METHODS

POLLINATION

Twenty-five 3-y-old plants were acquired as potted seedling stock from Oak Prairie Farm, Pardeeville, Wisconsin. Their seed originated with a 40-acre commercial production field started from a ½ kg pool of seed harvested from five prairie remnants in southern Wisconsin. At the Bee Biology and Systematics Lab in Logan Utah, the plants were transplanted into clay loam on a grid with 1-m spacing and periodic drip irrigation. Eight individuals of like size and vigor were chosen and tagged. Individual pollinator exclusion bags were not used because *Dalea purpurea* flowers are crowded into very dense spikes, for which inadvertent pollen transfer could result if rubbed against bag netting. To exclude pollinators, the entire array of plants was planted in a walk-in field cage (7 × 7 × 2 m) made of Lumite screening (Synthetic Industries, Chicopee, Georgia, U.S.A.). Three concurrently maturing floral spikes were tagged on each of the eight plants just before initial anthesis. Once flowers began to dehisce pollen, new flowers of each plant's three tagged spikes could be treated and counted every other day, as unpollinated flowers persisted for at least 3 d. The banner petals of counted flowers were finely marked using indelible ink.

During July 2004 three manual pollination treatments were repeatedly applied to each of the 8 plants: autogamy (unassisted autopollination), geitonogamy (transfer of self pollen) and xenogamy (outcrossing). Each plant's 3 tagged spikes was assigned a different treatment. Geitonogamous pollination involved rubbing recipient virgin stigmas with anthers from an untagged flower from the

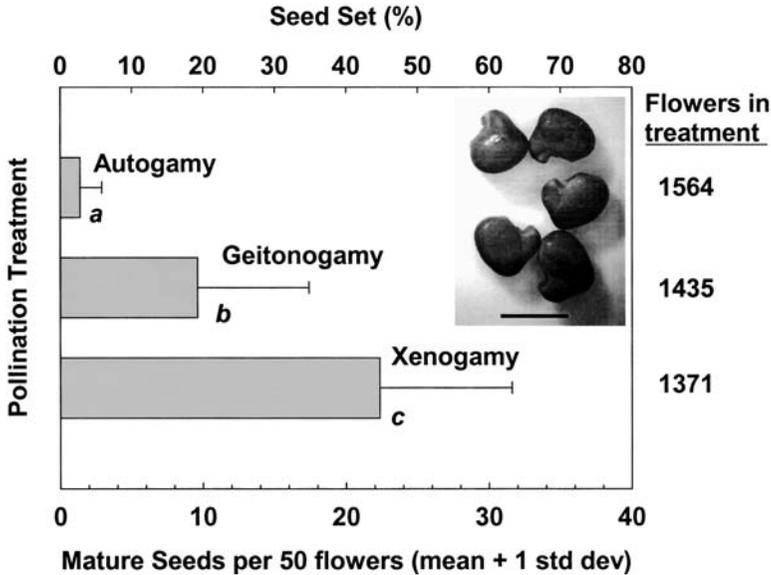


FIG. 1.—Three pollination treatments compared for the proportion of large seeds set per flowering spike of *Dalea purpurea*. Bars followed by different letters are statistically different from one another ($P \leq 0.05$). Inset: image of large *D. purpurea* seeds that contained endosperm, with a scale bar 2 mm long

same plant. Donor flowers for xenogamy were taken from untagged plants. Later, because the ink markers used to mark petals may have inadvertently transferred self pollen as well, I tagged an additional seed spike from each of 5 adjacent plants to serve as uncompromised autopollination controls. Since the floral calyx proved to be persistent, the original count of flowers on these spikes could be recovered. Optical visors were used to see each tiny stigma and confirm its applied pollen load. Manually pollinated floral spikes were, thus, pollinated every other day from the onset of bloom until their last flowers wilted.

SEED PRODUCTION

Plants remained caged after flowering in order to guard against seed predators and herbivores. Once seeds were mature, but before they were shed, spikes were individually bagged, clipped and returned to the laboratory. Although there are 2 ovules per flower, only 1 seed per flower matures (Barneby, 1977). After drying for >7 d, the entire complement of seeds and spent flowers was stripped from each spike. Fifty pods (or spent flowers) were then randomly subsampled from each spike's reproductive output, to be individually dissected for their complement of seeds (Fig. 1, inset). Seeds were sorted into two size classes and tallied for each spike and treatment. Endosperm fill of the visually scored large and small seeds was visualized by X-rays (HP 4380N Faxitron, 25 KV, 30 s exposure, medium grain industrial film) from a subsample of 90 seeds representing all pollination treatments. Germination was attempted using reported protocols (Baskin and Baskin, 1998), but proved inconsistent with this fresh seed.

In 2005 the array of plants was left uncaged, to allow open visitation by honeybees (*Apis mellifera* L.), nesting alfalfa leaf-cutting bees [*Megachile rotundata* (Fab.)] and diverse native bees. Resultant seed production was counted per each of 11 openly-pollinated plants (1 haphazardly chosen spike each). For one additional spike, seed count was related to its flower production.

The 3 pollination treatments from 2004 were compared by general linear models ANOVA tests for flower production and their yield of large seeds. A randomized complete block design was used with the 8 plants as blocks. Seed counts were square root transformed, rendering acceptable normality

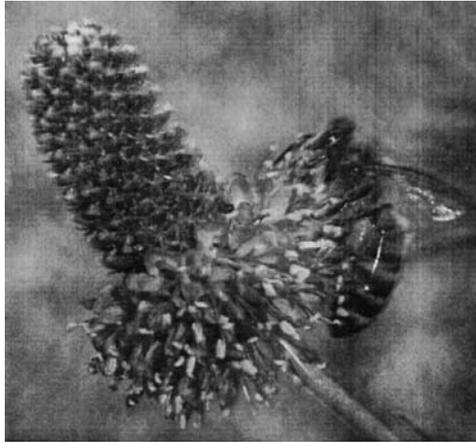


FIG. 2.—Honey bee (*Apis mellifera*) forager collecting pollen and nectar from flowering spike of *Dalea purpurea* at study plot

(Shapiro-Wilk statistic = 0.94, $P > 0.23$) and homogeneous variances for all three pollination treatments (Levene's test, $P > 0.18$). Where differences were found among treatments ($P \leq 0.05$), treatments were compared by Ryan-Enot-Gabriel-Welch (REGW) *a posteriori* tests (SAS Institute, 1989). A *t*-test for unequal variances was also used to compare seed production between the geitonogamy treatment and autopollination, spikes for the latter sampled from 5 neighboring plants uncompromised by manual floral marking.

RESULTS AND DISCUSSION

Manual outcrossing (xenogamy) between plants more than doubled seed production compared to manual selfing of the maternal plant (geitonogamy); actual autogamy (autopollination) yielded little or no seed (Fig. 1). Production of viable seed varied significantly among groups ($F_{[9,14]} = 5.13$, $P < 0.003$) with significant differences among the three fixed pollination treatments ($F_{[2,14]} = 10.81$, $P < 0.001$) and among the 8 blocks (plants) ($F_{[7,14]} = 3.51$, $P < 0.02$) (degrees of freedom are given in subscript brackets). Flower counts averaged 182 ± 64 flowers per spike and were similar among treatments ($F_{[2,14]} = 0.27$, $P > 0.7$). Autogamy treatments provided by a floral spike from each of five other plants (whose petals were not marked, thereby avoiding inadvertent pollen transfer) yielded significantly less seed than the geitonogamy treatments ($t_{[8]} = 2.9$, $P < 0.01$); the 10-fold difference (Fig. 1) is unlikely to be a mere plant effect. Endosperm fill of seeds was evident as underexposed areas in the negative images for 82 of 90 seeds that had been sub-sampled in equal numbers from each of the three treatments. The 8 sub-sampled seeds that lacked endosperm were all noticeably smaller and came from autogamy (3 seeds) and geitonogamy (5 seeds) treatments. Because these *Dalea purpurea* were grown from seed produced in a large field from seed sourced and mixed from five remnant prairie populations, inbreeding depression is an unlikely explanation for the superiority of the outcrossing treatment in producing seed (Gustafson *et al.*, 2002). Rather, it seems that *D. purpurea* is partially self-incompatible. Thus, pollinators are critical for sexual reproduction of *D. purpurea* and possibly for other *Dalea* species, for which reproductive biologies remain unknown.

Thorough pollination should produce prodigious quantities of seed from farmed *Dalea purpurea*. In the study plot, 11 sampled floral spikes (one per plant) openly visited by bees in 2005 produced an average of 199 ± 44 large seeds per spike. One big spike set large seeds from 80% of its 336 flowers. These plants averaged 110 ± 61 spikes each. Multiplied together, each young plant in the plot was producing an estimated 22,000 plump seeds when well-pollinated. Flowers of *D. purpurea* in this small Logan field plot were avidly visited by diverse bees, including: managed honey bees (Fig. 2), alfalfa leaf-cutting bees and

alkali bees (*Nomia melanderi* Ckll.); wild bumblebees [*B. fervidus* (Fab.) and *B. nevadensis* Cresson], and species of *Agapostemon*, *Anthophora*, *Dialictus*, *Halictus*, *Hoplitis* and *Megachile*. All collected the bright-orange pollen, which they daily stripped from the flowers by about 1100 MDST. Among these diverse bee species there were effective pollinators of *D. purpurea*, even when planted outside of its native range.

A few species of the Amorpheae have been surveyed for their native visitor faunas; they host diverse guilds of native bees, which are undoubtedly their primary pollinators. From 19 midsummer surveys of bees visiting *Dalea purpurea* at five reserves near Minneapolis Minnesota, USA, Reed (1995) reported >65 non-parasitic bee species, about one-fourth of the 287 bee species that she collected at all 59 forb species. No other prairie flower attracted as many bee species. Numerically abundant, taxonomically rich genera of bees at *D. purpurea* in that survey included bumblebees (*Bombus*, 10 spp.), *Colletes* (7 spp.), sweat bees (*Dialictus*, 16 spp.; *Halictus*, 4 spp.) and *Perdita* (unidentified, but numerous) (C. C. Reed, pers. comm.). Six of those bee species were taken only at *D. purpurea*, of which two are considered to be *Dalea* pollen specialists (oligoleges) (*C. susannae* Swenk and *C. wilmattae* Ckll.) (Swenk, 1925). The unknown species of *Perdita* are probably oligolectic as well. Bee faunas sampled at *Amorpha canescens* Pursh (also Amorpheae) during those surveys were quite comparable, with 41 bee species in 10 collections at five sites, with 1 apparent *Amorpha* specialist, *Hoplitis cylindrica* (Cress.).

A century earlier and 600 km southeastward around Carlinville, Illinois, U.S.A., exhaustive multi-year samples yielded 55 bee species at *Dalea purpurea* (Robertson, 1929). Species included 2 different *Dalea* specialists, *Colletes albescens* Cresson and *C. robertsonii* Dalla Torre, as well as diverse and abundant species of *Melissodes* bees. Both this and the Minnesota locations are at the juncture of the tallgrass prairie and eastern deciduous forest. In Alberta, a leaf-cutting bee *Megachile dentitarsus* Sladen was also reported at a *Dalea* sp. (Hobbs and Lilly, 1954); species of this manageable bee genus were rare or absent from the preceding surveys.

Collectively, these observations illustrate that native bees are abundant and apparently key for pollinating at least some members of this subtribe, and certainly *Dalea purpurea*. Most of these bees are ground-nesting species with little management potential. However, because *Dalea* are perennial forbs, some ground-nesting bee species should prosper and gradually increase on-farm over time, at least in untilled soil. At some farms, however, native bee numbers will be inadequate to maximize seed set. In the small Logan plot, hived European honey bees (*Apis mellifera* L.) were daily present, likely flying from an apiary about 1 km away. From a small nesting shelter adjacent to the plot, alfalfa leaf-cutting bees daily foraged for *Dalea* nectar and pollen to provision their nest cells. Both species are readily available, managed species that pollinate other summer blooming small-flowered legumes, and so have practical promise for pollinating *D. purpurea*. It is also clear that *D. purpurea* is highly attractive to diverse native bees. In prairie restorations, *D. purpurea* should be a valuable addition to seed mixes, in that it will support recovery of midsummer native bee communities that will be critical to sustained seed production in prairie restoration efforts.

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