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POLLINATION NEEDS OF ARROWLEAF BALSAMROOT, *BALSAMORHIZA SAGITTATA* (HELIANTHEAE: ASTERACEAE)

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ABSTRACT.—Arrowleaf balsamroot, *Balsamorhiza sagittata* (Pursh) Nutt, is a common, sometimes dominant, long-lived forb that flowers early in spring from the foothills to upper-montane areas of the northern Rocky Mountains and Intermountain West. Public land managers desire its seed for rangeland rehabilitation. Through manual pollination field trials, the species was found to have a mixed pollination system. It is primarily xenogamous (46% of ovules yielded plump achenes) but partially self-compatible (31% of achenes were plump). Unvisited flower heads formed virtually no mature achenes; only plump achenes contained seeds with endosperm. Freely visited flower heads in 2 populations produced as many achenes as manual outcross pollinations of flower heads, suggesting that seed production was not pollinator limited. Two species of *Osmia* bees rely mostly on *Balsamorhiza* and its close relative, *Wyethia,* for pollen. At least 165 females per hectare will need to be stocked to achieve thorough flower visitation in cultivated seed production fields.

Key words: Engelmanniinae, Osmia, *Apiformes, Apoidea, bees, seed set, self-incompatibility, pollinator limitation.*

The balsamroots (*Balsamorhiza,* 14 spp.) and mule's ears (*Wyethia,* 14 spp.) together form a monophyletic clade within the subtribe Engelmanniinae [largely equivalent to the former Ecliptinae (Heliantheae: Asteraceae); Robinson 1981, Urbatsch and Jansen 1995, Clevinger and Panero 2000, Moore and Bohs 2003]. They are restricted to western North America, where they are widespread and often abundant, ranging from valleys and foothills to subalpine habitats. Most members of their subtribe bloom in summer, but species of *Balsamorhiza* and *Wyethia* are unusual: their large taproots enable them to put forth large flower heads (= capitula) in early spring. At this time their young foliage and capitula are preferred forage of deer, elk, and both domestic and bighorn sheep (Burrell 1982, Wikeem and Pitt 1992). Local populations can be extensive, dense, and persistent, with cohorts of plants persisting for as many as 40 years (Treshow and Harper 1974). As a consequence of the ecological prevalence and forage utility of balsamroots, particularly *B. sagittata,* they have long been advocated for use in rangeland revegetation and rehabilitation. Wildland seed production is erratic and prohibitively expensive to harvest, however, prompting a call for agricultural production of *B. sagittata* seed.

Farming seed crops often requires pollinator supplementation. To evaluate pollination needs, a plant species' breeding biology must first be understood, but there are no published accounts for any species of *Balsamorhiza*, *Wyethia,* or any other species of their subtribe except *Echinacea angustifolia* (Leuszler et al. 1996). A cavity-nesting vernal solitary bee, *Osmia californica,* can be common at capitula of *Balsamorhiza;* museum label data and pollen constitution of larval provisions reveal it to be a specialist on the Asteraceae (Rust 1974, Torchio 1989). It occurs throughout the western USA, north of the warm deserts and south of Canada (Rust 1974), thus largely matching the geographic range of *B. sagittata.* This study's 2 objectives were (1) to characterize the breeding biology of *B. sagittata* to understand its relative dependence on pollinators; and (2) to estimate stocking densities for the native bee *O. californica* to achieve adequate floral visitation for commercial seed production.

MATERIALS AND METHODS

Pollination Treatments

My assistants and I chose and tagged 25 plants of *B. sagittata* at 2 separate populations near Logan, Cache Co., Utah, USA (Fig. 1). We

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Fig. 1. Arrowleaf balsamroot, *B. sagittata,* in flower. *Inset:* female *O. californica* foraging at flower of *B. sagittata.*

tagged 4 capitula on each plant just prior to anthesis; 3 were enclosed in drawstring bags made of white fine mesh "no-see-um" netting, 2 of which were used for manual pollinations. Once florets began to dehisce pollen, the same 2 capitula of each plant were manually pollinated every other day for 10 days. To manually pollinate capitula, a donor capitulum that was shedding pollen was gently but thoroughly rubbed against the open florets of the recipient capitulum. After each pollination event, recipient capitula were rebagged.

We applied 4 pollination treatments in the field: autogamy (unassisted autopollination),

geitonogamy (manual transfer of self-pollen), xenogamy (manual transfer of outcross pollen), and free-visitation (no bag). One capitulum remained bagged throughout bloom to test for autogamy. Geitonogamous pollination involved rubbing the recipient capitulum with an extra untagged blooming capitulum from the same plant. Donor capitula for xenogamy were clipped from plants growing >100 m distant. Manually pollinated capitula were thus pollinated every other day from the onset of bloom until the last central florets closed. Freely visited capitula were tagged but remained unbagged and accessible to pollinators, serving as a positive control for our manual pollinations while also revealing natural seed production.

The technique's efficacy for manually transferring pollen was assessed using a pollen surrogate. A light dusting of fluorescent powder was applied by brush to open florets of 4 capitula. Each of these capitula was then rubbed against a 2nd recipient in the manner used in the field. We then illuminated the 4 recipient capitula by ultraviolet light and viewed them microscopically. In this trial, 92% of 101 recipient florets picked up some fluorescent powder from donor capitula (range of 73%–100% transfer per capitulum).

Seed Production

After flowering ceased, bags were removed. We protected the 4 capitula of each plant from vertebrate seed predators by inserting each into a stiff cylinder made of coarse plastic mesh. These are marketed to protect young conifer saplings used for reforestation (Forestry Suppliers Inc., Jackson, MS). The top and bottom of each tube were drawn closed with a wire. Each mesh tube was supported by a stake.

Once achenes were mature, but before they were shed, capitula were individually bagged, clipped, and returned to the laboratory. After drying for 10 days, achenes were harvested. Visibly plump achenes and shrunken achenes (Fig. 2 inset) were sorted and tallied for each capitulum. Reproductive potentials of the visually scored plump and shrunken achenes were evaluated in 3 ways: (1) seed mass, (2) endosperm content, and (3) viability staining. Subsamples of 5 plump and 5 shrunken achenes were taken from 8 treatment plants, weighed, and compared by a *t* test. Endosperm content was visualized by X rays (HP 4380N Faxitron, 25 KV, 30 seconds, medium-grain industrial film). Viability of 100 X-rayed seeds was checked by the tetrazolium test (Grabe 1970). Germination was attempted using reported protocols (Young and Evans 1979), but proved unsatisfactory.

Many capitula in the autogamy treatment produced no plump seed, complicating statistical comparison. I first compared autogamy and geitonogamy for the proportion of capitula bearing >1 versus no plump achenes, using a G-test with Williamson's correction (Sokal and Rohlf 1995). General linear model ANOVA tests were then used to compare the 3 pollination treatments (excluding autogamy) for total achene production, sum of plump achenes, and the proportion of achenes that were plump. A randomized complete block design used plants as blocks. I square root transformed achene counts, rendering homogeneous variances for all 3 variables (Levene's test, $P > 0.6$). Where treatment differences were significant ($P \leq 0.05$), I compared treatments by Ryan-Enot-Gabriel-Welch (REGW) a posteriori tests (SAS Institute 1989). Degrees of freedom are given in subscript brackets for ANOVA tests.

Activity of *Osmia californica* Bees

A nesting shelter with overwintered nests of *Osmia californica* Cresson, a release box, and 4 drilled wooden nesting blocks was placed at the edge of several hectares of blooming *B. sagittata* (and budded *B. macrophylla* and *Wyethia amplexicaulis* Nutt.). Once nesting had commenced, nest entrance traffic was filmed for 45 minutes at midday. This video was later transcribed for durations of 14 pollen foraging trips, each of which ends when a returning female first enters her nest hole head first, regurgitates nectar, then walks out and backs into her nest to unload pollen (which differs from stray hole visits, nest partition manufacture, etc.). Concurrently, 21 females were timed and followed as they each visited a sequence of 5 *B. sagittata* capitula that were seen to be dehiscing pollen.

RESULTS AND DISCUSSION

Achene size proved to be a useful indicator of viability. From 91 capitula, we obtained 6739 achenes, 67% of which were plump. Achenes scored as plump (Fig. 2 inset) were 5-fold heavier than shrunken achenes (groups of 10

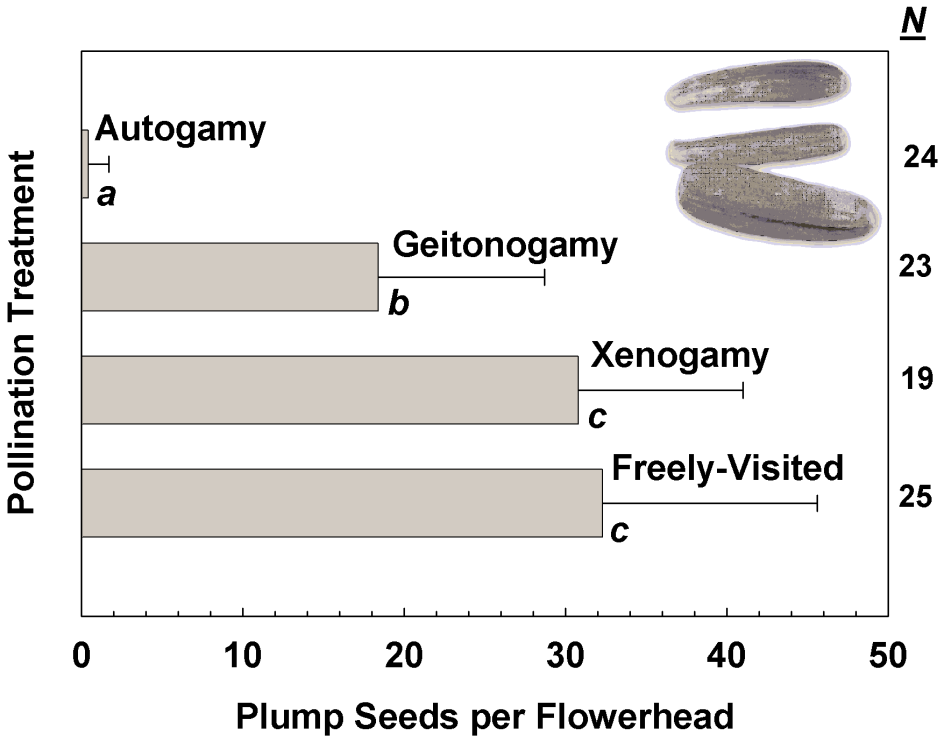

Fig. 2. Four pollination treatments compared for the sum of plump achenes produced per capitulum of *B. sagittata*. Bars followed by different letters are statistically different from one another ($P \leq 0.05$). Means and 95% confidence intervals are presented as their back-transformed values (Sokal and Rohlf 1995). Numbers of capitula are given in the column to the right of the graph. *Inset*: image of 1 plump (filled) and 2 shrunken (empty) achenes.

achenes, $\bar{x}$ = 75 mg vs. 15 mg; $t_{[14]} = 7.0$, $P < 0.0001$). Endosperm absorbs X rays; it was readily discerned as clear unexposed areas in the radiograph negatives of achenes. Endosperm was evident in 78% of visibly plump achenes ($n = 132$), whereas shrunken achenes lacked endosperm (69 of 70 achenes). For the subsamples of 10 achenes which were X-rayed and then tested using tetrazolium staining for viability, 64 of 67 achenes with apparent endosperm were scored as live, while all 33 achenes lacking apparent endosperm were scored as dead.

Autogamy treatments of *B. sagittata* capitula rarely produced any plump achenes compared with capitula from geitonogamous pollination (13% vs. 75% of capitula with ≥ 1 achene; n = 47 capitula; $G_{adj} = 19$, $P < 0.0001$). Of the 24 capitula in the autogamy treatment, 18 had only shrunken achenes, and the remaining 6 produced a sum of only 62 plump achenes (range 1–39). Autogamy yields little if any natural achene set in *B. sagittata* (Fig. 2).

Total achene production (combining plump and shrunken achenes) is an estimate of ovule number for individual capitula. For the 3 treatments other than autogamy, total achene production per capitula differed among plants ($F_{[24,66]} = 2.4$, $P < 0.008$) but not between pollination treatments ($\bar{x}$ = 73–79 achenes; $F_{[2,66]} = 0.3$, $P < 0.7$). Hence, there was no systematic size bias for ovule number in assignment of treatments to individual capitula within plants.

Production of plump achenes varied with treatment as well as maternal plant. The numbers of plump achenes produced differed both among plants ($F_{[24,66]} = 2.5$, $P < 0.005$) and among the 3 treatments (excluding autogamy; $F_{[2,66]} = 3.9$, $P < 0.03$) with no significant interaction (Fig. 2). Manual pollination using pollen from the maternal plant yielded significantly fewer plump achenes than either xenogamy or the freely visited treatments. Xenogamy and freely visited treatments resulted in the maximum set of plump achenes per capitula,

and were nearly identical (Fig. 2). When expressed as proportions, plump achene production differed among both plants ($F_{[24,66]} = 2.9$, $P < 0.0013$) and the 3 treatments ($F_{[2,66]} = 6.4$, $P < 0.004$) with no significant interaction. Capitula receiving self-pollen set proportionately fewer plump achenes (31%) than did xenogamy or open pollination, which were identical in the proportion of ovules producing plump achenes (46%). Evidently, achene production by *B. sagittata* was not pollinator limited at the study sites. Comparable contrasts between pollination treatments were reported for *Echinacea angustifolia* (Leuszler et al. 1996).

The importance of pollinator visitation for sexual reproduction of species in the Engelmanniinae has been demonstrated only for *Echinacea angustifolia* (Leuszler et al. 1996) and now *B. sagittata.* Autogamy is at most infrequent among other representatives of the Heliantheae (Sundberg and Stuessy 1990). Species of the Engelmanniinae host diverse native bees, but few if any other pollinating insects, even at a single locale. Bees are undoubtedly the primary pollinators, although we were unable to reliably track fates of rings of maturing (or empty) achenes that resulted from rings of receptive florets being individually visited by a bee. At Carlinville, Illinois, USA, exhaustive multiyear samples yielded 24–48 bee species visiting flowering species of Engelmaniinae (*Echinacea purpurea, Ratibita pinnata, Silphium perfoliatum,* and *Verbesina helianthoides;* Robertson 1929). From specimen labels at the USDA-ARS Pollinating Insect Research Unit in Logan, Utah, 35 species of native, nonparasitic bees had been taken from the western U.S. while visiting species of *Balsamorhiza,* although *Balsamorhiza* has never been the focus of a methodical pollinator survey. I have initiated such a survey and thus far have found that the cavity-nesting, nonsocial bee, *Osmia californica,* is always present and often prevalent at capitula of both *B. sagittata* and *B. macrophylla.* Collectively, these observations illustrate that native bees are abundant and key to pollination in this subtribe.

Pollinators will be essential for any agricultural production of *B. sagittata* achenes, an objective of a multiyear federal program to produce seed for wildland rehabilitation in the Intermountain West. It is useful to estimate the stocking densities of bees that will be needed to pollinate such seed fields. Estimated commercial seed production is 100 ± 25 lbs $\cdot$ acre^{-1} (= 112 kg $\cdot$ ha^{-1}; Stevens et al. 1996). We found there to be 133 achenes per gram, which would give an expected harvest of 15 million achenes per hectare. Our yield of 35 plump achenes per capitulum would therefore require 427,000 full capitula per hectare (= 43 $\cdot$ m^{-2} or 14 full capitula per plant). This estimate seems reasonable in light of recommended planting of 30,000 plants per hectare (calculated from Stevens et al. 1996).

The numbers of capitula visited in a female bee's lifetime can be calculated from either foraging and provisioning tempos, or rates of pollen acquisition and caching (Cane et al. 1996). Combined with pollination efficacy estimates, optimal stocking densities of nonsocial bees can be estimated that would maximize crop production (e.g., Cane et al. 1996, Vicens and Bosch 2000, Torchio 2003). Females of *O. californica* typically produce 1 offspring daily, each requiring pollen and nectar acquired during 25–30 foraging trips (Rust 1974, Torchio 1989). I found that midday pollen-foraging trips by *O. californica* in wild patches of *B. sagittata* lasted 8.4 ± 6.8 minutes (median = 5.5 minutes, $n = 14$ trips). At midday, pollen-laden females handled an average of 9.6 ± 4.7 capitula per minute ($n = 21$ bees, 105 capitula), and could be seen patting the florets with their abdominal venters to acquire pollen. Honeybees (von Frisch 1967) and alkali bees (Cane personal observation) both fly at about 23 km $\cdot$ hr^{-1}, so the comparably sized *O. californica* should need 30 seconds to travel an arbitrary average commute distance of 100 m out and back between nest and meadow. Multiplying average capitula handling rates by the median duration of foraging trips (minus commute time) and thence by the number of trips to provision a nest cell, gives an estimated 48 capitula that are visited per trip; thus, 1320 capitula would be visited daily for 1 nest cell. Each female reportedly produces as many as 30 progeny in the greenhouse (Torchio 1989), but field bees probably produce only a third as many, so each female might visit 13,200 *Balsamorhiza* capitula in her lifetime. Since we could fully pollinate capitula manually by pollinating them 5 times in 10 days, a female bee might fully pollinate 2640 capitula (13,200/5) in her lifetime. From these measures, I calculate that at least 165 nesting female *O. californica* would be needed to pollinate a single hectare of farmed

arrowleaf balsamroot, comparable to the 250–1100 females per hectare reportedly needed in several tree fruit and berry crops (Cane et al. 1996, Vicens and Bosch 2000, Torchio 2003). Nesting shelters probably can be widely spaced in seed fields; nesting females of the closely related, like-sized vernal composite specialist, *Osmia montana,* were seen foraging at a distance of ≥0.6 km to the nearest blooming suitable floral hosts (*Helianthella* and *Crepis;* personal observation). These rough guidelines will be useful for native seed growers interested in maximizing pollination in commercial fields of this slow-growing perennial wildflower.

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