Journal of the Torrey Botanical Society 144(2): 218–229, 2017.

Reproductive ecology of *Asclepias meadii* Torr. (Apocynaceae), a federally threatened species[1]

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Abstract. Reproductive success in the federally threatened species *Asclepias meadii* is limited by several factors, including the increasing age and fragmentation of populations, low fruit set, and potential disease. We investigated the reproductive ecology of three isolated populations of *A. meadii* over five seasons (2010–14) in Missouri and Kansas. Experimental hand-pollinations showed that flowers at all sites were incapable of automatic self-pollination

[1] The authors thank the US Fish and Wildlife Service for providing funding through the Endangered Species Section 6 Grant E-15-R-1 and the Missouri Department of Conservation for making this grant possible; Webster Groves Nature Study Society is thanked for providing funds for the Proffit Mountain research through the Bo Koster Grant. Peggy Horner, Melissa Briggler, and Mike Leahy are thanked for their professional assistance and encouragement. We are grateful to The Nature Conservancy and Dr. W. Dean Kettle for granting research permission and providing historical and phenological information; Helen Alexander and Steven Roels are thanked for herbivore and seedling ecology information. Lawrence Meier (ret.) is thanked for professional assistance. The Missouri Botanical Garden is thanked for allowing access to its herbarium and laboratory. Dr. David Bogler provided assistance on using the scanning electron microscope and laboratory cameras; thanks to Dr. George Yatskievych for accepting floral vouchers and botanical identification. Finally, we deeply appreciate photographic assistance provided by Craig Fowler of Creve Couer Camera.

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doi: 10.3159/TORREY-D-15-00069

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Received for publication October 25, 2015, and in revised form April 4, 2016; first published April 4, 2017.

(autogamy) and lacked early acting self-incompatibility. The average number of ovaries with pollen tubes in open (insect-mediated) pollination varied from 0.10 to 0.81 depending on site and season. The conversion rate of ovaries into fruits never exceeded more than two fruits per umbel with insect-mediated fruit set varying from 0% to 50% according to site and season. Nectar was the only reward with a per-flower volume as high as 5 μL containing dissolved sugars from 21% to $\geq$ 50%. The nectar was hexose dominant, and the amino acid content was limited to aspartic acid and arginine. Nectar-drinking insects were common visitors at all sites, but only three taxa in the family Apidae (*Anthophora abrupta*, *Apis mellifera*, and *Bombus* species) were consistent carriers of *A. meadii* pollinaria on their legs. Density and diversity of these three pollinaria carrying taxa also varied according to site and season. Although all three taxa were successful pollinaria vectors, the nonnative *A. mellifera* does not appear to be as efficient as the native species in the two other bee genera. Our findings offer evidence that *Bombus* species are especially successful in vectoring pollinaria between flowers of *A. meadii*; that pollen tubes are successfully reaching the ovaries in insect-mediated, hand-self, and hand-cross pollination events; and that automatic self-pollination (autogamy) does not occur.

Key words: *Asclepias meadii*, floral phenology, fruit, pollination, stigmatic slits

Mead's milkweed (*Asclepias meadii* Torr.: Apocynaceae, Asclepiadoideae *sensu* Endress and Bruyns 2000) is a globally imperiled species (NatureServe 2015) and was classified as a federally threatened species by the US Fish and Wildlife Service on September 1, 1988, under the Endangered Species Act of 1973, as amended (US Fish and Wildlife Service 1988). Missouri lists its status as "State-Endangered" (Missouri Department of Conservation 2015). A recovery plan by Bowles *et al*. (2003) identified populations persisting in Kansas, Missouri, Iowa, and Illinois. Populations once found in Wisconsin and Indiana are regarded as extirpated (US Fish and Wildlife Service 2003). Genetic sustainability of remnant populations of *A. meadii* is of continued importance to the conservation of this species because its populations are now so highly fragmented (Betz 1989, Hayworth *et al*. 2001). Furthermore, it has been shown that *A. meadii* can serve as an egg-laying site (Betz 1989) as well as a larval food source for *Danaus plexippus* (Roels 2011, Edens-Meier and Bernhardt 2013).

This herbaceous perennial is associated with tallgrass prairies and glades (Yatskievych 2006). Its fruits mature and release their seeds from late August through September (Morgan 1980, Kurz and Bowles 1981). Low fruit set appears to be characteristic of this species (Betz 1989, Kettle *et al*. 2000, Roels 2011). In Missouri, fruit set in remaining populations at most sites may be negligible (Bowles *et al*. 2001). Betz and Lamp (1990) found that low fruit set in 18 *Asclepias* species was not unique to *A. meadii* and was similar to *Asclepias exaltata, Asclepias variegata*, and *Asclepias lanuginosa* but far lower than *Asclepias incarnata* and *Asclepias syriaca*.

The reproductive ecology of *Asclepias* species has been of interest to botanists since the 19th century (Darwin 1877, Endress 2015). The functional floral morphology of this genus is so atypical that Brown (1833) compared *Asclepias* with Orchidaceae. *Asclepias* species do not release granular pollen. Instead, each anther consists of two pollen sacs, and each sac contains one pollinium. These two pollinia are attached via two translator arms converging into one bilabiate, clamp-like structure or corpusculum (Brown 1833, Corry 1883). A prospective pollinator removes a pollinarium (two pollinia + two translator arms + one corpusculum) during foraging (Betz *et al*. 1994; Fig. 1). In the gynoecium, two superior ovaries are free, whereas their styles fuse in a terminal, but sterile, compitum. The five receptive stigmatic slits are produced by adnation between the androecium and gynoecium. Similar to a number of flowers of terrestrial orchids (Darwin 1877), a single pollinium of an *Asclepias* pollinarium will not insert into a stigmatic slit until its translator arm dries and repositions at an appropriate angle. In most *Asclepias* species, only one side of a pollinium can fit into the stigmatic slit initiating pollen tube germination, growth through style tissue, and ovule penetration (Brown 1833).

Nectar secreted within the stigmatic chamber (Ollerton and Liede 1997) is the only edible reward in *A. meadii* and provides a liquid medium in which the pollen grains germinate (Kevan *et al*. 1989). Several studies showed that a variety of insects carry the pollinaria of different *Asclepias* species on their legs and/or mouthparts (Willson and Bertin 1979, Willson *et al*. 1979, Bookman 1981, Morse 1982, Broyles and Wyatt 1991, Betz *et al*. 1994). However, out of 12 *Asclepias* species studied, eight species, including *A*. meadii, were

FIG. 1. Scanning electron microscopy image showing the corpusculum clamped to the pulvillus of *Apis mellifera*. Note the angled position of the pollinium to its translator arm. (Photographer: Gary Brown)

visited by *Bombus* species and *Apis mellifera* (Betz *et al*. 1994).

Efforts to protect populations of *A. meadii* by reintroducing seedlings seem futile until the reproductive ecology of this species is understood. *Asclepias meadii* is classified as a federally threatened species, and much remains unknown regarding its pollination biology (Betz and Lamp 1990, Betz *et al*. 1994). Therefore, we provide a 5-yr investigation on reproductive efforts in *A. meadii* as it relates to site, flower phenology, nectar production, pollinator fidelity, fruit set, and pollinaria attachment sites on pollinia vectors.

Materials and Methods. STUDY SITES. Data were collected from three field sites, including flowering stem counts. The Anderson County Prairie Preserve site (2010–12) is located in Anderson County, KS, owned by The Nature Conservancy (Arlington, VA), and managed by the Kansas Biological Survey (Lawrence, KS). The habitat consists of approximately 303 ha of dry, tallgrass prairie. Management includes patch-burn, grazing, and hay-making regimes.

The Rockhill Prairie site (2011–12) is located in Benton County, MO and is owned by The Nature Conservancy. The habitat consists of 24 ha of dry chert prairie and is maintained through periodic controlled burning.

The Proffit Mountain site (2013–14) is located in Iron County, MO. The habitat is a glade on igneous strata, embedded within a *Quercus-Carya* woodland and is maintained by controlled burning.

FLORAL PHENOLOGY AND PRESENTATION. Selected flowering stems were tagged and flagged during 2011 and 2012 at the Rockhill and Anderson County Prairie Preserve sites. The following information was recorded for each umbel: the day on which the first flower bud opened, the day on which all flowers on each inflorescence were open, and the day on which the first flower on each umbel senesced. A Wilcoxon rank-sum test was used to compare floral lifespans between the two sites. The number of flowers on each umbel and variation in perianth color were recorded. Floral scent was detected by smelling the concentrated fragrance of an enclosed cut flower placed in a capped vial for 20–30 min. Phenology data were compared with herbarium collections of pressed flowering specimens at the Missouri Botanical Garden (St. Louis, MO). Herbarium sheets provided both the month and year of collection ($n =$ 14 sheets), collected from 1833 to 2012.

COMPATIBILITY EXPERIMENTS. Umbels were bagged using a single layer of tulle to exclude insects at the Rockhill and Anderson County Prairie Preserve sites. Individual flowers were hand-pollinated on the same day that all buds on the same umbel opened. Bagged inflorescences were included in one of the three following categories. In the first category, flowers were bagged but not manipulated. Those flowers provide the natural rate of automatic self-pollination (autogamy). In the second category, hand–self-pollination, the tulle was removed temporarily from the umbel. After selecting a flower, one pollarium was removed by inserting the point of an insect pin under the corpusculum and gently pulling. After flower selection, one pollinium was inserted into a stigmatic slit. The hood of the pollinated flower was inked with a permanent marker, and the tulle was then replaced. In the third category, hand–cross-pollination, the process was identical to that of self-pollination, except that the pollinarium was harvested from a second flowering stem a minimum of 10 m from the experimental plant.

HARVESTING, FIXATION, AND PRESERVATION. Flowers in all three categories were harvested 96-hr after treatment. Each flower was fixed and preserved using procedures by Edens-Meier *et al*. (2010). Only the first identified flower from a multiple-collection sample was included within the statistical analyses.

EPIFLUORESCENCE ANALYSIS. Preserved flowers were prepared for epifluorescence microscopy following Edens-Meier *et al*. (2010), with the following modifications, as developed by G. Brown. The styles with ovaries were separated from the compitum (a sterile, stigmatic-like structure), placed in separate vials, incubated in 0.10 sodium sulfite at 60 °C for 5 and 7 min respectively, and washed in deionized water. Before staining and squashing, the two pistils and their compitum were divided lengthwise using a scalpel, and the wall of each ovary was split and pulled back to reveal the ovules. Samples were observed a minimum of 24 hr after squashing using a Carl Zeiss (Jenna, Germany) incident fluorescence microscope with a violet exciter filter. Each specimen was examined to determine whether pollen grains had germinated and whether pollen tubes penetrated the tissues of either ovary.

COMPARATIVE RATES OF POLLINARIA REMAINING IN BAGGED *VS*. INSECT-VISITED (OPEN) FLOWERS AT ANDERSON COUNTY PRAIRIE PRESERVE AND ROCKHILL PRAIRIE (2011–12). One flower per inflorescence per plant was examined to determine the retention of whole pollinaria remaining in anthers in bagged and open flowers. The anthers in each flower were counted, and the number of remaining pollinaria was recorded.

AUTOMATIC SELF-POLLINATION (AUTOGAMY) *VS*. VECTOR-MEDIATED POLLINATION. Inflorescences were divided into two categories. When all the flower buds on an umbel opened, flowers were collected on the fourth day. For the first category of bagged, but not pollinated (unmanipulated) inflorescences, the bag was retained over the entire inflorescence for the duration of the floral lifespan. For the second category of open (insect-mediated pollination) inflorescences, the inflorescences were never bagged and were exposed to the guild of local pollinators.

NECTAR. Nectar samples were removed from bagged inflorescences using 5-µL microcapillary tubes at the Rockhill Prairie (2011) and Anderson County Prairie Preserve (2011–12) sites. These samples were used to measure nectar volume; percentage of dissolved sugars, using a hand-held, Eclipse sugar refractometer (0–50%; Paragon Scientific Ltd., Prenton, Wirral, UK), and nectar biochemistry, which was outsourced to the laboratory of Dr. Gary Booth at Brigham Young University (Provo, UT).

FIG. 2. Color variation in an umbel of *A. meadii* with reddish-bronze hoods. (Photographer: Retha Edens-Meier)

FRUIT SET. The presence or absence of maturing fruit per infructesence in bagged and unbagged umbels was observed and counted after the flowering period.

POLLINARIA VECTORS. During the 5-yr period, seven individuals observed foraging behavior and collected floral foragers over approximately 204 field hr. Observations were made between 7:00 am and sunset. Nocturnal observations were made during the first week of research. After observing insects landing on flowers, they were netted and euthanized in a kill jar with ethyl acetate or isopropyl alcohol. Each insect's length, width, and thorax depth were measured. The number of corpuscula and their attachment sites were also recorded.

Results. GENERAL FLORAL PHENOLOGY AND PRESENTATION. Flowers were nodding and produced a floral fragrance of cloves. Pigmentation of the perianth was green, with a few exceptions (Fig. 2). Flowering stem height of *A. meadii* was significantly greater at Proffit Mountain than it was at the Anderson County Prairie Preserve site ($t_{d.f.=73.31}$ = –9.69, $P < 0.001$; Table 1). Inflorescences of *A. meadii* produced significantly more flowers at the Proffit Mountain site than they did at the Anderson County Prairie Preserve and Rockhill Prairie sites ($\chi^2_{d.f.=2} \leq 30.87$, $P = 0.0001$; Table 2).

Pressed specimens from *A. meadii* were poorly represented within the herbarium at the Missouri Botanical Garden. Four sheets that were collected

Table 1. Descriptive statistics for the average heights (cm) of plants at Anderson County Prairie Preserve and Proffit Mountain.

Site	n	Mean	SD	Min	Max
Anderson County Prairie Preserve	101	38.58	6.78	27.0	56.0
Proffit Mountain	46	52.13	8.32	33.8	73.0

Table 3. Descriptive statistics for the floral lifespan (days) at the Anderson County Prairie Preserve and Rockhill Prairie sites.

Site	n	Mean	SD	Min	Max
Anderson County Prairie Preserve	116	5.78	1.10	3	8
Rockhill Prairie	7	6.14	0.38	6	7

before 1890 lacked the day of collection (acquisition numbers: 788887, 2762178, 2762179, and 2762180). Two of those sheets recorded flowering in May, and two recorded flowering in June. The remaining 10 sheets had labels listing the day of collection. Five were collected in May and five in June. The earliest collection was on May 20, 2012 (6382061), whereas the latest collection was on June 16, 1998 (2762177).

No difference in floral lifespan of *A. meadii* was found between the Anderson County Prairie Preserve and Rockhill Prairie sites ($W = 326.5$, $P = 0.37$; Table 3). Flower buds of *A. meadii* usually opened by the last week in May, except in 2012, when flowers were already blooming by May 10th and withered by May 20th. The number of flowering stems appeared to vary with climate at two of our research sites. In 2011, 98 flowering stems of *A. meadii* were found at the Rockhill Prairie site, whereas only 17 flowering stems were found during the early spring of 2012. The number of flowering stems also varied annually at the research plot at the Anderson County Prairie Preserve, producing 78 flowering stems in 2010, 121 in 2011, and 150 in 2012. The research population at Proffit Mountain from 2013 to 2014 consisted of approximately 150 flowering stems.

Compatibility Experiments. A positive response to a compatibility test required the presence of pollen tubes in at least one pistil. Results of the hand-pollination treatments varied annually at both sites (Table 4). Pollen tubes were never found in the compitum. Aberrant pollen tube growth was noted in some hand–self-pollination and hand–cross-pollination treatments. Characteristics of aberrant growth included low fluorescence, coiling, swollen tips, and growing backward toward the stigmatic slits.

Table 2. Descriptive statistics for the average number of flowers-per-inflorescence at each of the three research sites.

Site	n	Mean	SD	Min	Max
Anderson County Prairie Preserve	294	11.60	3.45	2	24
Proffit Mountain	48	14.31	3.18	4	23
Rockhill Prairie	16	10.81	3.41	3	15

Comparative Rates of Pollinaria Remaining in Bagged *vs.* Insect-Visited (Open) Flowers. Pollinaria removal rates from 2010 to 2015 show that more pollinaria were removed in open flowers than in bagged flowers (Table 5).

Automatic self-pollination (Autogamy) *vs.* Vector-Mediated Pollination. The greatest number of pollinia found in stigmatic slits producing tubes that reached the ovaries was at the Anderson County Prairie Preserve site in 2011. None of the bagged flowers at either site had pollinia in their stigmatic slits.

A paired t test was used to compare the number of pollinaria removed to the number of pollinia inserted in each flower. The mean of 0.36 pollinaria per flower (Table 6) was found to be significant at ($t = 3.67$, d.f. $= 49$, $P = 0.0006$).

Nectar. Nectar droplets emerged from the horns of bagged flowers the first morning that the petals opened, and nectar secretion was highest that day (Fig. 3). Visible nectar was no longer observed as flowers aged. Nectar volume was not significantly

Fig. 3. Unusually copious secretion of nectar in an umbel of *A. meadii*. (Photographer: Retha Edens-Meier)

Table 4. Descriptive statistics indicating the average number of pistils containing pollen tubes for each experimental treatment at the Anderson County Prairie Preserve (ACPP) and Rockhill (RH) Prairie sites.

Site	Treatment	Year	*n*	Mean	SD	Min	Max
ACPP	Bagged	2010	25	0	0	0	0
		2011	22	0	0	0	0
	Cross	2011	21	0.14	0.36	0	1
		2012	9	0.11	0.33	0	1
	Open	2010	53	0.09	0.30	0	1
		2011	21	0.81	0.40	0	1
		2012	10	0.10	0.32	0	1
	Self	2011	15	0.13	0.35	0	1
		2012	10	0.50	0.53	0	1
RH	Bagged	2011	17	0	0	0	0
	Cross	2011	17	0	0	0	0
	Open	2011	33	0.06	0.24	0	1
		2012	10	0.10	0.32	0	1
	Self	2011	18	0.06	0.24	0	1

different between the Anderson County Preserve and Rockhill Prairie sites ($t_{d.f.=32.46} = -0.59$, $P = 0.56$) but dissolved sugars were different ($t_{d.f.=47.01} = 7.95$, $P \leq 0.0001$; Table 7).

Biochemical analyses showed that nectar of *A. meadii* contained two amino acids, detected in 20 of the 26 sample spots. These were aspartic acid (55,450.07 pmol/μ: –288,385.77 pmol/μL) and arginine (1,811.76 pmol/μL –13,514.04 pmol/μL). Fructose, glucose, and sucrose were detected in nectar samples, but sucrose was not detected in one sample. The range of sucrose was from 0 to 0.03

Table 5. Descriptive statistics for the average number of (a) pollinaria removed, (b) stigmatic slits with pollinaria inserted, and (c) stigmatic slits with germinating pollen for each experimental treatment at the Anderson County Prairie Preserve (ACPP), Proffit Mountain (PM), and Rockhill (RH) Prairie research sites.

Measure	Site	Treatment	Year	*n*	Mean	SD	Min	Max
Average No. of pollinaria removed	ACPP	Bagged	2010	25	0	0	0	0
			2011	22	0.27	0.46	0	1
		Open	2010	53	0.25	0.48	0	2
			2011	21	1.57	1.25	0	4
			2012	10	1.40	1.17	0	3
	PM	Open	2013	25	0.32	0.56	0	2
			2014	23	0.70	0.88	0	2
	RH	Bagged	2011	17	0	0	0	0
		Open	2011	33	0.45	0.71	0	3
			2012	10	0.80	0.92	0	3
Average No. of stigmatic slits with pollinaria inserted	ACPP	Bagged	2010	25	0	0	0	0
			2011	22	0.5	0.21	0	1
		Open	2010	53	0	0	0	0
			2011	21	1.14	0.85	0	3
			2012	10	0.3	0.48	0	1
	PM	Open	2013	25	0.08	0.28	0	1
			2014	23	0.30	0.56	0	2
	RH	Bagged	2011	17	0	0	0	0
		Open	2011	33	0.12	0.33	0	1
			2012	10	0.70	1.25	0	4
Average No. of stigmatic slits with germinating pollen	ACPP	Bagged	2010	25	0	0	0	0
			2011	22	0	0	0	0
		Open	2010	53	0.11	0.32	0	1
			2011	21	1.00	0.71	0	2
			2012	10	0.30	0.48	0	1
	RH	Bagged	2011	17	0	0	0	0
		Open	2011	33	0.12	0.33	0	1
			2012	10	0.5	1.27	0	4

Table 6. Descriptive statistics for the average number of pollinaria removed, the average number of pollinia inserted, and the difference between the average number of pollinaria removed and inserted per flower at the Proffit Mountain site during the 2013 and 2014 research periods.

Measurement	*n*	Mean	SD	Min	Max
Average number of pollinaria removed	50	0.56	0.79	0	2
Average number of pollinaria inserted	50	0.20	0.45	0	2
Average number removed minus average number inserted	50	0.36	0.69	−1	2

FIG. 4. *Apis mellifera* with leg trapped in a stigmatic slit of *A. meadii*. Note also two pollinaria attached to proboscis. (Photographer: Retha Edens-Meier)

μg/μL. Fructose ranged from 0.01 to 0.13 μg/μL, whereas glucose ranged from 0.001 μg/μL to 0.28 μg/μL.

FRUIT SET. Bagged, unmanipulated flowers never set fruit. A single fruit was counted at the Rockhill Prairie site in 2010, and no fruit was set in 2011. In 2010, a 28% ($n = 58$) fruit set was documented at the Anderson County Prairie site, with two infructescences producing two fruits each. In 2011, only three flowering stems produced fruit ($n = 52$); one of these stems produced two fruits. In 2012, 50% ($n = 100$) produced fruit with six plants producing two fruits each. Fruit set also varied at the Proffit Mountain site. Twenty-four flagged stems (2013) produced eight fruits, while 24 flagged stems (2014) produced six fruits.

POLLINARIA VECTORS. Insect visits to flowers of *A. meadii* began the first day the perianth opened. Insects were first observed from 8:00 am to 9:00 am and usually concluded between noon and 1:30 pm. Workers of *Apis mellifera* and some wasps were observed as late as 3:00 pm. Nocturnal observations were discontinued after the first week because the few noctuid moths collected failed to carry pollinaria. A wide variety of insects foraged for nectar on these flowers at the three sites (Table 8). During 2011–14, insects visiting *A. meadii* flowers were selectively captured because of the presence of a large number of foraging *Apis mellifera* and small halictids and to preserve the number of *Bombus* gynes (potential or actual queens).

CORPUSCULUM LOAD ANALYSES. The average number of corpuscula found on legs of *A. abrupta*, *A. mellifera*, and *Bombus griseollis* varied (Table 9) as did the pair of legs the corpuscula were attached to (Table 10). The record number of corpuscula on one bee was held by an *A. mellifera* worker collected on May 11, 2011, wearing 23 corpuscula: one on the glossa, two on the first pair of legs, 13 on the second pair, and 7 on the third pair. Unlike the two other apids, when corpuscula were deposited on the tarsi of the third pair of legs they were not always confined to the claws. Corpuscula were found around the margins of the tarsi where the corbiculus attached to the tibia. Several *A. mellifera* were missing the entire tibia of one of the third pairs of legs. When members of *A. mellifera* were unable to free their trapped legs from the stigmatic slits, they died still attached to the flower (Fig. 4). In addition, unlike the two other apids, workers of *A. mellifera* at Anderson

Table 7. Descriptive statistics for average nectar volume (μL) and refraction (0%–50% of dissolved solutes) at the Anderson County Prairie Preserve (ACPP) and Rockhill (RH) study sites.

Measure	Site	*n*	Mean	SD	Min	Max
Volume (μL)	ACPP	45	3.52	1.47	1.00	5.00
	RH	19	3.76	1.54	1.00	5.00
Refraction (0%–50%)	ACPP	45	44	0.09	0.23	0.50
	RH	19	28	0.06	0.21	0.41

Table 8. Number of insects of each species collected at each site, each year (2010–14).

Species	Anderson 2010	Anderson 2011	Anderson 2012	Rockhill 2011	Proffit Mountain 2013	Proffit Mountain 2014
Achalarus lyciades	0	0	0	0	1	0
Anthophora abrupta	27	8	0	2	14	9
Apis mellifera	10	31	23	0	0	0
Augochlorella aurata	6	9	1	1	0	0
Augochlorella persimilis	1	1	1	0	0	2
Augochloropsis metallica	8	3	6	0	0	1
Bombus fraternus	0	0	1	0	0	0
Bombus griseocollis	1	0	18	6	7	1
Bombus impatiens	0	0	0	0	0	1
Everes comyntas	0	0	0	0	1	0
Lasioglossum hitchensi	0	0	0	0	0	1
Lasioglossum paradmirandum	0	0	0	0	0	13
Lasioglossum pruinosum	1	0	0	0	0	0
Lasioglossum roweri	1	0	0	0	0	0
Lasioglossum sp.	0	0	0	0	0	4
Megachile addenda	0	0	0	0	0	1
Megachile brevis	0	1	0	1	2	0
Polistes fuscatus	1	0	0	0	0	0
Sceliphron caementarium	1	0	0	0	0	0
Thorybes pylades	0	0	0	0	3	0

County Prairie Preserve site were more likely to carry one to two corpuscula on their mouthparts. They were found most often on the glossa and/or one of the two labial palps.

The number of corpuscula carried by *Anthophora abrupta* varied significantly among legs ($X^2_{df=2} = 8.89$, $P = 0.01$; Table 10). Post hoc analysis determined that significantly more corpuscula were found on the midlegs of *Anthophora abrupta* than the hindlegs, but no other pairwise comparisons for this species were significant. The number of corpuscula carried by *Apis mellifera* also varied significantly among legs ($\chi^2_{d.f.=2} = 9.09$, $P = 0.01$; Table 10). In this species, the forelegs carried fewer corpuscula than the middle and hindlegs did, but the number of corpuscula carried by the middle and hindlegs was not found to be significantly different. Corpuscula loads among legs of *Bombus spp*. (three species pooled) were not found to vary significantly ($\chi^2_{d.f.=2} = 0.02$, $P = 0.99$; Table 10; Fig. 5).

No differences in the number of corpuscula carried by male *vs*. female *Anthophora abrupta* were found ($W = 425$, $P = 0.7$; Table 10) or among worker and queen *Bombus* species ($W = 96$, $P = 0.17$; Table 11).

Discussion. As Betz (1989) found approximately 12 flowers per inflorescence, it is interesting to note that flower number has not changed significantly in the past 50 yr and does not vary extensively between extant populations. Recent literature emphasizes that some herbaceous plants now bloom earlier because of global warming, and that may interrupt flower-pollinator interactions (Fitter and Fitter 2002). However, as herbarium sheets (collected 1833–2012) did not indicate flowering onset in the population where the

Table 9. Descriptive statistics for the average number of corpuscula found on the legs of three bee species at the Anderson County Prairie Preserve (ACPP), Proffit Mountain (PM), and Rockhill Prairie (RH) sites.

Species	Site	*n*	Mean	SD	Min	Max
Anthophora abrupta	All sites	60	1.10	1.66	0	9
	ACPP	35	1.63	1.93	0	9
	PM	23	0.39	0.78	0	3
Apis mellifera	ACPP	64	3.28	4.59	0	23
Bombus griseocollis	All sites	33	2.58	2.84	0	11
	ACPP	19	2.26	2.98	0	11
	RH	6	0.83	0.98	0	2
	PM	8	4.62	2.39	2	9

Table 10. Descriptive statistics for the average number of corpuscula found on the leg pairs (fore, mid, and hind) of *Anthophora abrupta*, *Apis mellifera*, and *Bombus griseocollis*.

Species	Leg-pair	*n*	Mean	SD	Min	Max
Anthophora abrupta	Fore	60	0.30	0.74	0	4
	Mid	60	0.60	1.04	0	5
	Hind	60	0.20	0.61	0	3
Apis mellifera	Fore	64	0.58	1.37	0	8
	Mid	64	1.34	2.30	0	13
	Hind	64	1.36	2.11	0	9
Bombus griseocollis	Fore	33	1.00	1.84	0	8
	Mid	33	0.82	1.36	0	6
	Hind	33	0.76	1.09	0	4

specimens were collected, we were unable to determine whether the floral phenology of this species shifted toward earlier flowering from the 19th century to 20th century. Specimen collections did show that this species bloomed from late May through mid-June. The earliest bloom time recorded in our study was May 05, 2012, with peak flowering occurring at the Anderson County Prairie Preserve population on May 10, 2012.

Hand-pollination experiments indicate that self-incompatibility in *A. meadii* lacks an early acting recognition. If a pollinium is placed in a stigmatic slit via hand-mediated self-pollination, pollen grains germinate and produce pollen tubes that can grow into the ovules. Consequently, self-incompatibility within this species is either late acting as in *Acacia* (Kenrick *et al*. 1986) or postzygotic.

Automatic self-pollination was not apparent in any of the *A. meadii* flowers examined. In open pollination treatments, however, pollinaria removal and pollinia insertion varied among sites and seasons at all field sites. In all cases, inflorescences in open-pollination treatments had more of their pollinaria removed than inserted. Not all of the pollinia inserted into stigmatic slits germinated. Lack of germination could be attributed to incorrect insertion by insects (Brown 1833) and/or lack of germination time.

FIG. 5. Gyne of *Bombus griseocollis* carrying pollinaria on four feet. (Photographer: Retha Edens-Meier)

Flowers pollinated by large bees with long tongues should be sucrose rich–sucrose dominant and abundant in amino acids (Baker and Baker 1983). *Asclepias meadii* is neither. The sucrose ratio in *A. meadii* is so low, it parallels flowers pollinated by small, short-tongued bees, whereas the amino acid levels in its nectar are similar to species pollinated by hummingbirds. Members of three apid genera may patronize *A. meadii* because of the high volume of nectar secretions and its rich sugar concentration. Why were sugar concentrations so different between Anderson County Prairie Preserve and Rockhill Prairie? It is possible that the Rockhill Prairie population produces nectar with lower sugar concentrations in response to different modes of environmental stress, such as reduced population size and/or infection (Brown *et al*. 2015). It is also possible that the ratio of dissolved sugars is altered in nectar infected with microbes (Vannette *et al*. 2013). Regardless of the cause, the 16% reduction in dissolved sugar levels at Rockhill may account, in part, for the lower visitation rates of primary pollinators.

A discrepancy exists between rates of pollination *vs*. the conversion of flowers into fruits. Fruit set varied between populations and years. The conversion rate of flowering stems into fruiting stems was historically low or absent at the Rockhill Prairie site. The largest population of flowering stems was located at the Anderson County Prairie Preserve site. The Proffit Mountain population showed a conversion rate of flowering stems into fruits similar to other *Asclepias* species (Betz and Lamp 1990), and other obligately

Table 11. Descriptive statistics for the average number of corpuscula found on male and female *Anthophora abrupta* and queen and worker *Bombus griseocollis* bees. (Data pooled 2010–14.)

Species	Sex	*n*	Mean	SD	Min	Max
Anthophora abrupta	Female	29	0.97	1.35	0	5
	Male	31	1.23	1.93	0	9
Bombus griseocollis	Queen	9	2.89	3.48	0	11
	Worker	16	1.38	2.06	0	6

outcrossing angiosperms (Bernhardt 1983, Bernhardt and Dafni 2000, Edens-Meier *et al*. 2013).

At least three explanations exist for why fruit set varied among these populations, especially between Anderson County Prairie Preserve and the Rockhill site. First, plant stress–related issues (Brown *et al*. 2015) and predation could lower fruit set. Second, small populations (*e.g*., Rockhill Prairie) should have lower genetic variability than larger populations, thus lowering their fitness (Futuyma 2013). Third, pollinator vectors varied among years, and large apids appeared to prefer the flowers of *Penstemon digitalis* and *Rudbeckia* sp. at the Rockhill Prairie site.

The major vectors of pollinaria of *A. meadii* are represented by three genera in the family Apidae. However, deposition of corpuscula on legs varied with field site and bee taxon. *Anthophora abrupta* carried most of the corpuscula on its second pair of legs, as noted previously by Betz *et al*. (1994), but our specimens carried some pollinaria on the other two leg pairs. *Apis mellifera* carried most of its corpuscula on the second and third pair of legs. Betz *et al*. (1994) collected only one specimen of *A. mellifera* on *A. meadii*, and it carried no pollinaria. Corpuscula appeared to be distributed evenly on the legs of our *Bombus* species (gynes and workers), but Betz *et al*. (1994) collected only one specimen of *B. affinis* and one specimen of *B. griseocollis* on *A. meadii*, carrying four and two pollinia.

Workers of *A. mellifera* dominated observations and collections in 2011 at the Anderson County Prairie Preserve site. However, although stigmatic slits contained their highest rates of deposited pollinia in 2011, fruit set was far lower than they were in 2010 and 2012. We suggest that the two native species may be more efficient in the "correct" insertion of pollinia into stigmatic slits compared with *A. mellifera*. It is also likely that annual pollinator density and diversity levels were affected by variations in yearly weather patterns.

It appears that *Bombus* species are important pollinators of *A. meadii*. Gynes require nectar-rich flowers, whereas establishing nests and the abundant nectar of *A. meadii* is clearly attractive to them. The bloom time in 2012 for *A. meadii* was sufficiently early and coincided with the peak emergence of *Bombus griseocollis* gynes. *Bombus griseocollis* is a very common and abundant species within the range of *A. meadii*; it can nest on or below ground and does not appear to be declining, unlike some other Midwestern *Bombus* species (Grixti *et al*. 2009, Cameron *et al*. 2011, Williams *et al*. 2014). In *Bombus* species, the legs of both gynes and workers showed an equal distribution of corpuscula deposition. Furthermore, we did not observe any *Bombus* species trapped in *A. meadii* flowers or any signs of lost leg segments. This is in contrast to *A. mellifera*, where dead trapped bees and leg-segment losses were observed. In addition, records and observations of commercial *A. mellifera* dying while trapped by the flowers of *Asclepias* species date back to 1920 (Pellett 1976).

Despite earlier collections by Betz *et al*. (1994), the significance of *A. abrupta* as a dependable pollinator is unclear. Their appearance was sporadic, and they carried fewer corpuscula than *Bombus* and *A. mellifera*. Unlike *Bombus* species, *A. abrupta* females require water to construct underground nests (Rau 1926, Norden 1984), and if local water sources disappear during drought years, emerging females must disperse and build nests elsewhere. Thus, as climatic conditions vary, so does the dependability of *A. abrupta* pollination services. For example, in 2012, *A. meadii* finished blooming before *A. abrupta* emerged. Emergence times of *A. abrupta* vary greatly (as much as 1 mo) between years (Rau 1926, Norden 1984) but largely coincide with the flowering of *A. meadii*. Bloom times for *A. meadii* seem to be coordinated with the onset of spring. The Missouri collection data for *A. abrupta* (1985–2015; Rau 1926; M. Arduser, unpublished data) are similar to documented bloom times for *A. meadii* based on herbarium collections (see above).

Gaining an understanding of how weather conditions affect bloom time and nectar volume and/or quality and how those factors relate to pollinator visitation rates will be important in increasing the reproductive success of *A. meadii*. Additional research is needed to determine what and how various stress factors may influence fecundity via potential fungal infection of its flowers (Brown *et al*. 2015). In addition to current management regimes, introduction of bison and observing vegetative recruitment in wallows could prove beneficial (Barkley *et al*. 1993, Hartnett *et al*. 1997, McMillan *et al*. 2011). It is imperative for *A. meadii* fruits to reach maturity to allow for seed dispersal, which potentially increases genetic diversity within the populations. Therefore, haying must be delayed until after mature fruits dehisce. Finally, management strategies for *Bombus* species (Hines and Hendrix 2005, Hatfield et. al. 2012) on public and private lands near *A. meadii* populations will likely benefit this plant through increased pollination services, as well as other less-threatened, but nonetheless characteristic and important, tallgrass prairie forbs and the prairie life that depends on them.

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