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The floral ecology and breeding system of *Veratrum virginicum* (Melanthiaceae)[1]

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Abstract. We analyzed the floral biology of a population of the threatened/endangered *Veratrum virginicum* (L.) W.T. Aiton at the Shaw Nature Reserve (Missouri, USA), comparing our results to the first and only study of this species by Charles Robertson (1896). We confirmed most of Robertson's original descriptions regarding floral presentation, protandry, and insect pollination but found the following new information. Each flower lived 9–11 days and smelled of raw liver and latex. We discerned four floral stages based on gradual changes in style and stamen orientation, which might assist in insect-mediated pollination. We determined that stigmas became receptive by the fifth day of anthesis. The generalist pollination system included three *Lasioglossum* species (Halictidae) and the beetle *Chauliognathus marginatus* Fabr. (Cantharidae). Most floral foragers showed a geometric mean of body dimensions between 2–3 mm. Approximately 40% of foraging insects carried heterospecific pollen loads, combining the host flower's pollen with grains from up to seven co-blooming taxa. Field observations suggest that all bees foraged on multiple inflorescences whereas beetles remained on the same inflorescence for hours. Larger Apidae species showed symptoms of nectar poisoning. Epifluorescence showed that flowers exposed to visiting insects contained < 5 pollen tubes per gynoecium. Fruit set was 3% for bagged flowers and 51% for exposed flowers. However, the conversion rate of ovules into seeds was low in both bagged (2%) and exposed (6%) flowers. We interpret low reproductive success as one explanation for current declines in populations of this species.

Key words: bees, beetles, fruit/seed set, paracladia, stamen-style movements

Understanding floral biology and pollination ecology is necessary to understand why some plant populations are in decline and how they respond to environmental change. This is particularly important in rare and threatened taxa. The Virginia bunch flower, *Veratrum virginicum* (L.) W.T. Aiton (syn. *Melanthium virginicum* L.; Melanthiaceae s.s. or Liliaceae s.l.), is a rhizomatous-bulbous perennial of wet habitats and grasslands (Bodkin and Utech 2002). The species is distributed through the southeastern USA, excluding southern Appalachia. It is considered critically imperiled and/or endangered in Indiana and Tennessee and classified as threatened in Ohio (Crabtree 2016, Endangered, Threatened, Rare and Extirpated Plants of Indiana 2016, Ohio Department of Natural Resources 2018). This species is also regarded as either threatened or endangered in Illinois, Kentucky, New Jersey, and New York according to the USDA website in 2019 (USDA Plants Database 2019). However, even a century ago, botanical observations and scientific collections in America appear to be infrequent. By the end of the 19th century, Robertson (1896) noted it was rare in Illinois.

Little literature is available on the pollination and breeding system of this species. It is known to reproduce asexually via clonal reproduction of rhizomes (Hilty 2019). Sexual reproduction occurs in compound-racemose, terminal-paniculate, or open-paniculate inflorescences 0.6–2 m in height (Fig. 1). Most flowers are perfect, but terminal flowers on paracladia (reiterative, secondary, raceme branches; *sensu* Weberling 1983) can be staminate. Each flower has six tepals and six stamens surrounding a single superior or partially inferior trilocular ovary bearing three distinct styles. *Veratrum virginicum* blooms from late spring to summer (Bodkin and Utech 2002). Infrequent flowering has been described in other

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FIG. 1. Paniculate inflorescence of *Veratrum virginicum* showing greening of basal flowers and the absence of gynoecia in apical flowers. Photo by Retha Edens-Meier.

members of this genus. For instance, *Veratrum woodii* J.W. Robbins ex Alph. Wood blooms only every 4 to 8 yr (Ebinger 1993, 1996; Schaffner *et al.* 1994) and *V. tenuipetalum* A. Heller might not flower for > 6 yr (Iler and Inouye 2013). This sporadic mode of flowering has not been described in *V. virginicum* to date.

Regarding floral attractants and rewards in *V. virginicum*, each tepal is 5.5–13 mm in length with colors ranging from white or greenish yellow and turning either greenish yellow or reddish purple with age. Each tepal bears two yellow nectar glands at its base (Robertson 1896, Bodkin and Utech 2002). Descriptions of the floral scent remain unpublished in peer-reviewed journals, but anecdotal reports have described the scent as reminiscent of urine, wet horses, and cow dung (Floden 2013, McD 2013, Woodbury 2017).

Literature on the pollination ecology of this species is limited to Robertson (1896). He collected and identified 15 species of flies (Diptera) in the families Syrphidae, Tachinidae, Sarcophagidae, Muscidae, and Anthomyidae on flowers of *V. virginicum*. He also collected six beetle species (Coleoptera) in the families Lampyridae, Scarabaeidae, Mordellidae, and Curculionidae, and three members of the Order Hymenoptera in families Andrenidae, Sphecidae, and Chalcididae on flowers of *V. virginicum*. However, his identification of the Andrenidae was incorrect because *Halictus confusus* Smith belongs to the Halictidae. Robertson concluded that floral adaptations in *V. virginicum* represented a trend toward beetle pollination, but he did not examine or compare visiting insects for the presence of the host flower's pollen.

Robertson (1896) performed no hand-pollination experiments to determine breeding systems, but he did attempt to determine the extent of self-isolation mechanisms in *V. virginicum*. Besides recognizing staminate flowers in inflorescences (see above) Robertson interpreted perfect flowers as protandrous. Without any biochemical tests for stigmatic receptivity available at the time, Robertson concluded that the stigmas became receptive after the anthers detached from their filaments. He also noted that staminal filaments bent toward the stigmas as the flower aged, presuming that this development might allow for mechanical self-pollination. To date, self-compatibility remains undocumented in this species, but self-incompatibility was found in *Veratrum album* L. (Kleijn and Steinger 2002, Kato *et al.* 2009).

The purpose of this paper is to expand the initial work of Robertson (1896). By adding to the basic knowledge of the reproductive biology of this rare species, we provide useful information that can be applied to future conservation efforts. We want to answer the following questions: What is the flowering phenology of this species in Missouri and how long do individual flowers live? How are floral organs arranged spatially and temporally? Does this species utilize a generalist or specialist pollination system? Does this species employ mechanisms of cross-pollination and modes of self-isolation to promote greater fructification and seed set?

Methods. POPULATION AND SITE. Field research and observations were made from June 5 to July 21, 2018 at the Shaw Nature Reserve, Gray Summit, Saint Louis County, Missouri (38°28′14.8″N 90°49′04.4″W). The site was usually visited on alternate days. The focal population grew in a mesic prairie (swale) among

tall grasses. The small and dispersed population at Shaw consisted of approximately 30 flowering stems.

FLORAL PHENOLOGY AND LIFESPAN. To record the flowering period of the population, we observed floral buds on 85 tagged paracladia on 11 inflorescences each day we visited the site. We defined a paracladium as in bloom if it had a minimum of one flower in which the tepals were expanded and white in color. We recorded the end of the flowering period of the paracladium when all of its flowers turned green (see below).

To document the lifespan of individual flowers and their stamens and styles, two flower buds were selected at random and labelled with dated jeweler's tags on each inflorescence ($n = 22$ flowers on 11 inflorescences). After the perianth expanded, we recorded the number of days the flowers remained open before turning green. During this period, we also measured the length of one out of the three styles on each ovary. Each day that a tepal remained white we recorded the number of anthers that remained closed, open, or spent (empty of pollen and/or abscised) in 20–22 flowers. Because anthers bend toward the stigmas as the flower ages, we recorded whether the staminal filaments were straight or bent each day ($n = 18$–21 flowers). We also recorded when the three styles in a flower bent outward toward the anthers.

INFLORESCENCE AND FLORAL PRESENTATION. We recorded floral color and sniffed flowers *in situ* to record floral odor. Because tepals are presented in a radial and open perianth in this species, nectar secretion was recorded by directly observing the presence of a liquid on the glands. We also observed nectar glands in translucent tepals of flowers preserved in ethanol (see below). We mounted whole, excised, tepals under cover slips to observe nectar glands under a compound light microscope. Tepal length was measured in 42 flowers using digital calipers and the height of each inflorescence was measured from the soil level to the lowest paracladium with a measuring tape.

INSECT VISITOR COLLECTIONS AND MEASUREMENTS. Insects were collected from 30 flowering stems of *V. virginicum* between 10:00 am to 5:00 pm but only after they were observed foraging for nectar or pollen on host plant flowers. These specimens were euthanized in jars with fumes of ethyl acetate and stored in a freezer at −10 °C. To remove pollen from insects, each specimen was placed on a separate glass slide and washed with two to four drops of ethyl acetate. After the ethyl acetate had evaporated, we stained the pollen with Calberla's fluid and labelled pollen slides following Edens-Meier *et al.* (2011) except that insect bodies were dried under warm airflow after washing. Measuring the insect with digital calipers, pinning, and coreferencing the insect specimen followed Edens-Meier *et al.* (2011). Insects were identified by Dr. Michael Arduser and donated to the Billiken Bee Lab in the Department of Biology, Saint Louis University, MO (*DW001–DW155*).

POLLEN LOAD ANALYSES. The slides of pollen loads collected from insects were examined under a compound light microscope and the pollen morphotypes were recorded. A morphotype was recorded as present if more than 25 grains were counted. This was a precaution to correct for any pollen contamination occurring when more than one insect was euthanized in the same jar (see Bernhardt *et al.* 1984).

In order to identify pollen morphotypes, a library of pollen grains taken from co-flowering plants *in situ* was assembled. We identified the specimens, collected their pollen, and then pressed them as vouchers. Specimens were identified by Dr. James Trager (Shaw Nature Reserve). Pollen grains were stained and mounted in Calberla's fluid as with pollen taken from insects.

Flowering of *V. virginicum* co-occurred with nine other flowering species. Species collected for the pollen library included *Achillea millefolium* L., *Amorpha canescens* Pursh, *Asclepias syriaca* L., *Asclepias tuberosa* L., *Baptisia alba* (L.) Vent., *Penstemon digitalis* Nutt. ex Sims, *Rudbeckia hirta* L., *Tradescantia ohiensis* Raf., and *Trifolium repens* L. Vouchers were deposited in the herbarium of the Missouri Botanical Gardens (*DW14–DW23*).

BREEDING SYSTEMS. To determine whether *V. virginicum* is capable of self-pollination, we tested self-isolation mechanisms. To test for herkogamy, we tracked the movement of dehiscent anthers and receptive stigmas over the floral lifespan as described previously. To determine whether dichogamy occurred, we compared periods of anther dehiscence (above) against tests for stigmatic receptivity. Stigmatic receptivity was recorded using a modified version of the hydrogen peroxide

test (Armbruster *et al.* 2002) on 22 flowers from 11 inflorescences representing different floral ages. We segregated these flowers into four stages based on age and relative positions of their stamens (see below). We then removed their tepals and stamens before submerging their gynoecia (still attached to their pedicels) in 3% hydrogen peroxide. We sampled 4–7 gynoecia for each floral stage. If 1–3 stigmas on the same ovary produced bubbles, we recorded the gynoecium as receptive. We also observed whether foraging insects contacted dehiscent anthers and/or stigmas.

To determine natural rates of insect-mediated pollination in flowers of *V. virginicum,* we examined natural rates of pollen grain deposition and pollen tube growth in paracladia. We collected 36 tagged, open flowers (from 11 inflorescences) ranging in age and exposure to insects by 2–6 days. We decided not to collect flowers after 6 days because pollen tubes tend to disintegrate within aging styles, making them difficult to count under epifluorescence (Lipow *et al.* 2002). These flowers were prepared for epifluorescence microscopy following Bernhardt *et al.* (1980). However, we used a razor blade to butterfly the ovary wall and expose the ovules. We recorded the number of pollen grains adhering to the three stigmas and the number and lengths of pollen tubes in each of the three styles and within the compound ovary (see Vance *et al.* 2004) using a Zeiss Axio Imager.M2. We also observed fungal hyphae under autofluorescence because recent literature indicates that fungi can arrest growth of pollen tubes in the pistils of other angiosperms (see Brown *et al.* 2015, Domic *et al.* 2017).

To test Robertson's hypothesis that stigmas self-pollinated as they expanded toward anthers, we used organza bags to isolate 42 paracladia in bud and left 42 paracladia exposed to insects over their flowering periods on 11 inflorescences. We did not select terminal florescences (*sensu* Weberling 1983), because Robertson (1896) suspected they were infertile. Bagged and exposed flowers were given 40 days to set fruit and then bags, labels, and fruit were collected. The number of fruits on each exposed and bagged paracladium was counted and compared to the original number of perfect flowers on the same paracladium, not including flowers removed to determine natural rates of pollen tube penetration (see above). We then selected 20 fruits at random on exposed paracladia and because only nine fruits developed on bagged paracladia, we selected all nine bagged fruits. The fruits were dissected, and the number of seeds was counted. To calculate the conversion rate of ovules into seed, we used a dissecting microscope to count the average number of ovules in 15 randomly selected and fixed exposed flowers from 11 inflorescences.

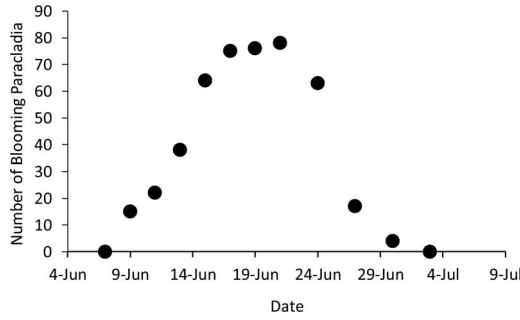

FIG. 2. The number of paracladia with at least one opened flower over the blooming period ($n = 11$ inflorescences observed).

STATISTICAL ANALYSES. To describe the expansion of styles, data were log-transformed to meet the assumptions of a linear regression model. Then we used a repeated measures ANOVA because the same flowers were measured each day in the field. To compare style length between particular dates, we used a pairwise *t*-test with a Holm adjustment. For fruit set, to account for within-individual variation, we used a nested design ANOVA in which individual treatments were assigned to entire paracladia and both sets of treatments were contained within each experimental unit (*i.e.*, each individual plant). We used a Kruskal-Wallis rank sum test to analyze seed set.

Results. FLORAL PHENOLOGY AND LIFESPAN. Flowers of *Veratrum virginicum* bloomed from June 7 to June 30, 2018. Flowers never withered or abscised. Instead, aging stamens abscised while tepals and gynoecia became stiff and green (Fig. 1). Peak bloom in 85 paracladia ($n = 11$ inflorescences under observation) occurred over a 5-day period from June 17 to 21 with 75–78 paracladia bearing open flowers (Fig. 2).

Each flower remained open for 9–11 days before turning green. Styles diverged and expanded in length increasing from a mean of 2.77 mm (SEM ± 0.12) to 3.17 mm (SEM ± 0.09) over 7–9 days. Expansion in style length as the flowers aged was significant overall ($F = 6.818$; df = 3, 54; $P =$

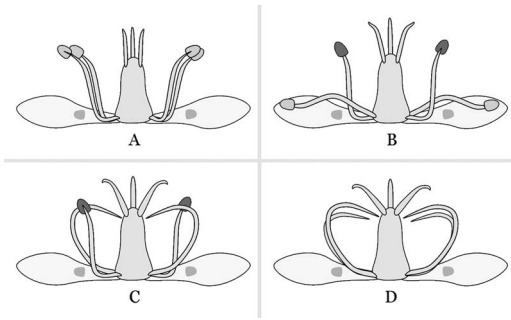

FIG. 3. Stages in the floral life-span of *Veratrum virginicum*: (A) Stage 1, day 1; (B) Stage 2, days 1–2; (C) Stage 3, days 3–4; (D) Stage 4, days 5–9. Darker shaded anthers signify dehiscence.

0.0006). However, differences between consecutive dates were not significant.

We identified four stages over the life of a flower based on movements of the six stamens in two whorls and the three styles (Fig. 3). In the first stage, following tepal expansion on the first day, the six staminal filaments pointed upward and anthers remained indehiscent (Fig. 3A) and the three styles were erect and parallel to each other. The second stage occurred toward the end of the first day and through the second (Fig. 3B). The anthers in the inner whorl dehisced on the first day after the tepals opened. By the second day, the inner stamens bent toward the three diverging styles until anthers were only 1–2 mm apart from the unopened stigmas (Fig. 4). The three outer anthers remained closed and bent downward, pressing against the tepals. By the second day, styles in 60% of observed flowers began to separate from each other. This divergence did not stop until the fourth day. By the third stage (between 3–4 days), the three, dehiscent, inner anthers detached from their filaments and these filaments continued to bend and sometimes contact the three, fully divergent styles. At this time, the three outer stamens moved back to their initial position and their anthers dehisced (Fig. 3C, 4). During the fourth stage (between 5–9 days), we observed abscission of the emptied anthers on the three outer stamens, and their staminal filaments continued to bend toward the styles (Fig. 3D, 4). We observed small bees perching on these inward bending staminal filaments as they foraged or crawled around the flower. Occasionally dehiscent anthers did not detach from their filament and contacted the stigma with any remaining exposed pollen. Examinations of preserved flowers showed that the stigmatic apices did not subdivide until the fifth day.

INFLORESCENCE AND FLORAL PRESENTATION. Floral dimensions included an average style length of 2.98 mm ($n = 84$; SEM ± 0.05) over the floral lifespan, an average tepal length of 8.70 mm ($n = 42$; SEM ± 0.18), and an average inflorescence height of 116 cm ($n = 11$; SEM ± 2.7). Flowers on

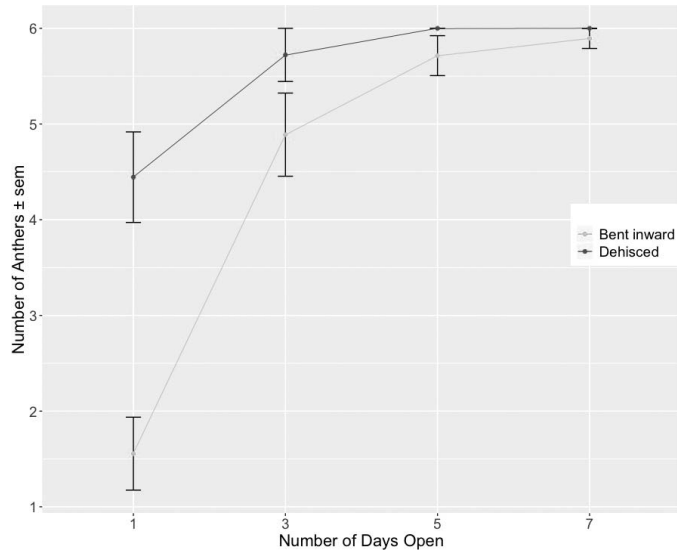

FIG. 4. Number of dehisced and/or abscised anthers ($n = 20–22$) and number of stamens bending inward toward pistils ($n = 18–21$) in *Veratrum virginicum* over time. Error bars are standard error of the mean.

FIG. 5. Two nectar glands at the base of the lamina of *Veratrum virginicum*. Each gland is positioned on either side of the midvein.

paracladia opened acropetally. Individual flowers were creamy white with two yellow nectar glands at the base of each lamina of each tepal on either side of the mid-vein. Nectar droplets were observed on these glands during field visitation periods (10:00 am to 5:00 pm). Under light microscopy, glands cleared of yellow pigment were dark and opaque compared to the now-translucent lamina tissue (Fig. 5). Flowers produced an uncommon scent that was neither honey-like nor putrid. We thought this odor was reminiscent of a combination of raw liver and latex. Toward the end of the flowering season, we noted that flowers produced by the terminal inflorescence and the apices of paracladia produced either reduced or no gynoecia (Fig. 1).

INSECT VISITOR COLLECTIONS, MEASUREMENTS, AND OBSERVATIONS. We collected and euthanized 163 foraging insects on *V. virginicum*. The most commonly collected insects were *Chauliognathus marginatus* (Cantharidae; $n = 59$), *Lasioglossum versatum* Robertson (Halictidae; $n = 38$) and *L. nymphaearum* Robertson ($n = 34$). Species abundance and geometric means of body dimensions are given in Table 1 with a histogram of the geometric means of body dimensions given in Fig. 6. Species with a body size of 2–3 mm were most common. Bees (Hymenoptera) showed the greatest diversity in geometric mean size, ranging from 2–11 mm. Beetles (Coleoptera) were more restricted, ranging between 2–4 mm. Flies (Diptera) were either very small (1–2 mm) or medium-sized (4–5 mm). True bugs (Hemiptera) were intermediate-sized (4–5 mm), and skippers (Lepidoptera) were larger (8–9 mm).

Chauliognathus marginatus foraged on nectar but also used flowers as copulation sites. More than half of the specimens collected were females. These beetles did not appear to eat pollen or anthers but passively contacted dehiscent anthers and receptive stigmas while foraging for nectar or

Table 1. Abundance and mean body sizes of pollinators found on *Veratrum virginicum*. NA = not assessed.

Insect Taxon	Number	% Females	Length (mm)	Width at widest part (mm)	Thoracic depth (mm)	Body size (mm^3)	Body size, geometric mean (mm)
Coleoptera							
Chauliognathus marginatus	59	58	10.91	2.99	1.06	34.58	3.26
Coleoptera (unidentified)	6	NA	7.09	2.12	1.02	15.33	2.48
Lucidota sp.	7	NA	6.53	2.11	0.78	10.75	2.21
Mordellidae (unidentified)	2	NA	4.51	1.76	1.03	8.18	2.01
Typocerus lunulatus	1	NA	8.59	2.55	2.43	53.23	3.76
Diptera							
Diptera (unidentified)	1	NA	3.97	1.23	1.2	5.86	1.8
Stratiomyidae (unidentified)	2	NA	12.01	4.46	3.55	190.15	5.75
Hemiptera							
Phymata sp.	4	NA	8.56	4.94	2.74	115.86	4.88
Hymenoptera							
Agridae (unidentified)	1	NA	10.84	4.39	2.72	129.44	5.06
Bombus auricomus Robertson	1	100	18.18	8.33	6.75	1022.2	10.07
Bombus griseocollis De Geer	1	100	14.99	6.67	6.07	606.9	8.47
Lasioglossum bruneri Crawford	3	100	7.12	2	1.73	24.64	2.91
Lasioglossum callidum Sandhouse	1	100	6.96	2.02	1.57	22.07	2.81
Lasioglossum nymphaearum	34	94	7.45	2.3	2	34.27	3.25
Lasioglossum versatum	38	100	6.4	1.99	1.75	22.29	2.81
Lepidoptera							
Epargyreus clarus Cramer	1	NA	21.1	4.81	5.6	568.35	8.28

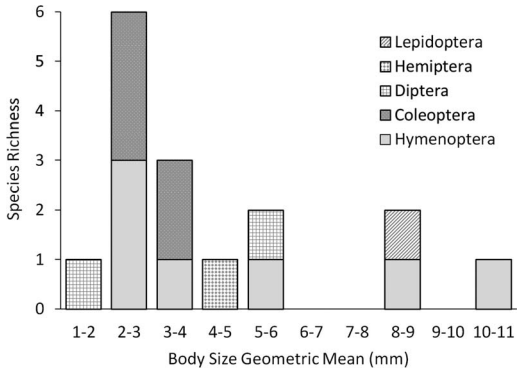

FIG. 6. Richness of pollinator species on *Veratrum virginicum* according to geometric means of body dimensions.

during copulation. Beetles remained on the same inflorescence for hours and we did not observe any flying between inflorescences.

With the exception of two male specimens of *Lasioglossum nymphaerum* and neuter female workers specimens of *Apis mellifera* and *Bombus,* all bees collected on *V. virginicum* were females. *Lasioglossum* species were observed actively collecting pollen and visiting nectar glands. Sometimes they landed directly onto the bent styles and filaments, and we observed these bees flying from inflorescence to inflorescence during the same foraging bout.

Bombus species and *Apis mellifera* L. (not collected) were also observed visiting more than one inflorescence during a single bout, but their movements appeared awkward and sluggish after consuming nectar. Their ability to cling to floral organs was poor. One unidentified *Bombus* species worker was seen probing a jeweler's tag used to identify a paracladium. We never observed these behaviors in the actively foraging and commonly collected *Lasioglossum* species.

POLLEN LOAD ANALYSES. Table 2 provides a breakdown of pollen morphotypes washed from insects collected on *V. virginicum*. The pollen of *V. virginicum* was present on 11 of 16 insect taxa. Pollen grains of *V. virginicum* were easily identified because they are monosulcate and approximately 35 μm in length (Fig. 7). Eight insect taxa (45 specimens, 28%) carried only pollen of *V. virginicum* whereas seven taxa (63 specimens, 39%) carried pollen of *V. virginicum* mixed with pollen from one or more co-blooming species. Separately, 48% of all insects collected carried pollen from at least one co-blooming species. Mixed loads of pollen from *V. virginicum* and co-blooming species were nearly 1.5 times more common than pure loads in total. The same trend is seen in specimens of *C. marginatus* and three species of *Lasioglossum*, which were the most common insects collected.

Table 2. Pollen load analysis for pollinators found on *Veratrum virginicum*. The total numbers of insect specimens collected are displayed against insect taxa and pollen load composition.

Insect taxon	*V. virginicum* only	*V. virginicum* + other	Other only	No pollen
Chauliognathus marginatus	15	24	**Coleoptera**	12
Coleoptera (unidentified)	1	1	—	4
Lucidota sp.	2	—	1	4
Mordellidae (unidentified)	—	—	1	1
Typocerus lunulatus	—	1	—	—
Diptera				
Diptera (unidentified)	—	—	—	1
Hemiptera				
Phymata sp.	4	—	—	—
Hymenoptera				
Agridae (unidentified)	—	1	—	—
Bombus auricomus	—	—	1	—
Bombus griseocollis	—	—	1	—
Lasioglossum bruneri	—	3	—	—
Lasioglossum callidum	—	—	1	—
Lasioglossum nymphaearum	10	18	3	3
Lasioglossum versatum	11	15	5	7
Stratiomyidae (unidentified)	1	—	1	—
Lepidoptera				
Epargyreus clarus	1	—	—	—
Total	45	63	22	32

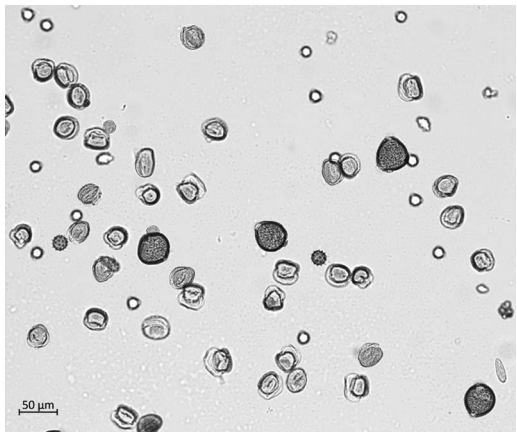

FIG. 7. A representative view of a pollen load (found on *Lasioglossum nymphaearum*) showing four pollen morphotypes. The grains of *Veratrum virginicum* are monosulcate and did not take up the basic fuchsin.

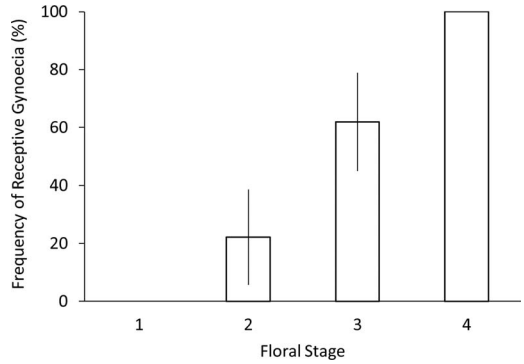

FIG. 8. Percentage of *Veratrum virginicum* gynoecia with 1–3 receptive stigmas at each floral stage (n = 4–7 gynoecia tested for each stage). Frequencies at stage 1 and 4 are absolute. Error bars are standard error of the mean.

Analyses of mixed pollen loads showed that grains of various, unidentified Asteraceae were the most common foreign morphotypes. Pollen grains from other species common at the study site, including *Baptisia alba*, *Penstemon digitalis*, and *Trifolium repens*, were found in pollen loads far less frequently than *V. virginicum* (Table 3). Of the floral foragers that carried pollen (n = 128), the majority (71) carried heterospecific pollen loads.

BREEDING SYSTEMS. Peroxidase tests showed that positive stigmatic responses indicating receptivity were most common in the 4th floral stage (100%), after stigma lobes opened (Fig. 8). At this time, most anthers in the same flower had abscised (Fig. 3, 4). Some stigmas responded positively to the peroxidase test as early as stage 2 (22%) when the first whorl of stamens dehisced. However, all gynoecia only responded positively by stage 4. Because pollen is released before stigmas become receptive in most flowers, this suggests a trend toward protandry.

Results of pollen deposition and pollen tube growth on flowers exposed to visiting insects are summarized in Table 4. Fluorescence microscopy showed that although each ovary has three styles, only a mean of 1.78 styles carried adherent pollen grains. Each gynoecium had a mean of 9.78 pollen grains of *V. virginicum* adhering to its stigmas (n = 27). We did not observe grain morphotypes belonging to co-blooming species on gynoecia. The average number of pollen tubes penetrating

Table 3. Mixed pollen load analysis of insects foraging on *Veratrum virginicum*. The total numbers of insect specimens collected with mixed loads are displayed against host insect taxa and plant taxa carried in their pollen loads. Vv = Veratrum virginicum, Pd = Penstemon digitalis, Ba = Baptisia alba, Tr = Trifolium repens, UA = Unidentified Asteraceae, UD = Unidentified eudicots, UM = Unidentified monocots, U = Unidentified angiosperms

Insect taxon	Pollen taxon							
	Vv[1]	*Pd*	*Ba*	*Tr*	UA	UD	UM	U
Coleoptera								
Chauliognathus marginatus	24	5	8	1	9	7	6	1
Coleoptera (unidentified)	1	—	—	—	—	—	—	1
Typocerus lunulatus	1	—	1	—	—	—	—	—
Hymenoptera								
Agridae (unidentified)	1	—	—	—	—	1	—	—
Lasioglossum bruneri	3	—	—	—	—	3	1	—
Lasioglossum nymphaearum	18	1	6	—	5	7	4	7
Lasioglossum versatum	15	1	3	—	6	7	6	2
Total	63	7	18	1	20	25	17	11

Table 4. Summary of pollen tube development in exposed *Veratrum virginicum* flowers using epifluorescence microscopy.

	Mean	n	SD	SEM	Range
Number of pollen grains per gynoecium	9.78	27	10.69	2.06	37
Number of stigmas per flower with pollen grains	1.78	27	1.15	0.22	3
Number of pollen tubes per gynoecium	4.74	27	6.58	1.27	23
Pollen grain germination rate	0.40	24	0.64	0.13	3
Proportion of flowers with pollen tubes	0.56	27	0.51	0.1	1
Proportion of longest tube length to style length	0.65	15	0.46	0.12	1

the styles and the ovary was 4.74 (Fig. 9B, C). Using these values, the rate of pollen grain germination was 40%. The proportion of pistils containing pollen tubes was 56%. Finally, mean length of the longest pollen tubes was 65% of the length of the corresponding style, although some tubes entered ovaries. Epifluorescence showed pollen tubes penetrating ovules (Fig. 9D). We found brown spores on the cuticles of some gynoecia. Autofluorescence also showed the development of fungal hyphae growing on style and ovary cuticles (Fig. 9A). Hyphae were also found penetrating pollen grains and within pistil tissue.

Paracladia with exposed bisexual flowers showed significantly higher rates of fruit set (51%, $n = 426$) than bagged flowers (3%, $n = 376$; Fig. 10), according to an ANOVA ($F = 24.04$; df = 10, 57; $P < 0.0001$). The mean number of ovules per ovary was 57.1 ($n = 15$). This was used to calculate the conversion ratio of ovules into seeds in 20 exposed and 9 bagged fruits. Seed set was extremely low in both groups. Exposed flowers produced on average 3.4 seeds per fruit (SEM ± 0.71; seed set was 6%), whereas bagged flowers produced 1.2 seeds per fruit (SEM ± 0.15; seed set was 2%) (Fig. 10). According to a Kruskal-Wallis rank sum test, exposed and bagged flowers had no significant differences in seed set (chi-squared = 0.21832; df = 1; $P = 0.6403$).

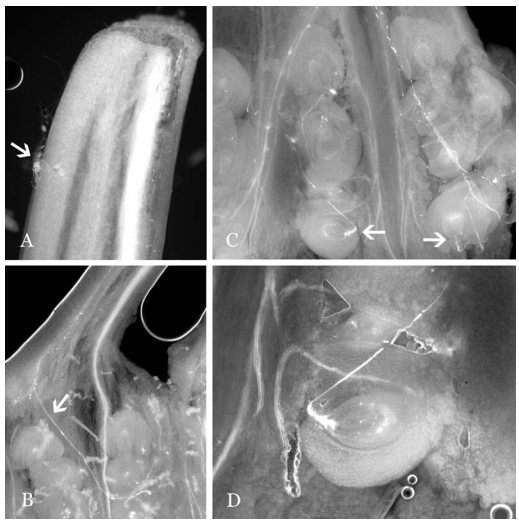

FIG. 9. Epifluorescent microscopic images of pistil squashes in exposed *Veratrum virginicum* flowers: (A) Stigma was not pollinated and shows the internal autoflorescent vein and hyphae on the cuticle; (B) One pollen tube penetrates the style of a second gynoecium and enters the ovary (note vein on right); (C) Multiple pollen tubes in ovary showing penetration of ovules (note callose plugs); (D) Pollen tube entering the micropyle of an embryo sac.

Discussion. FLORAL PHENOLOGY AND LIFESPAN. Anthesis in our population of *V. virginicum* began in June and lasted 3 weeks. This agrees with other descriptions of the phenology of this species, including Bodkin and Utech (2002). With individual floral lifespans lasting 9–11 days, a single flower can release and accept pollen throughout half of the flowering period. There is no explanation in the literature for the retention and greening of the tepals at the end of the floral lifespan. It is possible that the tepals are retained as part of the visual cue, in this mass-flowering herb, as receptive, nectar-secreting flowers diminish in number. Similar interpretations have been made for color changes in two, mass-flowering legumes (Gori 1989, Bernhardt 1990). A second possibility is that green tepals are photosynthetic, producing sugars to nourish seeds similar to the pedicellate spikelet of some grasses (AuBuchon-Elder *et al.* 2019). The potential adaptive significance of the bending staminal filaments is discussed below.

INFLORESCENCE AND FLORAL PRESENTATION. Bodkin and Utech (2002) reported that tepals are greenish yellow in this species and turn a dark reddish purple with age, but we did not observe this. It might be possible that coloration varies over the distribution range.

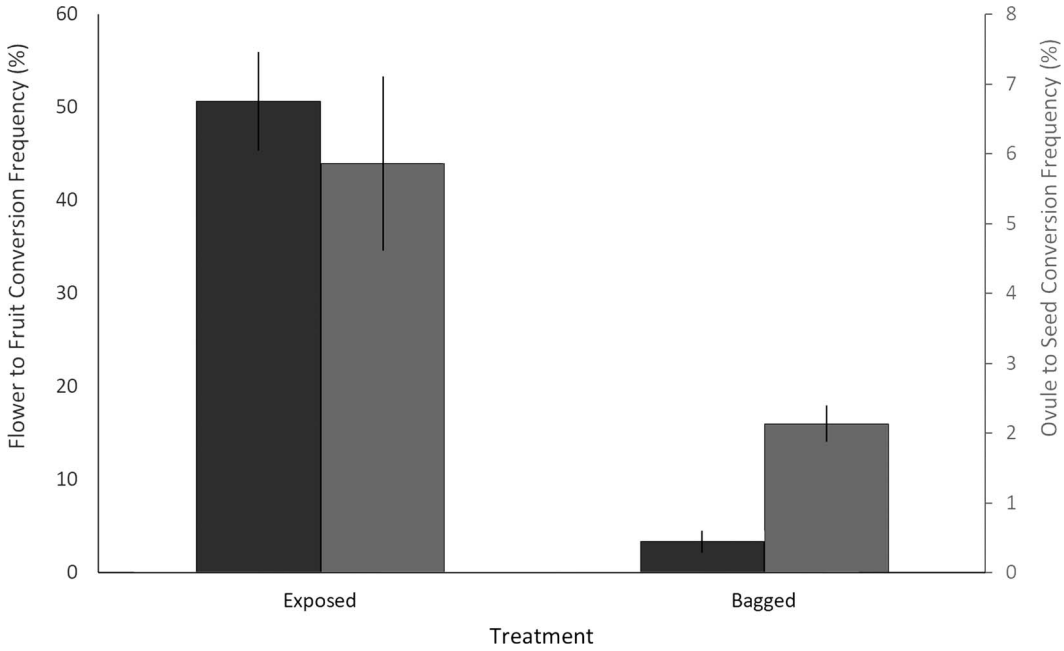

FIG. 10. Mean conversion frequency of *Veratrum virginicum* flowers into fruits per paracladia (left; $n = 15$–38) and ovules into seeds per flower (right; $n = 1$–20). Error bars are standard error of the mean.

This paper provides the first published description of the unusual floral scent. Floral odors that humans find unpleasant are considered classic characteristics of some beetle- and carrion fly-pollinated flowers (Faegri and van der Pijl 1979). Carrion flies were not observed or collected in this study, and the floral foraging preferences of *Chauliognathus* species remain understudied (Bernhardt 2000).

Most species in the Liliales have perigonal nectaries (Smets *et al.* 2000) and flowers with exposed, easily accessible nectaries such as these are usually interpreted as having beetle, fly, and short-tongue bee pollinators (Willmer 2011). Pigmentation of the nectar glands in lilioid monocots has also been documented in the allied genus *Toxicoscordion* (Melanthiaceae; Schwartz 2002). In the more distantly related genus, *Calochortus* there is vivid pigmentation and ornamentation of the same glands (Gerritsen and Parsons 2007). Because the large, swollen, yellow nectar glands in *V. virginicum* contrast distinctly with the white epidermis of the tepal, we interpret this pigmentation pattern as a nectar guide.

INSECT VISITOR COLLECTIONS, MEASUREMENTS, AND OBSERVATIONS. Beetles were major visitors to *V. virginicum*, with *Chauliognathus marginatus* (Cantharidae) being the most commonly collected insect in this study. In contrast to the original findings of Robertson (1896), flies were rare foragers whereas small bees were major cross pollinators at our site. We note that none of the species we collected on *V. virginicum* matched any of the species Robertson recorded in Carlinville, Illinois. Of course, insect community composition could naturally vary across relatively short distances and over a time frame of more than 120 years.

Flowers with radially symmetrical, flat (salverform) perianths and shallow nectar glands, like those of *V. virginicum*, tend to attract foragers with short mouthparts. This is indicative of generalist insect pollination as described by Faegri and van der Pijl (1979). This accounts for the 16 insect species we collected. However, we question whether *V. virginicum* consistently incorporates beetles, bees, and flies as pollinators in the same way that other generalist flowers do (see review by Bernhardt 2000). Our specific concern is whether the abundant beetle, *C. marginatus*, promotes outcrossing in *V. virginicum*. Although this beetle carried the study species' pollen, as well as grains of other co-blooming species, it foraged and copulated on the same inflorescence for hours. These slow, foraging beetles might be more likely

to facilitate vector-mediated, self-pollination. We note that *Xerophyllum tenax* (Pursh) Nutt. is also a member of the Melanthiaceae and is also visited by pollen-eating beetles (Vance *et al.* 2004). These beetles also remained on inflorescences for hours, and Vance *et al.* (2004) concluded that the dominant pollinators were flies in the family Syrphidae. Because our bagged flowers showed such low rates of fruit set by mechanical autogamy, we suspect that the primary agents of cross-pollination at our site are probably short-tongue bees in the genus *Lasioglossum* (Halictidae).

The majority of beetle and bee pollinators were small in size with a geometric mean of body dimensions between 2 mm and 3 mm. There could be two reasons why *V. virginicum* is not regularly pollinated by larger bees. First, the flowers lacked the "honey scent" associated with *Apis* and *Bombus* pollination (Faegri and van der Pijl 1979). Second, as described above, we suspect that some nectar constituents harm larger, long-tongue bees whereas smaller, native, short-tongue species are not adversely affected by the nectar. This can be corroborated elsewhere within the genus. Honeybees that were fed syrup mixed with pollen from *V. album* showed 39% mortality on the first day and 74% mortality by the third day (Perepelova 1949). Honeybees feeding on nectar from *Veratrum californicum* Durand also exhibited high mortality, with as many as 51 bees dying per minute observed at the flowers and in their hive (Vansell and Watkins 1933). The stems and leaves of *Veratrum viride* Aiton and *V. album* produce an alkaloid neurotoxin, veratradine, that can be fatal when consumed by livestock (Mulligan and Munro 1987, Schaffner *et al.* 2001). The nectar of *V. virginicum* should be analyzed for the presence of veratradine because it might be responsible for honeybee deaths across the genus.

POLLEN LOAD ANALYSES. Pollen load analyses indicates that the majority of floral foragers were polyphagic and female bees were usually polylectic. In fact, mixed pollen loads were far more common than pure loads of *V. virginicum* pollen on insect bodies. When we compared the pollen composition of pollen loads found on our specimens of *C. marginatus*, 68% of beetle specimens carried morphotypes of taxa other than pollen of the host flower (mixed and alien loads). Beetles carried a total of seven different morphotypes other than that of *V. virginicum*. When pollen loads of the three *Lasioglossum* species are combined, we also found that 68% carried morphotypes of taxa other than pollen of the host flower. These bees carried an additional six recognizable morphotypes. The prevalence of mixed loads could be due to the low abundance of *V. virginicum* at our site compared to other, mass-flowering species (*e.g.*, *Trifolium repens* and unidentified members of the Asteraceae).

PREPOLLINATION MODES OF SELF-ISOLATION. This population of *Veratrum virginicum* shows a trend toward protandry as described by Robertson (1896) with each sexual phase lasting approximately 50% of the floral lifespan. A notable feature in this taxon is that anther dehiscence is not synchronous. The inner whorl of stamens release pollen before the outer whorl. This might serve to increase the temporal availability of pollen for outcrossing throughout individual floral lifespans.

Robertson speculated that self-pollination could occur when stamens bent in toward stigmas during the transition to the female phase. We observed these same movements. However, results of bagged paracladia indicate that either rates of mechanical self-pollination are extremely low, or the species is self-incompatible to a large degree. We propose that the bending of anthers and stigmas toward each other is not a mechanism ensuring mechanical autogamy but rather assists in insect-mediated cross-pollination. The female phase is characterized by the divergence of the three styles, which might correlate with the small size of the primary pollinators, because smaller insects are less likely to contact tall, upright stigmas while foraging for nectar on outlying tepals. Moreover, the male phase is characterized by the inward bending of anthers. This arrangement formed a perch for pollinators; we observed bees landing on this framework of filaments. In this scenario, we expect insects to be more likely to contact the stigma, compared to bees that land directly on tepals.

Veratrum virginicum is andromonoecious as Robertson speculated. However, the lack of functional pistils in terminal flowers might be an adaptation to limited resources. We present two possible explanations. First, if these late-blooming, terminal flowers were bisexual and protandrous, producing gynoecia could be wasteful because pollen resources in the population would be diminished by the time stigmas in these final flowers reach receptivity. The flowers that bloom last would have a lower frequency of pollination

compared to flowers that bloomed prior. Furthermore, these final flowers are required only for the provision of pollen to gynoecia in other older, receptive flowers. Therefore, evolution toward staminate flowers at the end of the blooming period suggests that avoiding production of excess carpels is selectively advantageous. Second, when resources are limited, terminal flowers could receive fewer nutrients compared to basal flowers because nutrients must travel further up the scape and bypass other fruit (Stephenson 1981). A terminal fruit might be unable to acquire sufficient resources to mature over a 6-week period. We suggest that staminate flowers might be produced to avoid wasting resources (Cuevas and Polito 2004) if terminal developing seeds are not nurtured sufficiently by greening tepals (see AuBuchon-Elder *et al.* 2019).

Pollen Flow *vs.* Fruit and Seed Set. With an extremely low production of fruit in bagged, unmanipulated flowers, our results suggest that insect-mediated pollination is necessary for successful fertilization. This is corroborated by epifluorescence microscopy showing development of pollen tubes in exposed flowers. Bagging experiments showed that ovaries rarely set seed in the absence of insect visitations. However, with a 2% rate of seed set in bagged flowers, we could not conclude that this species is completely self-incompatible. The low rate of fruit set might have simply been caused by the equally low rate of mechanical self-pollination in a self-compatible system. Now, with the known floral age at which stigmas become receptive, future hand-pollination tests can be done to determine if a late-acting mode of self-rejection occurs in this species.

Fluorescence microscopy of pistil dissection also showed that many more pollen grains of *V. virginicum* were deposited on the stigma than pollen tubes penetrated pistil tissue. We know that allowing more time for pollen tubes to develop would not have greatly changed the observed frequency of pollen tubes (4.74 tubes per gynoecium in exposed flowers) because a low frequency of fertilization was corroborated by fruit dissections, which showed low seed set (3.4 seeds per fruit in exposed flowers). Low seed set seems contradictory, because fruit set was high (51% of flowers exposed to insects) but it agrees with a previous study on its congener, *Veratrum woodii*. Ebinger (1996) also found low seed set with over 75% of fruits containing 0–3 seeds. Taylor (1956) also found low seed production in *V. viride*, but with high seed production occurring intermittently every 4–5 years. Although we observed annual blooming outside of this study (2017–2020), a program of long-term monitoring is required to determine whether there are occasional years of high seed set in our population of *V. virginicum*.

We interpret the low conversion rate of ovules into seeds in our population of *Veratrum virginicum* as one possible reason why this species is classified as threatened and endangered. It is perplexing that a single ovary of *V. virginicum* should produce 57 ovules but fewer than four reach maturity. We suggest four possible explanations. First, the insects that we suggest are primary pollinators might be inefficient, delivering inadequate numbers of viable grains to receptive stigmas (Ashman *et al.* 2004). Second, pollinators might be delivering inadequate numbers of viable, cross-compatible grains. This was noted in a related member of the Melanthiaceae, *Xerophyllum tenax*. In a study of this species, 95% of dissected fruits showed low seed set because of a strong self-incompatibility mechanism, blocking the germination of grains deposited by mechanical and zoophilous self-pollination (Vance *et al.* 2004). Third, producing excessive ovules might be a vestigial character and/or a product of genetic constraint. Due to limitations in capsule size at maturity, it might be physically impossible for *V. virginicum* to develop more than four seeds per ovary, regardless of ovule number. This occurs in other nonrelated, multiovulate, herbaceous species, including *Paeonia brownii* Douglas ex Hook. (Bernhardt *et al.* 2013) and *Erythronium umbilicatum* Parks & Hardin (Motten 1983). A pollen supplementation study could be used to discern if this is the case. Fourth, a fungal pathology with hyphae penetrating pollen grains and ovaries might cause infertility, which occurred in *Polylepis* species (Domic *et al.* 2017) and possibly in *Asclepias meadii* Torr. Ex A. Gray (Brown *et al.* 2015). With many possible explanations, more research is required to understand why *V. virginicum* shows a rate of fruit set comparable to other perennial, andromonoecious herbs (Sutherland 1986) but shows such a low rate of seed set. If this low level of fecundity is found in other populations of this species it might help explain why it is considered threatened or endangered and contribute to future conservation research programs and subsequent policies.

Literature Cited

ARMBRUSTER, W. S., C. H. P. MULDER, B. G. BALDWIN, S. KALISZ, B. WESSA, AND H. NUTE. 2002. Comparative analysis of late floral development and mating-system evolution in tribe Collinsieae (Scrophulariaceae s.l.). American Journal of Botany 89: 37–49.

ASHMAN, T-L., T. M. KNIGHT, J. A. STEETS, P. AMARASEKARE, M. BURD, D. R. CAMPBELL, M. R. DUDASH, M. O. JOHNSTON, S. J. MAZER, R. J. MITCHELL, M. T. MORGAN, AND W. G. WILSON. 2004. Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. Ecology 85: 2408–2421.

AUBUCHON-ELDER, T., V. CONEVA, D. M. GOAD, D. K. ALLEN, AND E. A. KELLOGG. 2019. Sterile spikelets assimilate carbon in sorghum and related grasses. bioRxiv:396473.

BERNHARDT, P. 1990. Anthecology of *Schrankia nuttallii* (Mimosaceae) on the tallgrass prairie. Plant Systematics and Evolution 170: 247–255.

BERNHARDT, P. 2000. Convergent evolution and adaptive radiation of beetle-pollinated angiosperms. Plant Systematics and Evolution 222: 293–320.

BERNHARDT, P., J. KENRICK, AND R. B. KNOX. 1984. Pollination biology and the breeding system of *Acacia retinodes* (Leguminosae: Mimosoideae). Annals of the Missouri Botanical Garden 71: 17–29.

BERNHARDT, P., R. B. KNOX, AND D. M. CALDER. 1980. Floral biology and self-incompatibility in some Australian mistletoes of the genus *Amyema* (Loranthaceae). Australian Journal of Botany 28: 437–451.

BERNHARDT, P., R. MEIER, AND N. VANCE. 2013. Pollination ecology and floral function of Brown's peony (*Paeonia brownii*) in the Blue Mountains of northeastern Oregon. Journal of Pollination Ecology 2: 9–20.

BODKIN, N. AND F. UTECH. 2002. *Melanthium*, pp. 77–79. *In* Flora of North America Editorial committee, eds. 1993+. Flora of North America North of Mexico. Vol. 26. Oxford University Press, New York, NY.

BROWN, G., J. HERRERA, P. BERNHARDT, AND R. MEIER. 2015. Potential fungal pathogens of Mead's milkweed (*Asclepias meadii*: Apocynaceae). Natural Areas Journal 35: 599–605.

CRABTREE, T. 2016. Tennessee Natural Heritage Program Rare Plant List. https://www.tn.gov/content/dam/tn/environment/documents/na_rare-plant-list-2016.pdf. 52 pp. Retrieved April 5, 2019.

CUEVAS, J. AND V. POLITO. 2004. The role of staminate flowers in the breeding system of *Olea europaea* (Oleaceae): An andromonoecious, wind-pollinated taxon. Annals of Botany 93: 547–553.

DOMIC, A., P. BERNHARDT, R. MEIER, G. CAMILO, AND J. CAPRILES. 2017. Pollination ecology of *Polylepis tomentella* (Rosaceae), an Andean anemophilous tree presenting a potential floral fungal infection. International Journal of Plant Sciences 178: 512–521.

EBINGER, J. E. 1993. False hellebore (*Veratrum woodii*, Liliaceae) populations in Illinois. Transactions of the Illinois State Academy of Science 86: 85–91.

EBINGER, J. E. 1996. Flowering in false hellebore (*Veratrum woodii*, Liliaceae) populations in East-Central Illinois. Castanea 61: 46–48.

EDENS-MEIER, R., M. ARDUSER, E. WESTHUS, AND P. BERNHARDT. 2011. Pollination ecology of *Cypripedium reginae* Walter (Orchidaceae): Size matters. Telopea 13: 327–340.

ENDANGERED, THREATENED, RARE AND EXTIRPATED PLANTS OF INDIANA. 2016. Retrieved April 5, 2019. https://www.in.gov/dnr/naturepreserve/files/np-etrplants.pdf

FAEGRI, K. AND L. VAN DER PIJL. 1979. The Principles of Pollination Ecology, Third. Pergamon Press, New York, NY. 256 pp.

FLODEN, A. 2013. Bulbs for shade. https://www.pacificbulbsociety.org/pbslist/2013-July/26gra90cn8nmvoqk1gv8d6nkg1.html. Retrieved May 29, 2019.

GERRITSEN, M. AND R. PARSONS. 2007. *Calochortus*: Mariposa lilies and their relatives. Timber Press, Portland, Oregon. 232 pp.

GORI, D. F. 1989. Floral color change in *Lupinus argenteus* (Fabaceae): Why should plants advertise the location of unrewarding flowers to pollinators? Evolution 43: 870–881.

HILTY, J. 2019. Virginia Bunch-Flower. Illinois Wildflowers. Prairie Wildflowers of Illinois. http://www.illinoiswildflowers.info/prairie/plantx/bunchflower.html. Retrieved May 29, 2019.

ILER, A. M. AND D. W. INOUYE. 2013. Effects of climate change on mast-flowering cues in a clonal montane herb, *Veratrum tenuipetalum* (Melanthiaceae). American Journal of Botany 100: 519–525.

KATO, Y., K. ARAKI, AND M. OHARA. 2009. Breeding system and floral visitors of *Veratrum album* subsp. *oxysepalum* (Melanthiaceae). Plant Species Biology 24: 42–46.

KLEIJN, D. AND T. STEINGER. 2002. Contrasting effects of grazing and hay cutting on the spatial and genetic population structure of *Veratrum album*, an unpalatable, long-lived, clonal plant species. Journal of Ecology 90: 360–370.

LIPOW, S. R., P. BERNHARDT, AND N. VANCE. 2002. Comparative rates of pollination and fruit set in widely separated populations of a rare orchid (*Cypripedium fasciculatum*). International Journal of Plant Science 163: 775–782.

MCD, M. 2013. *Melanthium* - bunchflower. North American Rock Garden Society. https://www.nargs.org/forum/melanthium-bunchflower. Retrieved May 29, 2019.

MOTTEN, A. F. 1983. Reproduction of *Erythronium umbilicatum* (Liliaceae): Pollination success and pollinator effectiveness. Oecologia 59: 351–359.

MULLIGAN, G. A., AND D. B. MUNRO. 1987. The Biology of Canadian Weeds. 77.Veratrum viride Ait.. Canadian Journal of Plant Science 67: 777–786.

OHIO DEPARTMENT OF NATURAL RESOURCES. 2018. Rare Native Ohio Plants 2018–19 Status List. https://ohiodnr.gov/wps/wcm/connect/gov/9389b4eb-97a9-4e3e-ab18-19541f731e3e/2018-19+Ohio+Rare+Plants+Status+List+FINAL.pdf?MOD=AJPERES&CVID=nfZL7rX. Retrieved April 5, 2019.

PEREPELOVA, L. I. 1949. Effect of hellebore pollen on bees. Works. vet. Sect. Lenin Acad. Agric. Sci. Session 27: 55–65.

ROBERTSON, C. 1896. Flowers and Insects. XVI. Botanical Gazette 21: 266–274.

Schaffner, U., J-L. Boevé, H. Gfeller, and U. P. Schlunegger. 1994. Sequestration of *Veratrum* alkaloids by specialist *Rhadinoceraea nodicornis* Konow (Hymenoptera, Tenthredinidae) and its ecoethological implications. Journal of Chemical Ecology 20: 3233–3250.

Schaffner, U., D. Kleijn, V. Brown, and H. Müller-Schärer. 2001. *Veratrum album* in montane grasslands: A model system for implementing biological control in land management practices for high biodiversity habitats. 22: 19N–28N.

Schwartz, F. 2002. *Zigadenus,* pp. 81–88. *In* Flora of North America Editorial committee, eds. 1993+. Flora of North America North of Mexico. Vol. 26. Oxford University Press, New York, NY.

Smets, E. F., L-P. Ronse Decraene, P. Caris, and P. J. Rudall. 2000. Floral nectaries in monocotyledons: Distribution and evolution, pp 236–237. *In* K. L. Wilson and D. A. Morrison, eds. Monocots: Systematics and Evolution. CSIRO, Melbourne, Australia.

Stephenson, A. G. 1981. Flower and fruit abortion: Proximate causes and ultimate functions. Annual Review of Ecology and Systematics 12: 253–279.

Sutherland, S. 1986. Patterns of fruit-set: What controls fruit-flower ratios in plants? Evolution 40: 117–128.

Taylor, C. A. 1956. The culture of false hellebore. Economic Botany 10: 155–165.

USDA Plants Database. 2019. *Veratrum virginicum* (L.) W.T. Aiton Virginia bunchflower https://plants.usda.gov/core/profile?symbol=VEVI5. Retrieved May 29, 2019.

Vance, N. C., P. Bernhardt, and R. M. Edens-Meier. 2004. Pollination and seed production in *Xerophyllum tenax* (Melanthiaceae) in the Cascade Range of central Oregon. American Journal of Botany 91: 2060–2068.

Vansell, G. H. and W. G. Watkins. 1933. A plant poisonous to adult bees. Journal of Economic Entomology 26: 168–170.

Weberling, F. 1983. Fundamental features of modern inflorescence morphology. Bothalia 14: 917–922.

Willmer, P. 2011. Pollination and Floral Ecology. Princeton University Press, Princeton, NJ. 832 pp.

Woodbury, S. 2017. Glorious native lilies. http://www.gatewaygardener.com/native-plants/glorious-native-lilies. Retrieved May 29, 2019.