

The Pollination Biology of an Annual Endemic Herb, *Physaria filiformis* (Brassicaceae), in the Missouri Ozarks Following Controlled Burns

Author(s) :Retha Edens-Meier, Michael Joseph, Mike Arduser, Eric Westhus, and Peter Bernhardt

Source: The Journal of the Torrey Botanical Society, 138(3):287-297. 2011.

Published By: Torrey Botanical Society

DOI: 10.3159/TORREY-D-11-00024.1

URL: <http://www.bioone.org/doi/full/10.3159/TORREY-D-11-00024.1>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

The Pollination Biology of an Annual Endemic Herb, *Physaria filiformis* (Brassicaceae), in the Missouri Ozarks Following Controlled Burns¹

Retha Edens-Meier²

Department of Educational Studies, Saint Louis University, St. Louis, MO, USA 63103

Michael Joseph

Department of Biology, Saint Louis University, Saint Louis, MO 63103

Mike Arduser

Missouri Department of Conservation 2360 Highway D, St. Charles MO 63304

Eric Westhus

Department of Biology, Saint Louis University, Saint Louis, MO 63103

Peter Bernhardt

Department of Biology, Saint Louis University, Saint Louis, MO 63103

EDENS-MEIER, R. (Department of Educational Studies, Saint Louis University, St. Louis, MO, USA 63103), JOSEPH, M. (Department of Biology, Saint Louis University, Saint Louis, MO 63103), ARDUSER, M. (Missouri Department of Conservation 2360 Highway D, St. Charles MO 63304), WESTHUS, E. (Department of Biology, Saint Louis University, Saint Louis, MO 63103), AND BERNHARDT, P. (Department of Biology, Saint Louis University, Saint Louis, MO 63103). The pollination biology of an annual endemic herb, *Physaria filiformis* (Brassicaceae), in the Missouri Ozarks following controlled burns. *J. Torrey Bot. Soc.* 138: 287–297. 2011.—*Physaria filiformis*, a threatened species of calcareous glade communities, blooms profusely within the Rocky Barrens Conservation Area (Missouri) under a program of controlled burns. Flowers visited by insect foragers in 2008 and 2009 had a mean > 35 and > 50 germinating grains on their stigmas respectively and a mean of 1.96–2.0 pollen tubes entering their ovaries (mean of 4 ovules/ovary). Results of controlled hand-pollinations conducted *in situ* in 2009 showed that this species had a negligible rate of self-pollination (mechanical autogamy) and showed classic *Brassica*-type, early-acting self-incompatibility. Each dissected flower had nectar glands and each seasonal population was visited by insects representing four insect orders; Coleoptera, Diptera, Lepidoptera and Hymenoptera. The most commonly collected floral foragers found to carry the pollen of the host flower were male and female native bees representing 38 species in the Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae and the naturalized *Apis mellifera*. Only four of these species (11%) were collected in all three years, but these four accounted for slightly more than a third of all individual bees collected. None of the bees were known specialists on flowers of the Brassicaceae and only two species were habitat (glade) specialists. As *P. filiformis* is an obligate out-breeder but a generalist entomophile we suggest that future management plans include conservation and management of adjacent non-glade habitats to sustain the diverse range of pollinators.

Key words: annual herb, glade, native bees, pollen tubes, self-incompatibility.

Physaria (*Lesquerella*) *filiformis* (*sensu* Al-Shebaz and O’Kane 2002) is known as the Missouri bladderpod due to its inflated, globose fruits. It is an annual herb restricted to the Interior Highlands of North America (Ozark and Ouachita Mountains, Missouri

and Arkansas), where it grows on calcareous (typically limestone) glades (Nelson 2005). The government of the United States lists it as a threatened species (McKenzie 2003). In some glade reserves in Missouri, the Department of Conservation maintains populations of *P. filiformis* with controlled summer or early autumn burns. Seeds in burned sites germinate in autumn, overwinter as basal rosettes, flower in late April or early May, set fruit, and die in early summer. Sites maintained by burning usually produce dense and massive flowering populations of *P. filiformis* that can be seen with the naked eye from considerable distances, as they form thick yellow carpets (Fig. 1A).

¹ We sincerely thank Larry Meier for his assistance in this research and the Natural History Biologist, Mike Skinner, of the Missouri Department of Conservation for providing us with information on the management of Rocky Barrens.

² Author for correspondence. E-mail: rmeier3@gmail.com

Received for publication March 28, 2011, and in revised form July 5, 2011.

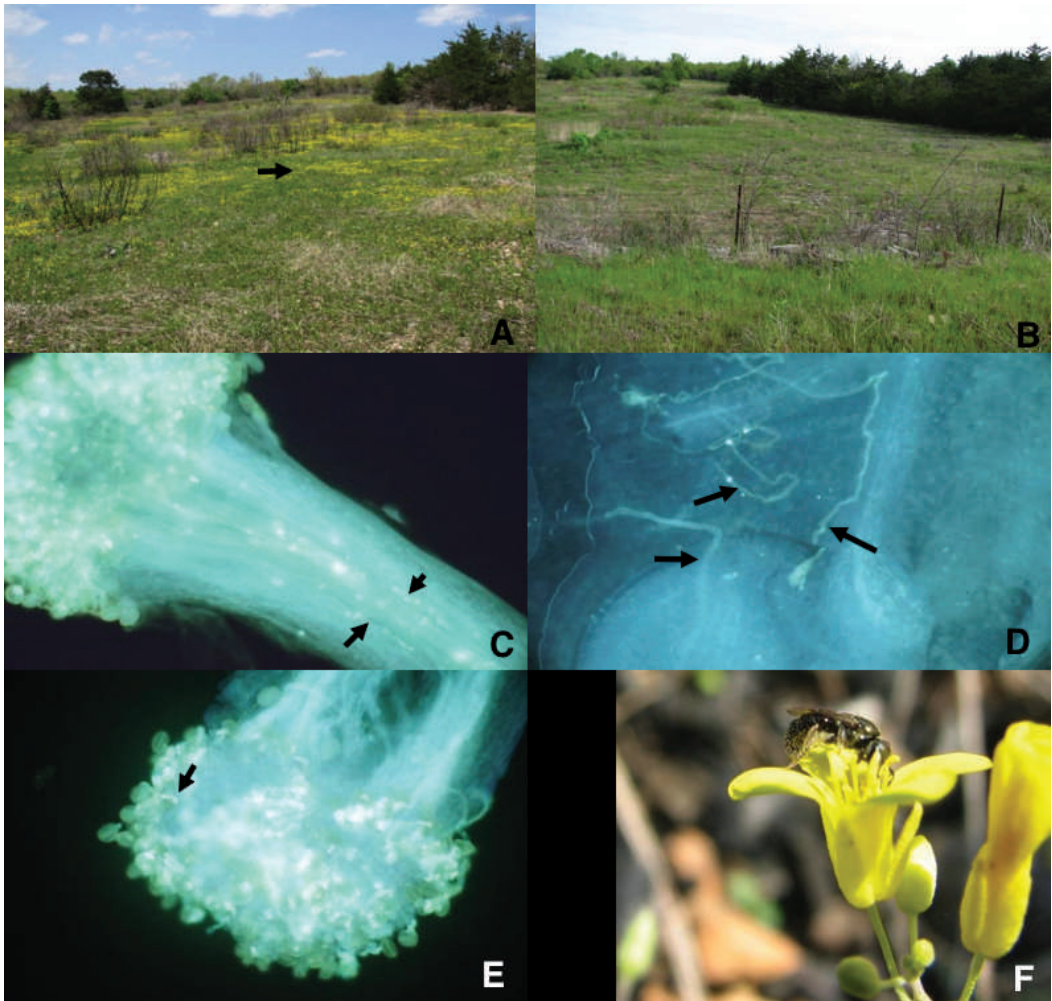


FIG. 1. A) *Physaria filiformis* (yellow) in full bloom growing on limestone glade (Rocky Barrens CA, Missouri, 2008). B) Absence of *Physaria filiformis*; same location, same month, different year; site was not burned (Rocky Barrens CA, Missouri, 2009). C) Cross-pollination showing pollen tubes in style. D) Cross-pollinated flower with pollen tubes in ovary. E) Self-pollinated flower showing self-incompatibility response (Note: ghostly pollen tubes on stigmatic surface failed to penetrate style tissue). F) A female *Lasioglossum* sp. visiting *Physaria filiformis* (Rocky Barrens CA, Missouri, 2009).

Blooming is so depressed in the absence of burning that the few plants in flower are difficult to find as this small herb is easily over-topped by grass culms and forb stems (Arduser, personal obs., Fig. 1B).

Although burning cycles maintain large and temporary flowering populations of *Physaria filiformis*, there is no complementary literature available on its pollination and breeding system. Pollen-pistil interactions are extremely variable within the Brassicaceae. Historically, *Brassica* spp. served as model organisms for the description and study of early-acting

(sporophytic) self-incompatibility systems (Stout 1931), and see review in Takayama and Isogai 2005). Self-incompatibility has also been shown following hand-pollination studies in the rare endemic *Physaria bellii* G. Mulligan (see review in Kothera et al. 2007), in *Physaria* (*Lesquerella*) *fendleri* (A. Gray) S. Watson (Mitchell and Marshall 1998), and in *Physaria ludoviciana* (Richardson) Rydb. (Claerbout et al. 2007). In other members of the Brassicaceae, self-incompatible and self-compatible species are found within the same genus. This includes the genera *Leavenworthia* (Solbrig

and Rollins 1977) and *Cardamine* (Kimata 1983). Cruden et al. (1996) found self-compatible and self-incompatible populations of invasive *Alliaria petiolata* (M. Bieb.) Cavara & Grande. In fact, *Alliaria petiolata* and *Hesperis matronalis* L. appear to produce allelopathic pollen that depresses the growth of intraspecific pollen tubes when their grains are deposited on stigmas of co-blooming flowers of *Alyssum alyssoides* (L.)L., *Lepidium campestre* (L.) W.T. Aiton and *Sisymbrium altissimum* L. (Murphy et al. 2009).

Therefore, when self-incompatibility dominates the breeding system of a population, cross pollination becomes essential for reproductive success because fruit set occurs only when a stigma receives sufficient pollen grains from genets that have two S alleles not shared with the prospective seed mother (i.e., sporophytic self-incompatibility; *sensu* Richards 1986, Wright and Barrett 2010). If not, fruit and seed set may decline by 77% if the surviving populations are pollinator-limited (Ariyoshi et al. 2006) and/or compatible pollen-limited (*sensu* Vance et al. 2004). Without faithful pollinators to maintain pollen flow, seed set may decline, and this is a common problem in flowering plant species (Burd 1994). Historically, pollination studies showed that insect-pollination dominated most lineages within the Brassicaceae of temperate zones (Proctor et al. 1996) but the diversity of prospective pollinators varied at the intraspecific and interspecific level. For example, different insects preferred to forage on different petal color morphs of *Raphanus raphanistrum* L. (Kay 1976) and different scent morphs of *Hesperis matronalis* (Majetic et al. 2009). At the interspecific level, the relatively widespread *Cardamine angustata* O.E. Schulz receives diverse pollen vectors (Motton 1986) while a population of the rare *Streptanthus bracteatus* A. Gray may be pollinated exclusively by the polylectic, long-tongue bee, *Megachile cornuta* (Dieringer 1991). *Physaria bellii* retains a generalist pollination system incorporating the naturalized honey bee (Kothera et al. 2007). While native bees and bumblebees pollinate *P. fendleri* (Mitchell and Marshall 1998), the rate of successful cross-pollination varies because mating is non-random. Seed set in this species is significantly higher when different genotypes are more densely packed together instead of broadly diffuse (Mitchell 1997, Mitchell and Marshall

1998). The role and diversity of insects as pollen vectors of *P. filiformis* has not been investigated to date.

Dixon (2009) considers the compatibility systems and pollination ecology of vulnerable populations in protected and restored sites. Additional information on the floral biology of *Physaria filiformis* should be obtained from areas subjected to controlled burns *in situ*. As *P. filiformis* survives within the drought and fire-cycle of restricted glade communities, the obvious questions that must be addressed are whether this species is self-compatible and mechanically self-pollinating and/or whether it is dependent upon insects for pollen dispersal. If the latter, then to what degree are the plant's pollinators restricted to or dependent on glades, and how do they respond to glade management?

Materials and Methods. **SITE.** Rocky Barrens Conservation Area (114 ha in Greene Co., MO) is co-owned by the Missouri Department of Conservation and The Nature Conservancy. The area includes approximately 50 ha of limestone glades and about 58 ha of early successional woodland, along with some old-field habitat. The Little Sac River borders a portion of the area. The area is managed with prescribed fire, thinning and brush removal, and occasional herbicide treatment for invasive exotics. The Missouri Department of Conservation conducted a series of small (8–12 ha) prescribed burns of the Area's glades in 2005 (September), 2006 (August), 2007 (August) and 2008 (two burns; July and August). We studied the inflorescences of *Physaria filiformis* and the anthophilous insects they attracted in April and May 2006, 2008 and 2009. Voucher specimens of *P. filiformis* were deposited in the herbarium of the Missouri Botanical Garden, St. Louis, and those of insect visitors were deposited in the American Museum of Natural History, New York.

INFLORESCENCE, FLORAL MORPHOLOGY, AND FLORAL DISPLAY. On May 4, 2008 we arbitrarily selected 33 plants. We counted the number of inflorescences/plant and the number of open flowers on each plant. Flowers and mature flower buds were dissected and observed under 10× magnification to determine the mode of anther dehiscence, location of nectar glands, and the number of ovules/ovary. Attempts were made to measure nectar

using a sugar refractometer measuring degrees Brix over a range of 0–50%.

RATES OF NATURAL INSECT POLLINATION. On May 4, 2008 and April 29, 2009, 8 h after sunrise we collected one open flower from each of 35 and 23 plants respectively. The flowers were fixed in 3:1, 0.95 ethanol: glacial acetic acid for ten hours, then decanted and preserved in 0.70 ethanol. To soften and clear pistil tissues to see the number of pollen tubes in each organ, whole flowers were incubated in a 0.10 solution of sodium sulfite at 45 °C for 25 min. The specimens were washed in de-ionized water, the pistils were excised from the floral receptacle and then each pistil was mounted on a glass slide. Each pistil was stained with several drops of decolorized aniline blue, then spread under a glass cover slip and stored in the refrigerator a minimum of 24 h before viewing under epifluorescence (see Vance et al. 2004). We counted the number of germinating grains on the stigma, pollen tubes penetrating the style, and pollen tubes in the ovaries. Due to the tangled nature of pollen tubes it was not possible to count accurately > 100 tubes in each stigma, or style. Consequently, due to this > 100 pollen tube category and the fact that the data's distribution was neither normal, nor homoskedastic, we elected to use the non-parametric Kruskal-Wallis test (Sokal and Rohlf 1995) to determine differences in pollen grain and pollen tube counts between years 2008 and 2009 on the stigma, in the style and in the ovaries. Analyses were conducted using the `kruskal.test` function in R (R Core Development Team 2010).

HAND POLLINATION EXPERIMENTS. In April 2009, we isolated 50 plants, removed all of their open flowers and enclosed the flowers under metal mesh strainers. The strainers were fixed to the ground with carpenter's nails. To compare rates of self-compatibility the remaining buds on each plant were sub divided into three experimental sections: Controls (mechanical self-pollination), Hand self-, and Hand cross-pollination. When Control flower buds opened we marked them with jeweler's tags without further manipulations. Self-pollinated flowers were marked with jeweler's tags but then we removed one or more dehiscent anthers from the same flower and pollinated the stigma in the same flower until the pollen was visible on the stigmatic surface when

viewed under a 3× optivisor. Cross-pollinated flowers were marked with jeweler's tags and then hand-pollinated with pollen from anthers of flowers found in bloom a minimum of one meter away. All flowers were harvested 48 hours after they were tagged. Pistil analysis and the counting of pollen tubes in pistil tissue were as above (see Vance et al. 2004). Counts of germinating pollen on the stigmas and pollen tubes within the styles and ovaries were compared among treatments. As above, we found that counts were neither normally distributed nor homoskedastic so we elected to use Kruskal-Wallis tests to determine if there were differences in pollen grain and pollen tube counts among treatment groups for the stigmas, styles and ovaries, and post-hoc Wilcoxon tests were used to identify the nature of those differences. Analyses were conducted using the `kruskal.test` and `pairwise.wilcox.test` functions in R (R Core Development Team 2010).

INSECT OBSERVATIONS, COLLECTIONS AND MEASUREMENTS. Insects were observed on flowers of *Physaria filiformis* on April 14, 2006, May 4, 2008, April 29, 2009, and May 2, 2009. Insects observed foraging actively for pollen and/or were observed to push their probosces or bodies into the floral throat were collected with butterfly nets and euthanized in jars with fumes of ethyl acetate. Specimens were pinned, labeled, and identified. As bees were the dominant visitors to *P. filiformis* we took three measurements of each specimen with digital calipers (see Li et al. 2008): Length from the frons to the tip of the abdomen, width between the widest points on the insect's body. For most bees this was the width of the segment at the base of the abdomen, and depth from the top to the bottom of the thorax.

POLLEN LOAD ANALYSES. To determine whether foraging insects collected pollen on co-blooming species before they were captured on *Physaria filiformis*, each euthanized insect was placed on a glass slide and bathed in 2–4 drops of ethyl acetate. The scopae or the corbiculae of each bee was also scraped with a probe to dislodge additional loads. After the insect was removed from the slide the corpse was air-dried, pinned and labeled to co-reference it with the label attached to the pollen slide. After the ethyl acetate evaporated on the slide, the residue was stained with

Table 1. Numbers of germinating pollen grains on stigmas, pollen tubes penetrating styles and ovaries, and ovules in ovaries open to insect pollination, sampled over two years.

	Mean		sd		<i>n</i>	
	2008	2009	2008	2009	2008	2009
Pollen grains/ Stigma	35.69	50.13	25.40	42.96	35	23
Pollen tubes/ Style	2.78	3.26	3.15	2.73	31	23
Pollen Tubes/ Ovary	1.96	2.00	2.22	1.91	28	23
Ovules/ Ovary	3.94	4.00	0.54	0.43	35	23

Calberla's fluid and mounted under a cover slip. As insects were killed in the same jar, contamination of pollen from one body to another was possible. Therefore, the pollen of a particular plant species was not counted as present unless we counted in excess of 25 grains of each morphotype on the same slide (see Bernhardt and Dafni 2000).

Results. FLORAL PRESENTATION AND FLORAL REWARDS. Individual plants in bloom produced one to 13 racemose inflorescences (mean = 3.90, sd = 2.17, *n* = 20). Flowering on a raceme was acropetal with a mean of 1.58 flowers open per scape at the time of counting (sd = 0.86, *n* = 67). Fructification was so rapid in this species that mature or near mature fruits were found at the bases of scapes while terminal flower buds were still opening. Each ovary connected to the receptacle via a columnar stipe. All ovaries contained a minimum of two and a maximum of seven ovules, although the majority contained four ovules (Table 1). Open petals and dehiscent anthers were bright yellow to the human eye. Anthers were dehiscent upon expansion of the petals. The four nectar glands on the receptacle were reduced to four, low, equidistant, mounds located between the ovary's stipe and petals, separated from each other by the broad bases of the staminal filaments. While nectar droplets were observed in freshly dissected flowers, quantities produced were inadequate for analysis using a sugar refractometer measuring degrees Brix over a range of 0–50 percent.

NATURAL RATES OF POLLINATION. In 2008 only one of 35 pistils collected on 35 flowering plants lacked germinating pollen grains on its stigma and pollen tubes penetrating its style tissue. In 2009, only three out of 47 pistils collected on 47 flowering plants lacked pollen tubes on its stigma or within style tissue. One pistil, collected in 2009, obviously lacked pollen grains and tubes as the stigma did not

develop and formed a non-receptive, acute terminus. Despite the large number of pollen grains deposited on the stigmas, ovaries contained a mean of < 4 pollen tubes penetrating a mean of four ovules in 2008 and 2009 (Table 1). No significant differences between 2008 and 2009 were found for the number of pollen grains on stigmas ($\chi^2 = 0.3773$, df = 1, *P* = 0.5391), the number of pollen tubes in the style ($\chi^2 = 0.992$, df = 1, *P* = 0.3192) and ovary ($\chi^2 = 0.0063$, df = 1, *P* = 0.937), or the ovules/ovaries ($\chi^2 = 0.0028$, df = 1, *P* = 0.9577) (Fig. 2).

HAND-POLLINATION EXPERIMENTS. The Control group had a negligible number of pollen grains on the stigmas, and a negligible number of tubes in the styles and ovaries (Fig. 3). The Hand Self-Pollinated (HSP) group had a large number of grains on the stigma, but a negligible number of tubes in the style and ovary (Fig. 3). The Hand Cross-Pollinated (HCP) group resulted in pollen tubes entering the style (Fig. 1C) as well as ovules within the ovary (Fig. 1D). This was the only treatment group with numerous grains/tubes present in all three pistil locations (Fig. 3). Kruskal-Wallis analyses detected significant differences among the treatment groups (Control, HCP, and HSP) in the number of tubes found on the stigmas ($\chi^2 = 50.11$, df = 2, *P* < 0.001), the styles ($\chi^2 = 67.37$, df = 2, *P* < 0.001), and the ovaries ($\chi^2 = 69.45$, df = 2, *P* < 0.001). Post-hoc analysis via pairwise Wilcoxon tests confirmed significant differences in the number of pollen grains on the stigmas between the Control and HCP (*P* < 0.001), Control and HSP (*P* < 0.001), and HCP and HSP (*P* = 0.016); differences in the number of pollen tubes in the styles between the control group and HCP (*P* < 0.001), and between HCP and HSP (*P* < 0.001); and finally differences in the number of pollen tubes in the ovaries between Control and HCP (*P* < 0.001) and HCP and HSP (*P* < 0.001). We reject the Null Hypothesis that rates of pollen grain deposi-

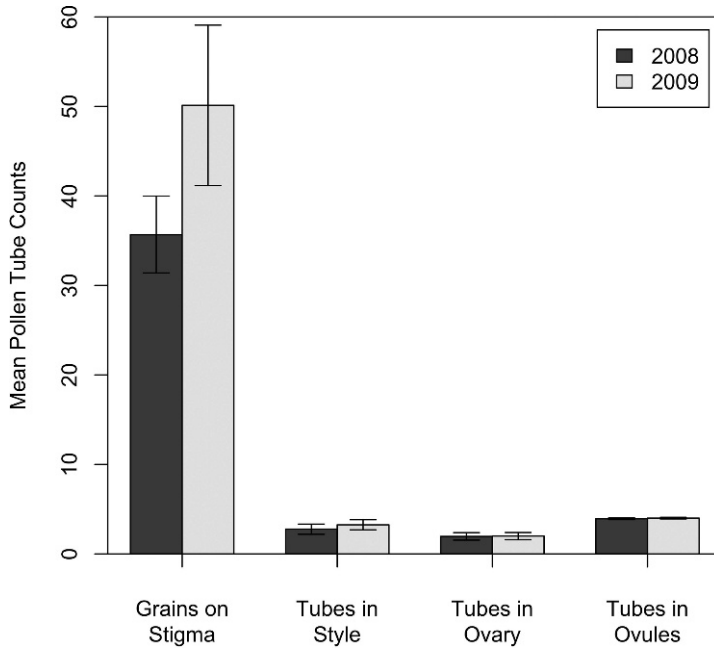


FIG. 2. Germinating pollen grains and pollen tubes penetrating styles vs. ovaries vs. ovules in Open (Insect-Mediated) Pollinations in 2008 vs. 2009 (means \pm standard error of the mean).

tion on stigmas and pollen tube penetration of pistil tissues was the same amongst the three experimental categories. Pistils self-pollinated by hand showed the same early-acting (Brassica-type) self-incompatibility response

(Fig. 3). Pollen grains hydrated on the stigmas produced short-coiling, pale-colored “ghost” tubes on or between stigmatic papillae, but penetration of the style was negligible (Fig. 1E).

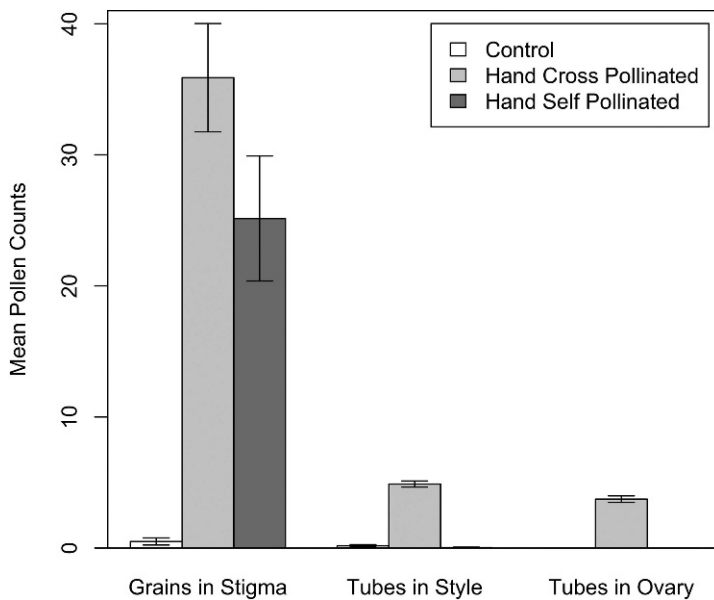


FIG. 3. Mean (\pm standard error of the mean) number of pollen tubes present on Stigmas, Styles, and Ovaries of Control, Hand Cross-Pollinated vs. Hand Self-Pollinated plants.

PROSPECTIVE POLLINATOR ACTIVITY AND DIVERSITY. Insects visited the flowers of *Physaria filiformis* in all three years. They were observed on warm, sunny days and represented four insect Orders. Small, black beetles (identified to the Mordellidae) were found in the floral tube and on the outer lobes of the petals but did not carry pollen of the host flower. Insects representing the Orders Diptera, Hymenoptera and Lepidoptera were observed flying from raceme to raceme on more than one plant. Lepidoptera in the families Lycaenidae (*Mitoura gyneus* Hübner), Nymphalidae (*Junonia coenia* Hübner) and Noctuidae (*Alypia octomaculata* Fabricius) probed flowers for nectar and carried pollen of the host flower. Pollen of *P. filiformis* was also found on true flies (Diptera) in several unidentified specimens belonging to the Syrphidae, and a single specimen that belonged to the Calliphoridae. The majority of floral foragers were true bees (Apoidea; Hymenoptera) representing 38 taxa in five families (Table 2). Females foraged for nectar and pollen but 25 bee specimens were males, representing nine species, and all of them carried significant quantities of host flower pollen. The majority of species (23 or 61%) were collected in only one of the three years; just four species (11%) were found in all three years. However, these four species, including *Lasioglossum paradmirandum* Knerer & Atwood, a species restricted in Missouri to Ozark glades, accounted for more than 1/3 (39%) of all individual bees collected over the three years. The great majority of the bee species (89%) were polylectic, and most of these were ground-nesting species. The grand total ($n = 150$) showed that $> 50\%$ of the bees collected belonged to members of the short-tongue family, Halictidae (Table 2, Fig. 1F). Pollen load analyses of 149 bees showed that 94% carried the pollen of *P. filiformis* but the majority (54%) carried pure loads of the pollen of *P. filiformis* exclusively while 40% carried *P. filiformis* grains mixed with the grains of one or two co-blooming species. We identified the pollen grains of *Camassia scilloides* (Raf.) Cory, *Oxalis* spp. (*O. cornuta* L. and/or *O. violacea* L.), *Sisyrinchium* sp., *Tradescantia* sp., *Verbena* sp. and several unidentified members of the Asteraceae in these mixed loads. In 2009 we commonly observed native bees visiting the flowers of *O. violacea* and *P. filiformis* during the same foraging bouts.

Despite the single collection of one *Bombus griseocollis* DeGeer gyne and workers of the naturalized *Apis mellifera* Linnaeus, the majority of native bees foraging on *P. filiformis* were small to medium-sized with a mean length of 7.11 mm ($n = 105$, $sd = 3.25$), a mean width of 2.42 mm ($n = 105$, $sd = 1.15$), and a mean thoracic depth of 1.90 mm ($n = 104$, $sd = 1.02$).

Discussion. Floral presentation in *Physaria filiformis* at the Rocky Barrens Conservation Area appears characteristic of an out-breeding herb despite its atypical life cycle. This includes a showy yellow corolla containing dehiscent anthers and four functional nectaries. After two seasons of random sampling of open flowers *in situ*, we conclude that their pistils were rarely pollen-limited (*sensu* Committee on the Status of Pollinators in North America 2007). In both years, the mean number of hydrated/germinating pollen grains on a stigma was far in excess of the mean number of four ovules/ovary.

Strong supporting evidence for obligate out-breeding in *Physaria filiformis* came from our hand-pollination experiments at three levels. First, these flowers did not self-pollinate in the absence of pollen vectors (mechanical autogamy) when plants were isolated from foraging insects under metal mesh. Second, pistils self-pollinated by hand showed early-acting (*Brassica*-type, *sensu* Takayama and Isogai 2005) self-incompatibility indicative of a homomorphic, multi-allelic breeding system (Richards 1986). Third, only hand-mediated cross-pollinations generated significant numbers of pollen tubes penetrating styles and ovaries. The classic literature maintains that annual herbs show a trend towards self-compatibility and facultative-obligate autogamy when flowering seasons are brief and/or growing conditions are stressful (Ornduff 1969, Solbrig and Rollins 1977, Richards 1986). Instead, the breeding system of *P. filiformis* parallels three of its self-incompatible congeners; *P. bellii* (Kothera et al. 2007), *P. fendleri* (Mitchell and Marshall 1998) and *P. ludoviciana* (Claerbout et al. 2007).

In fact, some lineages within the Brassicaceae show a trend toward self-pollination (Solbrig and Rollins 1977, Kimata 1983, Cruden et al. 1996). However, more recent publications suggest self-incompatibility is often well maintained within species in this

Table 2. The diversity of bees collected on *Physaria filiformis* at Rocky Barrens Conservation Area with selected pollen load analyses.

Insect taxon	Total Insects	Yearly Insects*			<i>Physaria</i> pollen only	<i>Physaria</i> & other spp.	Other spp. only	No pollen
		06	08	09				
Andrenidae								
<i>Andrena barbara</i>	2	0	2	0	0	2	0	0
<i>A. carlini</i>	2	1	1	0	0	1	0	1
<i>A. heraclei</i>	1	0	0	1	1	0	0	0
<i>A. lamelliterga</i> M**	2	1	0	1	1	1	0	0
<i>A. nasonii</i>	12	3	1	8	5	6	0	1
<i>A. nasonii</i> M	2	1	0	1	0	2	0	0
<i>A. nothoscordi</i>	3	0	3	0	1	2	0	0
<i>A. perplexa</i>	1	0	0	1	1	0	0	0
<i>A. personata</i> M	1	0	0	1	1	0	0	0
Subtotals	26	6	7	13	10	14	0	2
Apidae								
<i>Apis mellifera</i> W**	7	0	1	6	7	0	0	0
<i>Bombus griseocollis</i> G**	1	0	0	1	0	1	0	0
<i>Ceratina calcarata</i> **♦	4	2	0	2	3	1	0	0
<i>C. calcarata</i> M	5	3	0	2	1	3	0	1
<i>C. mikmaqi</i> M ♦	10	9	0	1	7	1	0	2
<i>C. strenua</i> M ♦	4	2	0	2	4	0	0	0
<i>C. strenua</i>	4	2	0	2	2	1	0	1
Subtotals	35	18	1	16	24	7	0	4
Colletidae								
<i>Hylaeus sparsus</i> M ♦	1	1	0	0	1	0	0	0
<i>H. (Prosopis) spp.</i> ♦	2	0	0	2	2	0	0	0
<i>H. modestus</i> M ♦	1	0	0	1	1	0	0	0
<i>Hylaeus sp.</i> A M	1	0	0	1	1	0	0	0
Subtotals	5	1	0	4	5	0	0	0
Halictidae								
<i>Agapostemon texanus</i>	1	1	0	0	NA**	NA	NA	NA
<i>Augochlora pura</i> ♦	1	0	0	1	1	0	0	0
<i>Aug. persimilis</i> M	1	0	0	1	1	0	0	0
<i>Aug. persimilis</i>	16	2	12	2	5	11	0	0
<i>Augochloropsis fulgida</i>	2	0	2	0	1	1	0	0
<i>Halictus ligatus</i>	3	0	0	3	2	1	0	0
<i>Ha. rubicundus</i>	1	1	0	0	0	0	1	0
<i>Ha. tripartitus</i>	1	0	0	1	1	0	0	0
<i>Lasioglossum anomalum</i>	2	1	0	1	1	1	0	0
<i>L. forbesii</i>	1	0	1	0	1	0	0	0
<i>L. foxii</i>	1	0	0	1	0	0	1	0
<i>L. imitatum</i>	1	0	1	0	1	0	0	0
<i>L. paradmirandum</i>	17	2	5	10	13	4	0	0
<i>L. pectorale</i>	1	0	1	0	1	0	0	0
<i>L. versatum</i>	13	13	0	0	3	8	1	1
<i>L. species nov.</i>	3	2	1	0	3	0	0	0
<i>L. tegulare</i>	11	6	4	1	8	3	0	0
<i>L. truncatum</i>	2	1	0	1	1	0	0	0
<i>L. callidum</i>	1	0	0	1	1	0	0	0
Subtotals	79	29	27	23	44	29	3	1
Megachilidae								
<i>Osmia conjuncta</i> ♦	1	1	0	0	NA	NA	NA	NA
<i>O. pumila</i> ♦	3	3	0	0	0	3	0	0
<i>Hoplitis simplex</i> M ♦	3	1	0	2	3	0	0	0
Subtotals	7	5	0	2	3	3	0	0
Grand Totals	152	59	35	58	86	53	3	7

* Collection Dates = April 14, 2006, May 4, 2008. April 29, 2009 and May 2, 2009

** All bee names refer to females unless otherwise marked G = gyne (queen), M = male, W = neuter worker. NA = pollen load was not assessed.

♦ Designates bees that nest in twigs, stems, dead wood, and cavities. Specific nesting sites are as follows: all 3 species of *Ceratina* (stems, twigs), all 3 species of *Hylaeus* (stems, twigs), *Osmia pumila* (twigs, existing cavities in dead wood), *Hoplitis simplex* (stems, twigs, existing cavities in dead wood), *Augochlora pura* (dead wood but not stems or twigs), and *Osmia conjuncta* (natural cavities; e.g., snail shells, but not wood).

family regardless of growing conditions and the length of the flowering period. In particular, Grundt et al. (2005) found that the herb, *Draba palanderiana* Kjellm. (Brassicaceae), while restricted to the Arctic region, maintains self-incompatibility over a short flowering season. Furthermore, the three, herbaceous and self-incompatible *Physaria* spp. mentioned in the previous paragraph also grow under highly stressful (xeric) conditions (Mitchell and Marshall 1998, Claerbout et al. 2007, Kothera et al. 2007).

However, we must still ask the question why any self-incompatibility alleles are retained over several often discontinuous generations in this species as mass flowering appears to be so dependent on infrequent/asynchronous cycles of severe, environmental disruption. The only obvious answer is that the unusually broad and generalist pollination system in *Physaria filiformis* is so efficient that it selects against any shift towards self-compatibility and self-pollination. A multi-allelic locus or loci is thus easily maintained. *Physaria filiformis* is not pollen-limited at the Rocky Barrens Conservation Area because it does not appear to be pollinator-limited (*sensu* Committee on the Status of Pollinators in North America 2007). In fact, only five days of collecting prospective pollen vectors suggests that *P. filiformis* attracts members of at least three anthophilous insect Orders (Diptera, Hymenoptera, Lepidoptera). In particular, when controlled burning stimulated mass flowering in *P. filiformis*, the annual population became a reliable source of nectar and/or pollen for polylectic female bees and polyphagic male bees. We note that Bernhardt (1990a) found that burning also stimulated the flowering of *Oxalis violacea* in a tall grass prairie site. As with *P. filiformis*, this vernal herb was also pollinated by small-medium sized bees but only 10 bee species were collected over the full 12 day flowering period of *O. violacea*. In 2009, we noted that *P. filiformis* and *O. violacea* bloomed together following the 2008 burn at the Rocky Barrens Conservation Area site. Although we observed native bees visiting both herbaceous species, only five native bees out of the 57 bees collected on *P. filiformis* in 2009, mixed the pollen of *P. filiformis* with the grains of *O. violacea*. This indicates that the floral presentation and reward system of *P. filiformis* is far more attractive to both male and female bees, compared to some other

small herbaceous angiosperms that also benefit from controlled burns, at least when *P. filiformis* blooms *en mass*.

Furthermore, the broad bee diversity visiting *Physaria filiformis* at the Rocky Barrens Conservation Area site remained consistent over three spring seasons at three levels. First, none of the bee species identified in this study were specialists on the flowers of the Brassicaceae (Krombein et al. 1979). In contrast, oligoleges of other unrelated plant taxa were attracted to *Physaria* flowers. We collected *Hoplitis simplex* Cresson and *Andrena lamelliterga* Ribble (males only), regarded as oligoleges of *Phacelia* (Boraginaceae) (Arduser 2002, Neff 2009). *Hylaeus sparsus* (single male), an oligolege of Apiaceae (Arduser 2002), was also collected in addition to several females of *Andrena nothoscordi* Robertson, which are primary oligoleges of *Nothoscordum* (Arduser 2002).

Second, the majority of bee species collected on *Physaria filiformis* were not restricted to glade communities with the exception of *Lasioglossum paradmirandum* and *L. sp. nova*. (Arduser 2002). All of the other bee species are either habitat generalists (occurring and nesting in a variety of habitat types, both disturbed and native, e.g., *Ceratina* spp.), more characteristic of forested areas (*Andrena nasonii* Robertson, *Andrena personata* Robertson), restricted to other habitat types (e.g., the two *Phacelia* oligoleges mentioned above are characteristic of mesic riparian corridors, not glades; riparian habitat occurred adjacent to the glades), occur exclusively on native prairies as well as glades (*Andrena nothoscordi*, *Halictus tripartitus* Cockerell). Some of these bees are clearly drawn to the glades from adjacent habitats when *P. filiformis* is in mass bloom. Consequently, *P. filiformis* at Rocky Barrens Conservation Area was never dependent on a narrow guild of glade endemics to effect cross-pollination even though some *L. paradmirandum* specimens were collected each season.

Third, the majority of native bee species visiting *Physaria filiformis* at Rocky Barrens are ground-nesting species, and are presumably sheltered from the direct effects of fire. However, at least ten bee species build their nests above ground in twigs, stems, dead/rotten wood, or other natural cavities (Table 2). Fire, at any time of the year, is more likely to have immediate negative effects on bee species nesting in combustible materials above ground exposed to fires (Arduser 2002). Woody stems

occurred on the margins of the glades, and some forb and grass stems occurred sporadically on the open glades. Numbers are too low to draw any conclusions other than the fact that many of the bee species present in 2006 were also present in 2009, persisting through several fire treatments. The small size of the burn units relative to the size of the preserve left extensive areas of unburned refugia, which may have served as source areas for recolonization of the burn units.

Only a small minority of the pollinator force servicing *Physaria filiformis* was consistently present in all three years (Table 2). However, these four species of bees (*Andrena nasonii*, *Augochlorella persimilis* Viereck, *Lasioglossum tegulare* Robertson and *L. paradmirationum*) comprised 39% of all individual bees collected, and appear to be the most dependable pollinators of Missouri bladderpod at Rocky Barrens. All four are ground-nesters, and at least the latter three of these species are known to nest on glades, not in adjacent forest or woodland habitats (Arduser 2002). We believe that the additional "boost" of pollinators drawn to the mass floral displays of *P. filiformis* from adjacent non-glade habitats is insurance that guarantees abundant pollen flow in this rare plant. Consequently, *P. filiformis* at Rocky Barrens Conservation Area is not exclusively dependent on a narrow guild of glade specialists to effect cross pollination. To maintain and maximize seed set in populations of this annual herb, we suggest that additional pollinator management strategies be incorporated into existing plans, providing that *P. filiformis* proves to be self-incompatible throughout its fragmented range. Specifically, plans should include the protection, management and restoration of natural communities adjacent to glades.

Literature Cited

- AL-SHEHBAZ, I. A. AND S. L. O'KANE. 2002. *Lesquerella* is united with *Physaria* (Brassicaceae). *Novon* 12: 319–329.
- ARDUSER, M. S. 2002. The bees of Missouri: A Preliminary report. Missouri Department of Conservation, Saint Louis, MO.
- ARIYOSHI, K., E. MAGNAGHI, AND M. FREY. 2006. Hand-pollination of *Cardamine californica* improves seed set. *Native Plants J.* 7: 248–252.
- BERNHARDT, P. 1990. Pollination ecology of *Oxalis violacea* (Oxalidaceae) following a controlled grass fire. *Plant Syst. Evol.* 171: 147–155.
- BERNHARDT, P. AND A. DAFNI. 2000. Breeding system and pollination biology of *Mandragora officinarum* L. (Solanaceae) in northern Israel. The Scandinavian Association for Pollination Ecology honours Knut Faegri. O. Totland, ed. Series 29. Det Norske Videnskaps-Akademi. Oslo 214–224.
- BURD, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Bot. Rev.* 60: 83–139.
- CLAERBOUT, A. E., J. M. COONS, H. R. OWEN, AND K. R. ROBERTSON. 2007. Floral biology of *Physaria ludoviciana* (Brassicaceae), a plant rare to the Midwest. *Castanea* 72: 130–137.
- COMMITTEE ON THE STATUS OF POLLINATORS IN NORTH AMERICA. 2007. Status of Pollinators in North America. National Research Council. The National Academies Press, Washington, DC. 322 p.
- CRUDEN, R. W., A. M. MCCLAIN, AND P. S. GOKARAN. 1996. Pollination biology and breeding system of *Alliaria petiolata* (Brassicaceae). *Bull. Torrey Bot. Club* 123: 273–280.
- DIERINGER, G. 1991. Pollination ecology of *Streptanthus bracteatus* (Brassicaceae): A rare central Texas endemic. *Southwestern Nat.* 36: 341–343.
- DIXON, K. W. 2009. Pollination and restoration. *Science* 325: 571–573.
- GRUNDT, H., R. ELVEN, AND C. BROACKMANN. 2005. A rare case of self-incompatibility in arctic plants: *Draba palanderiana* (Brassicaceae). *Flora* 200: 321–325.
- KAY, Q. O. N. 1976. Preferential pollination of yellow-flowered morphs of *Raphanus raphanistrum* by *Pieris* and *Eristalis* spp. *Nature* 261: 230–232.
- KIMATA, M. 1983. Comparative studies on the reproductive systems of *Cardamine flexuosa*, *C. impatiens*, *C. scutata* and *C. lyrata*, Cruciferae. *J. Plant Res.* 96: 299–312.
- KOTHEA, L., S. M. WARD, AND S. E. CARNEY. 2007. Assessing the threat from hybridization to the rare endemic *Physaria bellii* Mulligan (Brassicaceae). *Biol. Cons.* 140: 110–118.
- KROMBEIN, K. V., P. D. HURD, D. R. SMITH, AND B. D. BURKS. 1979. Catalog of Hymenoptera in America North of Mexico. Vol. 2. Smithsonian Institution Press, Washington, DC. 2209 p.
- LI, P., Y. LUO, P. BERNHARDT, Y. KUO, AND H. PERNER. 2008. Pollination of *Cypripedium plectrochilum* (Orchidaceae) by *Lasioglossum* spp. (Halictidae): the roles of generalist attractants versus restrictive floral architecture. *Plant Biol.* 10: 220–230.
- MAJETIC, C. J., R. A. RAGUSO, AND T. ASHMAN. 2009. The sweet smell of success: Floral scent affects pollinator attraction and seed fitness in *Hesperis matronalis*. *Funct. Ecol.* 23: 480–487.
- McKENZIE, P. M. 2003. 50 CFR Part 17. Endangered and threatened wildlife and plants: Reclassification of *Lesquerella filiformis* (Missouri bladderpod) from endangered to threatened. *Federal Register* 68(199): 59337–59345.
- MITCHELL, R. J. 1997. Effects of pollination intensity on *Lesquerella fendleri* seed set: variation among plants. *Oecologia* 109: 382–388.

- MITCHELL, R. J. AND D. L. MARSHALL. 1998. Nonrandom mating and sexual selection in a desert mustard: An experimental approach. *Am. J. Bot.* 85: 48–55.
- MOTTON, A. 1986. Pollination ecology of the spring wildflower community of a temperate deciduous forest. *Ecol. Monog.* 56: 21–42.
- MURPHY, S. D., I. SHERR, AND C. BULLOCK. 2009. Allelopathic pollen in Canadian invasive species: *Alliaria petiolata* and *Hesperis matronalis*. *Allelopathy J.* 23: 63–70.
- NEFF, J. L. 2009. The biology of *Hoplitis (Robertsonella) simplex* (Cresson), with a synopsis of the subgenus *Robertsonella* Titus. *J. Hymen. Res.* 18: 151–166.
- NELSON, P. M. 2005. The terrestrial natural communities of Missouri. The Missouri Natural Areas Committee, Jefferson City, MO. 500 p.
- ORNDUFF, R. 1969. Reproductive biology in relation to systematics. *Taxon* 18: 121–244.
- PROCTOR, M., P. YEO, AND A. LACK. 1996. The natural history of pollination. Timber Press Inc., Portland, OR. 487 p.
- R CORE DEVELOPMENT TEAM. 2010. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>
- RICHARDS, A. J. 1986. Plant breeding systems. George Allen and Unwin, London, UK. 544 p.
- SOKAL, R. R. AND F. J. ROHLF. 1995. Biometry: The principles and practice of statistics in biological research. W. H. Freeman and Co., New York, NY. 887 p.
- SOLBRIG, O. T. AND R. C. ROLLINS. 1977. The evolution of autogamy in species of the mustard genus *Leavenworthia*. *Evolution* 31: 265–281.
- STOUT, A. B. 1931. Pollen-tube behavior in *Brassica pekinensis* with reference to self-incompatibility in fertilization. *Am. J. Bot.* 18: 686–695.
- TAKAYAMA, S. AND A. ISOGAI. 2005. Self-incompatibility in plants. *Annu. Rev. Plant Biol.* 56: 467–489.
- VANCE, N. C., P. BERNHARDT, AND R. EDENS. 2004. Pollination and seed production in *Xerophyllum tenax* (Melanthiaceae) in the Cascade Range of Central Oregon. *Am. J. Bot.* 91: 2060–2068.
- WRIGHT, S. I. AND BARRETT, S. C. H. 2010. The long-term benefits of self-rejection. *Science* 330: 459–460.