Journal of Pollination Ecology, 16(14), 2015, pp 101-107

BREEDING SYSTEM OF *LINUM RIGIDUM* AND EFFECT OF HETEROSPECIFIC POLLEN FROM INTRODUCED *EUPHORBIA ESULA*

Benjamin R. Montgomery* and Caleb W. Phillips

Division of Natural Sciences and Engineering, University of South Carolina Upstate, Spartanburg, SC 29303, USA

Abstract—This study investigates the reproductive biology of, and effect of heterospecific pollen from introduced *Euphorbia esula* on, *Linum rigidum*, an annual plant native to western and central North America. Breeding-system studies revealed that *L. rigidum* is self-compatible, with similar pollination success, fruit set and seed set for flowers pollinated with self or outcrossed pollen. Untreated flowers not exposed to pollinators set seed, albeit at a lower rate than hand-pollinated flowers, indicating that *L. rigidum* can autonomously self pollinate. Experiments investigating whether heterospecific pollen transfer from *E. esula* interferes with pollination of *L. rigidum* indicated that large amounts of heterospecific pollen receipt 2 - 4 hours prior to conspecifc pollen receipt reduced fruit and seed production, but that small amounts of heterospecific pollen or larger amounts received immediately prior to conspecific pollen did not affect reproduction. Pollen of *E. esula* was observed to germinate on and penetrate into *L. rigidum* stigmas and styles. Nevertheless, *Euphorbia esula* is unlikely to interfere with *L. rigidum*'s reproduction because *L. rigidum* is self-compatible, capable of autonomous self-pollination, and unaffected by receipt of small amounts of *Euphorbia* pollen.

Keywords: Autogamy, breeding system, competition for pollination, interspecific pollen transfer, self-compatibility

INTRODUCTION

Plant reproduction may involve self-pollination (selfing), cross-pollination (outcrossing) or a combination of both (Barrett 2003), and multiple ecological and evolutionary factors influence which strategy is favoured (Goodwillie et al. 2005; Busch & Delph 2012). Outcrossing reduces inbreeding depression and increases the genetic diversity of offspring; however, selfing allows the plant to double its contribution of nuclear genes to its own seeds (Lloyd 1979). Additionally, selfing is associated with reproductive assurance, which may be favoured when receipt of outcrossed pollen is limited by a lack of mates or pollinator service (Darwin 1877; Lloyd 1979; Eckert 2000). Semelparous plant species, including annuals, are particularly sensitive to pollination failure because reproductive failure at one time cannot be compensated for by later reproductive bouts (Seed et al. 2006). Self-compatibility increases the chance of self-pollinated seed production, though a pollinator or autonomous mechanism of pollen delivery is necessary (Kalisz et al. 1999; Barrett 2003). Nonetheless, most angiosperm genera include self-incompatible species (Allen & Hiscock 2008), and self pollination cannot not provide reproductive assurance in these species.

Linum rigidum Pursh (Linaceae) is an annual (Rogers 1979) with anthesis in the morning and corolla abscission a few hours later. Flowers are visited by pollen-collecting solitary bees and syrphid flies (Montgomery & Rathcke 2012); nectar appears to be minimal or absent; and pollen is the primary floral reward. The *Linum* genus includes self-compatible homostylous and self-incompatible heterostylous species, with some members of the *Linopsis* clade, of which *L. rigidum* is most likely a part, reported to be homostylous (Armbruster et al. 2006; McDill et al. 2009). Dichogamy is not reported in *L. rigidum*, but self pollination may be limited by herkogamy (Mosquin & Hayley 1967). Flowers are generally upward facing, and the 5 anthers are inferior to and distal from the 5 stigma lobes, creating an average separation of more than 3 mm (Phillips & Montgomery, unpublished data). *Linum rigidum* has a non-restrictive floral morphology, indicative of a generalist pollination syndrome, receives visits from a variety of generalist pollinators, and receives a large quantity of heterospecific pollen from a variety of sources (Montgomery and Rathcke 2012).

Linum rigidum occurs in grasslands of central North America (Great Plains Association 1986) with its distribution in Iowa mostly restricted to dry prairies of the Loess Hills (Christiansen & Müller 1999). *Euphorbia esula* L. (leafy spurge), introduced from Eurasia, occurs commonly in the northern and eastern Great Plains (Watson 1985) and is frequent in the Loess Hills (Huerd & Taylor 1998). These species' flowering phenologies substantially overlap; both have yellow flowers and non-restrictive floral morphologies. As predicted by their floral similarity (Gibson et al. 2012), the composition of pollinators to each species substantially overlaps, with a variety of solitary bees comprising most visits to both species (Montgomery & Rathcke 2012). Consequently, *Euphorbia* could interfere with the pollination of *L. rigidum*.

One goal of this study is to evaluate the potential for *Euphorbia* to interfere with the pollination of *L. rigidum*. Harmful interactions between species mediated by shared

---

Received 27 December 2014, accepted 18 June 2015

*Corresponding author: bmontgomery@uscupstate.edu

pollination vectors (i.e. competition for pollination) can occur if pollinators visit one species in preference to another (Rathcke 1983), or if pollinators intersperse visits among species, in which case they may deposit less conspecific pollen on stigmas (e.g. Flanagan et al. 2009) and also deposit heterospecific pollen. Heterospecific pollen receipt reduces reproductive success in some systems by stigma clogging (Waser 1978; Galen & Gregory 1989; Brown & Mitchell 2001), allelopathy (Kanchan & Jayachandra 1980; Murphy 2000; Loughnan et al. 2014) or other mechanisms, yet some studies have detected no reduction in fecundity (Morales & Traveset 2008). In an area heavily invaded by *Euphorbia*, *L. rigidum* received an average of five *Euphorbia* pollen grains per flower (Montgomery & Rathcke 2012), with as many as 29 grains (Montgomery, unpublished data).

There is particular interest in whether introduced species interfere with pollination of native species for conservation reasons and because effects might be particularly strong if there has been insufficient time for evolution of traits that reduce interference (Traveset & Richardson 2006; Mitchell et al. 2009). Indeed, a meta-analysis found detrimental effects of aliens on pollinator visitation and fecundity of focal species, though aliens did not exert a stronger effect than native neighbours (Morales & Traveset 2009). Autonomously pollinated species may be less affected by reductions in visit quantity or quality than species more reliant on pollinators (Motten 1982; Fishman & Wyatt 1999). Petal abscission in *Linum rigidum* appears to cause self-pollination, and this mechanism could reduce the negative effects of competition for pollination with *Euphorbia* if *L. rigidum* is self-compatible.

To help evaluate the potential for introduced *Euphorbia* to interfere with the pollination of *L. rigidum*, this study investigates whether *L. rigidum* is self compatible, capable of autonomous seed production, and susceptible to reproductive interference from receipt of *Euphorbia* pollen. Breeding-system studies were performed on two varieties of *L. rigidum*, with an enclosed, unmanipulated treatment included in one study to test for autonomous pollination. Additionally, to assess whether *Euphorbia* pollen interferes with *L. rigidum*'s reproduction, heterospecific pollen transfer studies were performed. An initial study tested for effects of *Euphorbia* pollen received in large quantities and with a time delay prior to conspecific pollen; to test for effects under less severe conditions, follow-up studies were performed with varied timing or quantities of *Euphorbia* pollen receipt.

MATERIALS AND METHODS

Breeding system

Two breeding system studies were performed, the first in the field with a *L. rigidum var. rigidum* population in northwest Iowa, USA (Broken Kettle Grasslands Preserve, 42.697297N, 96.576875W) and the second with seeds of *L. rigidum var. berlandieri* in a growth room using seeds from a Texan native plant nursery (Native American Seed Company, Junction, Texas USA). The study of the Iowa population was conducted in May – June 2005 using plants enclosed with mesh bags supported by wire frames. The study included three pollination treatments: unmanipulated, cross-pollinated, and self-pollinated, with sample sizes of 20 to 21 flowers per treatment. In this and all subsequent studies, flowers were assigned to treatments randomly but subject to constraints such that all treatments were represented nearly equally on each plant. Up to one anther per flower from unmanipulated flowers and three anthers from self and cross-pollinated flowers were collected each day; other anthers were left intact, so self- and cross-pollinated flowers may have received some additional self pollen due to autogamy. Pollinations were performed by touching each stigma with an anther for self-pollinated flowers, or applying with a paintbrush a mixture of pollen from at least 3 donors to each stigma for cross-pollinated flowers. Fruits were collected at maturity and seeds were counted.

The growth-room breeding system study, initiated in January 2013, included self- and cross- pollination treatments. The study included 15 flowers per treatment performed on flowers of 7 plants, with a median of 6 flowers treated per plant (minimum = 1, maximum = 7), and treatments allocated as equally as possible among flowers on the same plant. For both treatments, all anthers were removed in the morning (09:00 – 10:00) and pollinations were performed by touching each stigma lobe with an anther. Fruit and seed set could be limited by either fertilization failure or lack of resources for seed maturation, so we considered the presence of germinated pollen and pollen tubes as well as fruit and seed set for the same set of flowers as response variables. The morning following pollinations, stigmas were excised and stored in 70% ethanol. To assay for pollen adherence and pollen-tube growth, styles were soaked in 6 M NaOH overnight, then soaked in a solution of 0.1% aniline blue and K_2HPO_4, after which styles were viewed under a fluorescence microscope. It was noted whether pollen was affixed to the stigma at this stage (all stigmas had pollen prior to soaking) and whether pollen tubes were present. Fruits were collected and seeds were counted from mature fruit. Seeds were stored at 7°C until January 2014, at which time seeds were soaked overnight in 1000 mg L^{-1} gibberellic acid, rinsed, then plated onto moistened filter paper in 5 cm diameter Petri dishes under a 12/12 hour light/dark cycle at 20°C. Germination was subsequently monitored over an 8-week period.

Effect of heterospecific pollen receipt on fruit and seed set

Field studies of effects of heterospecific pollen transfer from *Euphorbia* on the fruit and seed set of *L. rigidum* were conducted in 2005 and 2006. For the 2005 study, 24 *L. rigidum* plants were enclosed in mesh bags supported by wire frames. Newly opened flowers were assigned to one of three pollination treatments: enclosed and unmanipulated, cross-pollinated with conspecific pollen, or subjected to heterospecific pollen transfer (HPT) of *Euphorbia* pollen followed later by pollination with outcrossed *Linum* pollen. Between 62 and 65 flowers were assigned to each treatment, with a median of 9 flowers treated per plant (minimum = 4, maximum = 11). Pollinations were performed in the morning or early afternoon on rainless days from late May through early June. Flowers in the cross-pollination and HPT treatments were emasculated in the morning or early afternoon, and *Euphorbia* pollen was applied in the HPT treatment by touching all stigma lobes with anthers and gently spreading the pollen with forceps. Flowers in the cross-pollination treatment were similarly rubbed with forceps tips. Two to 4 hours later, flowers in the cross-pollination and HPT treatments were pollinated with a mixture of conspecific pollen from previously collected anthers, applied with a paintbrush. Fruit set was determined, and 27 – 43 fruits per treatment were dissected for seed counts.

In June 2006, two HPT studies were conducted in order to investigate whether effects of HPT were lessened if less *Euphorbia* pollen was received or if *Euphorbia* pollen was received with less time delay prior to conspecific pollen. Both studies included a cross-pollination (i.e. conspecific pollen only) and a heterospecific pollen transfer treatment with a large amount of *Euphorbia* pollen applied 2 – 4 hours prior to conspecific pollen, as in the 2005 study. The first experiment investigated the effects of quantity of heterospecific pollen by also including a third treatment of a smaller amount of *Euphorbia* pollen applied two to four hours prior to conspecific pollen. The transfer of a small amount of *Euphorbia* pollen (HPT-small) was accomplished by transferring *Euphorbia* pollen on the point of an insect pin. Conspecific pollen was applied to flowers in all treatments two to four hours later, in early afternoon. The HPT quantity study included 134 flowers from across 12 plants, with a median of 13 flowers used per plant (minimum = 1, maximum = 15), and a total of 43 – 47 flowers per treatment across all plants. Fruits were collected when mature, and seed counts were made for 17 – 20 fruits per treatment.

The second HPT study in 2006 aimed at investigating effects of timing of heterospecific pollen by including treatments of a large amount of *Euphorbia* pollen applied either 2 – 4 hours prior to conspecific pollen (HPT-hours-prior) or immediately prior to conspecific pollen (HPT-shortly-prior). Pollinations were timed such that all cross-pollinations were performed at the same time, regardless of when *Euphorbia* pollen was applied. The HPT timing study included 116 flowers from across 21 plants, with a median of 4 flowers treated per plant across all treatments (minimum = 1, maximum = 15) and a total of 37 – 41 flowers per treatment across all plants. Fruits were collected when mature, and seed counts were made for 18 – 27 fruits per treatment.

Euphorbia pollen could prevent *Linum* pollen from adhering to stigmas. In order to determine the quantities of *Euphorbia* and *Linum* pollen that adhered on stigmas, for both 2006 HPT studies, a subset of 11 – 15 stigmas per treatment was collected the day after pollination, by which time stigmas had senesced, and stored in 9:1 70% ethanol:glycerin for pollen counts. Stigmas were acetolyzed in microcentrifuge tubes with acetic anhydride and sulphuric acid, and the pollen was mounted in basic fuchsin jelly on slides for identification under compound microscopy (Kearns & Inouye 1993).

To investigate whether *Euphorbia* pollen could germinate and pollen tubes could elongate into *L. rigidum* stigmas, we observed a collection of 17 *L. rigidum* flowers under fluorescent microscopy that we had pollinated either with a large quantity of *Euphorbia* pollen only or with *Euphorbia* pollen followed 2 – 4 hours later with conspecific pollen. Stigmas were collected the day following pollination, and stored, softened and stained as described for pollen tubes in the growth-room breeding-system study. For each stigma, we noted whether *Euphorbia* pollen was observed to germinate and whether pollen tubes extended into the stigmatic surface.

Statistical analyses

For breeding system and HPT studies, the effect of the treatment variable on the dependent variable (fruit set or presence of pollen tubes) was tested with a generalised linear mixed effects model using GLMER with a binomial error distribution and including plant identity as a random effect. For many flowers in the field breeding-system and HPT studies, most ovules or all 10 ovules developed into seeds, such that seed set was not normally distributed even after a variety of transformations. Germination of all seeds in several fruit similarly resulted in a non-normal distribution. For these studies, seed set or seed germination was analyzed with a GLMER with binomial error distribution with the dependent variable being the number of ovules that set seed out of ten or the number of seeds that germinated out of the total seeds for that fruit, including plant identity as a random effect. For GLMERs with three treatments, statistical differences among treatments were determined by non-overlap of standard errors around regression coefficients. For the growth-room breeding system study, seed set was lower and normally distributed; consequently, seed set was analyzed with a LME, including treatment as a fixed effect and plant identity as a random effect. Residuals were inspected for normality and homogeneity of variance. Tukey's HSD test was used to determine the significance of differences between individual treatments. This same approach was used to analyze conspecific pollen receipt in the HPT studies.

RESULTS

Breeding system

In the field breeding-system study, fruit set varied significantly among treatments (X^2 = 6.904, *d.f.* = 2, P = 0.031), with similar fruit set for self pollination and cross pollination and significantly lower fruit set for unmanipulated flowers (Tab. 1). Seed set did not differ among treatments (X^2 = 4.936, *d.f.* = 2, P = 0.085), although there was a trend for lower seed set for unmanipulated flowers (Tab. 1). In the growth-room study, pollen still adhered to most stigmas after soaking in sodium hydroxide, and the proportion of flowers with pollen tubes did not significantly differ between treatments (X^2 = 0.238, *d.f.* = 1, P = 0.625; Tab. 1). Fruit set was the same in both treatments, and seed set did not significantly differ among treatments ($F_{1,19}$ = 0.189, P = 0.67; Tab. 1). Germination

TABLE 1. (A) Fruit and seed set (mean ± 1 SE) per fruit for enclosed flowers that were cross or self pollinated, or left enclosed and unmanipulated for the field breeding-system study, and (B) the proportion of flowers with pollen adhering to stigmas after soaking in sodium hydroxide (pollen adherence), the proportion of flowers with pollen tubes present, fruit set, and seed set for flowers that were cross or self pollinated in the growth-room study. For fruit set in the field study, different letters indicate a significant difference between treatments; other variables did not significantly differ among treatments. Rightmost column indicates the sample size or range of sample sizes per treatment.

Treatment	Cross pollination	Self pollination	Enclosed	Sample size
(A) Field study				
Fruit set	0.762 *a*	0.714 *a*	0.450 *b*	20 - 21
Seed set	9.1 ± 0.7	9.2 ± 0.6	7.9 ± 1.0	9 - 14
(B) Growth-room study				
Pollen adherence	0.938	0.938	--	16
Pollen tube presence	0.813	0.875	--	16
Fruit set	0.813	0.813	--	16
Seed set	5.77 ± 0.82	6.38 ± 0.78	--	13
Germination	0.760 ± 0.085	0.767 ± 0.108	--	10-13

rates were similar and not significantly different X^2 = 0.6956, *d.f.* = 1, *P* = 0.403; Tab. 1)

Effect of heterospecific pollen

For the 2005 HPT study, there was a significant effect of treatment ($F_{2,46}$ = 4.459, *P* = 0.017), with lower fruit set in the HPT and unmanipulated treatments compared to the cross-pollination treatment (Fig. 1). Seed set per fruit was also significantly affected by treatment (X^2 = 33.2, *d.f.* = 2, *P* < 0.001), with significantly lower seed set for flowers receiving *Euphorbia* pollen than cross-pollinated flowers and intermediate seed set for unmanipulated flowers (Fig. 1).

For the HPT quantity study, *Euphorbia* pollen receipt was near zero for flowers in the cross-pollination treatment (indicating a low level of contamination in the field or during analysis), intermediate for flowers in the HPT-small treatment and highest in the HPT-large treatment (Tab. 2). Conspecific pollen on stigmas tended to decrease with larger quantities of *Euphorbia* pollen (Tab. 2), but differences were only marginally significant ($F_{2,32}$ = 2.968, *P* = 0.066). For the HPT timing study, *Euphorbia* pollen receipt was similar whether it was added immediately prior or a few hours prior to conspecific pollen (Tab. 2). However, conspecific pollen receipt varied significantly with treatment ($F_{2,20}$ = 10.80, *P* < 0.001), with reduced conspecific pollen retention when *Euphorbia* pollen was applied 2 – 4 hours prior to conspecific pollen compared to the cross-pollination treatment and treatment with *Euphorbia* pollen applied immediately prior to conspecific pollen (HPT immediate). The cross-pollination and HPT immediate treatments did not differ significantly from each other in conspecific pollen detected on stigmas (Tab. 2).

In the HPT quantity study, fruit set did not vary significantly with treatment (X^2 = 0.585, *d.f.* = 2, *P* = 0.75, Fig. 1B). There was, however, significantly lower seed set for the HPT-large treatment than the cross-pollination and HPT-small treatments (X^2 = 34.21, *d.f.* = 2, *P* < 0.001, Fig. 1B). For the HPT timing study, there was not a statistically significant effect of treatment on fruit set (X^2 = 2.468, *d.f.* = 2, *P* = 0.29, Fig. 1C), but seed set was significantly lower for the HPT-hours-prior treatment relative to the cross-pollination treatment, and intermediate for the HPT-shortly-prior treatment (X^2 = 36.0, *d.f.* = 2, *P* < 0.001, Fig. 1B).

Euphorbia pollen was observed to germinate and extend pollen tubes into stigmas and occasionally upper portions of the style in all 17 flowers that were pollinated with *Euphorbia* pollen and examined under a fluorescence microscope.

DISCUSSION

Populations of both varieties of *L. rigidum* were self-compatible, with similar reproductive success for self- and cross-pollinated flowers; seeds resulting from self and cross pollination had similar germination success. Self compatibility in *L. rigidum* is consistent with most, but not all, members of the *Linopsis* section of *Linum* (Rogers 1982; McDill et al. 2009). *Linum rigidum* is an annual in grasslands dominated by perennials. In this context, self-compatibility may be advantageous if new populations are established by one or a few individuals following disturbances. Autogamy occurred in the enclosed, unmanipulated treatments in both the breeding system study and the 2005 HPT study, although fruit set was reduced compared to flowers hand pollinated with conspecific pollen in both cases, and seed set was significantly reduced compared to cross-pollinated flowers in the 2005 HPT study. This finding suggests that *L. rigidum* benefits from but is not completely reliant on pollinator visits for reproduction. Herkogamy probably prevents direct transfer of pollen from anthers to stigma lobes, and observations suggest that petal abscission may result in anthers being dragged across stigma lobes, allowing self pollination to occur (B. Montgomery, pers. obs.).

Linum rigidum overlaps in pollinators and receives pollen from introduced *Euphorbia* where the two species co-

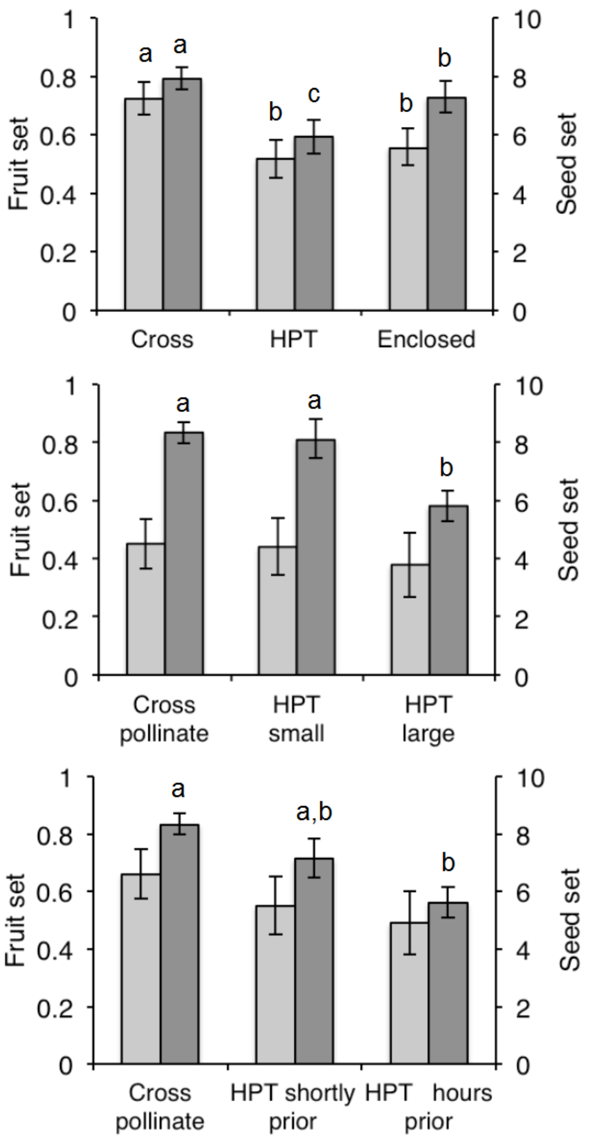

FIGURE 1. Mean (± SE) fruit set (light bars) and seed set (dark bars) for *Linum rigidum* flowers in field enclosures that were exposed to various pollination treatments. (A) 2005 study in which flowers were cross pollinated (Cross), exposed to heterospecific pollen transfer from *Euphorbia esula* then cross pollinated (HPT), or left without manipulation (Enclosed); (B) 2006 study in which flowers were cross pollinated (Cross pollinate), or pollinated with a small (HPT small) or large (HPT large) quantity of *E. esula* pollen, then cross pollinated 2 – 4 hours later; (C) 2006 study in which flowers were cross pollinated, pollinated with a large quantity of *E. esula* pollen then cross pollinated soon thereafter (HPT-shortly-prior), or cross pollinated 2 – 4 hours later (HPT-hours-prior). Different letters above bars indicate a significant difference among treatments.

occur (Montgomery and Rathcke 2012), which suggests that *Euphorbia* could interfere with the pollination of *L. rigidum*. However, heterospecific pollen transfer studies suggest that this pollen is unlikely to substantially reduce fruit or seed set under typical pollination conditions. Receipt of large quantities of *Euphorbia* pollen resulted in a weak, marginally significant trend for less conspecific pollen to adhere to stigmas, but even in the treatment with the most *Euphorbia* pollen applied, conspecific pollen receipt was on average more than 4-fold greater than the number of ovules per flower. Moreover, reductions in fruit and seed set were only significant when *Euphorbia* pollen was received in quantities substantially larger than the average (5 *Euphorbia* pollen grains) that Montgomery and Rathcke (2012) detected on *L. rigidum* stigmas and when there was a time delay prior to conspecific pollen receipt; removing the time delay or reducing the amount of *Euphorbia* pollen applied negated the deleterious effects. The amounts of *Euphorbia* pollen applied in this study were higher than amounts typically found on *L. rigidum* stigmas, but the quantity detected for the HPT-small treatment overlapped with amounts detected on open-pollinated stigmas. Other studies that have investigated effects of heterospecific pollen have also typically included larger quantities of heterospecific pollen than occur naturally (Morales & Traveset 2008). Studies of other systems have also found that a time delay after application of heterospecific is required for the pollen to reduce fecundity (Waser & Fugate 1986; Caruso & Alfaro 2000) and that effects may be dosage dependent (Thomson et al. 1982; Murphy & Aarssen 1995).

In its introduced range, pollen from *Euphorbia* has been found on a variety of species with a range of floral morphologies (Larson et al. 2006; Montgomery & Rathcke 2012), but there is limited evidence that this receipt substantially affects reproduction of other species. In this study, an effect of *Euphorbia* on *L. rigidum* fruit and seed set was only detected under the most severe conditions tested (with large quantities of *Euphorbia* pollen and a time delay prior to receipt of conspecific pollen). Occlusion of the stigmatic surface by *Euphorbia* pollen may have contributed to the observed reproductive interference, but the requirement of a time delay for interference suggests this is not the only mechanism at play. The finding that *Euphorbia* pollen germinates on and penetrates into *L. rigidum* stigmas means it may have changed conditions on the stigma. Fewer conspecific pollen grains adhered to stigmas when *Euphorbia* pollen was applied with a time delay prior to conspecific pollination, suggesting that the conspecific pollen was less able to anchor, possibly due to lower germination rates or failure of pollen tubes to penetrate the stigma. Other studies detected no effect of *Euphorbia* pollen on *Sisyrinchium campestre* even under similarly severe conditions (Montgomery 2009b), and effects of *Euphorbia* pollen on *Viola pedatifida* only when heterospecific pollen was applied prior to conspecific pollen (Montgomery 2009a). More generally, our results are in-keeping with the findings of a review that found pollen from alien species is not typically transferred in large enough quantities to heterospecific stigmas to result in stigma clogging or saturation of the stigmatic surface (Morales & Traveset 2008).

Perhaps as a result of its unrestrictive morphology, *L. rigidum* receives relatively large quantities of heterospecific pollen; consequently, heterospecific pollen may have little effect because selection would favour minimization of effects in species prone to heterospecific pollen receipt (Cruden & Millerward 1981; Kohn & Waser 1985; Jakobsson et al. 2008). Overall, this study indicates that *L. rigidum* is self-compatible, able to pollinate autonomously to some degree and capable of withstanding receipt of moderate amounts of

TABLE 2. Quantities (± 1 SE) of *Euphorbia* and conspecific pollen found on *L. rigidum* stigmas in two heterospecific pollen transfer (HPT) studies: (A) HPT-quantity study, including flowers that were cross pollinated (Cross pollination), or pollinated with a small (HPT-small) or large (HPT-large) quantity of *E. esula* pollen, then cross pollinated 2 – 4 hours later; and (B) HPT-timing study, including flowers that were cross pollinated (Cross pollination), pollinated with a large quantity of *E. esula* pollen then cross pollinated soon thereafter (HPT-shortly-prior), or cross pollinated 2 – 4 hours later (HPT-hours-prior). Different superscripts indicate a significant difference in conspecific pollen receipt within each study.

	N	*Euphorbia* pollen	Conspecific pollen
(A) HPT Quantity study			
Cross pollination	15	2.2 ± 0.62	64.3^a ± 6.67
HPT-small	15	47.1 ± 12.60	59.9^a ± 6.94
HPT-large	15	338.1 ± 28.38	44.9^a ± 6.65
(B) HPT Timing study			
Cross pollination	11	3.1 ± 0.82	61.2^a ± 7.62
HPT-shortly-prior	13	259.8 ± 23.18	71.0^a ± 8.11
HPT-hours-prior	13	272.4 ± 26.10	29.5^b ± 8.52

heterospecific pollen without a significant decrease in fruit or seed set. These traits of *L. rigidum* should reduce effects of interference competition via heterospecific pollen transfer from *Euphorbia*. Future studies should investigate whether the species compete via other pollinator-mediated mechanisms, including competition for pollinator visits and loss of conspecific pollen due to pollinator visitation of intervening heterospecific plants.

ACKNOWLEDGEMENTS

Comments of two reviewers substantially improved this manuscript. We thank the Explorers Club Exploration Fund, and the University of South Carolina Magellan Scholars program and for research funding, and the Nature Conservancy Iowa Chapter for access to Broken Kettle Grasslands Preserve.

REFERENCES

Allen AM, Hiscock SJ (2008) Evolution and phylogeny of self-incompatibility systems in angiosperms. In: Franklin-Tong VE (ed) Evolution and Phylogeny of Self-Incompatibility Systems in Angiosperms. Springer Berlin Heidelberg, pp. 73-101.

Armbruster WS, Perez-Barrales R, Arroyo J, Edwards ME, Vargas P (2006) Three-dimensional reciprocity of floral morphs in wild flax (*Linum suffruticosum*): a new twist on heterostyly. New Phytologist 171:581-590. doi: 10.1111/j.1469-8137.2006.01749.x

Association GPF (1986) Flora of the Great Plains. University Press of Kansas, Lawrence, Kansas.

Barrett SCH (2003) Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 358:991-1004. doi: 10.1098/rstb.2003.1301

Brown BJ, Mitchell RJ (2001) Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. Oecologia 129:43-49.

Busch JW, Delph LF (2012) The relative importance of reproductive assurance and automatic selection as hypotheses for the evolution of self-fertilization. Annals of Botany 109:553-562. doi: 10.1093/aob/mcr219

Caruso CM, Alfaro M (2000) Interspecific pollen transfer as a mechanism of competition: effect of *Castilleja linariaefolia* pollen on seed set of *Ipomopsis aggregata*. Canadian Journal of Botany 78:600-606.

Christiansen P, Müller M (1999) An Illustrated Guide to Iowa Prairie Plants. Iowa City Press, Iowa City.

Cruden RW, Millerward S (1981) Pollen-ovule ratio, pollen size, and the ratio of stigmatic area to the pollen-bearing area of the pollinator - an hypothesis. Evolution 35:964-974.

Darwin C (1877) The different forms of flowers on plants of the same species. Murray Press, London, United Kingdom.

Eckert CG (2000) Contributions of autogamy and geitonogamy to self-fertilization in a mass-flowering, clonal plant. Ecology 81:532-542. doi: 10.1890/0012-9658(2000)081[0532:coaagt]2.0.co;2

Fishman L, Wyatt R (1999) Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). Evolution 53:1723-1733. doi: 10.2307/2640435

Flanagan RJ, Mitchell RJ, Knutowski D, Karron JD (2009) Interspecific pollinator movements reduce pollen deposition and seed production in *Mimulus ringens* (Phrymaceae). American Journal of Botany 96:809-815. doi: 10.3732/ajb.0800317

Galen C, Gregory T (1989) Interspecific pollen transfer as a mechanism of competition: consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. Oecologia 81:120-123.

Gibson MR, Richardson DM, Pauw A (2012) Can floral traits predict an invasive plant's impact on native plant–pollinator communities? Journal of Ecology, 100: 1216-1223.

Goodwillie C, Kalisz S, Eckert CG (2005) The evolutionary enigma of mixed mating systems in plants: Occurrence, theoretical explanations, and empirical evidence. Annual Review of Ecology Evolution and Systematics 36:47-79. doi: 10.1146/annurev.ecolsys.36.091704.175539

Huerd SC, Taylor SE (1998) Leafy spurge (*Euphorbia esula* L.) spread in Iowa. Journal of the Iowa Academy of Sciences 105:27-29.

Jakobsson A, Padron B, Traveset A (2008) Pollen transfer from invasive *Carpobrotus* spp. to natives - A study of pollinator behaviour and reproduction success. Biological Conservation 141: 136-145.

Kalisz S et al. (1999) The mechanism of delayed selfing in *Collinsia verna* (Scrophulariaceae). American Journal of Botany 86:1239-1247. doi: 10.2307/2656771

Kanchan S, Jayachandra (1980) Pollen allelopathy - a new phenomenon. New Phytologist 84:739-746.

Kearns CA, Inouye DW (1993) Techniques for Pollination Biologists. University Press of Colorado, Niwot, Colorado.

Kohn JR, Waser NM (1985) The effect of *Delphinium nelsonii* pollen on seed set in *Ipomopsis aggregata*, a competitor for hummingbird pollination. American Journal of Botany 72:1144-1148.

Larson DL, Royer RA, Royer MR (2006) Insect visitation and pollen deposition in an invaded prairie plant community. Biological Conservation 130:148-159.

Lloyd DG (1979) Some reproductive factors affecting the selection of self-fertilization in plants. American Naturalist 113:67-79. doi: 10.1086/283365

Loughnan D, Thomson JD, Ogilvie JE, Gilbert B (2014) *Taraxacum officinale* pollen depresses seed set of montane wildflowers through pollen allelopathy. Journal of Pollination Ecology 13:146-150.

McDill J, Repplinger M, Simpson BB, Kadereit JW (2009) The phylogeny of *Linum* and Linaceae subfamily Linoideae, with implications for their systematics, biogeography, and evolution of heterostyly. Systematic Botany 34:386-405.

Mitchell RJ, Flanagan RJ, Brown BJ, Waser NM, Karron JD (2009) New frontiers in competition for pollination. Annals of Botany 103:1403-1413. doi: 10.1093/aob/mcp062.

Montgomery BR (2009a) Effect of introduced *Euphorbia esula* on the pollination of *Viola pedatifida*. Botany-Botanique 87:283-292. doi: 10.1139/b08-139.

Montgomery BR (2009b) Pollination of *Sisyrinchium campestre* (Iridaceae) in prairies invaded by the introduced plant *Euphorbia esula* (Euphorbiaceae). American Midland Naturalist 162:239-252.

Montgomery BR, Rathcke BJ (2012) Effects of floral restrictiveness and stigma size on heterospecific pollen receipt in a prairie community. Oecologia 168:449-458. doi: 10.1007/s00442-011-2094-x.

Morales CL, Traveset A (2008) Interspecific pollen transfer: Magnitude, prevalence and consequences for plant fitness. Critical Reviews in Plant Sciences 27:221-238. doi: 10.1080/07352680802205631.

Morales CL, Traveset A (2009) A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. Ecology Letters 12:716-728. doi: 10.1111/j.1461-0248.2009.01319.x.

Mosquin T, Hayley DE (1967) Evolutionary relationships of the southern Florida populations of *Linum rigidum* (Linaceae). Canadian Journal of Botany 45:1277-1283.

Motten AF (1982) Autogamy and competition for pollinators in *Hepatica americana* (Ranunculaceae). American Journal of Botany 69:1296-1305.

Murphy SD (2000) Field testing for pollen allelopathy - a review. Journal of Chemical Ecology 26:2155-2172.

Murphy SD, Aarssen LW (1995) In-vitro allelopathic effects of pollen from 3 *Hieracium* species (Asteraceae) and pollen transfer to sympatric fabaceae. American Journal of Botany 82:37-45.

Rathcke B (1983) Competition and facilitation among plants for pollination. In: Real L (ed) Pollination Biology. Academic Press, Inc., Orlando, pp 305-329.

Rogers CM (1979) Distyly and pollen dimorphism in *Linum suffruticosum* (Linaceae). Plant Systematics and Evolution 131:127-132. doi: 10.1007/bf00984126.

Rogers CM (1982) The Systematics of *Linum* sect. *Linopsis* (Linac*eae).* Plant Systematics and Evolution 140:225-234.

Seed L, Vaughton G, Ramsey M (2006) Delayed autonomous selfing and inbreeding depression in the Australian annual *Hibiscus trionum* var. *vesicarius* (Malvaceae). Australian Journal of Botany 54:27-34. doi: 10.1071/bt05017.

Thomson JD, Andrews BJ, Plowright RC (1982) The effect of a foreign pollen on ovule development in *Diervilla lonicera* (Caprifoliaceae). New Phytologist 90:777-783. doi: 10.1111/j.1469-8137.1982.tb03286.x.

Traveset A & Richardson DM (2006) Biological invasions as disruptors of plant reproductive mutualisms. Trends in Ecology and Evolution 21: 208-216.

Waser NM (1978) Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. Ecology 59:934-944.

Waser NM, Fugate ML (1986) Pollen precedence and stigma closure - a mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. Oecologia 70:573-577.

Watson AK (ed) (1985) Leafy Spurge. Weed Science Society of America, Champaign, IL.