RESEARCH ARTICLE

Pollination of a native plant changes with distance and density of invasive plants in a simulated biological invasion[1]

Daniela Bruckman and Diane R. Campbell[2]

PREMISE OF THE STUDY: Effects of an exotic plant on pollination may change as the invasive increases in density. Quantity of pollinator visits to a native may increase, decrease, or change nonlinearly, while visit quality is likely to decrease with greater interspecific pollen movement. How visit quantity and quality contribute to the effect on reproductive success at each invasion stage has not been measured.

METHODS: We simulated four stages of invasion by *Brassica nigra* by manipulating the neighborhood of potted plants of the native *Phacelia parryi* in a field experiment. Stages were far from the invasion, near the invasion, intermixed with the invasive at low density, and intermixed at high density. We measured pollinator visitation, conspecific and invasive pollen deposition, and seed set for *P. parryi* at each stage.

KEY RESULTS: Native individuals near invasive plants and within areas of low invasive density showed greatest seed production, as expected from concurrent changes in conspecific and invasive pollen deposition. Those plants experienced facilitation of visits and received more conspecific pollen relative to plants farther from invasives. Native individuals within high invasive density also received frequent visits by many pollinators (although not honeybees), but the larger receipt of invasive pollen predicted interference with pollen tubes that matched patterns in seed set.

CONCLUSIONS: Pollinator visitation was highest when exotic plants were nearby. Detrimental effects of heterospecific pollen deposition were highest at high exotic density. Our study quantified how reproduction benefits from near proximity to a showy invasive, but is still vulnerable when the invasive reaches high density.

KEY WORDS Boraginaceae; *Brassica nigra*; Brassicaceae; density; heterospecific pollen; invasion ecology; *Phacelia parryi*; pollinator visitation; reproductive ecology; seed production

Exotic plants may inhibit the growth of native species not only by competing for physical resources (Levine et al., 2003; Gioria and Osborne, 2014), but also by impacting pollination services (Traveset and Richardson, 2006; Bjerknes et al., 2007). Pollination may be impacted via two important mechanisms that were first studied in interactions between native plant species (e.g., Waser, 1978; Campbell and Motten, 1985). First, exotic plants may influence the quantity of pollinator visits native plants receive (e.g., Chittka and Schurkens, 2001; Brown et al., 2002; Totland et al., 2006). Second, as invasive species become more abundant, they may interfere with pollinator visit quality by increasing interspecific pollen transfer, resulting in

increased amounts of heterospecific pollen placement on native stigmas and loss of conspecific pollen to exotics (Lopezaraiza-Mikel et al., 2007; Morales and Traveset, 2008).

These impacts may change as invasion into a native ecosystem proceeds (Melbourne et al., 2007). During the initial period of exotic plant establishment, density is usually low and may have little influence on pollinator behavior and therefore reproductive success of native plants. However, as invasive density increases, pollinator-mediated interactions between natives and exotics may intensify. Pollinator visit quantity may simply decrease as a linear function of invasive plant density if competition gradually becomes more intense (e.g., Muñoz and Cavieres, 2008; Flanagan et al., 2010). Alternatively, invasive effects on pollinator visits may shift between facilitative and competitive when exotic plants act first as "magnet species" (Thomson, 1978) at low to moderate densities (Moeller, 2004; Molina-Montenegro et al., 2008), but eventually outcompete natives for pollinator attention once their abundance

becomes high (Mitchell et al., 2009), as originally suggested for native plants by Rathcke (1983). On the other hand, heterospecific pollen deposition, an aspect of the quality of a pollinator visit, is expected to increase monotonically with increasing invasive density (Morales and Traveset, 2008).

Distance of a native plant to the nearest exotic plant may also influence pollination. Since the mobility of animal pollinators allows plants to interact even when species are not completely intermixed, native plant pollination may be influenced by invasive species that have not yet infiltrated a plant community. Native plants near an invasion may experience greater fitness reductions than distant individuals if competition is strong, and similarly, facilitative interactions may be more pronounced near the leading edge of an invasion if interactions between species are positive. Moreover, interactions between species may involve competition and facilitation simultaneously, depending on spatial scale. For example, showy competitors could facilitate visitation by attracting pollinators from outside the floral neighborhood, yet might compete on a local scale via mechanisms like interspecific pollen transfer (Morales and Traveset, 2008; Seifan et al., 2014).

Recent studies have demonstrated that competition among sympatric species for pollination can be influenced by relative plant abundance (e.g., Caruso, 2002; Ghazoul, 2006; Takakura et al., 2009; Seifan et al., 2014), including that of invasive plants (Muñoz and Cavieres, 2008; Flanagan et al., 2010; Dietzsch et al., 2011; King and Sargent, 2012; Sun et al., 2013). Very few of these studies, however, examined how both density and distance to an invasive plant affects seed set as a measure of reproductive fitness (Cariveau and Norton, 2009). Importantly, none of those previous studies examined how invasive pollen receipt on stigmas was influenced by invasive plant density, leaving it unclear how the two mechanisms of changes in quantity and quality of pollinator visits contribute to effects on seed set. Quantifying interspecific pollen transfer between exotic and native plants is essential for identifying mechanisms of interaction since reductions in visit quality are possible even when the frequency of pollinator visits is not affected (Waser, 1983; Larson et al., 2006; Lopezaraiza-Mikel et al., 2007) and because heterospecific pollen loads can be high in nature (Arceo-Gómez et al., 2016).

In this study, we experimentally tested how variation in density and spatial position of the invasive species *Brassica nigra* (Brassicaceae) influences the reproductive success of the sympatric native, *Phacelia parryi* (Boraginaceae), and quantified the role of changes in pollinator visitation vs. heterospecific pollen receipt. By manipulating the density of *B. nigra* flowers around potted *P. parryi* individuals in a field setting, we simulated four stages in invasion: native plants far from the invasion, native plants near the invasion, the invasive species intermixed with the native species at low density, and the invasive species intermixed with the native species at high density. We asked the following questions: (1) How do distance to and density of an invasive influence pollinator visitation to a native species? (2) How do distance to and density of an invasive influence the extent of conspecific and invasive pollen deposition on native stigmas? (3) What are the predicted effects of those changes in pollen deposition on pollen tubes and relative success at reproduction, based on previous studies of how mixed pollen loads affect pollen tube growth (Bruckman and Campbell, 2016)? (4) Does seed set change with invasion stage in the pattern predicted by alterations in visitation and pollen loads?

MATERIALS AND METHODS

Study species—*Phacelia parryi* Torr. (Boraginaceae) is an annual herb, native to coastal sage scrub and chaparral ecosystems throughout southern and Baja California, and is especially common on open, recently burned slopes. Flowers are self-compatible but exhibit signs of inbreeding depression when self-pollinated, suggesting that this species relies on pollinators for transfer of outcross pollen (Bruckman and Campbell, 2014). Although many species of *Phacelia* have a low ovule number, *P. parryi* commonly produces 40–90 seeds (Jepson Flora Project, 2013). In Orange County, California, *P. parryi* flowers from late February to early May. The inflorescence is a helicoid cyme of bell-shaped flowers each 1–2 cm long. The showy violet to royal blue flowers last 3–4 d. Daily floral display size can vary from just a few individual flowers to several dozen.

Black mustard [*Brassica nigra* (L.) W.D.J.Koch] was used in this study as a model invasive species and is often found growing near *P. parryi*. It is an annual weed of Mediterranean origin that occurs commonly in disturbed areas throughout North America (Westman et al., 1999). Black mustard plants have numerous terminal inflorescences of yellow, hermaphroditic, self-incompatible flowers that attract a variety of insect pollinators (Conner and Neumeier, 1995). In the invasive range of *B. nigra*, stands are often large and dense and secrete allelochemicals that inhibit germination of neighboring plant species (Bell and Muller, 1973; Aicher et al., 2011). It is a winter annual forb and flowers primarily between the months of February and May in southern California, overlapping substantially with the phenology of *P. parryi*. Although individual *B. nigra* flowers are small, the floral display of a single plant may contain several hundred flowers. The two plant species overlap extensively in floral visitors, with most visits by solitary bees, bumblebees, syrphid flies, or introduced honeybees (Bruckman and Campbell, 2016).

Experimental design—Potted *P. parryi* plants were used in field experiments at the University of California Irvine Arboretum (33°39′49″N, 117°51′10″W) to determine the effects of *B. nigra* on native pollination. In spring of 2013, a 38 × 25 m area of bare ground was hand-seeded with *B. nigra*. It was then manually thinned to create patches that represented four interspersed invasive treatments (hereafter "high" or "low" density, "near" or "far" patches), each replicated four to five times (Appendix S1, see Supplemental Data with the online version of this article). Between one and three potted *P. parryi* plants were placed within each patch, separated by 3 m, with 36 potted plants in total in 2013 (Appendix S1). Plants were separated by 3 m to ensure that insect visitors treated them as separate individuals (online Appendix S2). With this small number of target plants, our design simulated invasions into small natural populations only, such as those often observed for *P. parryi* (Bruckman and Campbell, 2014), and may not apply to native species in large or dense stands. The design also assumed that pollinators would respond to small-scale manipulations, which was justified by previous responses to floral neighborhoods of 4 m² (Bruckman and Campbell, 2014). Low-density patches contained between 50 and 500 *B. nigra* flowers/m², while high-density patches had more *B. nigra* plants and flowers, specifically more than 1000 flowers/m² (Appendix S1). In both of those treatments, *P. parryi* pots were placed so that the appropriate *B. nigra* density surrounded them by at least 0.5 m on every side (Appendix S2). Although this procedure lessened edge effects, it did not eliminate them because insects may respond to flowers farther away. Near

patches contained no *B. nigra* plants within the patch but with the potted *P. parryi* within 3 m of a high patch (invasive at high density), and far patches also contained no *B. nigra* in the patch with the *P. parryi* pots ranging from 5 to 7 m from the invasive at high density. The purpose of the latter two treatments was to investigate whether distance from invasive patches influenced reproductive success in *P. parryi*. The number of open *P. parryi* flowers on an individual experimental plant ranged from 30 to 112, which was comparable to floral displays in natural patches studied by Bruckman and Campbell (2014). *Brassica nigra* densities were maintained by thinning and removing plants as needed throughout the study. We attempted to keep patch area as constant as possible within the requirements of maintaining *B. nigra* density, but area varied some due to patchiness in *B. nigra* germination and changes in *B. nigra* flower density over the season. Most patches were between 10 to 15 m^2, with extremes of 5 m^2 and 25 m^2, but the variation was random and not associated with treatment. Flowering plants other than *B. nigra* were weeded out of field plots to control for effects that other species may have on pollinator activity.

The experiment was repeated in spring of 2014 with 29 potted *P. parryi* plants. Twenty patches with four to six of each treatment were established in the same manner as described above. Due to severe drought conditions in 2014, the study site was irrigated to maintain flowering populations of *B. nigra* for the duration of our experiment. The addition of water in this year increased average *B. nigra* floral display size in high-density patches from 1250 flowers/m^2 (SE = 106) in 2013 to 1675 flowers/m^2 (SE = 318) in 2014. Invasive densities in the low-density treatment remained similar between years. For the 2 years combined, treatments did not vary in the average number of *P. parryi* plants per patch (one-way ANOVA, $F_{3, 34} = 1.13$, $P = 0.350$) or average open *P. parryi* flowers during observations ($F_{3, 34} = 0.56$, $P = 0.648$). So treatment effects were not confounded with conspecific density.

Pollinator visitation—To determine the effects on quantity of pollinator visits (question 1), pollinator observations were conducted between 8:00 and 15:00 hours on 12 d between 3 April and 1 May in 2013 and on 8 days between 8 April and 22 April in 2014. We did not conduct observations if it was raining or if temperatures were below 12°C. During each observation period, one individual *P. parryi* plant from each of the four invasion treatments was observed simultaneously by a different person to control for temporal variation in pollinator visitation. Each observation period lasted 30 min, and we defined a pollination event as any contact of an insect with anthers or stigmas on an individual *P. parryi* flower. Previous studies have shown that honeybees, bumblebees, and solitary bees are all effective pollinators of *P. parryi* (Bruckman and Campbell, 2014). We recorded the insect visitor to the lowest field-identifiable taxonomic category. At the end of the 30 min, we moved on to the next four *P. parryi* individuals, chosen at random, and the process was repeated. Each individual *P. parryi* plant was observed between 1 and 5 times for the duration of the study, depending on how long each plant remained in flower. We recorded 219 individual insect foraging bouts (775 flower visits) to 36 *P. parryi* plants over 128 half-hour observations periods in 2013 and 381 individual insect foraging bouts (2347 visits) to 29 *P. parryi* plants over 67 half-hour observation periods in 2014.

Honeybee (*Apis mellifera*), bumblebee (genus *Bombus*), solitary bee (families Halictidae and Apidae), syrphid fly (family Syrphidae) and total pollinator visitation rates were compared among invasive

density treatments. We calculated the mean visitation rates of each plant in each treatment as the number of visits per flower per hour. Plant means were averaged to obtain a mean visitation rate for each experimental patch. We analyzed the data separately for each year because of the extreme difference in visitor composition. Separate insect categories other than honeybees were analyzed in 2014 only, when these other insects were more common visitors. In each case, we used a one-way ANOVA supplemented with Tukey post hoc tests to compare the four treatments with patch as the unit of replication (Procedure GLM in SAS version 9.3; SAS Institute, Cary, North Carolina, USA). Residuals for patch means were always approximately normally distributed (Shapiro–Wilks $P > 0.05$). An alternative form of analysis would be a mixed model, with the random effect of plant nested within the fixed effect of treatment. We did not use this more complex analysis because in 2014 the residuals at the plant level were not normally distributed, and generalized linear models using other distributions led to estimated G matrices that were not positive definite.

Pollen deposition—To determine pollen deposition (question 2), two flowers from each of 27 individual *P. parryi* plants across all treatments (6 far, 8 near, 7 low, and 6 high) were tagged as mature virgin buds on 10 April 2013. These flowers were exposed to open pollination and harvested 48 h later, once flowers had opened and reached female phase. Stigmas were then stained with basic fuchsin gel (Kearns and Inouye, 1993) and examined with a compound light microscope to determine the number of conspecifc and *B. nigra* pollen grains that had been deposited by insect visitors. The same procedure was carried out on 18 April 2014, with four flowers sampled per plant on 21 *P. parryi* individuals.

Pollen deposition on *P. parryi* stigmas was compared among invasive density treatments using separate models for conspecific and *B. nigra* pollen grains. We used a mixed model with fixed crossed factors of year and treatment and a random effect of patch nested within year × treatment. For the analysis in Proc Glimmix of SAS version 9.3, we employed a Poisson distribution as that gave a better fit than a normal distribution judging by the residual log likelihood and Akaike information criterion (AIC). Tests of fixed effects were type III. Least squares means for the four treatments were compared with a Tukey post hoc adjustment.

Predictions of effects on pollen tubes—We used the amounts of conspecific and invasive pollen deposited in combination with data from a previous study (Bruckman and Campbell, 2016) to predict the impact of invasion treatment on pollen tube growth to the base of the ovary and hence relative reproductive success (question 3). Previous work compared *P. parryi* pollen tube growth in flowers pollinated with pure conspecific pollen and flowers pollinated with a combination of conspecific and *B. nigra* pollen loads (Bruckman and Campbell, 2016). The detrimental effects of *B. nigra* pollen on *P. parryi* pollen tube growth are strong when conspecific pollen deposition is also high, but weak when conspecific pollen loads are small, yielding the following equation for the expected number of pollen tubes as a function of pollen grains of the two types:

$$\text{Pollen tubes} = 0.541(\text{conspecific}) + 0.035(B.\ nigra)$$
$$- 0.026(\text{conspecific} \times B.\ nigra).$$

We used that equation to predict how successful pollen tube numbers would compare among invasive treatments from this study

given mean amounts of conspecific and *B. nigra* pollen received on the experimental *P. parryi* stigmas.

Seed set—To determine the net effect on seed set (question 4), three flower buds were tagged on each experimental *P. parryi* plant in each treatment. During a given field season, all buds were tagged on the same day to control for temporal variation. If tagged flowers set fruit, seed capsules were collected approximately 3 weeks later, and seeds were counted. The final number of fruits collected per plant varied from 0 to 3 due to the loss of individual plants or branches that occurred during the study. In 2013, we collected a total of 80 fruits from 31 *P. parryi* plants, and in 2014 we collected a total of 67 fruits from 23 *P. parryi* plants. Seed set was determined as the average number of seeds per flower, including zeroes, for a given plant. The plant level data were then analyzed as described above for the pollen deposition experiment. Briefly, we used a mixed model with year and treatment as crossed fixed factors and patch nested within year × treatment. Reported results are based on a Poisson distribution, which gave a better fit than a normal distribution.

RESULTS

Pollinator visitation—The European honeybee, *Apis mellifera*, dominated pollinator visitation to *P. parryi* in 2013, making approximately 95% of all flower visits (Table 1), with the remaining 5% of flower visits divided between native bees in the genera *Lasioglossum*, *Ceratina*, and *Bombus*. Pollinator visitation rates in 2013 were not significantly influenced by invasion treatment ($F_{3,14} = 1.97$, $P = 0.174$). In 2014, the pollinator assemblage was more diverse, with bumblebees most common and making up 53.4% of all flower visits, while honeybees, solitary bees, and syrphid flies made 35.1%, 8%, and 3.5% of the visits, respectively (Table 1). Invasion treatment had a significant effect on total pollinator visitation rate to *P. parryi* in 2014 (one way ANOVA; $F_{3,16} = 3.64$, $P = 0.035$) with plants in the near treatment receiving approximately three times as many visits/flower/hour as those in the far treatment (Tukey test, $P < 0.05$, Fig. 1). *Phacelia parryi* plants in the near treatment also received approximately 7 times more honeybee visits/flower/h as those in the high treatment (Tukey test, $P < 0.05$; Fig. 1), contributing to a tendency for overall visitation to fall off when *B. nigra* was at high density as well as when it was far away. Visitation separately by bumblebees, solitary bees, and syrphid flies did not differ significantly among invasive density treatments in 2014 ($P = 0.069$, 0.206, and 0.398, respectively).

TABLE 1. List of insect pollinator taxa, number of individual flower visits (*N*) and percentage (%) of total number of visits made by each insect taxon to experimental *Phacelia parryi* plants in 2013 and 2014.

Year	Insect type	*N*	Percentage of total visits
2013	*Apis mellifera*	738	95.2
	Bombus spp.	5	0.7
	Solitary bees	32	4.1
	Total	775	
2014	*Apis mellifera*	823	35.1
	Bombus spp.	1253	53.4
	Solitary bees	189	8
	Syrphid flies	82	3.5
	Total	2347	
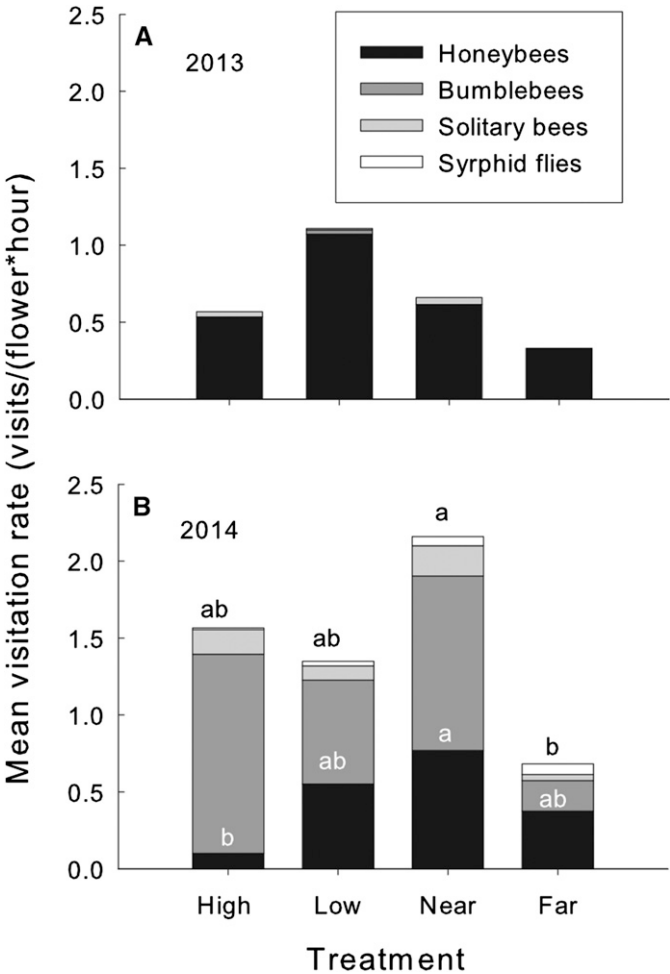
FIGURE 1 Effects of invasive treatment (high density, low density, near invasive, far from invasive) on mean visitation rates by different types of pollinators to *Phacelia parryi* in (A) 2013 and (B) 2014. Different lowercase black letters represent statistical differences for total visitation by all insects combined based on Tukey multiple comparisons. Different lowercase white letters represent statistically significant differences for honeybees based on Tukey multiple comparisons. Treatments did not differ significantly for visitation rate in 2013.

Pollen deposition—Invasive plant treatment influenced both conspecific and heterospecific pollen deposition. For conspecific deposition, there was a significant year by treatment interaction ($F_{3,25} = 5.26$, $P = 0.006$), so we also analyzed results separately by year, with patch nested within treatment. In 2013, no differences across treatment were detected ($P = 0.193$). In 2014, treatments differed in conspecific pollen deposition ($F_{3,11} = 6.69$, $P = 0.0078$) with flowers in the far treatment receiving approximately 10 times fewer conspecific pollen grains on average than flowers in either the near or low invasive density treatments (Fig. 2). High invasive density treatment plants showed a trend for lower conspecific pollen than the near and low treatments, but the differences were not statistically significant (Fig. 2). For *B. nigra* pollen deposition, the effect of treatment did not vary between years (interaction $F_{3,25} = 1.85$, $P = 0.164$). Deposition of invasive pollen increased monotonically from the far to near to low- to high-density treatment, with significant differences in all cases except between the far and near and between
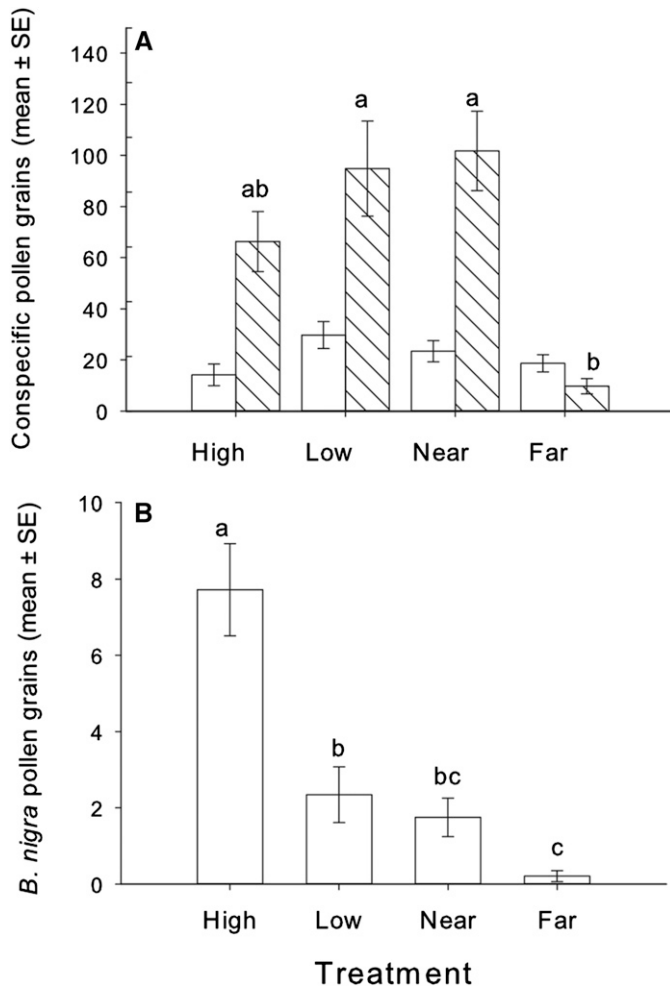
FIGURE 2 Effects of invasive treatment on pollen deposition on *Phacelia parryi* stigmas. Means and standard errors across the plot means are provided. (A) Conspecific pollen deposition separately in 2013 (open bars) and 2014 (hatched bars). Different lowercase letters represent statistically significant differences among treatments in 2014 based on Tukey multiple comparisons. Treatments did not differ significantly in 2013. (B) *Brassica nigra* pollen deposition in both years combined. Different lowercase letters represent statistically significant differences among treatments based on Tukey multiple comparisons.

the near and low treatments (Fig. 2). In the high-density treatment, 85% of *P. parryi* stigmas received some *B. nigra* pollen. Conspecific pollen showed higher deposition on *P. parryi* flowers in 2014 than in 2013 ($P = 0.0007$; Fig. 2), consistent with the higher pollinator visitation rates that year (Fig. 1).

Predicted effects on reproduction—Based on pollen loads, predicted pollen tube numbers were highest for *P. parryi* plants in the near and low treatments (mean = 23.8 and 23.9, respectively) and lowest in far treatment plants (mean = 7.4) (Fig. 3). We predicted an intermediate number of pollen tubes for *P. parryi* plants in the high invasive density treatment (mean = 12.2; Fig. 3).

Seed set—Seed set in *Phacelia parryi* varied both with invasive plant treatment and with year (treatment: $F_{3, 28} = 7.15$, $P = 0.0010$, year: $F_{1, 28} = 9.42$, $P = 0.0047$; Fig. 4). Flowers on plants in the low
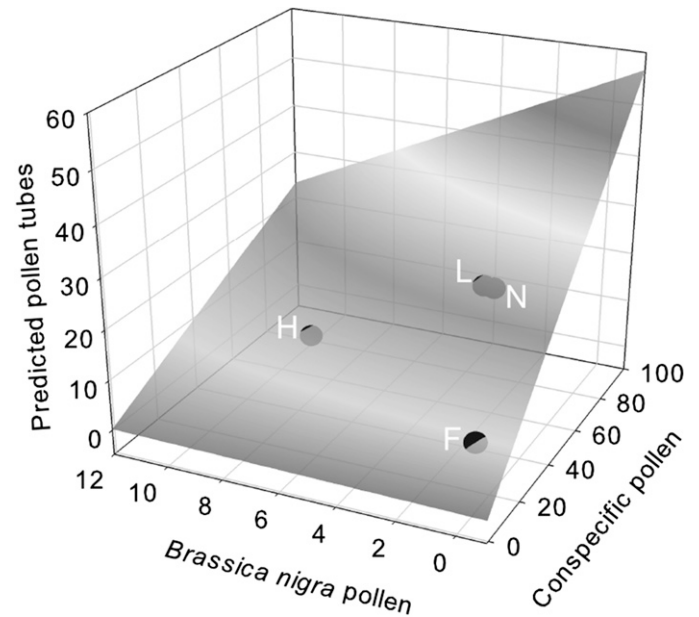

FIGURE 3 Predicted quantities of *Phacelia parryi* pollen tubes as a function of conspecific and *Brassica nigra* pollen deposition. The surface represents the function based on how pollen tubes responded to hand pollinations, as adapted from Bruckman and Campbell (2016). Letters represent predicted pollen tubes for high (H), low (L), near (N), and far (F) invasive density treatments based on the mean amounts of pollen received in the field experiment. The shading on the points occurs because a portion of each symbol is below the plane and a portion above.

and near treatments produced approximately 2.5 times as many seeds as those on plants in the far treatment (Fig. 4). High-density plants produced an intermediate number of seeds that was statistically indistinguishable from the other treatments (Fig. 4). This qualitative pattern, with seed set highest for plants in the low and near treatments, matched the pattern for pollen tubes expected from the levels of conspecific and heterospecific pollen (Fig. 3).

DISCUSSION

Pollinator-mediated interactions between native and invasive plant species may change over the course of a plant invasion (King and Sargent, 2012). As exotic plant populations establish and spread through host communities, reproductive success of native plants will likely be affected first by the distance to an invasion and later to increasing invasive densities. We found that pollinator visitation, pollen deposition, and seed set in a native plant were all influenced by two aspects of the simulated stage of exotic plant invasion: distance to the exotic, and density of the exotic when interspersed. Native individuals near invasive patches and within areas of low invasive density showed the highest net reproduction. By quantifying both quantity and quality of visits we were able to elucidate the underlying mechanisms. Plants in both of those situations benefited from facilitation of pollinator visits. In contrast, natives within areas of high invasive density received large amounts of heterospecific pollen, which tends to lower pollen tubes and seed set (Bruckman and Campbell, 2016). At the other extreme, isolation from an invasive patch was detrimental to fitness as individuals far
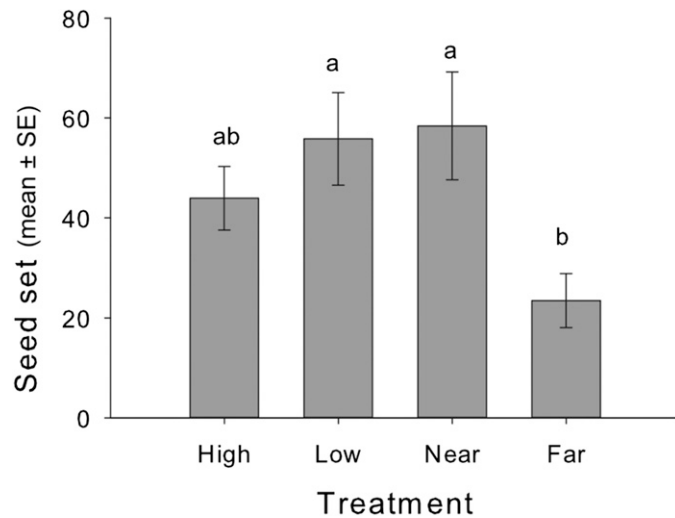
FIGURE 4 Effects of invasive treatment on mean seed set for *Phacelia parryi*. Means and standard errors across the plot means are provided for the combined 2013 and 2014 data. Different lowercase letters represent statistical differences among treatments in 2014 based on Tukey multiple comparisons.

from exotics suffered reduced seed set due to low pollinator visitation and conspecific pollen receipt.

Pollinator visitation rates to *P. parryi* were significantly influenced by invasive density treatment in our 2014 field season but not in 2013. Differences between years are likely attributable to the more diverse set of insect species in the assemblage of floral visitors to *P. parryi*, including a much higher abundance of native insects, in 2014 (Table 1). It is not clear what natural factors might have led to variation in pollinator assemblage composition between years; however, the addition of supplemental water to field plots in drought-stricken 2014 may have increased the overall attractiveness of our field sites by creating an oasis of green vegetation and more *P. parryi* flowers (Appendix S1) within an otherwise parched landscape.

In 2014, *P. parryi* individuals in the near treatment received significantly more pollinator visits than individuals in the far treatment. This finding suggests that *P. parryi* plants in close proximity to a patch of a showy invasive species, rather than far away, experience facilitation for pollinator visits, reaping the benefits of being within an attractive floral environment. On the other hand, visitation specifically by honeybees was lower to *P. parryi* individuals within high density patches than individuals in the near treatment, contributing to a tendency for visitation to decline again as exotic density increased to high levels. Honeybees foraged frequently on *B. nigra* flowers, and it is possible that in very high densities, *B. nigra* usurps the attention of honeybees, leading to more constant foraging on that species, as a result of overwhelming floral abundance or visual obstruction of the shorter *P. parryi* floral displays.

Although total pollinator visitation rate was not significantly reduced in the presence of a high density of invasives, other factors contribute to low reproductive success in that setting. In both of our field seasons, stigmas of *P. parryi* received more *B. nigra* pollen in the high invasive density treatment than in all other treatments (Fig. 2). Sun et al. (2013) found similar results when *Sonchus arvensis* visitation facilitated by high densities of invasive *Solidago canadensis*, yet seed set did not follow the same trend. The high

levels of invasive pollen and low levels of conspecific pollen they found on pollinator bodies suggested that high visitation rates were counteracted by interspecific pollen transfer, although it was not directly measured. Deposition of foreign pollen on stigmas can potentially reduce reproductive success by interfering with pollen germination, pollen tube growth or ovule fertilization (Morales and Traveset, 2008). In a previous study, we found that *B. nigra* pollen in amounts (mean = 6.5 pollen grains) similar to those seen in our high-density patches impairs seed production in *P. parryi* (Bruckman and Campbell, 2016). Pollen grains of *B. nigra* are much larger than those of *P. parryi,* and even a small number can occupy a large fraction of the stigma (Bruckman and Campbell, 2016). Some of the conspecific grains may be self-pollen, but those contribute to seed set as the species is self-compatible (Bruckman and Campbell, 2014). The simultaneous placement of small numbers of the much larger *B. nigra* grains and higher numbers of conspecific grains on *P. parryi* stigmas caused a decrease in both pollen tube growth and seed set compared to pure conspecific pollen loads, suggesting that visitation quality may be just as important as visitation quantity for *P. parryi.*

Conspecific pollen deposition in *P. parryi* was not influenced by invasive density treatment during our 2013 field season; however, in 2014, *P. parryi* stigmas in far treatments received significantly lower amounts of conspecific pollen grains, on average, compared with plants that were placed within or close to *B. nigra* patches. The reduction in conspecifc pollen receipt for flowers relatively isolated from patches of *B. nigra* appears to reflect the low pollinator visitation rates we recorded. Far treatment plants received particularly low visitation from native pollinators (Fig. 1) such as bumblebees and solitary bees, which are more effective at transferring *P. parryi* pollen than exotic honeybees (Bruckman and Campbell, 2014). The patterns of conspecific and heterospecific pollen received led us to predict that successful pollen tubes would be lowest for *P. parryi* far from the invasive, highest in the near and low-density treatments, and intermediate for plants surrounded by a high density of *B. nigra.* Seifan et al. (2014) also found that visitation can peak at intermediate densities of another plant species that shares pollinators, but previous studies were not able to link a decline in seed set at high density to the changes in conspecific and heterospecific pollen deposition.

Our predictions about combined effects of conspecific and heterospecific pollen were largely borne out by the patterns in seed set in *P. parryi*, showing how changes in both visit quantity and visit quality contribute to effects of the invasive species. In both field seasons, seed set was lowest for far *P. parryi* individuals, which received little conspecific pollen. Furthermore, individuals surrounded by a high density of exotics produced a quantity of seeds intermediate between far plants and near or low plants indicating the costs associated with high heterospecific pollen deposition in areas of high invasive infestation.

The facilitation we observed in this study appears to function through the effects of *B. nigra* on quantity of pollinator visits. In general, pollinators preferentially visit large flower displays that maximize their foraging efficiency (Schaffer and Schaffer, 1979; Eckhart, 1991; Kunin, 1993). Exotic plants with large, showy floral displays such as *B. nigra* may therefore attract pollinators from the surrounding habitat, leaving solitary plants less likely to receive pollinator services (Moragues and Traveset, 2005). In our study, *P. parryi* plants within or near a patch of *B. nigra* received more frequent insect visits, higher quantities of conspecific pollen

deposition and greater seed set than plants that were relatively isolated. Other studies investigating the effects of invasive density on native pollination have also found evidence of facilitation (Masters and Emery, 2015), particularly at low densities (Muñoz and Cavieres, 2008; Sun et al., 2013). Still, a few studies demonstrate decreases in pollination of native plants (measured variously as visitation, conspecific pollen deposition, or seed set) with increasing exotic density (Flanagan et al., 2010; Dietzsch et al., 2011; King and Sargent, 2012), suggesting the direction of these interactions is context-dependent.

Our study reveals how pollinator-mediated interactions between invasive and native plant species can change over the course of an invasion. *Phacelia parryi* suffers fitness reductions when relatively isolated from *B. nigra* and when surrounded by high densities of it. This finding suggests that exotic plants may impair pollination of native plants most in late stages of the invasion process through effects of heterospecific pollen deposition. However, other processes may contribute to different effects in a whole natural community than seen in the experimental populations studied here. For *P. parryi* in particular, loss of other native plant species in close proximity can lead to a replacement of more effective pollinators by the less effective honeybees (Bruckman and Campbell, 2014). The abundance of those other native plant species is in turn likely decreased by *B. nigra*, which is a competitive dominant in seed recruitment against some of those species (e.g., *Lupinus* spp., *Melilotus indicus*, and *Vicia villosa*; Aicher et al., 2011). Thus, there are likely other impacts of *B. nigra*, mediated indirectly through competition with other plant species that were not incorporated in our experiment. More generally, our experiment simulated impacts to native plants in very small populations. For native plants that form large populations, the native plant may initially exceed the invasive in density, forming a showy stand that is attractive to pollinators. In such a case, native plants far from an invasive stand might enjoy high conspecific pollen deposition and seed set. How initial abundance of a native plant affects the impacts of distance to and density of an invasive plant remains to be explored.

CONCLUSIONS

Our findings quantify how an ecological relationship can change during a biological invasion of small populations of a native plant. Pollination and seed set of the native plant *Phacelia parryi* varied under different simulated stages of plant invasion. Plants near the invasive plant *Brassica nigra* benefited from facilitation of pollinator visits, and those surrounded by the invasive at low density also had high seed set, but, under conditions of high infestation, high deposition of heterospecific pollen tended to reduce reproductive fitness again. The combined effects on seed set qualitatively matched those predicted by receipt of conspecific and heterospecific pollen and the effects of mixed pollen loads on pollen tube growth, showing that the balance can be predicted by understanding the mechanisms. Thus an invasive exotic can have both beneficial and detrimental effects on pollination of a native plant that depend on the density and degree of intermixing with the native species.

ACKNOWLEDGEMENTS

LITERATURE CITED

Aicher, R., L. Larios, and K. N. Suding. 2011. Seed supply, recruitment, and assembly: Quantifying relative seed and establishment limitation in a plant community context. *American Naturalist* 178: 464–477.

Arceo-Gómez, G., L. Abdala-Roberts, A. Jankowiak, C. Kohler, G. A. Meindl, C. M. Navarro-Fernández, V. Parra-Tabla, et al. 2016. Patterns of among- and within-species variation in heterospecific pollen receipt: The importance of ecological generalization. *American Journal of Botany* 103: 396–407.

Bell, D. T., and C. H. Muller. 1973. Dominance of California annual grasslands by *Brassica nigra*. *American Midland Naturalist* 90: 277–299.

Bjerknes, A. L., O. Totland, S. J. Hegland, and A. Nielsen. 2007. Do alien plant invasions really affect pollination success in native plant species? *Biological Conservation* 138: 1–12.

Brown, B. J., R. J. Mitchell, and S. A. Graham. 2002. Competition for pollination between an invasive species (purple loosestrife) and a native congener. *Ecology* 83: 2328–2336.

Bruckman, D., and D. R. Campbell. 2014. Floral neighborhood influences pollinator assemblages and effective pollination in a native plant. *Oecologia* 176: 465–476.

Bruckman, D., and D. R. Campbell. 2016. Timing of invasive pollen deposition influences pollen tube growth and seed set in a native plant. *Biological Invasions* 18: 1701–1711.

Campbell, D. R., and A. F. Motten. 1985. The mechanism of competition for pollination between two forest herbs. *Ecology* 66: 554–563.

Cariveau, D. P., and A. P. Norton. 2009. Spatially contingent interactions between an exotic and native plant mediated through flower visitors. *Oikos* 118: 107–114.

Caruso, C. M. 2002. Influence of plant abundance on pollination and selection on floral traits of *Ipomopsis aggregata*. *Ecology* 83: 241–254.

Chittka, L., and S. Schurkens. 2001. Successful invasion of a floral market—An exotic Asian plant has moved in on Europe's river-banks by bribing pollinators. *Nature* 411: 653.

Conner, J. K., and R. Neumeier. 1995. Effects of black mustard population-size on the taxonomic composition of pollinators. *Oecologia* 104: 218–224.

Dietzsch, A. C., D. A. Stanley, and J. C. Stout. 2011. Relative abundance of an invasive alien plant affects native pollination processes. *Oecologia* 167: 469–479.

Eckhart, V. M. 1991. The effects of floral display on pollinator visitation vary among populations of *Phacelia linearis* (Hydrophyllaceae). *Evolutionary Ecology* 5: 370–384.

Flanagan, R. J., R. J. Mitchell, and J. D. Karron. 2010. Increased relative abundance of an invasive competitor for pollination, *Lythrum salicaria*, reduces seed number in *Mimulus ringens*. *Oecologia* 164: 445–454.

Ghazoul, J. 2006. Floral diversity and the facilitation of pollination. *Journal of Ecology* 94: 295–304.

Gioria, M., and B. A. Osborne. 2014. Resource competition in plant invasions: Emerging patterns and research needs. *Frontiers in Plant Science* 5: 501.

Jepson Flora Project. 2013. Jepson eFlora. Jepson Herbarium, University of California, Berkeley, California, USA. Website http://ucjeps.berkeley,edu/IJM.html

Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Boulder, Colorado, USA.

King, V.M., and R. D. Sargent. 2012. Presence of an invasive plant species alters pollinator visitation to a native. *Biological Invasions* 14: 1809–1818.

Kunin, W. E. 1993. Sex and the single mustard: Population density and pollinator behavior effects on seed set. *Ecology* 74: 2145–2160.

Larson, D. L., R. A. Royer, and M. R. Royer. 2006. Insect visitation and pollen deposition in an invaded prairie plant community. *Biological Conservation* 130: 148–159.

Levine, J. M., M. Vilà, C. M. D'Antonio, J. S. Dukes, K. Grigulis, and S. Lavorel. 2003. Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society, B, Biological Sciences* 270: 775–781.

Lopezaraiza-Mikel, M. E., R. B. Hayes, M. R. Whalley, and J. Memmott. 2007. The impact of an alien plant on a native plant–pollinator network: An experimental approach. *Ecology Letters* 10: 539–550.

Masters, J. A., and S. M. Emery. 2015. The showy invasive plant *Ranunculus ficaria* facilitates pollinator activity, pollen deposition, but not always seed production for two native spring ephemeral plants. *Biological Invasions* 17: 2329–2337.

Melbourne, B. A., H. V. Cornell, K. F. Davies, C. J. Dugaw, S. Elmendorf, A. L. Freestone, R. J. Hall, et al. 2007. Invasion in a heterogeneous world: Resistance, coexistence or hostile takeover? *Ecology Letters* 10: 77–94.

Mitchell, R. J., R. J. Flanagan, B. J. Brown, N. M. Waser, and J. D. Karron. 2009. New frontiers in competition for pollination. *Annals of Botany* 103: 1403–1413.

Moeller, D. A. 2004. Facilitative interactions among plants via shared pollinators. *Ecology* 85: 3289–3301.

Molina-Montenegro, M. A., E. I. Badano, and L. A. Cavieres. 2008. Positive interactions among plant species for pollinator service: Assessing the 'magnet species' concept with invasive species. *Oikos* 117: 1833–1839.

Moragues, E., and A. Traveset. 2005. Effect of *Carpobrotus* spp. on the pollination success of native plant species of the Balearic Islands. *Biological Conservation* 122: 611–619.

Morales, C. L., and A. Traveset. 2008. Interspecific pollen transfer: Magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Sciences* 27: 221–238.

Muñoz, A. A., and L. A. Cavieres. 2008. The presence of a showy invasive plant disrupts pollinator service and reproductive output in native alpine species only at high densities. *Journal of Ecology* 96: 459–467.

Rathcke, B. 1983. Competition and facilitation among plants for pollination. *In* L. Real [ed.], Pollination biology, 305–329. Academic Press, Orlando, Florida, USA.

Schaffer, W. M., and M. V. Schaffer. 1979. Adaptive significance of variations in reproductive habit in the Agavaceae. 2. Pollinator foraging behavior and selection for increased reproductive expenditure. *Ecology* 60: 1051–1069.

Seifan, M., E.-M. Hoch, S. Hanoteaux, and K. Tielbörger. 2014. The outcome of shared pollination services is affected by the density and spatial pattern of an attractive neighbour. *Journal of Ecology* 102: 953–962.

Sun, S. G., B. R. Montgomery, and B. Li. 2013. Contrasting effects of plant invasion on pollination of two native species with similar morphologies. *Biological Invasions* 15: 2165–2177.

Takakura, K., T. Nishida, T. Matsumoto, and S. Nishida. 2009. Alien dandelion reduces the seed-set of a native congener through frequency dependent and one-sided effects. *Biological Invasions* 11: 973–981.

Thomson, J. D. 1978. Effect of stand composition on insect visitation in two-species mixtures of *Hieracium. American Midland Naturalist* 100: 431–440.

Totland, O., A. Nielsen, A. L. Bjerknes, and M. Ohlson. 2006. Effects of an exotic plant and habitat disturbance on pollinator visitation and reproduction in a boreal forest herb. *American Journal of Botany* 93: 868–873.

Traveset, A., and D. M. Richardson. 2006. Biological invasions as disruptors of plant reproductive mutualisms. *Trends in Ecology & Evolution* 21: 208–216.

Waser, N. M. 1978. Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. *Ecology* 59: 934–944.

Waser, N. M. 1983. Competition for pollination and floral character differences among sympatric plant species: A review of evidence. *In* C. E. Jones and R. J. Little [eds.], Handbook of experimental pollination ecology, 277–293. Van Nostrand Reinhold, New York, New York, USA.

Westman, A. L., S. Kresovich, and M. H. Dickson. 1999. Regional variation in *Brassica nigra* and other weedy crucifers for disease reaction to *Alternaria brassicicola* and *Xanthomonas campestris* pv. *campestris. Euphytica* 106: 253–259.