

TARAXACUM OFFICINALE POLLEN DEPRESSES SEED SET OF MONTANE WILDFLOWERS THROUGH POLLEN ALLELOPATHY

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Abstract—Plant species that share pollinators can suffer from interspecific pollen deposition. Male reproductive success is inevitably reduced by the loss of pollen to flowers of another species. Female reproductive success can be affected by reduced stigmatic area or, more strongly, through allelopathic effects by which the admixture of some foreign pollen reduces seed or fruit set. We tested for allelopathic effects of *Taraxacum officinale* (Asteraceae) pollen on the seed set of montane wildflowers *Erythronium grandiflorum* (Liliaceae) and *Erysimum capitatum* (Brassicaceae), by hand-pollinating plants with pollen mixtures. *Taraxacum* is a common invasive species, which produces allelopathic chemicals in its root and vegetative tissue, making it a likely candidate for pollen allelopathy. Flowers of both species produced fewer well-developed seeds when pollinated with pollen mixtures containing *Taraxacum* pollen. The pollen-allelopathic potential of weedy dandelion may add to its ability to disrupt communities that it invades.

Keywords: Pollen allelopathy, *Taraxacum officinale*, *Erythronium grandiflorum*, *Erysimum capitatum*, interspecific pollen transfer, phenological disruption

INTRODUCTION

Animal-pollinated plant species often occur in plant communities in which they share flowering times and pollinators with other plant species. A plant species can alter the pollination success of a co-flowering plant if its presence changes the flower-feeding behaviour of shared pollinators (Rathcke 1983; Mitchell et al. 2009). In particular, when pollinators move between the flowers of multiple plant species, they can cause interspecific pollen deposition (IPD) on plant stigmas. IPD can directly or indirectly reduce seed production and influence competitive dynamics among species (Levin & Anderson 1970; Waser 1978; Thomson et al. 1981). Phenological overlap in flowering is a necessary condition for IPD, raising the possibility that changes in phenological timing with climate change or changes in interacting species following invasion may lead to an increased incidence of IPD.

In the Rocky Mountains of the western United States, warmer spring temperatures and changing snowpack levels are causing earlier snowmelt and earlier onset of plant growth (Inouye 2008; Forrest et al. 2010). As a result, species that were once unlikely to co-occur, either within a season or habitat, are increasingly interacting; species that flower early in the spring are most affected, with more species co-flowering at this time (Forrest et al. 2010). This increased overlap among species may be exacerbated by the potential for increased colonization by exotic species in the area (Walther et al. 2009), with some exotic species such as

Taraxacum officinale also flowering in the early spring.

An adaptation believed to play a role in the superior competitive abilities and invasive nature of some exotic species is allelopathy, the release of chemicals that decrease the fitness of neighboring plants (Inderjit et al. 2008). These chemicals can be found in different plant tissues, including pollen grains. Through IPD on stigmas, pollen allelopathy has been inferred from reductions in pollen germination (Kanchan & Jayachandra 1980; Murphy & Aarssen 1995), pollen tube formation (Kanchan & Jayachandra 1980), and seed development (Thomson et al. 1981). The degree of pollen allelopathy depends first on the amount and frequency of interspecific pollen that is transferred to stigmas, which is governed by the flower constancy and visitation rate of pollinators when foraging, and the locations of pollen on a pollinator's body (Morales & Traveset 2008). Once pollen grains are deposited on stigmas, the degree of pollen allelopathy depends on the concentration of allelopathic chemicals in the donor pollen and the sensitivity of the recipient species to the chemicals (Inderjit et al. 2008).

We tested for pollen allelopathy in the common dandelion *Taraxacum officinale* (hereafter *Taraxacum*), a common, globally distributed invasive species capable of establishing under diverse environmental conditions (Holm et al. 1997). We hypothesized that this species would be likely to produce allelopathic pollen, as it is known to possess allelopathic chemicals in both its vegetative and root tissues (Jankowska et al. 2009). Anecdotal support for this hypothesis is mixed, however. Decreased seed set of *Erythronium grandiflorum* has been observed in the presence of *Taraxacum* pollen (J. D. Thomson, unpublished data), but the presence of neighboring *Taraxacum* plants had no

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detectable effect on seed set of *Delphinium nuttallianum* (Jones 2004).

To test whether allelopathic chemicals in *Taraxacum* affect the seed set of species native to the Colorado Rocky Mountains, we performed a hand pollination study on two species of animal-pollinated flowering plants that show some IPD with *Taraxacum*: *Erythronium grandiflorum* and *Erysimum capitatum* (hereafter *Erythronium* and *Erysimum*, respectively). We predicted that pollen mixtures including *Taraxacum* pollen would result in decreased seed set in the two native plant species, with greater proportions of *Taraxacum* pollen causing greater declines.

MATERIALS AND METHODS

Study site and plant species selection

We worked in subalpine meadows in the vicinity of the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA, in May-July 2012. Around the RMBL, *Taraxacum officinale* (Asteraceae) grows along trails and roads and in disturbed meadows, and flowered from May to July in 2012. We wished to study the effect of *Taraxacum* pollen on the seed set of focal plant species that receive *Taraxacum* pollen in the field. To search for candidate species, we sampled stigmas of seven plant species that flowered with and grew near *Taraxacum*. We collected stigmas from 10 plants of each species across multiple sites. The stigmas were stained and mounted using basic fuchsin jelly on microscope slides, to distinguish the pollen of different species present on a stigma (Kearns & Inouye 1993). *Taraxacum* pollen was not found on most of the species sampled, but was found at low amounts on the stigmas of three species, from which we selected our two focal species. We also carried out pollinator observations in areas where *Taraxacum* and the candidate species co-flowered, to see if the plants shared pollinators. These observations were carried out for ten-minute periods between 12:00 and 14:00 on multiple days. We selected *Erythronium* as a target species in part because of these observations, but also based on the promise of previous observations (J. D. Thomson, unpublished data). The presence of *Taraxacum* pollen on stigmas and observed shared visitors were taken to indicate the potential for pollen transfer to naturally occur between *Taraxacum* and *Erythronium* and *Erysimum*. *Erythronium grandiflorum* ('glacier lily'; Liliaceae) is a small perennial herb that flowers May to June and has yellow pendent flowers that are visited in the area principally by bumble bee queens (*Bombus* spp.) but also by hummingbirds and smaller solitary bees (Thomson 2010). *Erysimum capitatum* ('wallflower'; Brassicaceae) is a perennial herb that flowers June to July and typically has pale yellow upright flowers that are likely visited by a variety of insects including bumble bees. We observed visits by *Bombus bifarius*, *Bombus appositus* and halictid bees visiting flowers of *Taraxacum* and our two focal species.

Hand-pollination trials

Erythronium trials were conducted at four sites, using two-budded plants that grew in direct sunlight. We removed the second bud from each plant to ensure ample resource

allocation to the first flower. Shortly before the first bud opened, we separated the tepals and covered the pistil completely with a 2.5 cm length of plastic drinking straw. Straws with different diameters were used to ensure a snug friction fit around the ovary, without causing damage. We also placed sand-base mesh pollinator exclusion bags (Thomson et al. 2011) over the plant for the following two days, to ensure the plant was not pollinated in the event that the straw was dislodged. Due to the below freezing temperatures at night, plants were covered using inverted Styrofoam drinking cups. These cups were secured by piercing them with pin flags. The cups were removed early in the mornings and replaced over the flowers close to dusk.

Each plant used in the experiment was randomly assigned to one of the three groups as they matured within a site. We performed all pollinations during a twenty day period, from May 13 to June 1, 2012. Bagged flowers were pollinated using one of three *Taraxacum* pollen concentrations: a pure conspecific control including no *Taraxacum* pollen, and two mixtures that included 5-10% or 50% *Taraxacum* pollen, on a grain-count basis. The pollen of *Erythronium* came from buds collected the morning prior to pollination and left overnight for all anthers to dehisce. The buds of *Taraxacum* were collected the day of the pollination since they were unlikely to reopen if left overnight. Pollen mixtures were made by removing pollen from ten or more plants of *Erythronium* and *Taraxacum* and tapping pollen from the entire flower onto a pane of glass. We made fresh pollen mixtures daily and verified the proportions of pollen grains in each mixture by staining the pollen with fuchsin and counting the number of pollen grains of each species within two fields of view under a compound microscope. We made adjustments when necessary to produce the desired proportions. All mixtures for the lower concentration treatment contained between 5-10% *Taraxacum* pollen, while the 50% treatment was deemed suitable with a deviation of less than 2.5%, allowing for 5% error.

Each flower was hand pollinated using a Microbrush® disposable applicator dipped into the pollen mixture. Pollen was applied until a pollen layer was clearly visible on all three lobes of an *Erythronium* stigma. After a pollinated plant's ovaries began to swell, we removed the straws and allowed the fruits to develop and dry. At this time, fruits were collected and the well-developed seeds counted. We were able to quantify seed development for 25 of our control plants, 22 plants of the 5-10% treatment group, and 21 plants of the 50% treatment group.

We used a similar procedure for trials involving *Erysimum*. Plants at four sites were pollinated from June 14 to June 28. Because *Erysimum* produces inflorescences with numerous flowers, we removed 25% of the flowers in order to increase the resource allocation to those remaining. Two replicates of the three treatments were applied to individual flowers on a given plant. Depending on the height of the plant, we used either a swatch of bridal veil or an exclusion bag to exclude pollinators in these trials. Pollen mixtures were prepared as for *Erythronium* and applied the day after the buds first opened. We collected the fruits once they

developed, and counted the number of well-developed seeds. Some fruits were excluded from our analysis as insect larvae within the fruits had caused damage to the developing seeds. We were able to quantify the number of well-developed seeds for 29 of our control replicates, 28 replicates of the 5-10% treatment group, and 25 replicates of the 50% treatment group.

Data analysis

Seed counts for *Erythronium* treatments produced a zero-inflated dataset. Since we were unable to transform the data to create a normal distribution, we applied both a non-parametric test and a zero-inflated count data regression model. As both analyses produced similar results, we are only reporting the results of the Kruskal-Wallis test, a non-parametric test that is similar to an ANOVA. For *Erythronium*, we found a statistically significant depression of seed set for only the lowest treatment level of *Taraxacum* pollen; we therefore combined the data from the 5-10% and 50% treatments to increase the sample size and repeated the analysis to test whether the presence of *Taraxacum* pollen, at any level, had an effect. We performed a posteriori tests using the *kruskalmc* function in the *pgirmess* package of R version 3.0.0 (R Core Team 2013) to make pairwise comparisons between treatments.

The residuals produced by the seed counts for *Erysimum* were not normally distributed and were therefore square root transformed before analysis. We tested for effects of the pollen treatments using a linear mixed-effects model, using the *lme* function in the *nlme* package of R version 3.0.0 (R Core Team 2013). The model included the pollen treatment as a fixed effect. Individual plant identities were included as a random effect to account for the use of multiple treatments on single plants.

RESULTS

Taraxacum pollen significantly reduced seed set, but *Erythronium* and *Erysimum* differed in the patterns of their responses. *Taraxacum* had a significant effect on the development of seeds for *Erythronium* ($\chi^2 = 6.163$, $P = 0.04$, 2 df). By performing a posteriori comparisons, we found a significant effect from the application of the 5-10% *Taraxacum* pollen mixture on the development of *Erythronium* seeds ($P = 0.046$; Fig. 1), with a reduction of 50% in the number of well-developed seeds produced. Surprisingly, pollinating with 50% *Taraxacum* pollen produced a smaller mean reduction (31%) that did not differ from either the control group or the 5-10% treatment group (Fig. 1). Grouping the results from the two treatments, and testing the effect of the presence of *Taraxacum* pollen confirmed that there was a significant reduction in the seed set with the application of *Taraxacum* pollen ($\chi^2 = 5.126$, $P = 0.02$, 1 df).

The application of *Taraxacum* pollen had a significant effect on the seed set of *Erysimum* ($F_{2,55} = 7.7$, $P = 0.001$). Pair-wise contrasts of the least square means showed no significant difference in the seed set of the 0% *Taraxacum* and 5-10% *Taraxacum* pollen mixture treatments; however, both the control and 5-10% treatment groups had a

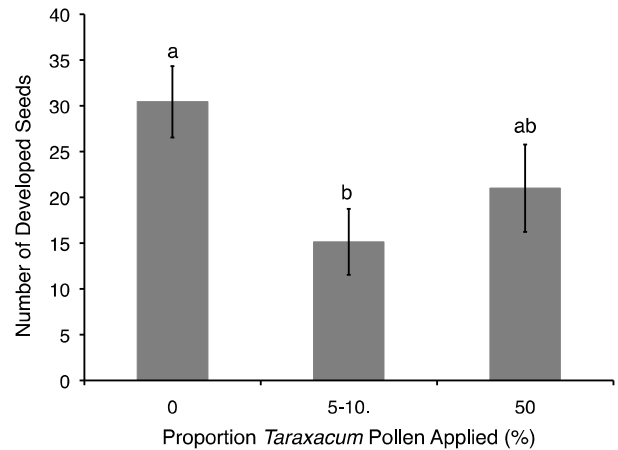


Figure 1. Seed set in *Erythronium grandiflorum* flowers when hand-pollinated with mixtures of conspecific and varying amounts of *Taraxacum officinale* pollen. Error bars represent standard errors. Different letters indicate significant differences between groups. $n=25$ for the 0% treatment group, $n=22$ for the 5-10% treatment group, and $n=21$ for the 50% treatment group.

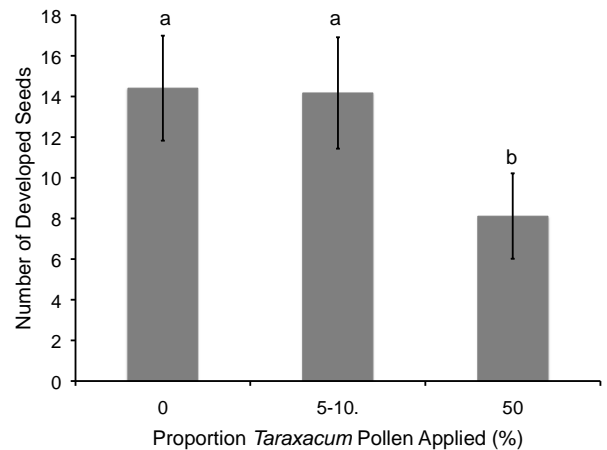


Figure 2. Seed set in *Erysimum capitatum* flowers when hand-pollinated with mixtures of conspecific and varying amounts of *Taraxacum officinale* pollen. Error bars represent the standard errors. Different letters indicate significant differences between groups. $n=29$ for the 0% treatment group, $n=28$ for the 5-10% treatment group, and $n=25$ for the 50% treatment group.

significantly higher seed set than that of the 50% *Taraxacum* pollen treatment ($P = 0.016$ and $P = 0.008$, respectively). The application of pollen containing 5-10% *Taraxacum* pollen resulted in a 12.2% decline in the number of well-developed seeds produced by this species; however, our analysis showed this to be a non significant decline. The deleterious effects of the *Taraxacum* pollen increased greatly when the applied pollen consisted of 50% *Taraxacum* grains, with seed production declining by 67.5% (Fig. 2).

DISCUSSION

In our two recipient species, seed set was depressed when stigmas were pollinated with mixtures containing *Taraxacum officinale* pollen in addition to sufficient conspecific pollen to fertilize all ovules. Presumably, this reduction arose from chemicals associated with the *Taraxacum* pollen itself,

although the mode of action also remains to be demonstrated. *Taraxacum* thus joins a few other genera known to produce potent pollen grains (Kanchan & Jayachandra 1980; Murphy & Aarssen 1995; Sukhada & Jayachandran 1980; Thomson et al. 1981). Interestingly, three of these genera (*Parthenium*, *Hieracium*, and *Taraxacum*) are Asteraceae that are also noted for apomixis (Powers & Rollins 1945; Richards 1973; Koltunow et al. 2011). Much broader taxonomic sampling would be necessary to determine whether this suggestive pattern is robust enough to need further investigation.

The more specific questions raised by our data concern dosage responses and possible ecological importance. First, the pattern of effects on *Erythronium* is perplexing: pollen mixtures with higher doses of *Taraxacum* pollen appeared to have weaker effects than milder doses. Because it is hard to imagine a mechanism for this pattern, and because the 50% treatment did not differ significantly from either of the others, we expect that this essentially represents a statistical anomaly. Uncontrolled environmental variation contributes to substantial variation in seed counts within treatments. More experiments in more controlled environments should be able to reduce the error variance and clarify the dosage response.

Second, the strength of inhibition shown by *Taraxacum* pollen on seed production in both of our recipient species is markedly less than in our earlier experiments with *Hieracium* pollen on *Diervilla lonicera* (Thomson et al. 1981). For example, the highest levels of seed reduction observed in this study were 67% (*Erysimum*) and 50% (*Erythronium*), whereas Thomson et al. (1981) showed that pollen mixtures with more than 20% *Hieracium* pollen often reduced seed set of *Diervilla* by more than 80%. This tentatively suggests that dandelion pollen may be more innocuous than hawkweed pollen, or alternatively, that some recipient species may be more sensitive to allelopathic pollen than others. Parallel trials of the two noxious pollens across a range of common recipients would be necessary to distinguish between these possibilities.

Does allelopathy from dandelion pollen play a role in this weedy plant's invasion of native communities? Murphy (2000) provided a rigorous experimental and observational framework necessary to demonstrate meaningful ecological effects of pollen allelopathy in plant-plant interactions in the field. Our results fall far short of such a demonstration, but our aim was simply to establish whether dandelion pollen might exert an allelopathic effect on other species, and our two recipient species were chosen partly for convenience. Our preliminary observations did detect *Taraxacum* pollen on stigmas of *Erythronium* and *Erysimum*, but we did not quantify the amount of IPD in this community and therefore cannot gauge the importance of the effect. Because of a tendency for flower-visiting animals to show flower constancy, two plant species can share pollinating species without much sharing of pollinating individuals. This may well contribute to Jones' (2004) finding that *Delphinium nuttallianum* flowers were unaffected by the presence of *Taraxacum officinale*. The flowers in Jones' study differ radically in both colour and form, which will tend to

encourage flower constancy and thus reduce IPD (Chittka et al. 1999; Gegear & Laverty 2005).

Our results do indicate that the nearly ubiquitous dandelion has at least the potential to affect its co-flowering neighbours through interspecific pollen deposition. Although *Taraxacum* pollen may be less toxic than that of *Hieracium*, concentrations as low as 5–10% suffice to depress seed set in *Erythronium*. Having established that modest admixtures of dandelion pollen can inhibit female reproductive success in other plants, we now can turn to investigating whether such effects are important in the field. If IPD occurs often enough to produce meaningful depression of reproductive output, future studies should examine the factors that determine the temporal and spatial proximity of dandelions to various recipient species. Moreover, species-specific changes in flowering times with climate change (Forrest et al. 2010) raise new possibilities about the potential for allelopathic interactions through IDP, as the overlaps between some species pairs intensify.

Pollen allelopathy is a previously unrecognized means by which *Taraxacum officinale* interacts with its surrounding plant community. Because this species is so widespread, and can be locally abundant, it is important to investigate whether this mode of interaction might have serious effects on vulnerable native taxa.

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