

POLLINATION ECOLOGY OF *POLYLEPIS TOMENTELLA* (ROSACEAE), AN ANDEAN ANEMOPHILOUS TREE PRESENTING A POTENTIAL FLORAL FUNGAL INFECTION

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Premise of research. Anemophily is considered to be a mechanism that evolved to promote pollination success. However, reproductive output can decrease if pollen loads are scarce due to low abundance of compatible mates or floral infections. Here we analyze the effects of breeding system, pollen limitation, and a potential floral fungal infection on the reproductive output of the Andean tree *Polylepis tomentella* (Rosaceae).

Methodology. We performed pollen supplementation experiments in the field to assess the extent of the effect of pollen limitation on fruit and seed set and to identify any pre- or postzygotic self-incompatibility responses within receptive pistils. Supplementary scanning electron microscopy and epifluorescence microscopy were used to observe the possible effects of a potential floral fungal infection on sexual reproduction.

Pivotal results. Pollen supplementation did not increase fruit and seed set, suggesting weak evidence of pollen limitation. Most stigmas from self- and cross-pollinated flowers showed germinated pollen grains and pollen tubes in the style, indicating that the species exhibits prezygotic self-compatibility. The presence of three Ascomycota genera in stigmas and styles appears to have negatively affected the germination of pollen grains by inhibiting pollen tube growth, but we did not find evidence suggesting that hyphae are able to penetrate the ovary.

Conclusions. This study showed that *P. tomentella* possesses a prezygotic self-compatible system and that fruit and seed set are not affected by pollen limitation. Therefore, reproductive assurance is achieved through pollen transfer between plants by wind and the role played by self-compatibility that allows selfing in the species. However, it is possible that the potential fungal infection could reduce the fecundity of its tree host, as hyphae are able to penetrate pollen grains.

Keywords: pollen limitation, potential floral fungal infection, prezygotic self-incompatibility, treeline, wind pollination.

Introduction

The tree genus *Polylepis* (Rosaceae) inhabits the Andean tree-line and is widely but fragmentally distributed through South America. Thanks to key morphological and physiological adaptations, such as small leaflets, multilayered bark, and high photosynthetic capacity, these trees have successfully colonized extreme elevations (Macek et al. 2009; Rada et al. 2009; Toivonen et al. 2014). Nevertheless, presently 60% of the species are undergoing some form of population decline, mostly attributed to direct and indirect human agency (Capriles and Flores Bedregal 2002; Gareca et al. 2010; Cuyckens et al. 2016). Conservation

efforts have been focused on reforestation; however, the success of these programs is variable due in part to low production of viable seeds. Causes of low viable seed yield have been broadly attributed to intrinsic factors, such as pollen limitation and parthenocarpy, as well as extrinsic factors, such as habitat degradation and resource limitation (Renison et al. 2005; Seltsmann et al. 2008; Domic 2012). Furthermore, the breeding system of *Polylepis* has not been systematically investigated, and it could include a wide variation in breeding systems, from obligate outcrossing species to autonomously selfing species, as has been shown in the other genera of the Rosaceae family (Ashman 1999). Thus, we focus on understanding the reproductive system of *Polylepis tomentella* by testing the hypothesis of pollen limitation, evaluating whether there is self-incompatibility and assessing a potential floral fungal infection, which might constitute a likely new external factor causing low seed set. The implications of the results are significant for understanding the reproductive systems of other endangered anemophilous trees around the world.

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Pollen limitation has important ecological and evolutionary implications because in many plant species pollen availability constrains sexual reproduction. Several meta-analyses of flowering plants have shown that pollen limitation is a widespread phenomenon that varies in magnitude (Ashman et al. 2004; Knight et al. 2005; Aguilar et al. 2006; García-Camacho and Tøtland 2009; Wolowski et al. 2014). Pollen limitation is particularly critical in small or fragmented populations (Knapp et al. 2001; Jump and Peñuelas 2006), in populations showing low frequencies of compatible mates (Steven and Waller 2007; Wagenius et al. 2007; Shelton 2008), and in newly colonized but outbreeding populations (Pannell and Barrett 1998; Pannell et al. 2008), as a reduction in seed yield can decrease both regeneration and population persistence.

From an evolutionary perspective, pollen limitation should promote selection of mechanisms that favor pollen dispersal. It has been suggested that wind pollination (anemophily) evolved from insect pollination (entomophily) as an alternative route to promote pollination success in response to pollinator limitation and changes in abiotic environment (Goodwillie 1999; Culley et al. 2002). Supporting this assertion, Friedman and Barrett (2009) showed that the sexual reproduction of only one out of 10 wind-pollinated herb species suffered from pollen limitation. However, an increasing number of studies show that available pollen loads may limit sexual reproduction in anemophilous species, such as masting trees (Pías and Guitián 2006; Pearse et al. 2015), marginal populations of invasive grasses (Davis et al. 2004), and endangered tree species with declining populations (Sanz and Pulido 2015).

The degree of pollen limitation depends not only on the dispersal of pollen grains but also on the self-incompatibility system. Self-incompatible species tend to produce fewer viable seeds when their intercompatible mates are absent (Agren 1996; Luijten et al. 2000). In this sense, although wind pollination may promote pollen movement, its effectiveness depends on the relative abundance of compatible pollen donors. Thus, self-incompatible species may be more likely to suffer from pollen limitation if prospective mates are rare or absent (Burd 1994; Knight et al. 2005).

A less explored issue is the effect of fungal infections on the sexual reproduction and fitness of the host. Fungi can reduce fertility by inhibiting inflorescence production, inducing flower loss, and even diverting resources from seed development and maturation (Carlsson and Elmqvist 1992; Marr 1998; Gilbert 2002; Ngugi et al. 2002; Pemberton 2010; Fontaine et al. 2013; Brown et al. 2015). In other cases, fungal parasites can modify host floral structures to enhance their own reproductive development. One such case is induced hermaphroditism in infected flowers of *Silene latifolia* with the anther smut fungus *Microbotryum violaceum* (Uchida et al. 2003).

In this article, we use the Andean anemophilous tree *P. tomentella* to test pollen limitation and the roles played by the compatibility system and a potential floral fungal infection in reproductive output. The species is endangered due to intensive anthropocentric disturbance (i.e., firewood extraction, farming, and burning), resulting in reduced population sizes and increasingly patchy distributions (Arrázola 2012; Domic et al. 2014). Specifically, we address three questions. First, are fruit and seed production limited by pollen availability? Second, which

compatibility system occurs in this species? And third, can a potential fungal infection affect fruit yield?

Material and Methods

Study System

Polylepis tomentella Weddell (Rosaceae) comprises evergreen trees and shrubs distributed in the semiarid Andean highlands of Peru, Bolivia, and Chile (Kessler 1995). In the Bolivian inter-Andean valleys, *P. tomentella* is the dominant tree species above 3000 m (Navarro et al. 2005). Plants produce erect-pendant, racemose inflorescences, bearing one to five flowers (up to eight). Flowers are bisexual, small (~2 mm in length), and show floral characters associated with wind pollination, including reduced inconspicuous corollas, big anthers, and large and dry stigmas that bear a single ovule (Simpson 1979; Kessler 1995). Flowers are protogynous, and the yellow stigma is receptive to incoming pollen (female phase) for up to 48 h before it collapses and the anthers dehisce (male phase; Domic et al. 2013). The species flowers annually between May and October and sets fruits from August to December. The fruits are winged achenes that contain one single seed, dispersed by wind on maturation and abscission. Seeds germinate readily in 7–15 d, but seed germination percentage is very low ($9\% \pm 7\%$) due to the large production of unviable seeds (Domic 2012).

We conducted fieldwork in forest stands where *P. tomentella* constitutes the only tree species. The species forms isolated forest patches over rocky hills with thin sandy-loam soils situated between 3100 and 4000 m near the towns of Tarabuco and Icla in Chuquisaca, Bolivia (Domic 2012). The region is characterized by a semiarid climate with a pronounced dry season between May and October (Ibisch et al. 2003). The mean temperature is 15.8°C, and the mean annual precipitation is 445 mm (SENAMHI 2011). The local vegetation is dominated by mixed shrubs (*Agalinis bangii*, *Baccharis dracunculifolia*, *Baccharis pappilosa*, *Colletia spinosissima*), scattered forbs (*Acicarpa tribuloides*, *Gamochaeta spicata*, *Plantago tomentosa*), and grasses (*Aristida adscensionis*, *Lamprothyrus hieronymi*, *Pennisetum villosum*).

Experimental Design

We scheduled field studies during the flowering season (June and July) of 2009 and 2010. In 2009, we carried out field experiments in three forest stands, but one site was vandalized. In 2010, we carried out field experiments in five forest stands, including all of the previously sampled locations. In each forest stand, we randomly selected 10 trees and subjected them to two pollination treatments: self-pollination with pollen of a different flower from the same tree, and outcross pollination with pollen from a different tree randomly selected within the same population. We collected anthers from several flowers of an individual and stored them in plastic Eppendorf containers. In each plant, we left two inflorescences as controls (open pollination), and we artificially pollinated four inflorescences by adding pollen to the surface of the stigma (two with self-pollen and two with outcross pollen) with designated high-precision tweezers. We did not emasculate hand-pollinated flowers be-

cause of their small size and the high risk of damaging them during the process.

Following hand-pollination, we covered the flowers with pollen-proof bags (glassine shoot bags manufactured by the Seedburo Equipment Company). In 2010, we also bagged open-pollinated flowers to prevent loss of flowers and fruits through wind dispersion. Samples were collected once fruits were completely mature (mid-October). To identify the potential effects of inbreeding, we measured fruits to the nearest 0.1 mm and weighed these fruits. We also cross-sectioned seeds to assess whether they were viable (i.e., they contained endosperm).

We used a self-incompatibility index (ISI) to determine the compatibility system (Zapata and Arroyo 1978). ISI was calculated as the mean fruit set from self-pollination divided by the mean fruit set from cross-pollination. We interpreted the results on the basis of the following scale: ISI > 1, self-compatible; $1 > \text{ISI} > 0.2$, partially self-compatible; ISI < 0.2, mostly self-incompatible; or ISI = 0, completely self-incompatible.

To evaluate rates of natural pollination, we collected flowers in the stigmatic receptive female phase in 2009 from two forest stands. Flowers were collected from 30 trees (15 from each forest stand) randomly selected during the flowering peak ($n = 300$). Whole flowers were fixed in a 3:1 acetic acid solution and 95% ethanol for 2 h and then stored in a solution of 70% ethanol. Individual flowers were softened in a 5% solution of sodium sulfite and incubated at 65°C for 15 min. Flowers were dissected on a glass slide to remove the remnants of the perianth and androecium. Carpels were stained in a solution of decolorized aniline blue. A coverslip was applied, and the female tissues were spread gently by tapping the coverslip with the tip of a probe (see Vance et al. 2004). To maximize penetration of the fluorochrome, slides were stored for a minimum of 24 h in a refrigerator and then observed under a Carl Zeiss incident fluorescence microscope using a violet exciter filter (see Bernhardt et al. 1980; Kearns and Inouye 1993). For each sample we recorded the number of (a) pollen grains on the surface of the stigma, (b) germinated pollen grains, and (c) pollen tubes reaching the base of the style. It was not possible to observe the number of pollen tubes entering the ovule due to the thickness of the ovary walls (Seltmann et al. 2008).

An additional hand-pollination experiment was performed to test for pollen-pistil compatibility. We hand-pollinated flowers from 30 trees from three forest stands in 2009 and from 50 trees from five forest stands in 2010. In each individual, we carried out pollen-addition treatments following the same procedure described for the pollen supplementation experiment (see above). Inflorescences were collected after 48 h, fixed, preserved, and analyzed under epifluorescence as described above. We also noted whether gynoecea contained fungal hyphae (see below).

To identify the presence of fungi in fresh tissue of *P. tomentella*, we collected samples of flowers, fruits, and leaves from the five study forest stands in 2010. Samples were sent to the laboratory of the National Institute for Agriculture and Forestry Innovation in Bolivia for identification. To assess the presence of floral fungi, we prepared five flowers showing the presence of fungi and fungal-free flowers for scanning electron microscopy. We air-dried flowers for 24 h, and a second set was dehydrated in a graded ethanol series (80%, 90%, and 100%) and critical-

point dried with CO₂. Samples were mounted on aluminum stubs, sputter-coated with gold, and observed under a scanning electron microscope (JEOL 5000 NeoScope) at the Missouri Botanical Garden.

Data Analysis

To test for differences in fruit set among hand-pollination treatments (hand pollination vs. control) and years, we used a nested mixed two-way ANOVA with the random factor “tree” nested within the random block factor “forest.” Differences in fruit size among hand-pollination treatments and control were tested using nested mixed two-way ANOVAs to identify which fruit traits were significantly different among groups. We applied Bonferroni corrections because we conducted multiple comparisons between fruit traits and treatments. A contingency test was used to identify the distribution of the number of pollen grains on stigmas from the natural pollination assessment. We performed a linear regression to identify the relationship between the number of pollen grains deposited on the stigma and the proportion of pollen tubes in the style. We used generalized additive models (GAMs) with a binomial distribution to test for differences in the proportion of germinated pollen grains and pollen tubes reaching the style between hand-pollination treatments and years. To test the prevalence of trees bearing potential fungi-infected flowers among years and populations, we performed a paired *t* test. We included only the populations studied in both 2009 and 2010. We also used GAMs to test for differences in the percentage of germinated pollen and the percentage of pollen tubes between those containing fungi and fungal-free flowers and hand-pollination treatment. All analyses were carried out using the R environment (ver. 2.15; R Development Core Team 2012).

Results

We found that pollen addition increased fruit set in only one year (table 1; treatment, $F_{2,49} = 0.36$, $P = 0.36$; year, $F_{1,49} = 37.56$, $P < 0.0001$; treatment \times year, $F_{2,49} = 6.41$, $P < 0.01$). In 2009, control-wind-pollinated flowers produced a lower proportion of fruits ($63\% \pm 5\%$ SE) than did hand-self-pollinated ($87\% \pm 4\%$) and hand-cross-pollinated ($84\% \pm 4\%$) flowers. In 2010, fruit set was similar among the two pollen-addition treatments (self-pollination, $96\% \pm 18\%$; cross-pollination, $95\% \pm 15\%$) and control ($97\% \pm 13\%$). Both hand-self- and hand-cross-pollinated flowers produced fruits and seeds. The

Table 1

Proportion (Mean \pm SE) of Fruit Set following Cross-Hand-Pollination (CP), Open Pollination (OP), and Self-Hand-Pollination (SP) in Forest Stands of *Polylepis tomentella* in South-Central Bolivia

Year	CP	OP	SP
2009 ($n = 20$)	84 \pm 4 ^A	63 \pm 5 ^B	87 \pm 4 ^A
2010 ($n = 38$)	95 \pm 15 ^A	97 \pm 13 ^A	96 \pm 18 ^A

Note. Data are %. Different letters indicate significant differences ($P < 0.05$). The total numbers of sampled trees are indicated in parentheses.

Table 2
Fruit Size and Percentage of Viable Seeds (Mean \pm SE) Produced by Cross-Hand-Pollinated (CP), Open-Pollinated (OP), and Self-Hand-Pollinated (SP) Flowers of *Polylepis tomentella* in South-Central Bolivia

Fruit trait	2009			2010		
	CP	OP	SP	CP	OP	SP
No. trees	16	11	15	32	34	33
No. fruits	178	128	188	317	333	248
Length	4.9 \pm .08 ^A	5.1 \pm .09 ^A	5.1 \pm .07 ^A	4.7 \pm .07 ^B	4.6 \pm .06 ^B	4.5 \pm .05 ^B
Width	3.5 \pm .08 ^A	3.7 \pm .09 ^A	3.4 \pm .07 ^A	3.7 \pm .07 ^A	3.5 \pm .05 ^A	3.5 \pm .05 ^A
Thickness	2.2 \pm .05 ^A	2.4 \pm .06 ^A	2.3 \pm .05 ^A	2.3 \pm .05 ^A	2.3 \pm .04 ^A	2.3 \pm .04 ^A
Width/length	.46 \pm .01 ^A	.48 \pm .01 ^A	.45 \pm .01 ^A	.8 \pm .01 ^B	.8 \pm .01 ^B	.8 \pm .01 ^B
Thickness/length	.46 \pm .01 ^A	.48 \pm .01 ^A	.45 \pm .01 ^A	.5 \pm .01 ^A	.5 \pm .01 ^A	.5 \pm .01 ^A
Viable seeds (%)	22.6 \pm 3.7 ^A	17.2 \pm 4.7 ^A	9.7 \pm 2.9 ^A	19.6 \pm 3.3 ^A	19.9 \pm 2.6 ^A	20.1 \pm 3.1 ^A

Note. Different letters indicate significant differences ($P < 0.05$).

ISI of *Polylepis tomentella* was 1.01 ± 0.02 , categorizing the species as self-compatible. A small proportion of plants (8%) from 2009 were not used to calculate the index because samples were vandalized in the field.

Fruit size was similar among hand-pollination treatments (table 2; treatment, $F_{2,1276} = 1.01$, $P = 0.39$; year, $F_{1,1276} = 13.6$, $P < 0.0001$; treatment \times year, $F_{2,1276} = 2.01$, $P = 0.02$), although fruits from 2010 exhibited a significantly higher width-to-length ratio than did fruits from 2009 independently of hand-pollination treatment. All fruits contained seed coats, but dissection showed that 80% of seeds were not viable, as they lacked endosperm and contained aborted embryos.

Pollen counts showed a high variation in the number of pollen grains deposited on the surface of stigmas collected randomly from two populations. Stigmas contained on average 19 ± 3 pollen grains (range, 0–125) and were observed on the majority of the flowers ($\chi^2 = 2838.15$, $df = 80$, $P < 0.0001$). A large proportion of the samples exhibited between one and 20 pollen grains (68%), and only two contained more than 100 grains. Pol-

len grains were absent on 13% of stigmas. Not all pollen grains on a stigma germinated; 34% of the stigmas retained only two germinated pollen grains, and only 17% had two or more pollen tubes reaching the style. The relationship between the number of pollen grains deposited on the stigma and the proportion of pollen tubes in the style was not significant (log number of pollen tubes = $0.9 - 0.05 \times \log$ number of pollen grains germinated; adjusted $r^2 = 0.02$, $P = 0.66$).

Hand-self-pollinated flowers exhibited 1.5 times more germinated pollen grains than did hand-cross-pollinated flowers (fig. 1A; year, $F_{1,262} = 0.03$, $P = 0.86$; treatment, $F_{2,262} = 4.57$, $P < 0.01$; year \times treatment, $F_{2,262} = 1.15$, $P = 0.31$). The percentage of pollen tubes penetrating style tissue of hand-self-pollinated flowers from 2010 was almost double that of hand-cross-pollinated flowers during both years (fig. 1B; year, $F_{1,262} = 5.2$, $P < 0.01$; treatment, $F_{2,262} = 6.03$, $P < 0.001$; year \times treatment, $F_{2,262} = 1.95$, $P = 0.14$).

Flowers with fungi present and fungal-free flowers did not show differences in shape or color, as hyphae were visible only

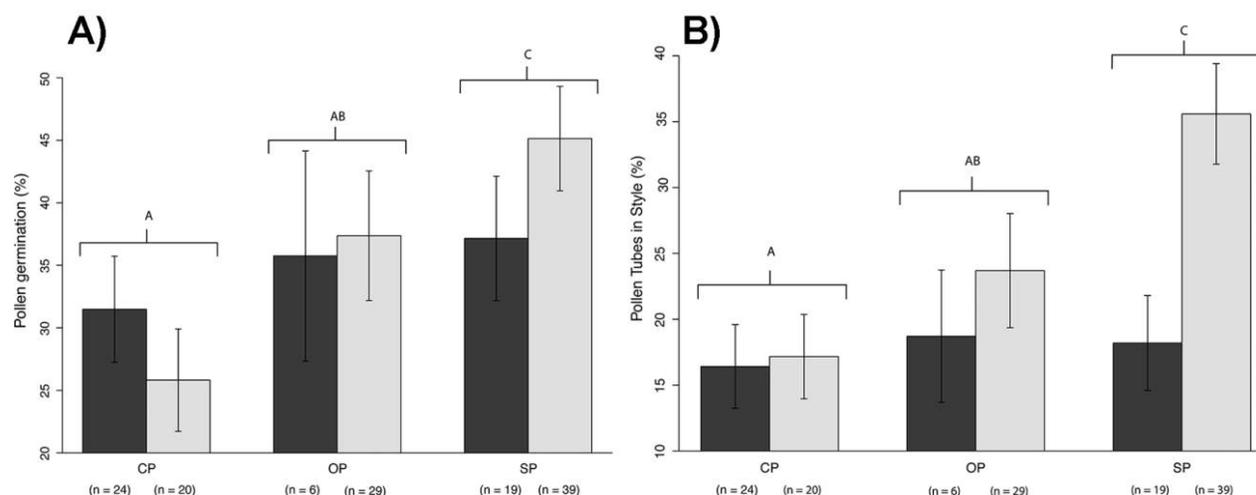


Fig. 1 Proportion of (A) germinated pollen grains on the stigma and (B) pollen tubes in the style following cross-hand-pollination (CP), open pollination (OP), and self-hand-pollination (SP) of *Polylepis tomentella* in 2009 (dark gray bars) and 2010 (light gray bars). Different letters indicate significant differences among treatments at $P < 0.001$ by ANOVA on arcsine-square root transformed data (untransformed means and SEs are presented). Numbers of sampled trees are shown in parentheses.

when observed under a microscope. The presence of fungi was noted in 32% of the samples ($n = 128$). Fungal hyphae were commonly observed on the surface of the stigma surrounding pollen grains (fig. 2) but were rarely observed on the style and anthers as well as the cuticle and epidermis of the ovary. We noted that pollen tubes either failed to contain cytoplasm that fluoresced under the Axioscope filter or germinated and produced pale and nonpenetrating ghost tubes (Edens-Meier et al. 2011). On the basis of mature morphological traits, the Ascomycota were placed in three genera: *Alternaria*, *Penicillium*, and *Aspergillus*.

Scanning electron microscopy of floral tissues also showed hyphal growth only on the stigmatic surface (fig. 3). Fungal hyphae were observed on the surface of the stigma, covering and penetrating pollen grains (fig. 4). In some cases hyphae grew on the surface of anthers, but these were less frequent.

Although we observed a slight increase in the percentage of trees bearing fungi-infected flowers in 2010 ($78.8\% \pm 13.2\%$) in comparison with 2009 ($43.3\% \pm 8.8\%$), this difference was not statistically significant ($t = -1.72$, $df = 2$, $P = 0.22$). In 2010, the six surveyed forest stands included trees bearing flowers with hyphae. We found only two forest stands where 40%

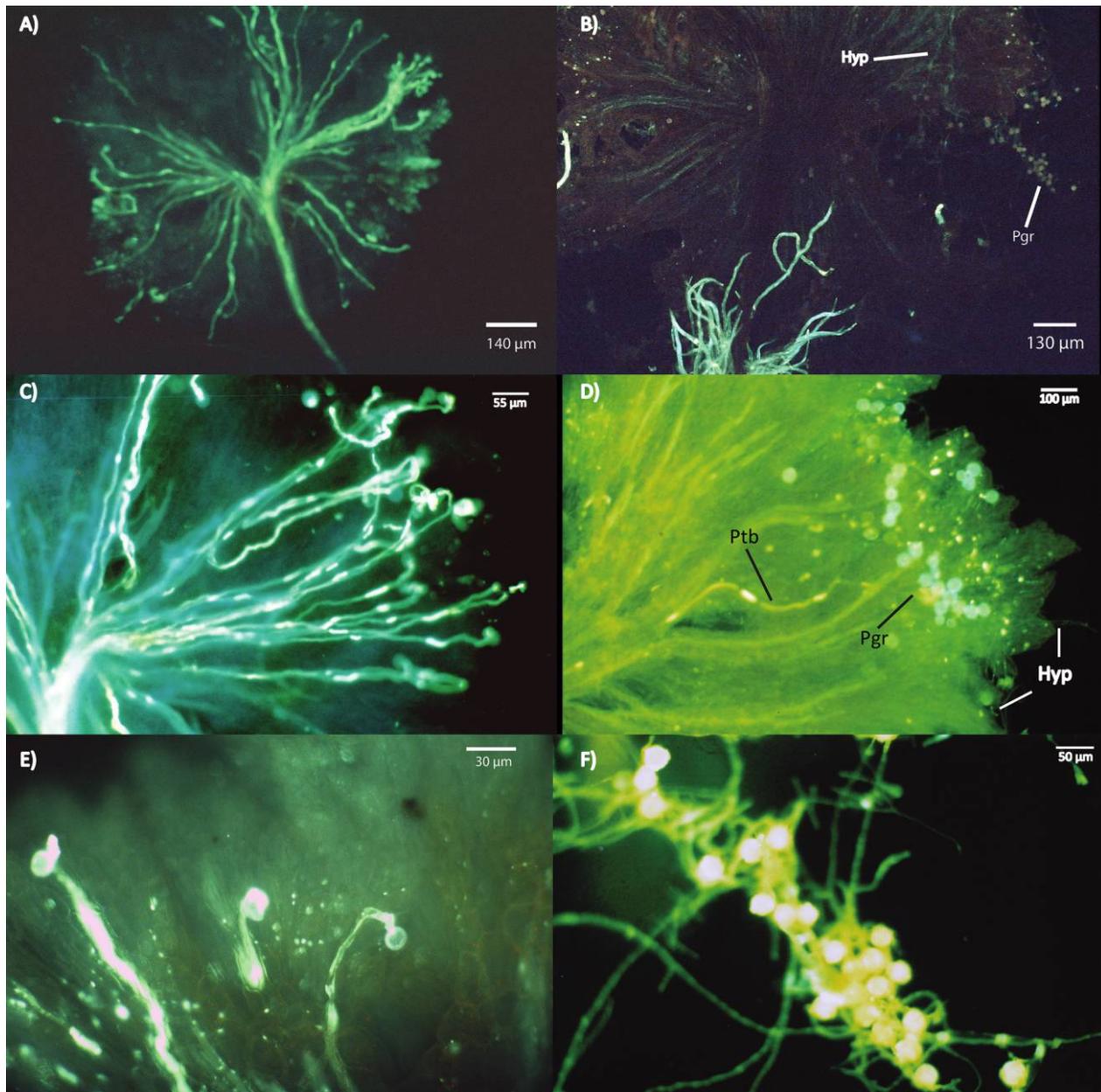


Fig. 2 Fluorescence microphotographs of uninfected (*left*) and fungi-infected (*right*) carpels of *Polylepis tomentella*. A, B, stigma and style; C, D, surface of stigma with pollen grains; E, germinated pollen grains; F, fungi-infected pollen grains. Hyp = hyphae; Pgr = pollen grain; Ptb = pollen tube.

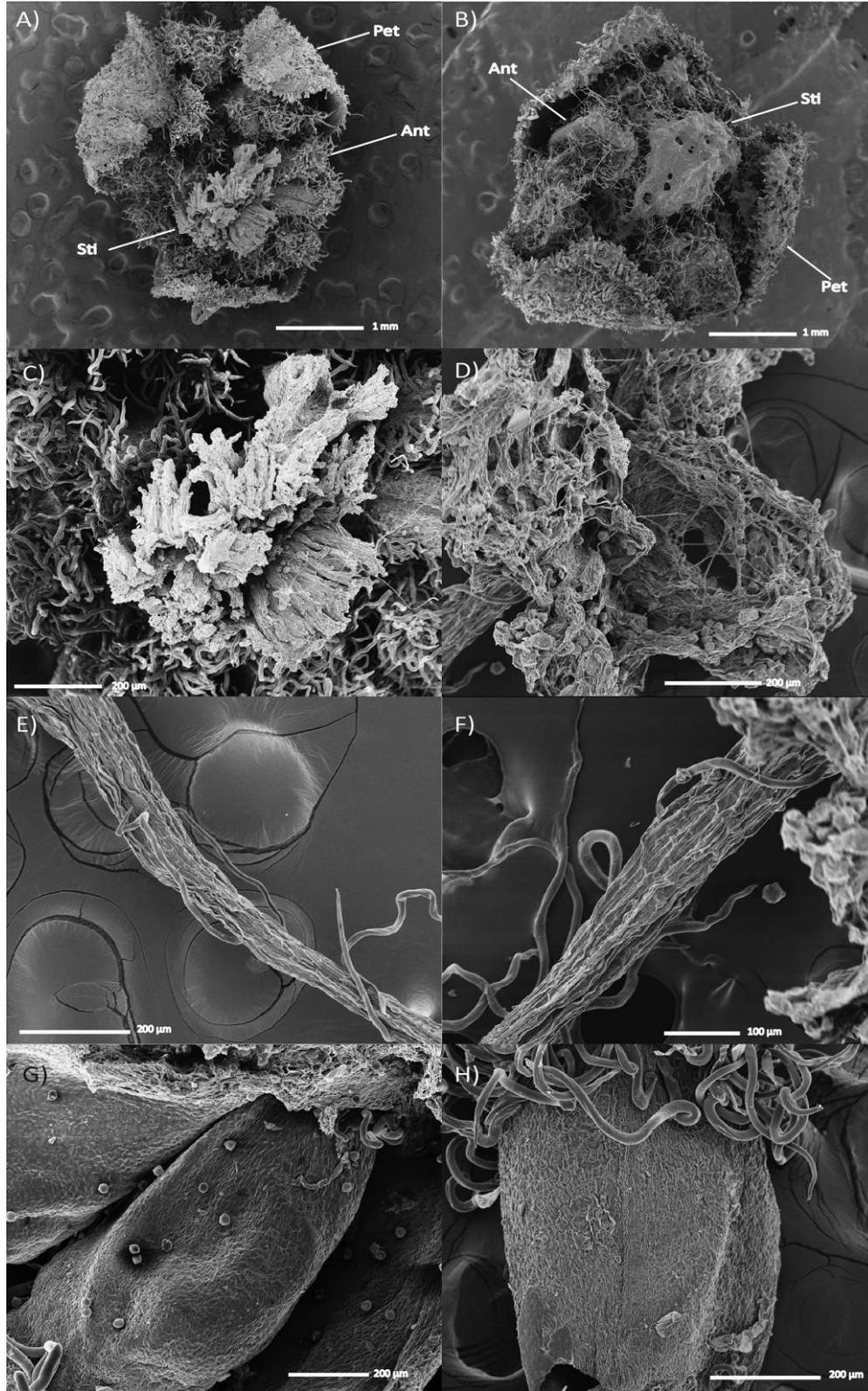


Fig. 3 Scanning electron microphotographs of uninfected (*left*) and fungi-infected (*right*) flowers of *Polylepis tomentella*. A, B, flowers; C, D, stigma; E, F, style; G, H, ovary. Ant = anthers; Pet = petals; Sti = stigma.

and 50% of the trees bore flowers with hyphae; in the other three populations, the percentage was much higher (80%–100%).

Flowers with fungal presence exhibited low numbers of germinated pollen grains (14 ± 2) in comparison with fungal-free flowers (35.4 ± 2), regardless of hand-pollination treatment (fig. 5A; treatment, $F_{2,406} = 4.79$, $P < 0.0001$; fungal presence, $F_{1,406} = 43.97$, $P < 0.0001$; treatment \times fungal presence, $F_{2,406} = 0.34$, $P = 0.71$). We observed a similar pattern in the proportion of pollen tubes in the styles. Flowers showing the presence of fungi had almost three times fewer pollen tubes in the style than did fungal-free flowers (fig. 5B; treatment, $F_{2,406} = 6.85$, $P < 0.001$; fungal presence, $F_{1,406} = 32.31$, $P < 0.0001$; treatment \times fungal presence, $F_{2,406} = 0.22$, $P = 0.79$).

Discussion

In this study, we have shown that reproductive output of the Andean tree *Polylepis tomentella* is not limited by pollen supply. Additionally, we found that the species exhibits a prezygotic self-compatible system and that flowers bearing fungal hyphae exhibited a lower percentage of germinated pollen grains. Reproductive assurance is achieved through pollen transfer between plants and self-compatible pollen. However, the potential fungal infection could reduce the fecundity of the tree host, with negative consequences for the regeneration of the species.

The effect of supplemental pollination varied between years for the surveyed forest stands of *P. tomentella*. In 2010, pollen supplementation did not increase fruit production, as control and experimentally hand-pollinated flowers produced similar rates of fruit sets. Although open-pollinated flowers experienced a relatively low fruit set in 2009, we suspect that this was caused by natural wind dispersion of fruits and not by fertilization failure, as they were not covered with pollination bags that year. Our results are consistent with those for *Polylepis australis*, which also failed to show a significant increase in reproductive output following pollen addition (Seltmann et al. 2009).

The results from the second year suggest weak experimental evidence of pollen limitation in *P. tomentella*. The species exhibits several demographic and reproductive traits that favor pollen movement among reproductive individuals (Whitehead 1969; Friedmant and Barret 2009), particularly high density of sexually mature trees (0.4–0.75 plants/100 m²), high phenological synchronicity during flowering, a large male-to-female phase ratio, and flowering during the dry season (e.g., reduced precipitation, low air humidity, and frequent winds; Domic et al. 2013, 2014). Current pollen loads could be sufficient to guarantee sexual reproduction in the studied populations because the majority of stigmas from open-pollinated flowers contained germinated pollen grains (Seltmann et al. 2008; Friedman and Barrett 2009).

We found no evidence of inbreeding depression, as fruit size did not differ among pollination treatments. Early advantages for seedlings from outcrossed seeds within a species are common, but in our study the direct effect of outcrossing on seed size was very weak. However, we did not conduct germination experiments, which limits our capacity to assess the effects of geitonogamous self-pollination on offspring fitness. In contrast,

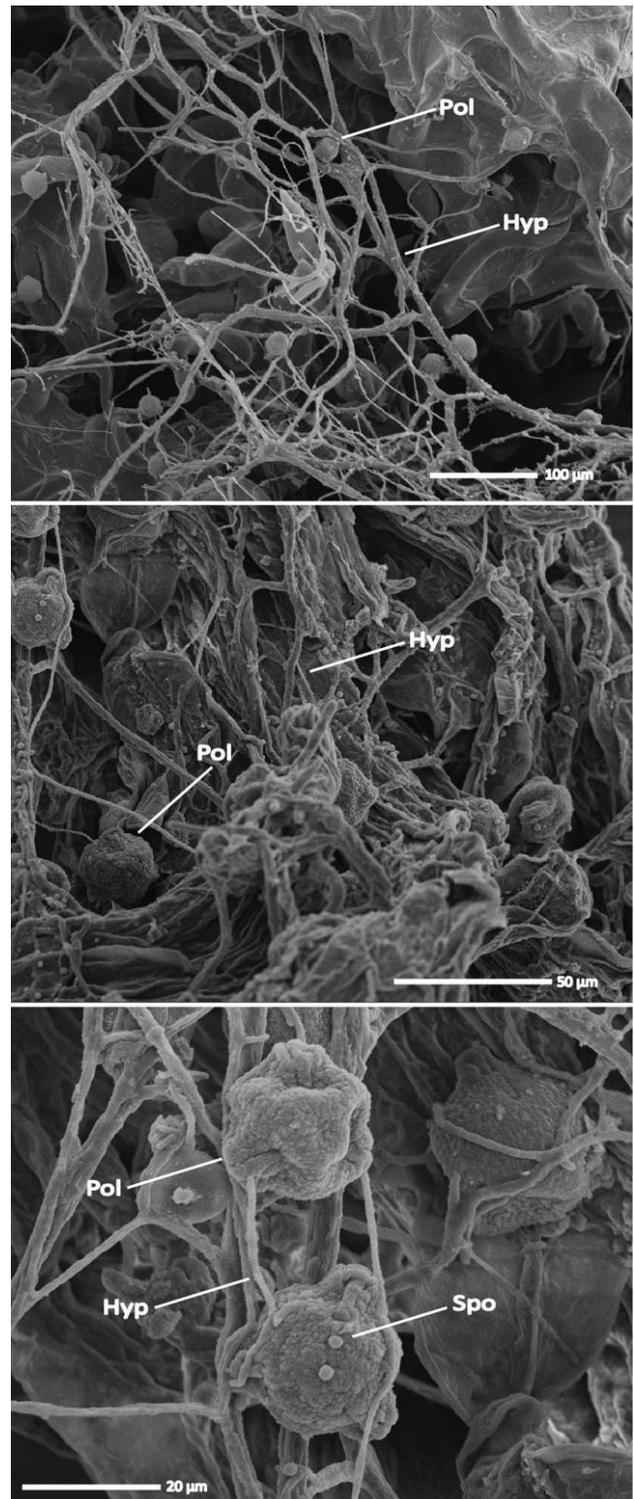


Fig. 4 Scanning electron microphotographs of stigma and pollen grains of *Polylepis tomentella* infected with fungal hyphae. Hyp = hyphae; Pol = pollen grain; Spo = fungal spore.

selfed seeds from *P. australis* experienced low germination rates, suggesting that early inbreeding depression (postzygotic incompatibility) may be common in some *Polylepis* species (Seltmann

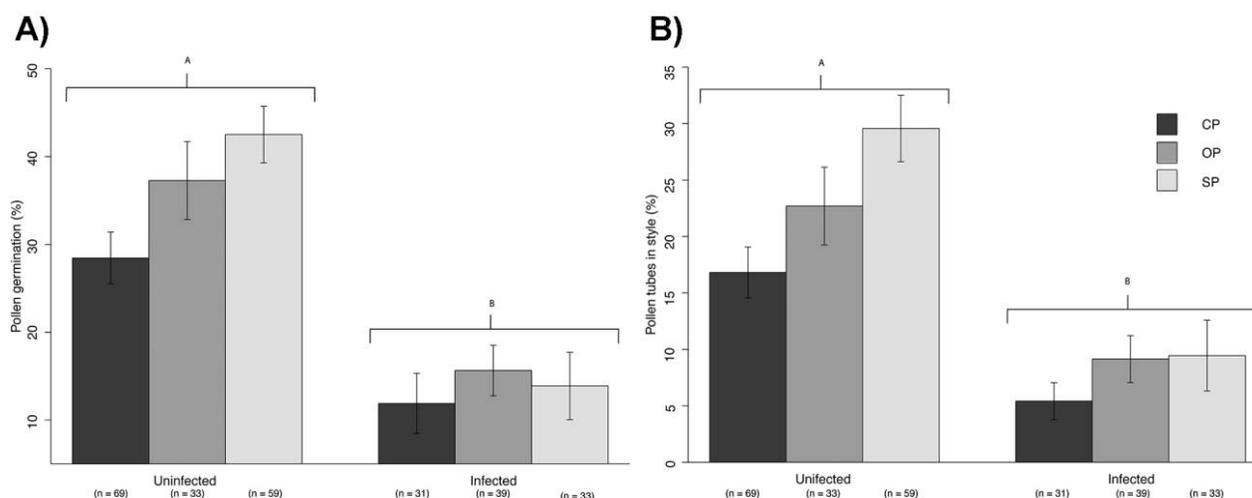


Fig. 5 Proportion of (A) germinated pollen grains and (B) pollen tubes in the style following cross-hand-pollination (CP), open pollination (OP), and self-hand-pollination (SP) of fungi-infected and uninfected flowers of *Polylepis tomentella*. Different letters indicate significant differences among treatments at $P < 0.0001$ by ANOVA on arcsine-square root transformed data (untransformed means and SEs are presented). Numbers of sampled trees are shown in parentheses.

et al. 2009). There is some evidence showing that inbreeding depression is expressed late in selfing species and may negatively affect growth and reproduction (Husband and Schemske 1996).

The results of our experimental hand-pollination and pollination efficiency assessment suggest that *P. tomentella* exhibits prezygotic self-compatibility, which has also been observed in *P. australis* (Setlmann et al. 2009). Flowers produced fruits regardless of whether they were experimentally pollinated with pollen from the same or a different plant. Additionally, a relatively larger percentage of pollen tubes reached the style in self-pollinated flowers, but we were unable to determine whether they were able to penetrate the ovary and whether a subsequent rejection response was induced (e.g., ovarian self-incompatibility). Similar results have been reported infrequently in other flowering species, including *Ariocarpus bravoanus* (Martínez-Peralta et al. 2014).

Stigmatic surfaces containing fungi were found to be detrimental to the sexual reproduction of *P. tomentella*. We noted a number of pollen grains penetrated by hyphae. These grains failed to show cytoplasmic fluorescence and/or produced ghost tubes, but we did not quantify our observations. We should note that, in other species, pollen grains deposited on receptive stigmas that fail to fluoresce fully and/or germinate weak tubes are often indicative of inviable grains (Bernhardt et al. 2013) or were rejected by stigmatic biochemistry (Edens-Meier et al. 2011). As this is a self-compatible tree, it seems reasonable to presume that hyphae penetrating pollen walls and growing on the stigmatic surface may have some deleterious or interrupting effect on the germination-penetration process.

The three genera of Ascomycota have been reported previously on flowers of other plant species (Kolpak et al. 1980). *Penicillium* and *Aspergillus* are not particularly associated with flower diseases, but spore exudates could inhibit pollen germination and pollen tube growth (Kimura et al. 1991; Roiz et al. 2000). In contrast, the genus *Alternaria* includes several patho-

genic species, some of which infect flowers (Li et al. 2007; Brown et al. 2015). In fact, a host-specific toxin (destruxin B) produced by *Alternaria brassicae* inhibits pollen tube growth in several species of *Brassica* (Shivanna et al. 1993). Brown et al. (2015) found that *Alternaria* constitutes a potential fungal pathogen that could inhibit the reproductive yield of *Asclepias meadii* by inducing tissue necrosis and floral abortion.

Fungal spores and hyphae were observed frequently on stigmas of *P. tomentella*. Flowers showing the presence of fungi exhibited a lower proportion of germinated pollen grains on stigmas and pollen tubes in the style in comparison with fungal-free flowers, which could be attributed to multiple factors. Hyphae could inhibit pollen tube development if they are able to multiply inside the style and occupy intracellular spaces used by pollen tubes to reach the ovary (Cisneros-López et al. 2010). If pistils of the host plant are unable to discriminate between pollen tubes and fungal hyphae (i.e., pollen mimicry), conidia could use stigmatic exudates to hydrate, germinate, and adhere to the style in order to reach the ovary (Ngugi and Scherm 2006a, 2006b). Finally, hyphae could kill pollen grains if they are able to penetrate pollen walls and then grow intensely, leading to the destruction of the grain's plasma membrane and cytoplasm (Huang et al. 1999; Hung-Chang et al. 2003).

Microscopic examination of flowers with fungal presence showed hyphal growth on the stigma and penetration of individual pollen grains but little evidence of growth within the style and ovary tissues. The occurrence of mycelium on the surface of the stigma suggested that fungal spores were able to germinate and grow but were unable to penetrate the ovary through the stigma-style pathway or through the ovary wall (Ngugi and Scherm 2006b). We did not conduct isolation or inoculation experiments in healthy flowers of *P. tomentella* to test whether they developed similar symptoms, but testing inoculation for pathogenicity and Koch's postulates could further verify the existence of a floral pathogenic infection (Brown et al. 2015).

This study has shown that the wind-pollinated Andean tree *P. tomentella* possesses a prezygotic self-compatible system. Fruit and seed set are not limited by natural depositions of pollen from coblooming genets in these forest stands. The large production of nonviable seeds, regardless of pollen origin, implies that other factors are responsible for seed abortion. We additionally report a potential fungal infection of the pollen grains that could reduce the fecundity of its tree host. We suggest that hyphae penetrate naturally deposited pollen grains on the stigmas by consuming the receptive protein pellicle and any available sugars on the stigmatic epidermis (Ngugi and Scherm 2006a, 2006b). This could disrupt the process of pollen recognition, hydration, and/or pollen tube growth and substantially reduce the fecundity of the host.

The low production of viable seeds of *P. tomentella* might not be the result of fecundity and pollen limitation exclusively but could result from other ontogenic and genetic factors that are poorly understood. Therefore, to ensure the long-term persistence of small and degraded forest patches, conservation programs should go beyond the current emphasis on reforestation and focus on habitat restoration. This involves assisted restoration to increase pollen movement and seed dispersal among remaining forest stands, improving habitat quality to increase

seed yield and support seedling establishment, and understanding and securing essential habitat requirements during critical life stages (e.g., seedling establishment, survival of reproductive plants). The results underscore the importance of the breeding system, along with a need to better understand the response of seed germination to selfing, potential fungal infections of sexual organs, and other reproductive constraints.

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