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Invasive conifers reduce seed set of a native Andean cedar through heterospecific pollination competition

Nicolás A. Martyniuk · Carolina L. Morales · Marcelo A. Aizen

Abstract The invasion of natural forest communities by introduced woody species may threat processes that are critical for ecosystem integrity, including plant pollination and reproduction. In particular, invasive conifers (e.g., *Pseudotsuga menziesii*, *Pinus* spp.) escaped from forest plantations may impair seed production of anemophilous native conifers by altering the dynamics of wind-borne pollen flow among trees. In this study, we analyzed the effects of invasive conifers on the pollination and sexual reproduction of Andean cedar (*Austrocedrus chilensis*), a dioecious conifer native to the temperate forests of southern South America. Wind-dispersed pollen captured in pollen traps revealed that the timing of pollen release between the invasive and native conifers overlapped extensively, and the pollen cloud surrounding female cedar trees in invaded forest was heavily contaminated with pollen from the invasive conifers. Increasing abundance of invasive conifers in the proximity of female cedars decreased the local density of air-borne cedar pollen and increased contamination of the pollen cloud with pollen from the invasive conifers. This increased the chance of finding heterospecific pollen from these invaders in the micropyles of the native cedar. In turn, the presence of pollen grains from invasive conifers, particularly of *P. menziesii*, in the cedar's micropyles was associated with a strong increase in the percentage of seed abortion. Our results clearly support the hypothesis that invasive conifers can interfere with the pollination and sexual reproduction of native wind-pollinated conifers. Thus, plantations of introduced and highly invasive conifers should not occur in the proximity of stands of native conifers in order to guarantee their reproduction and long-term population viability.

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Introduction

Forest plantations of introduced trees are often established close to native vegetation, which leads to scenarios where native and introduced species interact along contact edges (Vilà et al. 2010), and within natural and seminatural habitats when the introduced species are able to disperse and establish beyond the

plantation boundaries (Sullivan et al. 2005; Van Wilgen and Richardson 2012). In invaded communities, invasive species may outcompete natives by usurping different abiotic resources (e.g., space, light, water and different macro and micronutrients), which can impair native species growth and survivorship (Vitousek et al. 1996). However, more subtle competition can occur via plant reproduction, when invasive plants interfere with the pollination of native plants (Morales and Traveset 2009). In spite of the fact that most of studies of pollination-mediated competition have focused on interactions between native species, the increasing worldwide incidence of invasion by alien plant species raises the possibility that pollination of natives might suffer when they are sympatric with one or more invasive species. In particular, invasive plant species could affect the sexual reproduction of both wind- and animal-pollinated plants through reductions in both pollen quantity and quality (Aizen and Harder 2007; Burgess et al. 2008; Morales and Traveset 2009).

In the case of introduced conifers, massive pollen release from tree plantations or invasive trees escaped from those plantations can affect negatively the pollination of native, wind-pollinated species (Buonaccorsi et al. 2003). In particular, an extensive overlap in pollen release between invasive and native wind-pollinated gymnosperms can change the relative abundance of invasive versus native wind-borne pollen (Koenig and Ashley 2003), which may result in a high amount of heterospecific pollen grains deposited in the micropyles of native conifers. In turn, lower conspecific pollen deposition and/or mechanical and/or allelophatical interference by heterospecific pollen grains can limit the sexual reproduction of native plants (Morales and Traveset 2008; Dietzsch et al. 2011; Nishida et al. 2012). Even though wind-transported pollen can travel long distances (Koenig and Ashley 2003), this form of pollination-mediated competition is expected to become exacerbated in plant communities where the composition of former local pure conspecific neighborhoods become increasingly dominated by alien neighbors (Burgess et al. 2008, for an example on wind-pollinated angiosperms). In this situation, a focal tree of any wind-pollinated native species surrounded by alien wind-pollinated species will suffer the effects of being directly exposed to pollen flowing from its invasive neighbors. Although different impacts of conifer

invasions have been investigated (Simberloff et al. 2010; Van Wilgen and Richardson 2012), to our knowledge there is no previous study asking whether invasive conifers can impair seed output of native plants via pollination interference. Analyzing tree neighborhoods, here we evaluated the impact of invasive conifers on the pollination and seed output of a native conifer.

Plantations of introduced conifers, particularly of *Pinus radiata*, *Pinus ponderosa* and *Pseudotsuga menziesii*, have been established successfully in the forest-steppe ecotone of NW Patagonia, Argentina (Simberloff et al. 2010). These plantations have become important propagule sources for nearby, relatively open forests dominated by the native dioecious cedar *Austrocedrus chilensis* (Sarasola et al. 2006). This representative of a monospecific genus endemic to the temperate forests of southern South America, occurs at both sides of the Andes, occupying at present some 141,000 ha in Argentina (Bran et al. 2002) and 45,000 ha in Chile (CONAF 1999). This important regional timber species currently suffers from serious conservation problems and it has been included in the Red List of the World Conservation Union as vulnerable (Souto and Gardner 2013). In particular, many previously monospecific stands of the Andean cedar have now become heavily invaded by introduced conifers, increasing the isolation among potential mates by surrounding them with a barrier of invasive trees (Pastorino and Gallo 2009). This is especially worrisome for dioecious species, which necessarily rely on conspecific mates for reproduction.

We assessed the effect of invasive conifers on the reproduction of native *A. chilensis* via pollination. Because a prerequisite for the occurrence of a pollination-mediated effect on seed production between wind-pollinated species is substantial overlap in the timing of pollen release, we first characterized the phenology of pollen release of the invasive conifers and the native cedar. After corroborating that pollen release from both the native and alien conifers overlap extensively and that pollen clouds surrounding *A. chilensis* female trees are heavily contaminated with pollen from the invasive species, we asked: (1) does neighborhood composition affect the abundance of wind-borne heterospecific (i.e., from invasive conifers) versus conspecific (i.e., from *A. chilensis*) pollen in the proximity of focal female cedars (i.e., in their pollen clouds)?, (2) does a local increase in the

abundance of wind-borne pollen from invasive conifers also increase the likelihood of finding heterospecific pollen grains in the micropyles of *A. chilensis*?, and (3) do the presence and/or number of heterospecific pollen grains in the micropyles of *A. chilensis* relate to a decrease in cone and seed production? Here we report evidence that invasive conifers can strongly affect the sexual reproduction of native conifers by interfering with their pollination.

Methods

Study species and site

Austrocedrus chilensis is a dioecious conifer that extends along the foothills of the southern Andes between 32°S and 44°S. On the Argentine side, *A. chilensis* dominates a transitional, relatively xeric (about 700–1,000 mm annual rainfall) forest community that lies between the Patagonian steppe to the east and the more mesic *Nothofagus dombeyi* forest to the west. This Andean cedar can reach up to 30 m in height, 1 m in diameter, and ages >400 years (Pastorino and Gallo 2002).

Female and male trees can be easily distinguished by their sexual structures. Unfertilized cones are greenish, <1 mm and embedded in the vegetative bracts (Castor et al. 1996) in female trees. When mature, cones reach a size of 1.5–2 cm and the scales become wooden, each cone releasing up to four winged seeds. Aborted, seedless cones also become wooden but only grow to a size of ca. 1 cm. Pollen is borne in small (ca. 5 mm) aments in male trees. In masting years, those male trees loaded with thousands of aments take on a brownish hue (Brion et al. 1993). Female cone receptivity and pollen shedding occur from late September to mid-November and fruit maturation can extend to mid-May (Brion et al. 1993).

The study was carried out at the northern coast of Nahuel Huapi Lake, (40° 56′ 27.8″ S, 71° 22′ 16 0.5″ W) in an *A. chilensis* forest stand located to the E-SE of a 2 ha mixed plantation of introduced *P. menziesii*, *P. radiata* and *Pinus contorta*, during the 2010–2011 austral reproductive season (September–March). The plantation is about 50 year old and has remained unmanaged for the last 30 years (Fig. 1a). *P. menziesii and P. contorta* are the invaders with highest density, and reproductive trees of all three invasive coniferous species can be found invading the cedar stand up to

500 m from the plantation edge. Henceforth, we refer to these three species generically as "invasive conifers", to *P. menziesii* as "Douglas fir", and to the two species of *Pinus* as "pines". At the beginning of this study, we selected 32 female and 40 male focal cedars, about 6–18 cm in diameter at breast height (DBH) and 3–10 m in height scattered over an area of 17 ha (Fig. 1a).

Timing of pollen release

Following Brion et al. (1993) and Castor et al. (1996), we divided the reproductive phenology of male and female cedars on three stages, i.e., pre-reproductive, reproductive and post-reproductive, based on the morphology of reproductive structures. This allowed us to distinguish the period of cone receptivity and the period of pollen release. Every two weeks, we estimated the percentage of females bearing receptive cones and of males releasing pollen, inspecting reproductive bracts with a magnifying lens. We used information from pollen traps (see below) to characterize the duration of the period in which pollen of the invasive conifers can be found in the pollen cloud of female Andean cedars, potentially interfering with the pollination of this native conifer.

Pollen in female cedars' pollen cloud and micropyles

In each focal female cedar, we placed pollen traps to estimate the local availability of conspecific (*A. chilensis*) and heteroespecific (invasive conifers) pollen in the pollen cloud surrounding that tree (Fig. 1a). Each trap consisted of a wooden plate covered by an acrylic, sticky 4 × 10 cm adhesive film. Each trap was nailed on the trunk at a height between 2 and 2.5 m approximately, facing one of four cardinal directions (i.e., S, E, N, and W). The adhesive film of each trap was replaced every 2 weeks ($n = 5$ sampling periods) from September to December 2010. After removal, we cut a central portion of each adhesive film of the same area as a glass slide (i.e., 2.5 × 7.5 cm), mounted this portion after adding three drops of Alexander's stain (1969), and counted the number of pollen grains of the cedar, pines, and Douglas fir under a stereoscopic microscope at 100× in 15 randomly-chosen fields. Pollen of these three taxa can be easily identified. Andean cedar pollen is

Fig. 1 Map depicting the introduced conifer plantation and the distribution of study females of the Andean cedar *A. chilensis* in the nearby invaded native forest (**a**). Relative sizes of pollen grains of pines (*right*) and cedar (*left*) (**b**), and Douglas fir (*left*) and cedar (*right*) (**c**)
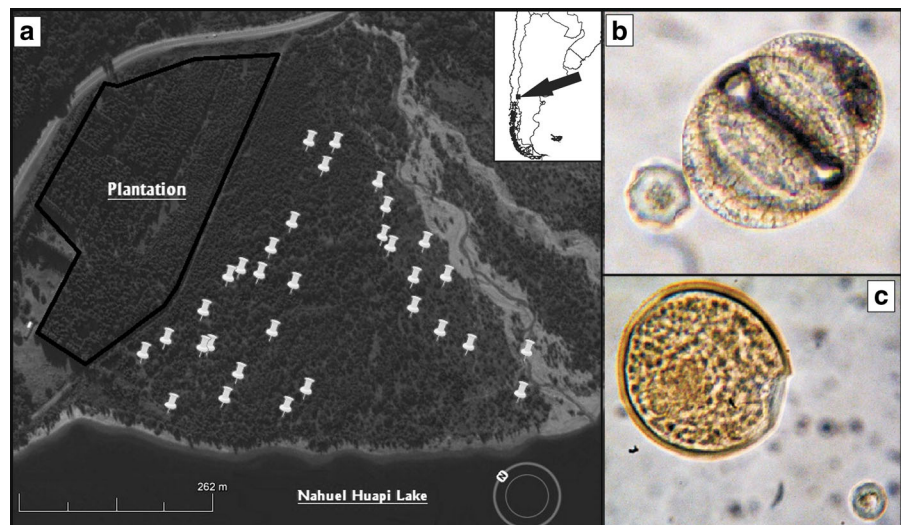
spherical, 20–24 μm in diameter, whereas pollen grains of Douglas fir and pines are much larger, 90–100 and 50–75 μm, respectively. Only the pollen from pines is winged (Fig. 1b, c see also Plate S2, S3 in Online Resource 1). Pollen of other species was very rarely detected (<0.5 %), and its occurrence was not taken into account in this study.

Cone and seed set

Between late February and early March 2011, we haphazardly harvested ten cones from ten reproductive branches (about 20 cm long) from each focal female cedar. Ripe cones contain between zero (i.e., aborted cones) and four seeds. From each ripe cone containing at least one seed, we counted seeds and dissected all ovules that did not develop into seeds. We estimated seed set as the mean ratio of seed per ovule averaged per tree. These undeveloped ovules were hydrated, mounted on glass slides, stained with 0.1 % (W/V) aniline blue (Currier and Strugger 1956), and observed under a dissecting microscope at 100×. We could only identify and count pollen from pines and Douglas fir on the cedar's micropyles. Thus, we are uncertain whether conspecific pollen grains were already dislodged or were never present.

Neighborhood influence on canopy openness and the pollen environment

Local neighborhoods of female *A. chilensis* were characterized by the identity (*A. chilensis*, *Pinus* spp.

or *P. menziesii*), DBH, and distance of the nearest 20 reproductive conifers to each focal female cedar, sexing the trees in the case of cedar neighbors. Next, for each female cedar we calculated a neighborhood index for each potential neighbor taxon (*A. chilensis*, *Pinus* spp. or *P. menziesii*), according to the following general formula (Daniels et al. 1986):

$$NI = \sum \frac{\left(\frac{DBH_n}{DBH_f}\right)}{DIST_{n-f}}$$

where DBH_n and DBH_f are the DBH of the neighbor tree and of the focal female cedar, respectively and $DIST_{n-f}$ is the distance between them. For each focal female cedar we calculated a neighborhood index (NI) for male cedars (i.e., pollen donors), a NI for pines and a NI for Douglas firs (i.e., pollen competitors). Thus, large NI values for a given neighbor taxon indicate the presence of numerous, large (i.e., larger than focal) and/or close neighbors, whereas small NI values indicate the presence of few, small and/or distant neighbors. This index combines the effect of DBH, number and distance of neighbors to focal tree, and although it does not allow to differentiate between few large or many small trees in the neighborhood, it can be considered a measure of density corrected by the size of the closest individuals (Tomé and Burkhart 1989).

Pollen production and seed set in cedar could be limited by resources, particularly light availability, a variable closely associated with canopy openness (Campoe et al. 2013). To estimate canopy openness

above each focal female cedar, we took four hemispherical pictures, one at each cardinal point, at 1.5 m away from the trunk of each focal tree with the camera pointing up and mounted on a tripod at a height of 1.30 m. We used a digital camera CoolPix 995, Nikon, with a fisheye lens that provides a view of 180° (FCE8, Nikon). The photographs were examined using WinSCANOPY^{TM}, where latitude was fixed for later analysis. As an estimator of canopy openness, we used the DSF (direct site factor fraction), defined as the proportion of direct radiation received below the canopy, which is closely related to other commonly used light estimates (Tercero-Bucardo et al. 2007). Canopy openness is maximum when DSF = 1 (i.e., full exposure), and minimum when DSF = 0 (i.e., full canopy cover).

Data analysis

First, we determined the phenology of pollen release in male ($n = 40$) and receptivity in female ($n = 32$) *A. chilensis* trees based on the percentage of male trees releasing pollen and of female trees bearing receptive cones. Next we estimated overlap in pollen shedding and cone receptivity between male and female cedars, and pollen shedding between male cedars and invasive conifers by tracking changes over time in the number of pollen grains of cedars, Douglas fir and pines counted from the pollen traps. Phenological overlap was estimated using Ausgpurger's index of synchrony (1983). For a certain individual i, this index (X_i) measures flowering overlap in terms of flowering days, weeks, or months with all other individuals in the sampled population. Perfect synchrony and asynchrony occur when $X = 1$ and $X = 0$, respectively (Augspurger 1983).

Before assessing neighborhood effects on cedar pollination, we checked for any potential spatial autocorrelation in NIs for each taxa (i.e., of cedar, Douglas fir, and pines), in pollen deposition in traps, and cedar seed set, by calculating Moran's I Index obtained with the SAM program, v.3.0 (Rangel et al. 2010). After corroborating the absence of any significant spatial autocorrelation (see Online Resource 1, Table S1), we performed a Kruskal–Wallis, one-way analysis of variance, to compare the NI values among the three taxa. Then we used multiple linear regressions to relate the canopy openness (as estimated from the DSF) with the NIs. We also tested through

multiple linear regressions whether canopy openness and the NI of a given taxon affected the number of pollen grains of each respective taxon found in the pollen traps. In the specific case of Andean cedar, we also included NIs of both invasive conifers in addition to cedar NI as predictors, in order to test for a potential barrier effect of pine and Douglas fir trees to conspecific pollen flow. After that we tested through multiple regressions whether the number of pollen grains cm^{-2} from the invasive conifers (pines and Douglas fir) in the traps was related to their presence in the micropyles of the native cedar using simple linear regressions. Finally, we tested whether canopy openness, the proportion of micropyles with at least one heterospecific pollen grain, and the mean number of heterospecific pollen grains on those micropyles with at least one pollen grain, as well as the number of pollen grains of cedar cm^{-2} detected in pollen traps, affected cedar seed set using a multiple linear regression. All predictive and response variables were standardized to make their effects comparable. Also, for all analyses the focal female cedar is the experimental unit, with all variables averaged at this level, when necessary.

To further understand how invasive conifers could interfere with the pollination and seed production of the native cedar, we summarized the results of the analyses above by proposing a causal model tested by means of path analysis (Shipley 1997). In the model, the NI of male cedars determines local availability of conspecific pollen in the pollen cloud (i.e., number of cedar pollen grains cm^{-2} in the pollen traps), which has a positive effect on seed set, through increasing the chance of deposition of conspecific pollen on cedar micropyles. This mechanistic explanation could not be tested because of the impossibility to detect conspecific pollen in micropyles. In turn, the NIs of Douglas fir and pines determine the extent of contamination of the local pollen cloud with alien pollen (i.e., number of Douglas fir pollen grains and pine pollen grains in the traps), which increases the chance of finding heterospecific pollen of these invaders on cedar micropyles. The presence of heterospecific pollen in the micropyles of female cedars could cause seed abortion through mechanical interference or allelophatic effects, thus reducing seed set (Fig. 2). Alternatively, an increase in the NIs of both cedar and invasive conifers decrease canopy openness (DSF), a factor that can limit seed set both directly via resource (i.e., light) availability, and
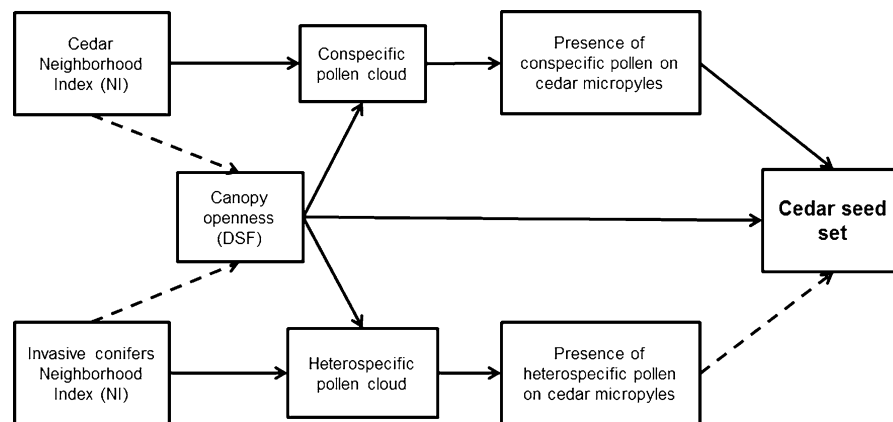
Fig. 2 Conceptual Model for the effects of the NI of male cedars and invasive conifers on canopy openness (DSF) and on the pollen density in the pollen cloud surrounding each focal female cedar. This model proposes that the relative abundance of pollen of each taxa in the pollen cloud will be reflected in the cedar's micropyles affecting seed set. *Solid* and *dashed arrows* indicate expected positive and negative effects, respectively of the independent variable on the dependent variable. Subsequently the effects of invasive conifers were tested for each species (pines and Douglas fir) separately

indicectly via pollination through reductions in pollen production and/or physical interference with pollen flow. To check the goodness-of-fit of the model we used three different indexes. Most statistical methods only require one statistical test to determine the significance of the analyses. However, in confirmatory analysis, several statistical tests and indexes are used to determine model fit (Suhr 2006). The first index, relative Chi square (CMIN/DF), is simply the Chi square fit index divided by the degrees of freedom. The criterion for acceptance varies across researchers, ranging from values <2 to <5 (Schumacker and Lomax 2004). The second, the Comparative Fit Index (CFI), is estimated from the difference between the observed and predicted covariance matrices and ranges from 0 to 1 (Hu and Bentler 1999), with values close to 1 indicating best fit. Finally, the Root Mean Square Error Approximation (RMSEA) is the square root of the variance of the residuals. This index indicates the absolute fit of the model to the data—how close the observed data points are to the model's predicted values. RMSEA should be <0.08 for an adequate model fit. We run all path analyses in AMOS 18.0 (Arbuckle 2003). Remaining analyses were run in Sigma Plot.12 (Systat Software Inc., San Jose, CA, USA). Figure 2 depicts the overall conceptual model, which was further expanded in the actual analyses to include the separate effects of both pines and Douglas fir (see "Results").

Results

Timing of pollen release

Female cone receptivity and male pollen release extended from mid-September to mid-December peaking during the first week of October. Cone receptivity in female trees and the dehiscence of pollen-producing aments in male trees were considerable synchronized (0.63 overlap, SD = 0.22) in Andean Cedar. Pollen traps showed extensive overlap in pollen release between the native cedar and invasive conifers (Fig. 3). More specifically, phenological overlap in pollen release between the cedar and Douglas fir was extensive, averaging 0.73 (SD = 0. 26), whereas between cedar and pines was much lower, 0.22 (SD = 0. 15). In addition to extensive overlap, the phenology of pollen release of the native cedar and Douglas fir peaked during the first fortnight of October. At the peak period (from second fortnight of September to second fortnight of October), we recorded a mean of 1.15 (SD = 0.59) and 1.87 (SD = 1.71) pollen grains cm^{-2}, of cedar and Douglas fir, respectively, at the pollen traps. On the other hand, pollen release in pines peaked 1 month later, during the first week of November (Fig. 3). At its peak, we recorded a mean of 15.99 (SD = 8.34) pine's pollen grains cm^{-2}, whereas at the earlier cedar's pollination peak we recorded only 0.75 (SD = 0.71) pine's pollen grains
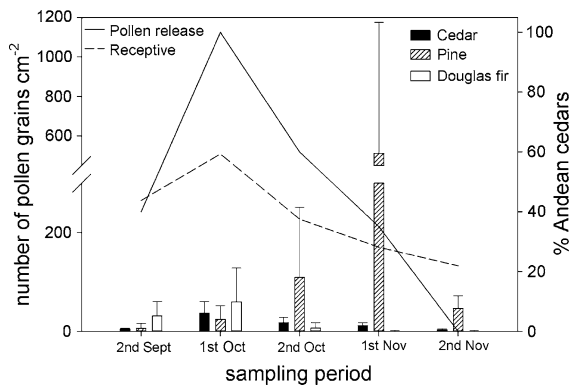
Fig. 3 Seasonal changes in pollen abundance estimated as mean number of pollen grains cm^{-2} of Andean cedar, pines and Douglas fir in pollen traps (*left axis*), and percentage of female cedars ($n = 32$) with receptive cones an male cedars ($n = 40$) with aments releasing pollen (*right axis*)

cm^{-2}. Thus, although pine pollen production seems to be one order of magnitude higher than that of either the cedar or of the Douglas fir, during the period of phenological overlap all three species release more or less comparable number of pollen grains.

Heterospecific pollen from all invasive conifers considered together represented a large fraction of the pollen cloud surrounding female cedars. The average percentage of alien pollen in traps ([heterospecific pollen/total] × 100) between September and November was 81.6 % (SD = 13.1 %), where 87.6 and 12.4 % was pollen from pines and Douglas fir, respectively. At the peak of pollen release period of the native cedar, the percentage of alien pollen was still high, 69.5 %, where 28.7 and 71.3 % corresponds to pollen from pines and Douglas firs, respectively. Contamination of the pollen cloud with alien pollen during the period of highest female cone receptivity of the native cedar (i.e., from second fortnight of September to second fortnight of October) was 78.7 %.

Neighborhood influence on canopy openness and the pollen environment

Native male cedars were the most important component of the tree neighborhoods surrounding the focal female cedars, followed by pine, as estimated by the NIs (Kruskal–Wallis test: $H = 58.907$, $p < 0.001$; a posteriori Tukey test $p < 0.05$ between all pairs). NIs

for male cedars, pines and Douglas fir averaged 0.033 (SD = 0.015), 0.006 (SD = 0.005) and 0.004 (SD = 0.003), respectively. NIs for all three taxa showed no significant spatial autocorrelation (Moran's I indexes ranged from -0.003 to -0.187, $p > 0.05$ in all cases; Online Resource 1, Table S1). Thus, each neighborhood can be considered as an independent sampling unit, and its associated variables as independent observations.

NI of male cedar was strongly influenced by density of neighboring male trees surrounding the focal female cedar (standardized partial regression coefficient, $b' = 0.57$, $p = 0.04$), but not significantly influenced by distance to focal female tree ($b' = 0.05, p = 0.8$) or DBH of the cedars ($b' = -0.03, p = 0.9$). For invasive conifers, both the density ($b' = 0.78$, $p < 0.001$ for Douglas fir and $b' = 0.912, p < 0.001$ for pine) and the distance ($b' = -0.64$, $p = 0.01$ for Douglas fir and $b' = -0.34$, $p = 0.004$ for pine) significantly influenced the NI of each taxon, whereas DBH of the trees had no significant influence ($b' = 0.35$, $p = 0.1$ for Douglas fir and $b' = 0.40$, $p = 0.4$ for pine). Thus, NI can be considered a proxy of the density or "crowdedness", of invasive conifers in the local neighborhood of female cedars.

Canopy openness, as estimated by the DSF, averaged 0.54 (SD = 0.20). Canopy openness (DSF) was influenced by all NIs (multiple regression, $df = 32$, $r^2 = 0.46$, $p = 0.032$). An equal increase in the NIs for all three taxa had comparable effects on canopy openness ($b' = -0.28$, $p = 0.024$ for cedar; $b' = -0.33$, $p = 0.008$ for Douglas fir and $b' = -0.32$, $p = 0.007$ for pines). Thus, the three taxa were comparable in their per-capita effect on light availability.

The amount of pollen sampled in the pollen cloud of female cedars from the native cedar, Douglas fir, and pines was greatly determined by the local abundance of each respective taxon. On one hand, we found a strong positive effect of the NI of male cedar ($b' = 0.66$, $p < 0.001$) and a positive, but less strong effect of canopy openness, as estimated by DSF ($b' = 0.30$, $p = 0.031$) on the mean number of conspecific pollen grains cm^{-2}. On the other hand, NIs of invasive conifers had a negative but weak effect on the mean number of conspecific pollen grains cm^{-2} (pines, $b' = -0.108$, $p = 0.040$; Douglas fir, $b' = -0.103$, $p = 0.043$). These four factors together explained 73 % of the variability (multiple regression

$F_{4,27} = 17.74$, $p < 0.001$) in the mean number of cedar pollen grains cm^{-2} in the pollen traps of focal female cedars. Thus, conspecific pollen availability in the pollen cloud seems to be primarily determined by the local density of male cedar trees, secondarily by canopy openness and to a lesser extent by the NI of invasive conifers (i.e., barrier effect).

Heterospecific pollen was also positively influenced by the local abundance of invasive conifers (Online resource 1, Fig. S1a, b). In particular, the number of pine pollen grains cm^{-2} was related to the NI of pines ($b' = 0.659$, $p < 0.001$) and secondarily to canopy openness ($b' = 0.256$, $p = 0.004$), these two factors explained 59.9 % ($F_{2,29} = 21.6$, $p < 0.001$) of the variation in the mean number of pine pollen grains cm^{-2} sampled in the traps. In turn, Douglas fir's pollen grains cm^{-2} was greatly affected by this species' NI ($b' = 0.568$, $p < 0.001$), but not by canopy openness (DSF, $b' = 0.159$, $p = 0.361$). The NI of Douglas fir explained 30.2 % of the variability in the number of pollen grains cm^{-2}. Thus, our analyses showed that the more invaded is the local neighborhood of a female cedar, the more contaminated with heterospecific pollen is the pollen cloud surrounding that tree.

Pollen in female cedars' pollen cloud and micropyles

In undeveloped ovules of *A. chilensis*, we could find pollen grains from pines and Douglas fir but not from the cedar (Online resource 1, Plate S1, S2 and S3). We found heterospecific pollen in 351 of the 722 undeveloped ovules we examined: 44.8 % of them had pine pollen only, 51.6 % had Douglas fir pollen only, and 3.6 % had pollen from both taxa. In micropyles with heterospecific pollen, we counted up to four pollen grains, with an average of 1.32 (SD = 0.34) pollen grains overall; 1.51 (SD = 0.63) for those with just pine pollen and 1.13 (SD = 0.56) for those with just Douglas fir pollen. Thus, pollen from both invasive conifers was equally represented in the micropyles of the native cedar.

At the tree level, the incidence of heterospecific pollen grains found in the micropyles of the native cedar increased with the amount of pollen from the alien conifers in the pollen cloud surrounding each focal female (Online resource 1, Fig. S1c, d) during the receptive period (second fortnight of September to second fortnight of October). The mean number of

heterospecific pollen grains on the micropyles of aborted ovules (including those micropyles with none pollen grains) related positively to the mean number of pollen grains found in the pollen traps ($b' = 0.62$, $p < 0.001$ for Douglas fir, Online resource 1, Fig. S1 c and $b' = 0.37$, $p < 0.001$ for pines, Online resource 1, Fig. S1d). The abundance of pollen from these two taxa in the local pollen cloud explained almost all the variation in the mean number of heterospecific pollen grains found in the micropyles of the native cedar ($r^2 = 0.964$, $F_{2,29} = 35.12$, $p < 0.001$).

Cone and seed set

Seed abortion occurred frequently as only 558 out of 1,280 (43.6 %) ovules developed into seeds. Individual cones ($n = 320$) had an average of 1.74 (SD = 1.17, range 0–4) seeds per cone. A multiple regression of canopy openness, as estimated by DSF, the frequency of micropyles with at least one heterospecific pollen grain (presence of alien pollen), and the mean number of heterospecific pollen grains per micropyles revealed that the presence ($b' = -0.830$, $p < 0.001$) rather than the number ($b' = -0.030$, $p = 0.796$) of heterospecific pollen grains in the micropyles was the most important determinant of seed set (mean of the proportion of seeds produced per cone per female cedar). Secondarily, canopy openness also positively influenced seed set ($b' = 0.36$, $p = 0.003$). Both, the strong negative effect of the alien pollen and the much weaker positive effect of light, explained 76 % of the variation in seed set ($F_{3,28} = 33.834$, $p < 0.001$). Considering in the analysis pollen from both alien taxa separately, revealed that the presence in the cedar's micropyles of pollen from Douglas fir had a much stronger effect than the presence of pollen from pine in reducing seed set ($b' = -0.59$, $p < 0.001$ vs. $b' = -0.24$, $p = 0.292$, respectively). The proportion of micropyles with at least one pollen grain of Douglas fir explained almost half of the variation in seed set across female cedar trees ($r^2 = 0.487$, $p < 0.001$).

Path analysis

Exploratory path analysis was used to summarize the proposed causal direct effects, through canopy openness (i.e., DSF), and indirect, through pollination, of the alien conifers on the seed set of the native cedar
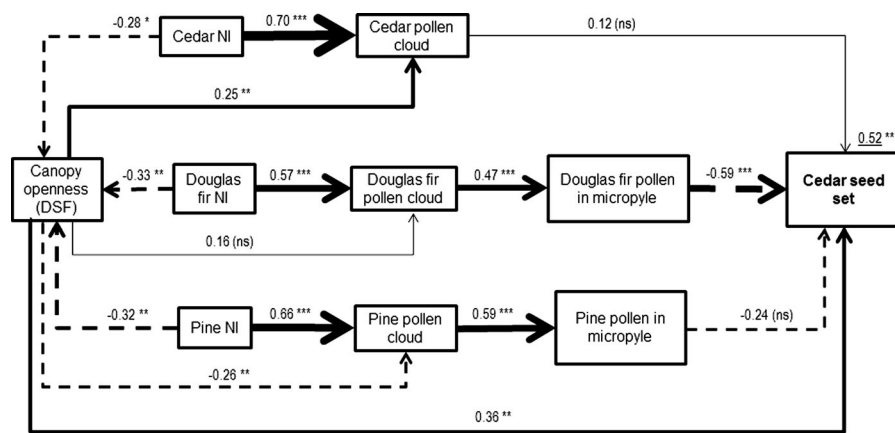
Fig. 4 Path analysis testing the effect of neighborhood composition on the cedar seed set mediated by pollination and resources, considering the effect of conspecific male cedars, and alien pines and Douglas fir. *Arrow* size is proportional to effect size. *Solid* and *dashed arrows* indicate positive and a negative effect, respectively. *ns* non-significant, $p > 0.05$, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

(Fig. 4). Overall, NIs of the native cedar and of each of the invasive conifers significantly and positively affected the amount of pollen of each respective taxon present in the pollen cloud surrounding each female cedar. Thus, the density, of pollen sources in the neighborhood, as estimated from the NI for male cedars, determined local conspecific pollen availability. The number of pollen grains of cedar in the pollen cloud was also affected by light availability as related to canopy openness, suggesting a decrease in local availability of cedar pollen in shadowed neighborhoods. Local increases in the abundance of cedar, Douglas fir and pine also reduced canopy openness (DSF). However, DSF was negatively, rather than positively related to pine pollen in the pollen cloud, whereas it was not related to Douglas fir pollen. An increase in the abundance of pollen from Douglas fir and pines in the pollen cloud increased the presence of pollen from these two alien taxa in the micropyles of the native cedar. However, although the presence of heterospecific pollen of both invasive taxa negatively affected seed set of focal cedars, only the effect of Douglas fir was strong and highly significant (Figs. 4, 5). Secondarily, canopy openness also positively affected seed set but to a much lesser extent than the presence of pollen from Douglas fir negatively affected it. The proposed model explained 52 % of the variance in seed set. Both a CMIN/DF = 1.388 and a CFI index = 0.898 suggest that the model adequately fits the data with the model explaining the correlations with an average error of 0.052 (RMSEA).

Discussion

Invasive conifers frequently outcompete native trees via resources usurpation (Richardson and Pyšek 2012), but their impact on the wind pollination of native trees has not been previously documented. In contrast, pollination-mediated interactions between animal–pollinated plant species sharing pollinators has been a topic that received much attention in the ecological literature, and more recently in the context of biological invasions (Burkle and Alarcon 2011; Gonzalez-Varo et al. 2013). For animal pollinated–plants, the mechanisms of this indirect type of plant–plant interactions are well known including impaired reproductive success of a focal plant species due to decreased pollinator visitation, conspecific pollen loss during visitation to intervening heterospecific flowers, and mechanical and chemical effects associated with alien pollen deposition (Traveset and Richardson 2006; Bjerknes et al. 2007; Morales and Traveset 2008, 2009). On the other hand, pollination-mediated interference has been a topic that has received much less attention among wind-pollinated plants despite that some of the most important plant invaders worldwide are conifers. Ours is, to the best of our
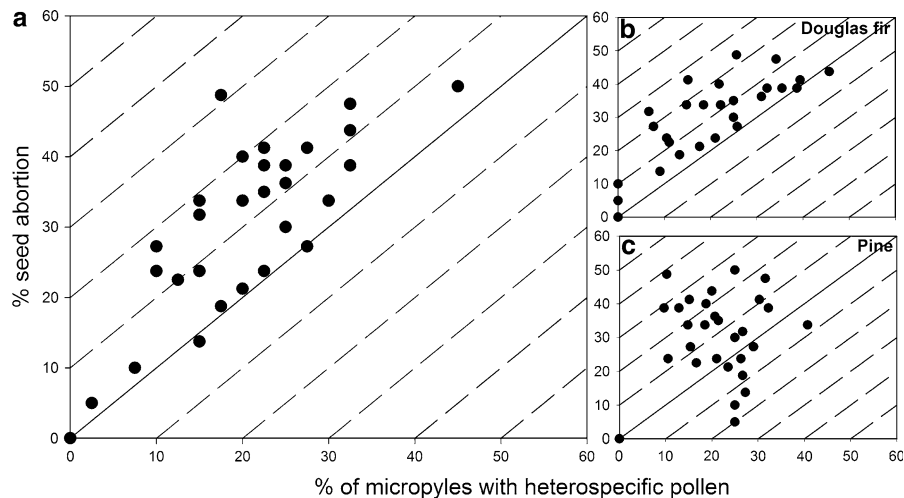
Fig. 5 Effect of alien pollen on cedar seed set. Relationship between the percentage of cedar seed abortion and **a** percentage of micropyles with heterospecific pollen grains (slope $\pm$ SE = 1.1394 ± 0.1340, $r^2 = 0.71$, $p < 0.0001$), **b** percentage of micropyles with Douglas fir pollen grains (slope $\pm$ SE = 0.7704 ± 0.2520, $r^2 = 0.67$, $p < 0.0001$) and **c** and percentage of micropyles with pine pollen grains (slope $\pm$ SE = 0.5601 ± 0.0989, $r^2 = 0.14$, $p = 0.03$). The isoline with slope of 1 and ordinate of 0 is indicated with a solid line, other isolines with slope of 1 and ordinates $\neq$ 0 are indicated with dashed lines. Slopes of regression lines estimated for heterospecific pollen in general and for Douglas fir do not differ significantly from 1 ($p > 0.05$), indicating that deposition of heterospecific pollen of this invasive conifer can explain seed abortion above basal levels

knowledge, the first study tackling this issue (but see Burgess et al. 2008 for angiosperms).

The synchrony of pollen and ovule production is an important contributor to plant reproductive success (Kelly and Sork 2002). In this study, we documented a high temporal synchronization (i.e., overlap) between the female cone receptivity and the aperture of pollen-producing aments in male trees. Interspecific pollination interference is then based on the assumption of an extensive overlap in the period of pollen release between native and alien plants. We indeed found that pollen release by the invasive conifers and the native cedar overlapped extensively (Fig. 3). Furthermore, pollen of invasive conifers saturated the pollen cloud surrounding female cedars, accounting for almost 80 % of pollen found in traps at the peak time of female-cone receptivity of the native cedar. Thus, the studied native cedar and the alien conifers meet all the conditions for the occurrence of pollination-mediated competition (Waser 1978; Bell et al. 2005; Mitchell et al. 2009): (1) sharing of the same pollination agent (i.e., wind), (2) extensive overlap in pollen release, and (3) heterospecific pollen transfer and eventual conspecific pollen loss associated with the dilution of

native pollen in a pollen cloud dominated by alien pollen.

Effective pollen dispersal in wind-pollinated species depends greatly on forest structure (Millerón et al. 2012), so that the mean effective pollen dispersal distance is lower under high density and/or when individuals are aggregated (Piotti et al. 2012). In these latter circumstances, pollen abundance in the pollen cloud will be much more affected by the reproductive biomass present in local neighborhoods rather than by the biomass of the entire forest stand or allochthonous pollen sources. Agreeing with this view, here we found that the amount of pollen of each of the three taxa (cedar, Douglas fir, and pines) in the pollen cloud surrounding each focal female cedar was strongly influenced by the density of nearby reproductive individuals. Only in the case of Douglas fir, we found an effect of distance to the plantation on the density of this alien species' pollen in the pollen cloud (Fig. 1) ($b' = -0.492$, $p = 0.036$), which was in any event of lower magnitude than the effect associated with the abundance of that taxon in the local neighborhood ($b' = 0.621$, $p < 0.001$). Thus, extirpation of the invasive conifers in invaded stands could be effective

in reducing the levels of pollen contamination despite the existence of nearby plantations.

Canopy openness also influenced positively the availability of cedar pollen in the pollen cloud surrounding female trees. Two non-mutually exclusive hypotheses might explain this relation. First, in forest ecosystems light is a highly limiting resource strongly affecting plant reproduction (McKinney and Goodell 2010). The Andean cedar is a light-demanding species, which depends greatly on light for growth and reproduction after establishment (Rovere 1991). Thus, it is plausible that light limits pollen production by male cedars particularly by regulating the number of male aments (Bloom et al. 1985). Second, trees in forest ecosystem may interfere with the transfer of wind-borne pollen grains by reducing wind speed and by representing a physical barrier that interrupts pollen flow (Gray et al. 2012). In any event, in relatively open pure cedar stands the high density that fast-growing invasive conifers can reach, might represent an important obstacle that interferes with the regular flow of cedar pollen from male to female trees (Oostermeijer and De Knegt 2004; Bacles and Ennos 2008). Thus, even if absolute distances between cedars remain unaltered, mates become physically isolated by the encroachment of alien trees. In this sense, intervening alien trees can play a similar role among wind-pollinated species as showy flowers of alien species among animal-pollinated species in causing conspecific pollen loss (Friedman and Barrett 2009; Mitchell et al. 2009). On the other hand, local abundance of pine pollen grains could be reduced in open, isolated neighborhoods, because their winged-appendices facilitate their transport away from the producing sources with increasing wind flow. In any event, in our study system light or environmental conditions associated with canopy openness played a minor role compared to neighborhood density and composition in determining pollen abundance and contamination of the pollen cloud surrounding a focal female tree.

Our study is one of the few (see also Burgess et al. 2008 for angiosperms) showing that detrimental impact of interspecific pollen transfer is not limited to animal-pollinated plants (Morales and Traveset 2008), and it can occur extensively in wind-pollination species. Specifically, we found that pollen clouds increasingly contaminated with alien pollen were tightly related to the proportion of undeveloped ovules containing alien pollen in their micropyles. Thus, we

can conclude that contamination of the pollen environment with alien pollen results in effective transfer of air-borne pollen between phylogenetically distant coniferous species. In turn, an increasing proportion of undeveloped ovules with the presence of heterospecific pollen in their micropyles were associated with decreasing seed set. This adds evidence to a growing literature showing the detrimental effect of heterospecific pollen on plant reproduction (Brown and Mitchell 2001; Matsumoto et al. 2010). However, establishing a causal relationship is difficult because the presence of heterospecific pollen may just indicate the absence of conspecific pollen and therefore of fertilization. After all, conspecific pollen could not be detected in any of the examined undeveloped ovules. Alternatively, the relatively small cedar pollen grains could easily become dislodged over time and during ovule preparation for examination compared to the much larger Douglas fir and pine pollen grains. Several lines of indirect evidence, however, point at the deposition of heterospecific pollen as a primary factor causing seed abortion. First, canopy openness seems to be more influential on seed set than cedar pollen availability (Fig. 4). This might indicate that seed production in the native cedar is light-rather than pollination-limited. There is evidence that at least cone production in light-demanding species, like ours, is highly limited by this resource (Bloom et al. 1985; Niesenbaum 1993; Greene et al. 2002). Second, seed abortion above 10–30 % fits suspiciously tight to the incidence of undeveloped ovules with heterospecific pollen in their micropyles (Fig. 5a). Third, the differential impact of the two taxa on seed set also suggests that is not the absence of conspecific pollen but the presence of particular heterospecific pollen that causes seed abortion (Fig. 5b, c). In this case, the presence of Douglas fir pollen, rather than the presence of pine pollen, in the micropyles of the native cedar seems to be the main factor triggering seed abortion in the native conifer. The fact that the effect was primarily associated with the presence rather than the number of pollen grains from Douglas fir, (i.e., for micropyles with >0 pollen grains) might point out to a physiological or allelophatical rather than a mechanical interference (Zenkteler and Relska-Roszak 2003), perhaps associated with a false biochemical signal delivered by this alien pollen.

Direct and indirect negative impacts of alien conifers on the reproduction of the native cedar, *A.*

chilensis, can be inferred from our study. On one hand, rapid invasive conifers growth and encroachment reduce canopy openness decreasing seed set of the native cedar directly via resource limitation (Fig. 4). On the other hand, invasion by more shade-tolerant Douglas fir decreases seed set indirectly via hetero-specific pollen transfer, due to the strong overlap in pollen release with native cedar. In the present study, this latter pollination-mediated effect was at least twice as strong as the resource-mediated effect. Interestingly, the fact that the NI of Douglas fir was on average one order of magnitude lower than that of cedar suggests that this strong pollination-mediated impact acts since early stages of invasion. In the extreme, severe reduction of seed set due to invasive conifers can compromise the long-term viability of the native cedar because of the disruption of the pollination process. These results support the recommendation of the urgent eradication of these alien conifers from invaded cedar stands. However, this measure will be ineffective in the long-term if the propagule sources represented by the plantations are also not properly managed.

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