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ORIGINAL PAPER

Quantifying how acquired interactions with native and invasive insects influence population growth rates of a non-indigenous plant

Wilfredo Falcón · **James D. Ackerman** · **Raymond L. Tremblay**

Abstract Non-native species often acquire novel interspecific interactions, which are central to several hypotheses of invasion success, including biotic resistance and invasional meltdown. However, the outcome of these interactions is not often linked with the demographic evidence based on the full life cycle of the species. The Philippine Ground Orchid (*Spathoglottis plicata*) has invaded Puerto Rico and has acquired both negative and positive interspecific interactions involving the native weevil *Stethobaris polita* and the invasive red fire ant *Solenopsis invicta*, respectively. We studied a population in the Rio Abajo Forest, and asked how these interactions affect population demography by using a combination of field, experimental and modelling approaches. Stage-structured matrix population models based on four years of field observations showed that the population of *S. plicata* is growing at a rate (λ) of 1.05 under natural conditions. When we modified fecundity values based on experimental exclusion of weevils and ants, the control treatment showed a similar λ. Excluding weevils increased λ to 1.20, whereas the exclusion of ants decreased λ to 1.03. When we incorporate demographic and environmental stochasticity in our models, exclusion of invasive red fire ants significantly reduces the orchid abundance over time. Although weevils offer some biotic resistance to *S. plicata*, these effects do not prevent orchid population growth and expansion. On the other hand, invasive red fire ants have a positive effect on the invasive orchid's λ, partially supporting the invasional meltdown hypothesis. This study presents a method that allows one to combine opposing mechanisms of species interactions within the same quantitative framework, and the results highlight the importance of considering acquired plant–animal interactions and stochastic processes when evaluating the population growth rates and dynamics of invasive plants.

Keywords Biotic resistance · Demography · Invasional meltdown · Invasive species · Orchidaceae · Plant–animal interactions · Population dynamics

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Introduction

Introduced non-native species often acquire novel interspecific interactions outside their native range, which affect the outcome of the invasion (Levine and D'Antonio 1999; Levine et al. 2004; Richardson et al. 2007). The effects of these biotic interactions on the population performance of non-native species may vary from negative, neutral, to positive, and serve as a basis for different hypotheses in invasion biology. For example, non-native species that are kept in check by predators or herbivores in their native range may experience a release from these antagonistic interactions in their introduced range, which positively influences the population performance (Elton 1977). On the other hand, receptive native communities may have local predators or herbivores that preferentially consume non-native species, and thus negatively affect their invasion success (Parker and Hay 2005). Moreover, biotic interactions between non-native species may have positive synergistic effects in a way that increases their invasion success, which in turn negatively affect the receptive communities (Simberloff and Von Holle 1999; Lach et al. 2010).

Understanding the demography of invasive species is essential to comprehend the processes that contribute to the establishment and spread of a particular species outside its native range, and to develop sound management strategies when needed. Many studies on species invasions measure specific life history traits to study the role of acquired interspecific interactions in the novel ecosystems (Levine et al. 2004; Mitchell et al. 2006). However, the outcomes of these interactions are not often linked with the demographic evidence which integrates an approximation of the full life cycle of the species, and measured at the population level.

Matrix population models provide the means to combine multiple vital rates based on life cycles, that allows one to evaluate how changes in these rates affect population dynamics (Caswell 2001; Sakai et al. 2001; Morris and Doak 2002). Although such models have been used to evaluate population viability, and to develop management strategies for the conservation or control of species populations (Hastings 1997; Caswell 2001; Morris and Doak 2002; Fieberg 2004), their use to understand the effects of acquired interspecific interactions on the population dynamics of invasive plants remains limited. Using these models could offer a more quantitative assessment to dissect the importance of processes regulated by biotic interactions in relation to other processes affecting the invasion success of a given species, and allows one to combine opposing mechanisms within the same quantitative framework.

In this study we assess how matrix population models can serve to quantify the effects of antagonistic and beneficial acquired interspecific interactions on the demography of invasive plants by coupling demographic data of the invasive Philippine Ground Orchid (*Spathoglottis plicata* Blume) with data from exclusion experiments in Puerto Rico. The Philippine Ground Orchid is one of several species of Orchidaceae that have escaped cultivation and established invasive populations in Puerto Rico, and was generally considered innocuous (Ackerman 2007; Ackerman et al. 2011; Recart et al. 2013; Ackerman et al. 2014). However, a recent study found that *S. plicata* is having negative ecological impacts on the native orchid *Bletia patula* Graham through apparent competition mediated by a native orchid-specialist weevil (*Stethobaris polita* Chevrolat) (Recart et al. 2013). Moreover, invasive red fire ants (*Solenopsis invicta* Buren) forage at the extrafloral nectaries present in the inflorescences of *S. plicata*, and may protect the inflorescences against weevil florivory and oviposition (Ackerman et al. 2014).

We first asked what life-cycle stages are more important for the invasive orchid's success, and what is the population growth rate (λ) under natural field conditions? By evaluating the resulting projection matrices based on the demographic field study carried out in a population of *S. plicata* for four continuous years, we expected to identify important life-cycle stages and transitions for the orchid's population performance. Moreover, and because *S. plicata* is an invasive species expanding its range in Puerto Rico, we expected our population to exhibit a positive λ. We then asked what are the effects of acquired interspecific interactions on the invasive orchid's population growth rate? We assessed this question by incorporating into our matrix population models the expected fecundity based on reproductive success data derived from ex situ weevil and ant exclusion experiments. We anticipated to find agreement between the resulting population performance metrics of both our field (observed demography) and control (exclusion experiment treatment)-derived matrices as a validation to

our approach (both include the three-way interactions). We expected to find that native weevils significantly reduce λ, but fail to control the invasive orchid, whereas invasive red fire ants increase λ by protecting the inflorescences. Finally, we sampled plants throughout their invasive range in Puerto Rico and Hawai'i, and compared their reproductive success in the presence or absence of antagonistic weevils. We expected to find significantly lower reproductive success in populations were weevils were present.

Methods

Study area

Our study site was located along Highway 10 in the Río Abajo State Forest (18°20.96N, 66°41.07W) in the municipality of Arecibo, which lies within the northern karst region of Puerto Rico, and is classified as Subtropical Moist Forest according to the Holdridge Life Zone system (Ewel and Whitmore 1973). The vegetation in the study area was characteristic of early secondary succession, with grasses and ferns dominating the area, followed by scattered shrubs (e.g., *Piper aduncum* L., *Gesneria pedunculosa* (DC.) Fritsch) and young trees (e.g., *Tabebuia heterophylla* (DC.) Britton). As a result, the area is open with high light availability, although local variation exists.

Study system

Spathoglottis plicata is a caespitose perennial herb that depends on seed production and wind dispersal for seedling establishment and population growth, with a mixture of autopollination (sensu Kearns and Inouye 1993) to outcrossing reproductive strategies within and between populations throughout its range (Ackerman et al. 2014). *Spathoglottis* has sympodial growth but because rhizomes are very short, the pseudobulbous shoots are caespitose. As pseudobulbs age they may lose their leaves, but new shoots form so that healthy plants are continuously photosynthetic (and lack a dormant stage at our site). *Spathoglottis plicata* can survive for over four years (this study), and rarely do plants become large enough to form independent ramets so clonal reproduction is unlikely to be a significant contributor to λ. Individual plants usually produce a single many-flowered inflorescence at a time, but will develop several over the course of a year. Flowers either develop into mature fruits approximately four weeks after pollination, or are either dropped (if not pollinated) or aborted, leaving a scar in the inflorescence. Fruits stay attached to the inflorescence long after dehiscence. Once seeds are dispersed, they may germinate in 1–2 weeks and flower within a year (Prakash and Leelee 1973; Thompson and Wright 1995; Ackerman et al. 2014).

The Philippine Ground Orchid is the most common and widespread species of the genus, with several forms cultivated and sold as ornamentals. The autopollinated forms of *S. plicata* have escaped cultivation and exotic populations have been reported in West Africa, Kenya, Mascarene Islands, Seychelles, Hawai'i, Florida, Cuba, Dominican Republic, Puerto Rico, US Virgin Islands, the Lesser Antilles, and Panama (Jolliffe 2010; Ackerman et al. 2014). In Puerto Rico, *S. plicata* mostly exploits naturally or anthropogenically disturbed (open) areas of moist and wet regions from near sea level to about 1000 m (Ackerman 1995; Ackerman et al. 2014). However, they can also be found inside mature forests, especially along riparian areas (WF personal observation). The first naturalized population was discovered in the mid 1970s within El Yunque National Forest in eastern Puerto Rico (R. Woodbury personal communication to JDA, 1981), and became noticeably common by the first decade of the new millennium. Based on local herbarium holdings (MAPR, UPR, and UPRRP), two specimens were collected in the 1980s, one in the 1990s, and 31 in the 2000s, and location records over time seem to indicate east to west spread. Collecting efforts were intense during these decades as floristic studies were in progress (Ackerman 1995; Axelrod 2011), so we may assume that the collection records reflect the lag-phase often observed in invading species. Populations of *S. plicata* have been established in the Río Abajo State Forest since at least 1995, when the first populations were reported (*Axelrod 9190*, UPRRP). Currently, white and pink flowered morphs are found on the island, and fruit set (FS; a measure of reproductive success given by the number of fruits divided by the sum of fruits and scars) can be over 50% (Recart et al. 2013). Seed set (proportion of seeds with visible embryos) in our study area can reach up to 90% (JDA personal observation). This is comparable to populations in Hawai'i, where

fruit set is 66% (Kores 1980), and the mean seed set is 76% (±19; N = 12).

In Puerto Rico, *S. plicata* has acquired several interspecific interactions (see Ackerman et al. 2014 for a detailed description of the system). *Stethobaris polita* is a relatively small black weevil, which is an orchid specialist native to Puerto Rico that feeds on flowers and oviposits in fruits, thus reducing the reproductive output of both invasive and native orchids (Recart et al. 2013; Ackerman et al. 2014). The average seed set of *S. plicata* fruits with oviposition is 8% ± 0.17 (N = 20 fruits), but it is unknown whether these seeds would ultimately be viable as we often found rotten fruits with fungal and/or bacterial infections. When weevil damage is severe, an inflorescence will exhibit rotting fruits, buds and apices (Ackerman et al. 2014).

In Puerto Rico, red fire ants (*Solenopsis*) forage for extrafloral nectar produced on buds and floral bracts of *S. plicata*. Ants of the genus *Solenopsis* have a cosmopolitan distribution, occupy a wide range of niches, and several species are native to Puerto Rico (Smith 1936). In the Rio Abajo State Forest, the invasive *S. invicta* was the only red fire ant observed foraging for extrafloral nectar, they outnumbered other species of ants visiting the inflorescences 2 to 1, and they can attack or deter weevils from feeding on the orchids (Ackerman et al. 2014). *Solenopsis invicta* is a common invasive species throughout the world, and the polygynous forms are widespread in disturbed and open areas in Puerto Rico and other Caribbean islands (Global Invasive Species Database; Davis et al. 2001; Wetterer and Snelling 2006). While they may be passengers rather than drivers of ecosystem change (King and Tschinkel 2008), they are known to cause negative impacts on community structure, particularly arthropods (e.g. Porter and Savignano 1990).

Field methods

To study the population biology of *S. plicata* in our study site, we established a total of 8 (1 × 3 m) plots (8–10 m distant from each other), from which we gathered demographic data. In each of the plots, we located and identified all individuals of *S. plicata* using unique-numbered metal tags (attached to the base of each plant). Four censuses were conducted: the first in January 2009, and subsequently in November of 2009–2011. As a consequence, the first time

interval is 11 months, and the other two were 12 months. After taking into account the differences in time intervals, we found that the effects of having an 11-month time interval for the first census did not affect our results and inference, so we used this time interval without correcting for time. The four different censuses conducted from early 2009 until late 2011 are referred to as t_0, t_1, t_2, and t_3, and the time transitions 2009–2009, 2009–2010, and 2010–2011 are transitions t_0–t_1, t_1–t_2, and t_2–t_3, respectively.

For every individual in each census, we measured the width and length of the longest leaf, as well as the reproductive status (i.e. presence of active inflorescences, number of opened flowers, the number of fruits, the proportion of damaged fruits, and the number of scars present). Beginning in the second census, we recorded the number of new recruits, as well as the number of dead individuals.

Model construction and parameterization

To project the population dynamics of *S. plicata* at this site, we used size-structured matrix population models (Lefkovitch 1965). The width of the longest leaf was used as a measure of size for the model. Previous studies have successfully used a variety of leaf measures as indicators of size for orchids (Montalvo and Ackerman 1987; Tremblay and Hutchings 2003), and preliminary analyses indicated that reproductive effort (RE; total number of flowers produced given by the sum of fruits and scars) was significantly correlated with this variable (Kendall $\tau = 0.28$, $p < 0.001$; for the final dataset). We employed the Vandermeer–Moloney algorithm for determination of size-stage categories (Vandermeer 1978; Moloney 1986). This algorithm minimizes both the sampling error, and the error of distribution (Vandermeer 1978). The sampling error measures choosing a category that is too small, while the error of distribution measures choosing a category that is too big. This method is appropriate when a species has no traits that distinctly define life history stages (Rosenberg et al. 2005).

Precise measures of fitness in the Orchidaceae are not practical because of the minute size of the seeds, the large number of seeds produced in a single fruit, and the difficulty in measuring the contribution of adult plants to seedling establishment. As an alternative, Tremblay and Hutchings (2003) suggested that fecundity values for this family may be calculated

using the number of fruits produced by flowering plants or the number of new recruits to the population (with the latter being more biologically relevant). Unfortunately, this method does not credit the production of recruits to specific parents (Tremblay and Hutchings 2003), which could be problematic when more than one size-stage contributes to the production of new recruits and that recruitment is not a linear function. However, the number of recruits to an orchid population is often assumed to be related to fruit-set (Tremblay and Hutchings 2003), which has been demonstrated in epiphytic orchids (Ackerman et al. 1996). Assuming that the number of new recruits added to a population is related to fruit-set, and that fruit production differs among plant size-stages, we calculated the recruitment rate as a ratio of new recruits added to the population to the total number of fruits produced in the previous year. We used this approach to determine the expected proportional contribution of a given size-stage as a function of the number of fruits produced at a given size-stage (see S1 for detailed methodology).

The Vandermeer–Moloney algorithm applied to the size distribution of the first transition (t_0–t_1) resulted in six size categories (Table 1). After establishing the size categories, we calculated the average life stage transition probabilities for each matrix (Caswell 2001) (see S2 for detailed methodology). The calculations of the demographic parameters and the asymptotic population growth rates (λ; maximum eigenvalue) from the projection matrices were done using the R package 'popbio' (Caswell 2001; Stubben and Milligan 2007).

We calculated the fecundities as the number of recruits produced per individual at a given size stage

Table 1 Size-stages and population size of *Spathoglottis plicata* divided by each size-category per census period in the Río Abajo State Forest, Utuado, Puerto Rico

Leaf width (mm)	Size-stage	t_0	t_1	t_2	t_3
0–11	1	24	13	10	15
≥11–20	2	47	35	8	13
≥20–35	3	89	83	67	68
≥35–40	4	23	31	32	19
≥40–50	5	23	37	46	49
≥50	6	11	22	36	50

"0" indicates absence of leaves in an active pseudobulb

under observed natural field conditions within our demography plots (from here on, "field conditions"; see S2 for details). This represents the matrix under natural conditions, which includes the influence of the interactions with weevils and ants. Thus, the resulting asymptotic population growth rates (λ), and the geometric mean population growth rates (λ_{geom}; mean λ between time transitions) obtained from the matrices derived from our field data should reflect what is happening in our study population. Figure 1 shows the simplified life cycle of *S. plicata* based on the observed transitions in our field study, and the interspecific interactions with native weevils and invasive red fire ants, which occur only on reproductive individuals.

We analyzed the contribution of each demographic parameter to the asymptotic population growth rate using the elasticity analyses (Caswell 2001). This analysis shows the relative contribution of the individual life-cycle transitions to the population growth rate (Caswell 2001). Elasticities can be used to determine which life-cycle transitions have the most influence on λ, and thus help delineate sound management strategies for the control (or conservation) of populations (Horvitz and Schemske 1995; Hastings 1997; Morris and Doak 2002; McCullough et al. 2006).

Effects of acquired interspecific interactions on λ

To investigate the effects of acquired plant–animal interactions on the orchid's λ, we used the approach described previously for calculating the fecundities, in combination with data from this study and exclusion experiments from Ackerman et al. (2014), whose study sites were approximately 100 m distant from ours, and whose study duration overlapped with ours. They performed four treatments that included: (a) control-unmanipulated inflorescences accessible to weevils and ants (0.145 ± 0.045 fruit set), (b) weevil exclusion (0.563 ± 0.050), and (c) ant exclusion (0.097 ± 0.047). To calculate the expected fecundity values for any given treatment, we used the reproductive effort (total number of flowers produced; given by the sum of the number of flowers, fruits and scars) of each plant during each census and the estimated fruit set according to each experimental treatment (see S2 for details). To incorporate the observed variation in fruit set for each treatment, we generated state-fate
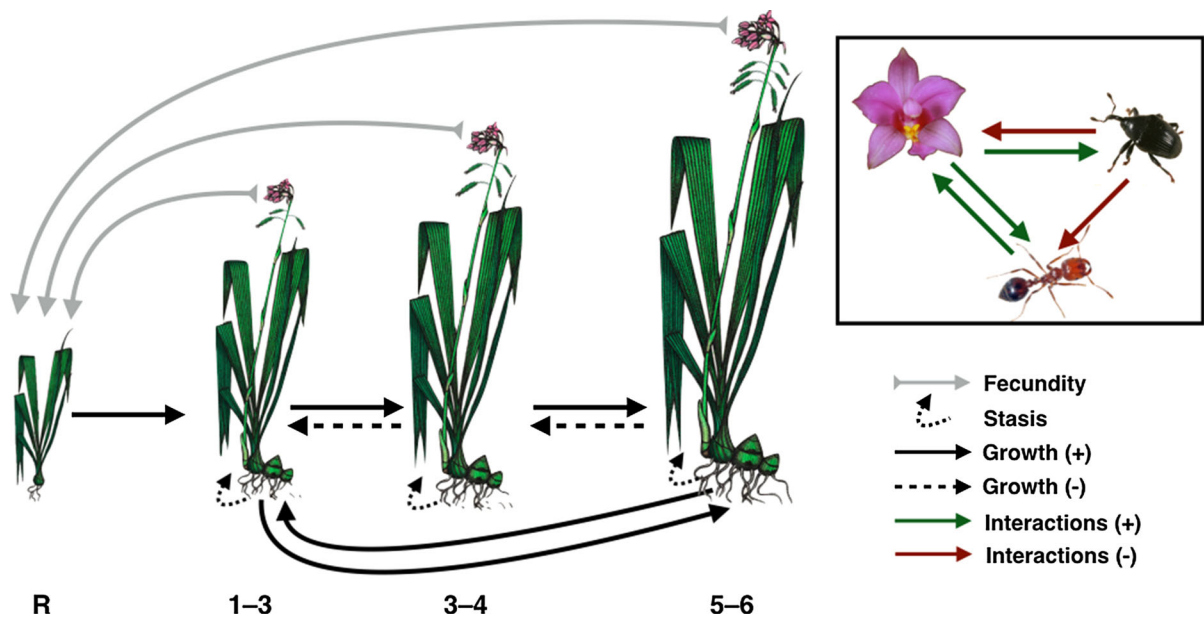
Fig. 1 Simplified life cycle of *Spathoglottis plicata* based on the observed stage-size transitions and fecundity contributions in our field demography study, and the acquired interactions with native weevils and invasive ants in Puerto Rico. In the life cycle, "*R*" represents recruits, and numbers represent the size-stages. Native weevils (*Stethobaris polita*) feed on orchid flowers and oviposit in fruits, while invasive red fire ants (*Solenopsis invicta*) forage at extrafloral nectaries and often attack weevils. Orchid drawing from Lewis Roberts (modified): [CC BY-SA 3.0 (http://creativecommons.org/licenses/by-sa/3. 0)], via Wikimedia Commons. Weevil image by Tom Murray, and ant image by WP Amstrong (modified and used with authorization)

transition data frames with estimated fruit sets by drawing random numbers from a normal distribution based on the mean and standard deviation of the fruit set for each treatment, and replicated this 100 times per treatment per time-transition.

Reproductive success throughout the invasive range

To put our results in context, we performed rapid surveys in different sites/populations of *S. plicata* outside our demography study area to collect information on reproductive success (fruit set) following the same methods indicated above (see S5 for locations). In Puerto Rico, we re-analyzed data of the 4 m^2 plots sampled in adjacent sites to our demographic study area in the Río Abajo State Forest in 2011 (during t_3, 100–500 m apart; Ackerman et al. 2014), and sampled three additional populations during 2012 found around El Yunque National Forest (east of the island, where *S. plicata* populations were first reported). Furthermore, we sampled populations in the Hawaiian Archipelago in 2010–2011 (Big Island, Kauai, Maui, and O'ahu islands), where *S. plicata* is invasive and

orchid-specialist weevils (*Orchidophilus sp.*) are rare on these plants.

Statistical analyses

All statistical analyses were run with R (v. 3.2.0, R Core Team 2015). Mean values ±SD are reported, unless otherwise stated. We selected and performed parametric and non-parametric tests based on the distribution of the data. To determine if there were population growth rate differences between time transitions and treatments, we calculated the bootstrap distribution of λ by randomly sampling with replacement from the stage-fate data of observed transitions, and observed and modified fecundities—based on the exclusion experiments (reported as λ_{boot}) (Caswell 2001; Morris and Doak 2002; Stubben and Milligan 2007). To project the long-term effects of the acquired interactions with weevils and ants, we projected the three transition matrices derived from this study (field conditions), and the matrices with the different fecundity values calculated based on the exclusion experiments (100 matrices per treatment per time

transition). We used the number of individuals at each of the six size-stages recorded in the first census (t_0; Table 1) as the initial population vector, and projected the matrices for 60 time steps, with demographic and environmental stochasticity. Because these projections are based on a stochastic population growth rate (λ_{sto}) rather than the asymptotic population growth rate (λ), we calculated the log stochastic population growth rate by Tuljapukar's approximation ($\lambda_{T\,approx}$) and by simulation (λ_{Sim}), and generated 95% CI for comparison from simulations using 5000 iterations (Morris and Doak 2002). Finally, we tested for differences between the reproductive success data from our demographic study and the other sites/ populations, and visualized the data using the R package 'yarrr' (ND Phillips 2016, https://github.com/ndphillips/yarrr), which allows to plot the raw data together with the frequency distribution, mean, and the 95% Bayesian Highest Density Interval (HDI).

Results

Demography of *Spathoglottis plicata*

We marked a total of 281 individuals of *S. plicata*, and found a tendency for plants to increase in size (width of the longest leaf) over time (Table 1). The mean growth per year was 4 mm $\pm$ 16, however, when we compare the change in plant size (growth) between years, we found no significant differences (N = 555 for all available pairs, $X^2 = 1.51$, df = 2, $p = 0.47$). During our study period, and based on analysis of the projection matrices derived from the observed field conditions, *S. plicata* was not at stable-stage distribution in our site. Intriguingly, there was a reduction in the number of individuals recruited to size categories 1–3 compared to previous and subsequent year at t_2 (Table 1).

The demographic parameters of the orchids exhibited variation throughout the study period (Table 2). Flowering probabilities ranged from 0.12 to 0.42 across years, and reproductive individuals produced a mean of 19–33 flowers per year. We found a strong positive correlation between reproductive effort and weevils damage, given by the sum of fruits with weevil oviposition and scars (Kendall$\tau = 0.84$, $p < 0.001$). Fruit set ranged from 21 to 46%, and weevil damage to fruits reduced the effective fruit set

Table 2 Summary of demographic data of *Spathoglottis plicata* collected in the Río Abajo State Forest for four continuous years

Parameter	t_0	t_1	t_2	t_3
ψ	0.12	0.38	0.42	0.34
RE	34	20	19	29
Fr	3	5	7	5
FS	0.21	0.46	0.44	0.27
EFS	0.07	0.26	0.25	0.14
Recruits	–	19	15	28
Deaths	–	9	12	13
Not found$_{(t)}$	2	11	8	30
Found$_{(\geq t+1)}$	2	8	7	–
Observed N	217	221	199	214
True N	219	229	206	–

t_0–t_3 stands for each census (year), ψ is the flowering probability, *RE* is the mean reproductive effort. *Fr* is the mean number of fruits produced per plant per census, *FS* is the mean fruit set given by the proportion of flowers that develop into fruits per inflorescence/plant while *EFS* is the effective fruit set, which discounts all fruits damaged by weevils (both are measures of reproductive success). *Recruits* is the total number of seedlings recorded each year, and *Deaths* is the total number of plants that died each year. *Not Found$_{(t)}$* indicates the number of marked individuals not found at time t and *Found$_{(\geq t+1)}$* indicate the number of individuals that were not found at time t but that were alive at $\geq t + 1$. Based on the latter, the observed population size (*Observed N*) was corrected to reflect the true population size (*True N*)

(EFS) by even more than half in the first census (i.e. the number of fruits undamaged by weevils). The total numbers of recruits ranged from 15 to 28 per census, and between 9 and 13 plants died each year. The mean plant density per year was 9.3/m^2, and ranged between 2 and 16 plants per m^2 (for all plots and censuses combined). We found no indication of density-dependent recruitment during the three time transitions (Kendallτ, $p = 0.70$) when we compared the association between the density of each plot during the census at time t, and the number of new recruits found at time $t + 1$. Moreover, in all censuses following t_0, some individuals were not found, but were recorded alive when observed in subsequent years (Table 2).

Differences in the size-stage projection matrices for the individual time transitions reflect the population's demographic variation over the years. Survival probability increases as the size-stage increases, and there

was considerable variation in size-stage 1 (see S4A). The most noticeable difference is the survival of individuals in size-stage class 1 in t_0–t_1, in comparison to the other time transitions. Moreover, as size-stage increased, so did the mean flowering probability, as well as the mean production of flowers and fruits (see S4B–D). The population projection matrices derived from our field study reflected these temporal differences (Table 3). Overall, the average projection matrix showed that plants tended to remain in stasis or increase in size—especially to size stages ≥3, and that plants from size-stages 5 to 6 contributed a disproportionately higher fecundity (Fig. 3a). This was supported by the elasticity analyses (Table 3) whereby the average elasticity matrix indicated that survival and stasis of plants in size stages 3 and 5–6, along with transitions between the two larger size classes contributed the most to λ (Fig. 3b). Moreover, the elasticity analysis (and average elasticity matrix) suggested that while the influence of survival and growth on λ was stronger than that of fecundity, the effects of the latter were also greatest in the two largest size stages (Fig. 3b).

Are there temporal differences in population growth rates of the invasive orchids? Does damage to flowers and fruits by the native weevil *S. polita* drive these differences? Under field conditions, the 95% CI of λ showed no differences between years (Table 4A). However, λ values within transitions do respond to weevil effects. When weevils were excluded, λ was higher than that produced under field conditions. Still, the 95% CI of the transition t_1–t_2 include values of λ <1 for both field and weevil exclusion conditions.

Effects of acquired interspecific interactions on λ

When the three-way interactions with weevils and ants occur, the invasive orchid population at our site exhibited a positive λ both under observed fecundities in the field as well as in those estimated based on the control treatment of the exclusion experiments (Table 4B; based on sampling with replacement from the stage-fate data). Here, the ant exclusion treatment is not significantly different from the controls, while the weevil exclusion treatment significantly increases λ. The stochastic population growth rates, on the other hand, showed significant differences between treatment types: under field and control conditions $\lambda_{sto} = 1.05$, without ant protection $\lambda_{sto} = 1.03$, and

without weevil damage $\lambda_{sto} = 1.21$ (Table 5). Similarly, when we projected populations over time, the exclusion of ants and weevils showed significantly different effects from field conditions and controls (Fig. 2). The projected abundance without ants after 60 time steps was negative (42% decrease) in relation to field conditions, whereas under weevil-free conditions it was positive (367 times greater).

Reproductive success throughout the invasive range

We found and sampled populations of *S. plicata* in different habitat types at different elevations, with presence or absence of antagonist weevils (Fig. 4; see S5 for locations and summary statistics). For reproductive plants, despite finding significant differences in terms of plant size (Kruskall-Wallis $X^2 = 213.46$, $df = 8$, $p < 0.01$), the mean reproductive effort was similar among all populations (Pairwise Wilcoxon with Bonferroni–Holm correction $p > 0.06$, for all pairs) (see Hochberg 1988 for details on Bonferroni-Holm correction). Moreover, reproductive success data from populations of the Philippine Ground Orchid throughout its invasive range shows a clear distinction with regards to the presence or absence of weevils (Wilcoxon rank sum test $W = 13,680$, $p < 0.01$). In Puerto Rico, when weevils are present, the mean fruit set was 0.31 ± 0.30, showing high variability. If fruits with weevil damage are excluded (effective fruit set), the mean reproductive success is reduced further to 0.19 ± 0.33. Moreover, damage to flowers and fruits by weevils in these populations seem to skew the reproductive success of invasive orchids towards the lower end of the distribution (Fig. 4). On the other hand, weevil-free populations in Puerto Rico and Hawai'i had a considerably higher mean reproductive success of 0.59 ± 0.30. These results were consistent even when we excluded the four years of data from our demography plots.

Discussion

The effects of novel interspecific interactions on introduced species may span from negative, neutral, to positive, and may involve both native and non-native species. Kelly and Dyer (2002) suggested that life cycle based models incorporating demographic

Table 3 Projection and elasticity matrices of *Spathoglottis plicata* in the Río Abajo State Forest, where the invasive orchid acquired novel interactions with weevils and ants

Stage	t_0-t_1						t_1-t_2						t_2-t_3					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Projection matrices																		
1	0.261	0.085	0.033	0.000	0.674	0.042	0.143	0.107	0.038	0.064	0.075	0.549	0.200	0.250	0.063	0.172	0.316	0.329
2	0.391	0.391	0.036	0.000	0.000	0.000	0.000	0.214	0.000	0.000	0.033	0.000	0.200	0.375	0.032	0.000	0.000	0.000
3	0.174	0.413	0.512	0.130	0.227	0.182	0.000	0.429	0.507	0.233	0.067	0.136	0.300	0.125	0.540	0.310	0.111	0.185
4	0.000	0.065	0.179	0.304	0.091	0.182	0.000	0.107	0.192	0.267	0.067	0.091	0.100	0.000	0.143	0.069	0.000	0.111
5	0.000	0.000	0.179	0.391	0.364	0.364	0.000	0.036	0.164	0.200	0.467	0.409	0.000	0.125	0.127	0.310	0.467	0.259
6	0.000	0.000	0.071	0.174	0.318	0.273	0.143	0.071	0.068	0.200	0.333	0.318	0.000	0.125	0.127	0.241	0.400	0.370
Elasticity matrices																		
1	0.024	0.005	0.005	0.000	0.065	0.003	0.006	0.000	0.001	0.002	0.005	0.029	0.023	0.010	0.010	0.008	0.036	0.038
2	0.048	0.030	0.007	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.009	0.000	0.030	0.019	0.007	0.000	0.000	0.000
3	0.028	0.042	0.125	0.014	0.039	0.020	0.000	0.006	0.094	0.026	0.020	0.035	0.052	0.008	0.132	0.022	0.019	0.032
4	0.000	0.008	0.049	0.037	0.018	0.023	0.000	0.001	0.036	0.030	0.021	0.023	0.019	0.000	0.039	0.005	0.000	0.021
5	0.000	0.000	0.064	0.061	0.091	0.058	0.000	0.001	0.035	0.026	0.164	0.120	0.000	0.010	0.040	0.029	0.104	0.059
6	0.000	0.000	0.020	0.021	0.062	0.034	0.037	0.001	0.015	0.028	0.126	0.100	0.000	0.009	0.038	0.021	0.083	0.078

Native weevils (*Stethobaris polita*) eat the flowers and oviposit on the fruits of the invasive orchids whereas invasive red fire ants (*Solenopsis invicta*) forage for extrafloral nectar and protect plants against weevils

Table 4 Population growth rates (λ) of *Spathoglottis plicata* for each time transition (t_0–t_3) and fecundity treatment (A), and summary metrics for all time transitions combined (B)

Treatment	λ	λ_{boot}	95% CI
A Independent transitions			
t_0–t_1			
Field	1.10	1.09	1.008–1.180
Control	1.14	1.13	1.055–1.222
Ant exclusion	1.10	1.10	1.036–1.167
Weevil exclusion	1.36	1.35	1.182–1.555
t_1–t_2			
Field	0.98	0.98	0.912–1.076
Control	0.98	0.98	0.912–1.069
Ant exclusion	0.97	0.97	0.911–1.037
Weevil exclusion	1.06	1.06	0.912–1.347
t_2–t_3			
Field	1.01	1.08	1.012–1.144
Control	1.00	1.05	0.994–1.089
Ant exclusion	0.98	1.02	0.979–1.061
Weevil exclusion	1.15	1.18	1.072–1.265
	λ_{geom}	λ_{boot}	95% CI
B Pooled transitions			
t_0–t_3			
Field	1.05	1.06	1.027–1.104
Control	1.05	1.06	1.024–1.103
Ant exclusion	1.03	1.04	1.006–1.068
Weevil exclusion	1.20	1.22	1.142–1.326

Field fecundities are those observed during the study; control, ant exclusion and weevil exclusion fecundities were calculated from exclusion experiments. λ is the asymptotic lambda calculated from the projection matrices of each treatment, and λ_{boot} is the population growth rate calculated by randomly sampling with replacement the transition stage-fate data, and its 95% Confidence Interval. λ_{boot} in B was calculated by pooling the transition stage-fate data for all time transitions (t_1–t_3)

Table 5 Stochastic population growth rates of *Spathoglottis plicata* calculated using fecundities representing observed field conditions, and modify fecundities based on exclusion experiments

Treatment	$\lambda_{T\ approx.}$	λ_{Sim}	95% CI
Field	1.05	1.05	1.052–1.056
Control	1.05	1.05	1.050–1.056
Ant exclusion	1.03	1.03	1.027–1.032
Weevil exclusion	1.21	1.21	1.199–1.215

The population growth rates were calculated by Tuljapukar's approximation ($\lambda_{T\ approx.}$), and by simulation (λ_{Sim}; 95% CI reported)

Demography of *Spathoglottis plicata*

As expected, our analyses showed that the Philippine Ground Orchid exhibits λ > 1 under natural conditions, despite acquiring antagonistic interactions with a native herbivorous weevil. Nevertheless, we found values of λ ≤ 1 during one of the census periods (t_1–t_2), even when fecundities were modified to reflect the exclusion of weevils. Elasticity analyses of the projection matrices for all the time intervals, with fecundities under field conditions, showed that small changes in the survival and size-stage transitions, and fecundities of size-stages 5–6, would have a greater effect on λ (see Table 3; Fig. 2b). Furthermore, when compared to the other time intervals, the recruitment rate per plot was 2–7% lower in t_2, and the overall survival rate of this time interval was also lower, especially for size-stage 1. This dramatically reduced the number of individuals in size-stages 2 and 3 on t_2 by approximately 77 and 19% respectively, which probably affected the recruitment to larger size-stages in subsequent years. The fact that there was a 12% increase of individuals in size-stages 1–3 recorded the next year (t_3) suggests that the reduction in the survival rate of size-stage 1 (during t_1–t_2) was the main cause of population decline.

What caused the reduction in survival rate for size-stage 1 during the t_1–t_2 time interval? While other factors may have been involved, we suspect that unusual weather was a major cause. Climatological data from the nearby Arecibo Observatory (18.349°N, 66.752°W; National Climatic Data Center; www.ncdc.noaa.gov) showed that total precipitation was above normal during the timespan of our study period.
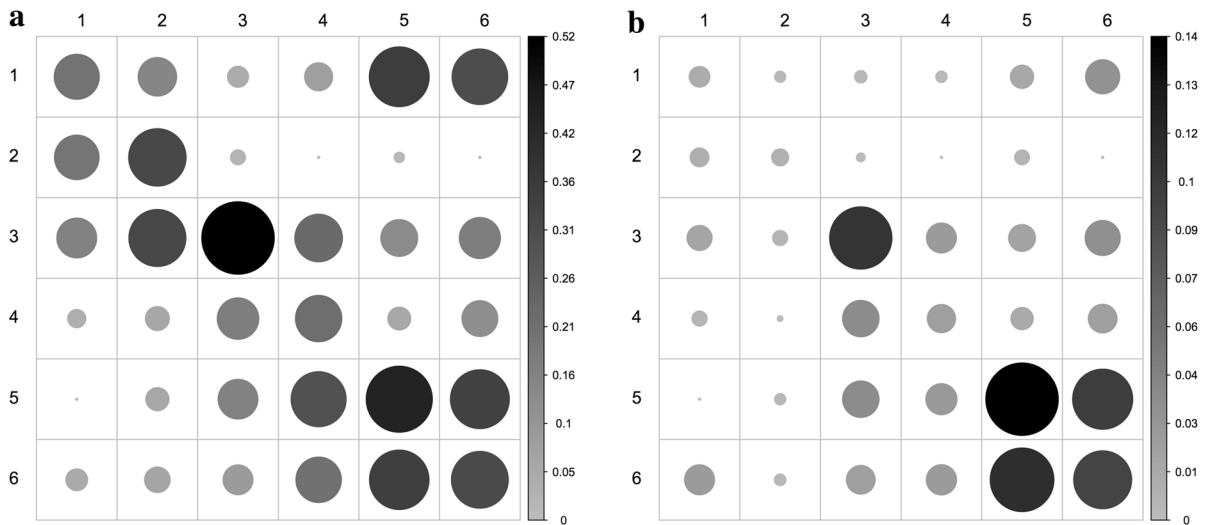
and environmental stochasticity would provide a better understanding of the population dynamics. Nevertheless, studies looking at the effects of novel interspecific interactions on introduced species seldom use population performance metrics. Here, we combined field demographic and fecundity data, as well as the results of the effects of exclusion experiments on fecundity to construct life-cycle based population models to quantify and assess the role of acquired interspecific interactions on the population performance (λ) of an invasive plant.

Fig. 2 Visual representation of the average population projection- (**a**) and elasticity (**b**) matrices of *Spathoglottis plicata* based on four continuous years of demographic data in the Río Abajo Forest, where the invasive orchid acquired novel interactions with weevils and ants. The average matrices reflect the effects of the acquired interactions on the population dynamics of *S. plicata* under natural field conditions during the study period

However, the precipitation during t_1–t_2 was not only much higher than the other periods, but it was also the most variable (t_0–t_1: 2176 mm ± 72; t_1–t_2: 2510 mm ± 110; t_2–t_3: 2348 mm ± 89). In other words, plants experienced very high precipitation events over short, intermittent periods of time during t_1–t_2 when compared to the other time transitions.

Although higher precipitation can be associated with increased recruitment rates in orchids (Zotz and Schmidt 2006; Diez 2007), the fact that the Río Abajo State Forest is a Subtropical Moist Forest suggests that water is not often a limiting factor. Excessive soil water moisture is known to cause increased mortality in cultured orchids and other plants due to susceptibility to disease and changes in the soil nutrient concentration and pH (Leonhardt and Sewake 1999). Two other mechanisms that could explain this are competitive exclusion of small individuals due to increased growth in larger plants or the disruption of mycorrhizal interactions. It should be noted that we are just beginning to understand how mycorrhizal associations affect the recruitment and survival of wild orchids (e.g. Bayman et al. 2002).

While our focus has been on biotic interactions with insects, fluctuations in weather certainly can play a role in the demography of orchid populations (Pfeifer et al. 2006). Our analysis showed that environmental fluctuations may result in uncertain survival and fecundity rates in smaller size-stages, that there is a trade-off between growth and reproduction (i.e. energy allocated to growth reduces reproductive success), and that fecundities from size-stages 5 to 6, although variable, are important vital rates contributing to population growth. While one may argue that our results are perhaps artifacts of the matrix, they are actually expected outcomes for iteroparous species with indeterminate growth inhabiting stochastic environments (Katsukawa et al. 2002), including invasive plants (Ramula et al. 2008). These traits and conditions are just what *S. plicata* possesses and experiences.

Effects of acquired interspecific interactions on λ

Biotic interactions are important factors determining the invasion success of exotic species (Mitchell et al. 2006), and the effects of plant–animal interactions, even at small time frames, may have long-term effects on plant populations (Strauss and Irwin 2004). When we projected the population of *S. plicata* over time assuming field conditions (all interactions included) and stochasticity, the average abundances predicted by the model increase as expected for an invasive species expanding its range. The model including demographic and environmental stochasticity showed a lag phase of ∼20–30 time-steps (equivalent to years in

our case; Fig. 2). While we have found that the ants and weevils interact with *S. plicata* throughout most of its distribution in Puerto Rico, the actual population growth curve in a given location is likely to be somewhere between weevil exclusion treatment and field and control conditions because of (1) resource discovery time lag of weevils (supported by the low frequencies of weevils on isolated plants and plant patches), and (2) a presumed weevil population growth lag-period. These processes may be linked to the estimated invasion lag phase recorded in Puerto Rico for this orchid (assuming an overall equal herbarium collection effort per decade, which is likely the case; see "Study system" section).

Here we showed that weevil-mediated biotic resistance proposed for *S. plicata* invasion in Puerto Rico (Ackerman et al. 2014) does indeed have demographic consequences. When weevils are excluded from inflorescences of *S. plicata*, λ significantly increased to 1.20, which underscores how detrimental *S. polita* can be to the population growth rates of this invasive orchid. Propagule pressure, the number or sets of individuals that are dispersed to new area, is an important factor in determining invasion success and invasion speed (e.g. D'Antonio et al. 2001; Von Holle and Simberloff 2005; Richardson and Pysek 2006). Although *S. plicata* produces thousands of seeds in each fruit, weevils dramatically reduce the number of mature fruits, thereby reducing propagule pressure. Without weevils, the expected population size is not only (on average) 367 times higher than projected with the influence of weevils, but the model also indicates a significantly higher stochastic population growth rate (λ_{sto}). Larger individuals of *S. plicata* in our study were associated with a higher reproductive effort, and the proportion of weevil damage to flowers and fruits was in turn positively correlated with reproductive effort. This is congruent with the results of the elasticity analyses, which revealed that only changes in the fecundities of the two larger size-stage classes had a substantial effect on λ (Fig. 2b). According to a meta-analysis of biotic resistance to exotic plant invasions, there is little evidence that plant–native herbivore interactions repelled invasions (Levine et al. 2004). Instead, herbivores limited the abundance of invasive plants once they become established (Levine et al. 2004). Similarly, we found support for biotic resistance imposed by native weevils, but this resistance is incapable of deterring the *S. plicata* invasion

since weevils alone do not reduce $\lambda \leq 1$, suggesting that propagule pressure is an important mechanism at play.

The invasional meltdown hypothesis predicts that biotic interactions between invaders will have positive synergistic effects, thus increasing the chances of success for both interacting species (Simberloff and Von Holle 1999). Is the *Spathoglottis plicata–Solenopsis invicta* interaction an example of invasional meltdown? Similar to the results of the ant exclusion experiments where the mean fruit set was reduced by 33%, our bootstrapped values of λ showed that although invasive ants tend to reduce λ, this reduction was not significant, suggesting they do not effectively protect the invasive orchids. Yet this modelling approach does not consider the observed variation of fruit set, and the effects of stochasticity (which is substantial in our system). Although the experimental exclusion of ants in particular did not showed statistically significant protection to the invasive orchid, the overall evidence from previous studies indicated that red fire ants are an important factor affecting positively the reproductive success of *S. plicata*, with fruit set increasing with increasing number of ants visiting the inflorescences (Ackerman et al. 2014). Ants are known to respond to the quality and quantity of resources (Mailleux et al. 2000; Schilman 2011), and presence of invasive red fire ants and abundance on *S. plicata* was quite variable within days (mornings and afternoons), and between weeks in sites close to our demography study site (Ackerman et al. 2014). When we included demographic and environmental stochasticity in our analyses, the absence of ant protection decreased the expected population size over time almost by half (compared to field conditions; Fig. 3), and the λ_{sto} values were significantly lower than those expected under both the control treatment and field conditions.

It is possible that the lack of statistically significant ant protection to invasive orchids based on exclusion experiments was due to the sample size, and the highly variable reproductive success when both weevils and ants have the potential to interact with the orchid. We believe that considering the variation associated to each exclusion treatment (rather than static mean values) and including stochasticity enabled the model to better capture the dynamics in the system, yielding results that are consistent with the overall evidence on *Solenopsis invicta* protection against weevils, and with
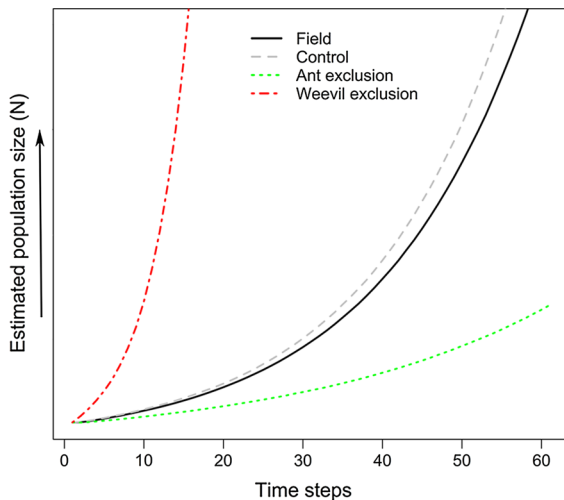
Fig. 3 The effects of acquired interspecific interactions on the projected population size of the invasive orchid (*Spathoglottis plicata*) over time. The population estimates were calculated by simulation (with 5000 iterations) based on the three projection matrices derived from the observed field conditions, and from the 100 matrices generated from each of the ex situ exclusion treatment (control, ant exclusion, and weevil exclusion; see methods). The initial population vector was based on the number of individuals at each size-stage observed at t_0 (N = 209)

protection of other plants throughout its invasive range (reviewed in Ness and Bronstein 2004). Furthermore, our results are consistent with the findings of Rico-Gray and Thien (1989), who had showed that (1) different species of ants foraging on extra-floral nectaries of *Schomburgkia tibicinis* in Mexico deterred the main herbivore, *Stethobaris sp.*, (2) that the prevalence of a given species varied with time, and (3) that the reproductive success of the orchid increased with increasing ant size (which was also related to species foraging dominance). Taken together, our results partially support the invasional meltdown hypothesis: invasive ants and invasive orchids are engaged in a mutually beneficial interaction that results in increased population growth for the orchids, and presumably fitness gains for ants (from energy derived from extrafloral nectar; Ness and Bronstein 2004, and references therein; Ackerman et al. 2014).

How prevalent are these interactions with ants across the invaded range? Plant–ant mutualisms, where ants protect the plants from herbivores and receive a reward from the plant, have been described or assumed for many plants (Rico-Gray and Thien

1989; Bronstein 1998; Chamberlain and Holland 2009). These interactions can be facultative and very generalized, not only for invasive species that have them, but for orchids as well (Fisher and Zimmerman 1988; Rico-Gray and Thien 1989; van der Cingel 2001). In Puerto Rico, there are at least seven native species of red fire ants (*Solenopsis*), and forest species may rely heavily on liquid foods (e.g. nectar, honeydew) (Torres 1984). Native *S. geminata* can be found in both disturbed, and relatively mature lowland dry to montane wet forests (50–600 m asl), and while it is considered invasive in Hawai'i (along with *S. papuana*), it can be displaced by invasive *S. invicta* when they co-occur (Porter et al. 1988). We have observed unidentified *Solenopsis* ants visiting the invasive orchid across the invaded range in Puerto Rico and Hawai'i, including relatively anthropogenically-undisturbed riparian areas inside mature forests (we only identified ants to the species level in our study site). Many other species of ants visit the inflorescences of the invasive orchid, and although there are several potentially aggressive species that specialize in liquid foods in Puerto Rico (Torres 1984), the only other species (very rarely) observed attacking and deterring weevils in our study area was the native *Wasmannia auropunctata*. This species can be found in mature forests from sea level up to 2400 m, is an aggressive invader elsewhere, and has been shown to increase the reproductive success of some plants (Ness and Bronstein 2004). Given the promiscuity of extrafloral nectaries-bearing plant and ant associations, and that many potential ant partners exist across Puerto Rico, we expect facultative mutualistic *Spathoglottis*-ant associations to be prevalent, but variable and governed by the orchid's residence time, plant (inflorescence) densities, disturbance regimes, as well as by physiological and behavioral aspects of ant species.

There are some limitations to this study, which need to be considered. Firstly, when evaluating the effects of interspecific interactions based on experimental exclusions, the calculated fecundities represent the relative effect of different treatments on population growth and projected abundances, rather than precise estimates, which limits the characterization of populations elsewhere. Secondly, our models lack the seed stage in the life cycle of *S. plicata*. However, we consider highly unlikely that there is an active seed bank that contributes to the population growth rate,
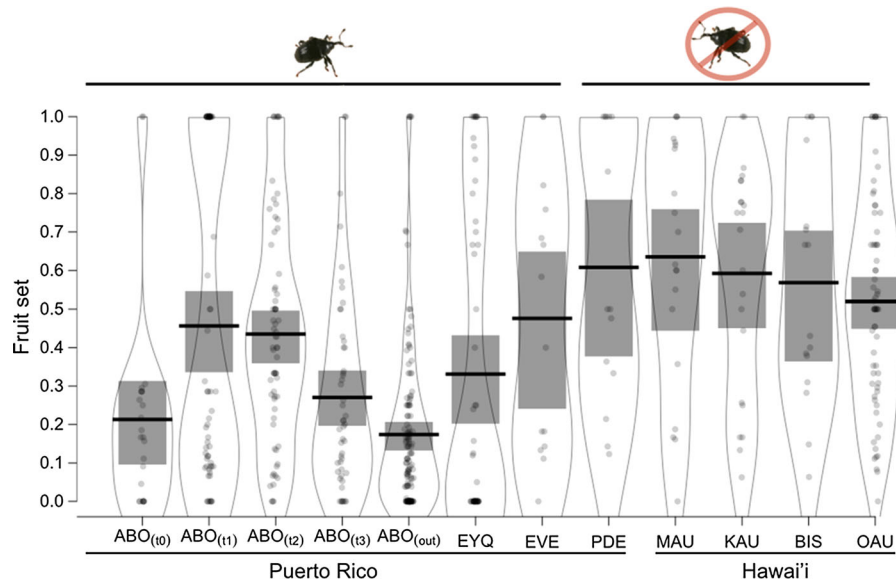
Fig. 4 Reproductive success (fruit set) of *Spathoglottis plicata* throughout its invasive range in Puerto Rico and Hawai'i in the presence or absence of herbivorous weevils. Points represent raw data (individual reproductive plants sampled), the frequency distribution of observations is denoted by the shape of the beans, the solid line represents the mean, and the grey bar represents the 95% Bayesian Highest Density Interval. Data from the Río Abajo State Forest population include fruit set from the demographic censuses per year, $ABO_{(t0-t3)}$, and two sites located 80–500 m, $ABO_{(out)}$, sampled at t_3. Data collected in El Yunque National Forest (northeast of PR) include three populations: EYQ, EVE and PDE—with the latter being weevil-free. Hawaiian populations, where weevils are also absent, were sampled in Maui (MAU), Kauai (KAU), the Big Island (BIS), and O'ahu (OAU). Weevil image by Tom Murray (modified and used with authorization)

especially because this is a tropical species (e.g. Batty et al. 2000). Also, *S. plicata* produces inflorescences throughout the year, yet we only gathered reproductive data during the census periods (i.e. once a year). Thus, we are assuming that this reproductive "snapshot" is proportional to- and represents the annual contribution of a given size-stage to fecundity. Moreover, our models assume that new recruits are the progeny of plants within the study plots. Seed dispersal may affect this assumption and produce biases in the sense that (1) fecundity could be overestimated if new recruits are from plants outside the study plots and (2) the fecundity could be underestimated if the plants within the study plots produce new recruits outside the plots. However, we believe that their overall effects are minimal because studies on orchid seed dispersal and seedling establishment have shown that despite having the potential for long distance dispersal (which is inherently important for range expansion), a high proportion of seeds fall in the vicinity of parent plants, and sharply decreases with distance (e.g. Ackerman et al. 1996; Murren and Ellison 1998; Jersáková and Malinova 2007). In addition, demographic studies that

have excluded the seed stages of orchids have successfully predicted future populations sizes (Tremblay and Hutchings 2003; Schödelbauerová et al. 2009).

Despite these limitations, the demographic consequences of interactions with native weevils and ants on the reproductive success of the invasive orchid observed in our demographic study seems to capture the variation observed throughout the invaded range in Puerto Rico (Fig. 4). Moreover, both the weevil-free population found in PR, as well as those in Hawai'i, have fruit sets close to that of plants in which weevils were experimentally excluded. Considering this, we expect the dynamics we revealed to be generally applicable across Puerto Rico because actively invading species usually have a $\lambda > 1$, and most *S. plicata* populations that we have surveyed in Puerto Rico have both ants and weevils. Because populations of *S. plicata* in our study area have been present for at least 15 years, the results derived from field conditions probably do not reflect what's occurring at the invasion front. We expect reproductive success (and λs) in weevil-free and newly established populations

to be similar to that of our weevil exclusion treatments, as observed in PDE (where weevils were absent). In agreement with the findings of Ramula et al. (2008) for invasive plant populations, and assuming populations of *S. plicata* across Puerto Rico exhibit similar dynamics to those in our study, simultaneous reductions in either survival or growth in combination with targeted reduction of fecundity for the two larger size-stages would be necessary to reduce λ.

Studies aiming to assess whether biotic interactions can affect the invasion success and/or management of introduced species should include multiple (and replicated) independent populations representing the different ecological conditions which the species experiences throughout the invasive range where (1) demographic censuses are conducted for at least three years (and designed based on the life-cycle of the species), (2) exclusion experiments are conducted preferably within the demographic monitoring timeframe and area, and (3) additional rapid surveys are conducted in additional areas to maximize capturing the variation present in the invaded range of the species.

To finalize, the fate and invasion success of non-native introduced species is ultimately determined by processes occurring at the population level, and their populations do not occur in a vacuum. As a result, focusing on single species populations while ignoring the array of interactions that they encounter in their introduced range hinders our understanding of biological invasions. Here we showed how the combination of field, experimental and matrix population modelling approaches provide a quantitative framework to evaluate the effects of multiple biotic interactions on the performance of invasive plants at the population level. The implementation of approaches such as this not only will contribute to our understanding of biological invasions, but it can also serve to explicitly identify critical life-stages to target for management and assess the efficacy of different management strategies when interactions with opposing outcomes are at play (Sakai et al. 2001; D'Antonio and Meyerson 2002; Kelly and Dyer 2002; Allendorf and Lundquist 2003).

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