CONSEQUENCES OF THE HIGH ABUNDANCE OF *BOMBUS TERRESTRIS* ON THE POLLINATION OF *VICIA FABA*

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Abstract—One of the main visitors to Fabia bean crops (*Vicia faba*) in South America is the invasive bumblebee species *Bombus terrestris*. This is particularly true in Chile, where *B. terrestris* was first introduced in 1997 and is now common over much of the country. In this study, we evaluated the activity of the principal pollinators of *V. faba* over two cropping seasons by assessing their visitation rates while distinguishing between legitimate visits, likely to lead to pollination, and nectar robbery. We then determined the net contribution of insect visitation on pod and seed set. We recorded seven species of floral visitors. Most visits (legitimate visits and robbery) were from the honeybee (*Apis mellifera*), with *B. terrestris* being the next most common visitor. In the case of *B. terrestris,* 87.19% of visits were nectar robbery. On average, the same flower perforation was visited 23 times by *B. terrestris* during the flower's lifespan. In general, the frequency of legitimate visits varied with pollinator identity and year. For *B. terrestris,* each flower received an average of 0.95 legitimate visits during its entire lifespan. The time spent by *B. terrestris* visiting flowers for both nectar robbery or pollen collection decreased after the first day of flowering suggesting resource depletion. The number of pods, total seed number, and seed weight were lower where self rather than open pollination. This suggests that open pollination increased reproductive success. We conclude that *B. terrestris* was likely to contribute relatively little to pollination while at the same time depleting floral resources throughout the flowers' lifespan. *Bombus terrestris* is likely to make *V. faba* flowers less attractive to other more effective pollinators. As a result, this may potentially drive interspecific competition for pollen and nectar within the crop.

Keywords—*Apis mellifera, Bombus dahlbomii, Bombus ruderatus, Bombus terrestris, Cadeguala*, nectar robbery

INTRODUCTION

Vicia faba (broad bean, faba) an important crop worldwide, with the main producers being China, Ethiopia and Egypt (FAO 2015). The annual world production is estimated to be approximately 200,000 tonnes (Ansoleaga & Salinas 2010). The seeds of this crop are rich in proteins and the plant is easily adaptable to different environmental conditions (Crepon et al. 2010). While the flower of this legume is partially self-pollinated, pod and seed production still benefit from insect cross-pollination (Suso & Maalouf 2009; Marzinzig et al. 2018). The dependency of *V. faba* on insect pollinators ranges from 4 to 84% (Aouar-sadil et al. 2008; Bommarco et al. 2012; Cunningham & Le Feuvre 2013; Bishop et al. 2016; Bond & Poulsen 1983; Suso et al. 2001), although (Bartomeus et al. 2014; Free 1966; Nayak et al. 2015) reported yield increases from 40 to 185% when pollinated by insects. Pollinators also promote self-pollination by stimulating the release of an exudate from the

stigmas which promotes pollen germination (Bond & Poulsen 1983). Typically, pollinators of *V. faba* will provide useful cross-pollination up until a threshold beyond which increased pollinator abundance results in no increase in yield (Suso et al. 2001).

Worldwide, bumblebees (*Bombus* spp.) and honeybees (*Apis mellifera*) are the main pollinators of *V. faba* (Kendall & Smith 1975; Marzinzig et al. 2018), although other pollinators include the bees *Xylocopa* spp., *Eucera* spp. (Aouar-sadil *et al.* 2008) as well as sweat bees (Halictidae) and cuckoo bees (Nomadinae) (Poulsen 1973; Kendall & Smith 1975; Tasei 1976; Marzinzig et al. 2018). Marzinzig et al. (2018) showed that cross-pollination of *V. faba* is mainly the result of long-tongued species, such as *B. hortorum*, although others have identified *A. mellifera* to be another important pollinator (Kyllönen 2018). Not all visitations by bees are legitimate, entering directly through the main opening of the flower and having at least the potential to lead to a seed set. For example, *B. terrestris* commonly robs nectar from flowers by biting holes at the base. This behaviour avoids the plant stigmas while still removing nectar (Aouar-sadli et al. 2008). *Apis mellifera* is also a secondary nectar robber that makes use of these holes already created by *B. terrestris* (Marzinzig et al. 2018).

Unlike the often-diverse faunas of bumblebees encountered in Europe, Southern South America has only one native long-tongued bumblebee, *B. dahlbomii*. The populations of this native species have declined substantially after the arrival of *B. terrestris*, with similar trends observed in both Argentina and Chile (Arbertman et al. 2006; Smith-Ramírez et al. 2014). The invasion of *B. terrestris*, native to Europe, has largely been a result of the importation of commercially produced colonies used to promote pollination in several economically important crops (Aizen et al. 2018; Naeem et al. 2018). It has been shown that this trade is responsible for *B. terrestris* being invasive in many areas of the world (Kenis et al. 2008). In Chile, *B. terrestris* was introduced in 1997 and since then has quickly become naturalised so that it is now overabundant (sensu Lunney et al. 2007) and widespread across the temperate zones of Chile and Argentina (Sáez et al. 2014; Aizen et al. 2018).

Although nectar robbery is common in *V. faba* flowers and has previously been suggested not to be detrimental to yields (Newton & Hill 1983), it can still have a negative effect by promoting less efficient self-pollination (Kendall & Smith 1975; Marzinzig 2018). Where high enough abundances of *B. terrestris* occur, the probability that some of them make legitimate visits facilitating successful pollination may indeed be the case. However, this is only likely to be the case where nectar is not a limiting resource. Where nectar production is limited, *B. terrestris* could be robbing this resource from flowers that would otherwise be self-pollinated or pollinated by other species over the flower's lifespan. As flowers' attractiveness to pollinators depends on there being sufficient pollen and nectar, resource depletion by robing *B. terrestris* might negatively affect legitimate flower visitations and lead to reduced yields (Sáez et al. 2017). Assessing this effect can be challenging, although time spent by insects gathering these resources has been used as a proxy to indicate whether nectar and pollen are limited (Hingston & McQuillan 1999). In addition to direct resource depletion in flowers, repeated visits by one pollinator (or robber) might damage the flower, as shown for raspberries (Sáez et al. 2017).

Here we study whether changes in abundance (as visit rate to flowers) of *B. terrestris* can affect the overall rates of *V. faba* visits from other pollinator species during the lifetime of a flower. We focused on assessing inter-specific competition for pollen and nectar resources to identify how this affects the pollination of *V. faba*. We assessed this effect over two years to account for interannual abundance variation in *B. terrestris* (Ramires 2019). Our specific objectives were: 1) To identify the percentage of flowers visited by pollinator species and the rates at which legitimate and robbery visits occurred; 2) To test if there was a correlation between the number of legitimate visits and robbery events during the life of the flowers; 3) To evaluate whether the time spent in legitimate and robbery visits differ over the flowers lifespan; 4) To assess interannual variation in each of the aforementioned parameters, and finally; 5) To quantify the extent of pollinator dependence for a *V. faba* cultivar commonly used in south-central Chile.

MATERIALS AND METHODS

STUDY AREA

We studied *V. faba* L. subsp. *faba* var. *major* cultivar "agua dulce" plants in a small field (0.4 hectares) located at 40.76° 99.5' (S), 73.20°10.9′ (W), approximately 1.5 km from Río Negro, in Osorno Province, Chile. In the field, an area of 1200 m^2 was planted with *V. faba*, with no insecticide applied to the crop. Other crops were also grown in the field, including other beans and peas, raspberries and some fruit trees attractive to pollinators. Native plants, such as blackberries, as well as ornamental plants attractive to pollinators, were also found within 2 to 10 m of the field margins. The landscape matrix surrounding the field was dominated by managed grasslands. As *B. terrestris* shows population fluctuations following a second order curve, it is common for the annual abundances to be highly variable (Smith-Ramirez et al. 2014). For this reason, we ran this study over two years (2018 and 2019). The average temperature during the flowering season of *V. faba* was 17.8 ºC in 2018 and 15.3 ºC in 2019. Average precipitation accumulated over the flowering period was respectively 17.8 mm and 14.1 mm in 2018 and 2019. These climate data were collected from the nearest meteorological station, "Cañal Bajo" (https://climatologia.meteochile.gob.cl).

SAMPLE FIELD

Vicia faba was sown in late August (austral winter) in the first year and in late September in the second year. Seeds used to establish the crop were purchased in the local market for 2018, while seeds harvested in the first year were then used to establish the crop grown in 2019. We delayed the sowing in the second year because of the rainy condition during that winter. No managed colonies of *B. terrestris* or *A. mellifera* were located within a radius of ca. 3 km of the studied garden. Observations of pollinator visitation rates, their behaviour, and their effect on seed sets were undertaken from the last weeks of November until the first week of December in both years. All seeds from the crop were harvested in January of the following year. To evaluate species pollinator diversity and their behaviour (legitimate and robbery visits), we conducted two types of observations, one with cameras and another one with observers in the field. Although cameras can

provide the necessary information, their static focus limits their range, and so it was considered important to include observers to complement and better assess the variation and behaviour of pollinators on this crop.

It was important to distinguish what day legitimate visits first occurred, as *V. faba* flowers cannot be fertilized during the first day of their onset (Stoddard 1986). We considered legitimate pollination to have occurred when the keel (the modified petals that cover the flower´s reproductive organs) was lowered by an insect placing its head and thorax over the anthers and stigma. When the insect stands on the keel petals, they are lowered by the first pair of legs and expose the anthers for pollen collection (we call this behaviour Downing the Keel=DK). In some rare legitimate visits, the insect did not push the keel sufficiently and flew away without collecting pollen (we refer to this behaviour as Non-Downing of the Keel =NDK). Due to the angle of the camera when viewing the flowers, or because of the insect physically obscuring reproductive parts of the flower, it was not always possible to distinguish between these two behaviours (NV). Robbing behaviour was recorded when the insect inserted its proboscides in the perforation at the base of the flower. We consider this behaviour in more detail below.

Camera method: We used four Brinno TLC200 Time Lapse cameras, with images recorded every second for two to five consecutive days, using a configuration of FPS: 5-20 fps and a shooting interval of 1 sec. The cameras were active between 07:00 – 21:00 hrs. Each camera was placed facing one plant, with one to three inflorescences with open flowers. The total observation time for the first year was 155.93 hrs and 334.70 hrs for the second year. We standardized legitimate visits and nectar robbery per flower, using the number of studied flowers recorded per camera during anthesis. The standardization per hour was made individually for each flower, reflecting that they opened at different times (Fig. 1A). Each flower bud takes around 30 minutes to open completely; we considered it open when the largest petal (the banner) starts to move.

We recorded two plants with a total of 27 buds and/or flowers during the first year and two plants with 37 buds and/or flowers during the second

year. We recorded the species that visited the flower, the type of visit made (legitimate, DK/NDK, NV or robbery), the exact time the pollinator appeared, the number of visits made and the number of flowers visited. We identified each bud and/or flower during the recordings and counted their visits separately. In addition, the cameras were used to record the visits of pollinators in a sample of 10 flowers (out of 27) for the first year and 13 flowers (out of 37) for the second year. These 23 flowers were chosen because the cameras recorded pollinator visits over the entire flowers' lifespan. For these cases, we recorded the daily visits of pollinators, as well as their behaviour up until a day after the last time the flowers were open. When we observed that a flower was closed for an entire day, we assumed that the flower had senescent the day before.

To estimate the available resource in nectar and pollen, we recorded the time spend by *A. mellifera*, in legitimate visits for each day during the flowering period (42 visits on day one, 32 visits on day two, and 38 visits on day three). On the first day, we only considered the visits that occurred after one or two hours following the flower opening (to give the time for pollen to dry). It was not possible to make this comparison for *B. terrestris* as the number of legitimate visits was too few by day 3 to be viable for robust statistical analysis. For 27 legitimate visits per day, and for the two main pollinator species, we recorded DK or NDK behaviour (total $N = 162$ visits), including the time spend undertaking each behaviour. We recorded the time spend undertaking robbery between 17:49 and 19:58 by *A. mellifera* and *B. terrestris* in a sample of 175 visits (day one), 224 visits (day two) and 104 visits (day three) for both years. We used this period because it corresponds to high nectar robbing activity for both main visitors probably related with nectar secretion.

On-site observer: Three people observed the activity of pollinators simultaneously over periods of 10 minutes (130 min in year one and 140 min in year two) on the flowers of 10 plants chosen randomly and located within a plot of around 2 x 2 m². Over two years, each 10-minute observation was made between 10:10 to 15:10 over three non-consecutive days. While in the first year, the flowers opened from around 10 am, early openings were unusual in the second year. As such, we started our observations at 12:00 hrs. We chose this observation time because *V. faba* replenishes its nectar level overnigh (Stoddard & Bond 1986). We would therefore expect an increase of robbery on nearby adult flowers in the morning and the afternoon. For each observation (observer and camera), we counted the total number of open flowers per plant to standardize the observations. For both methods, we recorded the same information, except for the time (seconds) spent in legitimate visits, nectar robbery and where the flower keel was moved (this only being documented from the camera data). We also monitored the activity of 12 randomly chosen individuals of *A. mellifera* and *B. terrestris* to document the frequency of change in their foraging behaviour. From these individuals, we recorded how many times they changed from legitimate visits to robbery by the next day for the same visited flower. We did not see any activity in extra-floral nectaries of *V. faba*.

Pollination and reproduction of *V. faba*: In order to measure the contribution of pollinators to the reproductive output of *V. faba*, we conducted hand-pollination experiments of self-pollination, artificial cross-pollination and open pollination in each year of our study. In both years, we self-pollinated three or four flowers per plant for 30 plants (118 flowers in total), as well as manually cross-pollinating one to three flowers per plant for another 52 plants. Once hand-pollination was undertaken, we covered the treated buds with bags to prevent legitimate visits and robbery. As a control, we also marked 90 flowers over 60 plants to study open pollination. Some of the plants in which we did the different treatments were the same. We lost part of the production of the cross-pollination experiment due to an unknown reason in the first year and an infestation of young pods by the fungus *Botrytis* in the second year. We quantified the number of pods, seeds, and seed weight produced in these experiments. Pods were collected and kept in a refrigerator for five days and weighed on an analytical balance KERN EMB 500-2S (precision 0.01 g).

STATISTICAL ANALYSIS

For each species, we calculated the rates of legitimate visits and robbery, but only for the period where the flowers were open. Nevertheless, we also analysed total robbery during the flower's

lifespan by including the occurrence of nectar robbery events when the flowers were closed. We used the Pearson correlation test to estimate the relationship between the flowers lifespan (hours) and the number of nectar robberies, as well as between the number of legitimate visits and the number of robbery events. Since the data were normally distributed, we used an ANOVA to compare the duration of legitimate visits recorded for *A. mellifera* across both days and years, comparing these effects a *posteriori* using a TukeyHSD test. We used a non-parametric pairwise Wilcoxon Rank Sum test to compare robbery time (seconds) among days and years as these data were not normally distributed. For nectar robbery, we merged the information for *A. mellifera* and *B. terrestris*, as the average time dedicated to robbery was comparable at 3.12 ± SE 0.12 seconds for *A. mellifera*, and 3.35 ± SE 0.16 seconds for *B. terrestris*. Because the lifespan of *V. faba* flowers varies between 2 and 4 days, we compared the number of legitimate visits and robbery events for each hour of life. We compared the number of DK = 1 and NDK = 0 behaviour per species and day of flower lifespan, using contingency tables and chi-square tests.

To test for variation in bee behaviour (i.e. legitimate visits or robbery as response variable) between years (numerical predictor) and bee species (*B. terrestris* and *A mellifera* used as fixed categorical predictor), we performed a General Linear Model (GLM). First, we used the entire dataset of all observed flowers (*N* = 64). In the second analysis, we focused on a subset of flowers (*N* = 23), selecting those with visitation information available for their entire flowering period. For each model, we selected the most appropriate distributions (Gaussian, Poisson and negative binomial distribution) by contrasting the Akaike Information Criterion (AIC) of alternative models. Tukey test was subsequently applied for multiple comparisons of the variables over both years. We applied one model for both *B. terrestris* and *A. mellifera,* because they were the two main visitors, and one model (total) for all the bee species. In these models, we included "year" as a fixed effect to account for interannual population variation of *A. mellifera* and *Bombus* spp., possibly due to them sharing common pathogens (see Smith-Ramírez et al. 2014 and Smith-Ramírez et al. in preparation). We assessed goodness-of-fit of the selected model

by running a likelihood ratio test comparing it to the null model, similar to Burnham & Anderson (2002) and Wagenmakers & Farrell (2004). GLMs were fitted using the R packages: MASS (4.0, Venables & Ripley 2002), Matrix (1.3-4, Bates et al. 2021), nlme (3.1-152, Pinheiro et al. 2021), Hmisc (4.5-0, Harrell 2021) and the MuMIn (1.43.17, Barton 2021) package for model averaging.

The numbers of pods per flower observed under open, self and cross-pollination were compared with a simple logistic regression, as the data were binomial. The number of seeds per pod and seed weight between open and artificial crossed visits was compared by Wilcoxon tests following a logistic distribution (no data were normal or able to be normalized). We analysed all data using R Studio, Inc. (Version 1.2.5033; R version 3.6.1 (R Core Team 2019)). The significance used by all the test was *P*-value < 0.05, we considered marginally significant p-value between 0.05 to 0.1.

RESULTS

DIVERSITY, ABUNDANCE AND GENERAL BEHAVIOUR

A total of seven species were recorded by camera and on-site observer methods visiting *V. faba* flowers in the two years (Table 1). We found four Apidae species: three introduced species *A. mellifera*, *B. terrestris* and *B. ruderatus,* and one native bumblebee *B. dahlbomii;* two native Colletidae: *Cadeguala occidentalis* and *Cadeguala albopilosa*, and one Halictidae *Corynura* spp. (the latter was recorded by human rather than camera observations (Table 1)). The main visitor of *V. faba* was *B. terrestris*, but the main pollinators in both years were *A. mellifera*, followed by *B. terrestris* (Table 1). *B. terrestris* was generally a primary nectar robber, and *A. mellifera* was a secondary robber. We observed neither native pollinators nor *B. ruderatus* robbing nectar (Table 1). The relative frequency of legitimate visits by *B. ruderatus* was low, 3.39% and 2.38% in years one and two, respectively, visiting 37.04% in 2018 and 45.95% in 2019 of the flowers observed by cameras. The frequency of legitimate visits recorded for *C. occidentalis* was also low, accounting for 0.56% of the visits and 16.22% of the flowers observed by cameras (Table 1). Legitimate visits and robbery from exotic Apidae occurred mainly from 16:00 to 20:00, slightly out of phase

Table 1. Legitimate visits and robbery activity (when flowers are open) in a *Vicia faba* field. The total number of flowers in anthesis and buds recorded with cameras was 27 in 2018 and 37 in 2019. The total time recorded was 155.93 hours of anthesis in year one and 334.70 h in year two. A. = *Apis*, B. = *Bombus*, C. = *Cadeguala*. Lv = Legitimately visited, rob = robbed. In the observations made by on-site observer method, to standardize the visit rate, the average number of flowers per plant was 14.08 ± 0.68.

Species	Only rob flowers	Onl Lv flowers	Lv + rob flowers	Total fv flowers	Lv flowers (hour-1)	Total n° of legitimate visits	Total n° of robberies	Legitimate visit rate (flower-1 hour-1)	Robbery rate (flower-1 hour-1)
Observations made by cameras									
Year 1　A. mellifera	5	0	21	21	0.13	100	142	0.02	0.03
B. terrestris	4	0	9	9	0.06	23	47	0.006	0.01
B. ruderatus	0	10	0	10	0.06	15	0	0.004	
B. dahlbomii	0	1	0	1	0.006	1	0	0.0002	
Year 2　A. mellifera	0	15	22	37	0.11	222	9	0.02	0.0007
B. terrestris	5	0	25	25	0.08	163	723	0.01	0.06
B. ruderatus	0	17	0	17	0.05	30	0	0.002	
C. occidentalis	0	6	0	6	0.03	7	0	0.0006	
Observations made by people									
Year 1　A. mellifera	-	-	-	-	-	4	38	0.0006	0.006
B. terrestris	-	-	-	-	-	1	70	0.0002	0.01
C. albopilosa						1	0	0.0002	
Year 2　A. mellifera	-	-	-	-	-	9	0	0.001	
B. terrestris	-	-	-	-	-	0	91		0.01
C. chilensis	-	-	-	-	-	4	0	0.0005	

with the period of maximum flower opening (Fig. 1A and B). The visits of the native bees recorded by cameras occurred between 18:00 to 19:00. However, on-site observers have noticed midday activity for native bee species. The percentage of flowers pollinated within ten plant plots, during the morning to midday, was 17.49% (N = 14 flowers in all the observation time over both years; Table 1). However, using camera methods, we found that 78.28% of the flowers were visited (52 from 64 flowers, Appendix IA), and 100% of them received at least one legitimate visit during their lifespan.

Adding both years and methods, we found 1,615 visits (legitimate visits plus robbery; Table 1). Approximately 1/3 (N = 580) were legitimate, and from this nearly 1/3 (N = 187) from *B. terrestris*. Of the total visits of *B. terrestris* (legitimate visits plus robbery, N = 1118), 83.27% were robbery when flowers were open (N = 937; Table 1, Appendix I

A). *Bombus terrestris* also robbed nectar 373 times while flowers were in bud (closed) and 24 times (robbed or tested there is nectar) in recently senescent flowers (Appendix I B). Overall, the percentage of visits by *B. terrestris* robbing nectar (open and close flowers) was 87.66%. In contrast, *A. mellifera* robbed open flowers 33.2% (N = 164), and robbed closed or recently senescent flowers principally in the first year (N = 112 times).

LEGITIMATE VISITS AND ROBBERY PER YEAR, AND DURING THE LIFETIME OF THE FLOWERS

In year one, 33.33% of studied flowers received at least one legitimate visit by *B. terrestris*. In year two, this number doubled to 67.57% (Table 1). Year one had notably less activity than year two, in both legitimate visits and nectar robbery. This difference was mainly due to *B. terrestris* which had almost twice the number of legitimate visits and six times more robbery in the second year (year one: 0.005 fv/h/flower (legitimate visits per
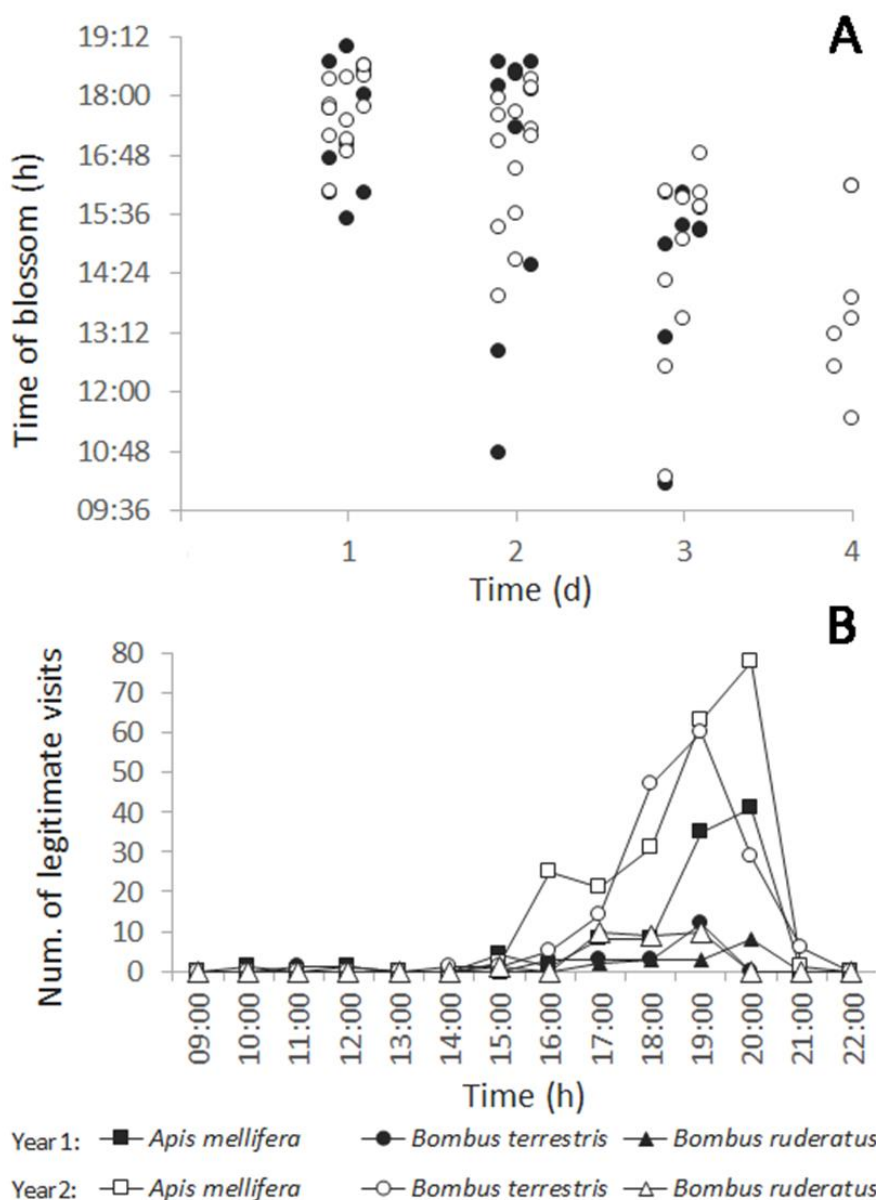
Figure 1. (A) Time of the day when flowers opened during the flower life for each study year. Black circles for year one; white for year two. (B) The number of legitimate visits for the main pollinator species of *Vicia faba*, for each hour and all flowers studied in both years.

hour per flower); 0.01 rob/h/flower; year two (robbery events per hour per flower): 0.01 fv/h/flower; 0.06 rob/h/flower) with many visits concentrated over a few flowers (Appendix I A; Table 1).

GLM analysis, using a negative binomial distribution, showed that the main legitimate visits were made by *A. mellifera*, especially in year one (Appendix II). Legitimate visiting behaviour was affected by the interaction between pollinator species and year, accounting for 77% of the observed variance by Akaike weight (Appendix II). Robbery behaviour was affected by pollinator species, year and their interaction. For robbery, the interaction accounted for 100% of the observed

variance (Appendix II). *Apis mellifera* was the main bee in terms of robbing behaviour in the first year (2018) (Table 1, Fig. 2B), while in year two, *B. terrestris* was the main bee robbing flowers.

The flowers were alive for a maximum of four days; on average, they were open at 11.97 ± 0.15 hrs, for a minimum of two and a maximum of eight hours per day. *Apis mellifera* typically made legitimate visits during the lifetime of the flowers. However, *B. terrestris* tended to show a reduction in the number of legitimate visits with the age of the flower in year two, which contrast with the pattern observed for nectar robbery (Figs. 2A and 3A; Kruskal-Wallis, df = 2, $P = 0.06$).
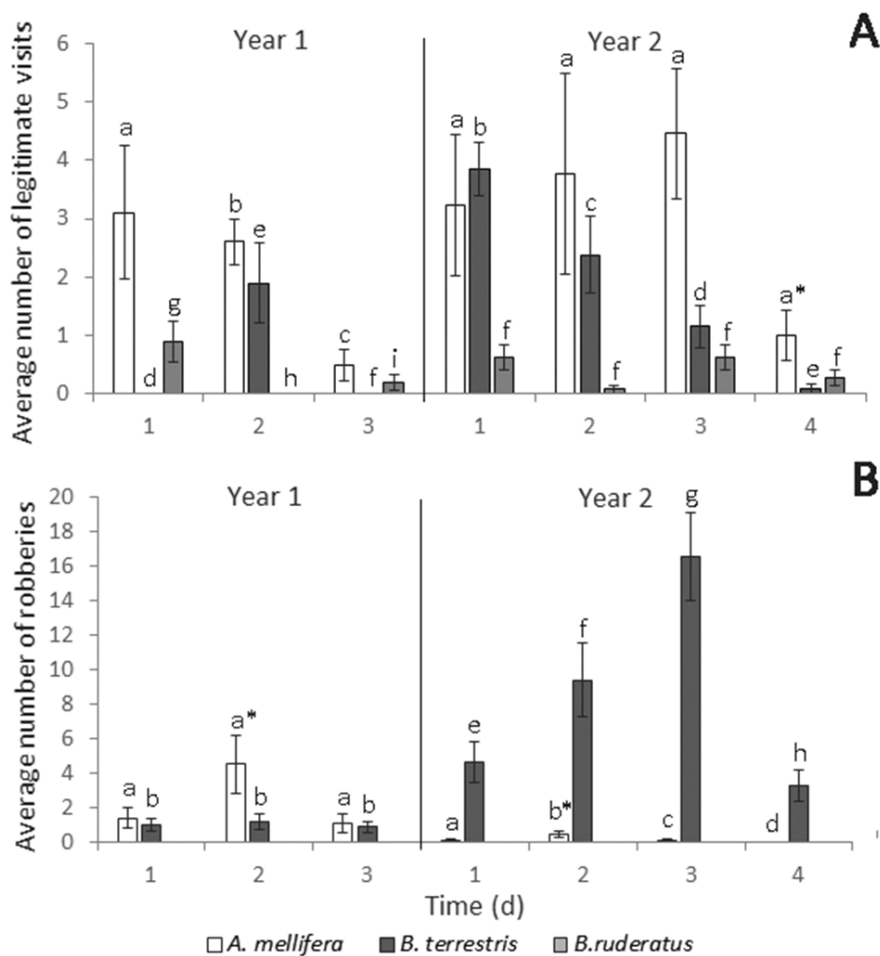
Figure 2. Average absolute number (± standard error) of (A) legitimate visits and (B) nectar robbery events by *Apis mellifera*, *Bombus terrestris* and *B. ruderatus* on open flowers of *Vicia faba* during each day of flower life in both study years (recorded by cameras). The same letters above bars mean there is no statistical differences, P < 0.05. Marginal p-values (between 0.05 to 0.1) are showed with an asterisk (*). Different letters mean significant differences.

Considering that legitimate visits produce fertilized ovules only from the second until last day of a *V. faba* flowers' lifespan, *A. mellifera* made on average 1.55 ± 0.8 legitimate visits per flower for these days in year one (ranging from 0 to 4 visits), and on average 3.19 ± 0.7 legitimate visits in year two (ranging from 0 to 20 visits). *Bombus terrestris* showed an average of 0.95 ± 0.4 legitimate visits per day in year one. However, counting only days two and three (there were no legitimate visits on day four), this was 0.86 ± 0.61 (0 to 6 visits), and in year two, the average was 1.27 ± 0.78 for days 2, 3 and 4, (0 to 7 visits). For *B. ruderatus*, we found that most of the pollination events were performed on the first day of flower opening (Fig. 2A). Considering all active pollinators between days two and four, the flowers received on average 2.60 ± 0.67 legitimate visits during year one and 4.46 ± 0.93 in year two. The number of robberies that each flower received during its life was 4 to 96, in average 23.70 ± 3.99, with more robbery events in year two (Table 1).

GLM analysis of the subset of flowers showed that legitimate visits were affected by the interaction between pollinator species and year (Appendix III). For days one, three and the total lifespan, Akaike weight accounted for 99%, 56% and 68% of the variability, respectively (Appendix III). Day two was influenced only by pollinator species, accounting for 43% of the observed data (Appendix III). The best-fit models for nectar robbery during the life of flowers always included the interaction term between pollinator species and year. For both day and total days, the interactions explained 100% of observed data by Akaike weight (Appendix III). Depending on the species there was a significant decrease in the legitimate visits on days three (Appendix III, Fig. 3B) or four.

There was a positive Pearson correlation between the number of legitimate visits and number of robbery visits during the flower lifespan (t = 2.56, *P* = 0.018). This significant correlation occurred over the first 14 hours of
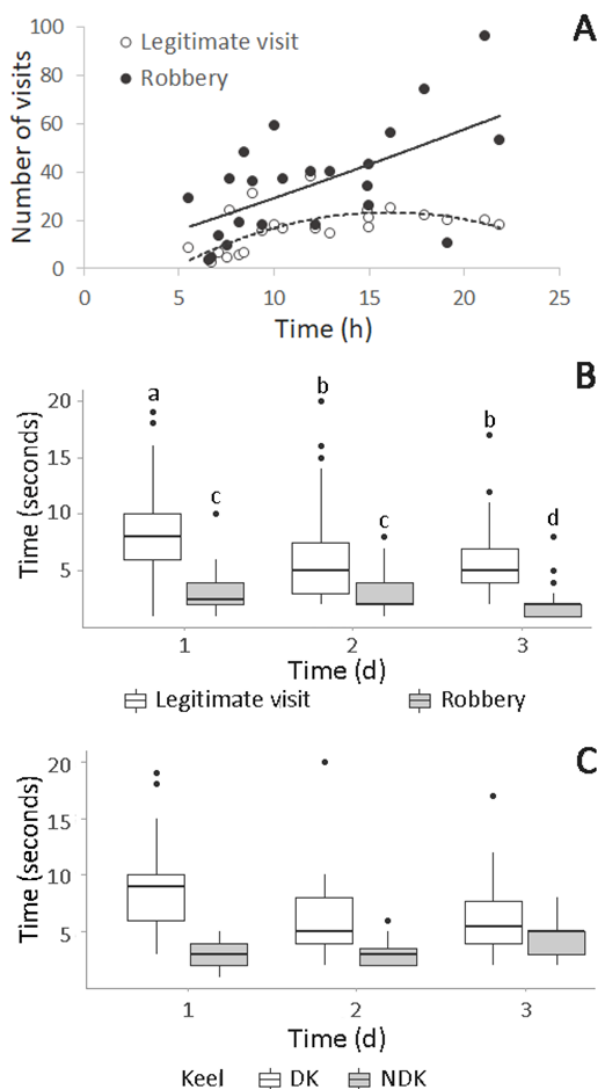
Figure 3. (A) Correlation between the time of a flower's life (hours) and the number of legitimate visits to the flower (white circles, tendency in dotted line) and nectar robbery per flower including robbery to buds, open, closed and dead flowers (black circles, tendency in continuous line). **(B)** Box plots of total time spent by pollinators on legitimate visits (*Apis mellifera*) and robbery (*A. mellifera* and *Bombus terrestris*) in a flower's life. Circles represent outliers. **(C)** Box plots of total time spent by *A. mellifera* down and non-down the keel (DK and NDK respectively) in a flower's life. Error standard is showed, and circles represent outliers.

flower life (Pearson correlation between the flower lifespan (hours) and robbery numbers: t = 3.39, *P* = 0.002). However, after 14 hours, we notice the opposite trend with robbery increasing while legitimate visits decrease (Fig. 3A).

The behaviour of both *A. mellifera* and *B. terrestris* was almost exclusively either foraging or collecting nectar by robbery, or for pollen by legitimate flower visits. Individuals rarely went for

both resources in the same visit to a flower or any other flower in close proximity. When following individual insects, we recorded up to 22 nectar robbery events without any pollination, and up to 12 legitimate visits without any robbery (after that, we lost the individuals of our view). On cameras, we recorded that from a sample of 102 visits; only in four cases (3.92%) did the visitors pollinate the same flower that they robbed nectar from.

TIME SPENT IN LEGITIMATE VISITS, POLLINATION VISITS AND IN NECTAR ROBBERY DURING THE LIFE OF THE FLOWER

The time spent by the main pollinator (*A. mellifera*) in legitimate visits during year one showed no significant difference between the days over which the flower was open, but there was some suggestion of a trend of decreasing visitation over time (Day 1: 10.60 ± 1.65, from 5 sec. to 19 sec; Day 2: 7.26 ± 1.28, from 2 sec. to 20 sec; Day 3: 5.50 ± 1.32, from 3 sec. to 9 sec; p-value Pairwise Wilcoxon Rank Sum test ranked from 0.13 to 0.97). In year two, however, the time spent was significantly higher on day one than the other days (Day 1: 8.31 ± 0.66, from 1 sec. to 18 sec; Day 2: 6.19 ± 0.71, from 2 sec. to 20 sec; Day 3: 5.60 ± 0.43, from 2 sec. to 17 sec; p-value for day one compared to day two was 0.007, 0.001 compared day 1 to day 3; but day 2 and 3 were similar *P* = 0.7). Adding data for both years together (Fig. 2A) showed on average that the trend was dominated by a year effect, with significant differences in visitation between days (ANOVA, F =11.67, df = 2, *P* < 0.0001). TukeyHSD showed that day 1 was different from days 2 and 3 (*P* = 0.03, diff = -0.33, lower = -0.63, upper = -0.02 and *P* < 0.0001, diff = -0.62, lower = -0.91, upper = -0.33, respectively).

We observed that for *A. mellifera* 29.87% of legitimate visits (average of the three days of flower life) did not lower the keel (NDK; Fig. 4A). These percentages were significantly different across days (chi-square test = 13.76, df = 3, *P* = 0.03). For NDK behaviour, the honeybee was in the wind pushing the banner gently, spending half the time (in average 3.87 seconds ± 0.38) than when the bee lowered the keel completely (in average 7.82 seconds ± 0.61). The average time where DK behaviour was performed was different from NDK (W = 59.5, *P* = 0.002; Fig. 3C). We also observed that, especially for days two and three, 25.96% of the visits of the honeybees were of only two to three seconds duration. NDK behaviour was observed
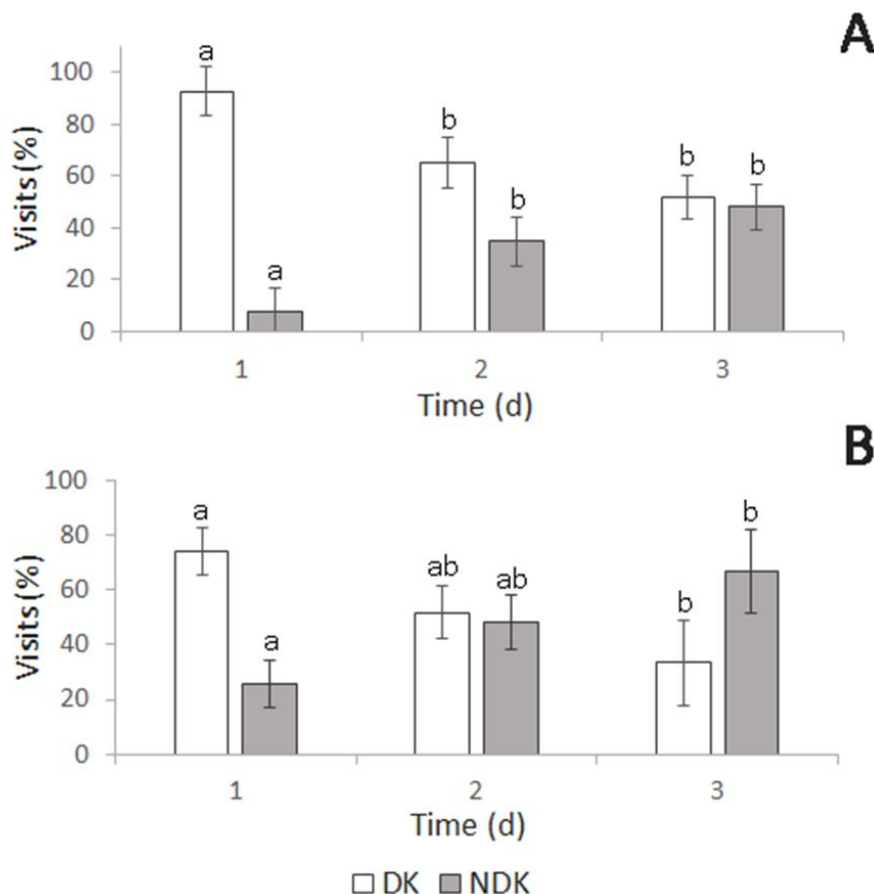
Figure 4. Percentage of the behaviour by *Apis mellifera* (A) and *Bombus terrestris* (B) to down completely (DK) and not to down completely the keel (NDK) of *V. faba* flowers along three days of its life. When the insect lowers the keel, he collects pollen. For *A. mellifera* and *B. terrestris* day was a significant factor using logistic regression (P = 0.0008 and P = 0.02, respectively). The first day was different for *A. mellifera* (Wilcoxon, P = 0.03 compared to day 2 and P = 0.003 compared to day 3), day 2 and 3 were similar (P = 0.3) and for *B. terrestris*, only day 3 is marginally different from day 1 (P = 0.09). The standard error is shown.

in *B. terrestris* 39.71% of the time (average of the three days; Fig. 4B), for a duration of one to five seconds.

The time spent by *B. terrestris* and *A. mellifera* in nectar robbery during year one were similar across the three days of the flower lifespan (Day 1: 2.78 ± 0.22, from 1 sec. to 6 sec; Day 2: 2.90 ± 0.21, from 1 sec. to 7 sec; Day 3: 2.62 ± 0.35, from 1 sec. to 8 sec; *P*-values using Pairwise Wilcoxon Rank Sum ranked from 0.45 to 0.95). However, the time spent each day undertaking nectar robbery in the second year was different. On day three, both bumblebee species spent significantly less time undertaking nectar robbery than days two and one (Day 1: 3.07 ± 0.47, from 1 sec. to 10 sec; Day 2: 2.82 ± 0.36, from 1 sec. to 8 sec; Day 3: 1.50 ± 0.15, from 1 sec. to 3 sec; *P*-value of day three was 0.01 for both comparisons, day one and two had a *P* = 0.94). We combined data across both years to produce Fig. 3B. In this case, the trend was significantly higher during the first and second day than the third (Kruskal-Wallis, df = 2, *P* = 0.0005, Wilcoxon pairwise day three compared with days one and two was similar, *P* = 0.001).

POLLINATION ASSAYS

We counted more pods, more seeds per pod and heavier seeds for those flowers that received open pollination compared to those with the self-pollination treatment. Overall, 33.33% (30 flowers) of marked flowers in open-pollinated assays and 5.08% of self-pollinated flowers (six flowers) produced pods. These differences were significant (W = 174, *P* < 0.001). On average, more seeds per pod (*N* = 0.53) were counted in open pollination compared to self-pollination assays, but the differences were marginally significant, mainly due to the low number of seeds observed in the self-pollination assay (W = 242.5, *P* = 0.074). Seeds in open pollination were heavier than self-pollination, although these differences were marginally significant (W = 1019, *P* = 0.059; Fig. 5).

DISCUSSION

In this study, we found that the main interaction behaviour of *B. terrestris* when foraging on *V. faba* flowers was nectar robbery, a result that was far higher in this system when compared to
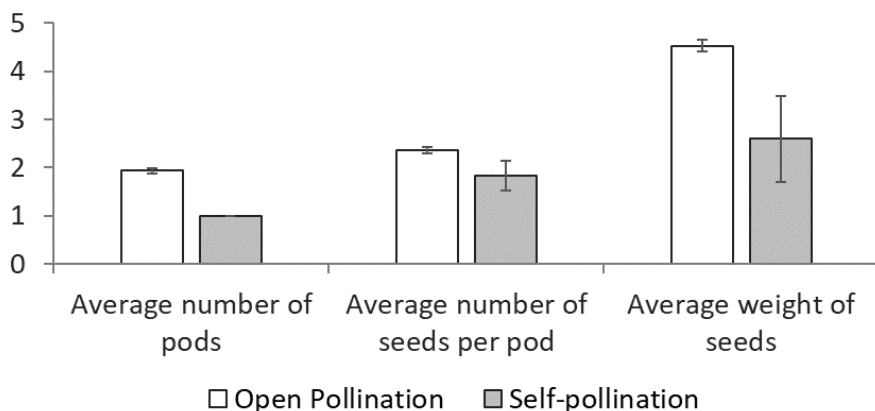
Fig. 5. The average number (± standard error; right), seed numbers (± standard error; middle), and weight (gr; ± standard error; left) of pods produced per inflorescence of *V. faba* in open pollination and self-pollination assays.

other studies. The visitation rate of *B. terrestris* varied significantly between years. We found evidence that suggested that competition for pollen and nectar is happening after the first and second day of the flower lifespan, respectively, especially in the year when *B. terrestris* is found in high abundance. *Bombus terrestris* was mainly a floral parasite of *V. faba*. In most cases, even when legitimate visits occur, the pollinator fails to move the flower keel (a necessary pre-requisite for successful pollination). In addition, the high demand of resources that this species needs is likely to prevent other pollinators from visiting and pollinating the flower.

Overall, there was a low diversity and abundance of native pollinators. It is likely that before the arrival of *B. terrestris*, the main pollinator of *V. faba* was the long-tongued bumblebee, *B. dahlbomii*. Interviews with Mapuche gardeners suggest that *B. dahlbomii* is or was the main pollinator of *V. faba* in rural areas 170 km north of the garden studied here (B. Chicori pers. Comm., indigenous leader). In 2019, we observed abundant *B. dahlbomii* on *V. faba* flowers in northern Chiloé Island (around 108 km south of the field studied). It is highly likely that *B. dahlbomii* pollination in *V. faba* has been replaced by both the long-tongued bumblebee *B. ruderatus* and the short-tongued *B. terrestris*.

In a garden experiment conducted in Germany, the frequency of nectar robbery by *B. terrestris* compared to legitimate visits was high at 67.4% (Marzinzig et al. 2018). In Finland, the frequency was nearly 60% (without considering extra-floral visits, Lönnqvist 2019). These percentages are lower than what we found in our study (87.19%),

and this is probably related to the high abundance of this invasive insect in south-central Chile and Argentina. We have no explication about why *A. mellifera* was identified as undertaking nectar robbing behaviour in the first but not in the second year. However, one possibility is that the higher abundance of *B. terrestris* during the second year could have altered the behaviour of *A. mellifera*, although it could be related with changes in resources. The high abundance of *B. terrestris* observed in 2019 was also found in eight other *V. faba* fields located in Osorno province (Rendón-Funes et al. manuscript in preparation), in Valdivia gardens (100 km north our study site (Ramires unpublish data)) and in Chiloé Island (200 km south our study site; Smith-Ramírez et al. manuscript in preparation).

High inter-annual changes in the relative abundance of *B. dahlbomii*, *Apis* and *B. terrestris* has been described in a long-term study by Smith-Ramírez et al. (2014). In the case of *B. terrestris*, while we see an overall increase in abundance (Montalva et al. 2017), there is an up and down (primary order) inter-annual dynamics (Ramires 2019). In 2018, the abundance of *B. terrestris* was lower than in 2019. Because our study sites and the surrounding landscape had only wild colonies of *B. terrestris* and *A. mellifera*, our observations are likely to be representative of natural population changes rather than the effect of importation of commercial colonies of either species.

Our results suggest that intra- and inter-specific competition for resources, both for pollen and nectar, occurs in years when *B. terrestris* is found in high abundance and pollen limitation precedes nectar limitation. Pollen is a more limited resource

than nectar because the former is not replenished over the lifetime of the flower. However, it is the occurrence of pollen that makes tubular flowers, including those of *V. faba*, attractive to short-tongued bees as *Apis* and *Cadeguala*. We also found that after the first day of the flower becoming a viable resource for bees, the time spent in legitimate visits by *A. mellifera* decreased, but only in year two. Evidence for some level of increase in the number of robbery visits during the lifespan of the flower probably may indicate that nectar production in *V. faba* has been stimulated by the ongoing extraction (as shown in Pierre et al. 1996). We found that pollinators spend less time in nectar robbery on the third day after flower onset, but only for the year with the highest abundance of *B. terrestris*. It is likely that there is a limit in the standing crop of nectar that a flower can produce, and after an average of 23 nectar robbery visits per flower (but up to 96 visits), continued nectar production is beyond the flower's capacity. Our results suggest that by the third day, visiting pollinators may still be searching for nectar, but after this, a reduction in nectar resources results in a decline in subsequent robbery events. Even during the first day, some pollinators spend as little as one second robbing some flowers. This may also be indicative that nectar has become a limited resource, and competition for nectar can occur at this stage in some years. Given the high rate of robbery observed in *B. terrestris*, it is unlikely that the number of legitimate visits can be sufficient to increase yields in this system.

It has been reported in Europe that *V. faba* flowers live six to seven days, being open 31.5 to 35.5 hours (Stoddard & Bond 1987; Pierryman & Marcellos 1988). In comparison, we found that the flowers of the cultivar studied lived three, and exceptionally four days, and that flowers were open for about 12 hours over this period in total. If we consider that the main activity of pollinators occurred in the portion of the day when *V. faba* flowers are open, then for the cultivar studied (one the most common in the Chilean family-gardens), this represents only a limited amount of time during which cross-pollination could occur (Stoddard 1986).

A low frequency of legitimate visits to *V. faba* has been reported by several authors as one of the reasons for the small and unstable yields of this legume (Scriven et al. 1961; Poulsen 1975; Mesquida et al. 1990; Suso et al. 2001 and many others). Suso et al (2001) studied six *V. faba* fields in Spain and six more in France, observing the flowers in periods of five minutes intervals over the entire flowering period. They found that on average, 1.9 to 8.5% of flowers in Spain, and 11.1 to 27.5% in France, received pollinator visits. We estimate that in our studied field, 70 to 100% of flowers received legitimate visits during the total observation period. This on-site result coincides with what we observed by cameras, with 100% of the flowers receiving legitimate visits during their lifespan. The average duration of a legitimate visit (excluding the first day) was 2.63 and 4.98 seconds in 2018 and 2019, respectively. However, one-third of them would have been unlikely to result in pollination. We do not know how many pollination visits are required for *V. faba* to be fertilized; however, our data suggest no over pollination of *V. faba* by *B. terrestris*, despite their high abundance. *Vicia faba* might even be pollinator-limited as the high abundance of *B. terrestris* may be displacing honeybees and native pollinators. We do not know why there is no increase in nectar foraging in the morning as *V. faba* flowers replenish the nectary early in the morning and afternoon (Stoddard & Bond 1986). It might be that the high foraging rate at sunset limits the capacity of the flower to refill the nectary by the next morning.

Although we did not obtain conclusive results from the cross-pollination experiment, we found that self-pollination decreases the production of pods, seed number and seed weight compared to open pollination. We propose that cross-pollination would probably be greater at lower abundance of *B. terrestris*, as this would allow greater abundances of *A. mellifera* and other pollinators to occur. Our results suggest that where high abundance of *B. terrestris* occurs, the resulting reduction in flower resources available for other pollinators could promote competitive displacement.

It has been proposed that *B. terrestris* contributes positively to the pollination of *V. faba* crops in Finland because the perforation in the base of nectary is necessary to induce legitimate visits (Kyllönen 2018). We think that in Chile this is not true, since *B. terrestris* is highly abundant,

which leaves little nectar available for the use of other pollinators, a result also found by Sáez et al (2017) in a South Argentina raspberries field. In our study, we also recorded some expulsions of *A. mellifera* from *V. faba* flowers by *B. terrestris* in the second year when *B. terrestris* was most abundant, but this was not frequent. Displacement of native pollinators (interference competition) by *B. terrestris* has also been mentioned by Dafni & Schmida (1996), but not found by Hingston & McQuillan (1999). Also, *B. terrestris* is not likely to be a good pollinator in this system despite its high abundances, reflecting the fact that approximately 50% of flowers did not receive any legitimate visits during their entire lifespan, with a further third of these legitimate visits being of a quality unlikely to lead to seed set (bees did not displace the flower keel).

In conclusion, this study suggests that in the case of *V. faba* in South-central Chile, the decrease in the time foraging for nectar or collecting pollen over the lifespan of the flower is evidence for some level of resource limitation. To our knowledge, only one other study has identified competitive displacement of other pollinators by *B. terrestris*, looking at the impact on two native Megachilidae in Tasmania and concluding that displacement happened because of competition for a limited resource (Hingston & McQuillan 1999). If such competitive exclusion continues to be an issue, it is likely that the continued increase of *B. terrestris* populations in Latin America (Montalva et al. 2017) will exacerbate resource competition with native pollinators. As much of the *V. faba* crops are grown for local consumption, this may negatively impact food resources, an unexpected result given the ever-increasing populations of this invasive pollinator species.

AUTHOR CONTRIBUTION

C.S.R., A.R. and R.B. wrote the manuscript; A.R. compiled the data. A.R., R.B. and W.M. led the statistical analysis; C.S.R., A.R., R.B. and W.M. did field samples; All the authors contributed critically to the draft and gave final approval to the publication.

APPENDICES

Additional supporting information may be found in the online version of this article:

Appendix I. Number of legitimate visits and nectar robberies when the flowers were open and closed.

Appendix II. Generalized linear models (GLM) used to test the effect of year and pollinator species

Appendix III.The results of GLM analysis with factors

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