

## Accepted Manuscript

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PII: S1439-1791(16)30084-6

DOI: <http://dx.doi.org/doi:10.1016/j.baae.2017.01.001>

Reference: BAAE 51012

To appear in:

Received date: 10-7-2016

Accepted date: 2-1-2017

Please cite this article as: <http://dx.doi.org/>

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# Invasive bumble bees reduce nectar availability for honey bees by robbing raspberry flower buds

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## Abstract

The introduction of exotic bees for crop pollination is an increasingly common practice worldwide. While beneficial for crop production, exotic species may become invasive with several deleterious ecological and economic impacts. We studied whether robbing of flower buds by a highly invasive bumble bee (*Bombus terrestris*) reduces nectar availability for managed honey bees (*Apis mellifera*). We assessed the proportion of robbed buds, and nectar availability in flowers and buds (robbed and non-robbed) in 16 raspberry fields along gradients of bumble bee and honey bee abundance in NW Patagonia, Argentina. We also measured nectar production and replenishment in flowers that developed from robbed and non-robbed buds in one experimental field. Across fields, the proportion of robbed buds increased with the abundance of bumble bees, but not of honey bees. At anthesis, flowers from buds that were robbed once contained half the nectar, and produced two thirds of the nectar compared to those flowers that had not been robbed. Therefore, high abundance of invasive bumble bees can reduce resources for managed honey bees by robbing flower buds. This study reveals a novel, negative impact of bumble bee invasions that could potentially affect honey production.

**Keywords:** honey bees, invasive bumble bee, flower buds, nectar production, nectar robbing, raspberry.

## Introduction

Biological invasions generate ecological, social and economic costs through their manifold impacts on natural- and agro-ecosystems (Pimentel 2001; Perrings et al. 2002; Simberloff et al. 2013). Invasion by bees has increased since their intentional introduction in most agro-ecosystems for crop pollination (Velthuis & van Doorn 2006). Bumble bees, most notably *Bombus terrestris*, are being increasingly reared and sold worldwide, becoming highly invasive in some regions (Matsumura et al. 2004; Schmid-Hempel et al. 2007, 2014; Dafni et al. 2010; Morales et al. 2013). Several studies have demonstrated the deleterious impacts of *B. terrestris* invasion on native bee fauna (Morales et al. 2013; Schmid-Hempel et al. 2014), wild plant reproduction (Dohzono et al. 2008), and even crop production and quality (Aizen et al. 2014). However, no previous research has addressed the potential impacts of invasive bumble bees on resource availability for managed bees, which could in turn impact apiculture.

Negative impacts of invasive species on different components of the native and introduced biota, are often related to their extremely high population densities (Kearns & Crawley 2002, Aizen et al. 2014, Saez et al. 2014). The high abundance reached by invasive bees, like *B. terrestris*, could increase resource competition with other bee species, including managed honey bees. Particularly, apiculture depends on floral resources, mainly nectar and pollen, gathered by honey-bee workers to produce honey and other products. The cooperative behavior of the social honey bee (*Apis mellifera*) makes it an efficient competitor for high-quality food resources that are highly clumped in space and time, like most flowering crops. However, the invasion of a floral competitor that is able to preempt nectar (by robbing behavior), could reduce nectar availability for managed honey bees. Although previous studies have shown that managed honey bees can inhibit resource gathering and reproduction of native bumble bees (Thomson 2004;

Goulson & Sparrow 2009), the reciprocal effect of invasive bumble bees on resource gathering by honey bees has been largely unstudied.

Many large bees, including several species of bumble bees (e.g. *B. terrestris*, *B. lapidarius*, *B. lucorum*), can make holes near the base of the perianth of tubular flowers to gain access to nectar, which otherwise would not be accessible to them owing to their short tongues (Inouye 1980; Inouye 1983, Garibaldi et al. 2015). This behavior, known as “nectar robbing” occurs more frequently when corollas are too long to reach the nectar produced in the nectaries through legitimate visitation, or when robbing is more energetically more efficient (Roubik et al. 1985, Soberón & Martínez del Río 1985). As a consequence, the holes produced at the flower base provide bumble bees a competitive advantage in the access to nectar over other flower visitors. Existing holes also provide an opportunity for visitors incapable of drilling them to steal nectar by using those made by the primary nectar robber (i.e. secondary robbing), a behavior frequently observed in honey bees (Maloot & Inouye 2000; Deleplane & Mayer 2000; Dedej & Delaplane 2005). However, the primary thief usually removes most of the nectar (Irwin 2000; Dedej & Delaplane 2005).

At the flower level, nectar robbing not only reduces instantaneously the amount of nectar available, but also can generate diverse responses in nectar production, from over-compensation (robbed flowers secrete more nectar than non-robbed ones; Roubik et al. 1985, see also Maloot & Inouye 2000) to under-compensation (robbed flowers secrete less nectar than non-robbed ones; González-Gómez & Valdivia 2005; Castro et al. 2008). On the one hand, the mechanisms behind over-compensation of nectar production in robbed flowers are still unclear, in part because of the confounding fact that robbers often visit flowers with higher intrinsic nectar production than average (Maloot & Inouye 2000). On the other hand, robbers can damage nectaries while robbing flowers leading to a

reduction (i.e. under-compensation) in nectar production (González-Gómez & Valdivia 2005; Traveset et al. 1998). Although there is an increasing amount of literature on the different evolutionary and ecological consequences of nectar robbing, the practical, economic implications for both agriculture and apiculture, particularly when crop flowers are intensively exploited by bees, have never been addressed before (see Dedej & Delaplane 2004). Here we asked whether primary nectar robbing of raspberry flower buds by an invasive bumble bee (*Bombus terrestris*) in NW Patagonia (Morales et al. 2013), could depress nectar availability for managed honey bees (*Apis mellifera*). If robbing produces over-compensation in nectar production, honey bees can be favored due to an increase in resource availability; however, if under-compensation occurs, honey bees could be negatively affected by decreased resource availability, which could subsequently reduce the income of beekeepers.

Nectar robbing of fully expanded flowers is a common phenomenon (Rojas-Nossa et al. 2015), and its consequences for subsequent nectar production and reproductive success has been extensively assessed (see Maloof & Inouye 2000 and references therein). However, nectar robbing of flower buds has been much less documented and, to our knowledge, its consequences for standing nectar crop and subsequent nectar production have never been addressed. Here, we studied how nectar robbing of raspberry (*Rubus idaeus*) flower buds by an invasive bumble bee (*B. terrestris*) affects nectar production, replenishment and standing crop. Because nectar from raspberry flowers is one of the main resources collected by managed honey bees, this strategy of resource preemption employed by *B. terrestris* might affect nectar availability. We hypothesized that robbing of raspberry flower buds by this invasive bumble bee could result in under-compensation and, consequently, in a reduction of nectar available to managed honey bees. Specifically, we asked the following questions: (i) Do bee abundance and identity (bumble bee or

honey bee) affect the intensity of bud robbing? (ii) Does bud robbing affect nectar standing crop and subsequent nectar production in open flowers? Finally, (iii) how much does bud robbing affect nectar availability for honey bees at the field scale?

## Material and methods

### *Study system*

Honey production is an important economic activity in inter-mountain valleys of the eastern slopes of Patagonian Andes, Argentina. Beekeepers place honey bee hives preferentially near raspberry plantations, the most important regional crop (see *Study crop* below), to increase honey production (Secretaría de Agricultura, Ganadería, Pesca y Alimentos 2006; Instituto Interamericano de Cooperación para la Agricultura 2012). Nectar and pollen from raspberry flowers represent one of the main floral resources collected by honey bees in these valleys, particularly late in the summer, when alternative floral resources are scarce (C. Morales, unpublished). In the Patagonian Andes, raspberry flowers are visited almost exclusively by two non-native bee species, the managed *A. mellifera* and the invasive *B. terrestris* (Morales, 2009; Sáez et al. 2014).

The bumble bee arrived in Argentine Patagonia in 2006 after being introduced in neighboring Chile for crop pollination in 1997 (Torretta, et al. 2006). Currently, *B. terrestris* is, by at least one order of magnitude, the most abundant flower visitor in the Patagonia region, where it robs nectar from over a dozen of native and non-native plant species (Morales et al. 2013; Schmid-Hempel et al. 2014; Geslin & Morales 2015, Morales et al. 2016). Although this bumble bee is a legitimate visitor of raspberry flowers, it was also observed robbing nectar from raspberry buds by drilling a hole through the calyx before flower anthesis (Sáez et al. 2014), and thus, obtaining floral resources before they become accessible to legitimate flower visitors.

*Study crop*

Raspberry, *Rubus idaeus* (Rosaceae), is a temperate-zone shrub cultivated for its fruit (Crane & Walker 1984). Flowers present numerous pistils in the centermost part, surrounded by a ring of nectary tissue, and an external ring of anthers. When flowers open, nectar is freely exposed to pollinators. The nectary produces large amounts of nectar with high sugar content (10-20  $\mu$ L and 35-60%, respectively) (Haragsimova-Neprasova 1960; Simidchiev 1976), and the rate of nectar secretion decreases along the ~3-day flower life span (Simidchiev 1976; Willmer 1994). Thus, bees find young raspberry flowers particularly attractive in terms of accessibility and reward.

- I. Do the abundance of bumble bees or honey bees affect the intensity of bud robbing?

Field work was conducted during the 2014 austral summer (January-February) in 16 raspberry fields located in north-west Patagonia, Argentina, near Lago Puelo National Park and other conservation areas. Therefore, these fields were surrounded by or near large extensions of temperate forests of the Sub-antarctic domain (Cabrera 1976). The sampled raspberry fields varied between 0.1 to 1 hectare, and all were planted with the “Autumn Bliss” variety. Distance between fields was always >1.5 km, averaging 12.2 km, which exceeds the expected mean foraging distance of most social bees (Walther-Hellwig & Frankl 2000; Steffan-Dewenter & Kuhn 2003; Osborne et al. 2008), and thus each field can be considered as an independent replicate regarded as an its bee fauna. All measures were recorded under fair weather conditions (i.e. sunny or slightly cloudy days with, at most, light wind) from early January to early March, between 10:00 and 20:00 h.

In order to quantify bud robbing, each raspberry field was surveyed on two different days during the flowering season, with collections made in the morning of one

day and in the afternoon of the other. On each sampling occasion, we collected about 15 buds which were in an advanced developmental stage (~3 h before anthesis), totaling 509 buds. Each bud was classified as robbed, if the perianth presented a hole, or non-robbed, if there was no hole.

To estimate the abundance of bees per raspberry field, we performed pollinator censuses during which we recorded the number of flowers and buds (non-robbed and robbed) visited by each pollinator species to a pair of neighboring raspberry stems (<20 cm apart) for a period of 5 minutes (i.e. no. visits · flower<sup>-1</sup> · 5 min<sup>-1</sup>). The number of open flowers, non-robbed buds and robbed buds per stem, and the number of observed flowers and buds varied among censuses ranging from 5 to 8 for receptive flowers, 0 to 3 for non-robbed buds, and 0 to 3 for robbed buds. Within a field, each census involved a different randomly selected pair of stems, and thus a different set of flowers and buds. Each field was surveyed on two different days over the flowering season, with 10 censuses performed in the morning (10-13 h) and 10 in the afternoon (15-19 h), totaling 320 censuses. Measurements were made during either morning or afternoon on each sampling day.

We also assessed whether bud robbing provides an opportunity for obtaining a higher nectar quantity in comparison with open flowers by measuring the quantity of nectar available (i.e. standing crop) in flowers and buds (both robbed and non-robbed). Nectar was extracted with microcapillary tubes repeatedly from the nectaries of randomly selected flowers and buds until no further nectar could be extracted. We used 0.5 µl microcapillary tubes for receptive flowers, and 2 µl for buds (robbed and non-robbed). To measure nectar in robbed buds, we used the hole already perforated by robbers to insert the microcapillary tube and reach the nectary. For non-robbed buds, we robbed the



flowers artificially by drilling a hole through the perianth with a microcapillary tube. In each field, we measured nectar in about 30 receptive flowers, 15 during the morning and 15 during the afternoon (totaling 575 measures), and in about 20 buds (about half of them robbed), 10 of them sampled during the morning and 10 during the afternoon (except for two fields where buds were not sampled), totaling 186 measures for non-robbed buds and 217 for robbed ones. Each field was surveyed on two different days over the flowering season, measuring nectar either during the morning or afternoon on each sampling day. We used nectar volume as a proxy of resource availability as it is closely related to total sugar content (see Appendix A for details).

We evaluated the influence of the abundance of *B. terrestris* and *A. mellifera* (estimated as visit frequency to flowers, see above) on the probability of a bud being robbed with a generalized linear mixed-effects model. Data analysis was carried out using the *lmer* function from the *lme4* package (Bates et al. 2015) of the R software (version 2.15.1). Because the response variable (i.e. “bud status”) follows a Bernoulli trial process (i.e. robbed vs non-robbed), the model assumed a Binomial error distribution with a *logit* link function. Visitation frequencies of *B. terrestris* and *A. mellifera* were included as additive fixed effects and each “field” as a random effect, allowing the intercept to vary among fields. Partial regression coefficients for the abundance of bumble bees and honey bees were used to discriminate between the differential effects of the two main flower visitor species. Although visit frequencies of *A. mellifera* and *B. terrestris* presented a weak negative correlation (see Results), the variance inflation factor was 1.38, showing no strong inflation effect on the SEs of the estimated coefficients (i.e. absence of multicollinearity: Neter et al. 1989; Hair et al. 1995; StataCorp 1997).

Because abundances of bumble bees and honey bees were negatively correlated, we evaluated which factor, i.e. abundance of bumble bee or presence/absence of bee

hives, had a stronger effect on the abundance of honey bees foraging in raspberry fields with a multiple regression model. This approach allows us to distinguish if bumble bees avoid or, on the contrary, displace honey bees. If bumble bees avoided honey bees, we should expect more honey bees and fewer bumble bees in fields with, or surrounded by, bee hives. If bumble bees displaced honey bees, we should expect a negative association between the abundances of foraging bumble bees and honey bees, but no additional effect of the presence of bee hives on honey-bee abundance. Data analysis was carried out using the *lmer* function from the *lme4* package (Bates et al. 2015) of the R software (version 2.15.1). Because the response variable (number of honey bee visits) were counts, the model assumed a Poisson error distribution with a *log*-link function. We included the number of raspberry flowers observed in each census as an *offset* (i.e. a fixed predictor known in advance to influence insect visitation) (Gelman & Hill 2007). Mean abundance of *B. terrestris* and the presence/absence of bee hives were included as additive fixed effects and each “field” as a random effect, allowing the intercept to vary among fields.

We also evaluated if the proportion of robbed buds at the field level modifies the foraging behavior of honey bees, increasing their visitation to robbed flower buds (i.e. secondary robbing). To do this, we analyzed the effect of the proportion of robbed buds on visit frequency to robbed buds by honey bees. Data analysis was carried out using the *lmer* function from the *lme4* package (Bates et al. 2015) of the R software (version 2.15.1). Because the response variable (number of honey bee visits) were counts, the model assumed a Poisson error distribution with a *log*-link function. We included the number of robbed buds observed in each census as an *offset* (i.e. a fixed predictor known in advance to influence insect visitation) (Gelman & Hill 2007). Mean proportion of robbed buds per field was included as a predictor fixed effect and each “field” as a random effect, allowing the intercept to vary among fields.

Finally, we evaluated the influence of three categories of flower status (i.e. receptive flowers, non-robbed buds, and robbed buds) on nectar availability with generalized linear mixed-effects models. Data analysis was carried out using the *lme* function from the *nlme* package (Pinheiro et al. 2015) of the R software (version 2.15.1), assuming a Gaussian error distribution. The flower status was included as fixed effect, and “field” as a random effect, allowing the intercept to vary among fields. Because our model did not achieve the assumptions of normally distributed errors and homogenous variance, we reran the analysis with the response variable ( $\mu\text{l}$  of nectar) transformed as  $\ln + 1$  and using a heterogeneous variance model (*varIdent* function in the *nlme* library), which increased model fit (lower AIC) and provided compliance with model assumptions. We made a multiple comparison of means with a Tukey post-hoc test using the *glht* function from the *multcomp* package (Hothorn et al. 2008).

## II. Does bud robbing affect nectar production and replenishment?

Field work was conducted during the 2015 austral summer (January-February) at San Felipe ( $42^{\circ} 0.582' \text{ S}$ ,  $71^{\circ} 31.677' \text{ W}$ ), one of the fields sampled the previous year. All measures were recorded under fair weather conditions for foraging bees (see above) from 10:00 to 20:00 h during the main raspberry blooming period from mid-January to mid-February.

We performed two separate trials to quantify the effects of bud robbing on nectar standing crop and replenishment. First, to estimate the amount of nectar gathered by the primary robber, we compared mean nectar volume from 30 non-robbed buds with mean nectar volume remaining in 15 robbed buds right after the first robbing by a bumble bee, extracting nectar in robbed and non-robbed buds as explained above. Second, to estimate the consequences of nectar robbing on subsequent nectar production, we bagged 20 non-robbed and 15 robbed buds with bridal mesh right after primary robbing. We then

measured nectar volume and sugar concentration with a refractometer at the moment of flower anthesis extracting nectar with 2  $\mu$ l microcapillary tubes repeatedly from the nectaries until no further nectar could be extracted. After nectar extraction, we bagged the flowers once again and re-sampled the same flowers after subsequent periods of 4 h, measuring quantity of nectar and sugar concentration to quantify nectar replenishment.

To estimate the amount of nectar removed by the first robber, we compared the volume of nectar in non-robbed buds *vs.* the volume remaining right after the first robbing with Student's *t* test by using the function *t.test* of the R software (version 2.15.1). The volume of nectar from both groups of data sets (non-robbed buds and robbed) was  $\ln + 1$  transformed to achieve homogeneity of variance.

To estimate the effect of bud robbing on nectar production and replenishment, we evaluated if the production and replenishment of nectar depends on whether the bud had been robbed or not by using the *lme* function from the *nlme* package (Pinheiro et al. 2015) of the R software (version 2.15.1), assuming a Gaussian error distribution. Because we took repeated measures from the same flowers we incorporated “flower” as a random effect in our model, allowing the intercept to vary among flowers. The response variable (i.e. volume of nectar) was  $\ln + 1$  transformed to comply with the normality assumption. Also, because we took repeated nectar measures on the same flowers over time (in sequences of 4 hours), we evaluated the presence of temporal auto-correlation in the normalized residuals from our mixed-effect model by using the auto-correlation function (ACF) (Zuur et al. 2009). Because the ACF plot showed no violation of the independence assumption (i.e. time lags had no patterns in the residuals), there was no need to incorporate a temporal correlation structure in our model.

**III.** Effects of bud robbing on resources availability for honey bees at the field scale.

We estimated nectar production per hectare available for free-foraging pollinators in raspberry fields with 0, 7.5, 40 and 80% of their buds robbed by *B. terrestris*. These values were chosen to represent a hypothetical field with no bud robbing and within the observed range of bud robbing intensity, being 7.5, 40, and 80% the minimum, mean, and maximum observed percent of robbed buds across fields, respectively. To achieve this goal, we combined data on estimated number of flowers produced per hectare, total quantity of nectar produced per flower over flower lifespan by flowers from buds that were robbed and non-robbed (see sub-section *II.* from M & M), and the probability of a bud being robbed (see sub-section *I.* from M & M). In each field, we counted the total number of flowers produced per stem (i.e. including buds, open flowers and developing fruits) and the number of stems per meter in a row, with eight random replicates per field. The number of flowers per hectare was then calculated by multiplying the mean number of flowers per meter by the total length of planted rows per hectare.

For each data set mentioned above (i.e. total number of raspberry flowers per hectare, and nectar production by flowers that were robbed and non-robbed before anthesis), we generated 1000 mean values using bootstrap with replacement using the *sample* function in R software (see Crawley 2007). Finally, we multiplied these three vectors of pseudo-means, estimating expected values of nectar production under different intensities of bud robbing as,

$$\text{Nectar production (ha)} = nF * [NRF * (1-p)] * [RF * p]$$

where  $nF$  is the mean number of raspberry flowers per hectare;  $NRF$ , nectar production in non-robbed flowers;  $RF$ , nectar production in robbed flowers after robbing, and  $p$  is the probability of a flower being robbed (0, 0.075, 0.4 and 0.8).

## Results

In total we observed 2,394 visits to receptive flowers of raspberry after accumulating a total of 26 h of pollinator observation. Managed honey bees (*A. mellifera*) and invasive bumble bees (*B. terrestris*) accounted for 52 and 46% of the visits, with a mean visit frequency of 0.83 and 0.73 visits · flower<sup>-1</sup> · 5 min<sup>-1</sup>, respectively. Wild bees accounted for the remaining 2% of the visits, with a mean visit frequency of 0.03 visits · flower<sup>-1</sup> · 5 min<sup>-1</sup>. Visit frequencies of honey bees and bumble bees were negatively correlated (Pearson's  $r = -0.60$ ,  $n = 16$ ,  $P = 0.01$ ) (Fig. 1), while the presence of bee hives, within or nearby raspberry fields, did not increase honey bee density ( $\beta = 0.32$ ,  $SE = 0.40$ ,  $z = 0.79$ ,  $P = 0.42$ ). Minimum, mean, and maximum total visit frequency to flowers across raspberry fields were 0.7, 1.5, and 2.6 visits · flower<sup>-1</sup> · 5 min<sup>-1</sup>, respectively. Mean ( $\pm$  SE) nectar volume available per flower (i.e. standing crop), regardless of robbing status, was  $0.057 \pm 0.006$   $\mu$ l.

Across raspberry fields, the probability of a flower bud being robbed increased significantly with bumble bee abundance, but not with honey bee abundance as estimated by visit frequency (in *logit* scale,  $\beta = 1.79$ ,  $SE = 0.68$ ,  $P = 0.008$ , and  $\beta = 0.48$ ,  $SE = 0.45$ ,  $P = 0.29$ , respectively) (Fig. 2). Indeed, during pollinator censuses we only observed bumble bees primarily robbing (i.e. piercing the sepals of non-robbed buds) to gain access to nectar. On average, 70% of the buds were robbed in fields with the highest bumble bees visit frequency, whereas only 15% of buds were robbed in fields with the lowest visitation (Fig. 2).

The standing volume of nectar sampled in non-robbed buds was, on average, 70 times higher than in receptive flowers (in *ln* scale,  $\beta = 1.27$ ,  $SE = 0.05$ ,  $z = 22.32$ ,  $P < 0.001$ ) with a mean ( $\pm$  SE) of  $4.0 \pm 0.29$   $\mu$ l of nectar per bud (Fig. 3). Once a bud was robbed, both honey bees and bumble bees kept harvesting nectar from the bud through

the hole drilled by the first robber (i.e. secondary robbing). However, bumble bees revisited robbed buds 2.2 times more frequently than honey bees. Across fields, honey-bee visitation to robbed buds (secondary robbing) was not significantly affected by the proportion of robbed buds ( $\beta = 1.79$ ,  $SE = 1.62$ ,  $z = 1.1$ ,  $P = 0.26$ ). The standing volume of nectar available in robbed buds was, on average, 15 times higher than in receptive flowers (in  $\ln$  scale,  $\beta = 0.44$ ,  $SE = 0.03$ ,  $z = 13.34$ ,  $P < 0.001$ ), but 4.5 times lower than in non-robbed buds, with a mean ( $\pm SE$ ) of  $0.89 \pm 0.08 \mu\text{l}$  of nectar per robbed bud (in  $\ln$  scale,  $\beta = 0.82$ ,  $SE = 0.06$ ,  $z = 12.51$ ,  $P < 0.001$ ) (Fig. 3).

Experiments of nectar consumption by the first robber (i.e. *Bombus terrestris*) showed that nectar availability decreased from (mean  $\pm SE$ )  $4.25 \pm 0.85 \mu\text{l}$  in non-robbed buds to  $1.13 \pm 0.51 \mu\text{l}$  after the first robbing ( $t = -3.27$ ,  $df = 31.44$ ,  $P = 0.002$ ), showing that, on average, the first robber removed  $3.12 \mu\text{l}$  of nectar per bud (Fig. 4, trial “a”). Interestingly, nectar accumulated at the moment of flower opening decreased from (mean  $\pm SE$ )  $21.24 \pm 1.24 \mu\text{l}$  when buds were non-robbed to  $11.22 \pm 1.02 \mu\text{l}$  in flowers whose buds were robbed once (in  $\ln$  scale,  $\beta = -0.56$ ,  $SE = 0.26$ ,  $t = -2.11$ ,  $P = 0.04$ ) (Fig. 4, trial “b”). Thus, flowers developing from buds that were robbed once offered, on average, half the amount of nectar (i.e.  $10.01 \mu\text{l}$  less nectar) than those flowers that were non-robbed, producing in total about 32.5% less nectar than non-robbed flowers (i.e.  $100 * \{1 - [(11.22 + 3.12) / 21.24]\}$ ) at the moment of anthesis. Nectar produced during the first 4 h, and between 4 and 8 h after flower anthesis was minimal and did not differ between flowers developing from buds that were non-robbed vs robbed ( $\beta = 0.64$ ,  $SE = 0.43$ ,  $t = 1.47$ ,  $P = 0.15$ , and  $\beta = 0.87$ ,  $SE = 0.63$ ,  $t = 1.38$ ,  $P = 0.17$ , respectively), with both types of buds experiencing a strong decline in nectar production after flower anthesis (Fig. 4, trail “b”).

Mean bootstrap estimation of nectar volume per hectare available for pollinators visiting open flowers in raspberry fields not experiencing bud robbing was, on average, 48.9 L/ha (Fig. 5). Taking into account the minimum, mean, and maximum values of bud robbing observed (i.e. 7.5, 40 and 80%) and regarding as a reference a hypothetical field with no bud robbing, estimated nectar production decreases, on average, 2.5%, with a mean production of 47.8 L/ha, for the minimum level of robbing intensity observed; 12%, with a mean production of 43 L/ha, for the average level of robbing; and 24%, with a mean production of 37.2 L/ha, for the maximum level of bud robbing observed (Fig. 5).

## Discussion

Bud piercing by invasive bumble bees to rob nectar has become a common phenomenon in raspberry fields of NW Patagonia. Despite high nectar production of raspberry flowers, this robbing behavior could relate to low nectar availability in open flowers due to extremely high visitation frequency (see Results, and also Sáez et al. 2014). Indeed, here we showed that the intensity of bud robbing increased with the abundance of *B. terrestris* foraging in raspberry fields. Furthermore, during more than 25 h of pollinator observation, we observed only bumble bees robbing buds, allowing them to reach higher quantities of nectar per visit compared to honey bees (bumble bees also visited robbed buds more frequently). The process of bud piercing generates a negative feedback, by reducing nectar availability at anthesis by half and total nectar production by about one third (i.e. under-compensation). In fields with the highest level of bud robbing (about 80%), we estimated that nectar available per ha for legitimate flower foragers decreases, on average, by 24% compared to the expected production without bud robbing. This is a conservative estimation because our calculations did not consider the impact of secondary robbing, which could exacerbate these differences.



Nectar robbing through corolla holes has been described for several bee species, and its fitness consequences have been studied for many plant species (Malooof & Inouye 2000 and references therein). But, why do bees rob flowers? First, robbing behavior could be the only way for short-tongued bee species to access floral rewards (Inouye 1980; Inouye 1983, Garibaldi et al. 2015, Rojas-Nossa et al. 2015). Flower robbing could also be promoted when the cost-benefit balance between handling time and resource intake favors this behavior (see Irwin et al. 2010). Pyke (1982) proposed that bees may adopt a robbing behavior as a resource preemption strategy due to competition with other bees for nectar resources; however, this hypothesis has never been experimentally tested (Irwin et al. 2010). Here, we present evidence that supports Pyke's (1982) hypothesis. Although we did not observe direct competitive interactions between honey bees and bumble bees at flowers or buds, abundance of *B. terrestris* foraging in raspberry fields was negatively associated with the abundance of foraging honey bees after controlling for the presence of bee hives. Because this bumble bee was able to preempt the nectar resource by robbing buds, whereas the honey bee was not, it is expected that here bumble bees displace competitively honey bees and not *vice versa*. In support of this interpretation, we found that addition of honey bee hives did not increase the abundance of foraging honey bees in raspberry fields exhibiting high visitation by *B. terrestris* as those surveyed here. Therefore, although honey bees are commonly good competitors for high quality resources due to their cooperative behavior (Patton 1990, 1996; Wills et al. 1990; Horskins & Turner 1999), in raspberry fields from NW Patagonia bumble bees are preempting an important nectar resource through their bud robbing behavior.

The bud robbing behavior exhibited by *B. terrestris* could partly explain why this alien bumble bee is a successful invader in this region. But more important, this bumble bee reduces the amount of nectar available for honey bees, and consequently for

beekeepers. This reduction caused by bud robbing occurs through two different mechanisms: (1) directly, through exploitative competition (i.e. resource preemption), and (2) indirectly, through a decrease in total nectar production (i.e. under-compensation). Although raspberry flowers represent one of the most important nectar sources for honey bees in NW Patagonia, particularly late in the flowering season, they also forage on many native and exotic plants species (C. Morales unpublished). Unfortunately, the exotic bumble bee is also a frequent robber of many of these species as well (Morales et al. 2013). For this reason, a thorough survey of nectar robbing at the plant community level will generate a more comprehensive understanding of the consequences of bumble bee invasion. However, the in-depth study reported here suggests that invasive bumble bees can have a sizable impact on the resources available for other flower visitors.

## **Conclusions**

Introduction of managed bees to supplement the pollination service provided by wild bees to crops is becoming a common practice worldwide (Velthuis & van Doorn 2006, and references therein). Although the introduction of non-native pollinators has increased the yields of some pollinator-dependent crops (Velthuis & van Doorn 2006; Southwick & Southwick 1992), in natural and agricultural ecosystems where these species become highly invasive the cost of such practice could exceed the benefits (Aizen et al. 2014). Besides the cost in terms of decreasing raspberry fruit quality via style damage (Sáez et al. 2014), here we described another overlooked cost, flower bud robbing, which by greatly reducing nectar availability might have negative consequences for the apicultural sector. For this reason, rather than introducing non-native species for crop pollination,

the implementation of friendly management practices that increase native pollinator abundance and diversity seems to be a more cost-effective and ecologically sound alternative to enhance pollination services and decrease competitive interactions with honey bees (Carvalho et al 2012; Garibaldi et al. 2013, 2014).

### **Acknowledgments**

The authors thank Nick Rosenberger, Rachel Dickson, Romina Rader, Lynn Dicks, Ray Callaway and his group, and two anonymous Reviewers for useful comments and suggestions in previous versions of this manuscript. We also thank the owners of 16 raspberry farms for allowing us to work in their property.

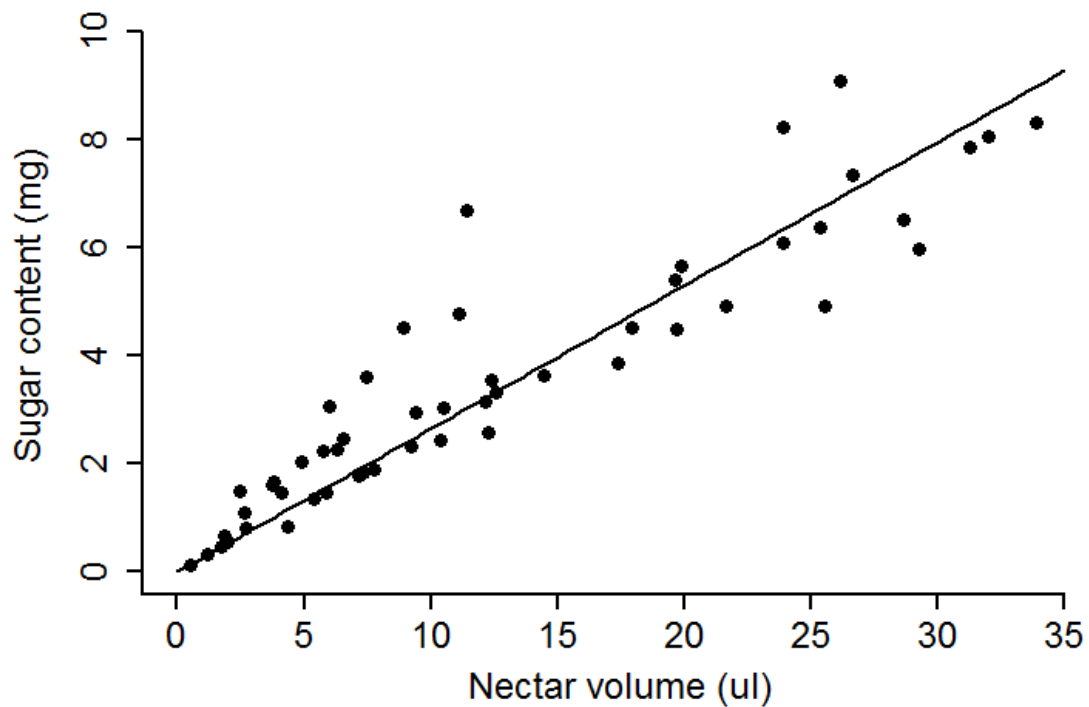
This work was supported by the GEF/UNEP/FAO Global Pollination Project, National Fund of Research PICT 2012-3015, and Universidad Nacional de Río Negro (PI 40-B-259, PI 40-B-399). AS holds a doctoral scholarship of the National Research Council of Argentina (CONICET), and CLM, LAG and AAM are researchers at the same institution.

## Appendix A. Nectar volume as a proxy of total sugar content.

Floral nectar consists of a water solution of sugars, mainly sucrose, glucose, and fructose. Nectar consumers take energy from the sugars, although the nectar as a source of water can also be important to nectarivores (Willmer, 1986; Lotz and Nicolson, 1999). The amount of sugar in nectar (e.g. milligrams of solute per microliter) of a flower is estimated from its total volume and sugar concentration (Kearns & Inouye 1993). While nectar volume quantification in flowers is relatively straightforward (e.g. by using calibrated microcapillary tubes), quantification of sugar content (e.g. by using a hand-held, temperature-compensated refractometer) present some methodological complications, mostly because refractometers need samples with a minimum volume to make the estimation. Some flowers offer minimum volumes of nectar (<0.1 mm), hampering proper sugar content quantification.

Here we estimated the amount of sugar content in nectar through a combination of nectar volume and sugar concentration (in sucrose equivalence units) in a sample of approx. 50 flowers and buds of raspberry (see Kearns & Inouye 1993). We explored if variation in sugar content in raspberry flowers and buds were more affected by observed variation in volume or in sugar concentration using multiple linear regression analysis. Although both volume and sugar concentration showed a significant relation with total sugar content ( $\beta_1 = 0.23$ ,  $SE = 0.009$ ,  $P < 0.001$ , and  $\beta_2 = 0.02$ ,  $SE = 0.005$ ,  $P < 0.001$ , respectively), nectar volume explained 95.1% of total variance (see Figure 1), while sugar concentration only 1.6%. Thus, in raspberry, nectar volume is a good proxy to quantify total sugar content in nectar.

The figure below shows the relation between total sugar content (in milligrams) and nectar volume. Points represent a sample from a flower and/or bud, and the thick line shows the fitted curve with parameters (mean  $\pm$  SE)  $a$  fixed to zero, and  $b = 0.23 \pm 0.009$ .



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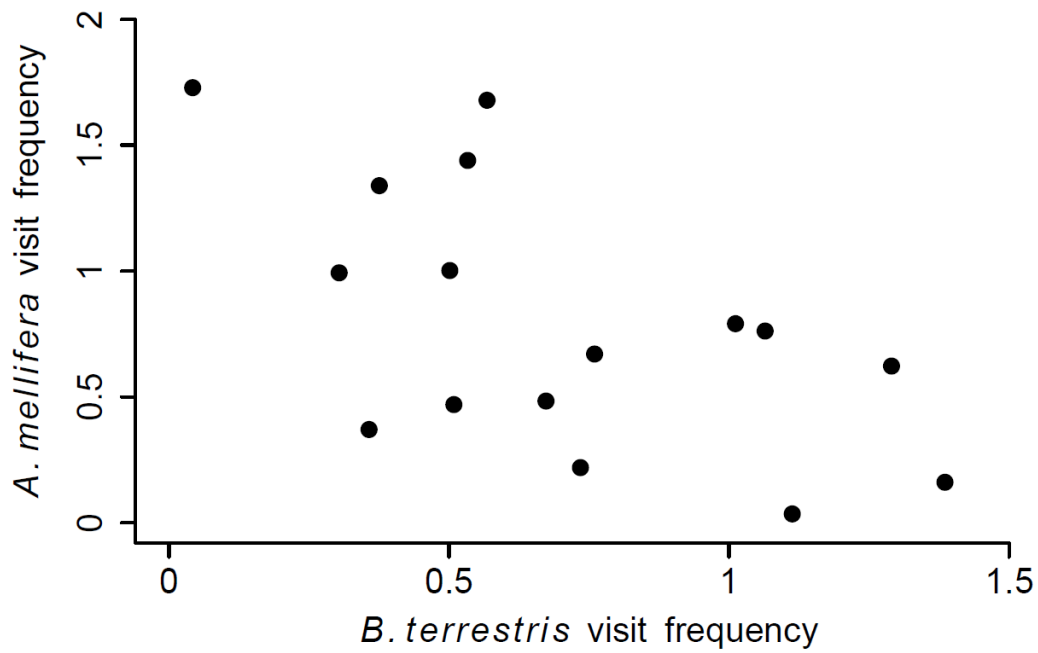
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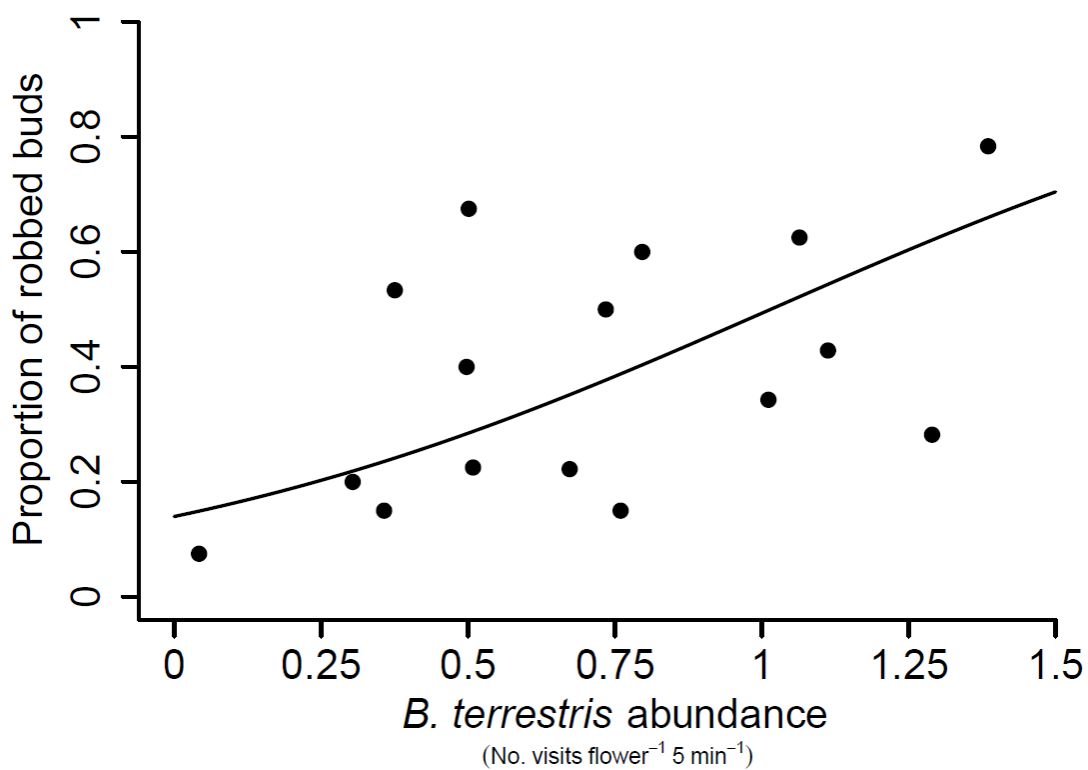
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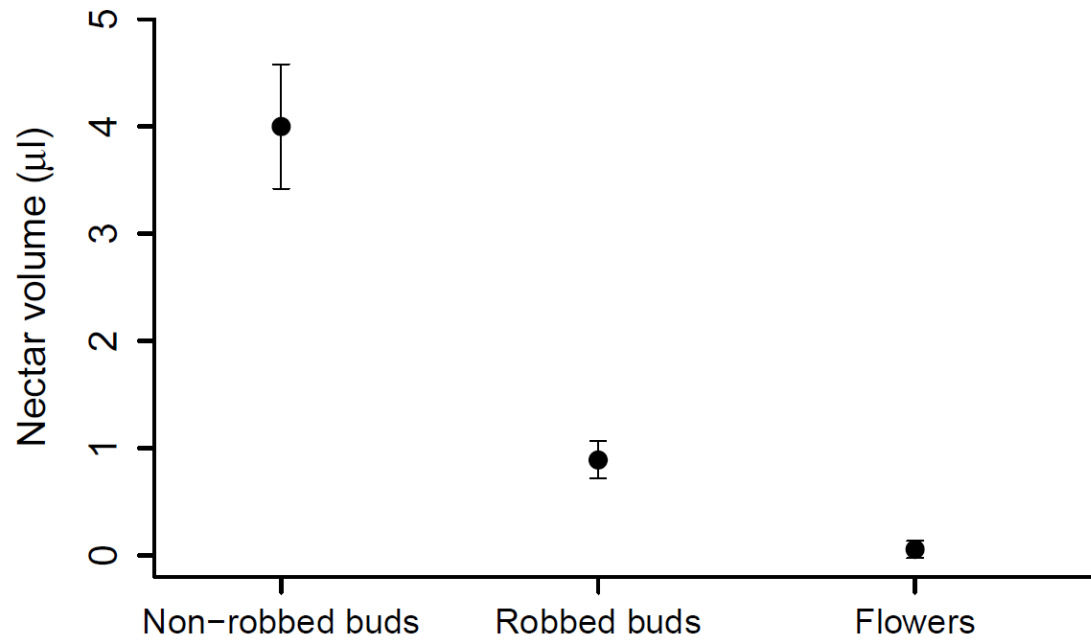
**Fig. 1.** The mean visit frequencies of honey bees and bumble bees were negatively correlated across 16 raspberry fields in NW Patagonia (Pearson's  $r=-0.60$ ,  $n=16$ ,  $P=0.01$ ). Mean visit frequency (no. visits  $\cdot$  flower $^{-1}$   $\cdot$  5 min $^{-1}$ ) was estimated from 20 pollinator censuses per field.



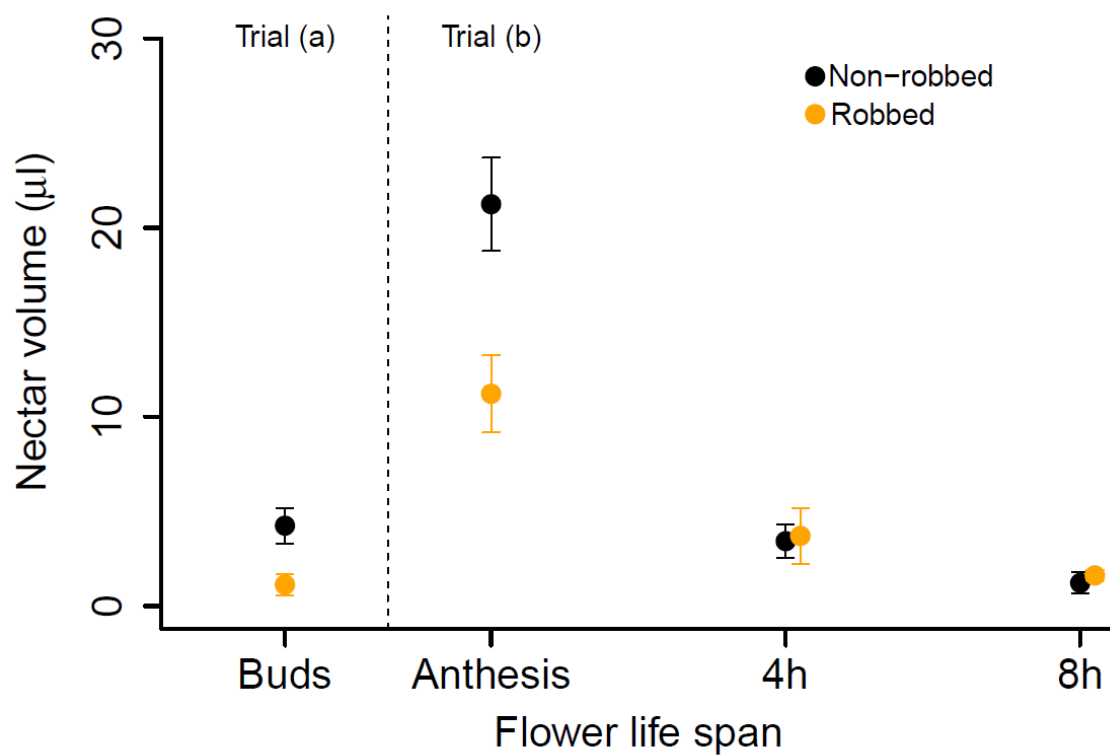
**Fig. 2.** The proportion of robbed buds increased with the abundance (i.e. visit frequency) of the invasive bumble bee (*Bombus terrestris*) across 16 raspberry fields. Proportion of robbed buds was estimated from a subsample of 30 buds per field. The solid curve depicts the regression equation estimated from a binomial model. Visit frequency is expressed as no. visits  $\cdot$  flower<sup>-1</sup>  $\cdot$  5 min<sup>-1</sup>.



**Fig. 3.** Nectar availability (standing crop) in non-robbed buds, robbed buds, and receptive flowers. Points and bars show mean  $\pm$  2 SE of nectar volume ( $\mu$ l) per bud or flower.



**Fig. 4.** Nectar availability and production. Nectar in non-robbed buds and nectar remaining after the first robbing (trial “a”). Nectar availability right after anthesis and nectar production during the first 4 h, and between 4 and 8 h after anthesis in flowers whose buds were non-robbed and robbed (trial “b”). Points show means  $\pm$  2 SE of nectar volume ( $\mu$ l) per bud or flower.





**Fig. 5.** Bootstrap estimations of nectar production (liters) per hectare under different levels of bud robbing. Points indicate the mean values of nectar production (and the bars the range of values defined by the 2.5 and 97.5% quantiles) in raspberry fields without nectar robbing, and those expected under the minimum, mean and maximum observed values of buds robbed at the study raspberry fields in NW Patagonia.

