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## **Landscape effects on wild *Bombus terrestris* (Hymenoptera; Apidae) queens visiting highbush blueberry fields in south-central Chile**

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Short title: Landscape effects in blueberry pollination

Abstract:

In this study pollinators visiting highbush blueberry fields set in landscapes with differing land use pattern in south-central Chile were investigated. Effects of spatial buffers from 0.5-8 km around each blueberry field on the abundance of the main wild pollinator, *Bombus terrestris* queens, were tested. Wild *B. terrestris* abundances were positively affected by natural forest area and negatively affected by high-food resource area, and these effects were strongest at a buffer radius of 1 and 3.5 km radii respectively. Possibly, continuous food resources provided by natural forest areas favor colony establishment and growth, and/or increase overwintering survival of bumblebee queens. Also, pollinator dependent crop area can generate a “transient dilution effect” by decreasing the density of bumblebees in simultaneously flowering crops. Management strategies might increase crop pollination services by considering the importance of nesting and overwintering habitat quality/amount and area of simultaneously flowering crops requiring insect pollination.

***Vaccinium corymbosum* / pollinators / bumblebee / natural forest areas / flowering crops**

### **1. Introduction**

Insect pollination is important to commercial blueberry production, and farmers usually rent honeybee (*Apis mellifera*) hives and, when possible, also buy *Bombus terrestris* L. colonies and place them in the field during the flowering season. Bumblebees are very effective pollinators for this crop compared to other bees (Javorek et al., 2002; Desjardins & de Oliveira, 2006). They can work relatively early in the season when most blueberries flower, at cool temperatures unfavorable to honeybee pollination (Corbet et al., 1993).

According to a review by Garibaldi et al. (2011) the proximity of natural areas can increase bumblebee density in crop areas since it can provide floral resources and/or undisturbed nesting/overwintering habitat. Floral resources provided by crop and non-crop areas can also increase bumblebee densities (Westphal *et al.*, 2003; Carvell *et al.*, 2006), but the temporal dynamics of flowering crops alters bumblebee densities as well. Previous studies reported a 'transient dilution effect' in which bumblebee densities decrease with increasing area of oilseed rape fields during flowering, both in this crop area and in nearby grassland areas (Holzschuh *et al.*, 2011). Only after the flowering events of oilseed rape bumblebee densities increased in nearby areas (Westpahl *et al.*, 2003; but see Diekötter *et al.*, 2010). Based on these observations, it is possible that bumblebee density in blueberry crops is positively influenced by natural areas and negatively by simultaneously flowering crops in the surrounding landscape. Research questions of this study were: (1) Are there any effects of surrounding natural areas and flowering crop areas on wild bumblebee abundance associated with blueberry fields? (2) What is the relevant spatial scale for these effects? Increasing the understanding of landscape effects on important wild pollinators can provide cues on possible management strategies to increase pollination services for blueberry production.

## 2. Methods

### *Field samples*

Eight blueberry farms in the central valley of the Region La Araucanía, Chile, were studied (Figure 1). Samples were collected from 'Briggita' cultivar of highbush blueberry (*Vaccinium corymbosum* L.). Distance between farms ranged from 7.4 to 97.8 km (mean  $\pm$  SE of  $42.6 \pm 21.5$  km). Blueberry fields varied in size from 0.5 to 120 ha (mean  $\pm$  SE of  $45.3 \pm 14.4$  ha). Commercial honeybee colonies were employed in 6 farms while 4 of those also had *B. terrestris* colonies. None of the surveyed farms applied pesticides during flowering. Weeds were suppressed in and around all of the fields.

Sampling of pollinators was performed on two different dates within the flowering season, between October 13<sup>th</sup> and November 5<sup>th</sup>, 2011, between 11:00 to 17:00 on days with favorable weather conditions (temperature  $>14$  °C, dry, wind speed  $< 5$  m/s). All sampled fields had the same plant density (1 x 3 m). Flower density was assumed to be relatively constant as blueberry variety was the same and plant age was relatively homogeneous (5-7 years). Depending on the field size, 4, 5 or 6 sites per farm were sampled; in one case only 1 site was sampled because of the limited field size (0.5 ha). Sampled sites were placed at different locations within the fields including field edges and field centers, in order to capture possible variation of pollinator densities. Each sampling event (n=68) consisted of separate counts of 4 successive rows in which a person walked through 10 consecutive plants in a row for 5 minutes, recording all insects that visited flowers. All row counts were summed to produce a site-level estimate of abundance.

Sampling was performed when the proportion of open flowers on 10 randomly chosen branches was  $\geq 0.2$ .

Bumblebees and honeybees were visually identified to the species level and other pollinators were recorded as other hymenopterans, syrphids, or other. During sampling wild bumblebee workers were not yet active and thus all sampled workers were assumed to be from commercial colonies placed by farmers. At the time of sampling no workers were observed in the area, except for those provided by commercial colonies.

#### *Land cover*

Natural forest areas and high-food-resources areas (i.e. blueberry or rapeseed crops flowering during the sampling period) surrounding the blueberry fields were mapped in a radius of 3.50 km from the center of the fields, based on orthorectified aerial photos acquired between 2008 and 2010 (2m spatial resolution) depending on the location, and high resolution imagery available from Google Earth (<http://earth.google.com>). The software ArcGIS 9.3 (ESRI Inc., Redland, CA) was used for this purpose. Natural forest areas correspond to unmanaged secondary forests and don't include exotic pine and eucalyptus plantations. High-food resources areas were mapped using the imagery and identified via visual inspection as food resource if flowering in the mapped area at the time of the surveys. The influence of landscape context on pollinator abundance was analyzed at different spatial scales using circular neighborhoods centered on the central point of sampled sites within the farm with radii of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 km.

To explore larger landscape effects circular areas of 5 and 8 km radius were used from on a land cover map of 15 m spatial resolution. This map was generated by classification of an ASTER satellite image from 2008 (Altamirano et al., unpubl. data) and was used to measure only natural forest areas at these scales. One farm at these scales of analysis was excluded because its buffer overlapped with neighboring farms.

The proportion of natural forest and high-food-resources areas varied across farms and both tended to decrease with increasing spatial scale (Table 1).

#### *Statistical analysis*

Analysis of landscape effects focused on the relationship between the proportion of forest area and high-food-resources area with the abundance of wild *B. terrestris* queens. This group was the most abundant among sampled pollinators.

Bumblebee abundances were averaged for each farm and across sampling dates. Linear regression models to predict farm-level bumblebee abundance were fitted as a function of the proportion of forest area and high-food-resources area, accounting for unequal number of sites per farm weighting each observation by the number of sites of that farm. Models with two covariates (forest areas and high-food resources areas) were tested at each scale of analysis considering also all combinations of different spatial scales. In all

models  $\log_e$ - $\log_e$  transformation of the data was used to improve linearity. Correlation between forest and high-food resources areas at each spatial scale was tested using Pearson correlation coefficient. R v.2.15.0 (R Development Core Team, 2012) was used for statistical analysis. The possible effect of commercial bumblebee and honeybee colonies was not evaluated. These colonies are placed in the field only during blueberry flowering and competition for food resources is unlikely given the over-abundance of this resource.

### 3. Results

Honeybees were the most abundant pollinator sampled, followed by naturally occurring *B. terrestris* queens and syrphids (Figure 2). Few individuals of *B. ruderatus* and *B. dahlbomii*, or other hymenopterans were found. In farms stocked with commercial bumblebee colonies *B. terrestris* workers were also observed.

The abundance of wild *B. terrestris* queens was positively associated with the area of natural forest and negatively associated with areas of high-food resources. These associations were significant at various spatial scales (Figure 3) and peaked at 1 and 3.5 km radii for forest and high-food resources respectively (Table 2). No correlation was found between natural forest area and high-food resources area at any spatial scale. However positive correlation of the proportion of natural forest and high-food resources areas between similar spatial scales (Pearson's  $r > 0.8$ ) was found.

### 4. Discussion

Among wild pollinators, *B. terrestris* queens were most abundant reflecting a successful spread of this exotic species in the study area. The only native bumblebee (*B. dahlbomii*) was almost absent which is consistent by previous reports on the decline of this species after the introduction of *B. ruderatus* and *B. terrestris* (Morales et al., 2013) in 1982-1983 and 1997-1998, respectively (Montalva et al., 2011; Smith-Ramírez et al., 2014).

The positive relationship between natural forest area and bumblebee abundance might reflect nesting suitability and/or hibernation habitat requirements of queen bumblebees. Undisturbed areas such as forests and forest edges can be suitable nesting habitat for bumblebees (Kells and Goulson, 2003; Osborne et al., 2008). These areas might be a source of continuous pollen and nectar resources throughout the foraging stage of bumblebees. While food supply in early spring can favor bumblebee colony establishment and initial growth (Westphal et al., 2009), the reproductive success of the colony seems to be determined by late-season food availability provided by surrounding natural areas (Persson & Smith, 2013).. Late-season food supply is crucial for hibernating queens since Beekman et al. (1998) found that body weight at the start of diapause positively affects its success, while environmental temperature has no effect. Thus, forests can favor the number of queens produced per colony and/or favor hibernation success by providing food supply for queens before they start diapause. Further studies should focus on the relevance of food

provision by natural forests and the type and quality of nesting/overwintering sites for bumblebees in south-central Chile.

The results of this study also suggest that the presence of nearby flowering crops used by these pollinators can decrease the abundance of queen bumblebees in blueberry fields. This negative effect of high-food-resources areas could correspond to a ‘transient dilution effect’ in which the bumblebee density decreases as they are spread across larger areas of flowering crops and is consistent with previous studies that document such effect on oilseed rape (Holzschuh et al., 2011). Simultaneously flowering areas probably compete for highly mobile pollinators such as bumblebees when they are available within the foraging range of the individuals. From the perspective of crop pollination services provision, this effect is important since the pollination service can decrease not only due to lack of neighboring natural areas but also nearby flowering crop area.

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**Tables:**

Table 1. Proportion of natural forest and high-food resources (flowering crops) areas (Mean[Min, Max]) for sampled farms at different radius distances. For simplicity some spatial scales are omitted.

Scale (m)	Natural forest	High-food resources
500	0.17 [0.02,0.31]	0.32 [0.06,0.73]
1500	0.13 [0.04,0.24]	0.08 [0.01, 0.36]
2500	0.13 [0.04,0.24]	0.04 [0.002, 0.14]
3500	0.12 [0.04,0.29]	0.02 [0.001,0.07]
5000	0.07 [0.02,0.20]	-
8000	0.08 [0.03, 0.22]	-

Table 2. Results from the best fitted regression model of queen *B. terrestris* abundances, weighted by the number of sites per farm sampled. Covariates and response variable were  $\log_e$  transformed. For each covariate (fraction of land cover type) the radius of circular areas used is given in parenthesis.

Variable	Estimat			R2
	e	t	P	
Intercept	-0.15	-0.33	0.76	0.89
Natural forest (1000m)	0.51	3.93	0.011	
High-food resources (3500m)	-0.24	-3.77	0.013	



**Figures:**

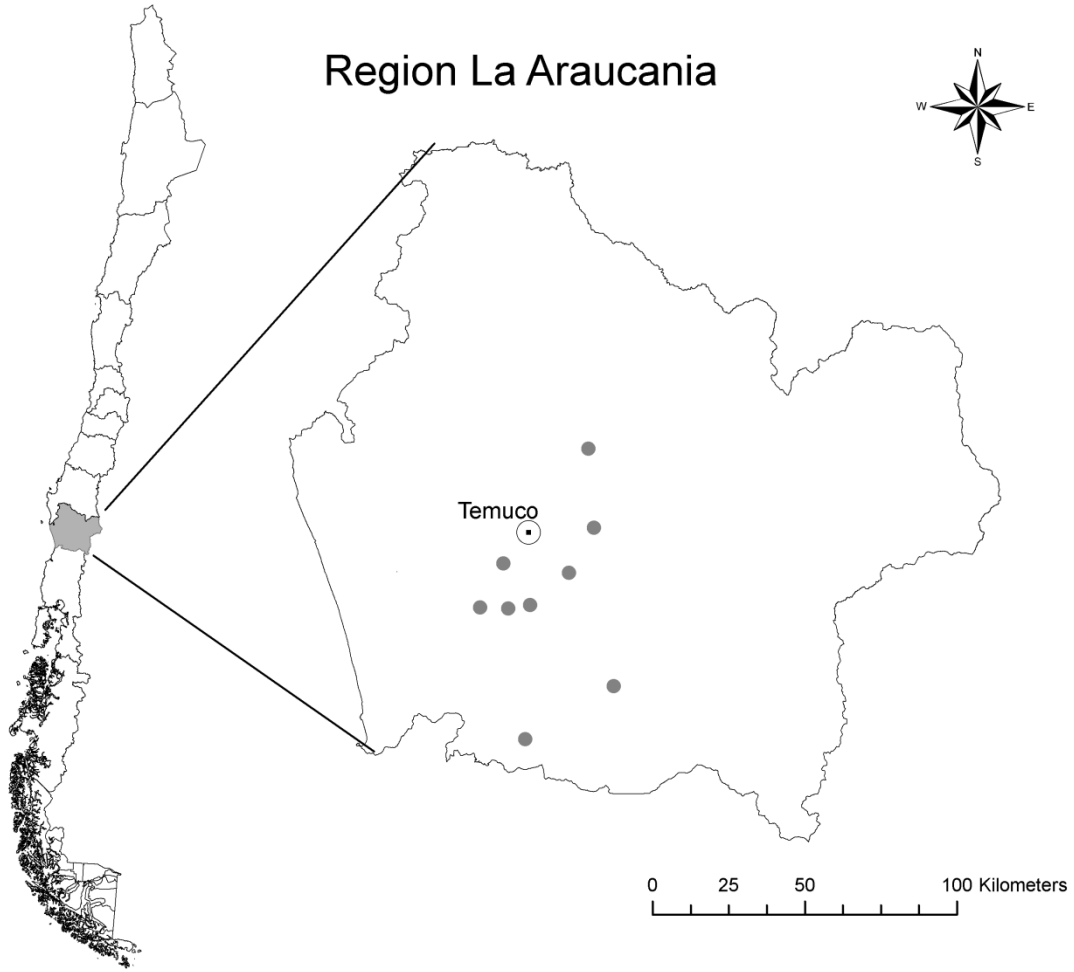


Figure 1. Sampled farm locations in south-central Chile.

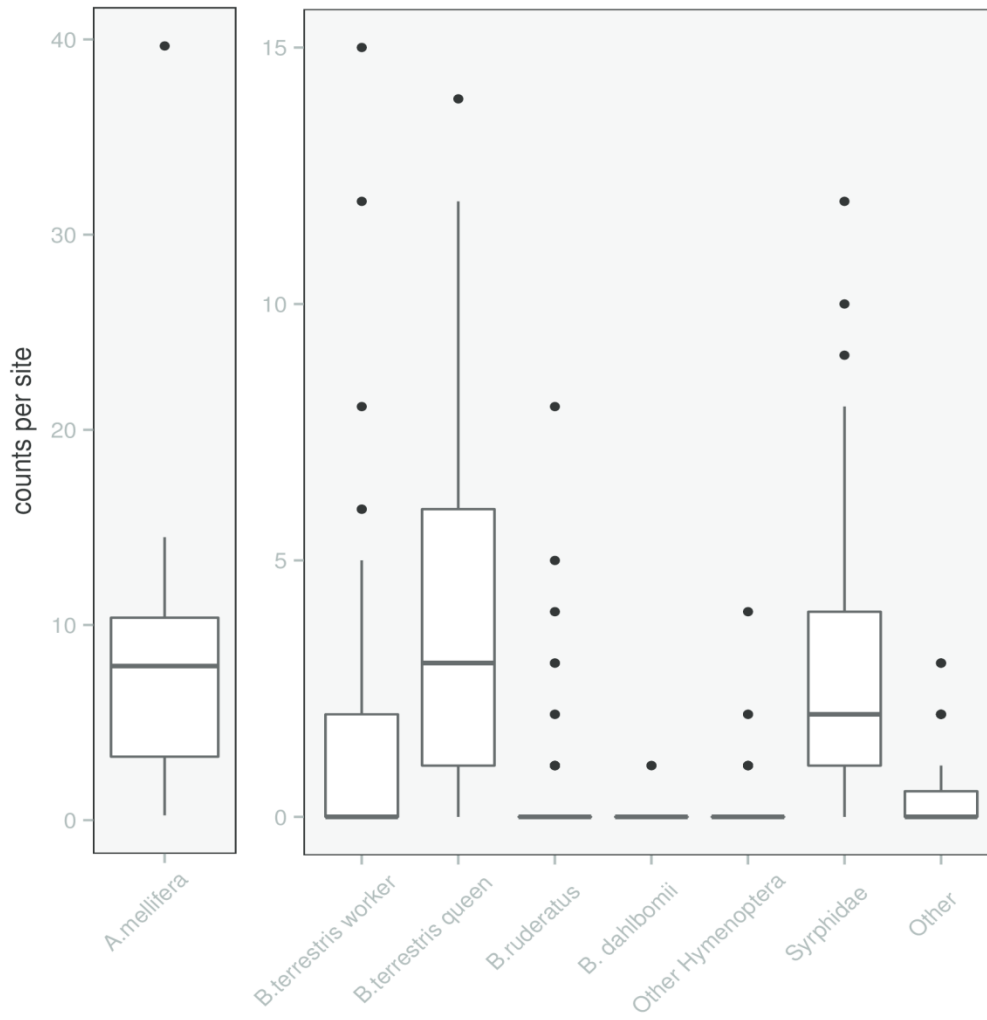


Figure 2. Abundance of different pollinators in sampled sites. Bars of the boxplot represent the median, and the 25% and 75% quartile boundaries, error bars represent range of values (excluding outliers) and points represent outliers.

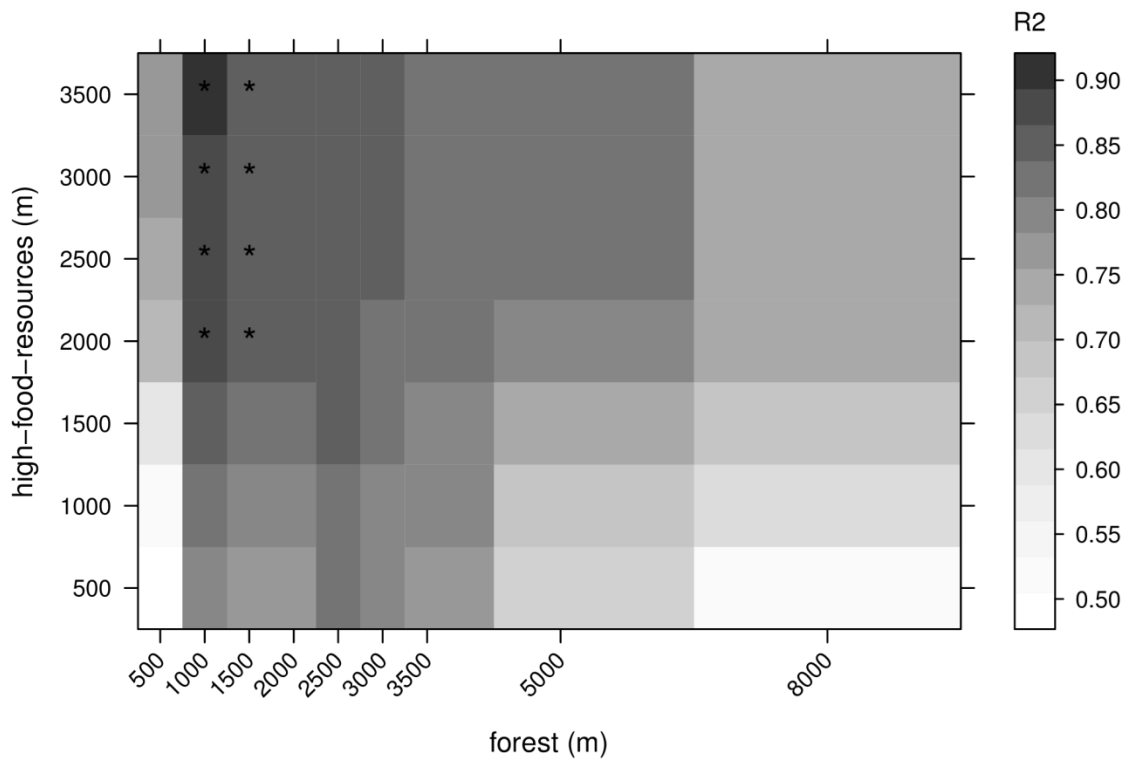


Figure 3. R2 value for linear models to explain queen bumblebee abundances. Covariates of fitted models are fraction of natural forest and high-food-resources in circular areas of different radius distances (represented by x and y axis respectively). An asterisk indicates that both covariates were significant at  $\alpha < 0.05$ .