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POLLINATION EFFICIENCY OF MANAGED BEE SPECIES (*APIS MELLIFERA* AND *BOMBUS PAULOENSIS*) IN HIGHBUSH BLUEBERRY (*VACCINIUM CORYMBOSUM*) PRODUCTIVITY

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ABSTRACT

Understanding how bees use the resources provided by crops of massive flowering is essential to develop meaningful agricultural management of plans to maximize the potential of pollination service. We assessed the effect of the pollination carried out by native species *Bombus pauloensis* and *Apis mellifera* on the production and quality of blueberry fruits. In this context, we tested the prediction that pollinator assemblages benefit fruit yield. Four treatments were performed: open pollination, *B. pauloensis* pollination, *A. mellifera* pollination, and autogamy. For each treatment, the frequency of floral visitors, fruit setting, yield, and quality were evaluated. The results showed that *Vaccinium corymbosum* L. 'Emerald' is highly dependent on entomophilous pollination to obtain optimal production and high-quality fruit, and that pollination with *A. mellifera* generated the highest proportion of fruit setting (0.80 ± 0.03). The highest seed number was found in open pollinated fruits. This study highlights the effect of the interactions among wild and managed pollinators on the productivity of commercial blueberry fields, and is the first report of *B. pauloensis* use in blueberry pollination.

Key words: blueberry, pollination, fruit quality, *Apis mellifera*, *Bombus pauloensis*

INTRODUCTION

Bees are some of the most important insect pollinators. The evaluation of how different bee species select and use certain resources within their environment is important to understand fundamental ecological processes in agroecosystems (Gill et al. 2016; Campbell et al. 2018). In intensive agricultural areas, it has been documented that several species of wild bees are threatened and declining (Garibaldi et al. 2013; Cariveau & Winfree 2015). At the same time, the abundance of populations of managed honey bees (mostly *Apis mellifera*) decreases to provide services in crops with high dependence on entomophilic pollination (Agüero et al. 2018; Requier et al. 2018). Understanding how bees use the resources provided by crops of massive flowering is essential to develop meaningful agricultural management plans that both sustain bee populations and maximize the potential pollination service they may provide to farmers (Pasquet et al. 2008).

Vaccinium is a large genus of plants with many cultivated species. Blueberry (*Vaccinium corymbosum* L.) is a very important crop in Argentina, with more than 2700 ha cultivated. This intensive agricultural practice reduces the heterogeneity of habitats; it helps to create a large mass of homogeneous flowers and, consequently, rapidly changes the composition and diversity of pollinators (Steffan-Dewenter et al. 2005; Chacoff &Aizen 2006; Tylianakis et al. 2008).

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Although some cultivars of blueberry are autogamous, cross-pollination is generally the rule. The bell-shaped pendant form of the flower discourages self-pollination via wind or gravity. Bee pollination is essential for a maximum blueberry production (Chiasson & Argall 1996) and honey bee, *A. mellifera* L., is the most widespread for blueberry pollination (Free 1993) as the introduction of honey bees' hives in orchard significantly increases the production (Aras et al. 1996; Basualdo et al. 2007). On the other hand, bumblebees probably coevolved with similar-type vegetation (Heinrich 2004) and a reciprocal adaptation has made bumblebees ideal pollinators of blueberry (Desjardins & De Oliveira 2006). The bumblebee's behavioral characteristic is buzz pollination, which induces anther dehiscence, and consequently, the release of a large amount of pollen (Goulson 2010; De Luca & Vallejo-Marín 2013), and their foraging capacity in a wide range of ambient temperature (Goulson et al. 2010), makes it an effective blueberry pollinator (Buchmann 1983). Several papers have demonstrated the pollinating effectiveness of *Bombus* spp.; most of them are from cold temperate climate (Tuell et al. 2008; Stephen et al. 2009), but utilization of the neotropical species, *Bombus pauloensis*, for blueberry pollination has not been documented. Methods for rearing of this native species from South America have been developed and the use of *B. pauloensis* commercial colonies has been evaluated in Argentina for blueberry (Basualdo et al. 2013), strawberry, kiwi, and tomato pollination (Basualdo & Rodríguez 2009).

Recent studies have demonstrated that wild pollinators enhance fruit setting of crops, where managed pollinators such as honey bees or bumblebees are being used (Garibaldi et al. 2013; Milfont et al. 2013; Breeze et al. 2014). The contribution of wild pollinators would be valuable for blueberry pollination because plants bloom at the end of winter with low temperatures.

The objective of this work is to assess the effect of pollination carried out by *B. pauloensis* and *A. mellifera* on fruit formation and physicochemical characteristics that determine the quality of the *V. corymbosum* 'Emerald' fruit. In this context, we tested the prediction that pollinator assemblages with more species benefit *V. corymbosum* 'Emerald' production and the quality of fruit. We also elucidated the relationship between the frequency of visits (FVs) of each pollinator individually and the production, weight, seed number, and the internal quality of berries.

MATERIALS AND METHODS

Study area

The experiment was conducted on a commercial blueberry (*V. corymbosum* L.) plantation situated in Entre Ríos Province (31°19′40.35″ S, 58°05′00.45″ W), Argentina. Six-year-old plants of 'Emerald' grown at a distance of 1 m between plants and 3 m between rows (3333 plants per 1 ha) were evaluated. Standard commercial management including pruning, fertilization, control of weed, pests and diseases, and frost protection was performed during the growing season. Blueberry production constitutes 1050 ha of the area, which is equivalent to 35% of the Argentine production (Engler et al. 2008).

Pollination experiment

This research was carried out during the blooming of blueberry, *V. corymbosum* 'Emerald', from 20 July to 30 August, on a 1 ha plot. Each treatment included four rows of length 15 m (a total of 60 plants). Four treatments were performed: open pollination, *B. pauloensis* F. pollination, *A. mellifera* L. pollination, and autogamy. The honey bee and bumblebee treatments were performed in insect-proof isolation cages (10 × 15 × 4 m). For *B. pauloensis* treatment, a commercial colony (Brometan®) with 100 individuals was used. For *A. mellifera*, one honey bee colony was kept in a single-standard Langstroth hive with seven frames covered by bees and four broods. In the treatment for autogamy evaluation, individual complete plants were surrounded by a special insect-proof mesh.

For each treatment, five plants were randomly chosen and the flowering stage was evaluated by tagging three branches per plant. Branches, located in different orientations, were selected to diminish the effect of shading on the formation of fruits. Fruit setting was calculated based on the proportion of flowers in the branches that developed into berries.

Frequency of floral visitors

In order to assess the pollination treatments, observations on the FVs were made for each treatment, monitoring 10–20 flowers of the tagging branches during 5 weeks over the flowering period. Records were made between 10 and 16 h during a 5-min period; 150 observations for a total of 12.5 h were performed. For open pollination, the number of insects visiting blueberry flowers was recorded whenever the weather was suitable for insects' activity. Results are expressed as the average number of bees per flower per 5 min.

Fruit setting and quality

In each treatment, a bucket of approximately 250 g of fruit per plant was collected and stored in a refrigerated chamber (Frutitec 15 m^3, Mod M 2500HT Coiron S.A.) at 1 °C for 24 h. At the time of analyzing their size and internal quality, the fruits were conditioned at 20 °C to standardize the measurements. For each sample, 15 berries were chosen from the initial pool, for which the size and reproductive variables were estimated. Firmness was assessed for each blueberry using a non-destructive compression test, simulating a very gentle squeeze with the fingers using a TA.XT Plus Texture Analyzer (Stable Micro Systems, UK) equipped with a 5-kg load cell and a 75-mm cylinder aluminum probe. Each blueberry was compressed 10% equatorially. Fruit mass was obtained with a digital scale (CS Series, OHAUS, USA) of 0.1 g of precision. The number of fertilized seeds per fruit was counted. The fruit moisture content was determined using an oven (DHG-924, PeetLab) at 65 °C for 24 h and an analytical scale (PA214, OHAUS, USA) with an accuracy of ±0.0001 g. At the same time, the internal quality of each sample from a fruit homogenate of 5 g was measured. The concentration of total soluble solids (TSS%) was estimated using an Abbe refractometer (ATAGO, 1-T, Tokyo, Japan). The total acidity of the fruit (TA%) was measured by titrating the juice with 0.1 M NaOH to an end point of pH 8.2 using Oakton series pH 11 pH meter. This procedure was repeated three times during the harvest season at 10%, 50%, and 80% fruit maturing of the lot studied.

Statistical analysis

Data were analyzed by generalized linear mixed model (GLMM). The variable responses for the number of fruits formed per branch, FVs during 5 min, fruit mass (g), compression force (g), number of seeds per fruit, TSS% (expressed as Brix), TA%, maturity index (MI, calculated as the quotient between TSS and TA), and the average dry mass were the fixed analyzed parameters used to characterize the treatments. For the fruits formed, the random variables "plant/branches" with a binomial-type error distribution were included. For the variables FV, mass, firmness, and number of seeds, the fixed character "date of harvest" was added and as a random variable, only "plant" was used and, in this case, a negative binomial-type error distribution was used. For the internal quality estimators, the time was included as the random variable, the best fit present was the one with gamma distribution. The glmer and glmer.nb function of the package "lme4" version 1.1-12 was used to estimate the models using the statistical software "R i368 3.5.1" (R Development Core Team 2013).

RESULTS

FVs of pollinators and fruit setting

FVs showed significant differences among the three treatments ($F = 15.78$, $p < 0.0001$). The FV in open pollination was 0.03 per flower per 5 min. In this treatment, the composition of observed visitor species was *A. mellifera* (89.09%), two species of Vespidae (5.45%) and Syrphidae (3.64%), and one of Zygaenidae. It has been found that the visiting frequency of *A. mellifera* under cage was about five times higher than under *B. pauloensis* cage and about 40% higher than under open pollination (Fig. 1).

To evaluate if there are differences in the percentage of fruits formed according to the different treatments, a total of 2672 flowers were monitored. Autogamy ranged from 0% to 0.38%. Fruit set was significantly smaller (~4.5 times) compared to the entomophilous pollination (Table 1).

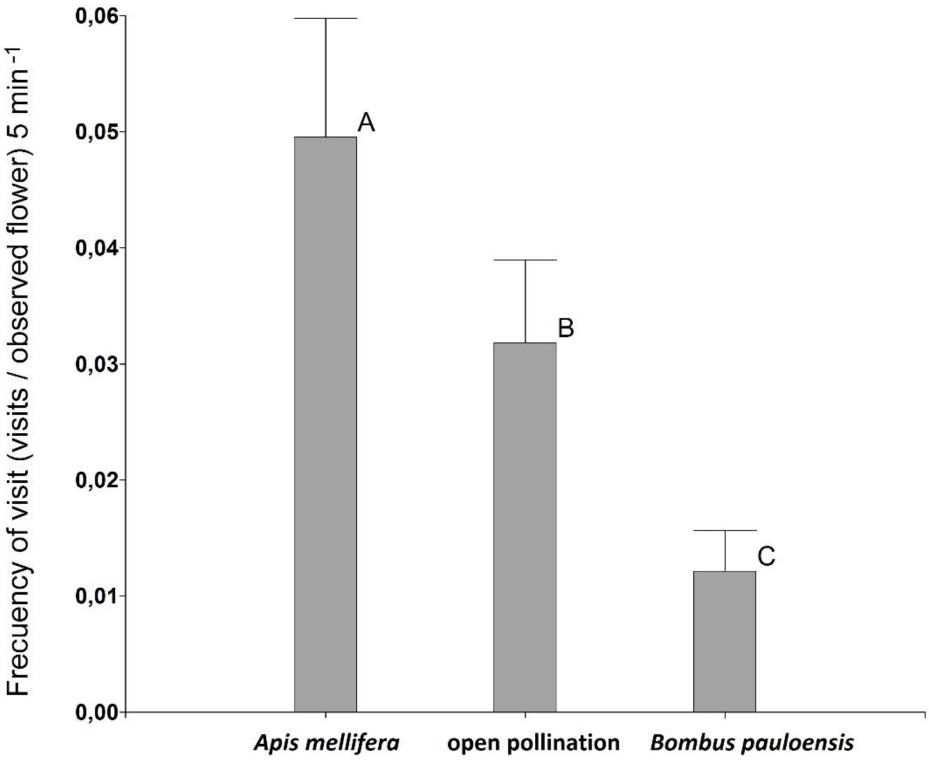

Fig. 1. Frequency of visits at entomophilous pollination. Different letters denote significant differences among treatments ($p < 0.05$)

Table 1. Fruit yield and quality parameters of blueberry depending on the type of pollination

Parameters	Autogamy	*Apis mellifera*	*Bombus pauloensis*	Open pollination	Wald test	
					F	P
Fruit setting	0.14 (±0.03) C	0.80 (±0.03) A	0.71 (±0.04) B	0.75 (±0.04) B	151.25	<0.0001
Seeds per fruit	1.09 (±0.11) C	10.97 (±0.84) B	11.73 (±0.89) B	15.00 (±1.08) A	157.83	<0.0001
Firmness (g)	183.38 (±3.73) B	273.34 (±5.21) A	268.13 (±5.11) A	268.13 (±4.47) A	55.45	<0.0001
Mass of fruit (g)	1.62 (±0.10) B	3.19 (±0.16) A	3.06 (±0.15) A	3.19 (±0.14) A	21.01	<0.0001
Average dry matter (%)	14.46 (±0.42) A	14.24 (±0.41) A	13.88 (±0.40) AB	13.16 (±0.35) B	3.49	0.0216
Total soluble solids (%)	13.81 (±0.35) A	12.69 (±0.33) B	13.01 (±0.34) A	12.07 (±0.31) C	18.61	0.0001
Total acidity (%)	0.62 (±0.07) A	0.73 (±0.08) A	0.70 (±0.08) A	0.72 (±0.07) A	0.49	0.6885
Maturity index	22.09 (±2.65) A	17.66 (±2.05) A	18.97 (±2.21) A	17.03 (±1.91) A	1.42	0.2464

Note: Values obtained through the best GLMM (generalized linear mixed model), using a criterion of lower AIC (Akaike information criterion), for the selected estimators to compare the effect of different types of entomophilous pollination. Fisher's LSD (least significant difference) test was used to represent the differences between treatments. The letter "A" denotes the highest average value. Means with a common letter are not significantly different ($p > 0.05$). The presented values include the temporal and inter-plant variability, being expressed as mean (±standard error).

Among the entomophilous pollination treatments, pollination with *A. mellifera* was significantly highest, reaching 0.8 fruits formed per branch. No significant differences were detected between an open pollination and bumble bee pollination.

Fruit quality parameters

Significant differences among treatments were detected in the number of seeds. The seed number produced at autogamy was significantly low and 10–15 times lower than under the other treatments. At the open pollination, the highest seed number per fruit, reaching an average of 15 seeds per fruit, was obtained, whereas in fruits obtained under cages, an average of 10.97 and 11.73 seeds per fruit were formed for honey bee and bumblebee, respectively (Table 1).

Comparing the entomophilous pollination treatments, there were no differences detected in the fruit mass (from 3.06 to 3.19 g) and fruit firmness, while the average fruit mass produced under autogamy was 1.62 g. The average dry matter of the fruit obtained under autogamy and pollination with *A. mellifera* was significantly higher as compared with the fruits obtained at open pollination. The percentage of soluble solids was the highest in fruits obtained under autogamy condition and on pollination by *B. pauloensis* (13.01 and 13.81, respectively), while the soluble solids of fruits pollinated in open system and by *A. mellifera* was 12.07 and 12.69%, respectively. Acidity and maturity index were not significantly different between the treatments.

DISCUSSION

Our work evaluated an important aspect of blueberry production – influence of different types of pollination on fruit set and quality. This study highlights the issue of a necessity to introduce specific pollinators on blueberry plantations.

The results show that *V. corymbosum* ‘Emerald’ is highly dependent on entomophilous pollination for optimal production and producing high-quality fruits. Similar results were reported for the cultivars ‘Northland’ and ‘Patriot’ (MacKenzie 1997), where both honey bees and bumblebees could provide adequate pollination service. Fruit setting did not increase at open pollination with wild insect visitation. This result disagrees with the data obtained by Garibaldi et al. (2013), who concluded that fruit setting could be maximized by wild pollinators in crops such as blueberry and almond, which are stocked with high densities of honey bee colonies. In the pollination mediated by *A. mellifera*, the highest number of fruits was set, which agrees with the results of Sampson and Cane (2000) and Javorek et al. (2002), who reported high fruit production of rabbiteye and lowbush blueberry due to pollination by honey bees. These results can be attributed to the high FVs of honey bees (0.05 visits per flower per 5 min) in caged plots, grown in conditions with no competing flowers of other plant species and wild insects.

Although we did not quantify the pollen deposition in different treatments, we considered the number of seeds as an indirect estimator of reproductive success (Dafni 1992). The number of seeds and, consequently, reproductive success was the highest on open pollination plots, where there was a stronger influence of honey bee visitation (89%) and a lower proportion of wild pollinators. The combined action of pollinators improved pollen deposition on the stigmas, this agrees with the data reported for other crops (Garibaldi et al. 2013, 2017). Despite the fact that the FVs on open pollinated plants was much lower than those made by honey bees under cages, the seed production was ~27% higher, evidencing a synergistic interaction between honey bees and wild pollinators. Bumblebees are considered the most efficient pollinators of blueberry due to the fact that they generate buzz pollination, through which the pollen is released. This non-*Apis* population carries a high amount of pollen in its body (De Luca & Vallejo-Marín 2013) and could enhance pollen deposition on the stigmas. Even though the stocking rate of bumblebees used in our cage resulted in adequate fruit setting, colonies with more individuals could even improve the productivity.

Berries pollinated in open system had more seeds, lower soluble solids, and consequently, higher water content resulting in better firmness, that is a component of a good fruit quality. Apparently, the relationship between seed setting and water content of fruit is regulated by gibberellins; therefore, both adequate pollination and nutrition are very important for the quality of fruit (Cano-Medrano & Darnell 1997).

This is the first report of the useful service of *B. pauloensis* in blueberry pollination. The use of native bees is relevant, since in some South American countries such as Chile, exotic bumblebee species such as *Bombus ruderatus* and *Bombus terrestris* were imported for commercial pollination; consequently, these species invaded Argentina, producing a considerable ecological impact on native bees (Morales 2007). Therefore, the incorporation of this native species would be an advantage, avoiding the use of invasive species and, furthermore, improving the productivity of *V. corymbosum* crops. Finally, we conclude that in the conditions of our trial, pollinator assemblages benefit some aspects of quality, but not the productivity of blueberries. Additional research is needed to understand interspecific behavioral relationships, such as competence or synergy, between *A. mellifera* and *B. pauloensis* in blueberry cultivation before incorporating them into blueberry production systems as an agent that provides an ecosystem service.

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