



RESEARCH ARTICLE - BEES

Pollination efficiency and foraging behavior of *Bombus pauloensis* (Hymenoptera: Apidae) on two highbush blueberry cultivars (*Vaccinium corymbosum*)

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Article History

Edited by

Denise Alves, USP, Brazil

Received 28 February 2023

Initial acceptance 27 March 2023

Final acceptance 07 November 2023

Publication date 06 March 2024

Keywords

Native bees, Bumble bees, Fruit quality, Palynology.

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Abstract

Bombus pauloensis Friese is a promising managed bumblebee that can pollinate crops in South America. Highbush blueberry (*Vaccinium corymbosum* L.) is a recently introduced and economically promising crop cultivated in open fields or greenhouses in Colombia. Although this crop is known to be pollinator-dependent, the efficiency of local pollinators in this geographic area has yet to be established. This study aimed to establish the pollination effectiveness and foraging behavior of *B. pauloensis* in two of the most common cultivars of highbush blueberries planted in a high Andean region of Colombia. We hand-reared and located *B. pauloensis* colonies in two different plantations of Sharpblue (open field) and Biloxi (greenhouse) cultivars. The time spent per flower and the number of flowers visited per minute of 300 foragers were registered to characterize the foraging behavior in both cultivars. Pollen analysis was performed once from corbicular loads, larvae, larval cells, and colony feces to identify the pollen sources collected by colonies located in the Sharpblue crop. Analysis of fruit quality was performed to establish the effect of *B. pauloensis* pollination. Foragers visited blueberry flowers with a corolla aperture of more than 3mm in both cultivars but spent more time visiting Biloxi than Sharpblue flowers. *B. pauloensis* pollination reduced the ripening time and increased the fresh and dry weight and the number of true seeds in both cultivars. Our results provide evidence that *B. pauloensis* pollination can improve the fruit quality in the blueberry cultivars evaluated here in Colombia, making it an efficient native pollinator for a promising commercial species.

Introduction

Bumblebees (*Bombus* spp.) colonies are essential pollinators of several crops because of their hairiness, large body size, buzz-pollination behavior, and they can be easily managed in crops (Velthuis & van Doorn, 2006). The benefits of bumblebees' use as managed pollinators favored the interest and introduction of some species out of their natural distribution, affecting native bees (Goulson, 2003; Morales, 2007; Madjidian et al., 2008). In response, some initiatives were driven to rear native bumblebees, including South

America (Abrahamovich et al., 2001; Aldana et al., 2007; Cruz et al., 2008). The Neotropical bumblebee *Bombus pauloensis* Friese, 1913 is the bumblebee species with the most significant potential as a managed pollinator of a variety of crops in South America (Cruz et al., 2008; Salvarrey et al., 2017). *Bombus pauloensis* is native to the Andean region and presents wide geographic distribution from Venezuela to Argentina (Abrahamovich & Diaz, 2002). Unlike populations of temperate zones of South America, *B. pauloensis* colonies are perennial in the tropical region, even lasting more than a year, alternating monogenic (mother queen) and polygenic



(several daughter queens) phases (Sakagami et al., 1967; Cameron & Jost, 1998). In Colombia, *B. pauloensis* has a wide altitudinal distribution (500–3000 m.a.s.l), is present all year round, and is a highly adaptable species to disturbed environments. Recent developments in the rearing of colonies at a small scale have allowed validating this bumblebee species as a key managed crop pollinator from Colombian high-tropical Andes (Almanza et al., 2007; Aldana et al., 2007; Riaño et al., 2015; Poveda et al., 2018).

Blueberry is an important crop grown predominantly in the United States, Chile, Argentina, México, Peru, and Canada (Brazelton, 2017; Basualdo et al., 2022). The Neotropical region and Latin America contributed with 23% of the global production in 2019 (FAOSTAT, 2020). In Colombia, some cultivars of highbush blueberries (*Vaccinium corymbosum* L.) have been introduced over the past eight years in some high Andean regions due to the optimal climatic conditions that favor production all year round (Cortez-Rojas et al., 2016). The most common cultivars in Colombia are Sharpblue, Legacy Misty, and Biloxi, which are cultivated in both open-field and greenhouse conditions. The highbush blueberries cultivars are self-compatible species, but cross-pollination mediated by bees improves fruit quality and crop productivity (MacKenzie, 1997; Dogterom M, 1999; Sampson & Spiers, 2000; Isaacs & Kirk, 2010; Da Silveira et al., 2011; Courcelles et al., 2013; Sagili et al., 2015; Ramírez-Mejía et al., 2023). Moreover, some studies have reported that the highbush blueberry is a highly pollinator-dependent crop (Basualdo et al., 2022; Ramírez-Mejía et al., 2023). Regarding pollinators, hummingbirds, wasps, moths and several bee species (i.e., managed and wild species) have been reported pollinating blueberry in Latin America, among them *Apis mellifera* L., *Bombus* spp., *Xylocopa* spp., *Plebeia* spp., among others (Raguse-Quadros et al., 2023; Ramírez-Mejía et al., 2023). However, the efficiency and foraging behavior of many pollinator species on blueberry remains to be studied in detail, especially in Colombia.

Several studies have shown the large variability of floral morphology in blueberry highbush cultivars, such as corolla length, aperture, or width, which could affect the pollinators' behavior and efficiency (Jacquemat & Thompson, 1996; Ritzinger & Lyrene, 1999; Willmer & Stone, 2004; Ne'eman et al., 2010; Courcelles et al., 2013; Sampson et al., 2013; Solís-Montero & Vallejo-Marín, 2017; Arrington & DeVetter, 2018). Thus, floral traits are essential to determining behavioral patterns of native floral visitors of blueberry and their trait-matching with floral morphology to establish their efficiency as pollinators in the new growing zones. Because of the novelty of blueberry crops in high Andean regions of Colombia, this work aimed to determine the pollination efficiency and foraging behavior of *B. pauloensis* in two widely cultivated highbush cultivars, Biloxi and Sharpblue planted in different conditions (open field and greenhouse).

Materials and Methods

Study area

The study was carried out in the municipality of Funza, Colombia (4°45'20.3 "N, 74°12'01.8 "W) on a farm with two highbush cultivars: Sharpblue and Biloxi cultivars, 1 ha of 2-yr old plants for each cultivar were present (2200 plants/ha, plants were 70 cm high). The Sharpblue cultivar was planted in the open field covered by a plastic net to prevent the entry of birds, the net had a height of ca. 4 meters and a mesh size of 2×2 cm. At the same time, the Biloxi cultivar was planted in a polyethylene greenhouse with a thickness of 8 mils (0.008 inch) and lateral side-wall curtains to provide constant ventilation. The greenhouse was of a chapel type with a height of 7 m.

Rearing and monitoring *B. pauloensis* activity in the two cultivars

Monogynic colonies were reared in the laboratory under controlled conditions from queens collected in natural zones near the study area and placed in the orchard of each cultivar according to protocols previously developed (Riaño et al., 2015; Poveda et al., 2018). Seven colonies were introduced in the open field of Sharpblue orchard: four colonies on July 31, 2014, and the remaining three on December 24, 2014, when the first four colonies were no longer in the orchard. Three colonies were introduced in the greenhouse Biloxi cultivar orchard on May 15, 2015 (Table 1). It is important to note that we observed that these cultivars in Colombia always produce flowers. Hence, the colonies always had resources to forage (Cleves & Sol, 2022). Colonies were placed in the middle of the orchard area at 60 cm height and 20 m apart (Stubbs & Drummond, 2001; Campell et al., 2017). All the colonies had a founder queen, abundant brood (eggs, larvae, and pupae), and workers (80-120 individuals). Individual colonies were observed weekly to record the presence/absence/mortality of the mother queen and the activity of the workers per colony.

Corolla morphology and foraging behavior of *B. pauloensis* in Sharpblue and Biloxi blueberry cultivars

We characterize some floral traits for each cultivar. Measurements were performed on 100 flowers during the anthesis of each cultivar as follows: (1) Corolla length, from the point of insertion of the petals on the hypanthium to the distal end; (2) Flower width, the equatorial diameter of the hypanthium; (3) Corolla aperture: equatorial diameter of the aperture of the corolla. The flowers (n = 200) were photographed in a top and lateral view with a Canon Eos 7D camera coupled to a Zeiss Stemi SV 11 (3X) stereoscope (Lyrene, 1994; Sampson et al., 2013). Then, the images were analyzed with the Image J software, considering the measurements proposed by Arrington and DeVetter (2018).

Table 1. Size and number of weeks of activity of colonies located in the two orchards of highbush blueberries (*V. corimbosum*).

Colony Introduction	Colony Serial	Number of Workers	Weeks of Activity	Cultivar
July 31, 2014	1	131	8	Sharpblue
	2	75	17	
	3	97*	17	
	4	117	8	
December 24, 2014	5	125	6	
	6	100*	13	
	7	92	10	
<i>MEAN ± SD</i>		<i>105.28 ± 19.9</i>	<i>11.28 ± 4.46</i>	
May 15, 2015	8	120	7	Biloxi
	9	77	2	
	10	76	2	
	<i>MEAN ± SD</i>		<i>91 ± 25.11</i>	

* colonies that produced queens.

To contrast the floral traits in two cultivars, tests of normality (Shapiro-Wilk), homoscedasticity (Levene), and independence (Durbin-Watson), the assumptions were met. T-tests were performed to compare floral morphology in both cultivars with the statistical software R v3.2.5 (R Core Team 2013). All the flowers selected in this section were taken from different plants (n = 100 individuals/cultivars).

To register the foraging behavior of *B. pauloensis* on each cultivar, we did direct observations of 150 foragers in each orchard. The observations were made weekly during the first two months, starting when the colonies were placed into the two orchards. The observations were made on non-rainy days, preferably in the morning (9 a.m. – 12 p.m.) when the colonies were more active. Time spent (s) per forager on a flower (from the moment it arrives to the flower until it leaves the flower) and the number of flowers visited per forager per minute were registered (Javorek et al., 2002; Courcelles et al., 2013; Riaño et al., 2015). The behavioral data were analyzed with the non-parametric Kruskal-Wallis test for each cultivar with the statistical software R v3.2.5 (R Core Team 2013).

Description of pollen resources used by *B. pauloensis* in Sharpblue cultivar

Because the colonies could forage freely outside the Sharpblue orchard (open field), pollen analyses were performed to identify the additional pollinic resources. The pollen samples were taken once from different colonies one month after the location of the colonies in the orchards. Five different samples were taken on the colonies: (a) corbicular loads of 4 foragers of different colonies, (b) 3 digestive tracts

of the larvae from 3 colonies (n = 9), (c) one larvae cell provision for 4 colonies (n = 4), and (d) one feces sample from three colonies (n = 3). All the pollen samples were kept in ethanol at 90%, processed using an acetolysis protocol (as per Erdtman 1960 with modifications from Silva et al., 2014), and mounted on microscope slides. All the samples were analyzed using an optical microscope (Zeiss Axio Lab, A1) for taxonomic identification to the highest taxonomic level using our Digital Palynological Collection from high Andean zones (available at <http://rcpol.org.br>). The proportion of pollen types (i.e., plant family) in each sample was estimated based on the first 400 pollen grains observed in each microscope slide (Silva et al., 2014).

Effect of pollination on the Sharpblue and Biloxi cultivars.

Four treatments were evaluated to establish the effect of pollination mediated by *B. pauloensis* of fruits on both cultivars: (1) wind pollination: 60 newly opened flowers selected randomly were emasculated and bagged for each cultivar; (2) *B. pauloensis* pollination: 60 flowers were bagged in each cultivar after being visited by a *B. pauloensis* forager, the effect of the number of visits was not considered in this study. Furthermore, we did not control the interaction of other floral visitors previously to the *B. pauloensis* visit; (3) apomixis: the stigma was removed carefully on 60 floral buds to establish if fruiting occurs without fertilization; (4) spontaneous pollination: 60 bud flowers were bagged to avoid any floral visitors and permit self-pollination. The bags were maintained in all treatments until the beginning of fruiting. The response variables were: (a) fresh weight (g): once ripe, the

fruits were harvested and weighed on an analytical balance; (b) diameter (mm): the equatorial diameter of each fruit was measured with a caliper; (c) dry weight (g): the fruits were dried at 80 °C for 24 h and then weighed in an analytical balance; (d) number and type of seeds per fruit: dry fruits were rehydrated during 24 hours in water, then the seeds were extracted and counted. In addition, the seeds were classified as true or false seeds, depending on their shape and size (Krebs & Hancock, 1991; Desjardins & Oliveira, 2006); (5) ripening time (days): the time fruits took to ripen were registered, from fruit set to harvesting. All the treatments were in the same plant, one flower per treatment, and then we worked with 60 plants chosen randomly. Statistical analysis was performed independently for each cultivar because the two cultivars were planted under different conditions. The fruit quality data were analyzed with the non-parametric Kruskal-Wallis test for each variety with the statistical software R v3.2.5 (R Core Team 2013).

Results

Development of *B. pauloensis* colonies in Sharpblue and Biloxi orchards

In the Sharpblue orchard (open field), all colonies (7/7) survived and lasted 11.3 ± 4 weeks. Two of those colonies reached the stage of producing males and new queens (Table 1). One colony in the Biloxi orchard (greenhouse) survived to the end of the observations (1/3), lasting seven weeks in the field. Although the queen of this colony survived for the first two weeks, the workers continued foraging and feeding the larvae for the remaining five weeks, then all died.

Corolla morphology and foraging behavior of *B. pauloensis* in Sharpblue and Biloxi blueberry cultivars

Sharpblue and Biloxi cultivars presented similar corolla lengths ($t = 0.041$, $df = 253$, $P = 0.96$) but differed in

corolla width and corolla aperture (Fig 1a). Corolla was wider in Biloxi (mean = 3.33 ± 0.36 mm) vs. Sharpblue (mean = 2.94 ± 0.32 mm) ($t = -3,044$, $df = 253$, $P = 0.002$). The corolla aperture had a larger diameter in Biloxi (mean = 3.61 ± 1.92 mm) vs. Sharpblue (mean = 3.07 ± 0.84 mm) ($t = -2.699$, $df = 132$, $P = 0.0039$). It is important to note that the corolla opening of both cultivars increases gradually during anthesis (Fig 1c). Moreover, we observed that the stigma is receptive throughout the open flower cycle and becomes more exposed as the corolla opening increases.

All the foragers extracted nectar from flowers, but the pollen adhered incidentally to the bee body during nectar drinking. During the observations, buzz pollination behavior was not reported in either cultivar. The foragers visited blueberry flowers with a corolla aperture of more than 3 mm in both cultivars, but the foraging behavior varied according to cultivar. Workers of *B. pauloensis* spent more time visiting Biloxi (mean = 8.83 ± 4.58 s) than Sharpblue flowers (mean = 5.61 ± 6.40 s) ($H_{1,387} = 97.1554$, $P < 0.00001$) (Fig 1b), by which the number of flowers visited per minute were higher in Sharpblue (mean = 8.6 ± 3.47 flowers/min) than Biloxi cultivar (mean = 5.9 ± 2.69 flowers/min) ($H_{1,500} = 82.3169$, $P < 0.00001$) (Fig 1b).

Pollen analysis of colonies located in Sharpblue orchard

Twenty-five pollen types were found in all the samples analyzed. Ten were identified up to the species level (including blueberry pollen), four at the genus level, and six at the family level. Five pollen types could not be identified and were cataloged as pollinic-types (Table 2).

Blueberry pollen was present in all samples but in different proportions, being the highest in corbicular loads and the lowest in feces (Table 2). Larvae samples presented the highest richness of pollen types (Table 2). The most abundant species were *Solanum americanum* Mill. in the digestive tract of larvae, as well as *Brassica napus* L. and *Raphanus sativus* L. in brood cell provisions.

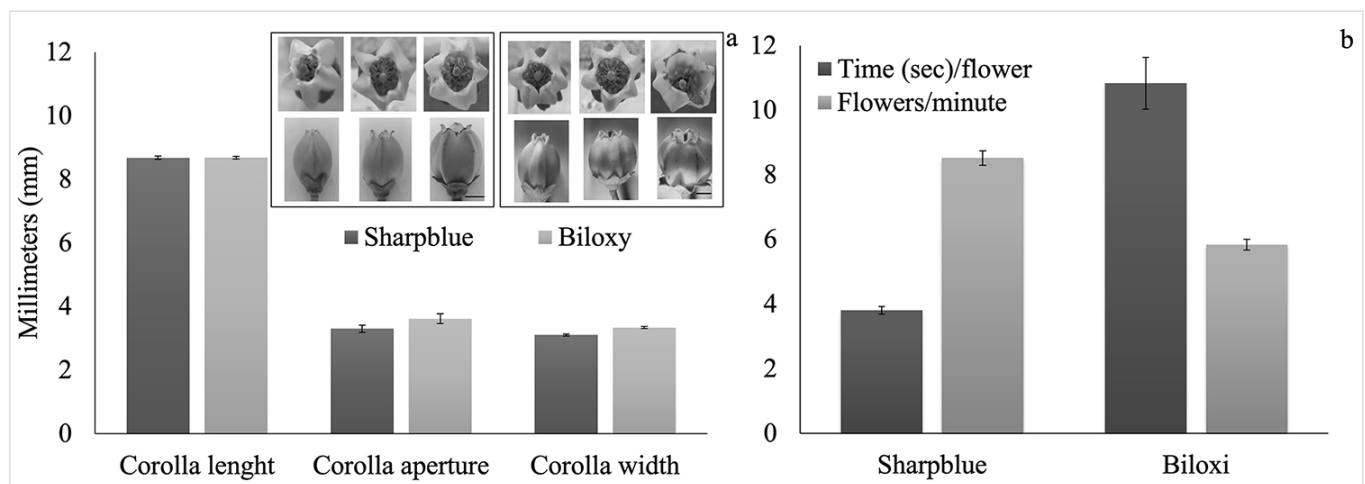


Fig 1. a) Floral measurements of two highbush blueberry cultivars (cv) Biloxi and Sharpblue, corolla length ($t = 0.041$, $df = 253$, $P = 0.96$), corolla width ($t = -3,044$, $df = 253$, $P = 0.002$), corolla aperture ($t = -2.699$, $df = 132$, $P = 0.0039$). b) Duration of a single visit per flower ($H_{1,387} = 97.1554$, $P < 0.00001$) and the number of flowers visited per minute by *B. pauloensis* workers in each cultivar ($H_{1,500} = 82.3169$, $P < 0.00001$). Error bars correspond to the standard error.

Table 2. Pollen sources identified from different samples of *B. pauloensis* colonies located in a Sharpblue blueberry crop. The values of indeterminate pollen types are not shown in the table. NF: that type was not found in the sample.

Family	Pollinic type or Species	Corbiculae	Cells provision	Larvae	Colony Feces
Ericaceae	<i>Vaccinium corymbosum</i>	81.9 %	17.6 %	11.8 %	5.2 %
	<i>Bidens sp.</i>				
	<i>Taraxacum sp</i>				
Asteraceae	<i>Carduus acanthoides</i>	1.4 %	18.9 %	3.8 %	24.2 %
	<i>Dahlia sp.</i>				
	Asteraceae ind.				
Fabaceae	<i>Trifolium pretense</i>	1.2 %	11 %	6.4 %	8.0 %
	<i>Acacia decurrens</i>				
	<i>Acacia melanoxylon</i>				
Solanaceae	<i>Solanum americanum</i>				
	Solanaceae ind.	3.2 %	9.9 %	49.7 %	10.6 %
Myrtaceae	<i>Eucalyptus sp.</i>	2.6 %	8.4 %	4.8 %	13.1 %
Poaceae	Poaceae ind.	NF	NF	3.5 %	NF
Polygonaceae	Polygonaceae ind.	NF	0.5 %	NF	0.8 %
Malvaceae	<i>Abutilon insigne</i>	NF	1.8 %	NF	1.1 %
Brassicaceae	<i>Brassica napus</i>				
	<i>Raphanus sativus</i>	8.6 %	18.8 %	11.8 %	26.2 %
Lamiaceae	Lamiaceae ind.	0.1 %	0.3 %	0.2 %	1 %
Euphorbiaceae	<i>Croton hibiscifolius</i>	NF	0.6 %	0.2 %	0.6 %
Rubiaceae	Rubiaceae ind.	NF	1.3 %	NF	0.3 %
Total per sample type		99 %	89.1 %	92.2 %	91.1 %

Effect of pollination on blueberry Sharpblue and Biloxi fruits

Apomixis and wind-mediated pollination treatments produced the lowest fruiting in both cultivars (1.6% and 5%, respectively), and fruits were too small, which were not included in the analyses. In contrast, spontaneous and *B. pauloensis* pollination favored fruit formation, but quality and ripening time varied significantly (Fig 2a and 3a).

In Sharpblue, *B. pauloensis* pollination favored ripening time, occurring 20 days earlier than fruits produced in spontaneous pollination treatment ($H_{1,85} = 30.7315$, $P < 0.00001$) (Fig 2a). In addition, *B. pauloensis* pollination increased the fresh weight in ca. 40% ($H_{1,85} = 15.2342$, $P < 0.00009$), the dry weight in ca. 60% ($H_{1,85} = 21.6281$, $P < 0.00001$) and the diameter of the fruits in ca. 15% ($H_{1,85} = 24.7176$, $P < 0.00001$) concerning spontaneous pollination (Fig 2b-c),

which was similar for the total number of seeds ($H_{1,85} = 5.9692$, $P < 0.01456$) and number of true seeds formed ($H_{1,85} = 21.4884$, $P < 0.00001$) (Fig 2d).

The effect of *B. pauloensis* pollination was also evident in the Biloxi cultivar (Fig 3). The ripening time occurred 30 days earlier than spontaneous pollination treatment ($H_{1,113} = 75.0749$, $P = 0.00001$) (Fig 3a). The fresh weight increased in ca. 30% ($H_{1,113} = 26.4956$, $P < 0.00001$), the dry weight in ca. 20% ($H_{1,113} = 24.1524$, $P = 0.00001$), and the diameter of fruits increased in ca. 50% ($H_{1,113} = 17.95$, $P = 0.00002$) concerning spontaneous pollination (Fig 3b-c). Although the number of total seeds formed per fruit did not vary ($P > 0.05$), *B. pauloensis* pollination favored the formation of true seeds ($H_{1,113} = 12.1823$, $P = 0.00048$) compared with spontaneous pollination (Fig 3d).

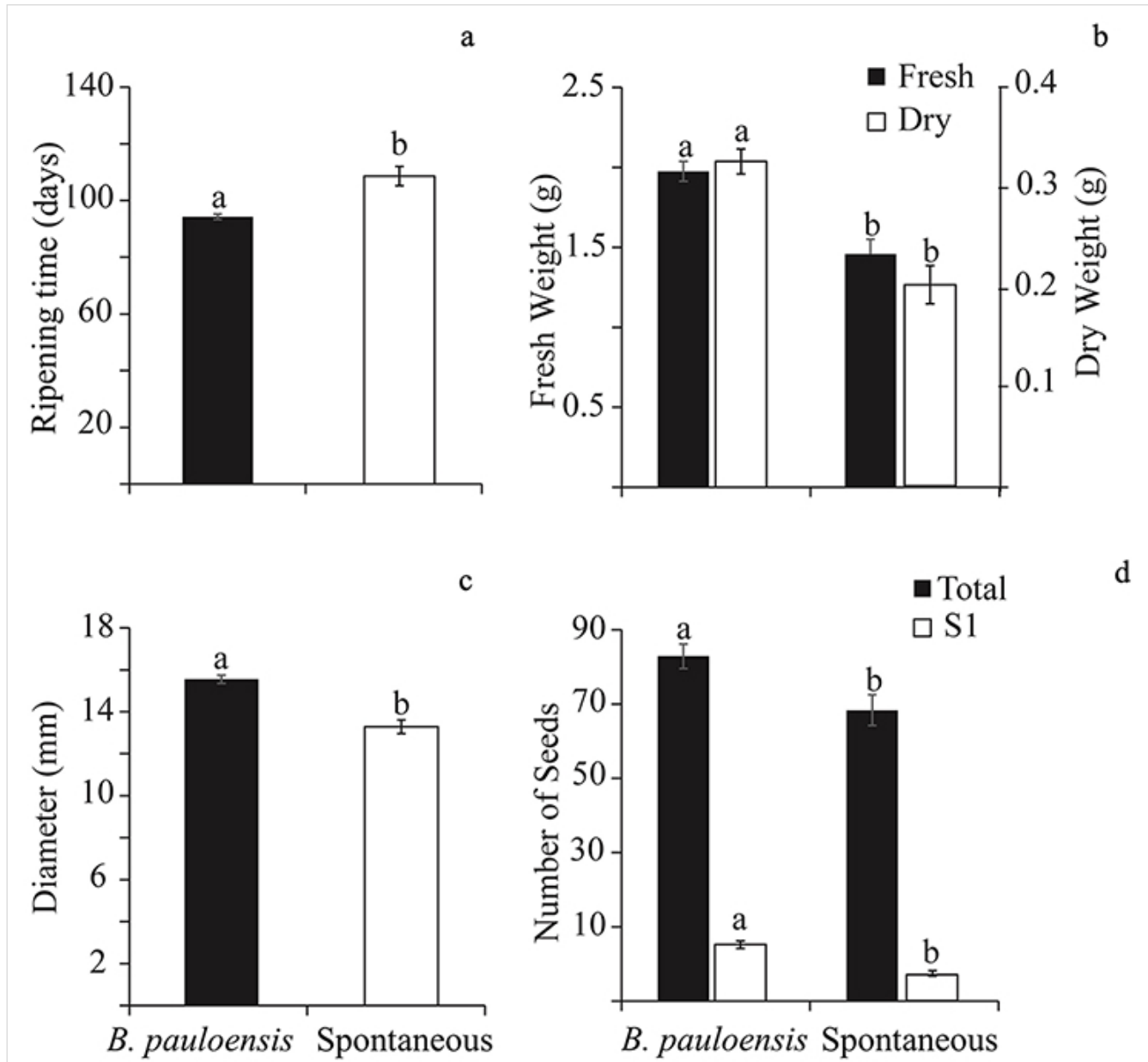


Fig 2. Effect of *B. pauloensis* pollination on the quality of fruits on the Sharpblue cultivar. *a*) Ripening time in days ($H_{1,85} = 30.7315$, $P < 0.00001$). *b*) Fresh weight ($H_{1,85} = 15.2342$, $P < 0.00009$) and dry weight ($H_{1,85} = 21.6281$, $P < 0.00001$). *c*) Equatorial diameter of the fruits ($H_{1,85} = 24.7176$, $P < 0.00001$). *d*) Total number of seeds ($H_{1,85} = 5.9692$, $P < 0.01456$) and number of true seeds (S1) formed per fruit ($H_{1,85} = 21.4884$, $P < 0.00001$). The lowercase letters above bars indicate if there are significant differences between treatments. Error bars correspond to the standard error.

Discussion

Here, we studied the effect of pollination of a native bumblebee species, *B. pauloensis*, on a promising commercial crop species in Colombia, the highbush blueberry, and some aspects of its foraging behavior on two of the most cultivated cultivars, Biloxi and Sharpblue. Our results showed a positive effect of *B. pauloensis* pollination on blueberry fruits, reducing the ripening time, increasing the fresh and dry weight, and the number of true seeds in both cultivars. The pollen analysis shows that bumblebee colonies used the blueberry pollen

in their diet. However, the colonies needed other sources of pollen and nectar. Additionally, we found that the time of flower manipulation and number of flowers per minute of *B. pauloensis* visits on blueberries varied depending on the cultivar. The results here represent a hint of the importance of *B. pauloensis* as a pollinator of *V. corymbosum*.

The native bumblebee, *B. pauloensis*, was an efficient pollinator of blueberries, improving fruit quality and shortening the ripening time. However, the foraging behavior of workers varied according to the floral traits of the cultivars, as observed previously in other blueberry cultivars (Lyrene, 1994; Courcelles et al., 2013; Sampson et al., 2013).

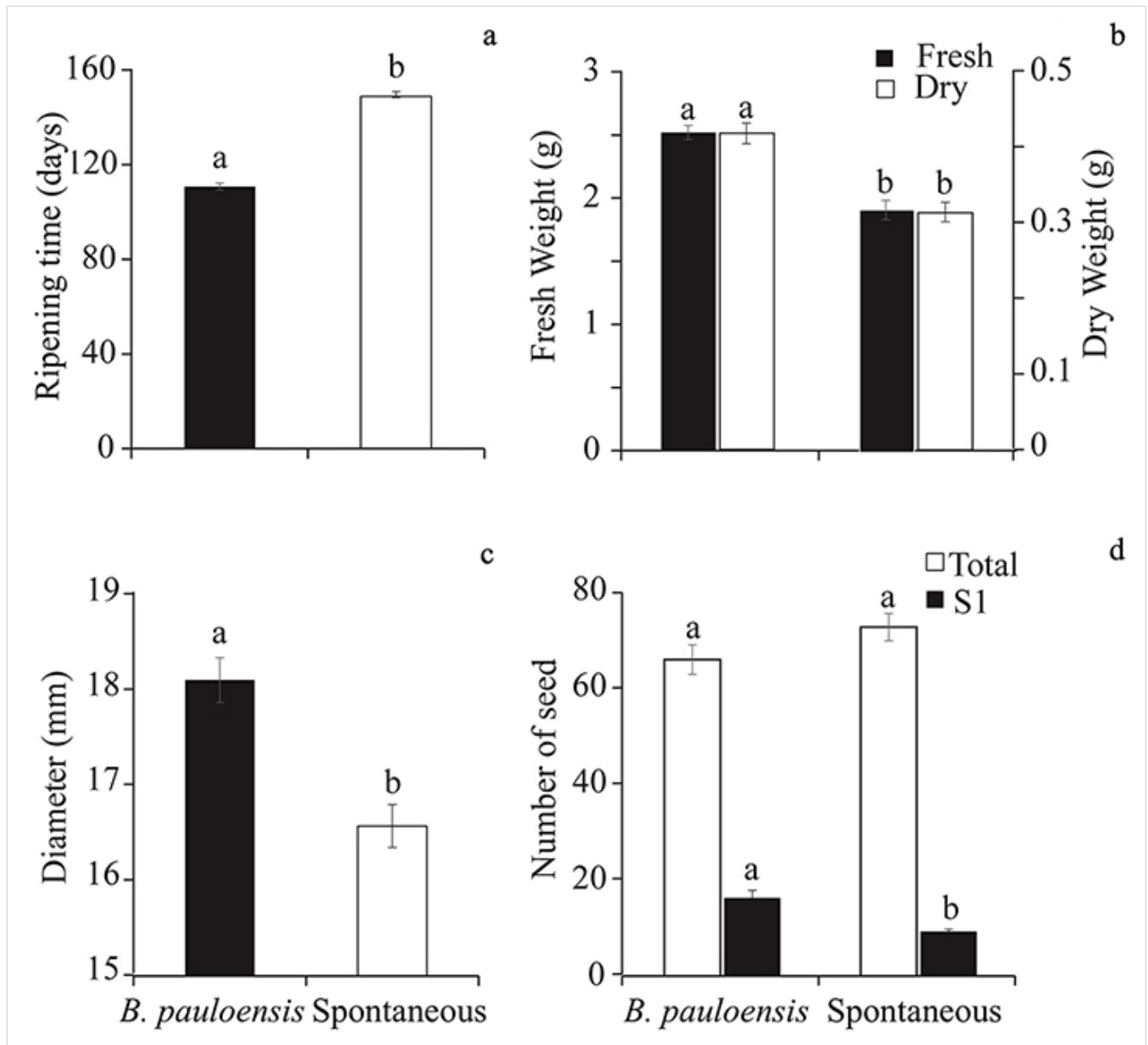


Fig 3. Effect of *B. pauloensis* pollination on the quality of fruits on the Biloxi cultivar. *a*) Ripening time in days ($H_{1,113} = 75.0749$, $P = 0.00001$). *b*) Fresh weight ($H_{1,113} = 26.4956$, $P < 0.00001$) and dry weight ($H_{1,113} = 24.1524$, $P = 0.00001$). *c*) Equatorial diameter of the fruits ($H_{1,113} = 17.95$, $P = 0.00002$). *d*) Total number of seeds ($P > 0.05$) and number of true seeds (S1) formed per fruit ($H_{1,113} = 12.1823$, $P = 0.00048$). The lowercase letters above bars indicate if there are significant differences between treatments Error bars correspond to the standard error.

For example, the cultivar Duke has a smaller corolla aperture. Only small and medium-sized bees such as *A. mellifera* can access the nectaries and pollinate the flower. In comparison, the Liberty cultivar has a wider corolla aperture, favoring bumblebee pollination (Courcelles et al., 2013).

The *B. pauloensis* colonies kept in the open field of Sharpblue orchard could forage on different pollen sources besides the blueberry, which let them supply their nutritional demands and allowed their growth and reproduction. Colonies kept in the Sharpblue orchard collected pollen from plant species with high protein content, particularly *B. napus*, *R. sativus*, and *S. americanum* (> 25% protein content), similar

to that was reported previously for colonies kept in suburban conditions (Riaño-Jimenez et al., 2020). Several studies have demonstrated the adverse effects of a protein-poor and low plant-diversity pollen diet in the survivorship and reproduction of bumblebee colonies (Génissel et al., 2002; Vanderplanck et al., 2014; Baloglu & Gurel, 2015; Crone & Williams, 2016; Vaudo et al., 2016; Spiesman et al., 2017; Riaño-Jimenez et al., 2020). The adverse effects of a low-quality diet could be related to the low performance of *B. pauloensis* colonies maintained in the greenhouse. The colonies had slow development, premature queen death, and no queen production. Although the colonies were not wholly

confined, the structure limited the foraging activity outside the greenhouse, hence the only source of flowers was the blueberry plants. In fact, according to the pollen analysis performed in colonies kept at Sharpblue orchard, blueberry pollen was present in low proportion in both provision pockets and larvae, which could indicate that it is not an essential resource for this bee species, probably because of their low protein content (13.9%) (Somerville, 2001), which is supported by the studies made by Leonhardt and Blüthgen (2011) since they found that the bumblebees gather pollen with high protein content and more essential amino acids. Our results agree with the results obtained by Cavigliasso *et al.* (2020), where they found *B. pauloensis* colonies forage on wild plants post-nest establishment. Although it is important to remark that we sampled pollen just once, the pollen sources accessed by the colonies could change depending on the blooming peaks of other plant species. It is necessary to develop new long-term studies on pollen use by *B. pauloensis* colonies during all the developmental phases to identify the important pollen sources to establish colony management strategies in the high Andean crops of Colombia. This type of study would help improve the management of colonies in blueberry crops, creating mixed crops in greenhouses or gardens and corridors with alternative floral resources near the orchards to meet the nutritional requirements of colonies (Riaño-Jimenez *et al.*, 2020).

The positive effect of *B. pauloensis* pollination has been reported previously in other crops such as tomato, lulo, and sweet pepper, increasing fruit quality by at least 30% (Aldana *et al.*, 2007; Almanza *et al.*, 2007; Riaño *et al.*, 2015). Cavigliasso *et al.* (2020) recently reported that *B. pauloensis* pollination increased the fruit quality of the highbush Emerald cultivar in Argentina. In our study, despite behavioral differences in both cultivars and the lack of buzz pollination with *B. pauloensis* pollination, we obtained a higher fruit quality and a shorter fruit ripening time than reported by Cortez-Rojas *et al.* (2016) in a crop without managed pollinators in Colombia. Furthermore, previous studies have reported *B. pauloensis* as a more efficient pollinator than other bee species for the blueberry cultivars evaluated here. Here, *B. pauloensis* pollination doubled the number of true seeds obtained by Lang and Danka (1991) in the Sharpblue cultivar with *A. mellifera* pollination, and the fresh weight of Sharpblue and Biloxi fruits compared with results obtained by Zee *et al.* (2006) in crops without pollinators.

Additionally, we found that pollination by *B. pauloensis* reduced fruit ripening time compared with that reported by Huang *et al.* (1997) in the Sharpblue cultivar, which was pollinated manually. Likewise, the fruit ripening time was similar to that obtained by Sampson and Spiers (2000) in the Misty cultivar, pollinated by *B. impatiens* Cresson. The relationship between pollinator efficiency and the number of true seeds per fruit has been reported previously in other blueberry cultivars (Huang *et al.*, 1997; Desjardins & Oliveira, 2006).

That is related to the synchrony between behavior and morphology (e.g., tongue length) of pollinators and morphological (e.g., corolla length) and phenological (e.g., anthesis, stigma receptivity) traits of the flower (Gorchov, 1985; Huang *et al.*, 1997; Desjardins & Oliveira, 2006; Campbell *et al.*, 2017; Wietzke *et al.*, 2018). Although it is worth clarifying that we could not control the presence of other floral visitors in the experiment, this could affect the perceived pollination effects by *B. pauloensis*. On the other hand, here, apomixis and wind-mediated pollination treatments produced small and deformed seeds, a high rate of floral abortion, and a low fruiting rate in both cultivars. These effects of deficient pollination have been reported by Stephenson (1981) for different plant species.

In conclusion, our results indicate that facilitating the bumblebee's access to different floral resources can be beneficial to ensure pollination activity and survival of *B. pauloensis* colonies and other pollinators in the crops. In addition, implementing mixed crops with different blueberry cultivars will favor the survival and growth of bumblebee colonies and the productivity of the crops (Parrie & Lang, 1992; Huang *et al.*, 1997; Chavez & Lyrene, 2009). Mixed crops through a more comprehensive floral offer will provide the necessary resources to meet the nutritional requirements of colonies in each developmental stage, favoring their pollinating activity in the crop (Riaño-Jimenez *et al.*, 2020; Ramírez-Mejía *et al.*, 2023).

However, we acknowledge that our study has some limitations, and the study was performed in only one location without replication. Additionally, we could not include a natural pollination treatment. Here, we only evaluated the *B. pauloensis* pollination. Finally, it is worth mentioning that commercial pollination of blueberries in the high Andean areas of Colombia using the native bumblebee *B. pauloensis* is possible, reducing the risks of introducing alien species. That requires changes in traditional pest management (i.e., chemical pest control) and agreements with the owners about schedules of pesticide applications to ensure the survival of bumblebee colonies and, thus the crop pollination (Cavigliasso *et al.*, 2021). Indeed, changes in traditional pest management added to the creation of gardens or corridors with alternative floral resources will also attract other wild bee species to the blueberry crop (Ramírez-Mejía *et al.*, 2023). Our study provides empirical evidence of the importance of the native bee species *B. pauloensis* on blueberry crop production, directly favoring some traits related to fruit quality, such as fruit size and weight.

Funding

This work was supported by the Governorate of Cundinamarca, the Colombian Association for the Advancement of Science, and Nueva Granada University under Grant number INV-CIAS 1655.

Disclosure statement

The authors report there are no competing interests to declare.

Acknowledgements

We thank the Asociación Colombiana para el Avance de la Ciencia (ACAC), the Professional Council of Biology (CPBIOL - 2015), the Cundinamarca Government, and Nueva Granada University for the financial support of this project. Thanks to Natali Plazas for data collection support, Margarita Lopez-Urbe and Romina Rader for reviewing the text.

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