Optimal pollination thresholds to maximize blueberry production

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ABSTRACT

Pollination management for highbush blueberry crops (*Vaccinium* spp.) generally depends on beehives stocked at variable densities, with little consideration given to optimal pollination levels dictated by the mating system of the crop. This approach limits our capability to accurately forecast the consequences of animal pollination on crop productivity and can result in pollination shortfalls. Using experimental and observational data, we estimated optimal pollination thresholds for blueberry crops that maximize fruit diameter. We manipulated stigmatic pollen loads and used Bayesian models to evaluate the effects on fruit diameter. In this way, we were able to define thresholds for deficient, optimal and supraoptimal pollen deposition in blueberries. These thresholds were then evaluated under field conditions in blueberry farms, and used simulations to estimate the minimum number of honeybee visits required for optimal blueberry pollen deposition. A quadratic relationship described fruit diameter in response to stigmatic pollen load, with optimal pollen deposition peaking at 192 pollen tetrads and ranging between 112 and 274. Our simulations showed that a flower visitation rate guaranteeing, on average, six to seven honeybee visits per flower (i.e. flower visitation rate of 0.6 visits per 100 flowers h^{-1}) would result in 60% of the plant flowers receiving optimum stigmatic pollen deposition. Higher numbers of honeybee visits increased the probability that blueberry stigmatic pollen loads were below the optimum and the probability that smaller berries were produced. We show that adverse pollination scenarios in blueberries can occur through different pathways, either because of a deficit or an excess of pollination that directly impacts the quality of the fruits produced. By identifying thresholds, we provide a pragmatic basis for adaptive management of honeybees based on average visitation rates that are most suitable for growers to manipulate. Our study provides new insights into the mechanisms behind pollination, fruit production, and the contribution of honeybee to blueberry crops. We highlight that systematic pollination management through flower visitation monitoring and clear optimal pollination targets can help prevent detrimental pollination scenarios.

1. Introduction

Highbush blueberry, *Vaccinium corymbosum* L., is a mass flowering crop dependent on animal-mediated pollination (Eeraerts et al., 2023). In general, increasing pollen deposition through flower visitation promotes blueberry production and reduces ripening time (Danka et al., 1993; Dogterom et al., 2000; Drummond, 2019; Nagasaka et al., 2022). This pollen demand is mostly covered by stocking honeybee hives —*Apis mellifera* L.— in blueberry fields (Bushmann and Drummond, 2020; Cavigliasso et al., 2021; Rollin and Garibaldi, 2019), with this species providing ~80% of flower visits to blueberry crop along its cultivation range (Eeraerts et al., 2023). In the USA alone, the contribution of honeybee pollination has an annual economic value of around 400 million USD for blueberry industry (Reilly et al., 2020). Even with its widely acknowledged importance, the management of honeybee pollination in blueberry crops remains simplistic an often relies on growers' intuition or rule-of-thumb for beehive stocking density (e.g., recommendations can range from 1 to 25 beehives ha^{-1} (Rollin and Garibaldi, 2019; Isaacs and Kirk, 2010). The absence of reactive management strategies based on target honeybee visitation to cover crop pollen deposition demand means that growers may fail to reach optimal pollination due to either a shortfall or over pollination (Aizen et al., 2014). At the very least, are wasting economic resources by stocking beehives at higher rates than needed. Moreover, such high levels of

honeybee abundance might have unexpected effects on wild pollinator communities through resources competition and transmission of pathogens (Mallinger et al., 2017).

Profits in pollinator-dependent crops depend on sufficient stigmatic pollen deposition to ensure ovule fertilization, seed development and outcrossing in plants (Aizen and Harder, 2007; Garibaldi et al., 2013; Drummond, 2019). For many crops, the demand for pollen transfer is covered by honeybees which are comparatively easy to manage and are estimated to pollinate about 80% of commercial flowering crop species (Aizen et al., 2020; Klein et al., 2007). To understand the contribution of honeybees to pollination service, researchers commonly use surrogate measures of pollination success, including abundance or visitation rate, and single-visit pollen deposition (Javorek et al., 2002; Benjamin et al., 2014). The levels of pollen deposited on crop flowers has received considerably less attention (Garibaldi et al., 2014), even though it is likely to represent a more accurate measure of pollination service delivery (Stavert et al., 2020). Pollen deposition is more closely linked to aspects of plant reproduction that determine seed set and, ultimately, fruit development and quality (Drummond, 2019). Linking pollen deposition and flower visitation in crops provides an opportunity to understand the mechanisms underlying pollinator contribution to crop productivity and could be the basis for developing management plans based on optimal pollination thresholds that can maximize crop productivity while reducing hives stocking costs (Garibaldi et al., 2020).

A precept of pollination management often focuses on saturating crop flowers with honeybees hoping to improve productivity profits (Eeraerts et al., 2020; Hevia et al., 2021). This assumption has serious potential implications for crop production if the relationship between honeybee numbers and pollination success is not a simple linear one. Instead of a non-saturated linear relationship, growing evidence indicates that the response of crop production to flower visitation rates may be best described by a quadratic function (i.e. a 'hump shaped' relationship, see (Aizen et al., 2020; Rollin and Garibaldi, 2019) reflecting an optimum beyond which additional flower visits might come at an interaction cost to plants reducing crop productivity (Aizen et al., 2020). Supraoptimal pollination levels may result from stigma damage, nectar robbing, reduced stigmatic pollen load or pollen tube stagnation (Aizen et al., 2014; Harder et al., 2016b; Young and Young, 1992). All those factors can negatively impact seed set, fruit set and fruit quality (Rollin and Garibaldi, 2019; Sáez et al., 2014). Reducing pollination to lower but optimal levels in this scenario would improve ovule fecundity and crop production (Rollin and Garibaldi, 2019). Information on plant reproduction and pollination effectiveness of main visitors, integrated into a pollination management program can, for example, help to decide on appropriate stocking densities by removing or relocating beehives based on objective evidence.

In this study, we aim to estimate levels of pollen deposition and honeybee visitation rates that optimize highbush blueberry production quality, focusing on fruit diameter as a key metric of the commercial value of this crop. We experimentally assessed the relationship between blueberry fruit diameter and stigmatic pollen load to define the function and potentially identify the thresholds for deficit, optimal, and supraoptimal pollen deposition. We also evaluated honeybee single-visit pollen deposition, and simulated random pollen deposition to estimate the number of visits needed to achieve optimal pollen delivery. This pollination threshold was then related to observed farm-level pollen deposition and honeybee abundance to assess its impact on pollination and crop productivity. We focused on honeybees as they account for more than 90% of floral visits in blueberry crops of Northern Argentina (Ramírez-Mejía et al., 2023a; Ramírez-Mejía et al., 2023b), and are the species currently most likely to be actively managed by growers. Because blueberry stigma should reach a pollen deposition saturation point (Parrie and Lang, 1992), we expect the relationship between fruit size and stigmatic pollen load to follow a non-linear function. Hence, if blueberries experience over-pollination due to an excess of pollen, we expect a quadratic relationship (Morris et al., 2010); otherwise we

predict that a saturating exponential function will be the best model. In any case, we expect the optimal pollination region to be located around the vertex or asymptotic point of the quadratic or the saturating exponential function, respectively. We predict that honeybee abundance —as a proxy for flower visitation rate— will have variable effects on field-level pollen deposition depending on extreme values (Aizen et al., 2014; Rollin and Garibaldi, 2019; Sáez et al., 2014) and that farms with pollen deposition closer to the optimum will produce fruits of higher quality.

2. Methods

2.1. Study site and system

We conducted the study during the blueberry bloom season of 2021 in northwestern Argentina (central point of the studied locality, 27°06′01″S – 65°34′32″W). We selected Snowchaser and Emerald cultivars for the experimental and observational research stages, respectively. Both cultivars are among the most commonly used by growers in the region (com. pers. APRATUC —NOA Blueberry Producers Association—). The flowering period (June – July) and the harvest season (August – November) of the Snowchaser cultivar start about one month earlier than most other cultivars. Emerald's bloom and harvest take place in August and September respectively. Both cultivars are self-compatible and produce a delayed harvest of small-sized berries in the absence of pollinators (Müller et al., 2013).

2.2. Finding optimal thresholds of pollen deposition

Our main objective was to assess the relationship between blueberry fruit size and pollen deposition. Therefore, we conducted a field experiment to maximize the variation in stigmatic pollen load. On one farm, we randomly selected 20 plants of the Snowchaser cultivar and for each plant we selected four flowering branches that were similar in terms of orientation, size and number of flowers. On these branches, we applied four treatments: 1) *pollination exclusion* – this treatment consisted of a nylon mesh bag surrounding the flowers to prevent animal pollination; 2) *partial pollination* – the flowers were excluded with a mesh bag, but hand-pollinated once with a brush; 3) *free pollination* – the flowers were exposed to natural pollination; 4) *supplemented pollination* – the flowers were exposed to natural pollination, but were also hand-pollinated once. The cross pollen used for hand-pollination was collected with a mechanical hand pollinator (VegiBee™, vegibee.com), a tool that we used to sonicate flowers and release pollen from flowers of randomly chosen plants from the same cultivar and block. When we conducted the experiment, Snowchaser was the only cultivar in bloom. We monitored three open flowers per treatment on each plant by labeling them with numbered tags attached to the pedicel (N_{total} = 240 flowers). From 12 to 24 h after flower anthesis, when the corolla fell off naturally and the extraction of the style had no effect on the probability of the flower setting fruit, we detached each style from the flower receptacle using small forceps and stored it in an Eppendorf tube containing 70% ethanol. In the laboratory, we used a stereoscope to do a transversal cut of the style at the stigma height and placed it on a microscope slide. We then positioned the stigma in polar view, covered it with a drop of Alexander's stain (Alexander, 1969), allowed it to saturate for one hour, crushed it with a coverslip and counted the pollen tetrads under a microscope (40x magnification). When the fruits were fully mature, we collected them and measured the equatorial diameter with a caliper of 0.05 mm accuracy. See photos illustrating the protocol in Appendix A Fig. S1.

2.2.1. Statistics

Using the data from the experiment, we fitted Bayesian quadratic and saturating exponential models to describe the relationship between fruit size and the number of pollen tetrads deposited on the stigma, and

used the lowest leave-one-out cross-validation information criterion (*looic*) to select the best-fitting model (Vehtari et al., 2017). For both models, we chose a normal distribution for the likelihood function. We also defined non-informative priors, Normal(0, 1), for the slope co-efficients of the quadratic model and Normal(5, 3) for its intercept. Regarding the prior probabilities of the saturating exponential function, we used a Normal(17, 3) for the parameter denoting the asymptote of the function and Normal(0, 1) for the parameters controlling the growth rate. In both models, we used an exponential(1) prior distribution for the dispersion parameters of the likelihood function. We estimated the parameters of each model using three chains, 6000 sampling and 500 to 1000 warmup iterations per model, with a thinning rate of 3. See the mathematical notation of the models and additional details on the prior probability used in Appendix B sections 1.1 and 1.2. We performed posterior predictive checks and used graphical analysis of the residuals to evaluate the quality of the models' fit. Based on the best-fitting model, we delineated three pollination categories: *pollination deficit*, *optimal pollination*, and *supraoptimal pollination*. We consider *pollination deficit* to occur when increasing stigmatic pollen load increases fruit size (i.e. $\beta >$ 0); *optimal pollination* when the relationship stabilizes and additional pollen deposition does not influence fruit size ($\beta \sim$ 0); and *supraoptimal pollination* when the increasing stigmatic pollen load reduces fruit size ($\beta <$ 0). The upper threshold of *pollination deficit* and the lower threshold of *supraoptimal pollination* delimit the range of *optimal pollination* and were estimated based on the first derivative of the curve, where the slope starts to decrease (maximum curvature knee point; Appendix B section 1.4). The analysis was conducted in R 4.1.2 (R Core Team, 2022) using: *base*, *dplyr*, *readxl*, *magrittr*, *cowplot* and *ggplot2* packages (Bache and Wickham, 2020; Wickham et al., 2021; Wickham, 2016; Wickham and Bryan, 2019; Wilke, 2020). We fitted the models using *Stan* 2.21.8 with the R packages *rstan*, *brms,* and *rethinking* (Bürkner, 2017; McElreath, 2021; Stan Development Team, 2023), estimated R^2 following Gelman et al. (2019), and estimated the first derivative knee points in the non-linear functions using the *kneearrower* package (Tseng 2020).

2.3. Number of honeybee visits to achieve optimal pollen deposition

In the same farm where we did the pollination experiment, we conducted another field experiment to evaluate the range of variation in per-visit pollen deposition by honeybees. The main objective was to estimate the minimum number of honeybee visits required to maximize fruit diameter. To do this, we placed nylon mesh bags over flowering branches with immature flowers to prevent pollen deposition by polli-nators. When the flowers were fully open, we removed the bag, cut the branch and kept it fresh in water. We then walked through the crop to offer the virgin flowers to honeybees. After a single honeybee-visit, we isolated the flower, tagged it, and brought the flowering branches to the laboratory for a period of at least 12 hours to ensure that the pollen tetrads had fully adhered to the stigma (Dogterom et al., 2000). We then collected the style and followed the procedure described above to esti-mate the amount of pollen deposition by honeybees on blueberry flowers in a single visit.

2.3.1. Statistics

We estimated the posterior distribution of honeybee pollen deposi-tion on blueberry flowers in a single visit using Bayesian quadratic approximation (Appendix B Sections 2–2.1). We then calculated 50 posterior predictive distributions and used them to conduct simulations to replicate the random process of pollen deposition during sequential honeybee visits (Appendix B Section 2.2). Using each posterior predic-tive distribution, we ran 2000 simulations per honeybee *visit level*. Each simulation can be considered as a flower —2000 flowers per honeybee *visit level* and per posterior predictive distribution— whose stigmatic pollen load is the additive result of random pollen deposition after *n* honeybee visits. We then calculated the proportion of stigmatic pollen load that falls within the optimum pollination interval for each level of

honeybee *visit* (Appendix B Section 2.2). Here we assume that tetrads are added at each successive visit and that no stigmatic pollen was removed in the process.

2.4. Field pollination levels and crop production

We selected seven blueberry farms (Emerald cultivar) ranging in size from 10.1 to 70.7 ha (mean $=$ 39.6 $\pm$ 20.9 SD) and with an average distance of 9.5 $\pm$ 2.4 km between each other. During the flowering and harvest season of 2021, we selected one plot per farm to record fruit diameter, field-level pollen deposition and honeybee abundance. (i) *Fruit diameter* — we randomly selected five plants from which we collected 10 mature fruits during the harvesting period to measure their equatorial diameter using a caliper with 0.05 mm accuracy ($N_{farm} = 50$, $N_{total} = 350$). (ii) *Field-level pollen deposition* — on five randomly chosen plants, we collected three styles of senescent flowers ($N_{farm} = 15$, $N_{total} = 105$) that we stored in 70% alcohol and estimated stigmatic pollen load following the protocol described above. (iii) *Honeybee abundance* — we conducted two honeybee counts during the flowering period and, each time, estimated the flowering percentage at the plot level by counting the ratio of open and closed/senescent flowers in two branches of ten plants. Then, on six farms we counted the number of honeybees visiting flowers during a period of 25 seconds on 25 five plants distributed in 50 m transects (N $=$ 4 transects). On one farm, we randomly selected five plants along a 50 m transect and conducted 5 min counts of honeybees visiting blueberry flowers. As the time spent on each honeybee count was higher on that farm, we adjusted the count for the time efforts by dividing the 5-minute count data by 12 (i.e. 25 s). We collected the data on sunny days, with low wind and temperatures above 15°C. We sampled approximately between 1000 and 1700 h, and randomized the hour of visit to each plot. We accumulated a total sampling observation of 8.4 h.

2.4.1. Statistics

We used Bayesian models to compare honeybee abundance, stig-matic pollen load, and fruit size across farms. In the honeybee abun-dance model, we included the flowering percentage as a predictor to account for the potential effect of flower availability. We also use the average estimate of the posterior distributions per farm to assess the relationship between fruit size and stigmatic pollen load (conditioned by honeybee abundance), and between stigmatic pollen load and honeybee abundance. We estimated the parameters of each model using three chains, 2000 sampling, and 500 warmup iterations per model. We used a Poisson distribution as the likelihood function for the model assessing differences in honeybee abundance across farms and a Normal distri-bution for the remaining models. Also, in the models comparing hon-eybee abundance, pollen deposition, and fruit size among farms, we applied contrast analyses on the posterior distributions to estimate the average variation between farms. We used a mixture of informative and semi-informative priors in all models. We provide specific details on the mathematical notation of the models, the prior probability used, and the coding procedure in Appendix B Section 3. Because we fitted these models using data from the Emerald cultivar only, but also considered the experiment model fitted with Snowchaser data to derive conclusions on fruit size (Section 2.2), we added a series of validation analyses to demonstrate that the Snowchacer model can be used to predict Emerald fruit size (see Appendix A Table S1). We fitted all models using the *rethinking* R package (McElreath, 2021) and evaluated the models' fit through posterior predictive checks.

3. Results

3.1. Optimal pollination thresholds

From the 240 flowers monitored in the four experimental treatments, we collected data from 72 of them where the fruits successfully ripened

($N_{exclussion}$ = 21, $N_{partial}$ = 8, N_{free} = 27, $N_{supplemented}$ = 16). The remainder were lost when the style went senescent or as a result of ripened berries being damaged by birds or during harvest. In the pollinator exclusion experiments, the stigmatic pollen load increased progressively, from pollination exclusion to partial pollination, to free pollination, and with the highest load in the supplemented pollination treatment. That is, flowers with supplemented pollination received on average 38 tetrads more than the flowers in the free pollination treatment, meaning stigmatic pollen loads ~24% higher (Appendix A Fig. S2). These increases corresponded to higher average fruit diameter, although the berries resulting from supplemented pollination were on average ~4% smaller than those in the free treatment (Appendix A Fig. S2).

A quadratic function best described the relationship between fruit diameter and stigmatic pollen load (R^2 quadratic model= 0.51

(0.38–0.60); R^2 saturating exponential model = 0.38 (0.23 - 0.50); Fig. 1a, Appendix B section 1.1.2). The number of pollen tetrads deposited on the stigma ranged from 1 to 370 (mean ± SD = 121.5 ± 98.5), and the stigmatic pollen load that maximized blueberry fruit diameter (the quadratic function vertex) was 191.7 tetrads (Fig. 1a). Optimal pollination of blueberry flowers was estimated to be between 112 and 274 tetrads deposited on the stigma (Fig. 1b, Appendix B section 1.4). That is, flowers pollinated with less than 112 or more than 274 pollen tetrads can be assigned to the deficit or supraoptimal pollination categories, respectively (Fig. 1b).

3.2. Optimal number of honeybee visits

We collected data on stigmatic pollen loads from 41 virgin flowers that received a single honeybee visit. We found that on average,
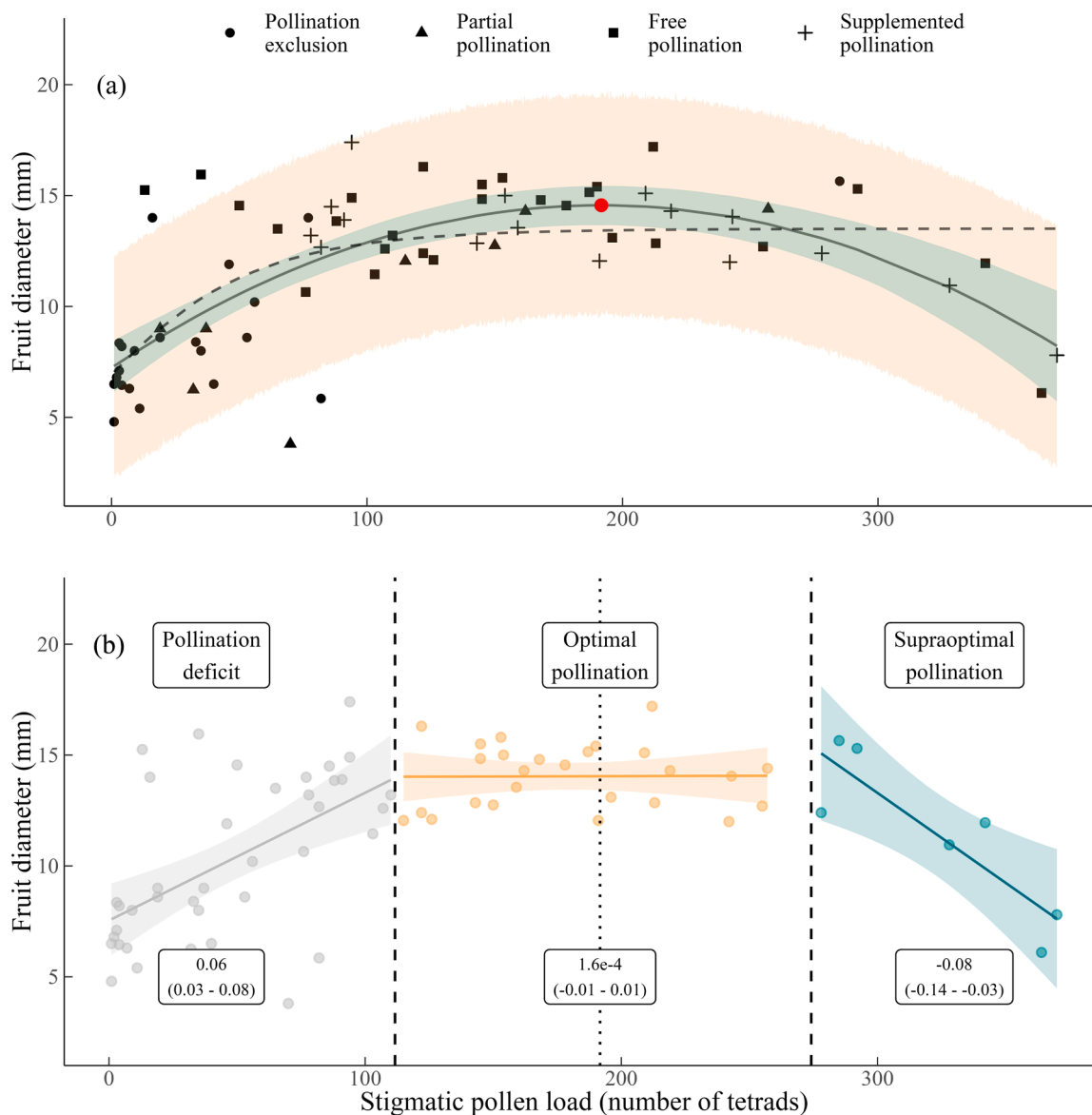

Fig. 1. Model predicting changes in blueberry fruit size as a function of the number of pollen tetrads deposited on the stigma. (a) Quadratic (solid) and saturating exponential (discontinuous) models; the red dot marks the vertex (coordinates: x = 191.2, y = 14.7) of the quadratic function; the inner green band indicates the 95% credibility intervals for average quadratic estimation, whereas the yellow band indicates the simulated 95% credibility intervals of the quadratic model prediction. The equation for the quadratic function is, *Fruit size = 7.23 − 1.9e^{-4} stigmatic pollen load^2 + 0.076 stigmatic pollen load*. (b) Thresholds of deficit, optimal, and supraoptimal pollination levels, given stigmatic pollen load deposition and respective fruit size outcome. Vertical discontinuous lines (x-axis values 112.4 and 274.1) are the thresholds for the optimal pollination interval, whereas the dotted line shows the stigmatic pollen load that maximizes fruit size (191.2 tetrads). The fitted lines are provided for each pollination level (bands denote 95% credibility intervals of the estimation), with the slope and its credibility intervals at the bottom.

honeybees deposit 18 pollen tetrads (95% CI= 12.7 – 25.1) after a single visit. We also found that six or seven honeybee visits are required to achieve the highest proportion of stigmatic pollen loads in the optimal range (Fig. 2). That is, our simulations indicate that when honeybee visitation rate ensures an average of six or seven visits to each flower during the receptivity period, 60% of the flowers on a blueberry bush will receive optimal pollen deposition.

3.3. Field pollination levels and crop production

The farms tended to vary in their abundance of honeybee, the field-level pollen deposition, and the size of the berries produced (Fig. 3a-c). The farms with the lowest honeybee abundance tended to have stigmatic pollen loads closer to the pollination optimum (Fig. 3a and b). That is, the stigmatic pollen loads were 82% higher on the farm with the lowest honeybee abundance (contrast of the posterior distributions between Acheral–Lucia; Fig. 3b), while farms with higher honeybee abundance tended to have stigmatic pollen loads closer to the pollination deficit threshold (Fig. 3a and b). Indeed, we found a 93% probability that honeybee abundance has a negative effect on the stigmatic pollen load on blueberry flowers (Fig. 3d, Appendix B sections 3.5 and 3.5.1). As

expected, increasing pollen deposition in blueberry flowers promoted the production of larger fruits (Fig. 3e), resulting in berries 36% larger on the farm where the stigmatic pollen loads were closer to the optimum (contrast of the posterior distributions between Acheral–Lucia; Fig. 3c). The posterior predictive checks indicated that all models were well fitted (Appendix B Sections 3.1 - 3.5).

4. Discussion

Typically, farmers do not consider target values for honeybee density (Garibaldi et al., 2020); instead, they seek to saturate crop flowers with pollinators (Aizen et al., 2014). Such logic would be unacceptable in the case of other agricultural inputs. By combining observational and experimental data with simulation methods, we have estimated accurate values of pollen deposition and honeybee visits required to maximize the production of export quality fruit. While these values have been derived for one region, they provide a tangible basis for the wider management of this economically important crop. We found that optimum pollen deposition to maximize blueberry size ranges between 112 and 274 pollen tetrads and that, on average, at least six or seven honeybee visits are required to ensure that 60% of the flowers receive optimum
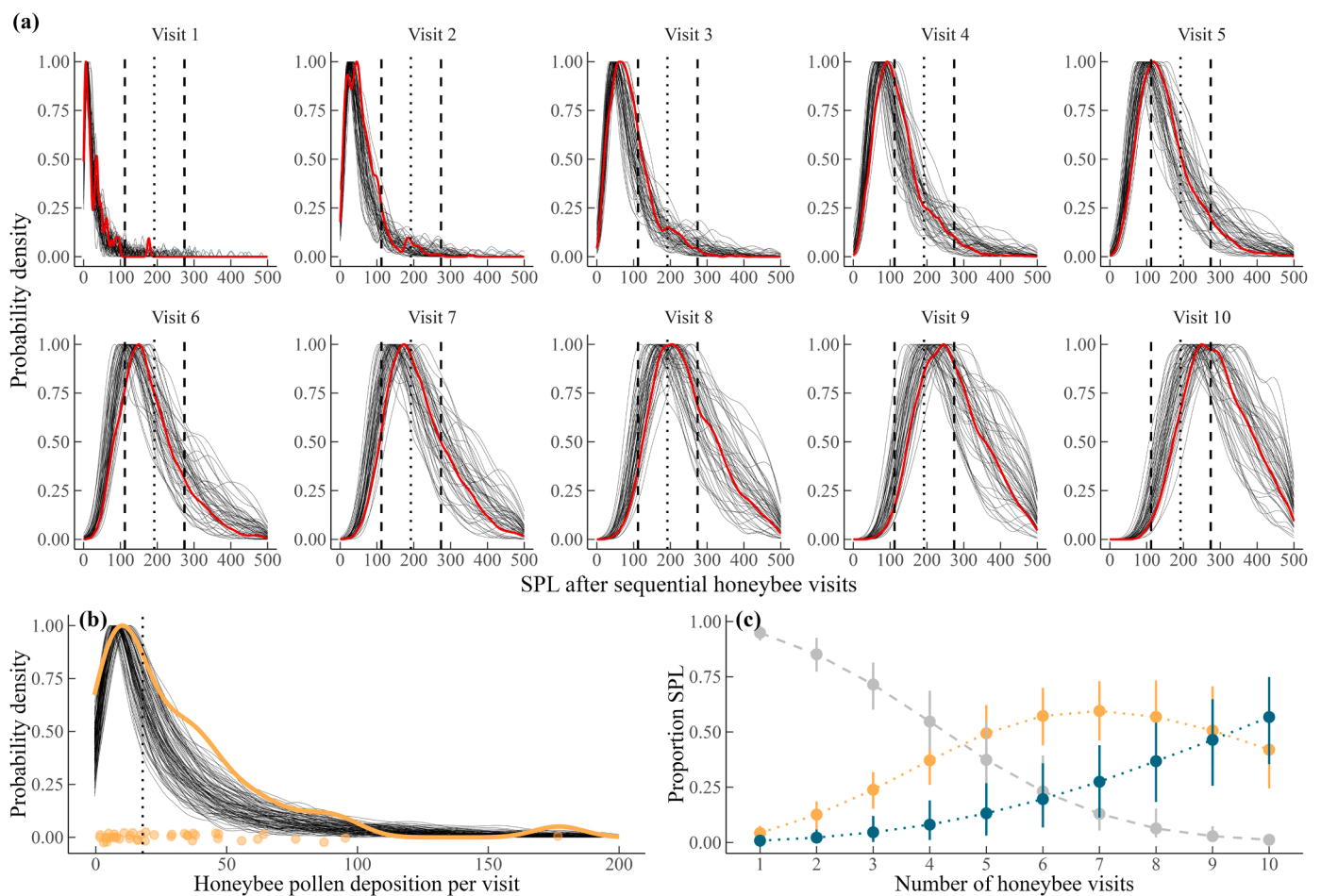

Fig. 2. Simulated stigmatic pollen loads (SPL) after random sequential honeybee visits (a), based on posterior predictive distributions from single-visits pollen deposition of honeybees on blueberry flowers (b), and (c) proportion of SPL in deficit (gray), optimal (orange), and supraoptimal (cyan) pollination ranges after *n* simulated visits. (a) From the first to the tenth visit, the density black lines correspond to random posterior predictive distributions —50 per panel— and the red line indicates the simulated value using the raw observations. Each density black line per panel resulted from 2000 simulations of honeybee pollen deposition through sequential random floral visits. The vertical dashed lines (x-axis values 112.4 and 274.1) are the thresholds for the optimal pollination interval, whereas the dotted line shows the stigmatic pollen load that maximizes fruit size (192 tetrads). (b) Observed values (orange density line and dots, N = 41) of single-visit honeybee pollen deposition in blueberry flower and its posterior predictive distribution (black density lines); the dotted line indicates the average value of ~18 (95% IC 12.7 – 25.1) pollen tetrads per visit. (c) Average proportion of SPL that falls within the three pollination interval for each honeybee visit; the vertical error bars denote the 0.025 and 0.975 quantiles of the simulated stigmatic pollen loads. The simulations do not account for possible pollen removal after each visit or pollen saturation on the stigma.
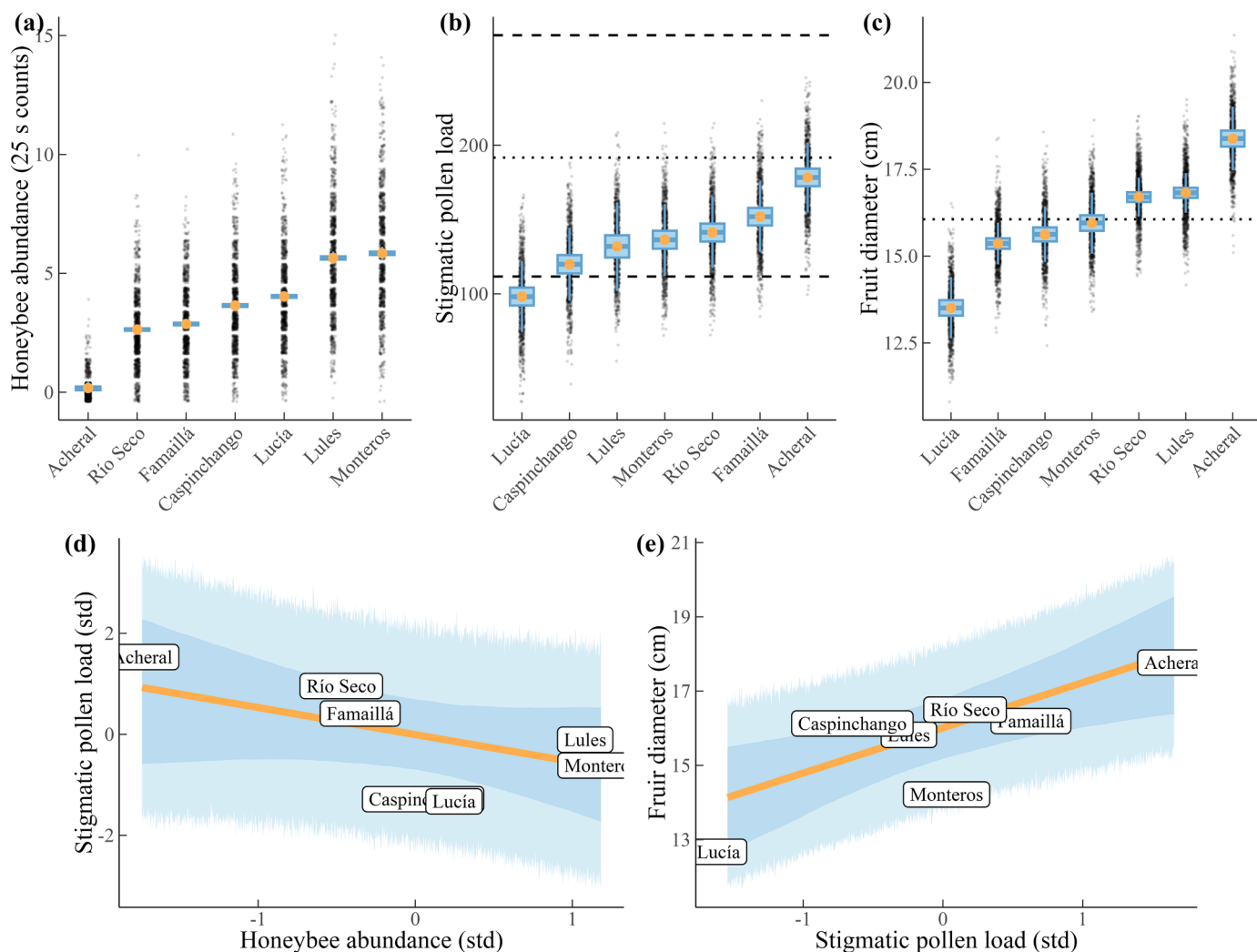
Fig. 3. Models comparing in seven blueberry farms: (a) honeybee abundance, (b) stigmatic pollen loads, (c) fruit size produced and their causal relationships (d and e). In a to c, the boxplots show the range of the posterior distribution of each estimated parameter per farm and its mean (yellow dots); background black dotes indicate random predicted values given the posterior distribution (black dots are jittered for visualization). In b, the horizontal dashed and dotted lines show the optimal pollination range. In c, the horizontal dotted line indicates the global average of fruit size (16.05 mm). In d and e the farm labels denote the X and Y values for each farm; the inner light blue band shows 95% credibility intervals of the regression coefficient (i.e. the yellow trend line), and the outer pale band indicates the simulated 95% credibility interval of the prediction.

stigmatic pollen loads. Also, we show that farms with the lower honeybee abundance tend to have a higher probability of receiving optimal pollen load and can produce fruits that are up to 36% larger than those produced on farms with higher honeybee abundance. In fact, our results indicate that excess honeybee visits can lead to lower pollen deposition and, ultimately, to the production of smaller berries.

This study provides new fine-grained information to precisely define the thresholds for deficient, optimal and supraoptimal pollen deposition for highbush blueberry. For instance, Dogterom et al. (2000) found that individual blueberry flowers reach their maximum fruit size when they receive 125 tetrads, but could not detect further changes beyond this level of pollen deposition. Similarly, our pollination experiment revealed that fruit size increased linearly up to the deposition of 112 tetrads; beyond that limit, an increase in stigmatic pollen load did not result in increased fruit size. However, we demonstrated that the relationship between fruit size and stigmatic pollen deposition is not asymptotic, as excess pollen can have a detrimental effect on berry diameter. In pollination mutualisms an interaction surplus can result in costs to the plants in terms of fitness (Morris et al., 2010). Excess pollen deposition can increase competition between male gametophytes within the style and promote stagnation of growing pollen tubes, leading to a

reduction in ovule fecundity (Harder et al., 2016a, 2016b). This may be occurring in blueberries when stigmatic pollen loads are artificially increased through, for instance, hand pollination experiments. However, we also found that supraoptimal stigmatic pollen loads are highly unlikely to occur under typical field conditions (results not shown in main text, see Appendix A Table S2). That is, on average, the observed probability of a blueberry flower receiving harmful levels of pollen deposition was close to zero. The lower probability of over-pollination occurring through this path is probably due to physiological controls that prevent additional pollen deposition after stigma saturation (Parrie and Lang, 1992). However, our results also show that over-pollination in blueberries can occur in the first stages of the pollination process (i.e., before ovule fecundation), when the pollinator contacts the stigma and deposits the pollen.

We found that six to seven honeybee visits are the minimum needed to ensure optimal pollen deposition in blueberry flowers, which is lower compared to the values reported in other works (~15 honeybee visits, see (Kendall et al., 2020; Devetter et al., 2022). Such disparity probably has two non-mutually exclusive explanations: (i) different pollination dependence of the cultivars used among studies and (ii) differences in the methodological approaches. That is, we simulated the proportion of

flowers that would receive optimal stigmatic pollen deposition after *n* honeybee visits, instead of directly evaluating the effect on fruit size of sequential visits on a single flower (Kendall et al., 2020). Still, our results agree that relatively few honeybee visits are needed to fully pollinate blueberry flowers (Devetter et al., 2022). Achieving the target of six to seven honeybee visits would require a visitation rate of 0.6 —visits per flower in a set of 100 flowers h^{-1}, assuming six hours of daily pollinator activity (Garibaldi et al., 2020) and two days of flower receptivity in blueberries (Ngugi et al., 2002). Previous studies have shown (see Danka et al., 1993) that maximum production gains are only achieved when flowers are exposed to "unlimited" honeybee visits. Using an "unlimited" number of honeybee visits as a basis for pollination service management ignores the risk associated with increasing interaction costs for blueberries. In our study, blueberry flowers on farms with higher honeybee abundance —a proxy of flower visitation rate— were less likely to receive optimal pollen deposition and had a lower probability of producing high quality fruits. This suggests that an excess of honeybee visits may come at a cost to blueberry plants in terms of the removal of previously deposited pollen and, ultimately, reduce reproductive success and fruit quality (i.e. size). Over-pollination leading to reduced yield or production quality has been documented for raspberry (Aizen et al., 2014; Sáez et al., 2014), grapefruits (Chacoff et al., 2008; Morris et al., 2010), apples (Garratt et al., 2021) and probably blueberry (Ramírez-Mejía et al., 2023a). Based on our data, we cannot infer a specific threshold above which additional honeybee visits will be detrimental to blueberry production. Rather, we have found a minimum number of visits that guarantees optimal pollination while reducing pollination deficit and potential over-pollination.

We have assessed the optimal pollination level for one productive metric, the fruit size. Different aspects of crop and fruit production might result in different values and thresholds for optimum pollination. For example, in wild blueberry (*V. angustifolium*), an average pollen deposition of 25 pollen tetrads is sufficient to reach a fruit set of about 70% (Drummond, 2019). Our results also demonstrate that flowers receiving small stigmatic pollen loads can set fruit, meaning that the pollen deposition demand for the quality (i.e., fruit size) and quantity (i.e., fruit set) components of blueberry production are not equal. Such variability should be taken into account when defining pollination targets to achieve specific production goals, avoid over-pollination risks and ensure pollinator effectiveness. We focused on honeybees because they are by far the most prevalent pollinator in blueberry crops in northwestern Argentina –91.4% (Ramírez-Mejía et al., 2023a) and 97.6% of the total visits (Ramírez-Mejía et al., 2023a). In other regions, however, bumblebees (Estravis-Barcala et al., 2021) or dipterans (Cook et al., 2020) may be the main pollen vectors for blueberries. The single-visit pollen deposition rates and the effectiveness of these different species need to be considered for these systems. However, unlike honeybees, whose hives can be easily moved across the landscape, reactive management of wild pollinators is probably not possible and will require long-term habitat management.

Based on our findings, we recommend that prior to the introduction of honeybee hives, blueberry growers establish an evidence-based management plan to optimize pollination services and production outcome. Such a management plan would benefit from a standardized pollinator monitoring protocol (Garibaldi et al., 2020) to estimate the effective number of honeybee visits and assess whether the visitation rate is sufficient to achieve optimal crop pollination (Garibaldi et al., 2020, Vaissière et al., 2011). Where the flowers receive less than six honeybee visits (i.e. flower visitation rate of 0.6 visits per 100 flower h^{-1}) during the maximum receptivity period, the addition of honeybee hives could help to ensure sufficient pollen transfer. Ideally, growers would follow a honeybee precision management protocol designed for blueberry crops (Cavigliasso et al., 2021). Based on our results, such protocols should avoid saturating flowers with honeybees as this could increase the probability of over-pollination and is not cost-effective. These recommendations are not intended as definitive guidelines for crop pollination management. Instead, we aim to provide a first step towards a more systematic and evidence-based approach for honeybees pollination management. A robust framework for farm-level pollination management requires (i) monitoring of hive strength and sanitary status (Geslin et al., 2017), (ii) knowledge of pollen deposition targets, (iii) a deeper understanding of pollination outcomes resulting from the interaction between field-level hive deployment and the landscape context of the farm (Ramírez-Mejia et al., 2023b; Eeraerts et al., 2022; Mallinger et al., 2021), and (iv) an understanding of how the spatial arrangement of beehives interacts with flower density to influence the frequency of pollinator-crop interactions and pollen accumulation in flowers across the farm (Santibañez et al., 2022).

5. Limitations and further steps

Understanding the net contribution of animal pollination to blueberries —and, more broadly, to any crop— implies assessing the multivariate nature of the production (Ramírez-Mejía et al., 2023a, 2023b; Kendall et al., 2020). That is, pollination effects in one productive metric can have underlying trade-offs with others (Ramírez-Mejía et al., 2023b), whereas the magnitude of pollination service benefits are contingent on the cultivar due to pollination dependence variation (Ramírez-Mejía et al., 2023a; Kendall et al., 2020). Therefore, more research is needed to define optimal pollination targets for other important production metrics (e.g. fruit set and nutritional content) and assess their consistency among cultivars. Computational models are a promising alternative for analyzing such dynamic systems (e.g., Santibañez et al., 2022). This technique can allow the incorporation of higher complexity from the honeybee–crop interaction (e.g. Kendall et al., 2022), the role of pollen quality (Parrie and Lang, 1992) and beehive stocking (Cavigliasso et al., 2021), to simulate scenarios where several productive metrics are jointly affected. Such a method would help to estimate the most parsimonious optimum that maximizes the net benefit to crop productivity, which would be the ultimate goal of a widely applicable pollination management protocol.

The need for animal pollination to promote blueberry productivity is broadly recognized (Eeraerts et al., 2023). Still, there is also evidence that the relationship between *production* and *honeybee flower visitation rate* might be negative (Miñarro et al., 2023; Ramírez-Mejía et al., 2023a, 2023b; Mallinger et al., 2021). Our data suggest that the removal of stigmatic pollen loads by extreme honeybee visits could be the underlying mechanism explaining such a pattern. However, we highlight that more research and replication are needed to assess how common over-pollination might occur in blueberries and the real risk for the overall productivity of the crop. Moreover, including pollen tube growth and seed set would improve our understanding of the mechanisms behind over-pollination.

Pollination service management based on optimal targets would not only help avoid undesired pollination scenarios but also offer a more cost-effective strategy for beehive management while reducing potential negative impacts on wild pollinators. That is, considering optimal pollination levels could maximize production gains while saving economic resources by avoiding a surplus beehive stocking. Future research should assess the benefit-cost ratio of implementing such pollination protocols. Moreover, reducing unnecessary honeybee hive densities in agroecosystems can benefit local pollinator communities by limiting the abundance of a dominant competitor and the probability of disease transmission (Mallinger et al., 2017).

6. Conclusions

Honeybees are crucial to ensure production profits of blueberries in many situations, and hive management provides the simplest system to manipulate pollinator density in a responsive manner (Aizen et al., 2009; Reilly et al., 2020). Our study provides new insights into: (i) the mechanisms underlying blueberry pollination and production, (ii) the

contribution of honeybees to blueberry crop pollination and yields, and (iii) the risks and opportunities to diagnose and prevent undesirable pollination outcomes. These findings are crucial to support the development of a protocol for detecting deficient, optimal or supraoptimal pollination levels based on flower visitor monitoring and target values (Garibaldi et al., 2020). The levels of fertilization, irrigation, pesticide or herbicide application are, generally, well established and rigorously implemented as part of regular agronomic management of pollinator-dependent crops. Although pollination is another agricultural input that is crucial for securing crop yields and profits, its management is generally based on much more ambiguous criteria, leading to uncertainties about its outcome and actual benefits. This study challenges the prevailing belief that 'more is better' when it comes to the management of pollination agents in pollinator-dependent crops and advocates for evidence-based management of pollination service to maximize production gains.

CRediT authorship contribution statement

Blendinger Pedro G.: Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization. **Schmucki Reto:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Chacoff Natacha P.:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization. **Ramírez-Mejía Andrés F.:** Writing – original draft, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Woodcock Ben A.:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Lomáscolo Silvia B.:** Writing – review & editing, Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2024.108903.

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