Assessing the effectiveness of honey bee pollinators for cultivated blueberries in South Africa

Keanu Martin[1*], Bruce Anderson[1], Corneile Minnaar[1], ML de Jager[1]

[1]Department of Botany and Zoology, Stellenbosch University, Matieland 7602, Cape Town, Western Cape, South Africa

*Corresponding author: keanum@sun.ac.za

Abstract

Globally, agricultural crops are often extremely dependent on insect pollination. Blueberries are an example of such a crop and owing to their proposed health benefits they are grown around the world, including locations where their native bumble bee pollinators do not occur. In the absence of bumble bees, blueberry pollination in South Africa and many other parts of the non-native, commercial range is performed primarily by honey bees. Despite this, the effectiveness of honey bee pollination on blueberries remains understudied. This study determined the effect of honey bee pollination on the fruit characteristics of five blueberry varieties that are extensively planted in South Africa. For each variety two metrics were calculated: 1) the benefit of bees - a comparison of fruit yields after exposure to honey bees and fruit yields after honey bee exclusion, 2) the pollination deficit - a comparison between yields after hand pollination and yields after exposure to honey bee pollinators. Honey bee pollination consistently resulted in improved yields, although the magnitude of this improvement was dependant on the variety considered. Similarly, the pollination deficit also varied considerably across varieties and while some varieties appeared to perform close to maximum potential, others appeared to perform relatively poorly under honey bee pollination. This study demonstrates that honey bees are functional pollinators of blueberries in areas where native blueberry pollinators are absent. However, in such areas, it is important that special focus be given to selecting blueberry varieties that perform well with honey bees as their sole pollinator. Further research is necessary to determine how the pollination deficit of blueberry varieties can be decreased as well as how to increase the effectiveness of honey bee pollination.

Keywords: Honey bee, blueberries, pollination, agriculture, pollination ecosystem service, yield

1. Introduction

Insect pollination is vital for numerous crops, not only to set fruit but also to increase fruit yield (Klein et al., 2007). Subsequently, this ecosystem service is said to be valued at €153 billion per year (Gallai et al., 2009). Although honey bees are globally the most widely used commercially available pollinator (Garibaldi et al., 2013), they are not always the most efficient pollinators and can be outperformed by wild insects (Garibaldi et al., 2013). In a comprehensive study using 41 crops, Garibaldi et al. (2013) found that wild insects were often more effective crop pollinators than honey bees. On average, fruit set doubled after increasing wild insect visitation compared to equivalent increases in honey bee visitation.

One crop where honey bees are thought to be less effective than other pollinators is blueberries. Wild and commercial blueberries are extremely reliant on insect pollination (Aras et al., 1996; MacKenzie, 1997; Dogterom et al., 2000; Fulton et al., 2015; Martin et al., 2021) and are typically pollinated by large bees. These include blueberry bees (Apidae) (Cane and Payne, 1993; Campbell et al., 2018), bumble bees (Apidae) (Mackenzie and Eickwort, 1996; Stubbs and Drummond, 2001; Javorek et al., 2002; Tuell et al., 2009; Campbell et al., 2017), and mining bees (Andrenidae) (Mackenzie and Eickwort, 1996; Javorek et al., 2002). These bees are thought to be effective pollinators of blueberry flowers due to their ability to buzz pollinate which causes the poricidal anthers to release their pollen (Cane and Payne, 1993). In comparison, honey bees cannot buzz pollinate and are less effective at releasing pollen from these specialised anthers. Consequently, bumble bees deposit about two-thirds more pollen per visit than honey bees (Javorek et al., 2002). However, recently Hoffmann et al. (2018) revealed that honey bees that foraged on blueberries had blueberry pollen on all body parts with the majority of blueberry pollen

being found on their legs and tarsi. These pollen-laden legs and tarsi regularly came into contact with blueberry stigmas during landing, grooming, and foraging and have the ability to effectively pollinate blueberry flowers. Moreover, Martin et al. (2021) showed that honey bees effectively pollinated the common South African blueberry variety, Ventura, significantly increasing both blueberry mass and diameter, as well as decreased ripening time.

The perceived inefficiency of honey bees as pollinators has resulted in pressure to import native blueberry pollinators, such as bumble bees. This is extremely problematic as introducing bumble bees into novel areas has the potential to disrupt local ecosystems. For example, introduced bumble bees can outcompete native pollinators, damage flowers, and rob nectar (Morales et al., 2013; Schmid-Hempel et al., 2014; Aizen et al., 2018). Consequently, it is important to quantify the benefits of honey bees for different blueberry varieties so that objective decisions can be made about the perceived needs of importing novel pollinators. This may also enable us to farm more intelligently with varieties that do better under honey bee pollination.

We aim to determine the effects of honey bee pollination on the fruit characteristics of five blueberry varieties that are extensively planted in South Africa. Particularly, we compare the benefits (in terms of fruit yield i.e. fruit mass, fruit diameter, percentage fruit set, and adjusted fruit mass) of honey bee pollination with situations when pollinators are absent. Although honey bees are often viewed as ineffective pollinators of blueberries, they still move pollen between flowers and often increase blueberry fruit production (Aras et al., 1996b; Mackenzie, 1997; Sampson and Cane, 2000; Hoffman et al., 2018). Accordingly, we hypothesize that honey bees considerably increase fruit yield (i.e. fruit mass, diameter,

percentage fruit set, adjusted fruit mass) and decrease fruit developmental period, compared to fruit produced in the absence of pollinators. Additionally, we establish if the pollination environment could be optimised so that fruit yield is improved relative to the present conditions where honey bees are the sole pollinators. Despite honey bees moving pollen between blueberry flowers (Hoffman et al., 2018), we predict considerable room for improvement, with hand pollination resulting in substantially larger fruit yields than honey bee pollination.

2. Methods

An experimental plot on Backsberg blueberry farm was used as the study site (Western Cape, South Africa, 33°48'30.7"S 18°54'09.8"E). Farmlands surround the experimental plot and there is also a nearby (2.98km) nature reserve consisting of Swartland shale renosterveld and fynbos. Hives were stocked at 15 hives per hectare which correlates to typical commercial blueberry farm hive density (Isaacs and Kirk, 2010; Gibbs et al., 2016; Hoffman et al., 2018). Hive densities on blueberries are often an order of magnitude greater than on crops such as apricots, peaches, and nectarines where one honey bee hive per hectare provides sufficient pollination (Allsopp et al., 2008). This experiment comprised of three treatments: pollinators excluded, open honey bee pollination, and hand pollination. We determined the effects of honey bee pollination on blueberry production and also whether there was scope to improve pollination conditions by comparing fruit yield (fruit mass, fruit set, and developmental period) among these three treatments. Varieties used were Emerald (*Vaccinium corymbosum* hybrid), Eureka (*Vaccinium corymbosum* hybrid), Snowchaser (*Vaccinium sp. hybrid*), Suziblue (*Vaccinium corymbosum* hybrid), and Twilight (*Vaccinium corymbosum* hybrid). Pollination combinations were repeated three times per plant and across nine plants, resulting in 27 pollination combination replicates. Moreover, we minimized any extrinsic factors which potentially influence fruit development by conducting our experiments across varieties in an experimental plot of only 50 m X 115 m.

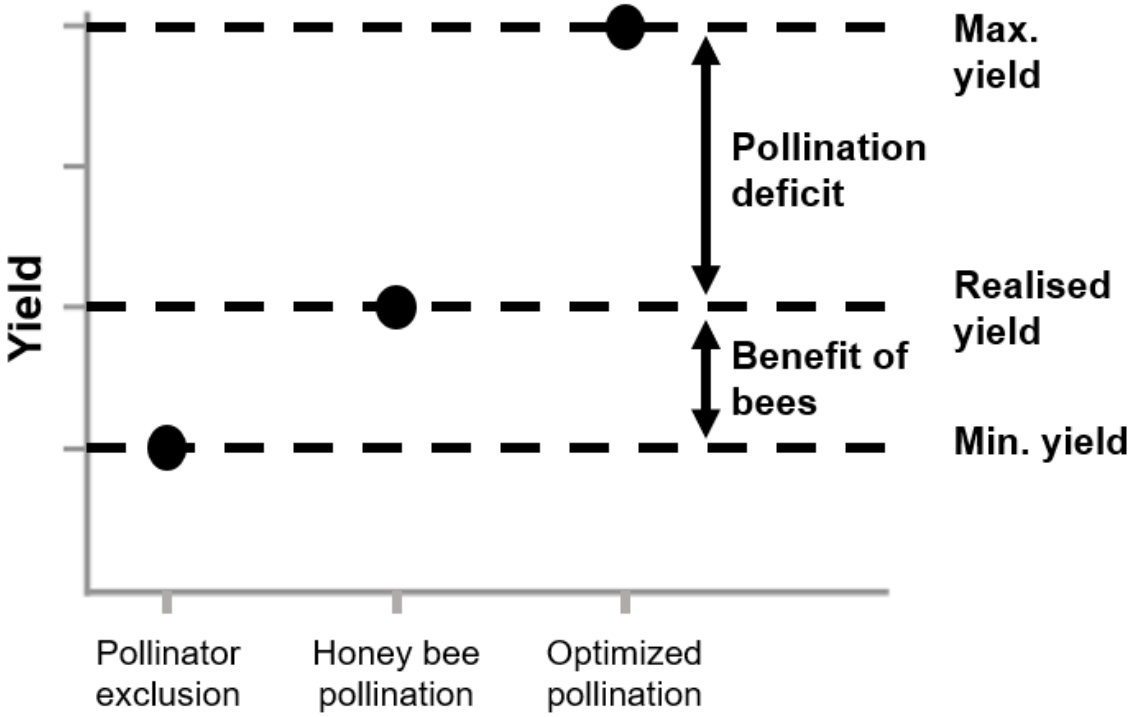

Fig 1: Hypothetical figure showing how the use of three treatments can be used to determine the benefits of honey bee pollination as well as how yields may potentially be improved upon (originally produced by Martin et al. 2021).

To determine pollination deficits and benefits of bees to the five varieties, we followed the methods of Martin et al. (2021). Below we provide a brief description.

We determined the benefit of bees by comparing blueberry fruit production as a result of honey bee pollination to blueberry fruit production in the absence of honey bees (pollinator exclusion. Pollinators were denied access to flowers by covering 27 individual virgin flowers with a fine mesh bag. Once flowers had wilted, these bags were removed to allow for normal fruit maturation. We estimated the fruit yield through honey bee pollination by exposing (unbagging) 27 flowers to bee pollinators. For the duration of the time spent at the

experimental plot (approximately 280 hours), honey bees were the only pollinator seen visiting blueberry flowers.

We calculated the pollination deficit for each variety by comparing the honey bee treatment to the optimised hand pollination treatment. Hand pollination is thought to result in the best fruit quality as it enhances both pollen deposition and quality of pollen. Results from Martin et al. (2019) were used to establish the most compatible pollen donor for each variety, and this was used to pollinate flowers. We then followed hand pollination protocols as set out by Martin et al. (2021).

2.3 Measurements of fruit quality

Following pollination treatments, fruit development was examined weekly to determine whether fruit were ready to be picked. Fruit were considered ready for picking when the entire berry had transformed to a uniform dark blue. Fruit were then harvested and the weight, diameter, and developmental period (weeks from pollination to harvesting) were determined on the harvest day. We also calculated the percentage fruit set per treatment. Fruit mass and percentage fruit set were combined to calculate the adjusted fruit mass for each treatment as follows:

$$Adjusted\ fruit\ mass = \text{Proportion fruit set X mean fruit mass} \qquad (\textit{Equation 1})$$

2.4 Statistical analyses

Linear mixed-effects models were used with fruit mass, developmental period, and adjusted fruit mass as functions of pollination treatment (fixed effect). To account for variation between individual plants, plant ID was used as a random effect. This increased the sensitivity of the model in identifying differences in fruit yield measures as a consequence of treatment alone. Linear contrasts (Tukey) were used to detect treatment differences.

Nakagawa R^2 values (Nakagawa and Schielzeth, 2013) were used to assess model fit, providing conditional variance (R^2c) and marginal variance (R^2m) estimates for linear mixed-effects models, which can be equated to traditional R^2 values. The conditional R^2 values (R^2c) explain the variance of the entire model (fixed effects and random effects), whereas marginal R^2 values (R^2m) explain the variance of the fixed effects alone.

All statistical analyses were conducted in R (version 3.3.2) (R Core Team, 2017) using the packages nlme (Pinheiro et al., 2017), multcomp (Hothorn et al., 2008), ggplot2 (Wickham, 2009), sjPlot (Lüdecke, 2017), car (Fox and Weisberg, 2011), lme4 (Bates et al., 2015), grid (R Core Team, 2017), gridExtra (Auguie, 2017), lattice (Deepayan, 2008), MuMIn (Barton, 2017), plyr (Wickham, 2011), and plotrix (Lemon, 2006).

3. Results

3.1 Emerald

3.1.1 Benefit of bees

We found strong statistical support indicating that honey bees increased blueberry production in Emerald compared to fruits produced in the absence of pollinators for three-quarters of the metrics measured. We provide further details on the four metrics below.

The conditional variance for the developmental period model was R^2c=0.471 and the marginal variance was R^2m=0.062. This shows that variation was mostly explained by the random effect (plant ID). The presence of honey bee pollinators appeared to decrease (statistically borderline) the developmental period of blueberry fruits by about 2 weeks (z=-2.085, p=0.089, Fig 2) compared to when they were absent.

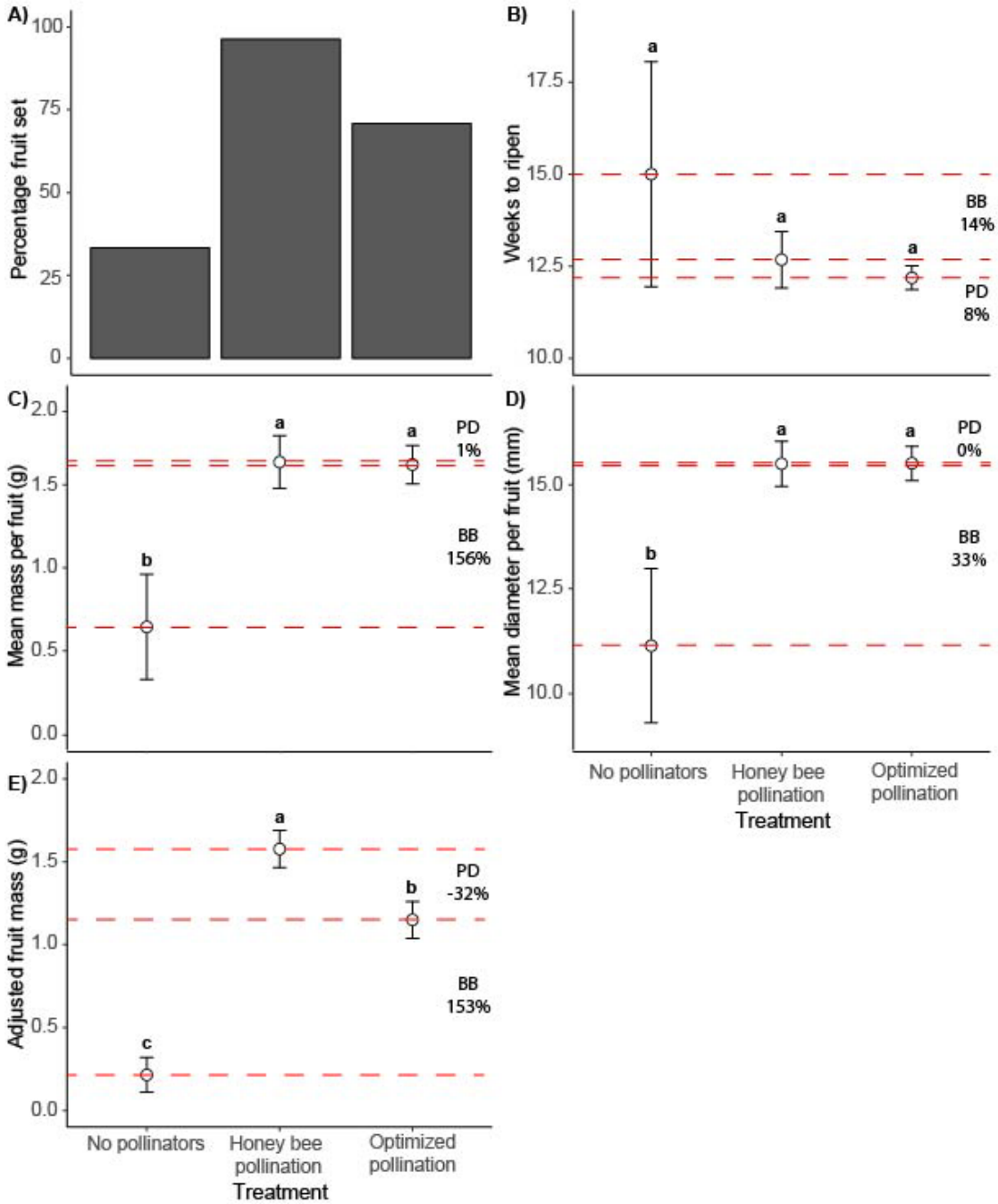

Fig 2: Pollination deficit and the benefit of bees for Emerald fruit. A) Percentage fruit set. B) Developmental period. C) Mean fruit mass. D) Mean fruit diameter. E) Adjusted fruit mass. Significance ($p<0.05$) of linear contrasts (Tukey HSD) are indicated by letters. Error bars indicate standard error. BB = benefit of bees, the difference (in percentage) between fruit produced in the absence of pollinators compared (n=27) to fruits produced by honey bees (n=27). PD = pollination deficit, the difference (in percentage) between fruits produced after honey bee exposure compared to fruits produced after hand pollination (n=27).

172 The conditional variance for the Emerald fruit mass model was R^2c=0.238 while the
173 marginal variance was R^2m=0.151. The conditional variance for the fruit diameter model
174 was R^2c=0.303 and the marginal variance was R^2m=0.210. This suggests that for both fruit
175 mass and diameter, variation is primarily explained by treatment. Fruit mass was
176 significantly increased by the presence of honey bees (mean ± SE fruit mass = 1.62g ± 0.12g,
177 z=2.799, p=0.013), resulting in a 156% mass increase compared to fruit produced in the
178 absence of pollinators (mean ± SE fruit mass = 0.64g ± 0.32g, Fig 2C). Likewise, honey bees
179 increased the fruit diameter by 4mm (33%) per fruit (mean ± SE = 15.51mm ± 0.41mm)
180 compared to treatments produced in the absence of pollinators (mean ± SE = 11.12mm ±
181 1.86mm, Fig 2D, z=3.412, p=0.002). There were significant differences in fruit set following
182 the three treatments (*Chi-square*=25.959, df=2, p<0.001, Fig 2A). Honey bees increased fruit
183 set by 63% relative to bagged plants. Similarly, honey bee pollination outperformed the
184 hand pollination treatment by 25%.

185 The conditional variance for the adjusted Emerald fruit mass model was R^2c=0.478
186 and marginal variance was R^2m=0.347. This suggests that adjusted fruit mass variation was
187 primarily explained by treatment. Honey bees led to a significant increase of adjusted fruit
188 mass (mean ± SE = 1.58g ± 0.11g, Fig 2E) of 153% compared to bagged treatments (mean ±
189 SE = 0.21g ± 0.11g, Fig 2E, z=4.639, p<0.001).

190 ***3.1.2 Pollination deficit***

191 The pollination deficit metric indicated that for Emerald, honey bee pollinators performed
192 equally or better than hand pollinations. Below we present those results.

193 Hand pollination resulted in a one week decrease in the developmental period of
194 blueberry fruit, however, this was not significant (z=-0.073, p=0.997), from 13 ± 0.32 weeks

195 (mean ± SE) when open to honey bee pollination to 12 ± 0.76 (mean ± SE) when hand-
196 pollinated (Fig 2B). Mass of fruit produced from hand pollination (1.62g ± 0.16g, mean ± SE),
197 and fruit produced from honey bee pollination (1.64g ± 0.12g, mean ± SE, Fig 2C) did not
198 differ significantly (z=-0.110, p=0.993). The diameter of hand-pollinated fruit (mean ± SE =
199 15.52mm ± 0.54mm) and fruit pollinated by honey bees (mean ± SE = 15.51mm ± 0.41mm),
200 also did not differ significantly (z=-0.026, p=0.999). Honey bees outperformed hand
201 pollination with regards to fruit set by 25%.

202 The adjusted fruit mass was decreased significantly (z=-2.471, p=0.034) by 32% when
203 hand-pollinated (mean ± SE = 1.15g ± 0.11g, Fig 2E) in comparison to fruit produced from
204 honey bee pollination (mean ± SE = 1.58g ± 0.11g, Fig 2E).

205

206 **3.2 Eureka**

207 ***3.2.1 Benefit of bees***

208 For all four metrics measured we found no statistical evidence suggesting that honey bees
209 improved Eureka fruit quality compared to fruits produced in the absence of pollinators. We
210 provide further details on each metric below.

211

212 For the developmental period model, the conditional variance was R^2c=0.405 and
213 the marginal variance was R^2m=0.370. The variation is therefore explained most by the fixed
214 effect (treatment). Honey bees did not change the developmental period substantially (z=-
215 0.042, p=0.999, Fig 3B).

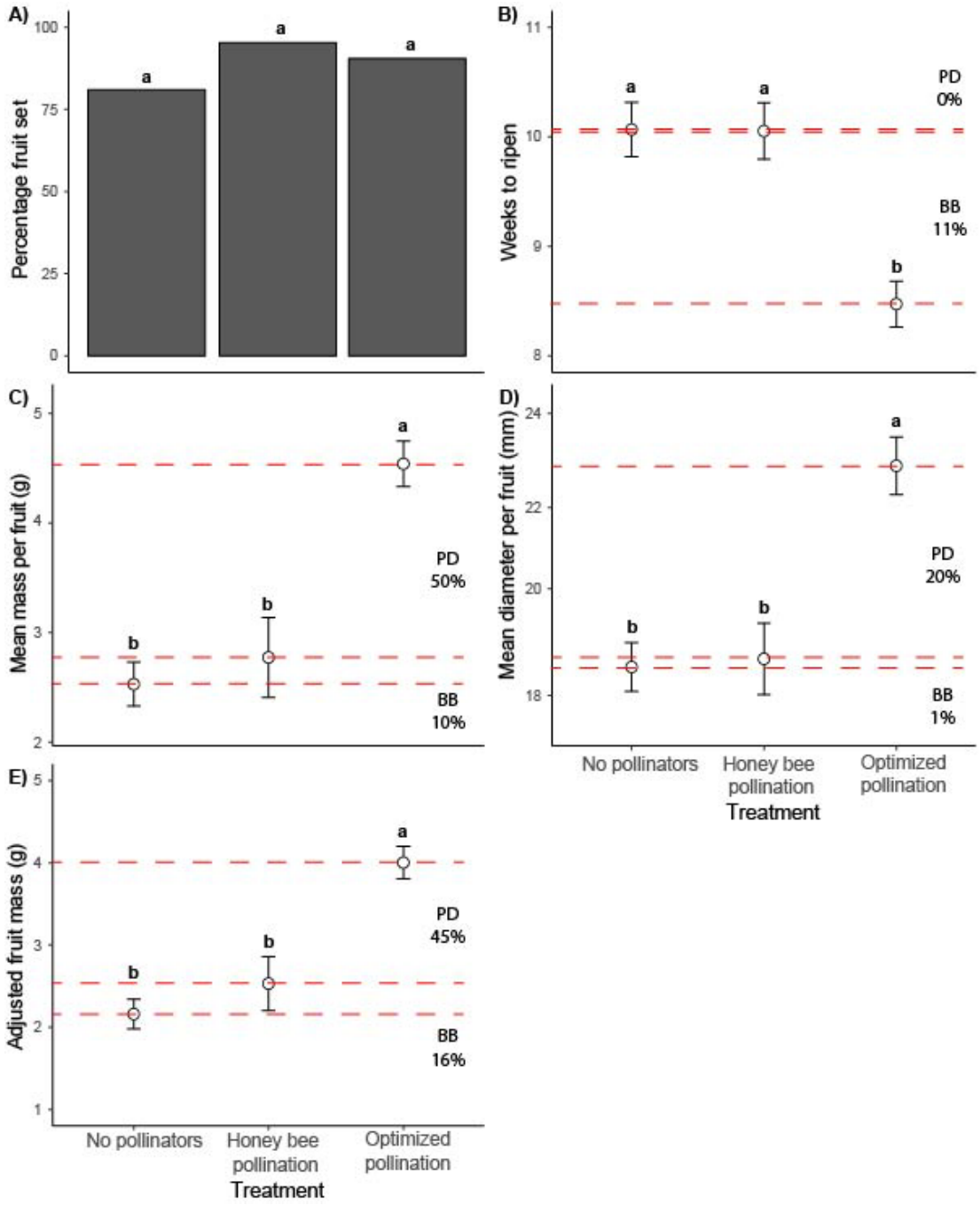

Fig 3: Pollination deficit and the benefit of bees for Eureka fruit. A) Percentage fruit set. B) Developmental period. C) Mean fruit mass. D) Mean fruit diameter. E) Adjusted fruit mass. Significance ($p<0.05$) of linear contrasts (Tukey HSD) are indicated by letters. Error bars indicate standard error. BB = benefit of bees, the difference (in percentage) between fruit produced in the absence of pollinators compared (n=27) to fruits produced by honey bees (n=27). PD = pollination deficit, the difference (in percentage) between fruits produced after honey bee exposure compared to fruits produced after hand pollination (n=27).

223 The fruit mass model resulted in a conditional variance of R^2c=0.693 and a marginal
224 variance of R^2m=0.398. The conditional variance was R^2c=0.569 for the fruit diameter
225 model, while the marginal variance was R^2m=0.371. This indicated that variance is primarily
226 explained by treatment for fruit mass and diameter. Honey bee presence did not increase
227 fruit mass (mean ± SE fruit mass = 2.66g ± 0.21g) significantly (z=0.815, p=0.684), compared
228 to when pollinators were absent (mean ± SE fruit mass = 2.41g ± 0.20g, Fig 3C). Likewise,
229 honey bees did not significantly increase (z=0.232, p=0.970) fruit diameter (mean ± SE =
230 18.50mm ± 0.61mm) compared to when they were absent (mean ± SE = 18.33mm ±
231 0.52mm, Fig 3D,). Honey bee pollination appeared to increase fruit set by 14%, however the
232 differences between the three treatments were not significantly different (*Chi-*
233 *square*=2.253, *df*=2, p=0.324, Fig 3A).

234 The conditional variance for Eureka adjusted fruit mass was R^2c=0.678 and the
235 marginal variance was R^2m=0.383. This indicated that for the adjusted fruit mass model,
236 most of the variance was explained by pollination treatment. The adjusted fruit mass of
237 honey bee pollination (mean ± SE = 2.53g ± 0.20g, Fig 3E), did not differ significantly
238 (z=1.334, p=0.363) from the adjusted fruit mass of fruit produced in the absence of honey
239 bees (mean ± SE = 2.16g ± 0.18g, Fig 3E).

240

241 ***3.2.2 Pollination deficit***

242 For all pollination deficit metrics measured for Eureka, hand pollinations outperformed
243 honey bee pollination. We provide further details on each metric below.

244 The developmental period of fruit produced by hand pollination was significantly
245 decreased by approximately two weeks (z=-4.521, p<0.001), from 10 ± 0.26 weeks (mean ±

246 SE) when honey bee pollinated to 8 ± 0.21 weeks (mean ± SE) when hand-pollinated (Fig
247 3B). Hand pollination significantly increased (z=3.239, p=0.003) the mass of fruit by 50%
248 (4.42g ± 0.36g, mean ± SE), compared to the fruit produced by flowers exposed to honey
249 bees (2.66g ± 0.21g, mean ± SE, Fig 3C). Furthermore, hand pollination also significantly
250 increased (z=3.657, p<0.001) fruit diameter (mean ± SE = 22.61mm ± 0.76mm) by 20% in
251 comparison to honey bee pollinated flowers (mean ± SE = 18.50mm ± 0.61mm, Fig 3D).
252 Hand pollination did not increase fruit set compared to honey bee pollination and in fact led
253 to a 4% decrease in fruit set.

254 The adjusted fruit mass increased significantly (z=3.008, p<0.007) by 45% after being
255 hand-pollinated (mean ± SE = 4.00g ± 0.33g, Fig 2E), compared to the adjusted fruit mass
256 after honey bee exposure (mean ± SE = 2.53g ± 0.20g, Fig 3E).

257

258 **3.3 Snowchaser**

259 ***3.3.1 Benefit of bees***

260 For all four metrics, access by honey bees substantially improved Snowchaser fruit
261 production compared to when they were excluded. We provide further details on each
262 metric below.

263 The conditional variance of the Snowchaser developmental period model was
264 R^2c=0.482, while the marginal variance was R^2m=0.460, suggesting that the variance in the
265 developmental period was explained primarily by treatment (fixed effect). Honey bee
266 pollinators significantly shortened the developmental period of blueberry fruits (mean ± SE
267 fruit weeks = 12 weeks ± 0.20 weeks, z=-3.533, p=0.001, Fig 4B) by approximately one week
268 compared to fruits produced in the absence of honey bees (mean ± SE fruit weeks = 13
269 weeks ± 0.80 weeks, Fig 4B).

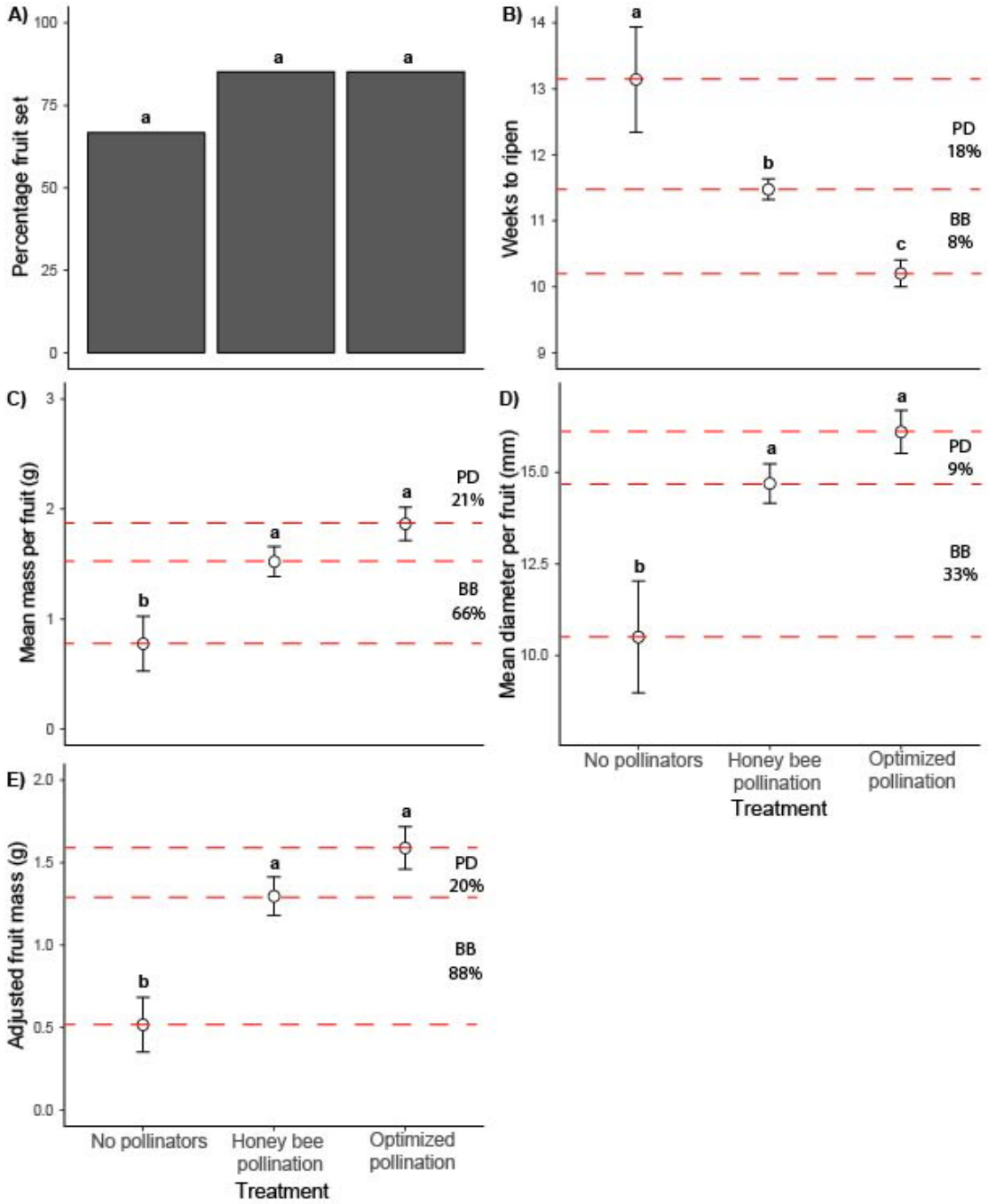

Fig 4: Pollination deficit and the benefit of bees for Snowchaser fruit. A) Percentage fruit set. B) Developmental period. C) Mean fruit mass. D) Mean fruit diameter. E) Adjusted fruit mass. Significance ($p<0.05$) of linear contrasts (Tukey HSD) are indicated by letters. Error bars indicate standard error. BB = benefit of bees, the difference (in percentage) between fruit produced in the absence of pollinators compared (n=27) to fruits produced by honey bees (n=27). PD = pollination deficit, the difference (in percentage) between fruits produced after honey bee exposure compared to fruits produced after hand pollination (n=27).

The conditional variance of the fruit mass model was R^2c=0.520 and the marginal variance was R^2m=0.203. Similarly, the conditional variance of the fruit diameter model was R^2c=0.469, while the marginal variance was R^2m=0.284. This suggests that variance in Snowchaser fruit mass and diameter was primarily explained by treatment. Here, honey bee presence significantly increased fruit mass (mean ± SE fruit mass = 1.52g ± 0.15g, z=2.362, p=0.047), by 66% compared to treatments where honey bees were excluded (mean ± SE fruit mass = 0.77g ± 0.25g, Fig 4C). Likewise, honey bee exposure resulted in a 4mm (33%) mean increase in fruit diameter per fruit (mean ± SE = 14.70mm ± 0.59mm) in comparison to fruit produced in the absence of honey bees (mean ± SE = 10.51mm ± 0.81mm, Fig 4D, z=3.229, p=0.003). Honey bee pollination appeared to increase fruit set by 18%, however the differences between the three treatments were not significantly different (*Chi-square*=3.559, df=2, p=0.169, Fig 4A).

The adjusted fruit mass model resulted in a conditional variance of R^2c=0.576 and a marginal variance of R^2m=0.273, suggesting that most of the variance in adjusted fruit mass is explained by treatment. Honey bee pollination significantly (z=3.062, p=0.006) increased adjusted fruit mass (mean ± SE = 1.30g ± 0.13g, Fig 4E), by 86% compared to the adjusted fruit mass of treatments where pollinators were excluded (mean ± SE = 0.52g ± 0.17g, Fig 4E).

3.3.2 Pollination deficit

Statistics indicated that honey bee pollinators and hand pollinations performed similarly for Snowchaser in three of the four metrics measured. We provide further details below.

299 Hand pollination resulted in a two week or 12% decrease in the developmental
300 period (z=-3.788, p<0.001), from 12 ± 0.20 weeks (mean ± SE) after honey bee exposure to
301 10 ± 0.16 weeks (mean ± SE) after hand pollination (Fig 4B). Hand-pollinated fruits had
302 similar fruit mass (mean ± SE fruit mass = 1.87g ± 0.14g, z=1.469, p=0.303) to fruit produced
303 after exposure to honey bees (1.52g ± 0.15g, mean ± SE, Fig 4C). Hand pollination slightly,
304 but not significantly (z=1.425, p=0.325) increased fruit diameter (mean ± SE = 16.10mm ±
305 0.54mm) by 9% in comparison to flowers exposed to honey bees (mean ± SE = 14.70mm ±
306 0.59mm, Fig 4D). Hand pollination did not lead to an increase in fruit set compared to honey
307 bee pollination.

308 The adjusted fruit mass appeared to increase by 20% for hand-pollinated flowers
309 (mean ± SE = 1.59g ± 0.12g, Fig 2E) compared to the adjusted fruit mass after honey bee
310 exposure (mean ± SE = 1.30g ± 0.13g, Fig 4E), however, this difference was not statistically
311 significant (z=1.486, p=0.295).

312

3.4 Suziblue

3.4.1 Benefit of bees

We found evidence suggesting that for Suziblue, honey bees do not improve blueberry fruit quality compared to when pollinators were excluded. We provide further details on each metric below.

Conditional variance for the developmental period model was R^2c=0.235 and the marginal variance was R^2m=0.235, suggesting that the variation was mostly explained by the fixed effect (treatment). Exposure to honey bees did not significantly affect the developmental period of Suziblue fruits (z=-0.957, p=0.604, Fig 5B).

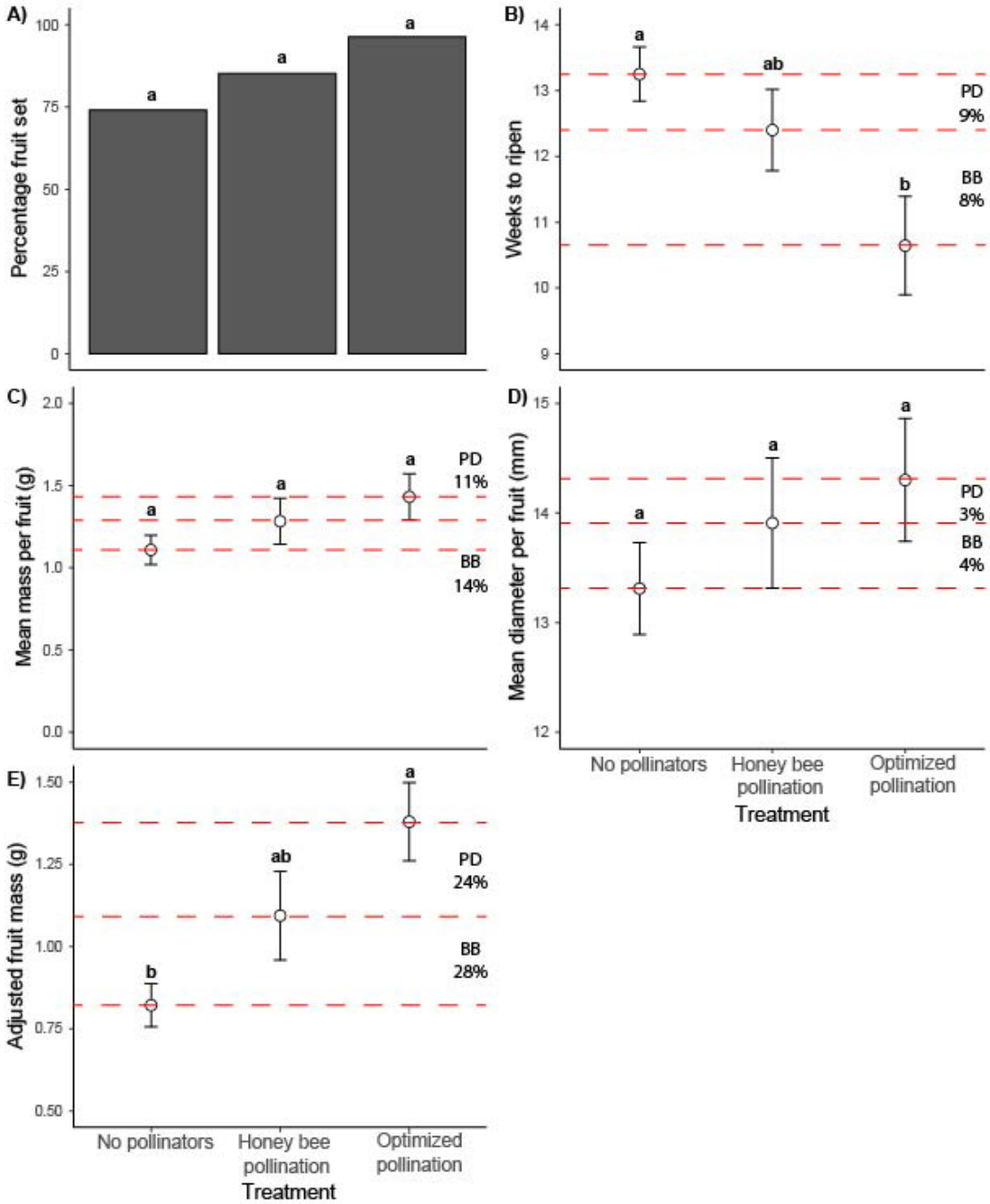

Fig 5: Pollination deficit and the benefit of bees for Suziblue fruit. A) Percentage fruit set. B) Developmental period. C) Mean fruit mass. D) Mean fruit diameter. E) Adjusted fruit mass. Significance ($p<0.05$) of linear contrasts (Tukey HSD) are indicated by letters. Error bars indicate standard error. BB = benefit of bees, the difference (in percentage) between fruit produced in the absence of pollinators compared (n=27) to fruits produced by honey bees (n=27). PD = pollination deficit, the difference (in percentage) between fruits produced after honey bee exposure compared to fruits produced after hand pollination (n=27).

329 The conditional variance of the fruit mass model was R^2c=0.645 and the marginal
330 variance was R^2m=0.144. For the fruit diameter model, the conditional variance was
331 R^2c=0.492 and the marginal variance was R^2m=0.088. Plant ID explained most of the
332 variance for fruit mass and diameter. Honey bee pollination had a minimal effect on fruit
333 mass (mean ± SE fruit mass = 1.28g ± 0.14g, z=1.137, p=0.485), in comparison to bagged
334 treatments (mean ± SE fruit mass = 1.11g ± 0.09g, Fig 5C). Honey bees also had a minimal
335 effect on fruit diameter (mean ± SE = 13.91mm ± 0.56mm) in comparison to bagged
336 treatments (mean ± SE = 13.31mm ± 0.42mm, Fig 5D, z=1.018, p=0.562). Significant
337 differences were found in fruit set following the three treatments (*Chi-square*=6.003, *df*=2,
338 p=0.049, Fig 5A). Honey bees increased fruit set by 11% relative to bagged plants, however,
339 this still left a pollination deficit of 11% (honey bee treatment compared to hand
340 pollination).

341 The adjusted fruit mass model resulted in a conditional variance of R^2c=0.757 and a
342 marginal variance of R^2m=0.330. The variance in the adjusted fruit mass model was
343 explained mostly by treatment (fixed effect). Flowers open to honey bee pollination resulted
344 in similar adjusted fruit masses (mean ± SE fruit mass = 1.09g ± 0.12g, z=1.816, p=0.160),
345 compared to honey bee exclusion treatments (mean ± SE = 0.82g ± 0.07g, Fig 5E).

346

347 ***3.4.2 Pollination deficit***

348 Below, we provide details showing that for all metrics measured, hand pollinations of
349 Suziblue flowers did not outperform honey bee pollination.

350 The developmental period of hand-pollinated fruit was similar (mean ± SE weeks =
351 12 ± 0.62 weeks, z=-2.047, p=0.101) to that of fruit produced from honey bee pollination (13

± 0.75, mean ± SE, Fig 5B). The fruit mass of hand-pollinated fruits was also similar (mean ± SE fruit mass = 1.43g ± 0.14g, z=0.786, p=0.707), to fruit produced from honey bee pollinated flowers (1.28g ± 0.14g, mean ± SE, Fig 5C). Hand pollination did not increase (z=0.579, p=0.830) fruit diameter (mean ± SE = 14.30mm ± 0.59mm) in comparison to the diameter of fruit produced by honey bee pollination (mean ± SE = 13.91mm ± 0.71mm, Fig 5D).

Adjusted fruit mass was similar and not significantly different (z=1.48, p=0.290) for hand-pollinated flowers (mean ± SE = 1.38g ± 0.14g, Fig 5E) and for flowers exposed to honey bees (mean ± SE = 1.09g ± 0.12g, Fig 5E).

362 **3.5 Twilight**

363 ***3.5.1 Benefit of bees***

364 For three out of the four metrics measures, honey bee presence enhanced blueberry
365 production compared to when pollinators were excluded. We provide further details on
366 each metric below.

367 The developmental period model had a conditional variance of R^2c=0.360 and a
368 marginal variance of R^2m=0.320. The fixed effect (treatment) explained most of the
369 variation. Honey bee pollination decreased the developmental period (mean ± SE weeks =
370 11 weeks ± 0.43 weeks, Fig 6B) of blueberry fruits by two weeks or 17% compared to fruits
371 produced in the absence of honey bees (mean ± SE fruit weeks = 11 weeks ± 0.49 weeks, Fig
372 2C). This was borderline-significant (z=-2.170, p=0.076, Fig 6B).

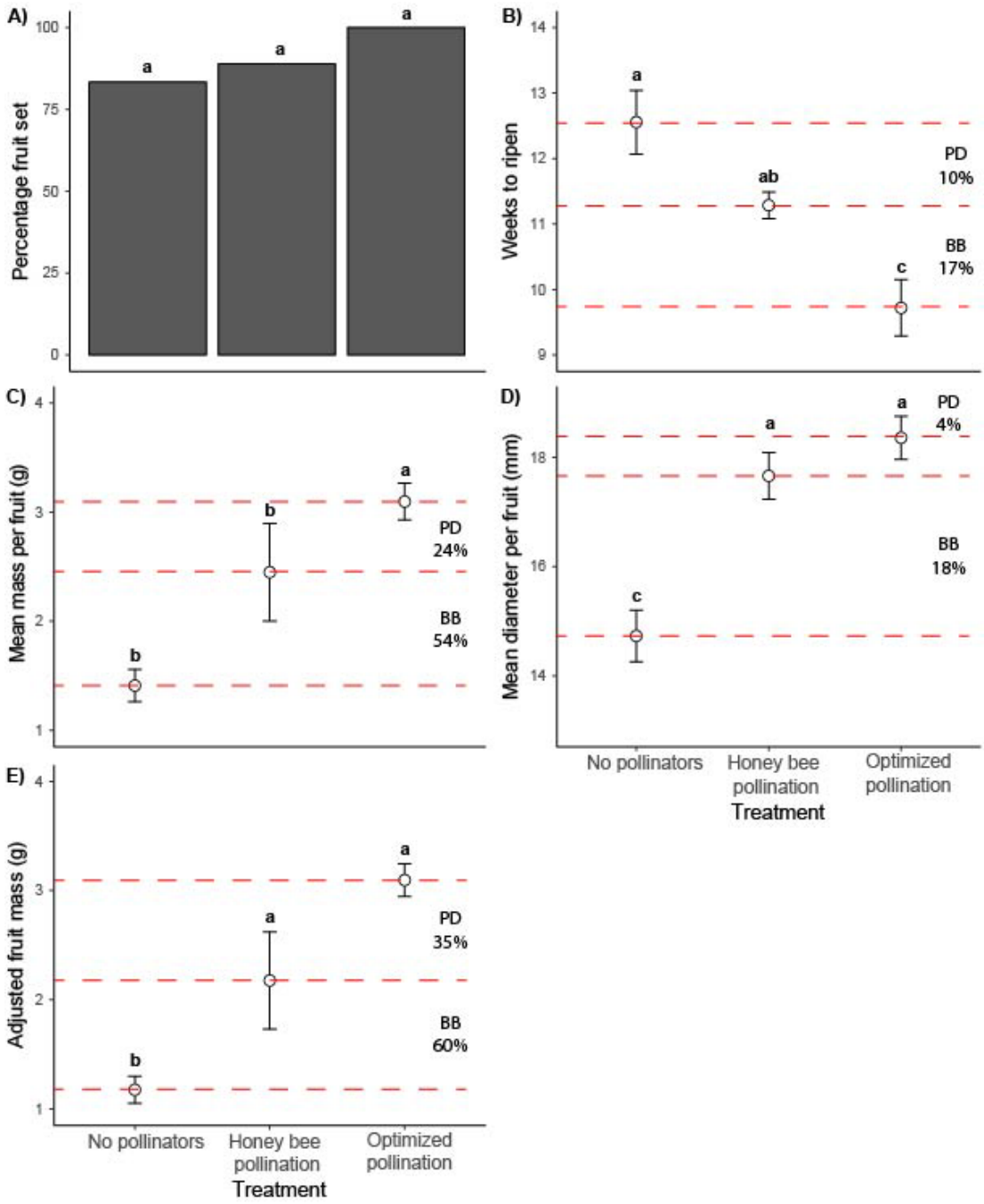

Fig 6: Pollination deficit and benefit of bees for Twilight fruit. A) Percentage fruit set. B) Developmental period. C) Mean fruit mass. D) Mean fruit diameter. E) Adjusted fruit mass. Significance ($p<0.05$) of linear contrasts (Tukey HSD) are indicated by letters. Error bars indicate standard error. BB = benefit of bees, the difference (in percentage) between fruit produced in the absence of pollinators compared (n=27) to fruits produced by honey bees (n=27). PD = pollination deficit, the difference (in percentage) between fruits produced after honey bee exposure compared to fruits produced after hand pollination (n=27).

380 The conditional variance for the Twilight fruit mass model was R^2c=0.337 and the
381 marginal variance was R^2m=0.170. For the fruit diameter model, the conditional variance
382 was R^2c=0.392, while the marginal variance was R^2m=0.369. This suggests that for both fruit
383 mass and diameter, the majority of the variance was explained by treatment. Fruit mass was
384 significantly increased after honey bee exposure (mean ± SE fruit mass = 2.45g ± 0.17g,
385 z=2.397, p=0.043), resulting in a 54% increase compared to bagged treatments (mean ± SE
386 fruit mass = 1.41g ± 0.17g, Fig 6C). Honey bee exposure similarly increased the mean fruit
387 diameter by 3mm (18%) per fruit (mean ± SE = 17.66mm ± 0.39mm) in comparison to
388 bagged treatments (mean ± SE = 14.73mm ± 0.47mm, Fig 6D, z=4.643, p<0.001). There were
389 significant differences in fruit set following the three treatments (*Chi-square*=13.176, *df*=2,
390 p=0.001, Fig 6A). Honey bees increased fruit set by 6% relative to bagged plants, however,
391 this still left a pollination deficit of 11% (honey bee treatment compared to hand
392 pollination).

393 The adjusted fruit mass model resulted in a conditional variance of R^2c=0.383 with
394 the marginal variance of R^2m=0.216 suggesting that fruit mass variance was primarily
395 explained by treatment. Honey bee presence significantly (z=2.368, p=0.046) increased
396 adjusted fruit mass (mean ± SE = 2.18g ± 0.15g, Fig 6E), resulting in a 60% increase
397 compared to pollinator exclusion treatments (mean ± SE = 1.18g ± 0.12g, Fig 6E).

398

399 ***3.5.2 Pollination deficit***

400 Hand pollinations resulted in improved Twilight fruit quality compared to honey bee
401 pollinations. However, these were only statistically supported for developmental period.
402 Below, we explain the details relating to each of the metrics measured.

403 The developmental period of blueberry fruit was significantly decreased by approximately
404 one week or 10% (z=-2.824, p=0.0132), from 11 ± 0.43 weeks (mean ± SE) when flowers
405 were open to honey bee pollination to 10 ± 0.20 (mean ± SE) when flowers were pollinated
406 by hand (Fig 6B). Hand-pollinated fruits appeared to be about 24% heavier (3.11g ± 0.45g,
407 mean ± SE), than fruit produced from flowers exposed to honey bees (2.45g ± 0.17g, mean ±
408 SE, Fig 6C), however, this relationship was non-significant (z=1.134, p=0.493). Hand
409 pollination did not significantly increase (z=1.134, p=0.493) the fruit diameter (mean ± SE =
410 18.36mm ± 0.43mm) when compared to fruit produced by honey bee pollinated flowers
411 (mean ± SE = 17.66mm ± 0.39mm, Fig 6D).

412 Hand pollination appeared to improve the adjusted fruit mass by 35% (mean ± SE =
413 3.10g ± 0.45g, Fig 2E) when compared to flowers exposed to honey bees (mean ± SE = 2.18g
414 ± 0.15g, Fig 2E), although this difference was not statistically significant (z=1.694, p=0.205).

415

4. Discussion

We provide a comprehensive comparison of honey bee effectiveness as pollinators of common commercial blueberry varieties in South Africa. Evidence suggests that the fruit quality of these southern highbush blueberry varieties are considerably affected by the pollination environment. Overall, we show that the absence of pollinators in highbush blueberries typically leads to reductions in fruit yield. This provides important information to blueberry growers as it shows that honey bees are usually extremely beneficial and can act as reliable blueberry pollinators, even in areas without access to native blueberry pollinators. Importantly, the study also highlights differences in the effectiveness of honey bees across different blueberry varieties. Finally, it also suggests that pollination conditions could be improved for certain varieties which appear not to be producing at their full production potential under honey bee pollination.

For comparative purposes, we have highlighted adjusted fruit mass as a summary table metric (Table 1) as it incorporates arguably the two most important yield metrics (fruit mass and fruit set), with fruit diameter being indirectly incorporated as it is highly correlated with fruit mass (Jorquera-fontena et al., 2017).

Table 1: Summary table comparing the benefit of bees and the pollination deficit for adjusted fruit mass in the five blueberry varieties from this study and Ventura, which was published by Martin et al. (2021). Positive benefit of bees imply that the addition of honey bees increases fruit yields compared to when pollinators are absent. Positive pollination deficit indicates that plants pollinated by honey bees produce lower yields than plants pollinated by hand. Statistical significance ($p < 0.05$) is indicated by bold fonts, while colour differences (red vs black) suggest differences in trend direction.

Variety	Benefit of bees	Pollination deficit
Emerald	**153%**	**-32%**
Eureka	16%	**45%**
Snowchaser	**86%**	20%
Suziblue	28%	24%
Twilight	**60%**	35%
Ventura (Martin et al. 2021)	**134%**	**37%**

In all cases, honey bees increase yield (usually statistically significant) compared to yield when pollinators were absent. This indicates that although honey bees lack the ability to buzz pollinate, they do move pollen between blueberry flowers and typically lead to increased blueberry yields. Martin et al. (2021) recently demonstrated this for Ventura, another southern highbush variety used in South Africa. In this study honey bees led to a 134% increase of adjusted fruit mass compared to fruits formed in the absence of honey bees, demonstrating that honey bees can effectively pollinate highbush blueberry varieties.

The addition of honey bees can result in very impressive yield increases for some varieties (i.e. Emerald, Snowchaser, Twilight, and Ventura), but for two varieties (Eureka and Suziblue), honey bee exposure had relatively small effects on fruit production. This may occur if a variety is good at self-pollination and does not need pollinators. Good self-pollinators should also have low pollination deficits because pollinators are not needed for

461 good fruit production. Suziblue appears to be a good self-pollinator because it is setting
462 close to its maximum possible yield (a small pollination deficit) and its yields are not strongly
463 increased by the presence of honey bees. We suggest that varieties such as Suziblue may be
464 good varieties to plant under conditions where pollinator visitation is extremely erratic.
465 Similarly, Eureka also did not benefit greatly (16%) from the presence of honey bees,
466 however, the high and significant pollination deficit (45%) for Eureka suggests that it is a
467 poor self-pollinator and that the marginal benefit of honey bee exposure is due to the fact
468 that this variety is extremely poorly adapted to honey bee pollination. Blueberry flowers
469 differ in terms of their morphological characters and it may be possible that it is difficult for
470 honey bees to access rewards in certain varieties. This may reduce pollinator visitation rates
471 to those varieties or possibly make it hard for pollen grains to be extracted. Studies on wild
472 pollinators have often found that traits such as floral tube length and diameter can have a
473 profound effect on the effectiveness of pollen transfer and also on whether pollinators are
474 able to access nectar or not (Campbell et al., 1996; Aigner, 2004; Fenster et al., 2006;
475 Martins and Johnson, 2013; Lázaro et al., 2015; Minnaar et al., 2019). We predict that
476 varieties with long tubes can exclude pollinators from accessing nectar, while varieties with
477 narrow entrances may exclude pollinators from accessing both nectar and pollen. Evidence
478 that some varieties have inaccessible or poor rewards for pollinators can be seen in studies
479 that show strong pollinator preferences for one blueberry variety over another (Dorr and
480 Matin, 1966; Courcelles et al., 2013).

481 The pollination deficit for Emerald was remarkable because it was so small (less than
482 zero), suggesting that honey bee pollination was better than hand pollination. The presence
483 of honey bees also had an enormous (153%) positive impact on yield, suggesting that this
484 variety is exceptionally well suited to honey bee pollination. Honey bees may be particularly

485 good at pollinating Emerald flowers due to the large flower openings (4.6 mm) which may
486 allow honey bees to more easily insert their bodies inside the flower. This may enhance the
487 probability of picking up pollen on each visit and it may also allow easier access to nectar,
488 which should make flowers more attractive to honey bees, potentially increasing visitation
489 rates. Emerald may therefore be an excellent variety to use in areas where there are no
490 bumble bees such as South Africa. Similarly, honey bee presence had a very positive impact
491 on Snowchaser yields and this, in combination with small (20%) pollination deficits suggests
492 that this variety is also quite well suited to honey bee pollination. Twilight and Ventura also
493 benefitted well from honey bee presence although the larger pollination deficits for these
494 varieties suggest that they are not as well suited to honey bee pollination as Emerald or
495 Snowchaser.

496 Honey bees are often thought to be poor pollinators of blueberries because of their
497 reluctance to forage in poor weather conditions (Tuell and Isaacs, 2010), slower foraging
498 times (Javorek et al., 2002), and decreased pollen deposition per visit (Javorek et al., 2002)
499 when compared to pollinators such as bumble bees. Despite this, we show that honey bees
500 are reliable pollinators of several blueberry varieties in regions that do not have access to
501 bumble bees. This is especially true for Emerald, where honey bee pollination is so effective
502 that it can potentially negate the need for other pollinators. Importantly the benefits of
503 honey bees can differ strongly between varieties, revealing the importance of conducting
504 research on the effectiveness of honey bee pollination on different highbush blueberry
505 varieties. Blueberry varieties Emerald, Snowchaser, and Twilight seem to do particularly well
506 under honey bee pollination and Eureka appears to be a very poor choice of variety for
507 environments that only have honey bee pollinators. However, it is also important to note
508 that without comparative studies on bumble bee pollination, it is difficult to say whether the

509 addition of bumble bees would significantly improve the pollination deficits observed. The
510 strong varietal differences in the effectiveness of honey bee pollination suggest that
511 considerable research is needed to identify which blueberry varieties do best under honey
512 bee pollination, what makes varieties successful or unsuccessful, which varieties can be
513 effectively pollinated by honey bees, and which varieties need other effective means to
514 increase fruit quality. Future research should therefore aim to determine how to decrease
515 the pollination deficit of blueberry varieties and increase the effectiveness of honey bees.

References

Aigner, P.A., 2004. Floral specialization without trade-offs: optimal corolla flare in contrasting pollination environments. Ecology 85, 2560–2569.

Aizen, M.A., Smith-Ramírez, C., Morales, C.L., Vieli, L., Sáez, A., Barahona-Segovia, R.M., Arbetman, M.P., Montalva, J., Garibaldi, L.A., Inouye, D.W., Harder, L.D., 2018. Coordinated species importation policies are needed to reduce serious invasions globally: The case of alien bumblebees in South America. J. Appl. Ecol. 1–7.

Aras, P., de Oliveira, D., Savoie, L., 1996a. Effect of a honey bee (Hymenoptera: Apidae) gradient on the pollination and yield of lowbush blueberry. J. Econ. Entomol. 89, 1080–1083.

Aras, P., de Oliveira, D., Savoie, L., 1996b. Effect of a honey bee (Hymenoptera: Apidae) gradient on the pollination and yield of lowbush blueberry. J. Econ. Entomol. 89, 1080–1083.

Auguie, B., 2017. GridExtra: miscellaneous functions for “grid” graphics.

Barton, K., 2017. MuMIn: multi-model inference.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48.

Campbell, D.R., Waser, N.M., Price, M. V, 1996. Mechanisms of hummingbird-mediated selection for flower width in *Ipomopsis aggregata*. Ecology 77, 1463–1472.

Campbell, J.W., Brien, J.O., Irvin, J.H., Kimmel, C.B., Daniels, J.C., Ellis, J.D., 2017. Pollinator ecology and management managed bumble bees (*Bombus impatiens*) (Hymenoptera:

Apidae) caged with blueberry bushes at high density did not increase fruit set or fruit weight compared to open pollination 46, 237–242.

Campbell, J.W., Kimmel, C.B., Bammer, M., Stanley-Stahr, C., Daniels, J.C., Ellis, J.D., 2018. Managed and wild bee flower visitors and their potential contribution to pollination services of low-chill highbush blueberry (*Vaccinium corymbosum* L.; Ericales: Ericaceae). J. Econ. Entomol. 111, 2011–2016.

Cane, J.H., Payne, J.A., 1993. Regional, annual, and seasonal variation in pollinator guilds: intrinsic traits of bees (Hymenoptera: Apoidea) underlie their patterns of abundance at *Vaccinium ashei* (Ericaceae). Ann. Entomol. Soc. Am. 56, 577–588.

Courcelles, D.M.M., Button, L., Elle, E., 2013. Bee visit rates vary with floral morphology among highbush blueberry cultivars (*Vaccinium corymbosum* L.). J. Appl. Entomol. 137,

Deepayan, S., 2008. Lattice: multivariate data visualization with R. Springer, New York.

Dogterom, M.H., Winston, M.L., Amy, M., 2000. Effect of pollen load size and source (self, outcross) on seed and fruit production in highbush blueberry cv. “Bluecrop” (*Vaccinium corymbosum*; Ericaceae). Am. J. Bot. 87, 1584–1591.

Dorr, J., Matin, E.C., 1966. Pollination studies on the high bush blueberry, *Vaccinium corymbosum* LQ, Bulletin of Michigan State University Agricultural Experiment Station.

Fenster, C.B., Cheely, G., Dudash, M.R., Reynolds, R.J., 2006. Nectar reward and advertisement in hummingbird-pollinated *Silene virginica* (Caryophyllaceae). Am. J. Bot. 93, 1800–1807.

Fox, J., Weisberg, S., 2011. An {R} companion to applied regression, Second Edi. ed. Sage, California.

559 Fulton, M., Jesson, L.K., Bobiwash, K., Schoen, D.J., 2015. Mitigation of pollen limitation in
560 the lowbush blueberry agroecosystem: effect of augmenting natural pollinators.
561 Ecosphere 6, 1–19.

562 Gallai, N., Salles, J.M., Settele, J., Vaissière, B.E., 2009. Economic valuation of the
563 vulnerability of world agriculture confronted with pollinator decline. Ecol. Econ. 68,
564 810–821.

565 Garibaldi, L.A., Steffan-dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham,
566 S.A., Kremen, C., Carvalheiro, L.G., 2013. Wild pollinators enhance fruit set of crops
567 regardless of honey bee abundance. Science (80). 339, 1608–1611.

568 Hoffman, G.D., Lande, C., Rao, S., 2018. A novel pollen transfer mechanism by honey bee
569 foragers on highbush blueberry (Ericales: Ericaceae). Environ. Entomol. 47, 1465–1470.

570 Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric
571 models. Biometrical J. 50, 346–363.

572 Javorek, S.K., Mackenzie, K.E., Vander Kloet, S.P., 2002. Comparative pollination
573 effectiveness among bees (Hymenoptera: Apoidea) on lowbush blueberry (Ericaceae:
574 *Vaccinium angustifolium*). Ann. Entomol. Soc. Am. 95, 345–351.

575 Jorquera-fontena, E., Génard, M., Franck, N., 2017. Analysis of blueberry (V*accinium*
576 *corymbosum* L.) fruit water dynamics during growth using an ecophysiological model. J.
577 Hortic. Sci. Biotechnol. 92, 646–659.

578 Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C.,
579 Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops.
580 Proc. R. Soc. London B Biol. Sci. 274, 303–313.

581 Lázaro, A., Vignolo, C., Santamaría, L., 2015. Long corollas as nectar barriers in *Lonicera*
582 *implexa*: interactions between corolla tube length and nectar volume. Evol. Ecol. 29,
583 419–435.

584 Lemon, J., 2006. Plotrix: a package in the red light district of R. R-News 6, 8–12.

585 Lüdecke, D., 2017. SjPlot: data visualization for statistics in social science.

586 MacKenzie, K., 1997. Pollination requirements of three highbush blueberry cultivars. J. Am.
587 Soc. Hortic. Sci.

588 Mackenzie, K.E., 1997. Pollination requirements of three highbush blueberry (*Vaccinium*
589 *corymbosum* L.) cultivars. J. Am. Soc. Hortic. Sci. 122, 891–896.

590 Mackenzie, K.E., Eickwort, G.C., 1996. Diversity and abundance of bees (Hymenoptera:
591 Apoidea) foraging on highbush blueberry (*Vaccinium corymbosum* L.) in central New
592 York. J. Kansas Entomol. Soc. 69, 185–194.

593 Martin, K., Minnaar, C., Jager, M. De, Anderson, B., 2019. Cross-compatibility of five
594 highbush blueberry varieties and ideal crossing combinations. bioRxiv 742114.

595 Martin, K., Anderson, B., Minnaar, C., Jager, M. De, 2021. Honey bees are important
596 pollinators of South African blueberries despite their inability to sonicate. South African
597 J. Bot. 137, 46–51.

598 Martins, D.J., Johnson, S.D., 2013. Interactions between hawkmoths and flowering plants in
599 East Africa: polyphagy and evolutionary specialization in an ecological context. Biol. J.
600 Linn. Soc. 110, 199–213.

601 Minnaar, C., de Jager, M.L., Anderson, B., 2019. Intraspecific divergence in floral-tube length

602 promotes asymmetric pollen movement and reproductive isolation. New Phytol. 224,
603 1160–1170.

604 Morales, C.L., Arbetman, M.P., Cameron, S.A., Aizen, M.A., 2013. Rapid ecological
605 replacement of a native bumble bee by invasive species. Front. Ecol. Environ. 11, 529–
606 534.

607 Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from
608 generalized linear mixed-effects models. Methods Ecol. Evol. 4, 133–142.

609 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C., 2017. Nlme: linear and nonlinear
610 mixed effects models.

611 R Core Team, 2017. R: a language and environment for statistical computing.

612 Sampson, B.J., Cane, J.H., 2000. Pollination efficiencies of three bee (Hymenoptera:
613 Apoidea) species visiting rabbiteye blueberry. J. Econ. Entomol. 93, 1726–1731.

614 Schmid-Hempel, R., Eckhardt, M., Goulson, D., Heinzmann, D., Lange, C., Plischuk, S.,
615 Escudero, L.R., Salathé, R., Scriven, J.J., Schmid-Hempel, P., 2014. The invasion of
616 southern South America by imported bumblebees and associated parasites. J. Anim.
617 Ecol. 83, 823–837.

618 Stubbs, C.S., Drummond, F.A., 2001. *Bombus impatiens* (Hymenoptera: Apidae): an
619 alternative to *Apis mellifera* (Hymenoptera: Apidae) for lowbush blueberry pollination.
620 J. Econ. Entomol. 94, 609–616.

621 Tuell, J., Ascher, J., Isaacs, R., 2009. Wild Bees (Hymenoptera: Apoidea: Anthophila) of the
622 Michigan highbush blueberry agroecosystem. Ann. Entomol. Soc. Am. 102, 275–287.

Tuell, J.K., Isaacs, R., 2010. Weather during bloom affects pollination and yield of highbush blueberry. J. Econ. Entomol. 103, 557–562.

Wickham, H., 2011. The split-apply-combine strategy for data analysis. J. Stat. Softw. 40, 1–29.

Wickham, H., 2009. Ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.