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# The impact of floral diversity on bumblebee colony development and pollination efficacy among foragers

Jennifer M. JANDT<sup>1</sup> , Barbara I. P. BARRATT<sup>2,3</sup> , Katharine J. M. DICKINSON<sup>2</sup> ,  
Georgia G. I. McCOMBE<sup>1</sup>, Jake TULLY<sup>1</sup>, and Janice M. LORD<sup>2</sup> 

<sup>1</sup> Department of Zoology, University of Otago, 340 Great King Street, P.O. Box 56, Dunedin 9054, New Zealand

<sup>2</sup> Department of Botany, University of Otago, Dunedin, New Zealand

<sup>3</sup> Invermay Research Centre, AgResearch, Mosgiel, New Zealand

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**Abstract** – The environment in which pollinators are reared may influence both their health and pollination performance. We compared the performance of 12 colonies of bumblebees (*Bombus terrestris*), each in their own simple (tomato monoculture) or florally diverse (tomatoes, borage, and lavender) arena inside a glasshouse. We found that colonies reared in florally diverse environments had a higher proportion of foragers overall and maintained slightly higher and less variable relative humidity inside the nest. Moreover, adding floral resources to a tomato crop resulted in increased total crop yield, whereas individual tomatoes reared in the simple monoculture environment were larger than those in the diverse. These results provide an important step in understanding the extent to which bumblebee health and flower-visiting behavior are influenced by the complexity of the foraging environment and how a diverse floral environment may enhance pollinator behavior toward a focal crop.

***Bombus terrestris* / Borage / Crop pollination / Greenhouse pollination / Semi-natural environment / tomato**

## 1. INTRODUCTION

Pollinator decline has been attributed to a number of human-induced factors, including habitat loss or modification, climate change, exotic species introductions, and pesticides (Goulson et al. 2015; Woodard and Jha 2017; Forister et al. 2019; Mathiasson and Rehan 2020; Wagner et al. 2021). Agricultural areas, particularly those that rely on active crop pollination, can be extreme examples of modified habitats with the lowest diversity—often monoculture—of floral landscapes. When pollinators

in these areas encounter multiple simultaneous stressors, such as pesticides, parasites, diseases, and reduced nutrient availability and/or diversity, even the most robust pollinators struggle to cope (Dolezal and Toth 2014). Adding or protecting flowering species, such as perennial shrubs or wildflowers, on the boundaries of these crop areas may help to mitigate the effects of these external stressors (Dolezal et al. 2016; Quinlan et al. 2021; Ohlinger et al. 2024) and increase pollinator populations overall (Cusser and Goodell 2013; Stein et al. 2020; Howlett et al. 2021).

Honeybee and bumblebee colonies are commonly managed for crop pollination services (Potts et al. 2016; Khalifa et al. 2021). Because bee colonies require both protein (pollen) and carbohydrates (nectar) for colony health and development (Vaudo et al. 2015; Watrous et al. 2019), managed colonies

Corresponding author: J. M. Jandt, [jjandt2@gmail.com](mailto:jjandt2@gmail.com)  
[jenny.jandt@otago.ac.nz](mailto:jenny.jandt@otago.ac.nz)  
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are often provided with artificial nectar or pollen (or both) supplements to ensure colony survival (DeGrandi-Hoffman et al. 2016). The quantity, quality, and diversity of nutrition brought into the nest can influence the development of the brood (Tasei and Aupinel 2008; Jandt et al. 2015) and overall colony health (Vaudo et al. 2015; 2016). Bee colonies that are managed to pollinate field crops can diversify the nutrients coming into the nest by collecting pollen from flowers in field margins and neighboring gardens. Moreover, crop pollination and fruit yield may increase when “weedy” flowers remain in the field (Kleiman et al. 2021).

Glasshouses provide an opportunity to increase crop production while decreasing water use compared to open-field crops (Maureira et al. 2022). In 2023, tomatoes had a farm gate value of over \$185 million in New Zealand, the majority of which are grown in glasshouses (TomatoesNZ 2024). Glasshouse crops differ from field crops in that plants are grown under more controlled conditions. Pollinators employed in these environments are therefore severely restricted in their options for nutrients. Bumblebees are effective pollinators of tomatoes, and colonies are available through commercial suppliers for use in glasshouse pollination services worldwide (Palma et al. 2008; Howlett and Donovan 2010; Torres-Ruiz and Jones 2012; Ahmad et al. 2015). Commercial bumblebee colonies are usually packaged and distributed with sugar syrup, a substitute for natural nectar sources. As tomato flowers only provide pollen, colonies employed for this service can still function as long as the sugar syrup remains available. This supplemented syrup is kept inside the colony—there is no evidence that providing this syrup outside of the nest affects foraging or pollination behavior (Orban et al. 2012). Therefore, not only are these glasshouse colonies limited in the types of pollen they can collect, but the colonies’ natural division of foraging tasks—nectar and pollen foragers—is interrupted since only pollen foragers will be successful in this environment (Hagbery and Nieh 2012). Therefore, these environmental restrictions are predicted to impact not only individual behaviors but colony phenotype as well (Bengston and Jandt 2014).

Tomato flowers do not require cross-pollination for fruit sets; however, they do require floral agitation in order to self-pollinate. When bumblebees “buzz-pollinate” a tomato flower, they vibrate their thorax at a frequency that allows the anthers to release pollen and fertilize the stigma. Devices have been designed to recreate this service artificially; however, buzz-pollination by a colony of bumblebees is still a cost- and time-effective method to ensure crop production (Dogterom et al. 1998). Therefore, it is a priority for tomato growers to maintain healthy and active colonies in their glasshouses to achieve optimal pollination (Morandin et al. 2001).

Most studies investigating how the addition of floral resources among crops affects managed pollinators have been conducted in the field, with mixed results (see Howlett et al. 2021). Glasshouse studies allow us the opportunity to manipulate the floral diversity that bees can access and ensure that other stressors (e.g., pesticides, parasites, diseases) are minimized while monitoring the individual behaviors and whole colony phenotypes. Here, we investigate how the addition of floral resources to a monoculture glasshouse tomato crop affects the overall health and behavior of commercial colonies of bumblebees (*Bombus terrestris*), as well as pollination efficacy and crop production. While including additional floral resources into a glasshouse system would incur additional costs to the grower in terms of resources (soil, water, space, and labor), we predict that increasing floral diversity will lead to more robust bumblebee colonies in the glasshouse, increased foraging activity, quality of pollination services, and crop production. However, if bees are distracted by the additional flowers in a florally diverse glasshouse environment, then crop production could decline.

## 2. METHODS

### 2.1. Tomato and flower planting

All tomatoes and flowering plants were initially grown from seed in a large glasshouse (12 m×40

m) at the Invermay Research Centre (Mosgiel, New Zealand). In Year 1, tomato (*Solanum lycopersicum*, Solanaceae, “Sweet 100”), borage (*Borago officinalis*, Boraginaceae, “Star Flower”), and lavender (*Lavender dentata*, Lamiaceae) seeds were sown in mid-spring (7 Oct 2019). In Year 2, tomato (“Sweet 100”) seeds were sown, and 114 lavender seedlings (*L. angustifolia* “Hidcote”) were purchased on 7 Oct 2020. Borage (“Star Flower”) seeds were sown a week later (13 Oct 2020) to coincide better with the start of tomato flowering. In Year 2, we also purchased 36 Sweet 100 tomato seedlings in late spring (6 November 2020) to ensure that each arena would have at least four well-grown plants by the start of the experiment. Tomatoes started from seed, and those purchased as seedlings were divided equally among the arenas.

All plants were grown in 4:1 potting soil to sharp sand mixtures. Seedlings were transplanted first into small containers (Year 1, 13 Nov 2019; Year 2, 11 Nov 2020) and then into 9-L plastic pots (Year 1, 6 Dec to 12 Dec 2019) or PB12 10-L bags (Year 2, 16 Nov to 7 Dec 2020). Tomatoes were tied to a 1.2-m wooden stake for support, and the bottom 20 cm of leaves were trimmed/removed to facilitate watering (Year 1, 9 Jan 2020; Year 2, 2 Dec 2020). Additional branches were tied up throughout the experiment to minimize stem bending and breaking. By the start of the experiment, tomato plants were 50–100 cm tall. All tomatoes received fertilizer once per week until the bumblebees were introduced (Year 1, Yates Thrive® Tomato Liquid Plant Food; Year 2, Yates Thrive® Tomato & Veggie Natural Fish Blood & Bone + Liquid Plant Food).

## 2.2. Simple and diverse treatment set-up

The glasshouse at the Invermay Research Centre was divided in the center to create two 12 m × 20 m subsections. Within each subsection, we constructed four (eight in total) 5 m × 8 m × 3 m arenas enclosed with insect-proof netting that each contained one bumblebee colony (*B. terrestris*, BioBees Ltd.) and 60 plants spread among 15 tables (four plants per table). These arenas reduced colony drift among foragers (Birmingham 2003; MacKenzie et al. 2021).

In Year 1 of data collection, only four arenas were complete ( $n=4$  replicates); in Year 2, all eight arenas were available ( $n=8$  replicates). Each year, in half of the arenas, we provided bees with a florally diverse environment (Fig. 1a): five tables contained four tomato plants, five contained four borage, and five contained four lavender. Borage and lavender were used because they are known to be visited by bumblebees and are readily available commercially. In the other arenas, we provided bees with a tomato monoculture environment (Fig. 1b): each of the 15 tables contained four tomato plants. Five tables of tomatoes in each arena (all tomatoes in the diverse treatment) were designated “focal tables.” Plants were set up in arenas prior to the introduction of bee colonies, and all dead flowers and/or developing fruits were removed (to encourage flower production and remove non-bee pollinated fruit). After bees were introduced to the arenas, focal tomatoes continued to receive fertilizer weekly, and their stems were continuously secured to stakes to minimize accidental fruit fall when walking through the arena. Non-focal plants (tomatoes, borage, or lavender) did not receive fertilizer but were re-tied to stakes when stems became too heavy. Throughout the study, we continued to dead-head borage and lavender. At the start of each week, we counted the number of new flowers on every plant (tomatoes, borage, and lavender) in each arena. Flowers were removed to ensure that arenas had similar numbers of focal tomato flowers and “other” flowers (borage, lavender, or non-focal tomatoes).

A syrup dispenser containing a sugar solution provided by BioBees was placed outside each colony. In Year 1, each focal table also had a syrup dispenser ( $n=6$  syrup dispensers per arena). In Year 2, the center table in the front and back of the arena contained additional syrup dispensers ( $n=3$  per arena). Syrup dispensers were refilled as needed to ensure bees had an ad lib sugar supply. All plants were watered daily.

## 2.3. Bumblebees

All bumblebee colonies were purchased through the New Zealand distributor BioBees



**Figure 1.** Arenas within the glasshouse showing **a** a florally diverse environment with borage (B), lavender (L), and tomato (T); **b** a simple monoculture environment with only tomato plants

Ltd. (Year 1, 4 colonies arrived on 21 January 2020; Year 2, 8 colonies arrived on 8 January 2021). Colonies were transferred into wooden nest boxes covered with glass (for viewing in-nest behavior and recording nest growth) before being introduced to the arena. The glass lids were covered with 5-cm-thick polystyrene when data were not being collected.

While transferring colonies to the boxes, we tagged each individual bee with a color-number-coded plastic bee tag to denote bee ID and colony of origin. In Year 1, we chilled the bees before tagging them to record thorax width using digital calipers. In Year 2, we did not chill bees while tagging and measured thorax width *postmortem*. After bees were introduced to the arena, we continued to tag newly emerging bees 3–4 times a week, chilling and measuring first in Year 1 only.

#### 2.4. Assessing colony performance

Colony performance was measured in terms of temperature/humidity maintenance, colony growth (number and size of workers), and proportion of workers engaged in foraging. In Year 1, we left the “excess” wax that bees produce to construct a waxy envelope over the nest in place for the duration of the study. In Year 2, we collected the wax that covered the brood area of the

nest each week, as it helped to facilitate bee tagging. Data on wax produced by colonies in Years 1 and 2 are available in Supplemental Material.

We placed three temperature and humidity data loggers (Hygrochron Temperature & Humidity iButtons, Maximus Technologies) in each arena: one on a central tomato table, one directly outside the bee colony, and one directly inside the bee colony (in the corner, not touching the brood). Data were logged every hour for 24 h each day. Data were downloaded from iButtons every 1–2 weeks.

To assess colony growth, we examined the body sizes among workers in each colony in two ways. First, because body size distribution among workers can vary across colonies (Couvillon et al. 2010), we compared the distribution of body sizes among those workers that were tagged before the colony was introduced to the glasshouse. Second, we explored the body sizes among workers that emerged from the colony after the colony was introduced to the glasshouse. For this, we used only those workers tagged after the colony was introduced to the glasshouses. To normalize the body sizes of those that emerged after the colony was introduced to the glasshouse relative to the average size of the original workers, we subtracted the body size of the “new” workers from the average size of the original workers.

To estimate the number of workers in the colony (colony size), every 1–3 days between 800 and 1300 h, we recorded which tagged

bees could be relocated inside the nest box or foraging. Dead bees were collected inside and outside the nest box. Bees that escaped from an arena were returned as quickly as possible.

To quantify foraging behavior, two researchers walked through each arena, scanned the plants on each table 4–5 times in an hour, and recorded the ID of each bee foraging on each table. In Year 1, scans were repeated five times with 10 min between scans each day. In Year 2, scans were repeated four times with 15 min between scans. Scan sampling was conducted 4 days between 800 and 1200 h, for each week that bees were in the arena. From these data, we determined the number of unique foragers in the arena during the scan, as well as the number foraging on each table (overall), and specifically on focal tomato plants.

Colony development and foraging behavior were collected for 4 weeks, after which bees were removed from the arenas.

## 2.5. Quantifying pollinator efficacy (crop yield)

The arenas ensured tomatoes were not visited by additional pollinators. Tomato fruits began to ripen ~2 weeks after bees were removed. Once a week for 4 weeks, we collected ripe fruit from each tomato plant, weighed all tomatoes from each plant together, and counted the number of tomatoes to calculate the average tomato weight produced per plant each week. Because the number of seeds produced per fruit can be more sensitive to pollinator visits than fruit size (Toni et al. 2021), in the last 2 weeks of tomato collection in Year 2, we selected one random ripe (red) tomato from each plant to dissect for seed count. This tomato was individually weighed, cut open, seeds removed, and photographed with the empty fruit and seeds in the image.

## 2.6. Data analyses

Data were analyzed with R, version 4.2.2 (R Core Team 2022). Data from Years 1 and 2 were combined to create 12 unique colony IDs

(hereafter “colony”), which were included as a random effect in all mixed effect models, using package “lme4” (Bates et al. 2015). To calculate *P*-values and effect sizes, we used the package “car” (Fox and Weisberg 2019), and when models included an interaction term, we used the package “emmeans” (Lenth 2023) to calculate pairwise comparisons.

We first confirmed that the number of flowers available to foraging bees was consistent across the different treatment types (diverse vs. monoculture). We ran two generalized linear mixed effects models (glmer) with a Poisson distribution (link = “log”) where the total number of flowers in bloom or the total number of tomatoes on focal tomatoes per arena was included as the response variable, treatment was included as the fixed effect, and the arena was included as the random effect.

### 2.6.1. Temperature and humidity

We calculated the average and variance for temperature and humidity for each day (000 to 2300 h); over the 4 weeks colonies were within arenas, to determine how microclimate compared inside vs. outside the nest, and across treatments. Temperature averages and variances fitted normal distributions, so we used linear mixed effects models (lmer) where either average or variance in temperature was included as the response variable, treatment, location (inside vs outside the nest), and their interactions were included as fixed effects.

Averages and variances in relative humidity fitted a log-normal distribution. We used a generalized linear mixed effects model (glmer) with a Gaussian distribution (link = “log”) where average relative humidity was included as the response variable, and fixed and random effects were the same as above. We log-transformed the variance of relative humidity, as the raw data failed to converge in the statistical model. We used a linear mixed effects model (lmer) where the log of the variance in relative humidity was included as the response variable, and the fixed effects were the same as above.

### 2.6.2. Workers and foragers per colony

To determine whether colonies assigned to each treatment differed in body size at the start of the experiment (i.e., whether one treatment received colonies with significantly larger workers), we used a linear mixed effects model (lmer) where the body size distribution of the original workers was included as the response, and treatment was included as the fixed effect. We then investigated how the distribution of body sizes might have been affected by the environment by analyzing the body sizes of workers that emerged after the colonies were introduced to the arenas. We used linear mixed effects models (lmer) where the body size distribution of the new workers or the normalized distribution of the new workers was included as the response, and treatment was included as the fixed effect.

To determine whether colony size (worker number) varied between treatments, we first normalized all colonies by subtracting the colony size on day 1 from the estimated colony size each day after that. Change in worker number fitted a normal distribution, so we used a linear mixed effects model (lmer) where the change in worker number was included as the response, and treatment and day since the start of the experiment were included as fixed effects.

To determine how foraging effort varied across treatments, we calculated the proportion of the colony that was foraging each day based on the number of individual foragers observed during scan sampling and the estimate of colony size for that day. These data also followed a normal distribution, so we used a linear mixed effects model (lmer) where the proportion of foragers was included as the response, and treatment and day since the start of the experiment were included as fixed effects.

### 2.6.3. Tomato pollination

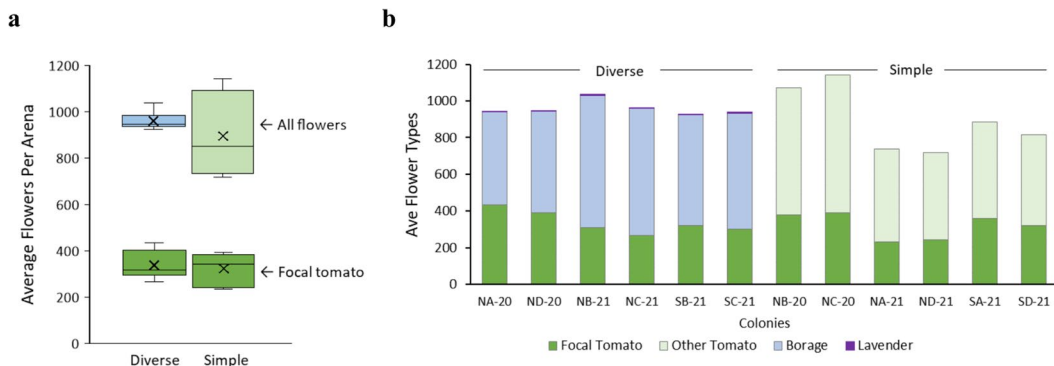
To quantify the pollination service provided by bees to tomatoes in the different treatments, we calculated the average number of bees foraging on every table, specifically on each of the focal tables

during scan sampling. Data were right-skewed and included zeroes, so we added 0.5 to each observation and log-transformed both sets of data. We used linear mixed effects models (lmer) where the number of foragers either on every table or on only focal tables was included as the response variable, and treatment and day since the start of the experiment were included as fixed effects.

We investigated pollination efficacy in four ways: the total number of tomatoes produced per plant, the total weight of tomatoes produced per plant, the average weight of tomatoes produced per plant (calculated as total weight divided by total number), and the total number of seeds produced in the randomly selected tomato from each plant. For all models assessing fruit production, we include treatment as the fixed effect and plant nested in the colony's arena as a random effect. The total number of tomatoes produced each week per plant fitted a Poisson distribution, so we used a generalized linear mixed model (glmer; family=poisson (link="log")). Total fruit weight produced per plant each week fitted a log-normal distribution, so we first added 0.001 to each value (to account for 0's in the dataset) and used a generalized linear mixed model (glmer; family=Gaussian (link="log")). The average weight of tomatoes produced each week fitted a normal distribution, so we used a linear mixed model (lmer). Finally, seed number per tomato fitted a normal distribution, so we used a linear mixed model (lmer) where treatment, the weight of the source tomato, and the interaction were included as fixed effects, and the plant nested in the colony's arena was included as a random effect.

## 3. RESULTS

We found no evidence that bees had access to a different total number of flowers across diverse or simple treatments (Fig. 2a; GLMM:  $\chi^2_1 = 0.25$ ,  $P = 0.61$ ). Moreover, there was no evidence that focal tomato plants varied in the number of flowers they produced in the two treatments (Fig. 2b; GLMM:  $\chi^2_1 = 1.32$ ,  $P = 0.25$ ). Lavender plants did not flower well, so floral diversity in the diverse treatment was provided largely by borage plants (Fig. 2b).



**Figure 2.** Flower numbers between treatments and across colonies within treatments. **a** There was no difference in the total number of flowers available overall (top) or flowers available on focal tomato plants only (bottom) across treatments. Boxes represent median (solid line)  $\pm$  25th percentiles. Lines represent the 10th percentile. X represents the mean. **b** Average of each flower type available to colonies within treatments

### 3.1. Temperature and humidity

All colonies maintained significantly higher temperatures inside nests compared to outside nests, regardless of the treatment (Fig. 3a; LMM: treatment  $\chi^2_1 = 0.003$ ,  $P = 0.96$ ; location  $\chi^2_1 = 18.94$ ,  $P < 0.0001$ ; interaction  $\chi^2_1 = 0.04$ ,  $P = 0.83$ ). The temperature variance inside nests was significantly lower compared to outside nests, regardless of treatment (Fig. 3b; LMM: treatment  $\chi^2_1 = 0.43$ ,  $P = 0.51$ ; location  $\chi^2_1 = 218.16$ ,  $P < 0.0001$ ; interaction  $\chi^2_1 = 0.45$ ,  $P = 0.50$ ).

Colonies overall maintained higher % relative humidity inside nests compared to outside nests (Fig. 3c; LMM: treatment  $\chi^2_1 = 1.42$ ,  $P = 0.23$ ; location  $\chi^2_1 = 340.59$ ,  $P < 0.0001$ ; interaction  $\chi^2_1 = 63.31$ ,  $P < 0.0001$ ); those in the diverse treatment maintained higher humidity compared to those raised in the simple treatment, although this difference was marginally non-significant ( $P = 0.068$ ). The variance in relative humidity inside nests was significantly lower compared to outside nests and lower overall (inside and outside the nest) for those colonies in diverse treatment (Fig. 3d; LMM: treatment  $\chi^2_1 = 4.20$ ,  $P = 0.04$ ; location  $\chi^2_1 = 813.23$ ,  $P < 0.0001$ ; interaction  $\chi^2_1 = 1.09$ ,  $P = 0.30$ ). Variance in relative humidity inside nests in the diverse treatment was lower compared to nests in the simple treatment, but not significantly so ( $P = 0.14$ ), whereas variance in relative humidity outside nests

in the diverse treatment did not differ from nests in the simple treatment ( $P = 0.64$ ).

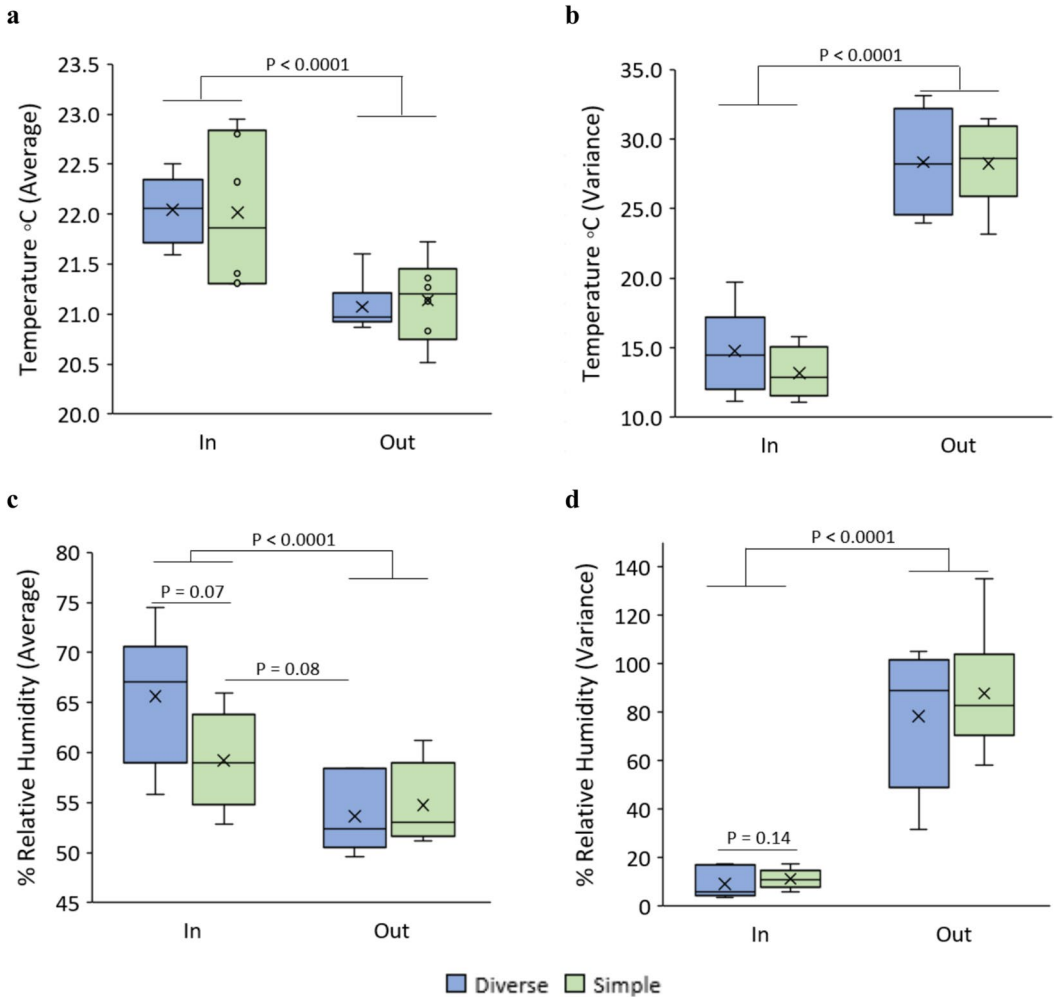
### 3.2. Workers and foragers per colony

There was no evidence that colonies assigned either treatment had larger or smaller workers (LMM:  $\chi^2_1 = 1.12$ ,  $P = 0.29$ ). After the colonies were introduced to the treatments, there was no evidence that workers that emerged from the nest varied in size across treatments (raw sizes: LMM:  $\chi^2_1 = 0.22$ ,  $P = 0.64$ ; normalized sizes:  $\chi^2_1 = 0.22$ ,  $P = 0.64$ ).

There was no evidence that the colony size, in terms of worker number, varied across treatment (Fig. 4a; LMM: treatment  $\chi^2_1 = 0.008$ ,  $P = 0.93$ ); colony size overall decreased over time (Fig. 4b; day  $\chi^2_1 = 21.42$ ,  $P < 0.0001$ ). Colonies raised in the diverse treatment had, on average, a higher proportion of the colony foraging each day (Fig. 4c; LMM: treatment  $\chi^2_1 = 69.30$ ,  $P < 0.0001$ ); proportion foraging declined over time for all colonies (Fig. 4d; day  $\chi^2_1 = 11.95$ ,  $P = 0.0005$ ).

### 3.3. Tomato pollination

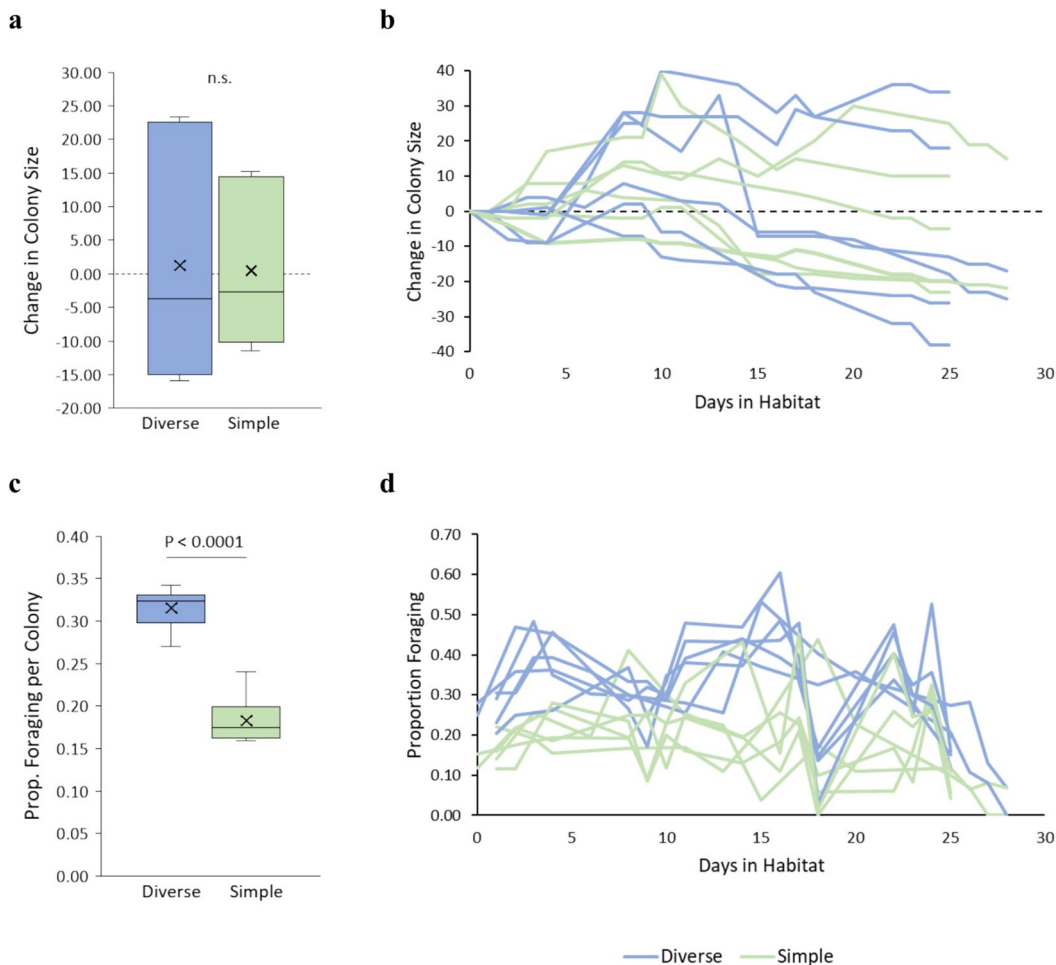
During the scan sampling, more foragers were observed on each table in the diverse treatment compared to the simple treatment (Fig. 5a; LMM:



**Figure 3.** Average and variance in temperature and % relative humidity recorded inside (In) and directly outside (Out) bumblebee nest boxes raised in florally diverse (dark grey in print, blue online) or simple (light grey in print, green online) treatments. Colonies consistently maintained **a** higher temperatures with **b** lower variance in temperature inside the nest compared to directly outside the nest. Colonies maintained **c** slightly higher relative humidity with **d** lower variance in humidity inside the nest compared to outside the nest. Boxes represent median (solid line)  $\pm$  25th percentiles. Lines represent the 10th percentile. X represents the mean

treatment  $\chi^2_1 = 24.92$ ,  $P < 0.0001$ ), with an overall decline in foragers observed during each scan over the course of the experiments (day  $\chi^2_1 = 222.24$ ,  $P < 0.0001$ ). There was no difference, however, in the number of foragers observed on focal tomato tables across treatments (Fig. 5b; LMM: treatment  $\chi^2_1 = 0.83$ ,  $P = 0.36$ ), though the number of foragers observed on focal tomatoes also declined over time (day  $\chi^2_1 = 34.25$ ,  $P < 0.0001$ ).

We found an increase in tomato production in the florally diverse environment. Plants produced more tomatoes (Fig. 6a; GLMM  $\chi^2_1 = 9.40$ ,  $P = 0.002$ ) and marginally higher total weight each week (Fig. 6b; GLMM  $\chi^2_1 = 3.09$ ,  $P = 0.079$ ). However, the average weight per tomato produced per plant was higher in the simple monoculture environment (Fig. 6c; GLMM  $\chi^2_1 = 0.94$ ,  $P = 0.003$ ).

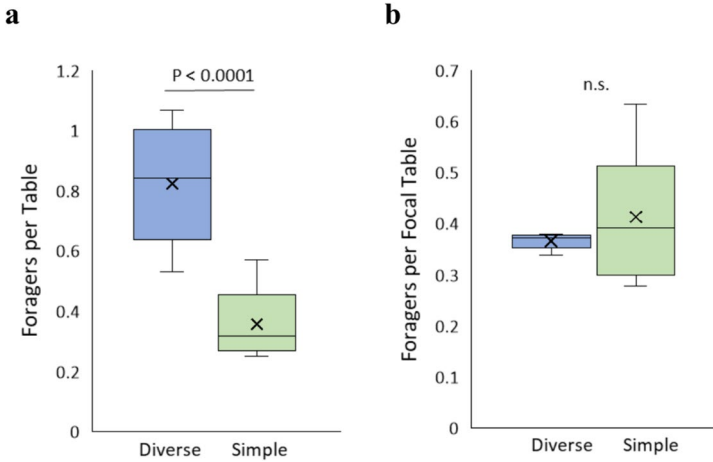


**Figure 4.** Change in colony size and proportion foraging across florally diverse (dark grey in print, blue online) and simple (light grey in print, green online) treatments. **a** There was no evidence that the change in colony size varied across treatments, though **b** colony size overall tended to decrease over time. Dashed lines represent no change in colony size. **c** Colonies raised in the floral diverse treatment had, on average, a higher proportion of the colony foraging each day. **d** The proportion of foragers in a colony decreased over time. **a**, **c** Boxes represent median (solid line)  $\pm$  25th percentiles. Lines represent the 10th percentile.  $X$  represents the mean. The average for each colony was calculated and used to create each boxplot.  $N=6$  colonies represented in each box. **b**, **d** Lines represent data for each colony in florally diverse (purple) or simple (red) treatments from 2020 and 2021 combined. Note that data were collected for 28 days in 2020 ( $n=2$  colonies in each environment) and 25 days in 2021 ( $n=4$  colonies in each environment)

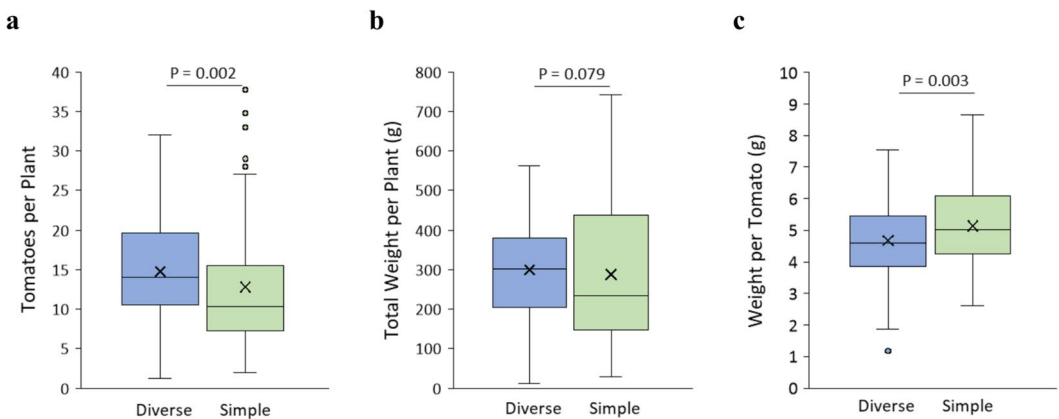
Tomatoes collected from plants in the diverse treatment tended to produce more seeds for their weight compared to tomatoes collected from plants in the simple treatment; a phenomenon that was most strongly observed among the smaller tomatoes (Fig. 7; LMM: weight  $\chi^2_1=528.08$ ,  $P < 0.0001$ ; treatment  $\chi^2_1=2.89$ ,  $P=0.089$ ; interaction  $\chi^2_1=4.12$ ,  $P=0.042$ ).

#### 4. DISCUSSION

Overall, our results support the hypothesis that increasing the floral diversity of a bumblebee's foraging environment can have positive effects on colony development and foraging behavior and also lead to an increase in fruit output. Instead of acting as a distraction,



**Figure 5.** Forager behavior on plants. **a** More bees were observed per table in the diverse treatment. **b** There was no difference in the number of bees observed per focal tomato table across the two treatments. Boxes represent median (solid line)  $\pm$  25th percentiles. Lines represent the 10th percentile. X represents the mean

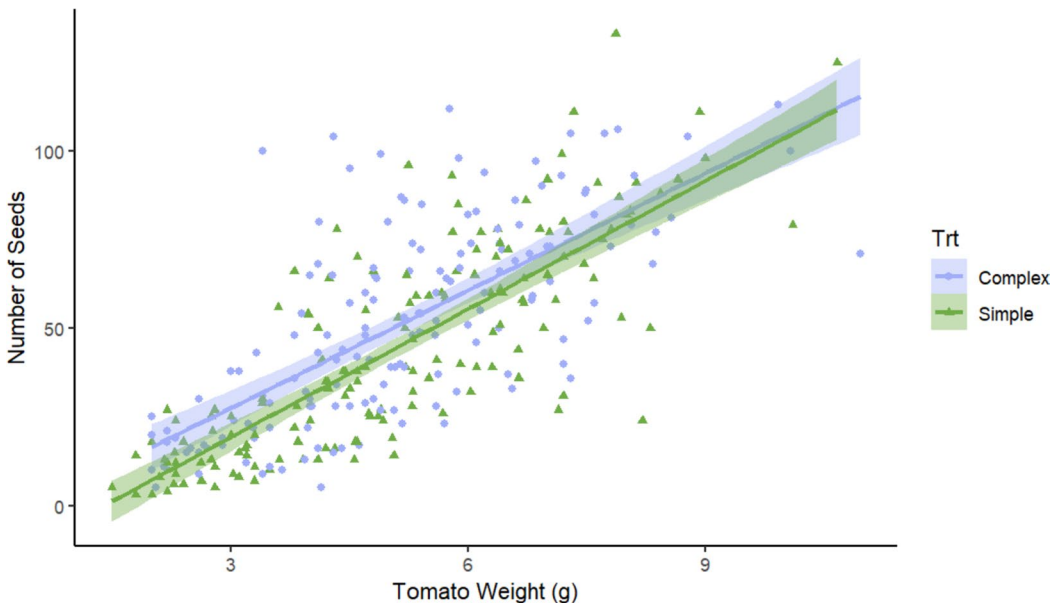


**Figure 6.** Tomatoes produced per plant across environments. **a** More tomatoes were produced in the florally diverse environments compared to simple environments, and **b** the average weight produced by each plant was slightly higher in the diverse environments. **c** On average, individual tomatoes were larger in the simple environment. Boxes represent median (solid line)  $\pm$  25th percentiles. Lines represent the 10th percentile. X represents the mean

the addition of borage and (to a smaller extent) lavender in the glasshouse led to an increase in pollination efficacy, quantified as an increase in overall tomato production and seeds developing per tomato. Fewer fruit and/or fewer seeds per fruit is a well-established outcome of pollen limitation, with larger fruit sizes among remaining fruit due to reallocating of resources (Stephenson 1981; Ashman et al. 2004). Given that only a few

of the lavender plants produced flowers during the experiment (Fig. 2B), this also implies that pollination outcomes, as well as colony phenotype—in terms of foraging effort and thermoregulation—improved simply by providing a single additional food source.

In our study, a stronger foraging cohort observed in the diverse environments may explain the slightly higher and significantly



**Figure 7.** Number of seeds compared to the weight of a randomly selected tomato from each plant. Among the smaller tomatoes, those raised in the diverse environment produced more seeds compared to those raised in the simple environment. This difference disappears among the larger tomatoes

more consistent relative humidity maintained inside the nest. High humidity and temperature are important for egg hatching and brood development (Doull 1976; Abou-Shaara et al. 2017). Temperature regulation inside bee nests is achieved when warm colonies are cooled down by an increase in the number of bees that fan in the nest, and cool colonies are warmed by an increase in incubating and vibrating bees (O'Donnell and Foster 2001; Weidenmüller et al. 2002; Jandt and Dornhaus 2014). High humidity may also be actively maintained by foragers returning to the nest with water or nectar that can be used for evapotranspiration (Human et al. 2006). Sugar water was available in both environments, so it is possible that access to natural nectar sources in the diverse environments (from borage and lavender flowers) may have played a role in nest thermoregulation, although this hypothesis requires further testing. In-nest workers may also assist in temperature and/or humidity regulation by producing or recycling wax that covers the nest or restricts air ventilation into the nest (Holland and Bourke 2015).

While we did not quantify airflow or wax build-up on the windows provided on the nest box for ventilation in this study, we did quantify excess wax produced that was used to cover the brood each week and found no evidence this varied across environment type (see Supplemental Material). Moreover, increase in humidity does not affect the number of bees fanning in the nest (Weidenmüller et al. 2002). While more direct evidence is needed, our results, taken with those of previous studies, suggest that an environment that supports higher foraging effort can have an impact on in-nest humidity regulation.

Our study supports the importance of pollen diversity in bumblebee diets as well as floral diversity benefiting crop yield. Though we did not see a difference in either worker size (thorax width) or colony size (number of workers), colonies in the florally diverse environments maintained a consistently higher proportion of foragers relative to colony size. Bumblebees that return to the nest with high-quality rewards will perform irregular runs through the colony and distribute a pheromone that motivates idle nestmates to leave the

nest to forage (Dornhaus and Chittka 2001, 2004). Bumblebees are commonly used for tomato pollination, and higher fruit production and seeds per fruit are evidence of increased pollinator efficacy (Toni et al. 2021). In our experiment, a consequence of having a higher proportion of the colony foraging was an increase in pollinator efficacy. Given that borage was the only regularly flowering plant in our diverse environment, we suggest that the presence of borage enhanced crop pollination rather than acted as a distraction or competition for pollination services, an important consideration when planting flowering species near crops (reviewed in Howlett et al. 2021).

Floral nectar is composed of more than sugars; the secondary metabolites present in nectar can reduce parasite infections (Richardson et al. 2015; Schmitt et al. 2021). Commercial bumblebees used for tomato pollination are provided with an adlib syrup they can access while remaining inside the box with their colony. It is not clear what ingredients were used to make this syrup; however, growers only need to open the box, and the bees can survive on the pollen provided by tomatoes and nectar provided by the syrup. Moving the syrup to a location outside the nest or far from the nest does not affect pollen foraging (Orban et al. 2012). In our study, bees were provided with a simple sugar water solution outside of the nest in both environments. Because borage and lavender produce both nectar and pollen, it is unclear whether the increase in foraging behavior in our study in florally diverse environments was due to a second type of pollen or the availability of a natural nectar source. Future studies will be designed to investigate this further.

Previous studies in the field that investigated the effects of providing additional floral resources on crop yield have found a mix of positive and negligible results (reviewed in Howlett et al. 2021). Our study is unique in that we not only conducted our research in a controlled glasshouse, but we constructed multiple screened arenas that contained the plants and bees, allowing us to have up to eight replicates running simultaneously. Isolating colonies to their own environments reduced the amount of worker drift often observed when multiple colonies share the same enclosed environment

(Birmingham and Winston 2004; Lefebvre and Pierre 2007; Terlinden 2019).

## 5. CONCLUSION

We showed that bumblebees, bred and marketed for tomato pollination, maintained a higher proportion of foragers, better regulated nest humidity, and contributed a higher efficacy in pollination when provided with an additional floral resource among a tomato crop. Glasshouses provide an effective way to maximize crop production while minimizing space and water use. Previous work has highlighted that adding bumblebees is an efficient and cost-effective method to increase crop pollination. Our results build on this by showing how the addition of an unrelated flowering species in a monocrop glasshouse environment can have major implications for growers hoping to maximize the health and foraging activity of the bee colonies they purchase for use in glasshouses, as well as fruit output.

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## AUTHOR CONTRIBUTION

JJ conceived research; all contributed to the study design. JJ, JT, and GM collected data. JJ conducted analyses and wrote the paper. All participated in revisions and read and approved the final manuscript.

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## DATA AVAILABILITY

The datasets generated or analyzed during the current study are available from the corresponding author upon reasonable request.

## DECLARATIONS

**Ethics approval** Not required. The work involved data collected on an unregulated and common insect species (*Bombus terrestris*).

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

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