Priming bumble bees with caffeine, odour of the target crop, and a food reward, has minimal effects on fruit crop pollination and yield in a semi-commercial setup

Sarah E J Arnold[1,2]*, Celine Xavier e Silva[1], Jan-Hendrik Dudenhöffer[1,3], David R Hall[1], Dudley I Farman[1], Felix L Wäckers[4], Philip C Stevenson[2,5], Michelle T Fountain[1]

[1] NIAB, East Malling, Kent, ME19 6BJ, UK

[2] Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent, ME4 4TB, UK

[3] Lincoln University, Lincoln 7647, Canterbury, New Zealand

[4] Biobest NV, Ilse Velden 18, 2260 Westerlo, Belgium

[5] Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, UK

*Lead Contact for correspondence: sarah.arnold@niab.com

Abstract

Caffeine is a widely occurring plant defence chemical that occurs in the nectar of some plants, e.g. *Coffea* or *Citrus* spp., where it may influence pollinator behaviour to enhance pollination. Previous laboratory work found inexperienced bumblebees (*Bombus terrestris*) located new food sources emitting a learned floral odour more consistently if they had been fed caffeine in association with the odour and a food reward. Inexperienced bees primed with caffeine also made more initial visits to target robotic flowers emitting the target odour, compared to control bees or those primed with odour alone.

We tested whether these behaviours could be replicated under semi-field conditions in strawberry crops, to improve crop pollination and hence marketable yield. In three trials in mini-polytunnels, odour/caffeine-primed and control bumblebee colonies were allowed to forage on strawberry crops with nectar-rich distractor flowers present. Some small effects of caffeine priming were observed (a slight increase in the proportion of visits to the target flowers in one trial), but after controlling for polytunnel identity, the priming treatment did not influence crop yield and quality. While caffeine priming of commercial bumblebee colonies may have potential to improve pollination in crops, further research is needed to optimise the system for in-field use.

Keywords

alkaloids, associative learning, bumblebees, flower constancy, soft fruit pollination, horticulture

1 Introduction

Strawberry is a major high-value crop grown across many global temperate zones. In the UK the value of the crop is nearly £400m (Defra 2022) with production across nearly 5000 ha nationally (Defra 2022). Fruit-set in strawberry benefits from insect pollination, with larger, higher quality fruit (Martin, et al. 2019; MacInnis and Forrest 2020) and a lower rate of mis-shapes where insect pollinators are deployed, compared to wind/self-pollinated control plants (Wietzke et al. 2018). Reducing mis-shapes is currently a high priority for growers in Europe (Ariza et al. 2012), as labour costs are high and even unsaleable fruit must often be picked to manage pest problems such as *Drosophila suzukii* (Leach et al. 2018). One route to reducing mis-shaped fruit is to optimise managed pollinators. Effective pollination translates into economic gains for growers (Castle et al. 2019).

Commercial bumblebees (primarily *Bombus terrestris* in Europe and *Bombus impatiens* in North America) are the main pollinator deployed in protected crops such as strawberry (Martin et al. 2019), raspberry (Lye et al. 2011), tomato (Cooley and Vallejo-Marín, 2021), blueberry (Drummond, 2012), and covered apple (Normandeau Bonneau et al., 2023). They can provide a significant benefit to crop productivity (Martin et al. 2019). A standard deployment rate is 3-10 “triple hives” (three colonies side-by-side) per hectare for pollination of soft fruit crops (Kiprijanovska et al. 2012; Hölzer and Hemmer, 2019), and the colonies normally provide pollination for 6-12 weeks. However, the performance can be inconsistent and is not always optimal. It is a particular challenge to ensure good pollination of crops that have moderate-to-high pollinator dependence, but also moderate-to-low attractiveness to pollinators, such as strawberry (Trillo et al. 2020), avocado (Afik et al 2006), kiwi (Estravis-Barcala et al. 2024) and tomato (Liu et al. 2022), as bees will actively seek alternative forage so often leave the crop to forage in hedgerows and on other non-crop vegetation (Foulis and Goulson, 2014; Trillo et al. 2020). Reducing non-crop foraging by commercial bumblebees benefits growers by providing better value for money, and the environment by reducing competition between wild and managed pollinators on non-crop vegetation.

Arnold et al. (2021) detailed the development a “priming system” to induce a preference for crop flowers in commercial bumblebees. This was based on previous studies showing that caffeine improved memory retention for floral odours in a honeybee assay (Wright et al., 2013). The priming system worked by pairing a reward (sucrose solution containing 0.1 mM caffeine) with a crop-related cue (an odour blend resembling the crop floral bouquet) in a feeder device inserted into the bumblebee nest. Exposure to this priming system in the nest caused the bees to form a positive memory association for the crop odour when foraging outside the nest. The caffeine was predicted to enhance the memory association to increase foraging efficacy. In the laboratory, priming in this way created a significant initial bias for *Bombus terrestris* workers to visit target artificial flowers, bearing crop floral odours, over distractor artificial flowers bearing a different odour. The ability to manipulate the behaviour of commercial bees to encourage them to forage more on the crop and less on non-crop flowers has potential value to fruit growers. This is different to increasing overall activity of bees by using caffeine as a stimulant, as that may simply result in more managed bees foraging in hedgerows and headlands. It is important to understand how this priming system performs in the field, where crops may vary in their floral odour production, and bees must deal with changing environmental and nutritional conditions.

Caffeine has various effects on bee behaviour, including changing activity patterns (Si, Zhang and Maleszka 2005), responses to reward (Couvillon et al. 2015), memory formation (Wright et al. 2013), and colour preference (Jones and Agrawal 2022). However, in particular, caffeine appears to enhance pollinators' memory of a previously rewarding experience associated with an odour (Wright et al. 2013), which could be relevant to flower-constancy (fidelity to a particular species of plant by a bee, improving conspecific pollen transfer). As a naturally occurring compound in the nectar of plants including *Coffea*, *Citrus* and sainfoin (*Onobrychis viciifolia*) (Kretschmar and Bauman 1999; Wright et al. 2013; Folly et al. 2021), it can conceivably be encountered by various foraging bee species and may offer the plant an adaptive advantage. At low doses, there is no evidence it is harmful to bees, who may consume it preferentially. We carried out a semi-field trial to test how previous joint exposure to caffeine and a floral odour, affects flower choices in a complex foraging environment with real crop plants. This enabled us to explore the potential for a caffeine-based priming system to keep bumblebees on the crop, increase their pollination activity, and ultimately improve fruit yields.

The trials sought to test a series of hypotheses: 1 Caffeine priming will increase the ratio of visits made by primed bees to the crop rather than the distractor plant, i.e. induce improved forage-focus (main research question); 2. Caffeine priming will (secondarily) increase the overall activity of primed bees foraging on the crop; 3. Crops pollinated by primed bees will produce larger and higher-grade fruit due to better pollination.

2 Materials and Methods

Semi-field trials were set up in Kent, UK (‘Ditton Rough’, N 51.289148°, E 0.455042°). Two trials were conducted in 2017 (henceforth: Trial 1a/b and Trial 2) and one trial in 2018 (Trial 3). For each trial, there were four replicate tunnels for each of three treatments (12 tunnels in total for each trial) (Fig. S1, S2B). Tunnels were 12 m × 2 m × 2.1 m and covered with bee-proof mesh (1 mm × 1 mm, Knowle Nets Ltd, Bridport, Dorset, UK). The arch of the tunnels was covered with a horticultural polythene and cut at 1 m above the ground to allow ventilation (Fig. S2A). The tunnels were orientated in a north–south direction.

In each tunnel, peat grow-bags containing the strawberry plants were placed down the centre (Fig. S2A,C). Plants were fertigated with drip irrigation, and runners and excess fruits removed and destroyed. This follows standard management of UK commercial strawberry crops. A white delta trap with a data logger was placed in each tunnel to record temperature and humidity every 30 minutes. No insecticides were used; occasional aphid outbreaks were managed by spot application of 5% detergent in water, and fungicides were applied at least 2 weeks before the experiment started, and again after flowering before fruiting, to manage mildew.

The distractor plants (plants that offered alternative food provision, with potential to distract bumblebees from strawberry flowers) were placed at the north end of the tunnels. This simulated a situation in which bumblebees in commercial tunnels would either be distracted by foraging on weeds within the crop or by leaving the tunnels and seek alternative wildflowers. Few suitable distractor species have a flowering period as long/flexible as strawberry; most of our replicates were carried out with potted lavender species as distractors as these grow well in pots and have robust, long-lasting flowering spikes. An overview of the 3 trials is shown in Table 1 with individual trial details described below.

Table 1. Details of the three field trials testing the optimisation off commercial bumblebee foraging of strawberry plants by priming with caffeine and an odour in the presence of distractor plants, including assessments.

Trial	**Parameters assessed**	**Colony Treatments (3)**	**Replicates**	**Strawberry Variety**	**Distractor plant**	**Dates**	**Analytical Model**
1	Flower visitation Colony growth	sugar sugar+odour sugar+odour+caffeine	4	Amesti	Phacelia	12 Jul - 13 Jul 2017	1a
					French lavender	18 Jul - 1 Aug 2017	1b
2	Flower visitation Colony growth Fruit quality	sugar sugar+odour sugar+odour+caffeine	4	Elsanta	French lavender	15 Aug - 24 Aug 2017	2

3	Flower visitation Fruit quality	sugar sugar+odour sugar+odour+caffeine	4	Elsanta	French + English lavender	30 May - 20 Jun 2018	3

2.1 Trial 1a/b (12/07/2017 – 01/08/2017)

Twelve tunnels were used, each with its own bee colony, 4 tunnels/treatment. Each tunnel contained 8 grow-bags, each planted with 9 cold-stored, runner cv. Driscoll's Amesti strawberry plants (Fig. S2D) (= 72 plants/tunnel). The distractor plants were 3 large (6.5L) potted *Phacelia tanacetifolia* plants (grown from seed) per tunnel in the first two assesment days (Trial 1a), followed by 2 potted lavender plants (3L, French lavender *Lavandula dentata*, B&Q, UK; Trial 1b), due to the *Phacelia* plants beginning to wilt.

2.2 Trial 2 (15/08/2017 – 24/08/2017):

Twelve tunnels were used, each with its own bee colony, 4 tunnels/treatment. Each tunnel contained 8 grow-bags, each planted with 9 cold-stored, runner cv. Elsanta strawberry plants (= 72 plants/tunnel), and 2 potted *Lavandula* x *intermedia* cv. Abrialii plants (9 cm pots, source Downderry Nursery, Kent, UK) as distractors. The strawberry variety was switched to a widespread commercial everbearing variety that produces high nectar volumes (Symington and Glover, 2024) to allow additional data collection when the Amesti stopped flowering. We used a lavender variety that was available and flowering in this time period.

2.3 Trial 3 (30/05/2018 – 20/06/2018):

Twelve tunnels were used, each with its own bee colony, 4 tunnels/treatment. Each tunnel contained 20 grow-bags, 10 in two rows per tunnel, each planted with 10 cold-stored, runner cv. Elsanta strawberry plants each (= 200 plants/tunnel), as a larger number of plants were expected to reduce risk of over-pollination observed at some points in 2017. Distractor plants in this trial were 2 French lavender, *Lavandula dentata,* and 2 English lavender, *Lavandula angustifolia*, grown in 3L pots, sourced from Downderry Nursery, Kent, UK, as these were in flower during the trial period.

2.4 Hives

Bombus terrestris audax nests ("hives") were supplied by Biobest NV (Westerlo, Netherlands), without nesting material, and new hives were used in each trial. Individuals in hives were reduced to 30 at the beginning of Trial 1 and 24.8 ± 0.27 (mean ± s.e.m.) at the beginning of Trial 2, with excess workers euthanised by being frozen at -20°C. Bumblebee hives used in Trial 3 were all of similar starting size 64.6 ± 1.4 bees per colony, but three subsequent rounds of worker-removal took place during the experiment, of average 20, 20 and 7 workers (numbers/hive recorded), to prevent over-pollination, which would damage the flowers. Hives were placed inside the tunnel doorway at the south end. Bees had not been exposed to strawberry flowers prior to the first assessment date.

2.5 Treatments

In all trials, there were 3 bumblebee colony treatments: A) sugar and odour (henceforth referred to as "sugar+odour" or "odour-only", i.e. odour but no caffeine), following the same principle as Molet et al. (2009) that exposure to odour inside the nest affects preferences for bumblebees foraging outside it, B) sugar plus caffeine and odour ("sugar+odour+caffeine"), anticipated to work like sugar+odour, but with a stronger and more persistent preference induced, C) sugar only ("control"). In all trials, 4 tunnels (and thus bee colonies) allocated to each treatment, meaning a total of 12 colonies were tested under each treatment.

We prepared 600 ml of the sugar solution at 37.5% sucrose (w/w) for each priming event (360 g of sucrose in 600 ml distilled water). For the caffeinated priming treatment (sugar+odour+caffeine), 0.1 mM caffeine sucrose solution was prepared adding 2 ml of the 0.01 M stock solution of caffeine to 198 ml of the sugar solution prepared previously and dispensed from an inverted glass specimen tube inserted into a 50 ml standard Falcon conical tube, with 16 x 1 mm holes drilled into the lid (Figure 1A-D); this formed the gravity feeder. The device was then inserted into a pre-prepared hole in the lid of the hive allowing the bees to feed from the holes (Figure 1E-F). Solutions were refrigerated until used. Each hive was supplied with 40 ml of the treatment solutions, sugar (A and C) or sugar and caffeine (B) in a gravity feeder in the nest 24 to 48 hours before the first assessment and replenished every Monday for the duration of each trial.

The odour solution was formulated separately from the sugar solution. The floral blend was the same as that used in Arnold et al. (2021), based on analyses of floral odour from Elsanta strawberry flowers, but omitting (*E*,*E*)-α-farnesene, which was not available in sufficient quantities (Table 2). The blend was formulated in paraffin oil. In trial 1, for treatments A and B, four odour applications were used, comprising 50 µl of the odour solution on a 27 mm filter paper at the bottom of the feeder (Figure 1A-E; Table 2). These were anticipated to evaporate relatively quickly, meaning that the exposure of the bees would decrease over time. In Trials 2 and 3, two odour applications were utilized for treatments A and B, as a pipette with odour solution attached to the side of the feeder (Figure 1G; Table 2). These were expected to evaporate more slowly, meaning a more consistent exposure to the odour. Floral odour dispensers were constructed from opaque, polypropylene pipette tips (1 ml; Fisher Scientific, part number FB34621) with a 0.2 mm aperture. The blend (100 µl) was impregnated onto a cellulose acetate cigarette filter (14 × 6 mm; Swan, High Wycombe, Bucks., UK) placed in the pipette tip. The latter was sealed with a Teflon-lined crimp seal (11 mm; Chromacol, Welwyn Garden City, UK).

Release rates from the pipette tip dispensers were measured as described in Arnold *et al.* (2021). Dispensers were maintained in a wind tunnel at 27°C and 8 km/h windspeed. At intervals, volatiles emitted were collected on Porapak resin and analysed by gas chromatography. The composition of the blend released changed with time, but the results confirmed the dispensers continued releasing during the experiments lasting up to 21 days.

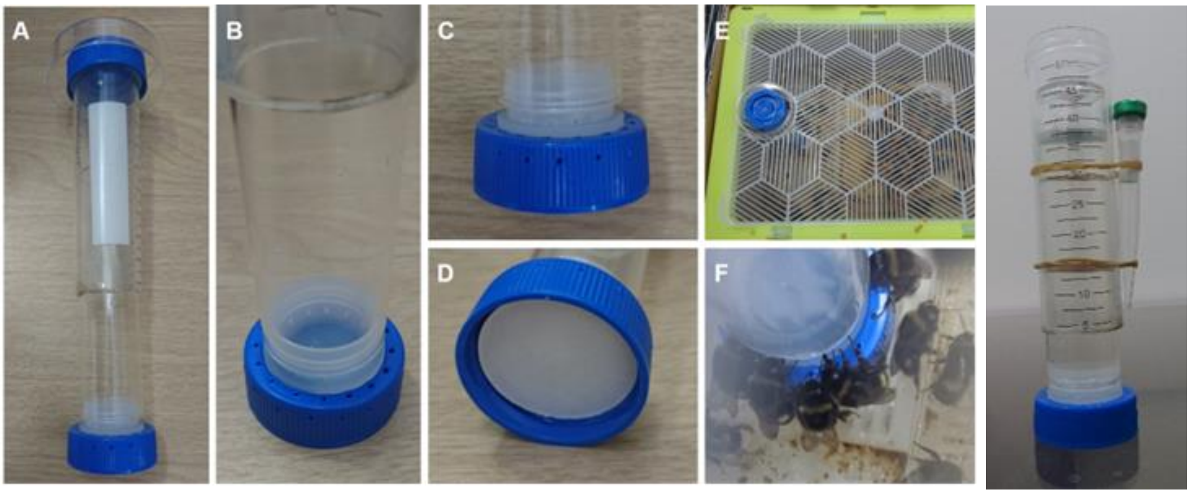

Figure 1. Gravity feeders for the application of priming solutions. A. General layout of the feeder, B. glass tube holding sugar solution, C. holes for odour release (blue lid) and sugar solution release (white lid), D. odour filter positioned on the bottom of the feeder tube, E. gravity feeder inserted in the colony, F. feeding bumblebees, G. odour pipette positioned on the side of the feeder tube.

Table 2. Blend of floral volatiles in paraffin oil used in experiments and release rates from pipette tip dispensers measured at 27°C and 8 km/h windspeed

		Mean release rate (ng/h; 27°C; *N* = 2)	
	Loading (mg/100 µl)	0 d	21 d
(*Z*)-ocimene	0.7	594	272
(*E*)-ocimene	1.3	1250	582
(Z)-3-hexenyl acetate	1.0	824	354
nonanal	1.2	449	255
decanal	0.3	245	196
benzaldehyde	1.8	293	176
methyl salicylate	2.1	99	146
p-anisaldehyde	15.0	153	377

2.6 Assessments

2.6.1 Bumblebee foraging behaviour – forage-focus and overall activity (hypotheses 1-2)

At least 20 hours before the first assessment, the door to the bumblebee hive was opened to allow bees to forage on the strawberry plants. Behavioural assessments took place as shown in Table 3.

In trial T2018, the exit door to the nest box was periodically closed to prevent flower damage (as the colonies grew and foraged enthusiastically, even on flowers that had not yet dehisced, and this can result in misshapen fruit (Mommaerts et al. 2011)). In between assessments, the feeder device was also removed to allow access to the hive sugar reservoir, and a teaspoon of pollen added to provide supplementary protein for the colony. The hive's exit door was reopened 30 min before the next assessment to allow bees to forage and then closed after each assessment.

Table 3. Behaviour assessment dates for the 3 trials (Trials 1a/b, 2 and 3).

Assessment	Assessment code	Trial 1a/b	Trial 2	Trial 3
1	a	12/7	15/8	30/5 am
2	b	13/7	16/8	31/5 am
3	c	18/7	17/8	31/5 pm
4	d	19/7	18/8	01/6 am
5	e	20/7	22/8	05/6 am
6	f	21/7	23/8	06/6 am
7	g	25/7	24/8	12/6 am
8	h	26/7	-	12/6 pm
9	i	27/7	-	13/6 am
10	j	28/7	-	13/6 pm
11	k	31/7	-	19/6 am
12	l	01/8	-	19/6 pm
13	m	-	-	20/6 am
14	n	-	-	20/6 pm

15	o	-	-	26/6 am
16	p	-	-	26/6 pm
17	q	-	-	27/6 am

Assessments took place as follows: In each tunnel, an experimenter stood at the south end of the tunnel and observed activity along the whole crop, and distractor plants beyond. The experimenter assessed the number of bee visits to strawberry and distractor plants (classed as landing on a flower) during 10 min observation windows (hypothesis 1). They also recorded the number of bees outside the hive, the number of in- and out-flights (hypothesis 2), and the number of strawberry and distractor flowers/inflorescences. In each assessment session, two or three experimenters worked in parallel in adjacent tunnels, e.g. the assessment of one tunnel would be: 14:00-14:10 - counting bees out of nest box and number of distractor plant and strawberry viable and total flowers in the tunnel; 14:10-14:20 - bumblebee numbers in and out of hive;14:20-14:30 - number of visits to distractor plant and strawberry flowers, before all experimenters moved to the next tunnel until all had been assessed. The order in which the twelve tunnels were assessed was randomised between visits.

Twelve foraging behaviour observations were made in trial 1, 7 observations in Trial 2 and 17 observations in Trial 3.

<u>2.6.3 Fruit quality assessments (hypothesis 3)</u>

In order to test hypothesis 3, harvested fruit was assessed for score, fresh mass (g) and diameter (mm) in trial 2 and 3. Aditionally the number of fertile seeds was also assessed in Trial 3.

Open receptive (stigmas accessible and at least 75% visible, flower not senesced or with black brown anthers) flowers (30-50) were tagged during the period of each field trial. Fruits were picked and frozen immediately after picking once they began to turn red, until all tagged fruit had been picked. Fruit mass was measured on a scale to the nearest 0.01 g , width of each fruit measured with callipers to the nearest 0.01 mm and then fruit was catagorised into 4 different classes depending on shape symmetry and achene distribution as described in Table 4 (following the approach in e.g. Hodgkiss *et al.*, (2018)). In T2018, flowers were protected by a cage mesh that covered up to 9 plants or netting bags that covered individual flower until fruits start to set. This prevented bumblebees from overworking the flowers, leading to deformed fruit.

Table 4. Criteria used for fruit quality scoring in Trials 2 and 3

Score	0	1	2	3
Definition	evenly-spaced achenes with radial symmetry	evenly-spaced achenes with nearly radial or bilateral symmetry	areas of tightly-clustered achenes and asymmetrical but not majorly malformed	areas of tightly-clustered achenes and majorly malformed

2.7 Statistical analyses

We assessed how the priming treatment affected the total number of flower visits and the bee's flower choices between the strawberry flowers and the distractor flowers. We applied general linear mixed models (r-package 'lme4' (Bates et al. 2015)) and fitted the total number of visits recorded in one observation round to a poisson distribution using a log link function and the proportion of strawberry visits to a binomial distribution using a logit link function. In addition to the priming treatment, we included the numbers of strawberry and distractor flowers as fixed effects to account for varying flower abundances across the trial period (flower abundances were scaled to zero mean and unit variance). We ran separate models for each trial. As the distractor plant was changed from *Phacelia* to lavender in the first trial (Trial 1), we split the data and used two separate models accordingly (models 1a and 1b as referenced in Table 1). We included the observation day as random intercept in the models for the trials in 2017 (coded as 1a and 1b (both of which referred to data within Trial 1) and 2 (Trial 2) respectively). In Trial 3 (model 3 in Table 1), we performed assesment rounds in the morning and the afternoon on some days (Table 4) and included the daytime nested in the assessment day as random intercept. To account for overdispersion in both, the binomial and the poisson models, we included an observation level random intercept in all models (Harrison 2014).

The models estimate the fixed effect of the priming treatments as difference in log number of visits (poisson model) and the difference in log odds (binomial model) of visiting strawberry flowers against the distractor flowers relative to the control treatment. We assessed the uncertainty of the fitted fixed effects using non-parametric boostrap and calculated 95% confidence intervals based on 10,000 bootstrap resamples (r-package 'boot' (Canty and Ripley, 2021; Davison and Hinkley 1997)). To visualize the total number of flower visits and the proportion of strawberry flower visits across the assessment periods we calculated the estimated marginal means adjusted for different strawberry and distractor flower abundances (r-package 'emmeans' (Lenth 2022)).The effect of the priming treatments on colony performance in the trials in 2017 was assessed using generalized linear models where the numbers of bees was fitted in the colonies to a poisson distribution. Following a Chisquare test on the significance of the main treatment effect, we tested for pairwise differences between the priming treatment using Tukey's HSD test (r-package: 'emmeans' (Lenth 2022)).

Fruit quality was characterised as fruit mass and shape. We tested for the effect of the priming treatments on fruit mass using a linear-mixed effect model (r-package 'lme4' (Bates et al. 2015)) and accounted for the non-independence of fruits sampled from the same polytunnel incorporating tunnel as random intercept. To test for treatment effects on the fruit shape scores, we applied a cumulative link mixed model (r-package 'ordinal' (Christensen, 2019)). Analogue to the model on fruit mass we accounted for tunnel as random intercept. Significance of the priming treatment was assesed using a likelihood ratio test against the intercept only models.

3 Results

3.1 Visitation activity and flower focus (hypotheses 1-2)

Overall, the bees in the sugar+odour+caffeine treatment visited fewer flowers in the 10-minute assesment periods during Trial 1, when lavender was the distractor plant (Figure 2 B,E), compared to bees in other treatment groups (contradicting our second hypothesis). In 2018 only, the sugar+odour+caffeine treatment conversely resulted in overall higher flower visitation (Figure 2 D,E) (so in one replicate, this hypothesis was supported). Overall (Figure 2F) the behaviour was similar.

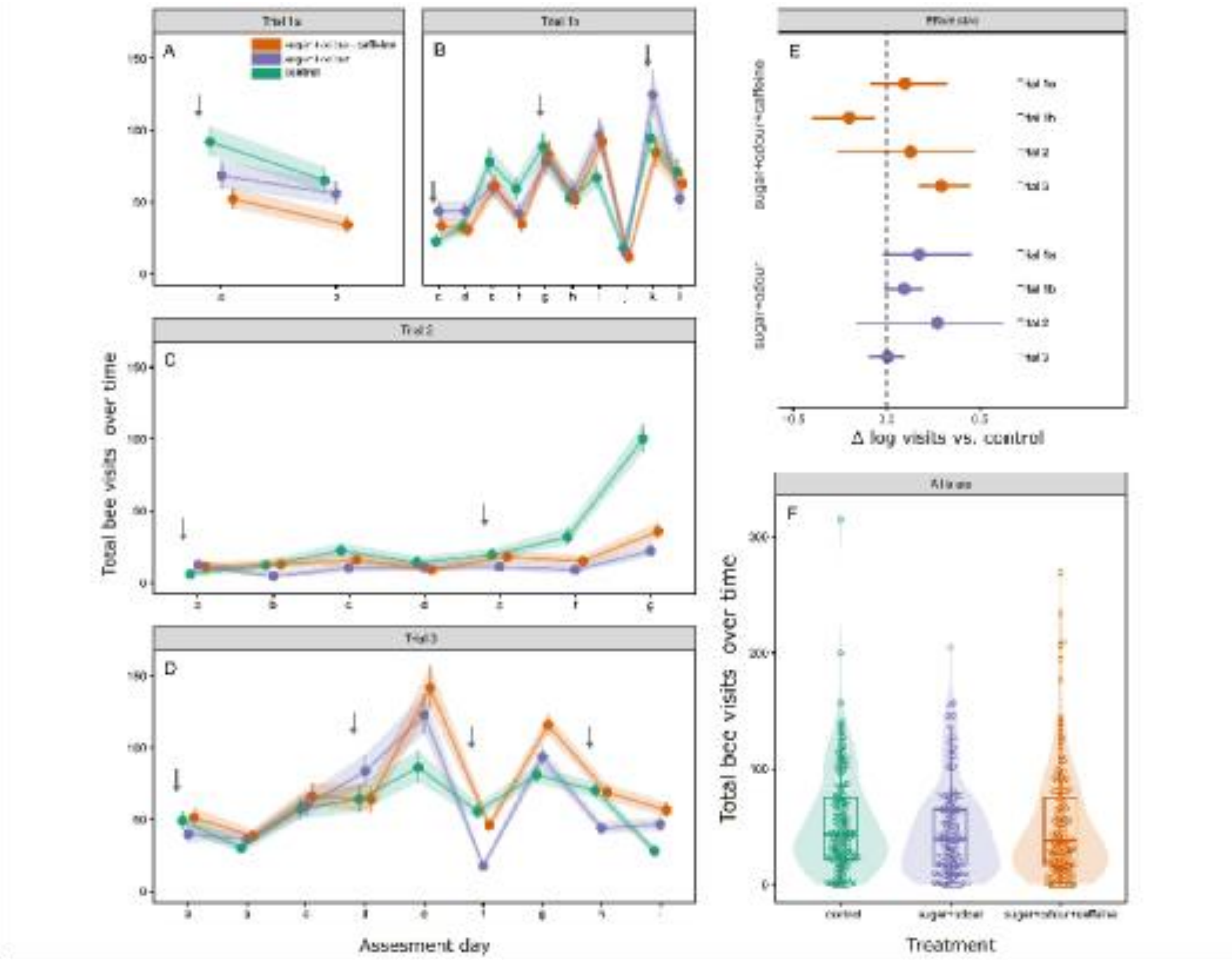

Figure 2. Treatment effects on the **total** number of bumblebee flower visits recorded in 10 minutes. Panels A, B, C and D: Shown are the estimated marginal means (adjusted for the covariates strawberry flowers and distractor inflorescenses) for each assessment day (see Table 4) in each year. Covariate means (number of flowers present) are provided in Table S1. Arrows indicate when the priming treatments were applied. The shaded area and the error bars indicate the pointwise 95 % confidence limits of the estimated marginal means. Panel E shows the effect sizes as difference in log visits to the control treatment (dashed line) for the sugar+odour and the sugar+odour+caffeine treatment for all trials. Error bars indicate the 95% bootstrap confidence interval of the effect size. Numbers after a year indicate the trial number, pha = *Phacelia*, lav = lavender plants.

In the first trial (T2017A), where the bees could choose between the strawberry variety Amesti and *Phacelia* (Figure 3A) and later French lavender (Figure 3B) as distractor plants, the sugar+odour+caffeine priming shifted their preference towards strawberry flowers compared to the control treatment (Figure 3C). The sugar+odour priming treatment did not result in a shift of bee flower preferences (Figure 3E, lower bars). In the second trial (T2017B), the bees could choose between the strawberry variety Elsanta and French lavender (Figure 3A). Neither the sugar+odour+caffeine nor the sugar+odour priming treatments affected the bee flower preferences compared to the control treatment (Figure 3E). In Trial 3, bees had to choose between the strawberry variety Elsanta and French/English lavender as distractor plants (Figure 3E). Bees in the sugar+odour+caffeine priming treatment visited relatively more distractor flowers compared to the control treatment, whereas the sugar+odour priming treatment showed no effect on bee preferences (Figure 3E). Thus, there was little support for hypothesis 1, that priming treatments including caffeine increased bee foraging on crop relative to non-crop flowers. Overall (Figure 3F) the behaviour was similar between treatment groups.

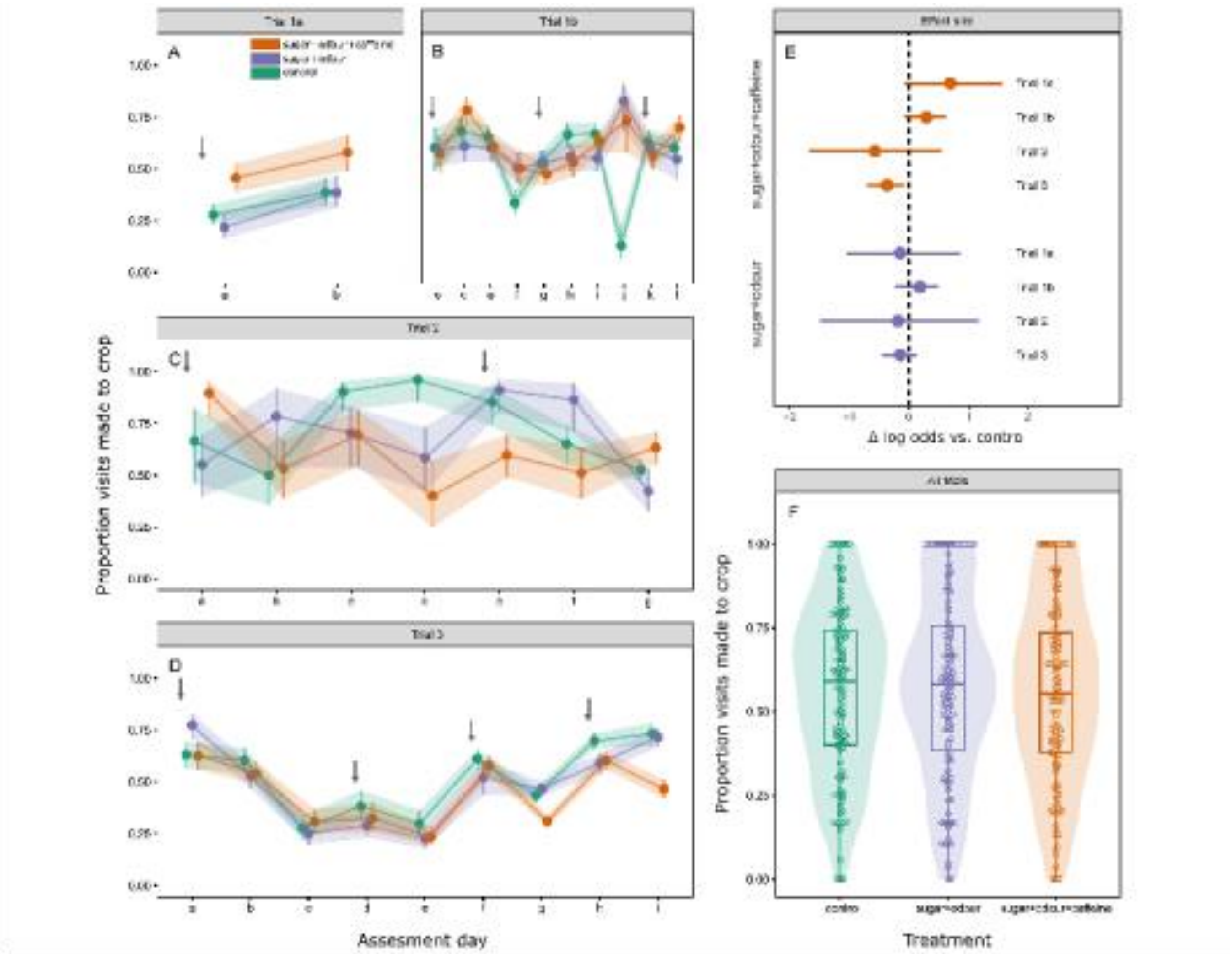

Figure 3. Treatment effects on the **proportion** of bumblebee visits to **strawberry flowers**. Panels A, B, C and D: Shown are the estimated marginal means (adjusted for the covariates strawberry flowers and distractor inflorescenses) for each assesment day (see Table 3; covariate means in Table S1). Arrows indicate when the priming treatments were applied. The shaded area and the error bars indicate the pointwise 95 % confidence limits of the estimated marginal means. Panel E: shows the effect sizes as difference in log odds to the control treatment (dashed line) for the sugar+odour and the sugar+odour+caffeine treatment for all trials. Error bars indicate the 95% bootstrap confidence interval of the effect size. Numbers after a year indicate the trial number, pha = *Phacelia*, lav = lavender plants.

3.3 Fruit quality (hypothesis 3)

We assessed fruit quality in Trials 2 and 3 and found no effects of the priming treatment on strawberry fruit mass or shape (Figure 4A-D) after controlling for polytunnel identity, indicating no support for hypothesis 3, that tunnels containing bees exposed to caffeine priming would produce larger/better fruit.

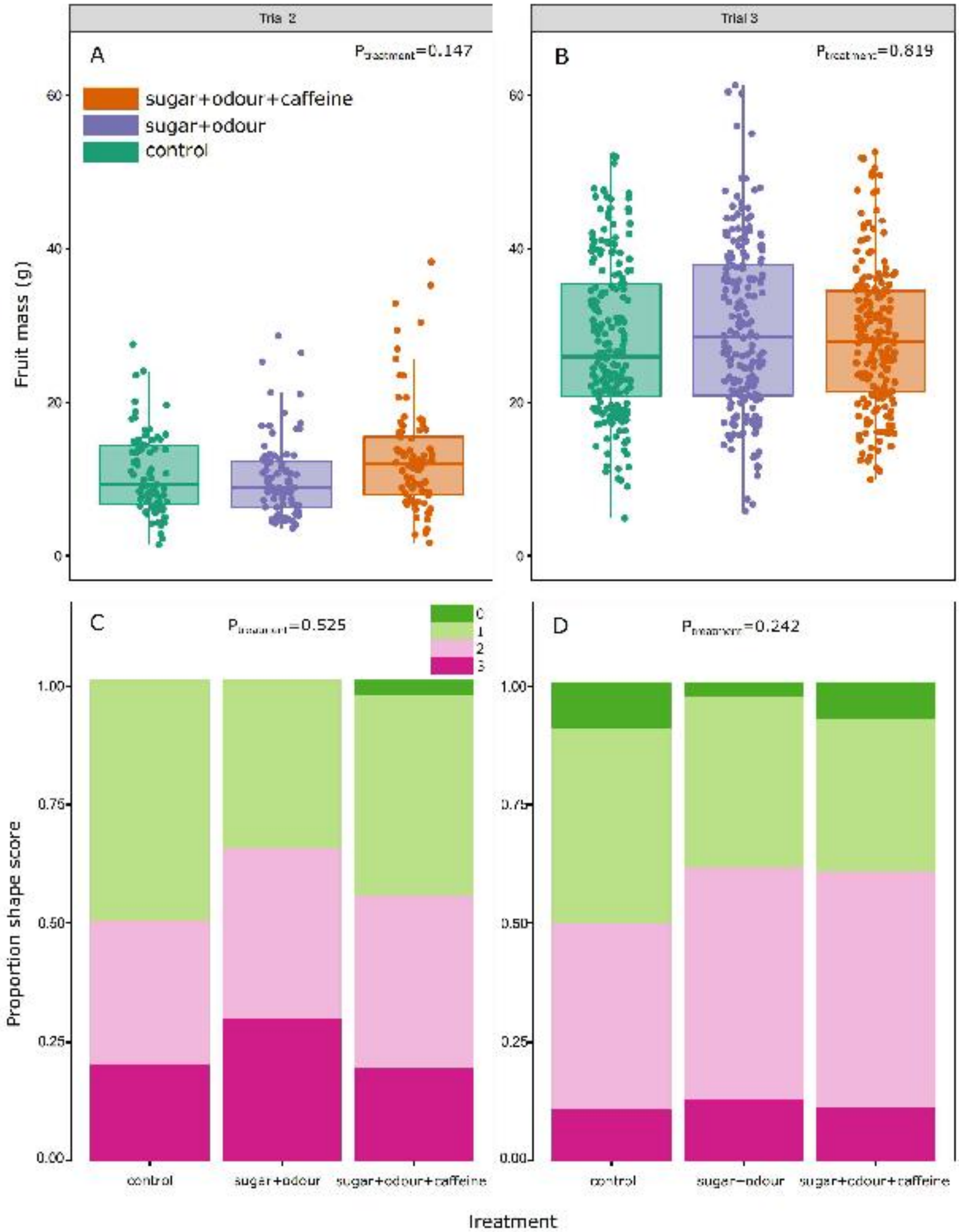

Figure 4. Fruit mass and shape scores from Trials 2 (A and C) and 3 (B and D).

4 Discussion

We hypothesised that, as in the laboratory, priming bumblebees with caffeine in combination with a target floral odour and sugar solution would lead to increased visits to crop flowers compared to non-crop flowers in mini-polytunnels. However, we did not find compelling evidence for a meaningful effect of caffeine priming on bumblebees in semi-field trials. Superficially, it seemed there was a link between caffeine priming and higher fruit grading. However, this effect disappeared once tunnel identity was controlled for, indicating that conditions within the tunnels more likely influenced fruit quality than the caffeine treatment. However, in some trials the priming did improve the ratio of target to distractor visits, and in no trial was the priming treatment detrimental to the crop overall. Thus, we suggest that this system has potential for further research and development.

In Trial 1, caffeinated bees visited more target flowers relative to distractor flowers. Conversely, in Trial 3, caffeinated bees visited more distractor flowers. English lavender is a highly attractive flower to bumblebees (Garbuzov and Ratnieks, 2014), and Elsanta variety of strawberries (used in Trials 2 and 3) is reportedly not very appealing to bumblebees (Ceuppens *et al.*, 2015) (there is little evidence about Amesti's attractiveness); strawberries in general are not considered highly bee-attractive. Consequently, it may be that the combination of relatively unappealing target and highly appealing distractor was not offset by the relatively subtle effect of the priming device. Some strawberry varieties have a more complex odour profile that bees may find more naturally attractive (Ceuppens *et al.*, 2015), and the priming device may perform better in those contexts.

Over time, organisms' responses to sensory stimuli can decrease (habituation) if they are constantly or repeatedly exposed to the stimulus (Chandra and Singh 2005), specifically if the stimulus is not a reliable predictor of reward. In our setup, with constant odour in the nest, the bees may learn to disregard it. Similarly, receptors sensitive to caffeine may downregulate over time if an organism is exposed to caffeine constantly (Glendinning *et al.*, 2001). As a result, the bees may have reduced their response to both the odour and the caffeine with the extended exposure in our experiment. It is possible that a pulsed or intermittent exposure would see a stronger behavioural effect.

Another consideration is that real flowers vary in their volatile organic compound (VOC) emission profile over time (Delle-Vedove *et al.*, 2017), and as a result the odour in the nest will not always be a good match for that on strawberry flowers. The extent to which bees generalise odours is not completely understood, whereas the understanding of how bees generalise and categorise colours and patterns is better characterised (Giurfa 2021).

We found no evidence that the caffeine treatment was harmful to bumblebees (following similar observations in honeybees from Marchi, Palottini and Farina (2021)) (see supplementary material, Fig. S3). While commercial bumblebees are sold for single-use deployment in the crop, and are removed once the colony comes to the end of their limited natural lifespan, one can still ask ethical questions around modifying their behaviour (Boppré and Vane-Wright, 2019). Our trials saw no difference in performance (growth, reproduction) in colonies where caffeine was provided compared to controls, and therefore no evidence that it has toxic effects or sublethal implications (Supplementary Information).

This study could conceivably serve as a cautionary tale, that promising laboratory results in insect behavioural assays do not always translate into ecologically-relevant behavioural outcomes. While there is evidence that caffeine can modify bee behaviour (e.g. Si, Zhang and Maleszka 200; Couvillon et al. 2015; Jones and Agrawal 2022)), the practical applications in pollination ecology require more refinement. This is in contrast to some comparable work with odour-priming honeybees that showed crop-relevant field results (Farina et al. 2020).

The priming odour used in these experiments was an approximation to the common components found to be emitted by three varieties of strawberry flowers (Arnold et al. 2021).

In the laboratory bioassays reported by Arnold et al. (2021), pipette tip dispensers containing this blend were used to prime the bumblebees and also as the attractive odour in artificial flowers. These bioassays demonstrated that inexperienced bees primed with caffeine made more initial visits to target artificial flowers emitting the target odour, compared to control bees or those primed with odour alone. However, in the field situation, the natural odours from strawberry flowers will likely differ from the artificial odour used to prime the bees in the hive. The odour blends emitted by flowers of different varieties of strawberry are extremely variable, as demonstrated by Arnold et al. (2021) and other authors (e.g. Klatt et al. 2013; Cueppens et al. 2015; Mozūraitis et al. 2020). For example, in some varieties (*E,E*)-α-farnesene (not present in our blend) was reported to be the major component, and in other varieties *p*-anisaldehyde (present in our blend) was the major component. (*E,E*)-α-farnesene is a difficult compound to obtain in pure form and so is not practical to include in field-scale priming; it is not known whether it is important in mediating recognition of strawberry flowers by bumblebees. It is present in variety Elsanta (Arnold et al. 2021), and preliminary data suggest that Amesti is broadly similar to Elsanta in volatile profile, so it is possible that the bees treated the real flowers as different; this may also be a constraint for this type of technology in strawberries specifically. The odours may also be influenced by the floral microbiome (Crowley-Gall et al. 2021), although with the foraging effort we observed and the low nectar secretion rates in strawberry (Symington and Glover, 2024) it is unlikely that the crop had nectar standing in flowers for extended periods during the day.

In the trials reported here, both Amesti and Elsanta varieties of the strawberry crop were used but neither showed strong evidence of a change in preference by the bumblebees, implying that the result was not just a result of using a “poor” variety. On the other hand, linalool and linalyl acetate, the major components of the odour of the lavender flowers used as distractor plants, were not present in the priming odour, and the priming odour was more similar to the crop than the distractor. However, a more accurate matching of synthetic priming blend and natural target flower odour may be necessary to realise the full potential of caffeine to enhance target flower visitation.

4.1 Economic evaluation

The priming device can be created relatively cheaply – the majority of chemical components in the odour blend are easily obtained from standard suppliers and the other elements are typical laboratory consumables. As a result, we anticipate that the cost of fitting a priming device to commercial hives would add no more than a few pounds to the overall purchase price. However, the results obtained here do not currently justify this modification. Further work with different odour blends and release rates, more investigation of pulsed versus continuous application, and perofrmance under a wider range of conditions needs to be evaluated.

4.2 Conclusion

While priming bumblebee colonies with caffeine and floral odour to increase forage-focus showed promise in the laboratory, semi-field trials at present do not show economically- or ecologically-relevant changes in behaviour. Some slight changes in the activity patterns and ratios of target to distractor flower visit numbers were seen in bees receiving caffeine treatments, but these were inconsistnt and hard to interpet.

Author contributions

Designed the experiments: MTF/CS/SEJA/JHD/FLW/PCS; Developed and prepared odour dispensers: DRH/DIF; Collected data: JHD/CS; Analysed data: JHD; Discussed the results: SEJA/JHD/MTF/FLW/PCS; Wrote the paper: MTF/SEJA/JHD/CS; Commented critically on the manuscript: All authors.

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Data availability

Raw data sets are available via OSF (https://osf.io/573w6/?view_only=95f5a994248b46e8a249bc83d9c885bd pre-publication, data set to be made public on manuscript acceptance)

Declaration of competing interests

FLW is an employee of Biobest NV. FLW was involved in the study design and interpretation but the funding bodies themselves were not involved in the design, data collection, analysis or decision to publish. At time of submission the authors hold no patents related to this work.

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