

Supplementary Material to 'Flowering resources modulate the sensitivity of bumblebees to a common fungicide'

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Appendix A. Experimental set-up and site preparation.

The experimental field was ploughed and harrowed on 13 March 2020. In April, the frames of a total of 40 metal cages were erected uniformly across the field (4 rows with 10 cages) and the soil was tilled. Of the cages, 13 were randomly selected to be sown with *Phacelia*, 13 with buckwheat, and 14 with a custom seed mixture consisting of six bumblebee-attractive plant species all from different plant families (Table S1). *Phacelia* was sown on 19 April at a seeding rate of 13 kg ha⁻¹ and on the following day, the seed mix was sown at a rate of 11 kg ha⁻¹. To achieve approximately simultaneous flowering, buckwheat was sown about two weeks later at a rate of 22 kg ha⁻¹. To ensure optimal plant growth, cages were irrigated twice in May with approximately 20 L m⁻² water. In addition, sporadic spots with low plant density in the *Phacelia* or seed mix cages were re-seeded on 30 May and one *Phacelia* cage was relocated to a plot with a denser cover of *Phacelia* that germinated as the field was in the previous year sown with this plant species. Due to an initially lower than expected plant density, all buckwheat cages were re-

sown at the initial seeding rate (22 kg ha⁻¹) on 16 June. One buckwheat cage with a low plant density was removed and its plants were relocated to the other buckwheat cages.

A stratified random sampling approach based on flower density was used to assign cages to the fungicide or control (water) spray treatment. Due to the uneven number of *Phacelia* cages, one *Phacelia* cage was randomly selected to be treated with the fungicide. All other cages were paired based on the flower density and then one of each pair was assigned to be treated with Amistar.

A similar approach based on initial number of living bees was chosen to assign colonies to cages. The first three randomly drawn colonies were assigned to floral mix-control, floral mix-Amistar, and *Phacelia* Amistar, respectively. The remaining 36 colonies were divided into six equally strong groups, which were then randomly assigned to the six flowering resource-treatment combinations. Within each combination, colony assignment to cages was completely random.

Table S1. Composition of the seed mix used in this experiment.

Common name	Scientific name	Plant family	Percentage (w/w)
Common buckwheat	<i>Fagopyrum esculentum</i>	Polygonaceae	40
<i>Phacelia</i>	<i>Phacelia tanacetifolia</i>	Boraginaceae	10
Cornflower	<i>Centaurea cyanus</i>	Asteraceae	20
Field mustard	<i>Sinapis arvensis</i>	Brassicaceae	10
Common mallow	<i>Malva sylvestris</i>	Malvaceae	10
Persian clover	<i>Trifolium resupinatum</i>	Fabacea	10

Table S2. Number of bumblebee colonies that lost their queen during the exposure period, and from which foragers for residue analysis were sampled. Number of pupae and adults with disease signs. Pupal sex ratio. The ratios are stated in respect to either all colonies or all adults or pupae examined in the final assessment.

Parameter	<i>Phacelia</i>		Buckwheat		Floral mix	
	Control	Amistar	Control	Amistar	Control	Amistar
Queen losses in exposure period	1/6	1/7	1/6	2/6	2/7	1/7
Colonies from which foragers for residue analysis were taken	4/6	7/7	3/6	5/6	6/7	7/7
Adults with deformed wings	5/142	2/131	0/99	2/91	3/114	0/133
Parasitized pupae	0/189	1/226	0/135	0/124	0/181	0/227
Parasitized adults	0/142	2/131	1/99	1/91	0/114	0/133
Male pupae	8/189	5/226	1/135	1/124	4/181	0/227

Table S3. Developmental stage of *B. terrestris* pupae based on body colour, eye colour, presence of body hair, and wings.

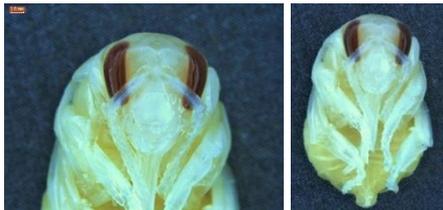
Stage	Eye colour	Body colour	Hair	Wings	Example photos
1	White	White	No	No	
2	Pink-brown	White	No	No	
3	Brown	Light brown	No	No	
4	Brown	Brown	No	No	
5	Black	Black	Yes	No	
6	Black	Black	Yes	Yes	

Table S4. Sample size. *N* indicates the number of bumblebee colonies/cages and *n* the number of observations used in modelling colony development (colony weight, number of living adults, number of dead adults), flower density, numbers of individuals (adult workers, adult males, worker/male cocoons), body size (adult worker intertegular distance, adult worker body mass, pupal worker body mass) and individual foraging performance (number of flowers visited per bumblebee and 2 min).

Parameter	Resource	N		n	
		Control	Amistar	Control	Amistar
Colony development in pre-exposure period	<i>Phacelia</i>	6	7	18	21
	Buckwheat	6	6	16	18
	Floral mix	7	7	21	21
Colony development in exposure period	<i>Phacelia</i>	6	7	28	34
	Buckwheat	6	5	28	23
	Floral mix	7	7	28	33
Individual foraging performance	<i>Phacelia</i>	6	7	35	37
	Buckwheat	5	6	25	32
	Floral mix	7	7	36	40
Individual foraging performance	<i>Phacelia</i>	6	7	51	62
	Buckwheat	5	5	47	39
	Floral mix	6	7	49	58
Flower density	<i>Phacelia</i>	6	7	36	42
	Buckwheat	6	6	36	36
	Floral mix	7	7	42	42
Bee numbers	<i>Phacelia</i>	5	6	5	6
	Buckwheat	5	4	5	4
	Floral mix	5	6	5	6
Adult worker ITD	<i>Phacelia</i>	5	6	71	83
	Buckwheat	5	4	75	50
	Floral mix	5	6	72	95
Adult worker body mass	<i>Phacelia</i>	5	6	71	83
	Buckwheat	5	4	75	50
	Floral mix	5	6	71	93
Pupal worker body mass	<i>Phacelia</i>	5	6	87	89
	Buckwheat	4	4	14	38
	Floral mix	5	6	90	165

Table S5 Effect of flower density. Estimated size of the effect of 1000 flowers (including 95%-confidence interval and *P*-value) on bumblebee parameters based on selected models. Where different effect sizes depending on the resource are reported, the interaction between flower density and resource was retained. Otherwise only the main effect of flower density was retained (Resource = any). Effects with *P*<0.05 are shown in bold.

Parameter	Resource	Effect size	95%-CI		<i>P</i>-value
Colony weight in pre-exposure period (g)	Buckwheat	0.48	-8.27	9.22	0.914
	Floral mix	-0.31	-3.98	3.37	0.870
	<i>Phacelia</i>	11.12	4.17	18.06	0.002
Colony weight in exposure period (g)	Buckwheat	-1.33	-11.16	8.51	0.790
	Floral mix	5.22	2.59	7.85	<0.001
	<i>Phacelia</i>	9.50	3.44	15.57	0.002
Final number of cocoons	Buckwheat	-0.66	-1.34	0.02	0.058
	Floral mix	-0.17	-0.29	-0.05	0.004
	<i>Phacelia</i>	0.31	-0.1	0.72	0.135

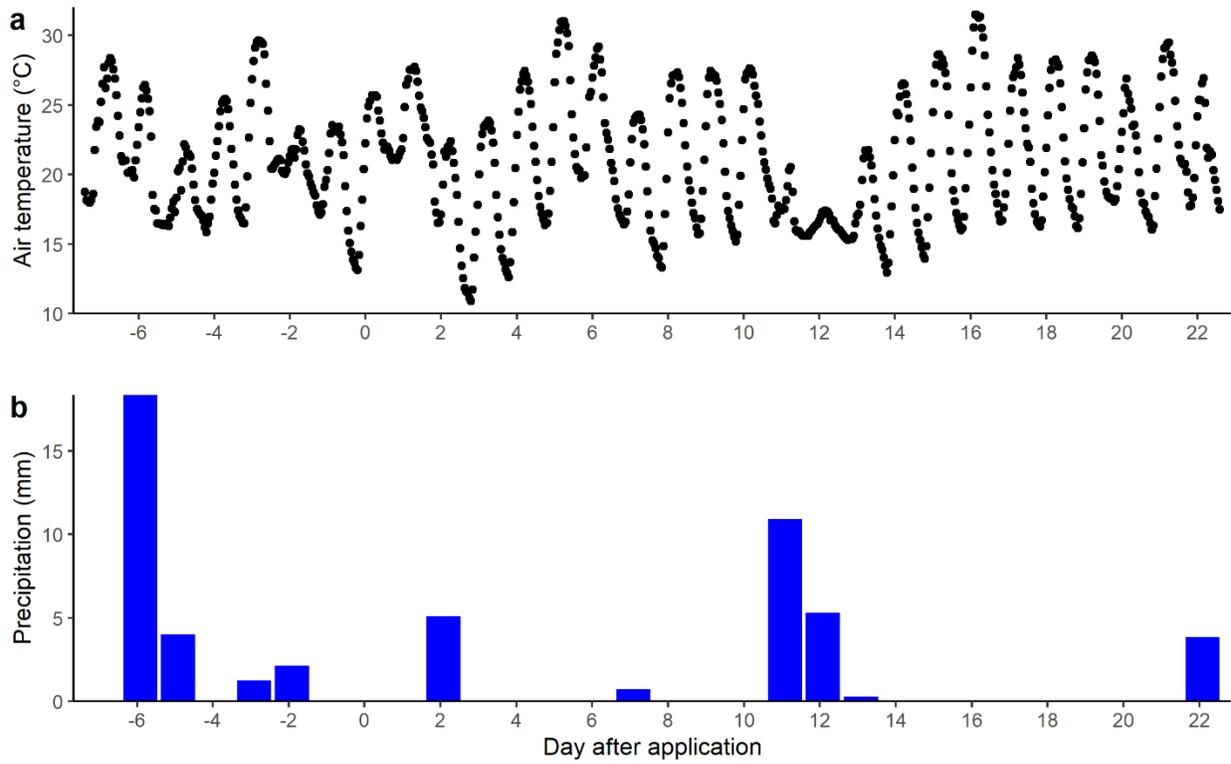


Figure S1. Air temperature (hourly, C°, **a**) and precipitation (daily, mm, **b**) over the study period.

Data are derived from the meteorological city station of the University of Freiburg at 2 m (air temperature) and 53 m (precipitation) above ground.

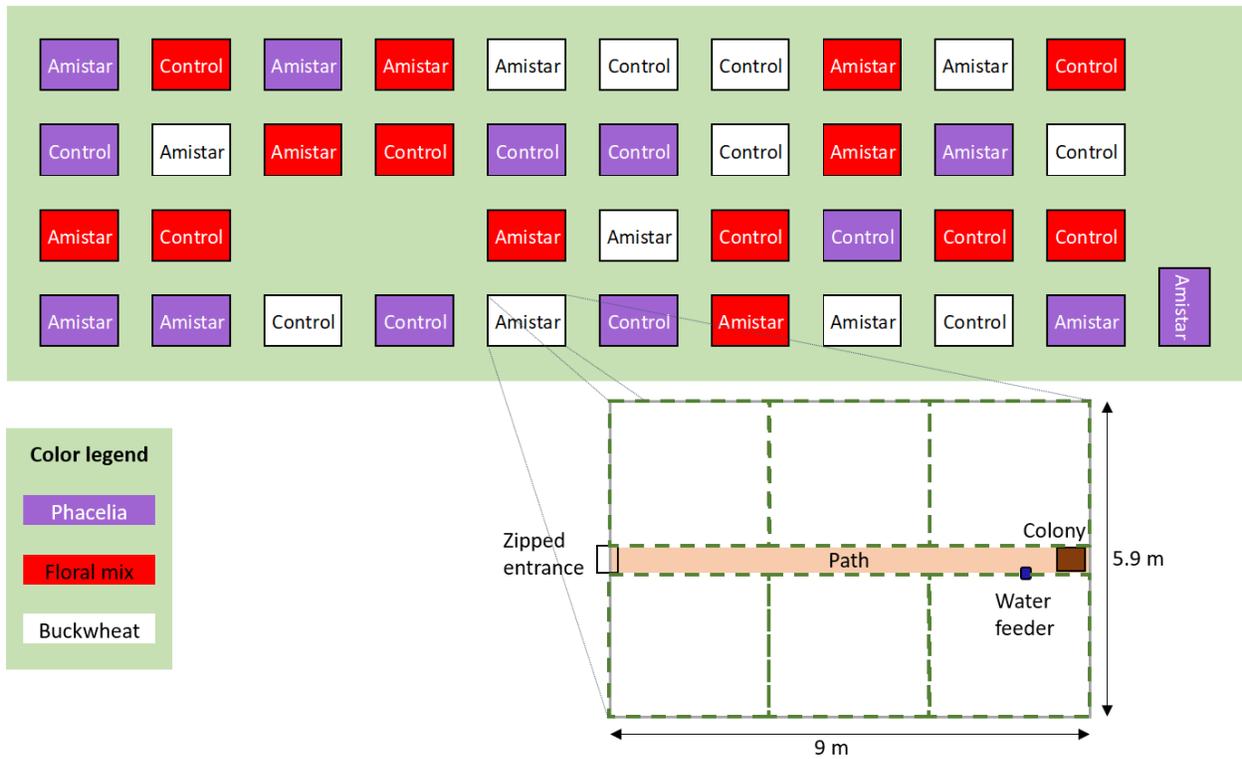


Figure S2. Sketch of the study site (a) and an individual flight cage (b). Flight cages are colour-coded according to flowering resource type. Green dashed rectangles within b are separations based on the poles of the cage and were used for random sampling of plots where flower density was determined.

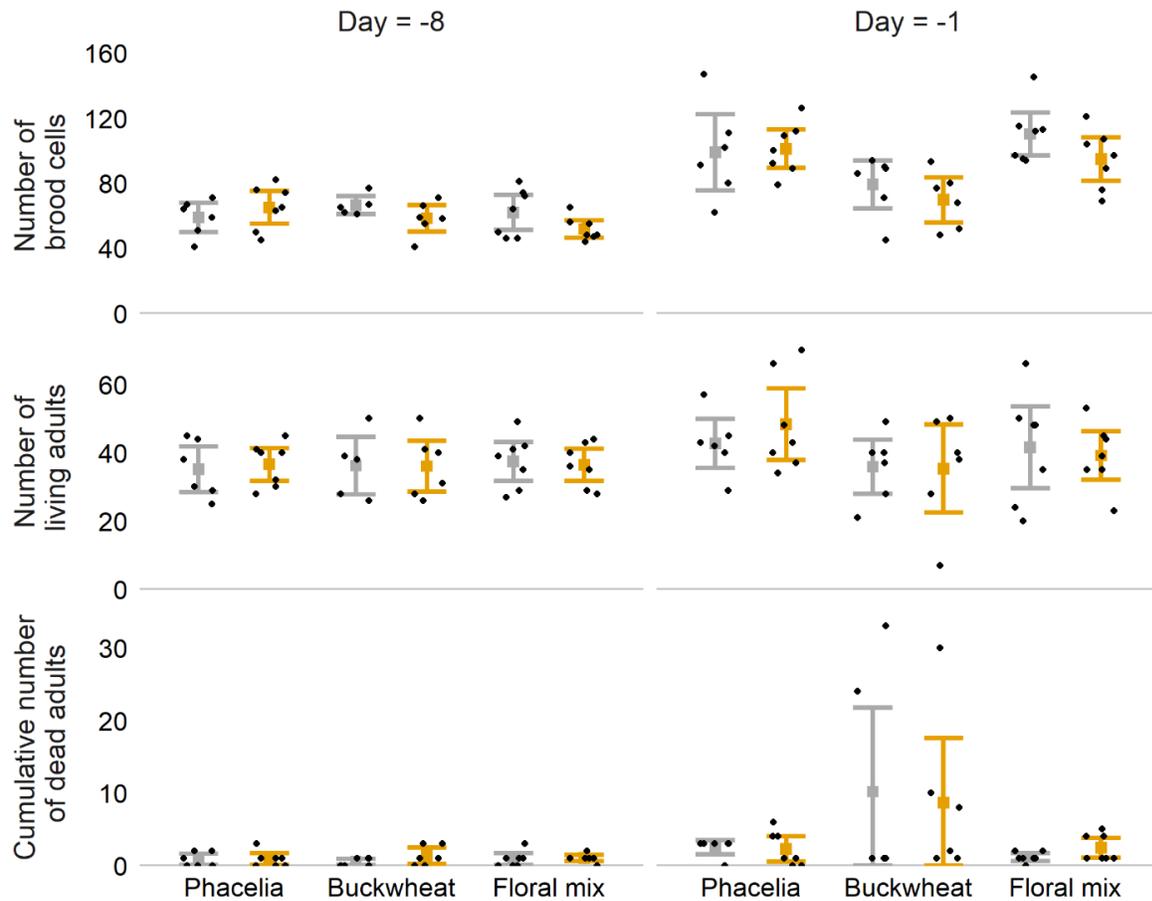


Figure S3. Bumblebee colony strength at the beginning of the experiment (day -8) and at the end of the pre-exposure period (day -1). Mean numbers of brood cells, living adults, dead adults in relation to flowering resource type and the spray treatment they were assigned to (but not yet exposed to; grey: control, orange: Amistar). Error bars indicate 95% confidence intervals. While number of brood cells on day -8 includes only brood cells seen and counted on a photo, number of brood cells on day -1 include an estimate of brood cells under a wax cover if colonies had built these (subjective extrapolation from count of seen brood cells). Error bars show 95% confidence intervals and dots depict observations.

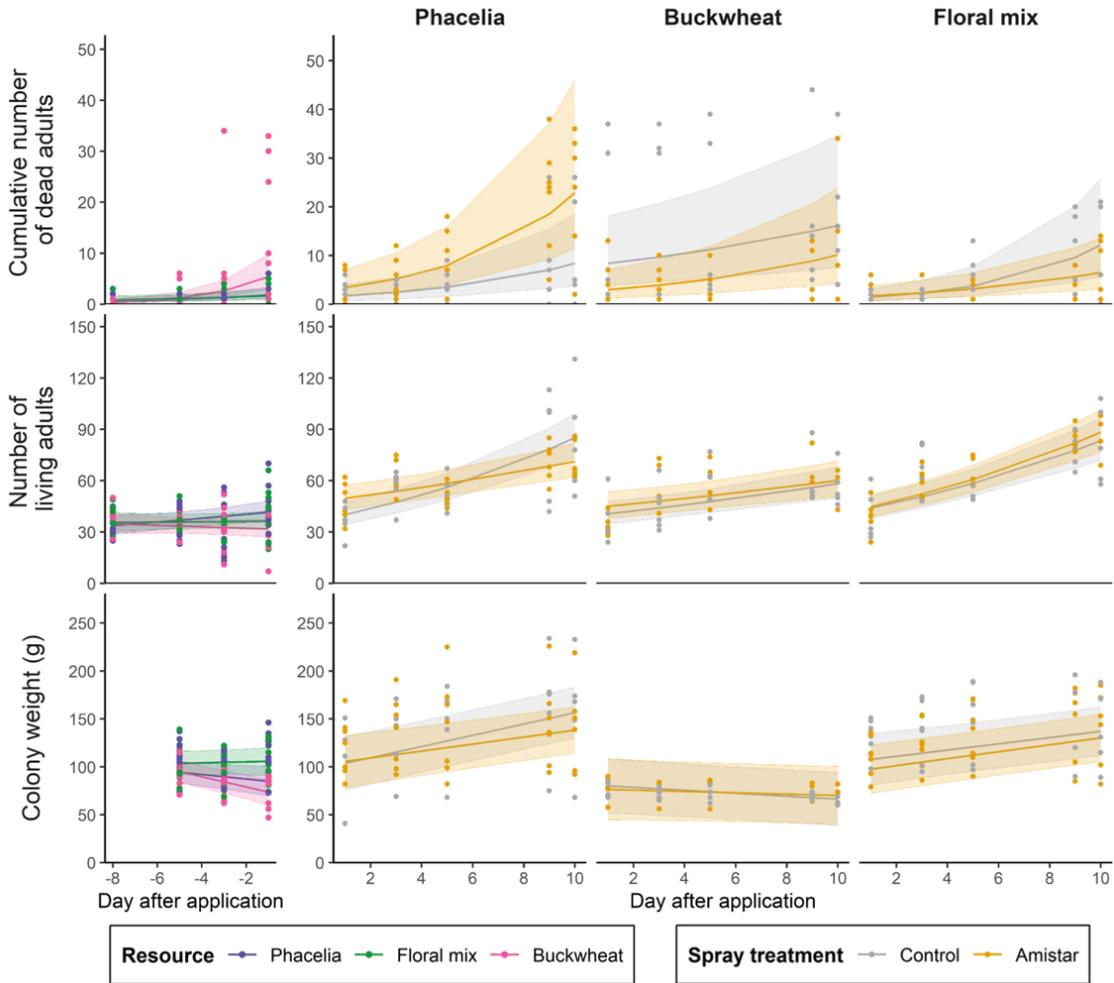


Figure S4. Bumblebee colony development. Development of colony weight and the numbers of living and dead adult bees during the pre-exposure and exposure period are shown in relation to flowering resource type and spray treatment. Flowering resource type is colour-coded in the pre-exposure period and separate plots for different resource types are shown for the exposure period with spray treatment being colour-coded. Lines represent (back-transformed) estimated marginal means and shaded areas depict 95% confidence interval obtained from (G)LMMs. Dots represent observations.

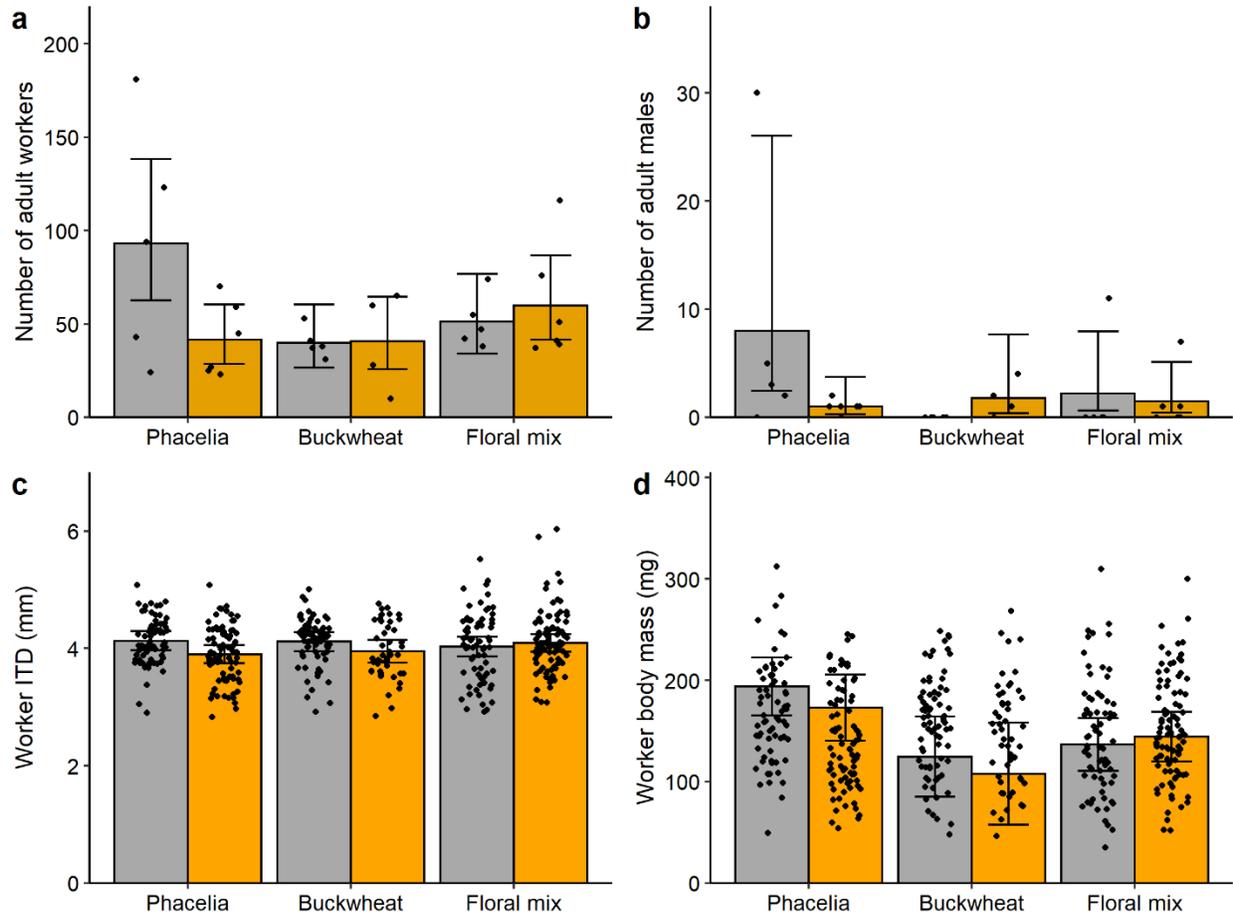


Figure S5. Final number and size of adult bumblebees. Number of adult workers and males as well as intertegular distance (ITD) and body mass of individual adult workers at the end of the experiment in relation to flowering resource type and spray treatment (grey: control, orange: Amistar). Bars represent (back-transformed) estimated marginal means and error bars depict 95% confidence interval obtained from (G)LMMs. Dots represent observations.

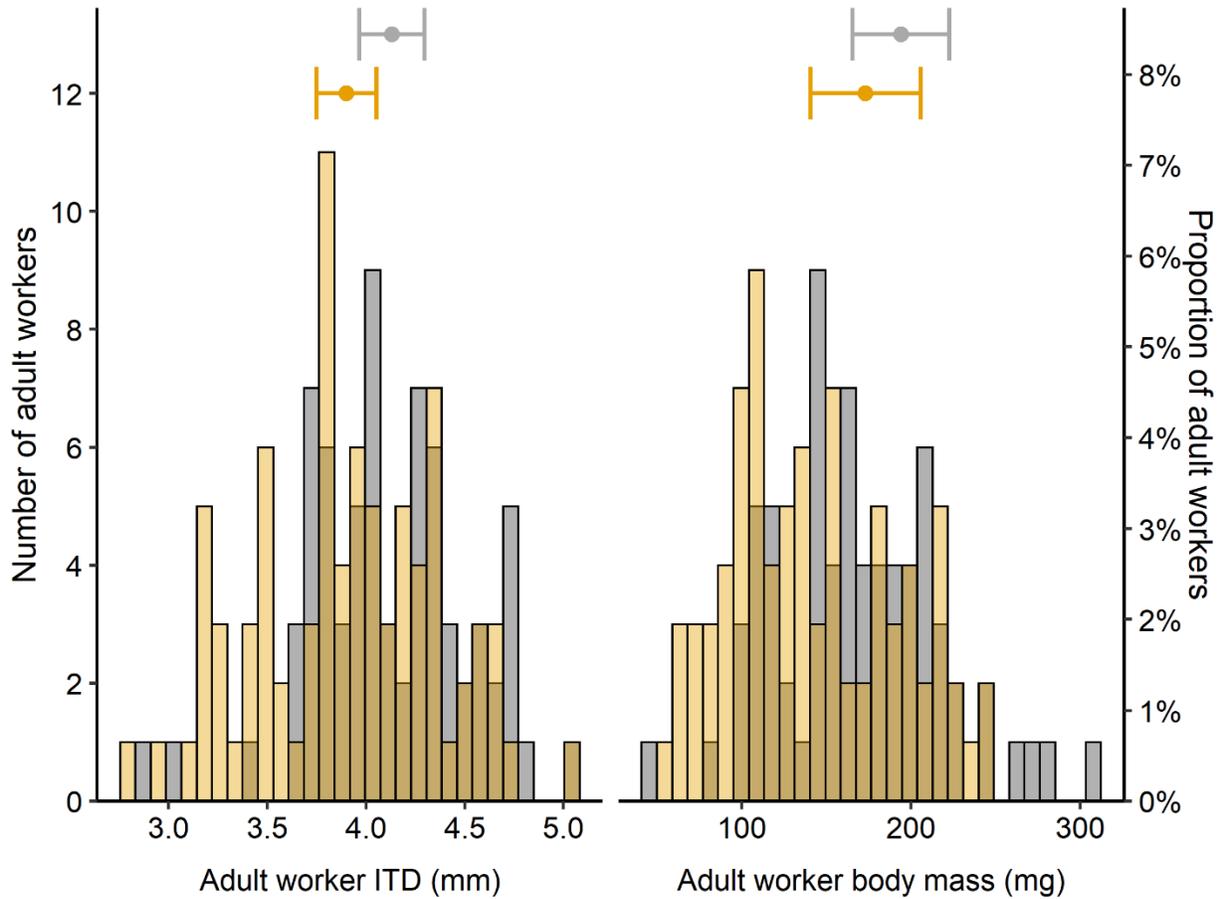


Figure S6. Size of adult workers in *Phacelia* cages. Histograms of the intertegular distance and body mass or adult workers in *Phacelia* cages in relation to spray treatment (grey: control, orange: Amistar). Dots indicate estimate marginal means and error bars show 95% confidence intervals obtained from LMMs.