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SUPPLEMENTARY INFORMATION

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Sulfoxaflor exposure reduces bumblebee reproductive success

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Supplementary Analyses

Colony mass: We found no evidence to suggest an overall effect of sulfoxaflor exposure on colony mass (lme: treatment parameter estimate = -0.00, 95% CI: -0.02 to 0.01; Figure S1), or on the number of nectar stores and pollen stores found within the nest (Nectar: glmmadmb: treatment: week parameter estimate = -0.01, 95% CL: -0.06 to 0.04; Pollen: glmmadmb: treatment:week parameter estimate = -0.00, 95% CL: -0.04 to 0.03); Figure S2A & B). When observing returning workers, we recorded whether they carried small pollen loads or large pollen loads relative to the size of their body and found no differences in the proportion of large (glmer: treatment: week parameter estimate = -0.02, 95% CL: -0.19 to 0.16; Extended Data Fig.3) and small pollen loads (glmer: treatment: week parameter estimate = 0.02, 95% CL: -0.18 to 0.21) being brought back to the nest by workers.

In contrast to other pesticide studies, we used wild-caught queens, so colonies were placed in the field over a six-week period according to colony development. We included the date of placement ("week started") within all analyses, and found that week started significantly influenced the number of sexual offspring produced (Zero-Inflated Count Model: week started, parameter estimate = 0.08, 95% CL: 0.04 to 0.13; Figure S3). Colonies moved into the field during weeks two and three of the experiment produced fewer sexual offspring, most likely due to poor weather conditions during these placement weeks. Note that colonies were always moved into the field in pairs, and so treatments were balanced across weeks. There was no effect of week started on the number of nectar stores contained within the nest (glmmadmb: week started, parameter estimate = -0.06, 95% CL: -0.03 to 0.14;Figure S4A) but there was a significant effect on pollen stores (glmmadmb: week started, parameter estimate = 0.16, 95% CL: 0.08 to 0.24; Figure S4B) with colonies put out during weeks 3, 4 & 5 containing on average more pollen stores.

Our results also showed that a greater proportion of workers returned to the colony with pollen in the morning than in the afternoon (glm, time of day, parameter estimate = -0.36, CL: -0.65 to -0.08; Figure S5). Temperature also had a significant effect on the proportion of workers returning with pollen (glm, temperature, parameter estimate = 0.10, CL: 0.05 to 0.15).

References

1. Whitehorn, P. R., O'Connor, S., Wackers, F. L. & Goulson, D. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* **336,** 351–352 (2012).

2. United States Environmental Protection Agency. *2016 Addendum to the Environmental Fate and Ecological Risk Assessment for Sulfoxaflor Registration*. (2016).

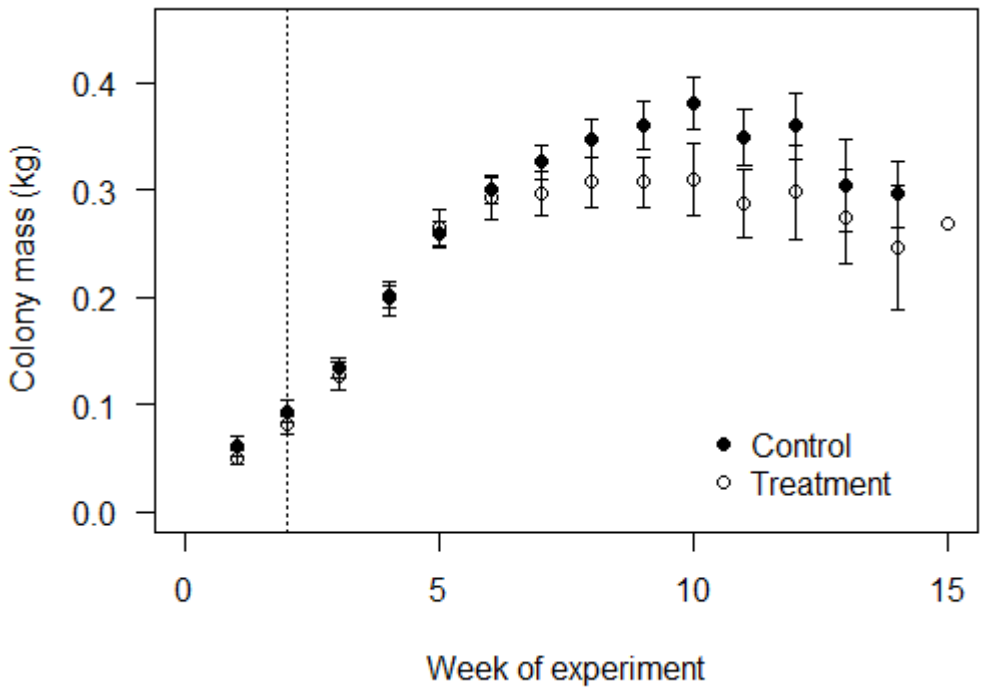

Figure S1: Changes in colony mass across the experimental period. Mean mass in kilograms (± SE) of control and treated colonies

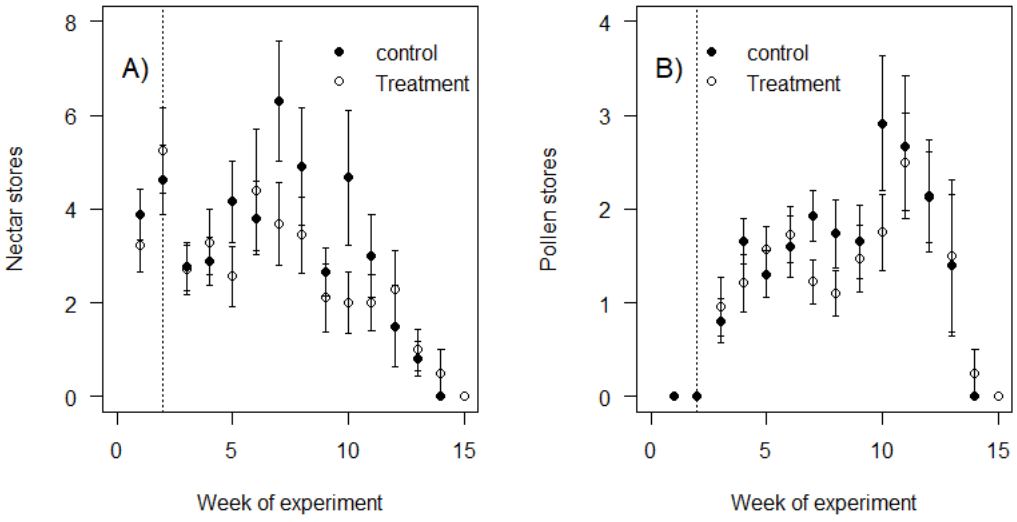

Figure S2: Nectar and pollen stores. The number (mean ± SE) of (A) nectar and (B) pollen pots in the nests of control and treated colonies.

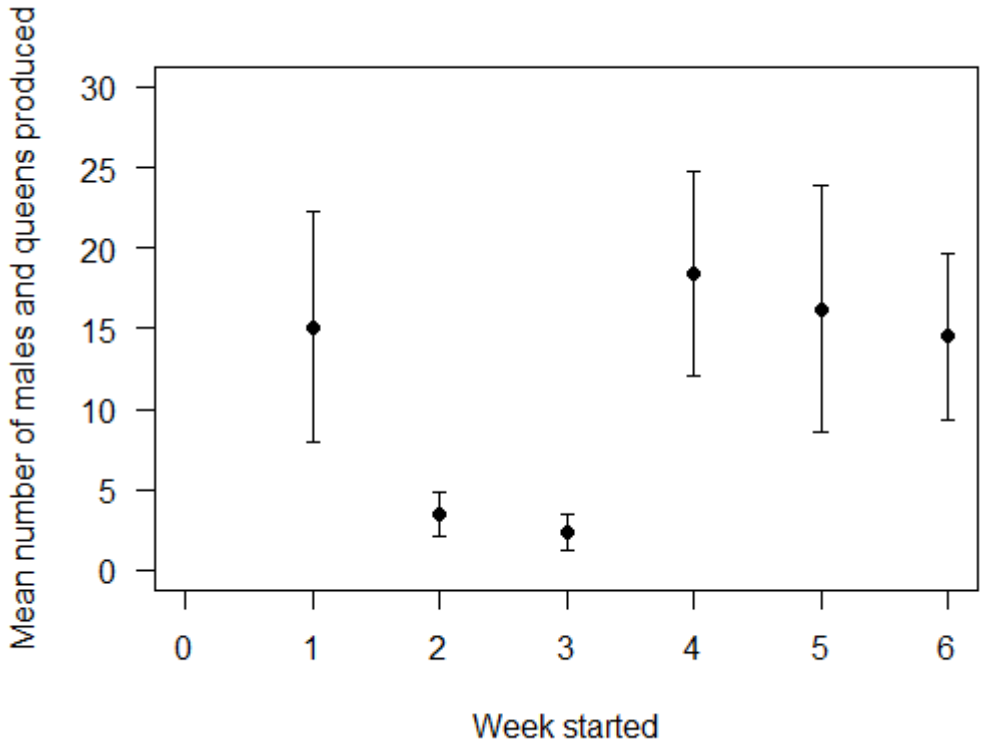

Figure S3 : Number of males and queens produced in relation to experimental timing: Impact of the week in which the colony was first placed in the field on the mean number (± SE) of sexual offspring produced by colonies.

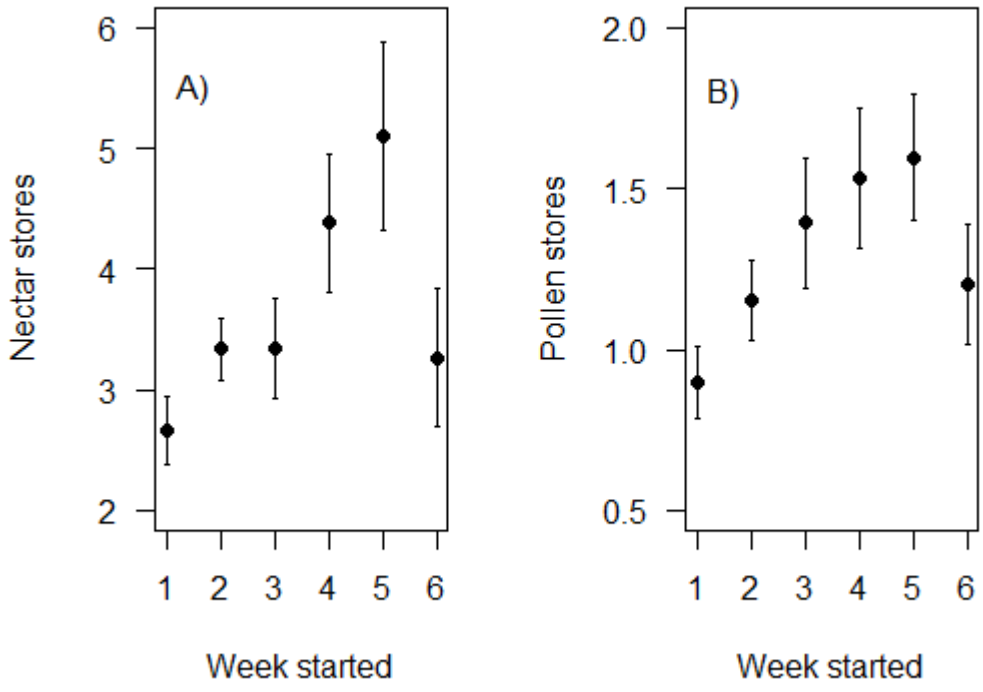

Figure S4: Nectar and pollen stores in relation to experimental timing. Mean number (± SE) of (A) nectar and (B) pollen pots produced, in relation to the week in which the colony was first placed in the field.

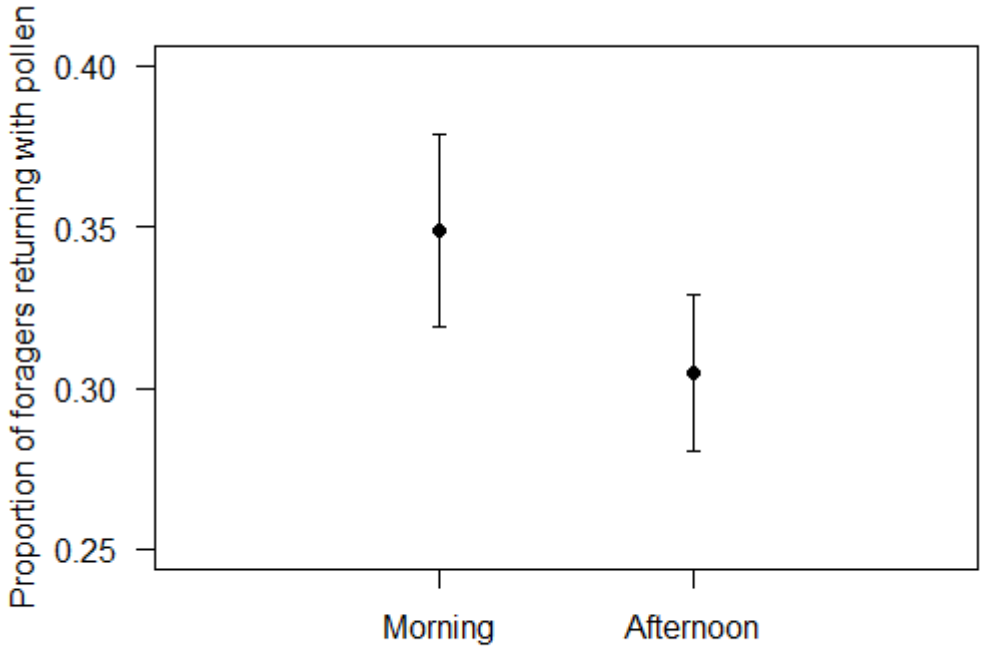

Figure S5: Pollen foraging across the day: Mean proportion (± SE) of worker bees returning to the nest with pollen in the morning compared to the afternoon.

Table S1) All model sets included the full model (below), all subsets and a basic model (that included the intercept and random factors).'Week colony' refers to the week of experiment (eg week 4 represents the fourth week of the experiment, four weeks after exposure started and two weeks after it ended) and week^2 was included when including week as a quadratic factor improved the model fit. 'Week started' refers to the week that the colony was first put into field.
A) male size (biomass); analysed with a linear mixed model; colony and pair included as random factors
Full model = week + treatment + week started + treatment:week + treatment:week started
B) colony growth rates (weight gained); fitted linear model; colony and pair included as random factors
Full model = week colony + treatment + week started + treatment:week colony + treatment:week started + week^2 + week^2:treatment
C) sucrose consumed during exposure period; fitted linear model with pair included as a random factor
Full model = treatment + week started
D) number of workers; generalized linear mixed model with colony and pair included as random factors, poisson distribution
Full model = Treatment + scale(week started) + scale(week colony) + week^2 + treatment:week started + treatment:week colony + treatment:week^2
E) proportion of pollen foragers; generalized linear mixed model with colony and pair included as random factors, binomial distribution
Full model = treatment + week colony + week started + time of day + temperature + treatment*week colony
F) total reproductive output (males & queens); zero inflated count regressions
Full model = treatment + week started
G) males produced; zero inflated count regressions
Full model = treatment + week started
H) number of returning workers; generalized linear mixed model, using AD model builders with colony included as a random factor, negative binomial distribution
Full model = treatment + week colony + week started + temperature
I) nectar stores counted within nest; generalized linear mixed model, using AD model builders with colony and pair included as random factors, negative binomial distribution
Full model = treatment + week started + week colony + treatment:week colony + treatment:week started
J) pollen stores counted within nest; generalized linear mixed model, using AD model builders with colony included as a random factor, poisson distribution
Full model = treatment + week started + week colony + treatment:week colony + treatment:week started
K) reproductive onset (week colonies first produced sexuals); coxph survival analysis
Full model = treatment + week started
L) number of weeks queen survived; coxph survival analysis
Full model = treatment + week started
M) number of weeks colony survived; coxph survival analysis
Full model = treatment + week started
N) proportion of pollen foragers returning with large pollen loads; generalized linear mixed model with colony and pair included as random factors, binomial distribution
Full model = treatment + week colony + week started + time of day + average temperature + treatment*week colony
o) proportion of pollen foragers returning with small pollen loads; generalized linear mixed model with colony and pair included as random factors, binomial distribution
Full model = treatment + week colony + week started + time of day + average temperature + treatment*week colony

Table S2. Parameter estimates and 95% confidence intervals derived by model averaging across the confidence set of models. Parameters highlighted in bold have 95% confidence intervals that do not cross zero.

A) male size (biomass)	Estimate	Std. Error		
Intercept	0.29	0.01		
B) colony growth rate (weight gained)	Estimate	Std. Error	Lower CI	Upper CI
(Intercept)	0.12	0.01	0.09	0.14
week colony	**0.10**	**0.00**	**0.09**	**0.11**
week colony^2	**-0.01**	**0.00**	**-0.01**	**0.00**
treatment	0.00	0.01	-0.02	0.01
C) sucrose consumed during exposure period	Estimate	Std. Error		
(Intercept)	0.15	0.01		
D) number of workers	Estimate	Std. Error	Lower CI	Upper CI
(Intercept)	**3.37**	**0.15**	3.11	3.44
week colony	**-0.12**	**0.02**	**-0.15**	**-0.08**
week^2	**-0.46**	**0.02**	**-0.50**	**-0.42**
week started	-0.03	0.04	-0.18	0.08
treatment	**-0.28**	**0.17**	**-0.48**	**-0.01**
treatment:week started	0.01	0.04	-0.11	0.15
treatment:week colony	**-0.06**	**0.03**	**-0.11**	**-0.01**
treatment:week^2	**0.11**	**0.03**	**0.05**	**0.16**
E) proportion of pollen foragers	Estimate	Std. Error	Lower CI	Upper CI
(Intercept)	-2.10	0.43	-2.94	-1.26
treatment	0.46	0.43	-0.38	1.31
week colony	-0.02	0.05	-0.12	0.08
time of day	**-0.36**	**0.15**	**-0.65**	**-0.08**
average temperature	**0.10**	**0.03**	**0.05**	**0.15**
treatment:week colony	-0.14	0.07	-0.29	0.00
week started	0.01	0.03	-0.05	0.06
F) total reproductive output (males & queens)	Estimate	Std. Error	Lower CI	Upper CI
count (Intercept)	2.66	0.09	2.48	2.84
count treatment	**-0.64**	**0.09**	**-0.81**	**-0.46**
count week started	**0.08**	**0.02**	**0.04**	**0.13**
zero (Intercept)	-1.30	0.80	-2.86	0.27
zero treatment	0.71	0.70	-0.67	2.09
zero week started	-0.14	0.20	-0.53	0.26
G) males produced	Estimate	Std. Error	Lower CI	Upper CI
count (Intercept)	2.66	0.12	2.42	2.89
count treatment	**-0.54**	**0.09**	**-0.72**	**-0.37**
count week started	0.05	0.03	-0.01	0.12
zero (Intercept)	-1.37	0.77	-2.89	0.15
zero treatment	0.71	0.70	-0.67	2.09
zero week started	-0.11	0.19	-0.49	0.26
H) number of returning foragers	Estimate	Std. Error	Lower CI	Upper CI
(Intercept)	1.52	0.25	1.03	2.01
week colony	**-0.19**	**0.02**	**-0.23**	**-0.15**
temperature	-0.01	0.01	-0.04	0.02
week started	-0.02	0.04	-0.11	0.06
treatment	-0.07	0.13	-0.32	0.19
I) nectar stores counted within nest	Estimate	Std. Error	Lower CI	Upper CI
(Intercept)	1.46	0.20	1.07	1.86
treatment	-0.10	0.20	-0.50	0.30
week started	0.06	0.04	-0.03	0.14
week colony	-0.02	0.02	-0.06	0.02
treatment:week colony	-0.01	0.02	-0.06	0.04
treatment:week started	0.01	0.04	-0.06	0.08
J) pollen stores counted within nest	Estimate	Std. Error	Lower CI	Upper CI
(Intercept)	-0.97	0.21	-1.39	-0.55
week started	**0.16**	**0.04**	**0.08**	**0.24**
week colony	**0.14**	**0.02**	**0.11**	**0.18**
treatment	-0.03	0.18	-0.38	0.33
treatment:week colony	0.00	0.02	-0.04	0.03
treatment:week started	-0.01	0.03	-0.08	0.06
K) reproductive onset (week colonies first produced sexuals)	Estimate	Std. Error	Lower CI	Upper CI
week started	0.14	0.12	-0.10	0.38
treatment	-0.05	0.18	-0.41	0.31
L) number of weeks queen survived	Estimate	Std. Error	Lower CI	Upper CI
week started	0.23	0.12	0.00	0.46
treatment	-0.07	0.20	-0.47	0.33
M) number of weeks colony survived	Estimate	Std. Error	Lower CI	Upper CI
week started	0.15	0.15	-0.14	0.44
treatment	-0.03	0.21	-0.43	0.38
N) proportion of pollen foragers returning with large pollen load	Estimate	Std. Error	Lower CI	Upper CI
Intercept	-0.94	0.54	-2.00	0.13
average temperature	0.02	0.03	-0.04	0.09
week colony	0.01	0.02	-0.04	0.05
time of day	-0.01	0.08	-0.17	0.15
treatment	-0.02	0.09	-0.19	0.16
week started	0.00	0.02	-0.05	0.05
O) proportion of pollen foragers returning with a small pollen load	Estimate	Std. Error	Lower CI	Upper CI
Intercept	-0.04	0.64	-1.29	1.22
average temperature	-0.05	0.04	-0.13	0.03
treatment	0.02	0.10	-0.18	0.21
time of day	0.01	0.09	-0.16	0.19
week started	0.00	0.03	-0.05	0.05
week colony	-0.01	0.03	-0.07	0.05