**Supplementary Information**

**No evidence for impaired solitary bee fitness following pre-flowering sulfoxaflor application alone or in combination with a common fungicide in a semi-field experiment**

Janine Melanie Schwarza,b, \*, Anina C. Knauera, Matthew J. Allanc, Robin R. Deand, Jaboury Ghazoulb, Giovanni Tamburinie,f, Dimitry Wintermantele, Alexandra-Maria Kleine and Matthias Albrechta

aAgroscope, Agroecology and Environment, Zurich, Switzerland

bETH Zurich, Institute for Terrestrial Ecosystems, Ecosystem Management, Zurich, Switzerland

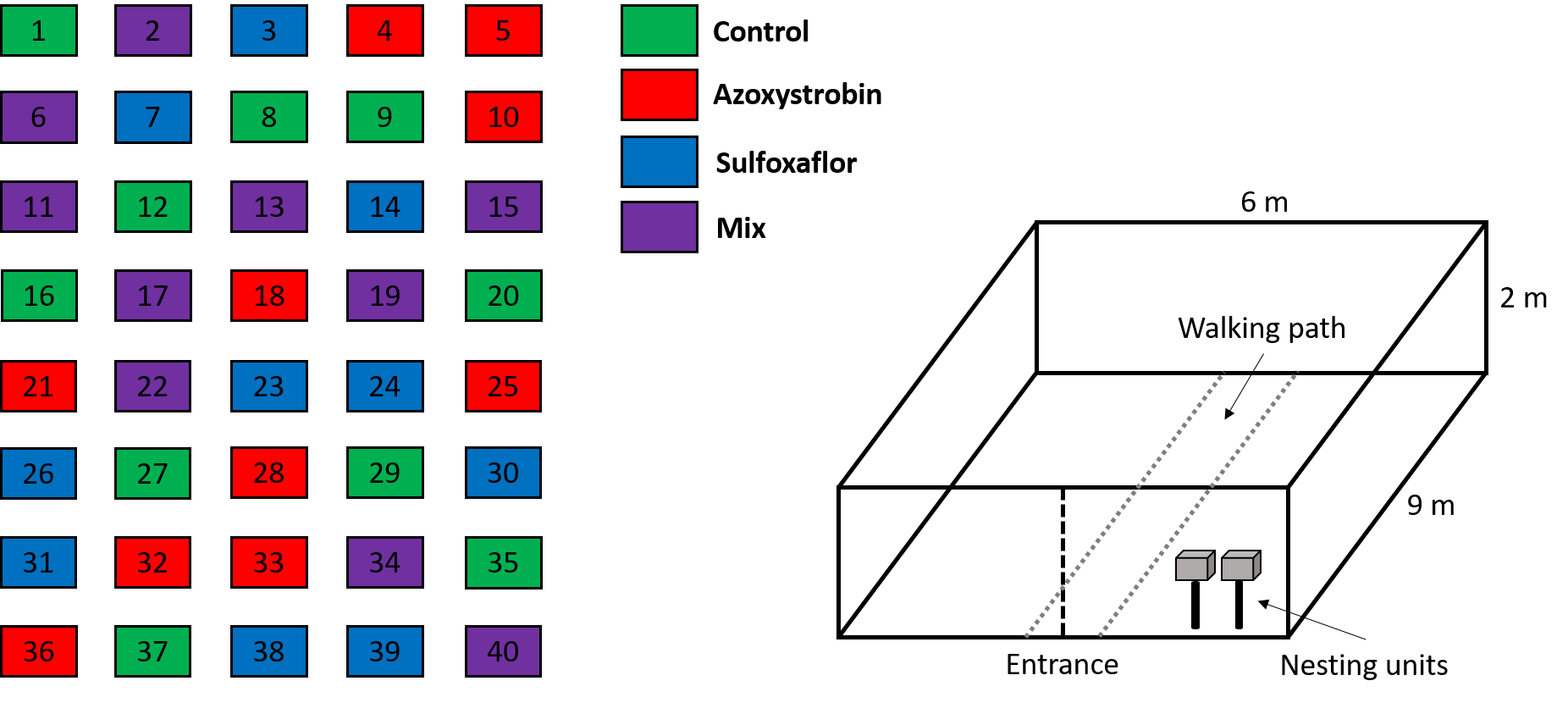
cAtlantic Pollination Ltd, Eastleigh, United Kingdom

dRed Beehive Company, Bishops Waltham, United Kingdom

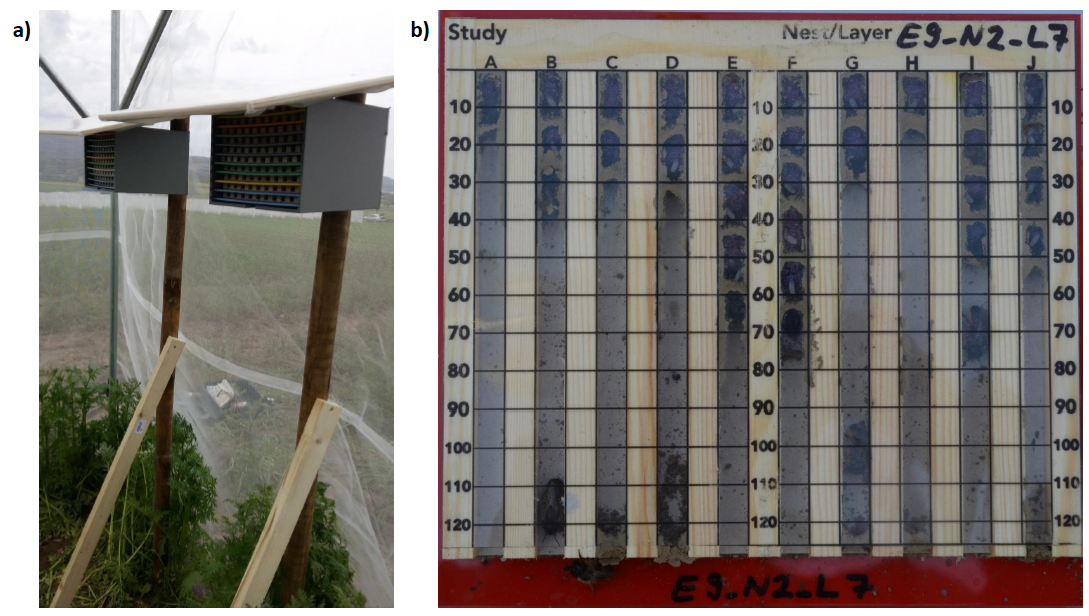
eUniversity of Freiburg, Nature Conservation and Landscape Ecology, Freiburg, Germany

fUniversity of Bari, Department of Soil, Plant and Food Sciences (DiSSPA - Entomology), Bari, Italy

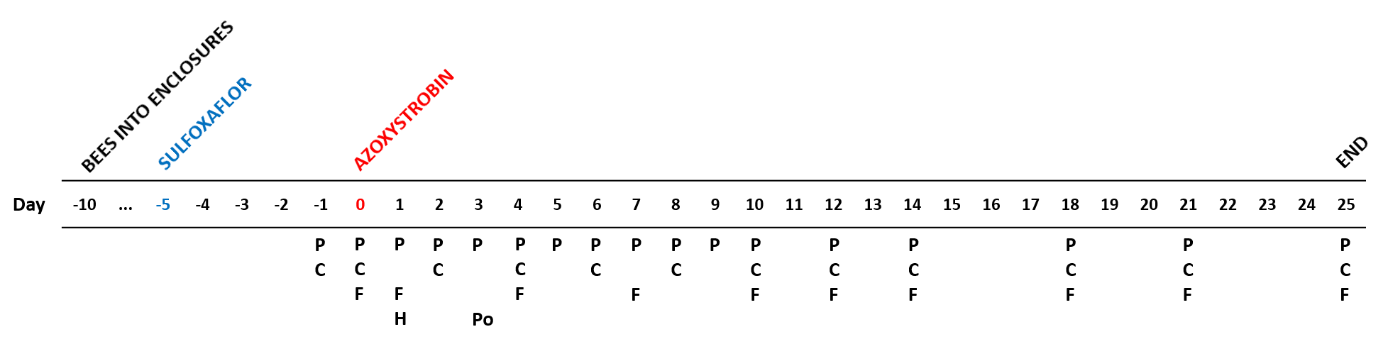
\* Corresponding author: Janine Melanie Schwarz, Agroscope, Agroecology and Environment, Zurich, Switzerland. *E-Mail*: janine.schwarz130790@gmail.com*, Phone*: +41584687086



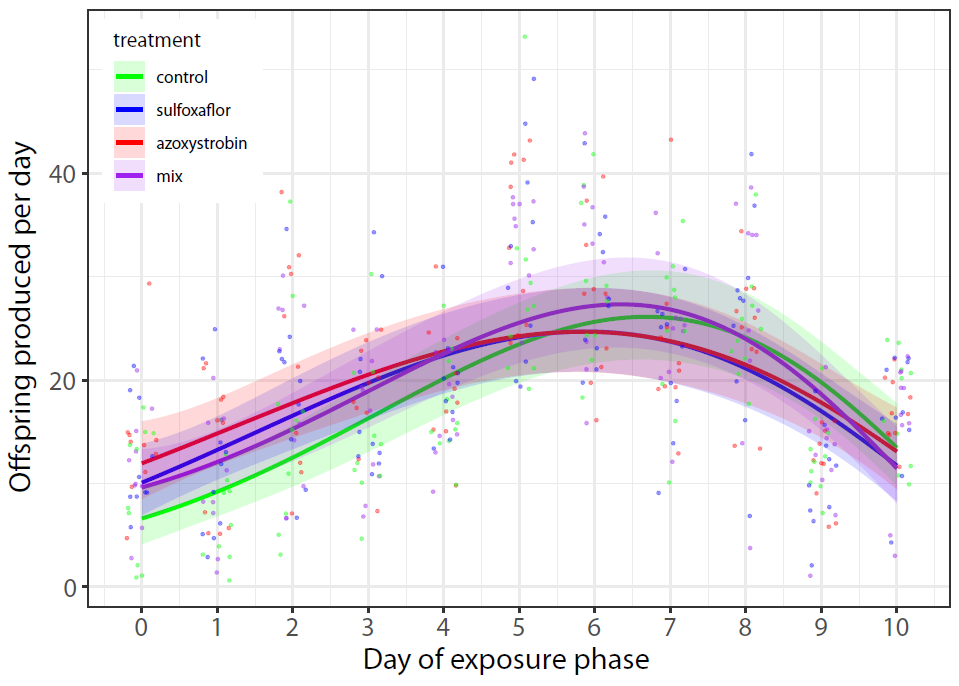
**Supplementary Figure 1: Random distribution of cages on the field site and scheme of the structure of an individual cage.** Cages were spaced at least 5 m apart from each other and from field margins. Entrances and nesting units were oriented always in the same direction.



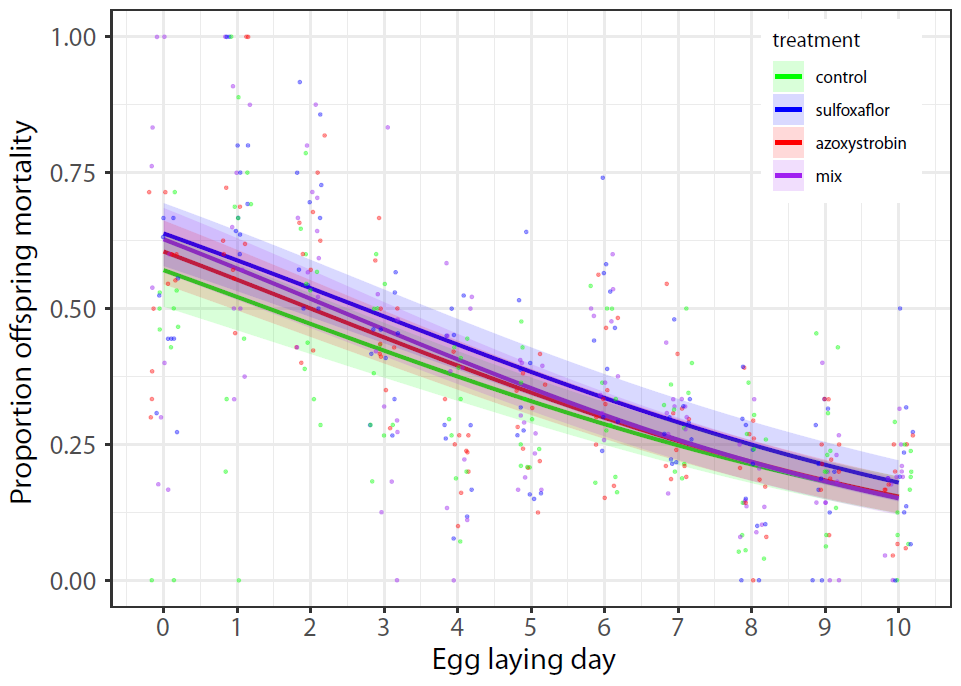
**Supplementary Figure 2: Experimental setup**. **(a)** Nesting units installed in each cage and **(b)** insight into a nesting layer filled with *O. bicornis* brood cells produced during the exposure phase.



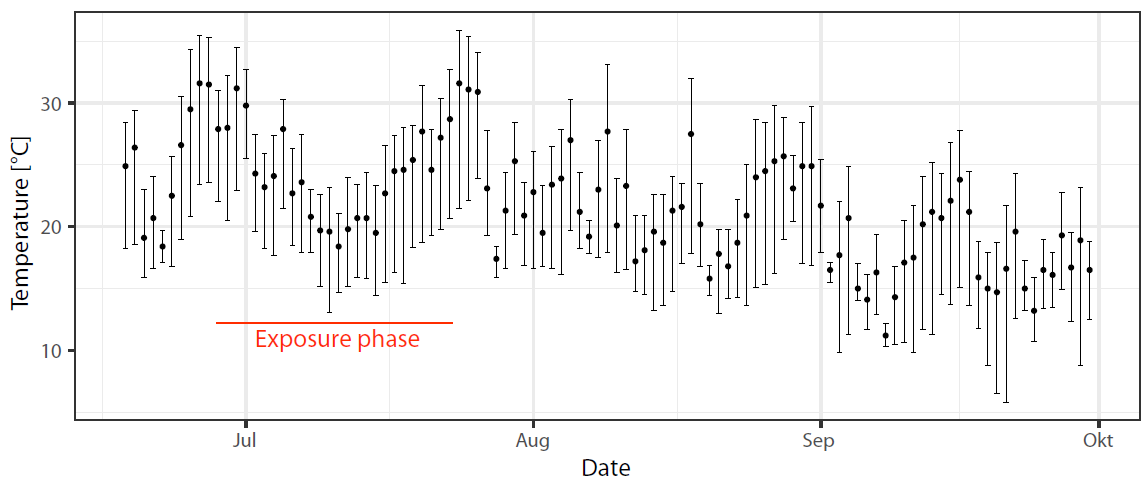
**Supplementary Figure 3: Timeline of the experiment**. The exposure phase lasted from day 0 (28 June) until day 25 (23 July). Bees were introduced 10 days before the start of the exposure phase, sulfoxaflor (product Closer) was applied 5 days before the start of the exposure phase as pre-flowering application, azoxystrobin (product Amistar) was applied at the beginning of the flowering and marked the beginning of the exposure phase. **P**: Pictures of brood cells taken after 4 pm; **C**: Count of roosting female *O. bicornis* in nesting units after nightfall; **F**: Flower abundance estimation; **Po**: Pollen store sampling; **H**: Sampling of three female bees per cage for a separate study.



**Supplementary Figure 4:** **Predicted number of offspring produced per cage during days 0-10 of the exposure phase**. Model prediction from a linear mixed effects model (LMM) including raw data (dots) and 95% confidence intervals as shaded areas.



**Supplementary Figure 5:** **Predicted proportions of offspring mortality per cage of offspring produced during days 0-10 of the exposure phase**. Model prediction from a generalized linear mixed effects model (GLMM) including raw data (dots) and 95% confidence intervals as shaded areas.



**Supplementary Figure 6: Temperature during the experimental period 2019.** The graph shows the daily mean temperature (dots) between 6 am and 6 pm. Error bars indicate the minimum and the maximum temperature between 6 am and 6 pm. The exposure phase in the cages lasted from 28 June until 23 July 2019. Data from Bundesamt für Meteorologie und Klimatologie, MeteoSchweiz, Switzerland, GPS coordinates of the weather station: 47.427694 / 8.517953.

**Supplementary Table 1: Survival of adult *O. bicornis* females.** Results of the mixed-effects cox proportional hazards model of adult female *O. bicornis* survival during the exposure phase of the experiment analysed with the 4-level factor treatment(levels: sulfoxaflor (product Closer), azoxystrobin (product Amistar), mix (Closer + Amistar), water control (control). Cage ID was included as random factor.

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| --- | --- | --- | --- | --- | --- |
| **Survival of adult female *O. bicornis* during the exposure phase** | | | | | |
| **Coefficients** | **Hazard ratio** | **Lower 95% CI** | **Upper 95% CI** | ***Z*** | ***P-*value** |
| sulfoxaflor  azoxystrobin  mix | 1.31  1.13  1.28 | 0.93  0.80  0.90 | 1.86  1.60  1.81 | 1.58  0.73  1.42 | 0.11  0.47  0.16 |

**Supplementary Table 2:** Results of linear models (LMs) and (generalized) linear mixed effects models ((G)LMMs) on offspring production and mortality for testing the impact of pesticides. Analyses were conducted with the 4-level factor treatment (levels: sulfoxaflor (product Closer), azoxystrobin (product Amistar), mix (Closer + Amistar), water control). Flower abundance per m2 was included as co-variate. LMs were used to test for differences in total numbers of offspring produced during the entire exposure phase and numbers of offspring that reached the cocoon stage. An LMM with the additional continuous variable day of exposure phase (day) was used to analyse differences in the number of offspring produced per day (square root-transformed to obtain normal distribution of residuals). A third order polynomial term was added for day to improve model fit (function poly). Offspring mortality (i.e. the proportion of offspring that died as egg or larva) was analysed with a binomial GLMM and included egg laying day as co-variate. Likelihood ratio tests were used for statistical inference of (G)LMMs. The (G)LMMs included cage ID as random factor. Significant effects (*P* ≤ 0.05) are indicated in bold.

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| --- | --- | --- | --- |
| **Reproduction and offspring mortality** | | | |
| **Variables** | **Sum of squares** | ***F*-value** | ***P-*value** |
| ***Total number of offspring produced (days 0-25)*** | | | |
| treatment  mean flower abundance | 1882  1366 | *F3,35*= 0.12  *F1,35*= 0.26 | 0.949  0.615 |
|  |  |  |  |
| ***Total number of offspring reaching cocoon stage (days 0-25)*** | | | |
| treatment  mean flower abundance | 3284  740 | *F3,35*= 0.47  *F1,35*= 0.31 | 0.708  0.578 |
|  |  |  |  |
|  | **λLR** | **df** | ***P-*value** |
| ***Number of offspring produced per day (days 0-10)*** | | | |
| treatment  **day+day2+day3**  **flower abundance**  **treatment × (day+day2+day3)** | 0.93  **218.98**  **7.05**  **20.04** | 3  **3**  **1**  **9** | 0.817  **< 0.001**  **0.008**  **0.018** |
|  |  |  |  |
| ***Offspring mortality (days 0-10)*** | | | |
| treatment  **egg laying day**  **flower abundance**  treatment × egg laying day | 2.90  **605.58**  **5.15**  1.05 | 3  **1**  **1**  3 | 0.407  **< 0.001**  **0.023**  0.789 |

**Supplementary Table 3: Offspring sex ratio and size.** Results of linear mixed effects models (LMMs) and a generalized linear model (GLM) on offspring size (cocoon volume) and sex ratio (proportion of female offspring) for testing the impact of pesticides. Analyses were conducted with the 4-level factor treatment (levels: sulfoxaflor (product Closer), azoxystrobin (product Amistar), mix (Closer + Amistar), water control). Flower abundance per m2 was included as co-variate. The cocoon volumes of un-emerged female and male offspring were separately analysed with LMMs. Cage ID was included as random factor and likelihood ratio tests were used for statistical inference of LMMs. A quasi-binomial GLM was used to analyse differences in the proportions of female offspring produced during days 0-5 of the exposure phase.

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| **Offspring size and sex ratio** | | | |
| **Variables** | **λLR** | **df** | ***P-*value** |
| ***Cocoon volume of female offspring (days 0-5)*** | | | |
| treatment  mean flower abundance | 0.77  1.51 | 3  1 | 0.858  0.219 |
| ***Cocoon volume of male offspring (days 0-5)*** | | | |
| treatment  **mean flower abundance** | 3.76  **4.19** | 3  **1** | 0.288  **0.041** |
|  |  |  |  |
|  | **Sum of squares** | ***F*-value** | ***P-*value** |
| ***Overall offspring sex ratio (days 0-5)*** | | | |
| treatment  mean flower abundance | 5.61  0.06 | *F3,34*= 1.52  *F1,34*= 0.05 | 0.228  0.832 |

**Supplementary Table 4:** Results of a linear model (LM) testing for differences in initial numbers *O. bicornis* females in cages assigned to the different treatments on day -1 (the night before the start of the exposure phase). In total, 1,785 female bees were alive on day -1 (control: 450, sulfoxaflor: 438, azoxystrobin: 455, mix: 442). The analysis was conducted with the 4-level factor treatment (levels: sulfoxaflor (product Closer), azoxystrobin (product Amistar), mix (Closer + Amistar), water control) as explanatory variables.

|  |  |  |  |
| --- | --- | --- | --- |
| **Initial conditions in cages at the start of the exposure phase** | | | |
| **Variables** | **Sum of squares** | ***F*-value** | ***P-*value** |
| ***Number of female O. bicornis in cages on day -1*** | | | |
| treatment | 17.67 | *F3,36*= 0.41 | 0.750 |

**Supplementary Table 5**: Results of a linear mixed effects model (LMM) testing for differences in flower abundance in cages of different treatments during the exposure phase (days 0-25). The flower abundance in every cage (n = 40, 10 cages per treatment) was assessed ten times during the experiment (resulting in 100 data points per treatment condition and 400 data points in total). The response variable was log-transformed to obtain normal distribution of residuals. The model included the 4-level factor treatment (levels: sulfoxaflor (product Closer), azoxystrobin (product Amistar), mix (Closer + Amistar), water control)and its interaction with day (continuous) as explanatory variables. The model included cage ID as random factor. A third order polynomial term was added for day to improve model fit (function poly). Likelihood ratio tests were used for statistical inference. Significant effects (*P* ≤ 0.05) are indicated in bold.

|  |  |  |  |
| --- | --- | --- | --- |
| **Flower abundance during the exposure phase** | | | |
| **Variables** | **λLR** | **df** | ***P*-value** |
| ***Number of open flowers per m2 during the exposure phase (days 0 - 25)*** | | | |
| treatment  **day+day2+day3**  treatment × (day+day2+day3) | 3.86  **634.24**  6.51 | 3  **3**  9 | 0.277  **< 0.001**  0.688 |