Manuscript on agrochemical and pathogen effects on bumble bee health at the colony level

Deliverable D6.3

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PoshBee

Pan-european assessment, monitoring, and mitigation of stressors on the health of bees

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Preface

Bees in the wild are exposed to a myriad of concurrent stressors. These include climatic conditions, nutritional deprivation and parasites (Goulson et al., 2015). If these stressors interact with pesticides, which are wholly anthropogenic stressors, bees could be under more pressure from pesticides than previously thought. It is thus critical that we conduct multi-stressor testing such that these interactions can be quantified. If large scale synergies occur, than pesticide risk assessment will drastically underestimate the level of risk pesticides pose to pollinators (Siviter et al., 2021).

Parasites, one common stressor on bee health, have co-existed with bees for millennia (Schmid-Hempel, 1998). However, emerging infectious diseases, transported globally by human activity, are presenting novel stress to bee health. Of the established diseases in bumble bees, perhaps none is as prevalent as *Crithidia bombi*, a trypanosome gut parasite (Shykoff & Schmid-Hempel, 1991). Work on *Crithidia bombi* has found it to be relatively harmless to healthy, unstressed bees, but very impactful for stressed bees, or at sensitive life history stages (Brown et al., 2000, Brown et al., 2003). For this reason it is possible that *Crithidia bombi* would interact with a pesticide stressor to the detriment of bee health.

The true metric of bee health is fitness, which is the evolutionary metric of success (Straub et al., 2020). However, measuring this is nearly impossible, as it requires tracking the reproductive success of offspring (and, so, a multi-generational experiment) and a more tangible metric, which serves as a good proxy measure, is colony development. A larger colony will be able to produce more sexuals (offspring queens and male) and thus have a higher fitness. Larger colonies will also contribute more ecosystem services and thus benefit humanity more.

Sulfoxaflor, a novel insecticide touted as a successor to the neonicotinoids, has recently emerged onto world markets. Given delays in data reporting, little information is present on its uptake within Europe. However, as farmers struggle to control insect pests in the wake of the neonicotinoid ban, and with growing resistance to and a dwindling pool of approved insecticides, it is thought sulfoxaflor may become a more prevalent part of farmers' toolkits (Brown et al., 2016). Sulfoxaflor has previously been found to be harmful to bumble bees, through impairments to bee reproduction, reducing investment in sexuals and in egg laying when queens were chronically exposed (Siviter et al., 2018, Siviter et al., 2019).

Here we tested if field realistic exposure to sulfoxaflor, a novel insecticide successor to the neonicotinoids, harms bumble bee colony development, and whether it interacts with the common bumble bee parasite *Crithidia bombi*.

Summary

We conducted a whole colony, fully-crossed experiment to determine the individual and combined impacts of two stressors – the agrochemical sulfoxaflor and the parasite *Crithidia bombi* – on bumble bee colony health. Young colonies were exposed either to the agrochemical, the parasite, both, or neither of the stressors. Colonies were then allowed to live for approximately 8 weeks from the initial treatment (parasite exposure) before sacrifice and assessment of colony development. The results suggest that field realistic exposure of young bumble bee colonies to sulfoxaflor is not harmful to their development, nor does it exacerbate *Crithidia bombi* infection. This develops our understanding of the safe use of sulfoxaflor. Ultimately, this research does not suggest that any changes need to be made to sulfoxaflor's registration, nor to the risk assessment process, with respect to its potential interactions with a common parasite stressor.

1. Methods

15-25 worker queenright commercial bumble bee research colonies, *Bombus terrestris audax*, were ordered from a local supplier to be used in the experiments.

On arrival, 20 bees per colony were removed from the colony, and then induced to defecate in plastic tubes with time and light agitation. DNA was extracted from the faeces, pooled and screened for micro-parasites (Rutrecht & Brown, 2009). None were detected across the experiment, enabling all colonies to be moved forward for experimental use.

Colonies were weighed whole, and weights were used to rank allocate them to treatments. This involved ordering colonies by weight, and then allocating them one-by-one to each of the treatment groups (control, sulfoxaflor only, *Crithidia bombi* only, combined) until all colonies had been allocated.

In order to equalize colony size, all bees, bar the queen, were then removed from each colony. A random 20 bees (from the same colony) were returned to each colony, to make a total of 21 bees including the queen (the queen was left unmarked to minimize stress).

Table 1: The treatments used in the experiment

Control	***Crithidia bombi* only**
Sulfoxaflor only	**Sulfoxaflor and *Crithidia bombi***

A *Crithidia bombi* inoculum was prepared for each colony following Cole (1970). This contained 210,000 cells of *C. bombi*, equaling 10,000 cells per bee, a dose that is known to cause reliable infection (Ruiz-González & Brown, 2006). This was then diluted in sugar water and fed to the colonies over 12 hours using a petri dish. Control colonies and sulfoxaflor only colonies were given an equivalent petri dish with a sham inoculum.

Colonies were then left to develop for a week, allowing the infection to take hold and spread evenly through the colony. To assess this, 30 bees were removed from each colony at the end of the week, moved into plastic specimen tubes, and induced to defecate. A DNA extract of these faecal samples were then screened individually for *C. bombi*, as used to screen colonies for background infection. The investigator screening the bees was blind to the treatment. A minimum infection rate in the screened bees of 25% was required for an infection to be deemed successful. In colonies not deliberately exposed to the parasite, our tolerance for *C. bombi* was zero, meaning any colony with even a single infected individual was removed from the experiment.
Colonies were then shipped to a collaborating team at the University of Reading for sulfoxaflor exposure. Here bees were exposed to a field realistic exposure of sulfoxaflor diluted in 30% w/w sucrose solution lasting for 4 days. The exposure pattern mimicked the realistic degradation of sulfoxaflor within nectar, with the concentrations on day one being 161ppb, dropping down to 4ppb by day 4. Colonies had *ad libitum* access to this spiked solution.

Colonies were entered into a pollination experiment not reported here. All manipulations carried out were evenly applied to all treatments, and experimenters were blind to the conditions. Colonies spent 7 days outside as part of this experiment.

After the pollination work, colonies were moved into a temperature and humidity-controlled room for 6 weeks with *ad libitum* sucrose and pollen topped up weekly. After this, they were frozen and shipped back to RHUL. At RHUL colonies were dissected, queen presence was noted, and the

following metrics were recorded: larval number and weight, pupal number, male number and weight, worker number and weight.

These data were analysed using mixed effects models (package lme4) in R.

2. Results

Across all of the metrics measured, there were no statistically significant effects of either individual treatments or of the combined treatment. Here we report results for worker, larval, and male (drone) weight, as composite indices of productivity that combine number and investment into individuals, and pupal number, as an index of investment into sexual production. Specifically, total weight of worker was not affected by treatment (linear mixed effects model, sulfoxaflor, $p = 0.67$, *C. bombi*, $p = 0.532$, sulfoxaflor and *C. bombi*, $p = 0.747$; Fig. 1), total weight of larvae was not affected by treatment, albeit there was a trend for it to be marginally higher in the Sulfoxaflor treatment (linear mixed effects model, sulfoxaflor, $p = 0.05$, *C. bombi*, $p = 0.18$, sulfoxaflor and *C. bombi*, $p = 0.62$; Fig. 2), total weight of drones was not affected by treatment (Kruskal-Wallace model, sulfoxaflor, $p = 0.92$, *C. bombi*, $p = 0.94$, sulfoxaflor and *C. bombi*, $p = 0.94$; Fig. 3), and pupal number was not affected by treatment (linear mixed effects model, sulfoxaflor, $p = 0.73$, *C. bombi*, $p = 0.48$, sulfoxaflor and *C. bombi*, $p = 0.57$; Fig. 4).

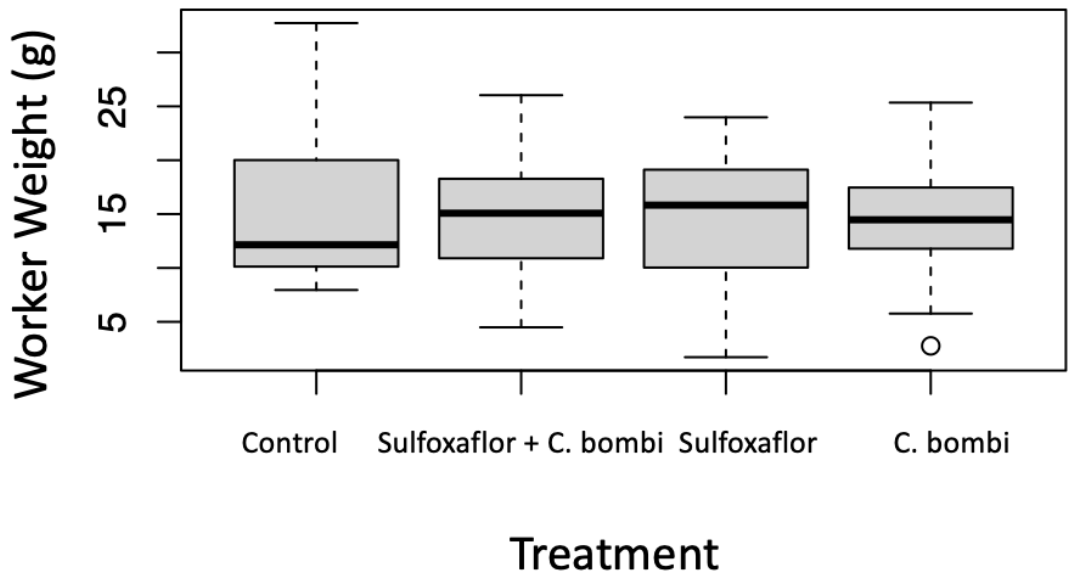

Figure 1. No effect of treatment on a colony's total weight of workers (boxplots showing median, interquartile range, min/max values, and outliers).

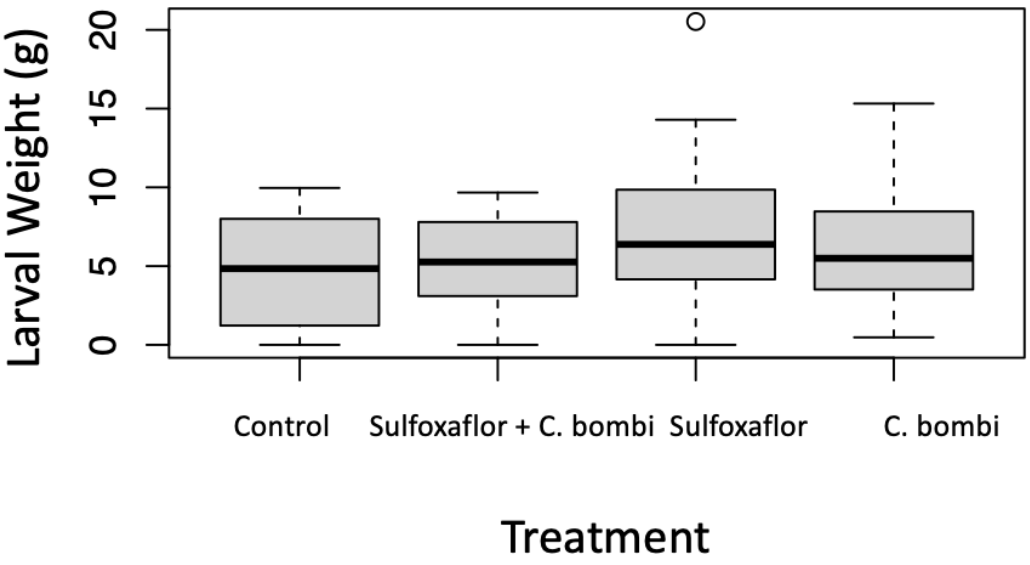

Figure 2. No effect of treatment on a colony's total weight of larvae (boxplots showing median, interquartile range, min/max values, and outliers).

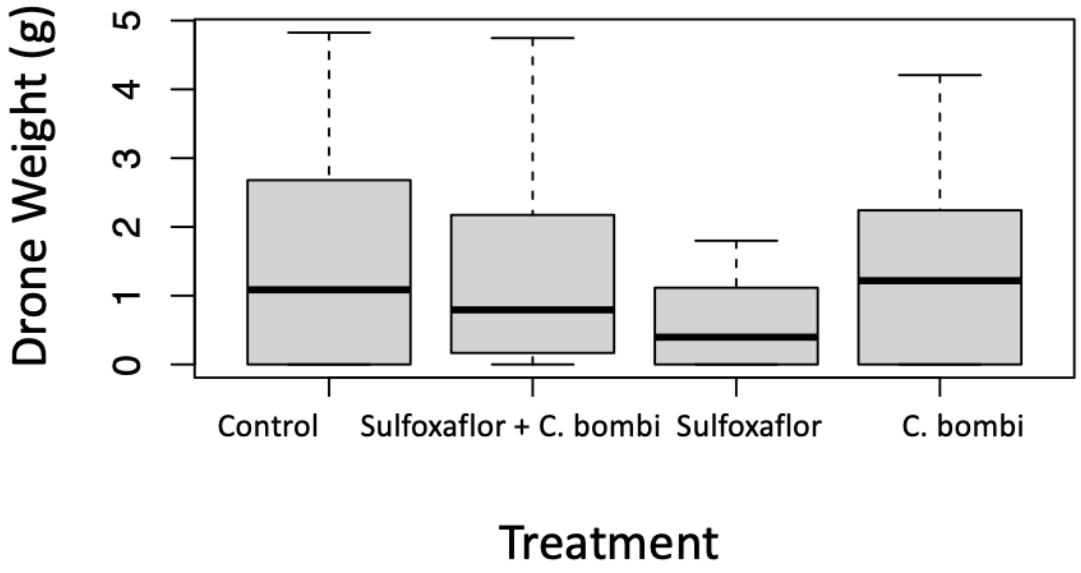

Figure 3. No effect of treatment on a colony's total weight of drones (boxplots showing median, interquartile range, min/max values, and outliers).

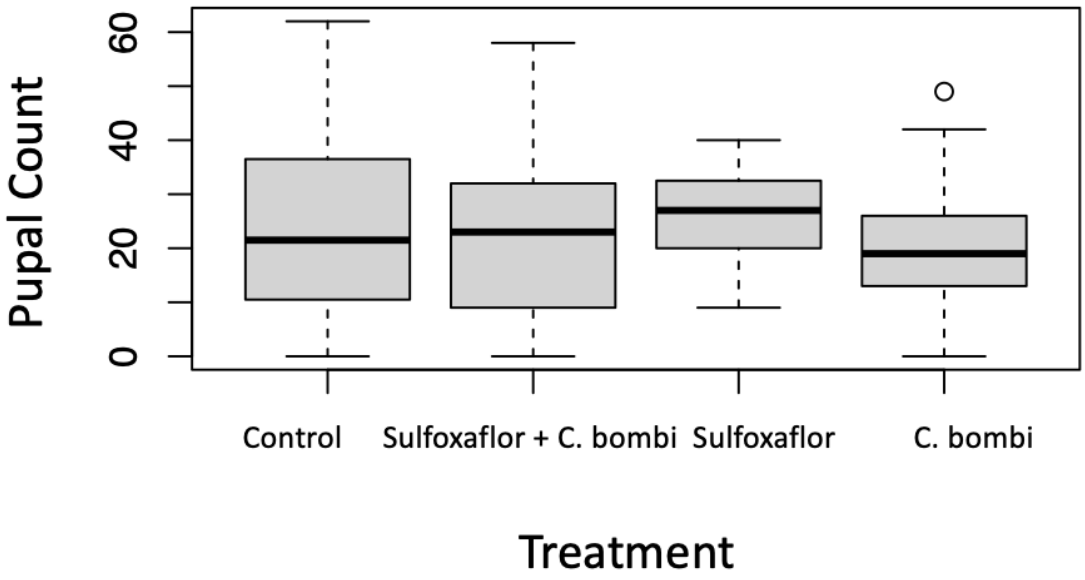

Figure 4. No effect of treatment on the number of pupae per colony (boxplots showing median, interquartile range, min/max values, and outliers).

3. Discussion

Here we found no effects of either individual or combined stressors – specifically, the agrochemical sulfoxaflor and the parasite *C. bombi* – on the health of bumble bee colonies. Consequently, this work suggests that field realistic exposure of young bumble bee colonies to sulfoxaflor is not harmful to their development, either in the presence or absence of a common parasite. This significantly develops our understanding of the safe use of sulfoxaflor.

That there is no interaction between a common parasite and sulfoxaflor is encouraging. However, there are a range of other parasites, including more harmful species like *Nosema bombi*, which may still have the potential to interact with agrochemicals like sulfoxaflor due to the parasite's more sever effects on bee health (Rutrecht & Brown, 2009). Further, it is possible that at more sensitive points in the bee lifecycle, where *Crithidia bombi* has a greater impact (Brown et al., 2003), additive or synergistic interactions might still exist. However, the research here finds that, for the colony stage at which exposure took place, there is little consequence for colony health irrespective of exposure.

Interestingly, our results contrast with prior work. Siviter et al. (2018) exposed younger colonies (at the 8 worker stage) to a 14 day 5ppb dose of sulfoxaflor through *ad libitum* sugar water and found a significant effect on colony development. While this is a lower overall exposure to sulfoxaflor than used in the experiment reported here, exposure in Siviter et al. (2018) was for a longer period and at a more sensitive life history stage. This suggests that the effects of sulfoxaflor are worse when colonies are at the incipient stage, lacking the social robustness of older colonies (Bryden et al., 2013), and thus exposure puts them under greater physiological stress. This would indicate that summer use of sulfoxaflor is likely safe for bees, while springtime use is potentially damaging. The conditions in which a queen is exposed to 5ppb for 14 days would depend on the timing of agricultural applications of sulfoxaflor, which are not well reported in the literature. However, we

note that as the colonies in Siviter et al. (2018) were placed in the field post-treatment, this additional stress may have enabled the impacts of sulfoxaflor to be seen. In contrast, our colonies – after the pollination treatment – were kept under non-stressful laboratory conditions, which may have enabled colonies to compensate for impacts of our treatments (but see Brown et al., 2003, where impacts of *C. bombi* infection were seen despite non-stressful laboratory conditions).

Ultimately, this research does not suggest that any changes need to be made to sulfoxaflor's registration, nor to the risk assessment process.

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