Results

n.s



Bees, Pesticides and Environmental Conservation:

Defining the Sublethal Effects of Sulfoxaflor on Honeybees

Aims

• To observe alterations in response to Queen Mandibular Pheromone (QMP) and Brood Pheromone (BP), resulting from Sulfoxaflor exposure

• To investigate the molecular mechanisms, explaining why Sulfoxaflor has

sublethal effects on honeybees

<u>Importance of the Research</u>

Honeybees are essential pollinators, with an important role in the growth of food crops (Hung et al., 2018). Moreover, there has been evidence that honeybee interaction with wild bees can enhance pollination natural areas (Greenleaf and Kremen, 2006). Recently, global honeybee colonies have been experiencing significant declines.

Many pesticides interact with pheromones that are key to maintaining development within the hive, as shown by research done previously in my supervisor's (Elizabeth Duncan) lab. As bees grow, they respond differently to pheromones such as QMP and BP, allowing for division of labour. This creates groups to look after the queen, clean the hive, forage for food e.t.c.. The way that pesticides can affect behaviour means that this system can be disrupted, leading to disorganisation which negatively impacts colony health.

There have been some studies on how chronic exposure of Sulfoxaflor may be having effects on honeybees. This project was designed to further investigate this. Currently, in the UK the use of Sulfoxamine-based pesticides is permitted in greenhouse conditions, with it being suggested they pose little threat to honeybees. The ideal outcome of this project was to provide evidence proving Sulfoxaflor's ability to cause harm to honeybees.

A: Average proboscis extension response scores of bees treated with a control

(water), 50ppb Sulfoxaflorand 150ppb Sulfoxaflor. Sample size was 40 bees per group.

Statistical significance was tested using a Mann Whitney U Test. ** displays a significant

statistical significance (P < 0.05). n.s displays no significant statistical significance.



<u>Methodology</u>

Caging Bees:

Experimental cages were set up, consisting of newly emerged bees (Duncan et al., 2016). These bees were either exposed to water, 50ppb Sulfoxaflor (Ibrahim et al., 2023).

Proboscis Extension Response Assay:

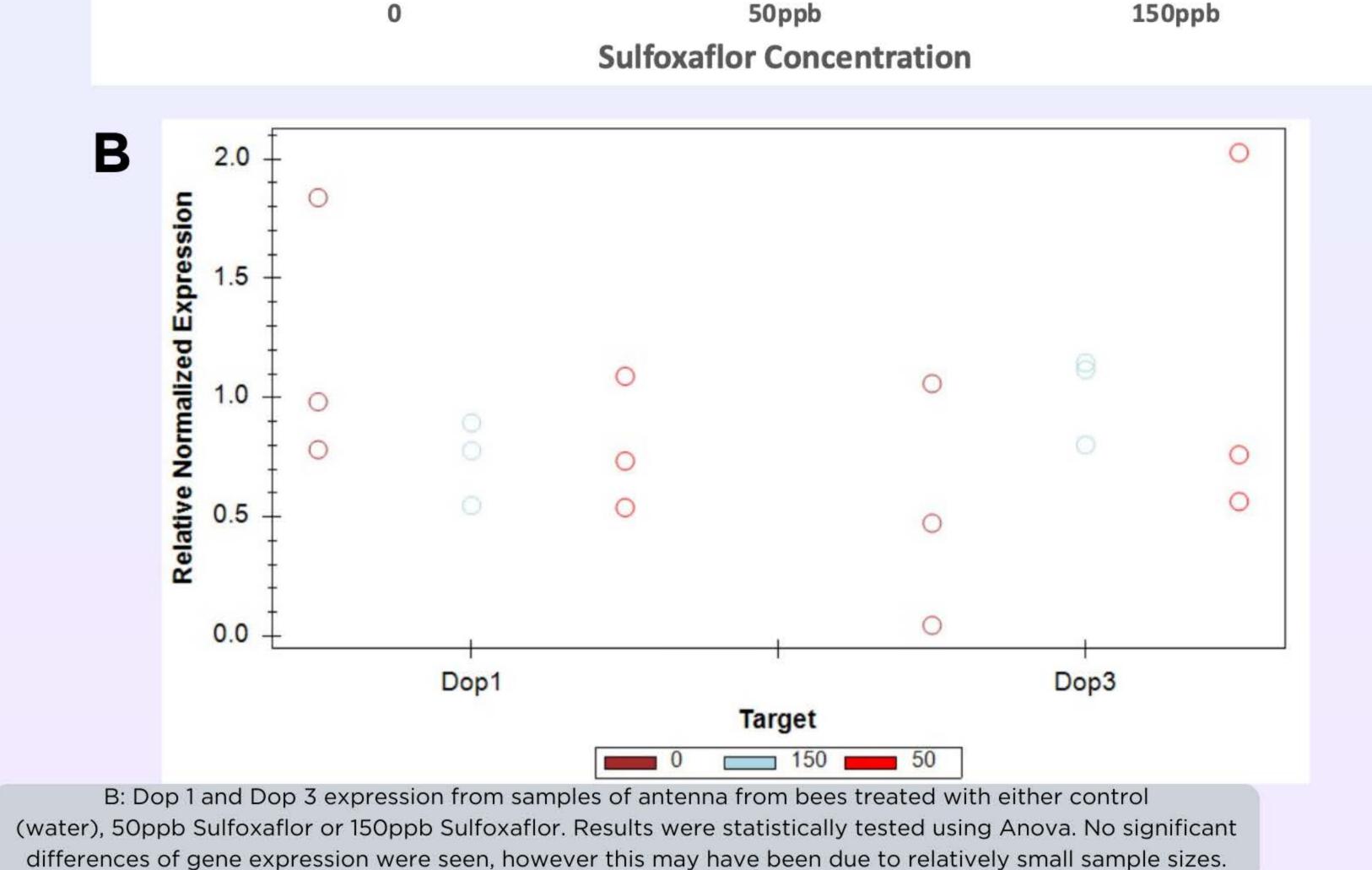
Investigates bee's responses to increasing concentrations of sucrose solutions, allowing for assumptions on foraging behaviour of bees (Matsumoto et al., 2012).

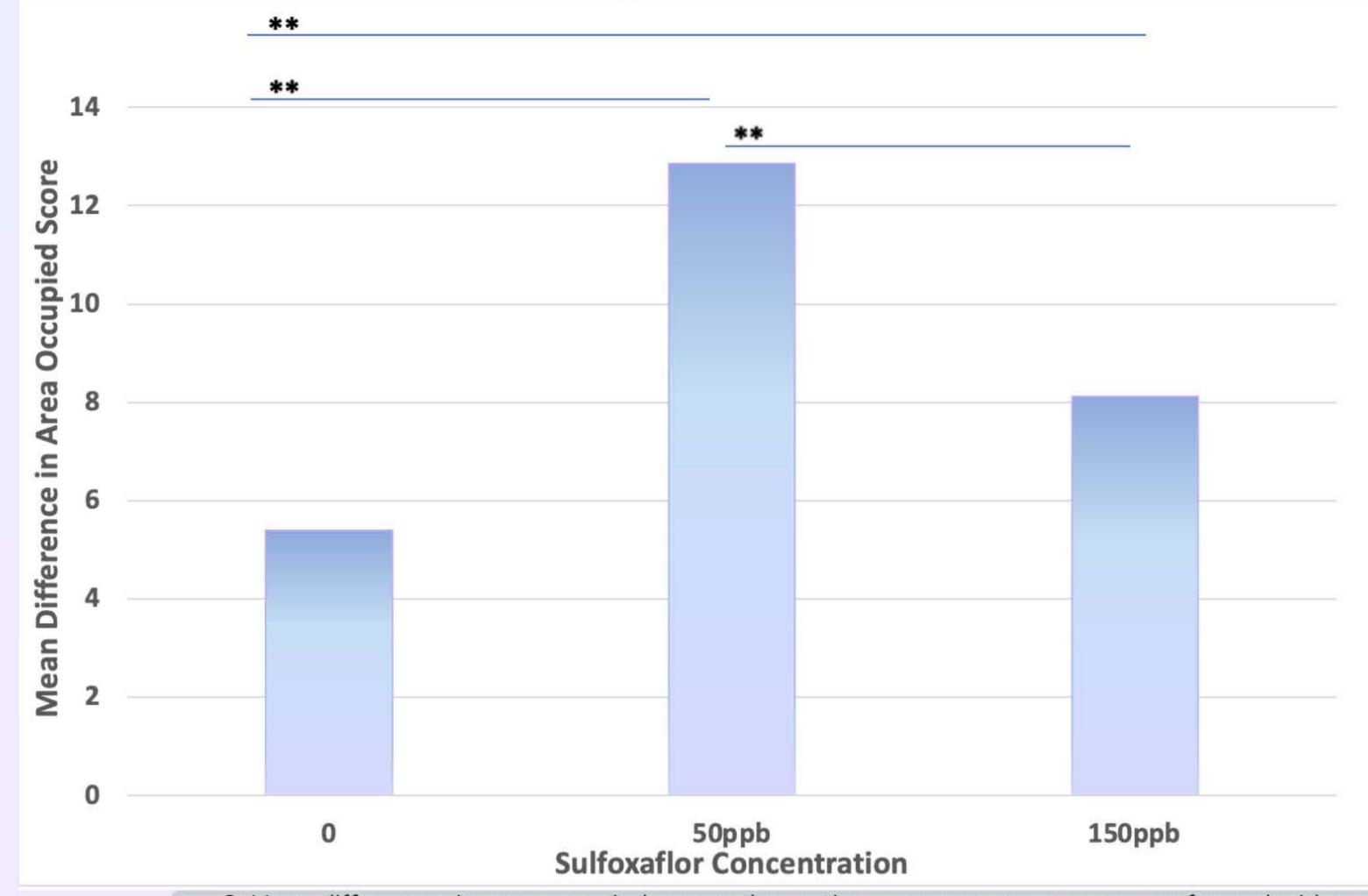
Retinue Response Assay:

Investigates bee's responses to QMP, in order to make assumptions surrounding brooding behaviour (Knapp, 2022).

RT-qPCR:

Antenna were dissected to analyse how Sulfoxaflor exposure may affect expression of the genes Dop1 and Dop3 (genes associated with dopamine release within QMP response (Vergoz et al., 2009)) RNA was extracted, cDNA created and RT-qPCR carried out.





C: Mean differences in area occupied scoreswhen retinue response assays were performed with a control (ethanol) and 0.01qe QMP. Groups are divided by treatments given (either control (water), 50ppb Sulfoxaflor or 150ppb Sulfoxaflor. Increased scores indicate greater attraction to QMP. Statistical significance was tested using Paired T Testing. ** displays a significant statistical significance (P < 0.05).

Conclusions

This is the first project done proving Sulfoxaflor's interaction with pheromone response. From the various experiments used within this investigation, it can be concluded that doses of 50ppb and 150ppb Sulfoxaflor resulted in increased response to QMP and reduced sucrose response thresholds. Increased attraction to QMP (a pheromone produced by the Queen bee) would cause bees to maintain their position looking after the Queen for longer. Reduced sucrose response thresholds result in bees only

consuming foods with high sucrose content, these conditions are found within hives. Therefore, for bees to begin foraging, their sucrose response threshold must lower. The interaction of these factors influence development, slowing the transformation of newly emerged bees into workers. The consideration of chronic, sub-lethal effects must be incorporated into pesticide approval testing by Governments, to prevent damage to both agricultural and natural land.

References: Duncan, E.J., F. Greenleaf, S.S.

, E.J., Hyink, O. and Dearden, P.K. 2016. Notch signalling mediates reproductive constraint in the adult worker honeybee. Nature Communications. 7(1).

af, S.S. and Kremen, C. 2006. Wild bees enhance honey bees' pollination of hybrid sunflower. Proceedings of the National Academy of Sciences. 103(37), pp.13890–13895.

L-L.J., Kingston, J.M., Albrecht, M., Holway, D.A. and Kohn, J.R. 2018. The worldwide importance of honey bees as pollinators in natural habitats. Proceedings of the Royal Society B: Biological Sciences. 285(1870), p.20172140.

E-D.S., alla, El-Masarawy, M.S., Salem, R.A., Hassan, N.N. and Moustafa, M. 2023. Sulfoxaflor influences the biochemical and histological changes on honeybees (Apis mellifera L.). Ecotoxicology. 32(5), pp.674–681.

R.A. 2022. Biogenic amines as mediators of queen mandibular pheromone's control of worker reproduction in the honey bee, Apis mellifera. [Online] The University of Leeds. [Accessed 11 October 2023]. Available from: https://etheses.whiterose.ac.uk/31433/1/THESIS%20CORRECTIONS%20-%20Floots, Y., Menzel, R., Sandoz, J.-C. and Giurfa, M. 2012. Revisiting olfactory classical conditioning of the proboscis extension response in honey bees: A step toward standardized procedures. Journal of Neuroscience Methods. 211(1), pp.159–167.