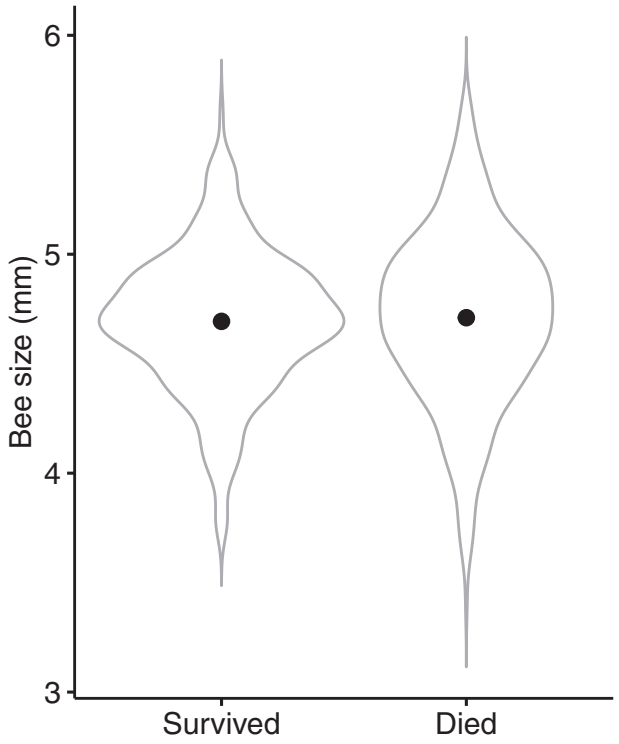

Fig. 2. Violin plots depicting the average size (mm) of bumblebees that either survived or died during the experiment (96 h). Mortality risk was higher for both smaller and larger bees.

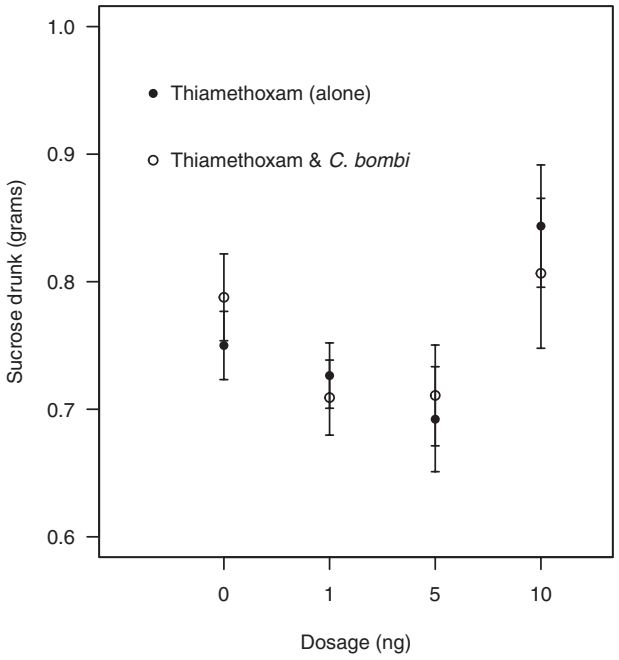

Fig. 3. The mean amount (grams) of sucrose drunk (±SE) over 96 h from parasitized and unparasitized bumblebees (*C. bombi*) acutely exposed to varying dosages of thiamethoxam. Subjects that did not survive the experiments were excluded from this analysis.

Discussion

Previous studies with bumblebees have shown that the LD_{50} of thiamethoxam is 5 ng of active ingredient per bumblebee (EFSA 2015),

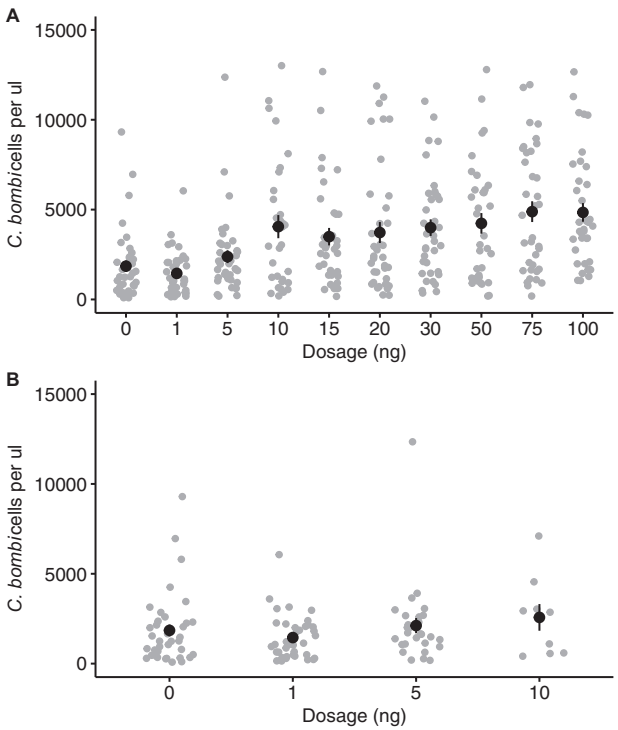

Fig. 4. The mean (±SE) number of *C. bombi* cells per µl found in the hindgut of all bumblebee workers from the experiment (A) and only bumblebees that survived until the end of the experiment (B).

and our results were similar (6.63 ng when exposed to thiamethoxam in isolation and 6.82 ng for bumblebees exposed to both thiamethoxam and *C. bombi*). This suggests that contrary to our original hypothesis, the parasite *C. bombi* had no impact on the LD_{50} of thiamethoxam on bumblebees (*B. terrestris*). This is surprising, as the effects of this parasite on bumblebees are context-dependent, and emerge most obviously when bees are exposed to other stressors (Brown et al. 2000, 2003; Yourth et al. 2008). Interestingly, and in contrast to previously observed results (Kessler et al. 2015, Arce et al. 2018) (but see [Muth et al. 2020]), we found no effect of thiamethoxam exposure on sucrose consumption. Finally, thiamethoxam exposure was seen to increase *C. bombi* intensity, but only at lethal dosages as there was no effect at sub-lethal levels. Our results demonstrate that methodologies currently used within the regulatory process can be modified to consider the interaction effects between multiple environmental stressors on wild bees.

We found no evidence of interaction effects between thiamethoxam and *C. bombi* on bumblebee mortality. This contrasts with previous studies that have shown that simultaneous exposure to both thiamethoxam and *C. bombi* can reduce bumblebee survival (Fauser-Misslin et al. 2014). However, Fauser-Misslin et al. (2014) assessed the impact of chronic, sub-lethal thiamethoxam concentrations over 9 wk on queen bumblebee survival, while here we used acute dosages, in a toxicity test with workers. Toxicity tests, such as LD_{50} experiments, are important in determining the lethal consequences of agrochemical use, but are not designed to detect more subtle, sub-lethal impacts of agrochemical exposure (Gill et al. 2012, Siviter et al. 2020b, Siviter et al. 2021b). While our modified LD_{50} protocol can be used to assess how parasites and agrochemicals interact at higher dosages, a failure to conduct sub-lethal assessments of chronic exposure in bumblebees alongside toxicity tests will clearly result in a failure to detect sub-lethal, but significant,

interactions between agrochemicals and parasites (Fauser-Misslin et al. 2014, Siviter et al. 2021a). While our methodology could be used within the regulatory process, future research should be focused on developing methodologies that assess the potential sub-lethal interactions between agrochemicals and parasites on bees.

We found that *C. bombi* intensity was significantly higher in bumblebees that had been fed high dosages of thiamethoxam and that had subsequently died. *C. bombi* intensity typically increases for up to 7 d after inoculation and plateaus between 7- and 10-days post inoculation (Logan et al. 2005). We exposed bumblebees to thiamethoxam 7 d post inoculation and found that bees that died (on day 7) had a higher intensity of *C. bombi* than bees that survived (Fig. 4). One explanation for this is that acute exposure to thiamethoxam exerts long-term inhibition on the growth of *C. bombi*, and that this could only occur in bees that survived exposure. Alternatively, higher *C. bombi* counts in bumblebees exposed to lethal acute doses could be due to rapidly enhanced production or release of the parasite from the gut lining (Koch et al. 2019). Future experiments are needed to determine the mechanism behind this interaction. However, as we found no effect of sub-lethal thiamethoxam dosages on *C. bombi* intensity, this suggests that at field-realistic levels, thiamethoxam is unlikely to impact *C. bombi* intensity.

Neonicotinoids are the most commonly used insecticides in the world and understanding the interaction between them and bumblebee pathogens is therefore vitally important. However, as the number of insect pests that are resistant to neonicotinoids increase, and bans/restrictions on their use increase globally, novel insecticides such as sulfoxaflor or flupyradifurone could replace them over large geographical areas (Brown et al. 2016, Siviter and Muth 2020). Sulfoxaflor exposure can have significant sub-lethal impacts on bumblebee (*B. terrestris*) reproduction (Siviter et al. 2018a, Siviter et al. 2020 a,b; Linguadoca et al. 2021) (but see [Siviter et al. 2019]) and flupyradifurone exposure can impair honeybee larval development (Tan et al. 2017, Al Naggar and Baer 2019), and adult behaviour (Tosi and Nieh 2019, Tong et al. 2019, Hesselbach et al. 2020) (recently reviewed in [Siviter and Muth 2020]). Novel insecticides could also interact with bee pathogens, for example, bumblebee larvae fed sulfoxaflor in isolation showed no evidence of an increase in larval mortality, but when coexposed to sulfoxaflor, and the common bumblebee parasite *Nosema bombi*, there was a significant increase in larval mortality (Siviter et al. 2020a). Similarly, honeybees (*A. mellifera*) fed flupyradifurone and inoculated with *N. ceranae* had lower survival than unexposed bees, and those exposed to each stressor in isolation (Al Naggar and Baer 2019). While we found no interaction between *C. bombi* and the neonicotinoid thiamethoxam on bee mortality, future research should focus on understanding how novel insecticides, such as sulfoxaflor and flupyradifurone, interact with common bee parasites (Siviter and Muth 2020).

Global bee declines are thought to be driven by multiple anthropogenic stressors, including agrochemicals and parasites (Vanbergen & Insect Pollinators Initiative 2013, Goulson et al. 2015, Siviter et al. 2021a) which suggests that the agrochemical regulatory process should consider how insecticides interact with commonly occurring bee parasites (Siviter and Muth 2020). Here we show how toxicity tests, such as LD_{50} experiments, can be modified to consider the interactions between agrochemicals and parasites. This methodology could easily be modified to test other parasites depending on the life history of the parasites. However, the sheer number of bee parasites (known and unknown), and the range of different agrochemicals used in intensive agriculture means that testing every potential interaction between parasites and agrochemicals is impractical, and in some cases, when we do not have an understanding of the parasite life-history, impossible. In these cases, post-authorisation monitoring observations, which are currently nonexistent (Milner and Boyd 2017), should be carried out that monitor interactions between pesticides and pathogens. More broadly a move towards a more holistic approach to environmental risk assessment, that considers the interactions between multiple stressors, and models their impact on wild bees, is required to better safe-guard bees, and other pollinators, from the potential harm of agrochemicals (Siviter and Muth 2020, Topping et al. 2021).

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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Data Availability

Raw data available here https://osf.io/vautc/.

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