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NOTES AND COMMENTS

A short note on extreme sex ratio in solitary bees *Osmia cornuta* in semi-field trials testing the impact of neonicotinoids

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The widespread prophylactic use of neonicotinoids has severe negative effects on non-target organisms that provide ecosystem services. Chronic neonicotinoid exposure led to a male-biased offspring sex ratio in the solitary bee species *Osmia bicornis* in laboratory experiments, but semi-field or field data are largely lacking. Here, we used semi-field experiments to investigate the potential impact of the neonicotinoid clothianidin on the sex ratio of the closely related species *Osmia cornuta*. Male and female individuals of *O. cornuta* were held in flight cages with fully blooming winter oilseed rape, treated or not with clothianidin. After hibernation, sex ratio of emerging offspring and overwintering mortality was determined. A total of 191 male and 4 female, and 203 male and 2 female *O. cornuta* emerged in the control and the clothianidin treatment, respectively, thereby clearly showing an extremely male-biased sex ratio. However, there was no significant clothianidin treatment effect. As sex ratio was strongly biased in both, the clothianidin and the control treatment, our data can obviously not explain the underlying mechanism. Such extreme sex ratios require more attention in semi-field studies assessing the impacts of pesticides and other stressors on solitary bees.

Keywords: Solitary bees; *Osmia* spp.; sex ratio; pesticides; neonicotinoid

Solitary bees/*Osmia* spp./sex ratio/pesticides/neonicotinoid

The widespread prophylactic use of neonicotinoids has severe negative effects on non-target organisms that provide ecosystem services including pollination (e.g., Neumann et al., 2015; Rundlöf et al., 2015). In laboratory experiments, chronic neonicotinoid exposure led to a male-biased offspring sex ratio in solitary bees *Osmia bicornis* (Sandrock et al., 2014), but semi-field or field data are largely lacking. Here, we used semi-field experiments to investigate the potential impact of the neonicotinoid clothianidin on the sex ratio of *Osmia cornuta*.

In April 2015, male and female *O. cornuta* (WAB Mauerbienenzucht, Germany) were held for 21 days in flight cages ($N = 20$, $1.5 \times 1.5 \times 2$ m; 5 males and 10 mated females per enclosure) during the peak blooming of winter oilseed rape (OSR), with nesting tubes (paper straws of 8 mm diameter), and plastic dishes containing moist clay for nest construction. According to a randomized split-plot design, OSR was treated or not (= controls) with clothianidin (seed coated; Modesto: clothianidin 400 g/L, Bayer CropScience AG, Germany). Residue analysis of pollen-nectar provisions confirmed field-realistic exposure in the clothianidin treatments (0.56 ng/g on average, $N = 13$) and its absence (or concentrations < detection threshold) in the controls ($N = 11$). After mimicked hibernation from November to March at 2 °C, sex ratio of emerging offspring and overwintering mortality were determined.

A total of 191 male and four female, and 203 male and two female *O. cornuta* emerged in the control and the clothianidin treatment, respectively, thereby showing an extremely male-biased sex ratio (control: 98% males, clothianidin treatment: 99% males). However, no significant treatment effects (Table 1), were observed in either sex ratio (Chi-square test: $\chi^2 = 0.78$, $df = 1$, $p = 0.38$) or overwintering mortality ($p = 0.867$, Mantel–Cox test). Average mortality rates (controls: 11.36%, treatments: 10.87%) showed no significant difference to previously observed winter mortalities for free flying *O. cornuta* (~11%; Torchio & Asensio, 1985; Torchio, Asensio, & Thorp, 1987).

Our data can obviously not explain the underlying mechanisms for the observed extreme sex ratio. The results are generally in line with previous reports of a male shifted offspring sex ratio in *O. cornuta* in the second half of their nesting period (~71% males in April, Bosch & Vicens, 2005). However, low quantity of available food resources (e.g., Goodell, 2003; Bosch & Vicens, 2005) can be excluded as a major driver, because floral resources in our study were considerable (controls: 93 flowers/m^2, treatments: 97 flowers/m^2), while low quality of monofloral OSR diet contributing to our results is possible but unlikely (e.g., Bukovinszky

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Table 1. Reproduction, sex ratio, and winter mortality of *O. cornuta* in controls and neonicotinoid treatments.

	Control	Clothianidin
Number of produced cocoons	220	230
Winter mortality [%]	11.36	10.87
Number of hatched adults	195 (98% males)	205 (99% males)
Offspring sex ratio (males:females)	191:4	203:2

The number of produced cocoons, hatched adults, the winter mortality, and the offspring sex ratio are shown.

et al., 2017). Insufficient matings (Seidelmann, 2014) and/or reduced sperm quality due to neonicotinoids (Straub et al., 2016) might otherwise explain a male biased offspring sex ratio. However, this seems highly unlikely as only mated females were used and additional males were inserted into each enclosure to enhance mating opportunities. Since the weather conditions were also favorable for *O. cornuta* (see Vicens & Bosch, 2000), other unidentified stress factors have apparently contributed to our results. As the sex ratio was extremely male-biased in both the control and clothianidin treatments, the finding can obviously not be explained by the neonicotinoid. In conclusion, such extreme sex ratios require more attention in semi-field studies assessing the impacts of pesticides and other treatments on solitary bees.

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