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INTRODUCTION

Pesticide residues found in nectar and pollen are usually not lethal for pollinators, but can have complex sub-lethal effects that decrease the fitness and health of bee pollinators. Multiple studies have demonstrated sub-lethal effects of pesticides on honey bee foraging and orientation (Fischer et al, 2014; Schneider et al, 2012). However, no studies have yet examined the detailed effects of pesticides on honey bee flight ability. Our goal was thus to determine the acute and chronic sub-lethal field-realistic doses of thiamethoxam (a common neonicotinoid pesticide) influence honey bee flight performances.

MATERIALS AND METHODS

We conducted two experiments. In each experiment each honey bee performed two successive flights. The first flight is an exhaustion flight designed to equalize the energy levels of bees, which were then fed 10 μ l of glucose for the second flight. Honey bee flight performances were evaluated using a flight mill (Fig. 1) that measured duration, distance, average velocity and maximum velocity. of the flights. We used field-realistic doses of thiamethoxam (EFSA, 2012; Henry et al, 2012).

Acute exposure experiment

Honey bees were fed with 1.8 M sucrose solution and received a single sub-lethal dose of pesticide (1.34 ng/bee) between the first and second flight. Bees rested 1 hr between flights to allow full absorption of the pesticide.

Chronic exposure experiment

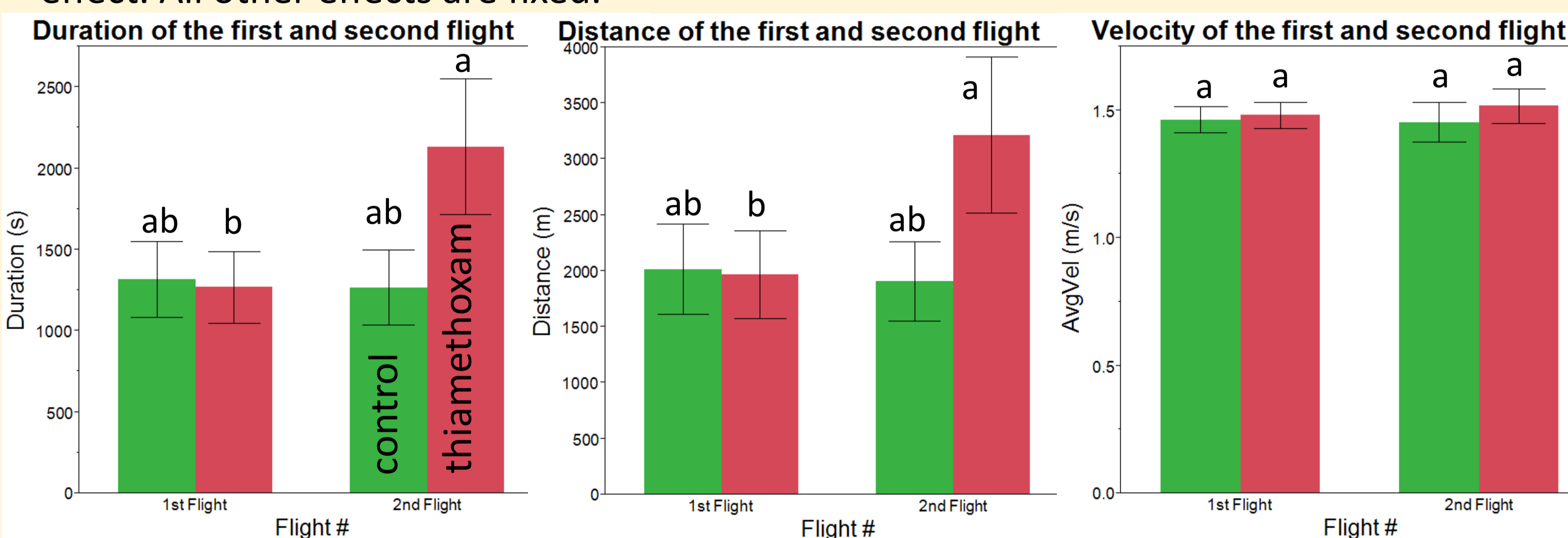
Honey bees were fed with 1.8 M sucrose solution containing either 0 ppb (control), 33 ppb, or 45 ppb of thiamethoxam for the 2 days before the test flight. Thus, unlike the acute exposure experiment, bees ingested the test solution before both flights. The bees received an average of 3.03 ± 0.09 and 4.24 ± 0.09 ng/day of thiamethoxam when fed the 33 and 45 ppb solution, respectively. These are field-realistic doses (EFSA, 2012).



Figure 1. Flight mill used in the test, with a bee flying attached to one extremity (upper one) of the flight mill arm. Design based upon the plans of Teah Smith (WSU TFREC).

RESULTS AND DISCUSSION

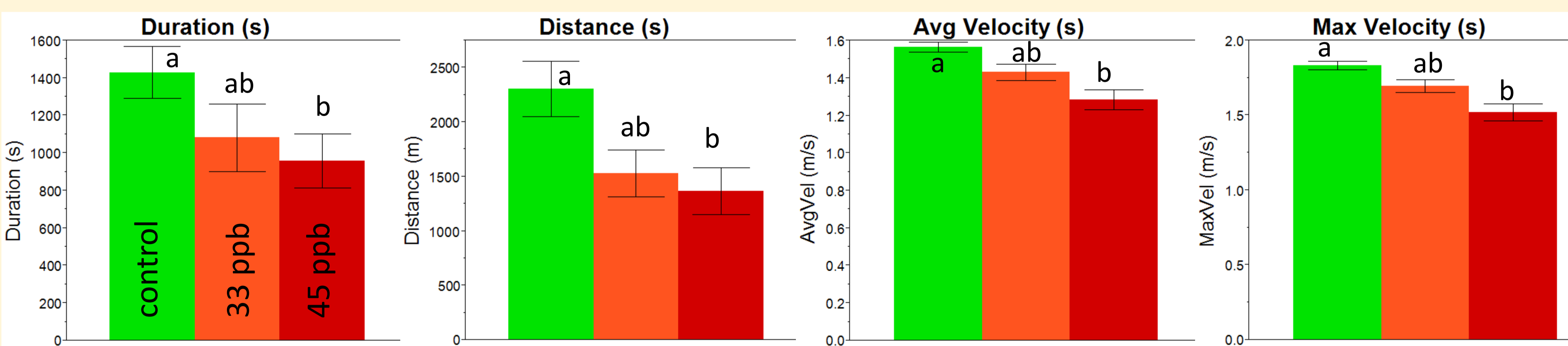
In both experiments we used repeated measures Analysis of Variance (ANOVA, REML Algorithm) and Tukey HSD tests ($p < 0.05$). Colony is a random effect. All other effects are fixed.



Acute exposure to thiamethoxam caused temporary excitation: a significant increase of the duration and distance flown by the bees caused by the pesticide treatment (Fig. 2).

Figure 2. Flight performances of the acute exposure experiment. The pesticide/control dose was administered *after* the first flight, the exhaustion flight.

A. Treatment*Phase, $F_{1,43} = 4.63$, $p = 0.0371$
 B. Treatment*Phase, $F_{1,43} = 4.43$, $p = 0.0413$
 C. Treatment*Phase, $F_{1,43} = 0.19$, $p = 0.6640$



The pesticide treatment in the **chronic exposure** experiment significantly decreased the duration, distance, average velocity, and maximum velocity. This is opposite of acute exposure effects, but is consistent with temporary neonicotinoid excitation effect followed by longer term impairment.

Figure 3. Flight performances of the chronic exposure experiment. The pesticide/control dose was administered *before* the first flight.
 A. Treatment, $F_{2,85} = 6.26$, $p = 0.0029$; B. Treatment, $F_{2,86} = 8.77$, $p = 0.0003$; C. Treatment, $F_{2,83} = 12.47$, $p < 0.0001$; D. Treatment, $F_{2,77} = 12.47$, $p < 0.0001$

Summary. We provide the first detailed demonstration that a sub-lethal neonicotinoid dose can alter actual honey bee flight ability. Neonicotinoids have an excitatory effect on insect neurons (Tan et al, 2007). A short-term exposure (1 hr) increased flight duration and distance by 40%. This agrees with the longer zig-zag flights of bees exposed to neonicotinoids (Fischer et al. 2014). Long term exposure (2 days) impaired flights, reducing all measures (i.e. duration, distance, average and maximum velocity were reduced by 33, 41, 18, 17%, respectively). Thus, thiamethoxam has complex effects upon bee flight that depend upon dose and exposure time. Coupled with the navigational disruption, these effects could explain why fewer exposed bees return to the nest, gradually decreasing the colony workforce and thus its health over time.

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