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Effects of a neonicotinoid pesticide on thermoregulation of African honey bees (*Apis mellifera scutellata*)

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ABSTRACT

Thiamethoxam is a widely used neonicotinoid pesticide that, as agonist of the nicotinic acetylcholine receptors, has been shown to elicit a variety of sublethal effects in honey bees. However, information concerning neonicotinoid effects on honey bee thermoregulation is lacking. Thermoregulation is an essential ability for the honey bee that guarantees the success of foraging and many in-hive tasks, especially brood rearing. We tested the effects of acute exposure to thiamethoxam (0.2, 1, 2 ng/bee) on the thorax temperatures of foragers exposed to low (22 °C) and high (33 °C) temperature environments. Thiamethoxam significantly altered honey bee thorax temperature at all doses tested; the effects elicited varied depending on the environmental temperature and pesticide dose to which individuals were exposed. When bees were exposed to the high temperature environment, the high dose of thiamethoxam increased their thorax temperature 1–2 h after exposure. When bees were exposed to the low temperature, the higher doses of the neonicotinoid reduced bee thorax temperatures 60–90 min after treatment. In both experiments, the neonicotinoid decreased the temperature of bees the day following the exposure. After a cold shock (5 min at 4 °C), the two higher doses elicited a decrease of the thorax temperature, while the lower dose caused an increase, compared to the control. These alterations in thermoregulation caused by thiamethoxam may affect bee foraging activity and a variety of in-hive tasks, likely leading to negative consequences at the colony level. Our results shed light on sublethal effect of pesticides which our bees have to deal with.

1. Introduction

Pesticides are widely used in agriculture and veterinary medicine, however they also affect the health of numerous non-target organisms (Desneux et al., 2007; Schäfer et al., 2012). Beside the numerous factors playing a role in colony losses (Vanbergen et al., 2013), recently attention has focused on the effects of neonicotinoid pesticides on honey bees and their possible role in declining health of honey bee colonies (for reviews see Godfray et al., 2014 and Pisa et al., 2014). These systemic insecticides are potent agonists of the nicotinic acetylcholine receptors (nAChR), largely distributed in the insect central nervous system, and can disrupt processes involving cholinergic neurotransmission, such as olfaction, learning and memory (Jones et al., 2006; Armengaud et al., 2002; Williamson and Wright, 2013). Currently, 30% of the insecticides used worldwide are neonicotinoids (Simon-Delso et al., 2015) and honey bees are exposed to them both in the field (see Krupke and Long, 2015 for review) and inside the hive (Chauzat et al., 2006; Lambert et al., 2013; Mullin et al., 2010; Smodis Skerl et al., 2009). The sublethal effects of neonicotinoids on honey bees have been extensively studied at many different physiological levels, but thermoregulation has been surprisingly overlooked.

Thermoregulation is essential for honey bees both at individual and colony levels and is achieved by both physiological and behavioural processes (Heinrich, 1980a,b; Stabentheiner et al., 1995). Honey bees need to maintain the optimal temperature of the brood during the whole active season (~35 °C, Himmer, 1932; Human et al., 2006) and the winter cluster during the cold periods (Heinrich and Esch, 1994; Jones et al., 2004; Stabentheiner,

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2003). Forager bees under colder environmental conditions maintain their thorax above ambient temperature during the foraging cycle using the flight muscles (Coelho, 1991), while under warmer conditions foragers use evaporative cooling to maintain their thorax temperature (Heinrich, 1980a; Nicolson and Human, 2008). In fact, honey bee flight ability and consequent foraging behaviour depend on thorax temperature (Esch, 1988; Schmaranzer, 2000; Schmaranzer and Stabentheiner, 1988; Stabentheiner, 2001). Foragers control thorax temperature not only during foraging activity outside the hive but also when performing the recruitment dance and unloading food inside the colony (Stabentheiner et al., 1995; Stabentheiner and Hagmuller, 1991). The priority during unloading of foragers is related to their thorax temperature; in general, the hotter the thorax of the forager the more valuable and profitable the receiver perceives the food source to be, so that the forager has to wait less than a forager with low thorax temperature to be unloaded (Stabentheiner and Hagmuller, 1991). Therefore, any interference with thermoregulation has implications at both individual and colony levels.

African honey bees have a higher metabolic rate than European honey bees, but their smaller size facilitates heat loss and their thorax temperatures are similar (Heinrich, 1979, 1980a). Because African honey bees have a significantly greater (15%) engine (thorax) to bee mass ratio (Hepburn et al., 1999), they provide a more sensitive model to test the effects of temperature on thorax muscle activity. Honey bee thermoregulation is impaired by insecticides like organophosphates and pyrethroids that act upon the cholinergic and adrenergic pathways (Belzunces et al., 1996; Schmaranzer et al., 1987; Vandame and Belzunces, 1998), but the effect of neonicotinoids on honey bee body temperature and thermoregulation abilities has not yet been investigated.

In this study, we tested the effects of three acute doses of the neonicotinoid thiamethoxam, a commonly used second-generation neonicotinoid that succeeded the first-generation imidacloprid (Maienfisch et al., 2001; Simon-Delso et al., 2015), on the thorax temperature of individual honey bees (*Apis mellifera scutellata*). Forager bees, compared to in-hive bees, are more frequently exposed to neonicotinoids (*i.e.* via spray and food contamination) and exposed to a wider variety of environmental temperatures (Hepburn and Radloff, 1998; Kovac et al., 2010). Therefore, we tested the effects of thiamethoxam on foragers exposed to either low (22 °C, Experiment 1) or high (33 °C, Experiment 2) temperatures to mimic outside/inside hive temperatures. During neonicotinoid seed-dressed maize sowing (*i.e.* early spring), early flying foragers can be exposed to both low temperatures and neonicotinoids used for seed treatments, such as thiamethoxam (Nuyttens et al., 2013; Tremolada et al., 2010). Hence, we also investigated the effects of the neonicotinoid on the ability of foragers to recover from critically low temperatures (cold shock). We predicted that the pesticide, by activating the nAChR, may act upon cholinergic pathways and alter thermoregulation. Since flight behaviour and thermoregulation are closely related (Esch, 1976; Schmaranzer, 2000), changes in forager body temperatures could be one of the reasons behind the various sublethal effects elicited by thiamethoxam and other neonicotinoids involving homing and foraging behaviour of bees (Bortolotti et al., 2003; Henry et al., 2012, 2015).

2. Materials and methods

2.1. Honey bee preparation

Returning *Apis mellifera scutellata* nectar foragers were collected from four colonies at the experimental farm of the University of Pretoria, South Africa, in September 2014. After collection, bees were immediately brought to the laboratory. Each individual bee was chilled using ice, then inserted into a Plexiglas tube (6 mm diameter) and held in place with a beeswax-colophony mixture applied on the dorsal surface between the thorax and the abdomen. The thorax remained free of any material, since its temperature was the principal endpoint assessed in this study (Fig. 1).

Tubes with honey bees were placed upright in a holding rack at a distance that did not allow trophallaxis between individuals. Honey bees were individually fed 3 µl of 25% (w/w) sucrose solution and then placed in an incubator at 33 °C (Memmert HCP 108, GmbH + Co. KG, Schwabach, Germany) for one hour to help them recover from the stress of manipulation. The administration of this low sucrose concentration followed by the 1-h incubation with no food ensured the immediate and full consumption of the test solution provided later.

2.2. Administration of the test solutions

After the 1-h incubation period, bees were placed at room temperature (22 °C) and individually fed 10 µl of 50% (w/w) sucrose solution, according to the guidelines for pesticide toxicity testing on honey bees (OEPP/EPPO, 2010). The test solutions contained pure sucrose (*control*) or thiamethoxam (Dr. Ehrenstorfer GmbH, 98.0% purity, CAS# 153719-23-4) at 0.2 ng/bee (*low*), 1 ng/bee (*medium*) or 2 ng/bee (*high* dose treatments), corresponding to 20 ppb, 100 ppb and 200 ppb respectively (acetone was used as solvent, <0.02% in all treatments and controls). The doses consumed by the bees were respectively 25, 5 and 2.5 times lower than the LD_{50} of thiamethoxam for honey bees, which is considered to be 5 ng/bee (EFSA, 2013). Each bee received a randomly assigned treatment and those that did not consume the test solution completely were excluded from the experiment.

2.3. Temperature assessment

The thorax temperature of the harnessed honey bees was recorded using a FLIR SC325 thermal camera (FLIR USA). The camera was positioned vertically above the bees for simultaneous recording of the temperature of 80 individuals (4 treatments and 20 bees per treatment). The thorax temperature of each bee was targeted and defined by a unique identification code throughout the whole experiment (Fig. 1). The first temperature recording was done before treatment (Day 1 at time 0, between 12:00 h and 13:00 h) followed by subsequent recording, all carried out in a laboratory maintained at 22 °C. The Day 2 measurements started between 08:00 h and 10:00 h. Two experiments were conducted to test the effect of acute oral exposures of thiamethoxam on honey bee thermoregulation.

2.3.1. Experiment 1: low constant temperature and cold shock

After administration of the test solution, the bees were maintained at 22 °C. On this day (Day 1) their thorax temperature was recorded every 30 min for a total of 4 h (from time 0 to time 240). Additional recordings were made 1 and 2 h after treatment: 70, 80, 130 and 140 min post-treatment. When the recording on Day 1 was completed, the bees were fed 50% (w/w) sucrose *ad libitum* and maintained in the incubator at 33 °C to guarantee their survival overnight. On the second day (Day 2), bees were removed from the incubator and fed with 50% sucrose solution. This procedure is routinely used in long-term memory experiments on harnessed bees to avoid starvation (Wüstenberg et al., 1998; El Hassani et al., 2012). Their thorax temperature was recorded for a total of 1 h: every 10 min for 30 min and then at 60 min (time 1200, 1210, 1220, 1230, 1260). Thorax temperatures of a total of 302 bees were recorded during four experimental replicates.