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The effects of ingested aqueous aluminum on floral fidelity and foraging strategy in honey bees (*Apis mellifera)*

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ABSTRACT

Pollinator decline is of international concern because of the economic services these organisms provide. Commonly cited sources of decline are toxicants, habitat fragmentation, and parasites. Toxicant exposure can occur through uptake and distribution from plant tissues and resources such as pollen and nectar. Metals such as aluminum can be distributed to pollinators and other herbivores through this route especially in acidified or mined areas. A free-flying artificial flower patch apparatus was used to understand how two concentrations of aluminum (2 mg/L and 20 mg/L) may affect the learning, orientation, and foraging behaviors of honey bees (*Apis mellifera*) in Turkey. The results show that a single dose of aluminum immediately affects the floral decision making of honey bees potentially by altering sucrose perception, increasing activity level, or reducing the likelihood of foraging on safer or uncontaminated resource patches. We conclude that aluminum exposure may be detrimental to foraging behaviors and potentially to other ecologically relevant behaviors.

1. Introduction

Secondary consequences of anthropogenic change can have important ecosystem effects. One example is substrate acidification through acid rain and carbon dioxide emission (Andrews and Schlesinger, 2001; Bonan, 2008). Acidification can ionize potentially harmful compounds and is of particular concern regarding uptake of metals by plants (Andrews and Schlesinger, 2001; Peralta-Videa et al., 2009; Pourrut et al., 2011). Uptake of potentially harmful species of metals such as aluminum can cause both direct damage to plants as well as ecosystem consequences through the food chain (Nagajyoti et al., 2010; Rout et al., 2009).

Heavy metals and excess intake of micronutrient metals can cause direct damage through protein modification, competition with essential micronutrients, and acute and chronic negative behavioral effects (Bouraoui et al., 2008; Leal et al., 2012; Needleman et al., 1990; Ragunathan et al., 2010; Rivera-Mancía et al., 2010). The micronutrient metals zinc and iron are known to contribute to neurodegeneration outside of their biologic range (Ayton et al., 2014; Leal et al., 2012). These metals may also work in tandem with other metals and increase toxicity (Mizuno and Kawahara, 2017). Metals that negatively interact with micronutrients may also cause damage on their own. For example, species of aluminum can be taken up and distributed through tissues causing food-web wide disturbance (Delhaize and Ryan, 1995; Kaizer et al., 2008). Despite this disturbance and a growing body of literature that aluminum is harmful, it has been classified as biologically unimportant (Exley and Mold, 2015; Mirza et al., 2017).

Aluminum (Al) occurs in variable concentrations in soils and may be increasingly bioavailable to organisms from mining activity, soil acidification, and carbon emissions (Andrews and Schlesinger, 2001; Bonan, 2008; Rabajczyk and Namieśnik, 2010). Bioavailable aluminum can then be absorbed through plant roots, stunting growth, and disrupting photosynthetic processes (Delhaize and Ryan, 1995; Tahara et al., 2008). The metal can then spread up the food chain through herbivory, pollen, and nectar collection (Delhaize and Ryan, 1995). Once ingested, aluminum cannot be excreted and builds up in cells (Exley and Mold, 2015). In animals, the effect of aluminum intoxication is conflictive and understudied, however literature suggests that this metal can affect the ecology of aquatic animals and is not a deterrent to pollinators (Alexopoulos et al., 2003; Meindl and Ashman, 2013; Sparling and Lowe, 1996). There is some evidence that aluminum contamination alters the cholinergic system, but the me-

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chanism and direction of such contamination is still unknown (Exley and Vickers, 2014; Mirza et al., 2017; Yellamma et al., 2010).

Aluminum contamination of the cholinergic system is expected to inhibit acetylcholinesterase the regulatory enzyme for the neurotransmitter acetylcholine (Jackson et al., 2011; Yellamma et al., 2010). The inhibition of this enzyme interferes with the regulatory breakdown of acetylcholine and causes overstimulation of the post-synaptic neuron, potentially resulting in memory deficits, hyperkinesia and an overactive autonomic nervous system (Čolović et al., 2013; Hasselmo, 2006; Williamson et al., 2013). Disruption of the cholinergic system in organisms that have direct interaction with aluminum contaminated food sources may suffer severe consequences (Williamson et al., 2013; Yellamma et al., 2010). Of particular concern when considering aluminum exposure are organisms that are already at risk, such as pollinators, which directly use pollen and nectar resources and are in decline partially as a result of known toxicants, pathogens, and habitat fragmentation/food stress (Bekić et al., 2014; Ellis et al., 2010; Potts et al., 2010).

For the purpose of this study we focused on honey bees as these organisms are easily reared, economically important, have been previously used for learning and toxicological study, but have not been investigated in terms of aluminum (Burden et al., 2016; Gallai et al., 2009; Williamson and Wright, 2013). One of the first concentrated research programs on learning in honey bees was started by Von Frisch (1919) with less organized work starting even earlier (Maeterlinck and Sutro, 2003). One learning methodology, the proboscis extension response, has been used to study the sub-lethal effects of toxicants specifically on learning (Abramson et al., 2012; Burden et al., 2016; Hladun et al., 2012). Similarly, free-flying experiments have been used to understand how honey bees behave under the influence of toxicants in more natural conditions (Craig et al., 2014; Karahan et al., 2015). Both free-flying and laboratory methods can be used to understand how toxicants may affect honey bee behavior (Burden et al., 2016; Karahan et al., 2015).

Foraging behaviors are integral to individual bee and hive success and will likely be affected by aluminum exposure. Patches must be found and effectively utilized, then bees must successfully return to the hive, expel their crop and communicate to other bees the location of the floral patch (Henry et al., 2012; Von Frisch, 1967). These behaviors are also required in other pollinators such as solitary bees and Lepidoptera in which successful forage is essential to survival (Badgett and Davis, 2015; Cameron et al., 2011). Chemical exposure can affect any foraging behavior and produce ecological effects as well as economic effects on humans. To lessen this risk we must attempt to understand the sub-lethal and ecologically relevant behavioral effects of chemical exposure to bees.

The purpose of this study is to determine how aluminum ingestion may sub-lethally affect honey bees. Specifically we use foraging choice as a measure of sub-lethal behavioral change using the research design of Karahan et al. (2015). We expect that foraging efficiency will be reduced by aluminum contamination resulting in reduced return-rate or feeding on low-carbohydrate quality resources.

2. Methods

2.1. Study species

Apis mellifera spp. were from the Namık Kemal Üniversitesi apiary in Tekirdağ, Turkey during the summer of 2016. Experimental bees were from two subspecies, *Apis mellifera caucasica* and *Apis mellifera carnica*, with a bias favoring *carnica* subspecies. All experimental bees were foragers and therefore assumed to be of approximately 3–4 weeks old (Huang and Robinson, 1996; Huang et al., 1994; Robinson, 1987). Colonies had equal access to food resources and contained ten hive-frames per super.

2.2. Flower Patch Construction

Flowers were constructed following Cakmak et al. (2009), Giray et al. (2015), and Karahan et al. (2015). The underside of Plexiglas flowers were painted with blue and white Testors enamel paint (Vernon Hills, IL, 1208C and 1245C, respectively). We used clear plastic dowels rather than wooden dowels for the stems. We assume that the stem change did not affect the apparatus as the stems are not visible from the top angle that the bees primarily see. During the experiment, flowers were placed so that they protruded from a large flat brown board approximately 0.5 m off the ground.

2.3. Pre-training

Before the experiment began, honey bees were trained to visit a scented 1 M sucrose solution feeder located approximately 2 m from the experimental setup. Scents were only used for pre-training and were removed for the experimental procedures. The olfactory stimulus provided a secondary cue for bees to find the flower patch while they established landmarks and flight patterns for quick returns. Several scents were used over the course of the experiment, including clove and peppermint. However, these scents did not present a competitive advantage over the local flora and were replaced mid-summer with distilled sunflower oil from locally acquired flowers. Approximately 1 mL of the sunflower solution was added to 500 mL of 1 M sucrose solution. The scented feeder was refilled before each experiment.

Once the feeder attracted approximately 50–100 bees, a petri dish filled with the same scented solution was placed in the center of an empty flower patch board to begin pre-training to the experimental patch. After consistent visitation (defined as approximately 5 bees simultaneously on the region being observed), the petri dish was exchanged for 4 artificial flowers (2 white and 2 blue, see Flower Patch Construction). Consistent visitation was defined after experimenters noted 5 simultaneously visiting bees created enough potential for additional recruitment to the patch. The 4 artificial flowers were manually filled with 10 μL of the scented solution using an Eppendorf Repeater Pipette (Hauppauge, NY). After consistent visitation to the scented flowers they were removed and 54 unscented flowers (27 white and 27 blue) were randomly placed equidistant on the board. Each of the flowers was then filled with 10 μL of unscented 1 M aqueous sucrose solution. Bees that visited unscented flowers were marked with enamel paint (Testors: 9115X) on the thorax, abdomen or a combination of the two. After approximately 10 bees were marked, the flowers were cleaned and refilled for phase one of the experiment.

2.4. Flower patch phases

Each experiment consisted of 3 phases loosely following Karahan et al. (2015). During each phase, flower color choice, and number of returning trips to the hive were recorded. Bees that did not visit a minimum of 10 flowers per phase were removed from the primary dataset and those that did not complete phase two (post-treatment) were analyzed in a drop-out dataset (n = 38). Visitation was defined as landing on a flower and extending the proboscis into the sucrose well. The first phase was 30 min with phases two and three each lasting 45 min following the procedure of Karahan et al. (2015) (Table 1). Phases were terminated when bees that had returned before the time period ended completed their visitation and left the flower patch area. During the first phase all 54 flowers, regardless of color, were filled with 4 μL of unscented 1 M aqueous sucrose solution. Bees that completed phase one were caught in matchboxes the next time that they landed on a flower after termination of the phase (see Aluminum Distribution). The flower patch was kept in phase one setup until the last bee was released from digestion holding to minimize drop-out due to empty flowers and maintain standard experimental phase (phases two and three) time lengths. Digestion holding was 15 min for each bee