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Nectar alkaloids of tree tobacco can reduce Palestine sunbird foraging performance in a colour discrimination task

Rainee L. Kaczorowski, Shai Markman*

Department of Biology & Environment, University of Haifa – Oranim, Tivon, Israel

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Many plant species contain plant secondary metabolites (PSMs), such as alkaloids, in their tissues for protection against herbivore attack, but PSMs can also be found in floral nectar. Some pollinators have been shown to discriminate against floral nectar with PSMs and consuming PSMs may have negative fitness effects on pollinators. However, only a few studies have investigated the effects of ecologically relevant levels of PSMs on pollinator foraging performance. Here, we addressed the question of whether the natural concentrations of the alkaloids, nicotine and anabasine, found in tree tobacco, *Nicotiana glauca*, nectar affect foraging performance in Palestine sunbird, *Nectarinia osea*, pollinators that use the plant's nectar as a food source. We trained foraging sunbirds to discriminate between rewarding and nonrewarding artificial flowers based on colour. We measured sunbird foraging performance through their accuracy at distinguishing the two colours immediately after training (pretreatment), and again the following day after consuming sucrose solutions with or without alkaloids (post-treatment). We also explored other potential effects of PSM consumption by assessing bird activity level and flower visit rate. Birds that consumed alkaloids did not significantly change their activity level or flower visit rate across time (pre- and post-treatment) compared to birds that did not consume alkaloids (no significant time by treatment interaction). However, alkaloid consumption significantly decreased sunbird foraging performance in terms of their accuracy in distinguishing the rewarding colour, potentially due to reduced memory retention and/or other cognitive or physiological impairments following alkaloid consumption. We also found that sunbirds discriminated against higher, in favour of lower, ecologically relevant alkaloid concentrations in the nectar of tree tobacco and that previous exposure to alkaloids reduced overall consumption of alkaloid solutions. Reduced foraging performance due to PSM ingestion could greatly affect a pollinator's foraging efficiency, which could, in turn, affect both pollinator and plant reproductive fitness.

Plant secondary metabolites (PSMs), such as alkaloids, can be found in many plant species (Irwin, Cook, Richardson, Manson, & Gardner, 2014) and are important for deterring herbivores from consuming plant tissues (Bennett & Wallsgrove, 1994; Rosenthal & Berenbaum, 1992; Wink, 1998, 2010). PSMs are also found in the nectar of many flowers (Adler, 2000; Baker, 1977; Irwin, Adler, & Brody, 2004; Irwin et al., 2014), exposing mutualist pollinators to various levels of PSMs (Irwin et al., 2014; Kessler et al., 2012). Pollinators are often found to discriminate against nectars containing PSMs (Detzel & Wink, 1993; Kessler, Gase, & Baldwin, 2008; Köhler, Pirk, & Nicolson, 2012; Tadmor-Melamed et al., 2004). However, PSM concentrations in nectar are typically much lower than in other plant tissues (Adler & Irwin, 2012; Cook, Manson, Gardner, Welch, & Irwin, 2013; Detzel & Wink, 1993; Irwin et al., 2014; Manson, Rasmann, Halitschke, Thomson, & Agrawal, 2012, 2013), and may be less than concentrations that affect pollinator foraging behaviour (Elliott, Irwin, Adler, & Williams, 2008; Manson et al., 2013; Singaravelan, Nee'man, Inbar, & Izhaki, 2005; Tiedeken, Stout, Stevenson, & Wright, 2014).

In addition to potential effects on pollinator foraging behaviour, PSM consumption has also been found to limit activity (Cook et al., 2013; Manson et al., 2013), reduce oocyte development (Manson & Thomson, 2009) and increase mortality (Detzel & Wink, 1993; Köhler et al., 2012; Singaravelan et al., 2006; Tan et al., 2007) in different bee species. In Palestine sunbird pollinators, PSMs have been shown to reduce gut transit time and sugar assimilation, even at nondeterrent concentrations (Tadmor-Melamed et al., 2004), illustrating that PSM consumption can have physiological effects even when they apparently do not affect foraging behaviour.

* Correspondence: S. Markman, Department of Biology & Environment, University of Haifa – Oranim, Tivon, 36006, Israel.
E-mail address: markmans@research.haifa.ac.il (S. Markman).

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Few studies have investigated the effects of ecologically relevant PSM concentrations on pollinator foraging performance (Mustard, Dews, Brugato, Dey, & Wright, 2012; Thany & Gauthier, 2005; Wright et al., 2013) that goes beyond simple discrimination (i.e. ability of the pollinator to distinguish between food types containing various levels of PSMs, e.g. Tadmor-Melamed et al., 2004). Here, we considered a pollinator's ability to perform a specific foraging-related experimental task as representative of potential foraging performance. We are unaware of studies that focus on the effects of PSM consumption on a foraging-related experimental task of a vertebrate pollinator, as these effects have only been examined in bee species thus far (e.g. Mustard et al., 2012; Wright et al., 2013). Recently, Wright et al. (2013) showed that the consumption of caffeine, found in the floral nectar of *Coffea* and *Citrus* species, slightly increased learning rate and greatly enhanced memory retention in honeybees, *Apis mellifera*. However, a different study found that certain concentrations of caffeine slightly decreased learning rate and immediate recall in honeybees without affecting 24 h memory retention (Mustard et al., 2012). Another study showed that nicotine increased memory retention in honeybees, although the nicotine was not consumed, but rather injected into the antennal lobes (Thany & Gauthier, 2005). Effects of PSMs on the foraging performance and cognitive abilities of pollinators will probably depend upon many factors, including the method used to score cognitive ability, the concentrations of both the PSM and reward, when the PSM is administered with regard to experience and status of the forager, and how much is consumed. We, therefore, have much knowledge to gain by exploring the effects of PSMs on pollinator cognition and foraging behaviour under various scenarios.

In this study, we explored the effects of ecologically relevant levels of alkaloid PSMs (nicotine and anabasine) found in tree tobacco, *Nicotiana glauca*, nectar on the foraging performance of Palestine sunbird, *Nectarinia osea*, pollinators that use the plant as a food source. Generally, both acute and chronic (at least for a limited time) nicotine administration (by injections or skin patches) tends to have positive effects on cognition, through increased attention, improved learning or enhanced memory, in various animals, including humans (reviewed in Levin, 1992; Levin, McClernon, & Rezvani, 2006; Levin & Simon, 1998; Rezvani & Levin, 2001). Nevertheless, some studies have found no or negative effects on certain aspects of cognition (Attaway, Compton, & Turner, 1999; Kangas & Branch, 2012; Moragrega, Carrasco, Vicens, & Redolat, 2003; Vicens, Carrasco, & Redolat, 2003). The cognitive effects of anabasine have seldom been studied, although anabasine was found to have no effect on memory when administered alone, but reversed the negative effects of dizocilpine (a cognitive impairing drug) on memory (Levin et al., 2014).

Different PSMs can have very distinct effects on cognition, as they can vary in how they affect the function of receptors regulating neurotransmitters (Nasehi et al., 2010; Wright et al., 2013) and signalling compounds (Nasehi, Piri, Abdollahian, & Zarrindast, 2013). We predicted that the PSMs may have an effect on sunbird cognition (i.e. memory) that would alter how well they perform in an experimental foraging task. However, it was unknown whether a nicotine and anabasine mixture (mimicking tree tobacco nectar) would increase or decrease sunbird foraging performance, potentially by enhancing or reducing cognitive function (i.e. memory). Here, we tested how alkaloids affect sunbird foraging performance in a colour discrimination task. Sunbirds can use colour as a cue to make foraging decisions (Heystek, Geerts, Barnard, & Pauw, 2014; Whitfield, Köhler, & Nicolson, 2014), making it a useful cue to examine cognitive abilities and foraging performance. Effects on foraging performance due to PSM ingestion could greatly affect a pollinator's foraging efficiency, which could, in turn, affect both pollinator and plant reproductive fitness.

METHODS

Study System

The Palestine sunbird is a common pollinator of tree tobacco in eastern Mediterranean regions (e.g. Israel and Sinai), but may also pierce the base of the corollas and rob nectar (Tadmor-Melamed, 2004). In our previous study, we found low concentrations of nicotine (0.005 ppm) and anabasine (0.07 ppm) in the nectar of tree tobacco flowers and simulating nectar robbing by sunbirds significantly increased the amount of anabasine (to approximately 0.35 ppm), but not nicotine, compared to intact control flowers (Kaczorowski, Koplovich, Sporer, Wink, & Markman, 2014).

Here, we were interested in determining whether there were costs to sunbirds, in terms of foraging performance, when they consumed baseline levels of nicotine (0.005 ppm) and anabasine (0.07 ppm) found in the undamaged flowers of tree tobacco (see Kaczorowski et al., 2014), using a colour discrimination task (see experiment 1 below). When that experiment was complete, we explored whether sunbirds discriminated against the higher concentration of alkaloids (0.35 ppm anabasine and 0.005 ppm nicotine) previously found in damaged flowers over the lower concentration of alkaloids (0.07 ppm anabasine and 0.005 ppm nicotine) previously found in undamaged flowers (see experiment 2 below).

Ethical Note

Permits to capture and house wild sunbirds were provided by the Israeli Nature and Parks Authority (permit 2014/40225) and The University of Haifa Animal Experimentation Ethics Committee (permit 228/11). Adult Palestine sunbirds were caught on Oranim campus, University of Haifa, Kiryat Tivon, Israel, using a trap cage with a decoy bird. Most birds in the first two experiments (12 of 16) were caught in the spring of 2014 (February–May), but four birds (two males, two females) were in captivity for just under 2 years. The 12 birds in the third experiment were caught in the spring of 2015 (April–July). The experiments were conducted at least 3 weeks after the birds were captured. Following their capture, the birds were immediately (on average within 10 min) housed separately in painted metal cages (45 × 75 cm and 85 cm high) inside an air-conditioned room with continuous daytime lighting (12:12 h, day:night) on Oranim campus. Birds had a continuous supply of 10% sucrose solution with supplements (0.25% Orlux Lori; Versele-Laga, Belgium), made fresh daily and provided in two white commercially coloured dispensers with a transparent reservoir containing the solution. Birds were also regularly fed with fruit flies, *Drosophila melanogaster*, as an additional protein source.

Birds in this study were presented with treatment solutions that may (or may not) have contained alkaloids at low concentrations (see details below). We used alkaloid concentrations equal to those found in tree tobacco nectar (the average amount from undamaged [0.07 ppm anabasine and 0.005 ppm nicotine] or damaged flowers [0.35 ppm anabasine and 0.005 ppm nicotine]) in our previous experiment (Kaczorowski et al., 2014). These concentrations were well below the average concentrations previously found in tree tobacco nectar and used in sunbird feeding experiments (0.5 ppm nicotine and 5.0 ppm anabasine; Tadmor-Melamed et al., 2004). Treatment solutions were consumed by the birds voluntarily and were only available to the birds for 1 h (sunbirds can easily go without food for this amount of time in nature; S. Markman, personal observation). Birds did not show any apparent signs of discomfort or intoxication. They were weighed after the experiments were complete to minimize stress-related effects of weighing them before or during the experiments, and were released, at