Journal of Insect Physiology 86 (2016) 11–16

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

The fungicide Pristine® inhibits mitochondrial function *in vitro* but not flight metabolic rates in honey bees

Jacob B. Campbell [a,*,1], Rachna Nath [a,1], Juergen Gadau [a], Trevor Fox [a], Gloria DeGrandi-Hoffman [b], Jon F. Harrison [a]

[a] *School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA*
[b] *USDA, ARS, Honey Bee Research, 2000 East Allen Road, Tucson, AZ 85719, USA*

ARTICLE INFO

Article history:
Received 27 August 2015
Received in revised form 16 November 2015
Accepted 9 December 2015
Available online 10 December 2015

Keywords:
Mitochondria
Pristine®
Honey bee
Flight
Fungicide

ABSTRACT

Honey bees and other pollinators are exposed to fungicides that act by inhibiting fungal mitochondria. Here we test whether a common fungicide (Pristine®) inhibits the function of mitochondria of honeybees, and whether consumption of ecologically-realistic concentrations can cause negative effects on the mitochondria of flight muscles, or the capability for flight, as judged by CO_2 emission rates and thorax temperatures during flight. Direct exposure of mitochondria to Pristine® levels above 5 ppm strongly inhibited mitochondrial oxidation rates *in vitro*. However, bees that consumed pollen containing Pristine® at ecologically-realistic concentrations (≈1 ppm) had normal flight CO_2 emission rates and thorax temperatures. Mitochondria isolated from the flight muscles of the Pristine®-consuming bees had higher state 3 oxygen consumption rates than control bees, suggesting that possibly Pristine®-consumption caused compensatory changes in mitochondria. It is likely that the lack of a strong functional effect of Pristine®-consumption on flight performance and the *in vitro* function of flight muscle mitochondria results from maintenance of Pristine® levels in the flight muscles at much lower levels than occur in the food, probably due to metabolism and detoxification. As Pristine® has been shown to negatively affect feeding rates and protein digestion of honey bees, it is plausible that Pristine® consumption negatively affects gut wall function (where mitochondria may be exposed to higher concentrations of Pristine®).

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Pollination by insects is vital for the health of terrestrial ecosystems and for agriculture (Gallai et al., 2009). The value of crops in the United States pollinated by honey bees exceeds $12 billion and is trending upwards (Calderone, 2012). There is substantial evidence that species and population numbers of managed and wild pollinators are declining in Europe and the Americas, but the causes of such declines remains controversial (Goulson et al., 2015). An emerging theme is that complex interactions among stressors including pesticides, fungicides, parasites and habitat loss are responsible (Goulson et al., 2015). For example, the ergosterol biosynthesis inhibitor fungicides have little toxicity by themselves, but they increase the toxicity of some neonicotinoids by 1000× (Iwasa et al., 2004). However, the underlying molecular and physiological mechanisms of such interactions remain poorly studied and controversial.

Fungicides are perhaps the most common class of synthetic chemicals that honey bees and other crop pollinators encounter, because these are sprayed onto blooming flowers of crops such as almonds, cherries, and strawberries that are highly attractive to foraging bees (Legard et al., 2001). Fungicides are often used on grasses, providing additional exposure routes to pollinators and other organisms when they collect water as dew. These fungicides are also quite stable, and significant concentrations have been found in many aquatic environments, providing exposure to aquatic animals (Smalling et al., 2013). Pollen stores and wax combs of honey bees are often contaminated with fungicides (Mullin et al., 2010; Pettis et al., 2012). Exposure to fungicide is correlated with a variety of colony disorders including poor brood rearing, queen replacement, colony weakening, reduced pollen consumption and digestion, and increased virus titers (DeGrandi-Hoffman et al., 2013, 2015; Simon-Delso et al., 2014; Zhu et al., 2014).

* Corresponding author.
E-mail addresses: Jacob.Campbell.1@asu.edu (J.B. Campbell), rnath@asu.edu (R. Nath), Juergen.Gadau@asu.edu (J. Gadau), tpfox1@asu.edu (T. Fox), Gloria.Hoffman@ars.usda.gov (G. DeGrandi-Hoffman), j.harrison@asu.edu (J.F. Harrison).
[1] Jacob Campbell and Rachna Nath contributed equally to this paper.

http://dx.doi.org/10.1016/j.jinsphys.2015.12.003
0022-1910/© 2015 Elsevier Ltd. All rights reserved.

Unlike insecticides that often target neural function, fungicides affect basic cellular machinery such as nucleic acids and protein synthesis, cell membrane structure and function, signal transduction, respiration, mitosis and cell division (Yang et al., 2011). One class of common fungicides (strobilurins) are respiratory inhibitors targeting fungal mitochondria. If these fungicides can also affect the mitochondria of insects, this could provide a plausible mechanism to explain the observed synergy with other stresses, since even partial inhibition of ATP production could compromise growth, immune function and activity.

In this study, we focused on the effects of oral consumption of the strobilurin fungicide Pristine® (BASF, Research Triangle Park, NC, USA) on mitochondrial function and flight physiology of honey bees, as flight is the most energy-demanding activity of bees. Foraging bees can be exposed to Pristine® because it is sprayed during bloom to prevent crop loss from fungal diseases such as brown rot (Janousek and Gubler, 2010). Pristine® is composed of two fungicides: boscalid and pyraclostrobin (methyl *N*-[2-[[[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxy]methyl]phenyl]-*N*-methoxy-carbamate). Boscalid itself is composed of two carboxamide compounds, 3-pyridinecarboxamide and 2-chloro-N-(4′-chloro[1, 1′-biphenyl]-2-yl) (EPA Pesticide Fact sheet). Boscalid is a broad-spectrum fungicide that inhibits succinate dehydrogenase, a component of the tricarboxylic acid cycle and the electron transport chain (Spiegel and Stammler, 2006). Pyraclostrobin is a broad-spectrum fungicide that inhibits respiration by binding to the cytochrome bc1 complex (Spiegel and Stammler, 2006). These compounds have been found in pollen samples from broad surveys of honey bee colonies (Mullin et al., 2010; Simon-Delso et al., 2014).

In a recent study of fungicide residues in Belgian honey bee colonies, boscalid residues averaged 0.3, 0.7 and 0.02 ppm in wax, bee bread and honey respectively, while pyraclostrobin was undetected (Simon-Delso et al., 2014). High levels of boscalid residues were strongly associated with colonies classified as weak (Simon-Delso et al., 2014). Similarly, feeding honey bees pollen with field relevant concentrations of Pristine® decreased feeding rates, reduced protein digestion, lowered ATP levels in flight muscles and increased the levels of several viruses (DeGrandi-Hoffman et al., 2015). In particular, the depression of flight muscle ATP levels was suggestive of an effect of Pristine® on mitochondria.

Flight metabolic rates of honey bees are very high, among the highest observed metabolic rates in the animal kingdom (Harrison and Fewell, 2002). Over 40% of the flight muscles are filled with mitochondria (Suarez et al., 2000), thus flight provides a good test for functional effects of Pristine® consumption on mitochondrial function. The oxidative capacity of the flight muscles of honey bees increases with age, with flight muscles from more mature bees showing increases in the volume density of mitochondria, increased activities of glycolytic (hexokinase, phosphofructokinase, pyruvate kinase) and oxidative enzymes (citrate synthase, cytochrome c oxidase) and increased concentrations of cytochromes (Harrison, 1986; Herold, 1965; Herold and Borei, 1963; Hersch et al., 1978; Moritz, 1988; Schippers et al., 2006, 2010). These biochemical changes allow strong increases in flight performance and flight metabolic rate during a point in the lives of honeybees that are characterized by intensive foraging (Harrison, 1986; Schippers et al., 2006, 2010; Vance et al., 2009). Mitochondrial function and dysfunction is most often assessed by measuring the oxygen consumption rate of isolated mitochondria consuming preferred substrates, as well as the respiratory control ratio (RCR), which reports the increase in oxygen consumption rates in response to ADP (Brand and Nicholls, 2011). Conceivably, Pristine® consumption could affect the development of mitochondrial function in insects. We assessed mitochondrial function and flight performance in adult worker bees across a range of ages. Since high flight metabolic rates support high thoracic temperatures during flight (Harrison and Fewell, 2002), and high thorax temperatures allow the flight muscle to produce maximal power (Coelho, 1991), we also measured thorax temperatures as an additional index of flight performance.

2. Materials and methods

2.1. Animals and experimental design

All bees used in the study were from European honey bee (*Apis mellifera ligustica*) colonies headed by commercially produced and mated European queens (Pendall Apiaries, Stonyford, CA). With the exception of the direct mitochondrial dosing experiment, bees were maintained at the Carl Hayden Bee Research Center, Tucson, Arizona. Frames of sealed brood were taken from multiple source colonies and incubated overnight at 32–34 °C so that bees would eclose. Workers were collected on the day of eclosion, and kept as groups of approximately 100 workers in Plexiglas cages (dimensions: 11.5 cm × 7.5 cm × 16.5 cm) at 32–34 °C. Food for the bees in the cages consisted of three 2-cm-diameter-plastic tubes containing a 10 g pollen patty. Vials containing 30 ml of 50% sucrose, and 30 ml of water were also provided. All food was replaced weekly.

Pollen patties were made by mixing 4.5 kg of pollen from the Sonoran Desert in southern Arizona, USA, with 4.5 kg of sucrose, 4.5 kg of Bakers Drivert® sugar, and 1.2 L of water in an industrial mixer (Blakeslee Kitchen Machines model CC80). Pristine® was added to treatment patties by dissolving 0.1 g of Pristine® into 0.4 g of distilled water. The mixture was combined and thoroughly mixed with 9.5 g of pollen patty. The "treatment" pollen patty had a final Pristine® concentration 6.6 ppm. As Pristine® is 25.2% boscalid and 12.8% pyraclostrobin, the patties contained 1.6 ppm boscalid and 0.84 ppm pyraclostrobin. The control patty was prepared similarly, with 0.5 g of distilled water added to 9.5 g of pollen patty. Note that these experiments tested the effect of Pristine®, not the fungicides individually or in complex, as the control bees did not receive the inactive ingredients in Pristine® (kaolin (<5%), sodium-di-ethyl-hexyl-sulfosuccinate (0.1–1%), ammonium sulfate (10–15%), and proprietary ingredients (<41%)).

Three replicates were tested during the summer of 2014. Each replicate consisted of three cages (as described above) with bees fed control patties and three cages with bees fed treatment patties. After 14 days, workers do not consume large amounts of pollen (Crailsheim et al., 1992) so Pristine® was added to the sugar syrup (10 ppm) for an additional 10 days. Bees in control cages were provided sugar syrup without added Pristine. Bees in the cages could and did fly, though clearly could not fly as extensively as in an open hive. We measured the metabolic rates and thorax temperatures of hovering bees, and the respiration rates of mitochondria isolated from the flight muscle of workers from treatment and control cages at 4, 7 and 21–24 days after eclosion.

In a separate set of experiments, we derived a dose response curve describing the effect of Pristine® on mitochondrial respiration by adding Pristine® directly to isolated mitochondria. Bees used for this study were European honey bees (*A. mellifera ligustica*) collected as outgoing foragers from colonies maintained at Arizona State University (ASU); to our knowledge, these bees had had no prior contact with Pristine®.

2.2. Mitochondrial isolation

Mitochondria were isolated from thoraxes using methods adapted from Syromyatnikov et al. (2013). Thoraxes of three workers from each treatment group were separated from the head and abdomen, weighed, and homogenized together in 400 μl ice-cold

Download English Version:

https://daneshyari.com/en/article/2840301

Download Persian Version:

https://daneshyari.com/article/2840301

Daneshyari.com