Journal of Invertebrate Pathology 138 (2016) 24–29

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

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

Metarhizium anisopliae infection alters feeding and trophallactic behavior in the ant *Solenopsis invicta*

Hua-Long Qiu [a], Li-Hua Lu [b], M.P. Zalucki [c], Yu-Rong He [a,*]

[a] College of Resources and Environment, South China Agricultural University, Guangdong, Guangzhou 510642, China
[b] Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Guangdong, Guangzhou 510640, China
[c] School of Biological Sciences, The University of Queensland, St. Lucia, Brisbane, QLD 4072, Australia

ARTICLE INFO

Article history:
Received 19 May 2015
Revised 13 May 2016
Accepted 23 May 2016
Available online 24 May 2016

Keywords:
Entomopathogenic fungi
Red imported fire ant
Social behavior
Immunity

ABSTRACT

In social insects, social behavior may be changed in a way that preventing the spread of pathogens. We infected workers of the ant *Solenopsis invicta* with an entomopathogenic fungus *Metarhizium anisopliae* and then videotaped and/or measured worker feeding and trophallactic behavior. Results showed that fungal infected *S. invicta* enhanced their preference for bitter alkaloid chemical quinine on 3 days after inoculation, which might be self-medication of *S. invicta* by ingesting more alkaloid substances in response to pathogenic infection. Furthermore, infected ants devoted more time to trophallactic behavior with their nestmates on 3 days post inoculation, in return receiving more food. Increased interactions between exposed ants and their naive nestmates suggest the existence of social immunity in *S. invicta*. Overall, our study indicates that *S. invicta* may use behavioral defenses such as self-medication and social immunity in response to a *M. anisopliae* infection.

1. Introduction

The evolutionary success of ants has been attributed to their complex social behavior (Hölldobler, 1990; Lach et al., 2010). As ants live in close proximity to one another and are genetically closely related they are susceptible to parasites and pathogens, which may jeopardize the survival of the entire colony (Schmid-Hempel, 1998). In response ants have evolved specific behavioral and physiological strategies to cope with the increased risk of contracting an infection (Oi and Pereira, 1993; Cremer et al., 2007; Bos et al., 2012). Some ants are able to remove fungi and spores from their bodies and other nestmates through self and mutual grooming (Okuno et al., 2011). Some ants use chemical defense mechanisms, as exemplified by alkaloids in the venom of fire ants, which decrease conidial germination of entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea* (*Paecilomyces fumosoroseus*) (Storey et al., 1991). Metapleural gland secretions can also inhibit *M. anisopliae* growth and germination (Poulsen et al., 2002). These behavioral and physiological defenses undoubtedly have a significant impact on fungal infection in ant colonies. Additionally, parasites and pathogens regularly compete for nutrition with their hosts, resulting in energetic stress to the host (Mayack and Naug, 2009). Such energetic stress could reduce the ability of the host to ward off other pathogens. Continued energetic stress may also increase the level of hunger in the host, which would exert changes in host feeding behavior to compensate for the energetic stress (de Souza et al., 2008; Povey et al., 2014). Moreover, several parasitized insects also feed more antimicrobial compounds such as plant secondary metabolites as a defensive mechanism called self-medication (Shurkin, 2014). Therefore, we used the fire ant *Solenopsis invicta* as a model species because it is a well-established model system for studying ant behavior (Qiu et al., 2015). Like any other social insects, *S. invicta* colonies, with their rich store of food as well as suitable humidity and temperature, suffer attack from numerous pathogens and parasites (Siebeneicher et al., 1992). Given the fact that pathogenic infection cause behavioral alterations in other invertebrates as mentioned above, we hypothesized that *S. invicta*, being a dietary generalist, may change their feeding behavior after infection to defense against pathogens.

Sociality is based on a trade-off between costs and benefits. For example, social insect exchange their food with their nestmates by trophallaxis (mutual feeding), which might favor the transmission of pathogens in colonies (Jouvenaz, 1986). However, there was evidence that social insect increase their resistance against pathogens by trophallaxis, through which antimicrobial droplet in regurgitation was spread from fungal exposed individual to naive nestmates, resulting in social immunity (Hamilton et al., 2011).

* Corresponding author.
E-mail address: yrhe@scau.edu.cn (Y.-R. He).

http://dx.doi.org/10.1016/j.jip.2016.05.005
0022-2011/

Trophallaxis behavior result in social immunity in a colony is considered to be an important defensive line as it may increase the resistance of individuals and suppress the survival of pathogen. There were some studies suggested that the trophallactic behavior of ants would change after infection. For example, Bos et al. (2012) reported that *M. anisopliae* infected ant *Camponotus aethiops* spent significantly less time performing trophallaxis than their uninfected counterparts. However, in ant *Camponotus fellah*, immune challenged workers did not decrease the level of interactions with their nestmates but devoted more time to trophallaxis (de Souza et al., 2008). Due to the fact that no literature was focused on trophallactic behavior alteration in *S. invicta* induced by fungal infection, we tested the impact of fungal infection on trophallaxis in *S. invicta* in this study.

To throw more light on these questions, we investigated the feeding and trophallaxis of *S. invicta* following infection with a natural pathogen, *M. anisopliae*. *M. anisopliae* has also widely been used in experiments to study disease resistance in leaf-cutting ants *Acromyrmex echinatior* (Hughes et al., 2002), inducing of antifungal grooming behavior in *Formica selysi* as well as its role in activating social immunization in unicolonial ant species *Lasius neglectus* (Reber et al., 2011; Konrad et al., 2012). First, the fungal strain we used was identified with comparing the sequence of internal transcribed spacer (ITS). Second, we test whether fungal infection would induce *S. invicta* to ingest more alkaloid quinine. Quinine was selected because it is a bitter chemical widely distributed in nature and was often selected for studies on behavior of ants and other invertebrates (Dupuy et al., 2006; El-Keredy et al., 2012; Liscia and Solari, 2000). Finally, the trophallactic behavior between infected workers and their nestmates was videotaped, and the amount of food exchanged between them was also measured. To our knowledge, this is the first study focused on the impact of fungal infection on feeding and trophallaxis behavior of *S. invicta*.

2. Methods

2.1. Ants: origin and rearing

Three polygyne colonies of *S. invicta* were collected from the campus of South China Agricultural University (SCAU) in Guangzhou City, South China (PRC). They were reared in plastic boxes (50 cm × 40 cm × 15 cm) and kept at 25 ± 1 °C and 85 ± 1% relative humidity (RH), with a constant photoperiod of 12 h d^{-1}. Colonies were fed *Tenebrio molitor* larvae (purchased from the farmers' market) and 25% sucrose water every other day *ad libitum*. Medium-sized worker ants (*medias*) were selected for the experiments to eliminate the influence of body size on behavior. All ants were anesthetized with CO_2 and then filtered with two different screens, one with a mesh size of 14 units and the other with a mesh size of 18 units (Shanghai Zhenchun Powder Equipment Co., Ltd., China), in which majors were retained on the size 18 mesh sieve and the rest were minors which dropped to the size 18 mesh sieve; the latter two were discarded. Only *medias* retained on the 14 mesh sieve were kept for experiments; the mean ± standard error of the *medias*' head width was 1.15 ± 0.1 mm (N = 10), which was measured under a microscope (Zeiss, Jena, Germany) fitted with a graticule.

2.2. Identification of Metarhizium strain and preparation of conidial suspension

Phylogenetic studies of ITS sequence of our *Metarhizium* isolate proved it to belong to *M. anisopliae* (Fig. S1). *M. anisopliae* was cultured in Petri dishes (9 cm diam.) containing potato dextrose agar medium (PDA, 200 g L^{-1} potato, 20 g L^{-1} dextrose and 20 g L^{-1} agar). *M. anisopliae* was incubated in a constant temperature incubator at 25 ± 2 °C and 75 ± 5 RH for 10 days. The conidia were then brushed from PDA medium and suspended in a 0.01% aqueous solution of Tween-80. The concentration of the solution was measured using a haemocytometer (Hughes et al., 2002). Conidial preparations were adjusted to 5×10^6 conidia mL^{-1} for the following experiments.

2.3. Treatments

Treated ants (*media* workers) were submerged in 5×10^6 conidia mL^{-1} conidial suspension for 10 s. We chose this concentration because the medial lethal concentration (LC_{50}) of *M. anisopliae* against *S. invicta* workers on the fifth day post inoculation was 5×10^6 conidia mL^{-1}. Small percentages (about 10%) of fungus exposed workers begin to die about three days post inoculation (unpublished preliminary data). Ants were then removed from the solution and allowed to walk freely on filter papers for 10 min to remove surface liquid before being placed back in the foraging arena of their sub-colony. Control ants were submerged in 0.01% Tween-80 solution.

2.4. Effect of infection status on feeding preference for quinine

2.4.1. Find suitable assay duration

To find a suitable duration of the assay for testing the avoidance of a bitter substance by workers, we modified the methods described by El-Keredy et al. (2012) to incorporate different time points. Briefly, the day before experiments, a line was drawn on the back of each Petri dish (60 mm diam.) with a black marker pen, dividing it into two equal halves. One side of each dish was filled with only 1% agarose (henceforward called PURE; electrophoresis grade; Roth, Karlsruhe, Germany) while the other side was filled with 1% agarose treated with 5 mM of quinine hemisulfate (henceforward called QUI; purity: 98%, Powder; CAS: 6119-70-6, Sigma-Aldrich). This concentration of QUI was selected because it can repel invertebrates (El-Keredy et al., 2012). PURE were placed on both sides of the dish, serving as the control. About 20–40 workers were introduced in the middle of each dish. The number of workers on either side of the dish was recorded and the voidance index (VIs) was calculated from the following equation (El-Keredy et al., 2012):

$$VIs = (N_{QUI} - N_{PURE})/(N_{QUI} + N_{PURE}),$$

where N represents the number of workers on the respective side of the dish. Thus, positive values of PREF indicate workers prefer QUI and negative values represent aversion to QUI. Scores were calculated at 1, 2, 4, 8 min after the workers were introduced into the dish. There were 4 dishes established for each time point.

2.4.2. Quinine voidance among various concentrations

To test whether the concentration of QUI influences choice, the method described above was used: one Petri dish with PURE agarose on both sides was set up as the control condition (N = four replications for each of the three colonies), and four Petri dishes with one side PURE and the other side with QUI treatment. A series of QUI concentrations were set up: 0 mM, 0.05 mM, 0.5 mM and 5 mM. The above equation was used to calculate the voidance values for workers.

2.4.3. QUI preference after inoculation

The QUI preference of *S. invicta* infected by *M. anisopliae* was tested by food intake of 1% agarose mixed with 5 mM QUI and 2 mg mL^{-1} of brilliant blue. Each day post inoculation (dpi), two infected and two control workers were placed in a box and allowed to feed on agarose food for 24 h for each dpi, for a total of 4 dpi. Each treatment was repeated 12 times (N = four replications for