ORIGINAL ARTICLE

The effect of ergosterol on the allogrooming behavior of termites in response to the entomopathogenic fungus *Metarhizium anisopliae*

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Abstract Termites have physiological and behavioral immunities that make them highly resistant to pathogen infections, which complicates biocontrol efforts. However, the stimuli that trigger the pathogen-avoidance behaviors of termites are still unclear. Our study shows that workers of *Coptotermes formosanus* exposed to the conidia of *Metarhizium anisopliae* exhibited a significantly higher frequency and longer duration of allogrooming behaviors compared with untreated termites. Volatile compounds in the cuticle of control termites and termites previously exposed to a suspension of *M. anisopliae* conidia were analyzed and compared using a gas chromatography-mass spectrometer (GC-MS). Our results showed that the amount of ergosterol differed between the fungus-exposed and control termites. Choice tests showed that termites significantly preferred to stay on filter paper treated with ergosterol (0.05, 0.1, or 1.0 mg/mL) compared with control filter paper. In addition, termites exposed to ergosterol followed by *M. anisopliae* conidia were allogroomed at a significantly higher frequency and for a longer duration than termites exposed to alcohol (the solvent used with the ergosterol in the ergosterol trials) alone followed by *M. anisopliae* conidia. These results showed that ergosterol may enhance the allogrooming behavior of termites in the presence of entomopathogenic fungi.

Key words allogrooming behavior; entomopathogenic fungus; ergosterol; recognition; social immunity; termite

Introduction

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) is one of the most destructive pests of wooden structures and living

plants worldwide (Lax & Osbrink, 2003; Ahmad *et al.*, 2021; Blumenfeld *et al.*, 2021). These termites are often controlled using entomopathogenic fungi as alternative agents to chemical controls; however, these agents have not always been as successful as the chemical methods (Chouvenc *et al.*, 2011). This is because of a series of social immunity mechanisms evolved by the termites that promote pathogen resistance via a number of behaviors, including chemical communication, hygienic behavior, and physiological immune responses (Liu *et al.*, 2019a, 2020; Zhao *et al.*, 2020). With hygienic behaviors, the pathogens can be directly eliminated from individuals' cuticles by allogrooming, thereby improving the disease resistance of the entire termite colony

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(Shimizu & Yamaji, 2003; Yanagawa & Shimizu, 2007; Liu *et al*., 2019b). Additionally, the allogrooming contact causes the pathogens to quickly spread throughout the nest; as a result, termite individuals obtain only a few fungal conidia, triggering mild infections and an increase in immune gene expression and enhancement of antifungal activity (Konrad *et al*., 2012; Liu *et al*., 2015). Therefore, identifying the cues that stimulate the termites to allogroom fungi-infected termites may assist in developing a reliable method of biocontrol.

Worker termites are blind, sensing nest-mates and the environment through chemical communication (Watson & Gay, 1991; Funaro *et al*., 2018). Recent studies suggest that termites can distinguish fungi and respond sensitively to the musty odors of many species of entomopathogenic fungi (Yanagawa *et al*., 2009, 2010). These studies have identified certain components of the fungal odor, such as 1-octen-3-ol, 3-octanone, 3-methyl-1-butanol, and 3-octanol (Yanagawa *et al*., 2011, 2012). A study by Yanagawa *et al*. (2012) confirmed the termites demonstrate a significant aversion to the odor of 3-octanone and indifference to the odors of 1-octen-3-ol and 3-methyl-1-butanol. Although the termites showed inconsistent aversion to the odor of the fungi, they groomed the termites infected with fungal conidia (Yanagawa *et al*., 2012, 2015). However, recent studies have all focused exclusively on the fungi rather than the infected termites; therefore, identification of the odor components that trigger the termites to allogroom entomopathogenic fungi-infected termites may be incomplete.

The entomopathogenic fungus *Metarhizium anisopliae* is a widely used agent for controlling biological pests and an ideal experimental fungus for studying immunity in social insects (Liu *et al*., 2015; Konrad *et al*., 2018; Zhou *et al*., 2021). Here, the workers of *C. formosanus* and the fungal conidia of *M. anisopliae* were used as a host-pathogen system to identify the chemical components that can trigger the termites' allogrooming by focusing directly on the infected termites. First, the cuticle components of termites that had either been exposed or unexposed to *M. anisopliae* conidia were measured by gas chromatograph-mass spectrometry (GC-MS), and second, the effect of the chosen components was assessed based on the result of the GC-MS.

Materials and methods

Termite collection and rearing

In this study, we used colonies of *C. formosanus* collected using a lure collection device (a plastic basket

with pine blocks) in Longdong Forest Park, Guangzhou, China. These colonies were fed with wet pinewood and maintained in a greenhouse (26 ± 1 °C, 70%–80% relative humidity) under total darkness.

Preparation of the fungal conidial suspensions

The entomopathogenic fungus, *M. anisopliae* (strain no.: IBCCM321.93), was purchased from the Guangdong Microbial Culture Collection Center, Guangzhou, China. The fungus was cultivated on potato dextrose agar (PDA, HuanKai Microbial, Guangzhou, China) at 25 ± 1 °C for approximately 10 d. The conidia were harvested by washing each plate with sterile 0.1% Tween 80 solution and stored at 4 °C for a maximum of 10 d before using. The number of conidia was counted using a Thoma hemocytometer (Sail Brand, Yancheng, China) and diluted to the required concentration (0, 1×10^5, 5×10^5, 1×10^6, 5×10^6, and 1×10^8 conidia/mL) using 0.1% Tween 80.

Termite susceptibility to different concentrations of M. anisopliae

To select 2 significantly different concentrations of *M. anisopliae* conidial suspensions, susceptibility of the termites to different concentrations of the fungus was tested. Twenty-eight workers were each placed separately into a 2.0-mL microcentrifuge tube containing a conidial suspension (500 μL) and submerged in the suspension for 10 s; these termites were then transferred onto filter paper and dried for 60 s to absorb excess fluid (Bulmer *et al*., 2019). The bioassay arena was a 24-well plate (Corning-Costar, Beijing, China; diameter = 15.6 mm) with a piece of small circular filter paper (diameter = 15.6 mm) moistened with 20 μL of water on the bottom of each well. A worker was put in each well, and a total of 28 workers were used for each concentration of conidial suspension. A total of 504 wells (28 workers × 6 concentrations × 3 colonies) from 21 24-well plates were used. Termite deaths were recorded daily for 10 d. The sensitivity of the termites to the concentration of *M. anisopliae* was assessed in median lethal time (LT50). Three colonies (28 termites for each colony) were used.

Termites allogrooming behavior toward M. anisopliae-exposed termites

The allogrooming behavior of the termites responding to 2 concentrations (1×10^6 or 1×10^8 conidia/mL,

selected from the above experiment) of *M. anisopliae* were evaluated. The 0.1% Tween 80 solution was used as the control. Eight workers from each colony were treated with 150 μL of *M. anisopliae* conidial suspension or a control suspension in a 2.0-mL microcentrifuge tube. The method of treating termites was as outlined above. Sixteen workers from 2 colonies were used for each concentration. Each worker was then placed in a single well of a 24-well plate containing filter paper for 1 h of exposure. After exposure, 1 worker termite previously exposed to *M. anisopliae* conidia was brushed using green pigment on the dorsal surface of the abdomen and placed with 1 control worker (previously exposed to a control suspension) that had been brushed with red pigment, 1 blank worker (without any treatment), and 1 blank soldier (without any treatment). These termites were placed on a 5.5-cm diameter Petri dish with a piece of wet filter paper (diameter = 5.5 cm) and allowed to acclimatize for 15 min (Yanagawa *et al*., 2010). The behaviors of the termites were recorded for 30 min using a video camera. When the body surface of the worker was touched by the mouthpart of another worker, this was recorded as 1 allogrooming event (Liu *et al*., 2019b). Allogrooming events were counted by recording allogrooming frequency and total duration (the sum of each allogrooming event) of each worker. The experiment was repeated 16 times (8 times × 2 colonies).

Identification of volatiles in the *M. anisopliae*-exposed termites

To identify the chemical volatiles on the surface of *M. anisopliae* conidia-treated termites, *N*-hexane was used to extract the volatiles directly. Our preliminary experiment showed that it was difficult to detect the difference between samples with a small number of termites ($n < 100$). Therefore, the number of treated termites was increased to 300. Sixty workers from each colony were treated with 1 mL of $1 × 10^8$ conidia/mL *M. anisopliae* conidial suspension or 0.1% Tween 80 in a 2.0-mL microcentrifuge tube. The method of treating termites was as outlined above, and a total of 300 workers from each colony was used for each treatment. Then, each worker was placed in a single well of a 24-well plate containing filter paper for 1 h of exposure. A total of 1 800 wells (300 workers × 3 treatments × 2 colonies) from 75 24-well plates was used. After exposure, a total of 300 workers that had been exposed to *M. anisopliae* conidia was considered as the treatment group, 300 termites treated with 0.1% Tween 80 as the control group, and 300 untreated

termites as the blank group. Then, 300 termites from each group were put into a 4-mL sample injection bottle and 1.2 mL of *N*-hexane were added to extract the volatiles. After 10 min, 200 μL of supernatant were drawn up into a 2-mL sample injection bottle and blow-dried using nitrogen gas, followed by the addition of 100 μL of *N*-hexane to resolubilize the sample. Fifty microliters of resolubilized sample were drawn up into a glass-lined pipe in a 2-mL sample injection bottle, and the chemical volatiles on the surface of the treated termites were identified using GC-MS (Agilent 7890B-5977B, Wilmington, USA). The column oven was programmed to hold at 45 °C for 3 min, followed by an increase of 4 °C/min to 200 °C and hold for 3 min, then 6 °C/min to 300 °C, finally holding for 2 min at 300 °C. Each group was repeated for 2 colonies.

Termite preference for ergosterol

The ergosterol reference substance (Macklin, Shanghai, China) was dissolved in pure alcohol, and 5 concentrations (0, 0.01, 0.05, 0.1, and 1.0 mg/mL) of ergosterol were obtained. Two pieces of filter paper (diameter = 3.5 cm) were symmetrically pasted on the 2 sides of a Petri dish (diameter = 9.0 cm) with double-sided tape. One filter paper was soaked with 40 μL of alcohol, and the other one was soaked with 40 μL of ergosterol (0, 0.01, 0.05, 0.1, or 1.0 mg/mL). After 5 min, 30 workers were placed into the center of each Petri dish. Photographs were taken every 30 s for a 15-min period, and the numbers of termites that stayed on each filter paper were counted. The experiment for each concentration of ergosterol was repeated 16 times (8 times × 2 colonies).

Termite allogrooming behavior in response to ergosterol

This experiment was used to verify whether ergosterol enhanced termite allogrooming in response to *M. anisopliae*. Because alcohol can affect the activity of termites and *M. anisopliae*, we used a 2-step spray method to treat termites. Workers were randomly divided into 4 groups (Table 1), and (1) exposed to ergosterol followed by *M. anisopliae* conidial suspensions; 16 termites from each colony were placed into a 5.5-cm diameter Petri dish padded with a piece of wet filter paper and sprayed with 300 μL of ergosterol at a concentration of 0.1 mg/mL using a plastic sprinkling can. Then, each termite was transferred to 1 well in the 24-well plate for 30 min of exposure. Subsequently, all termites were transferred to

Table 1 Experimental treatments of allogrooming behavior of termites *Coptotermes formosanus* in response to ergosterol.

Treatment	Abbreviation	Method				
		First spraying	Exposing time	Second spraying	Exposing time	Marked color
Ergosterol + *Metarhizium anisopliae* conidia	E + M	300 μL 0.1 mg/mL ergosterol	Each termite 1 well in the 24-well plate for 30 min	300 μL 1 × 10^8 conidia/mL *M. anisopliae*	Each termite 1 well in the 24-well plate for 30 min	Green
Alcohol + *M. anisopliae* conidia	A + M	300 μL alcohol	Same as above	300 μL 1 × 10^8 conidia/mL *M. anisopliae*	Same as above	Black
Alcohol + 0.1% Tween 80	A + T	300 μL alcohol	Same as above	300 μL 0.1% Tween 80	Same as above	Red
The untreated termites	CK	Untreated	Same as above	Untreated	Same as above	Unmarked

the same Petri dish sprayed with 300 μL of 1×10^8 conidia/ml *M. anisopliae* conidial suspension, and then each termite was transferred to 1 well in a 24-well plate for 30 min of exposure. Finally, we selected the most active 8 termites of each group of 16 termites, and brushed them with green pigment for subsequent experiments. (2) The remaining termites were exposed to alcohol followed by *M. anisopliae* conidial suspensions: the method was similar to that outlined above, but the termites were placed on the Petri dish sprayed with alcohol first. All termites were brushed with black pigment, and (3) exposed to alcohol followed by 0.1% Tween 80: a similar method to that outlined above was used, but the termites were placed on the Petri dish sprayed with alcohol first, and then placed on the Petri dish sprayed with 0.1% Tween 80. All termites were brushed with red pigment. (4) Four untreated termites from the 4 treatment groups were placed on a 5.5-cm diameter Petri dish with a piece of wet filter paper (diameter = 5.5 cm) and allowed to acclimatize for 15 min. The allogrooming behavior (total frequency and total duration of each allogrooming event) of each termite was recorded for 30 min using a video camera. This experiment was repeated 16 times (8 times × 2 colonies).

Data analysis

The total frequency of allogrooming behavior in the behavioral experiments was analyzed using a generalized linear model (Poisson error structure, log link function). The termite mortality data were binary; therefore, a generalized linear model (binomial error structure, log link function) was used to examine termite mortality in the susceptibility experiment. Additionally, the time of 50% termite death (LT50) was used for predicting the doses for the binomial assay model and analyzed using the R package MASS (Venables & Ripley, 2002). A one-way analysis of variance (ANOVA) was used to examine the total duration of allogrooming. To determine the preference of the termites for the piece of paper containing ergosterol or the control in each Petri dish, the data were divided into 2 treatment groups. One group comprised the ergosterol concentrations (0, 0.01, 0.05, 0.1, and 1.0 mg/mL), while the other group comprised ergosterol and alcohol (alcohol and alcohol + ergosterol). The data on preferences of the termites (Poisson error structure, log link function) were analyzed using a generalized linear mixed-effects model (GLMM), and the random intercepts constituted the recording of the time-by-Petri dish repeat interaction. The R package lme4 was used to construct the GLMM (Bates *et al.*, 2015). A visual inspection of
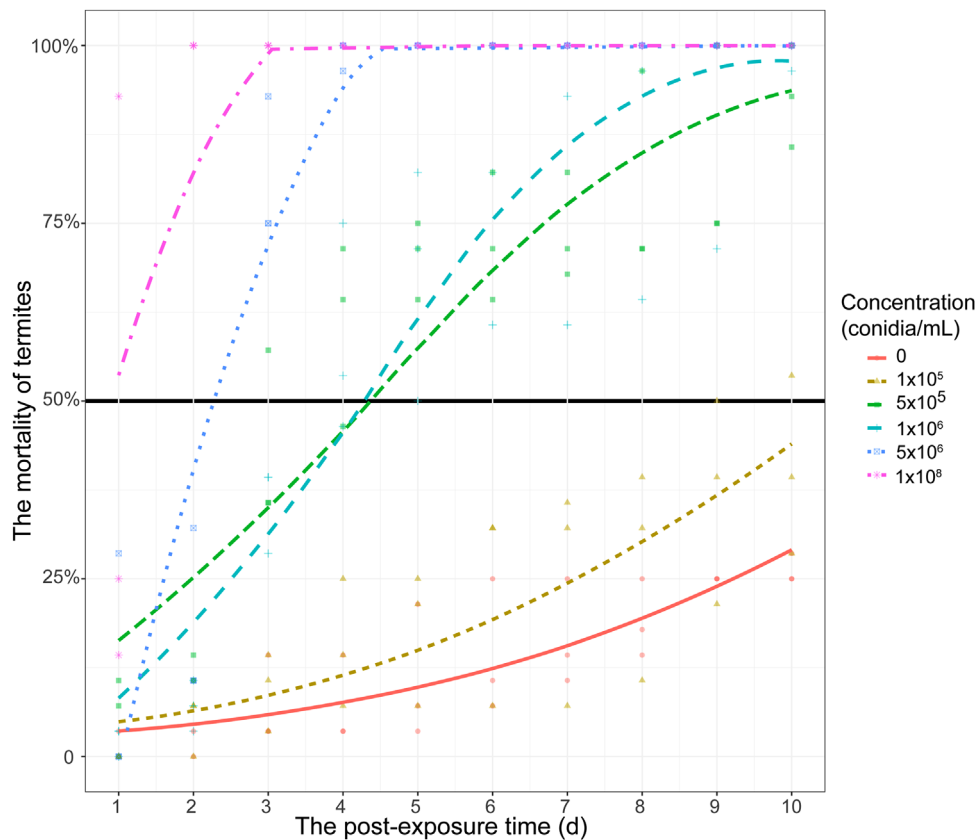
Fig. 1 Termite (*Coptotermes formosanus*) mortality at different concentrations of *Metarhizium anisopliae*. The day of intersection of each colored line and the black line represents the LT50 of termites in each treatment.

the residual plots did not reveal any obvious deviations from homoscedasticity or normality. When a significant effect was detected, the pairwise contrasts between the treatments were made using the Tukey post hoc comparison procedure with the emmeans R package (Lenth, 2018). All the data analyses were conducted using R 4.0.2 statistical software (R Development Core Team, 2020), and the figures were created using the R package ggplot2 (Wickham, 2016).

Results

Termite mortality at different M. anisopliae concentrations

At all 6 concentrations of *M. anisopliae* conidial suspensions, termite mortality increased as the postexposure time increased ($\chi^2 = 48.85$, $P < 0.001$; $\chi^2 = 85.07$, $P < 0.001$; $\chi^2 = 266.85$, $P < 0.001$; $\chi^2 = 413.32$, $P < 0.001$; $\chi^2 = 587.95$, $P < 0.001$; $\chi^2 = 247.09$,

$P < 0.001$ for 0, 1×10^5, 5×10^5, 1×10^6, 5×10^6, and 1×10^8 conidia/mL, respectively). As the concentration increased, the time of termite death gradually advanced (LT50: $0 > 1 \times 10^5 > 5 \times 10^5 > 1 \times 10^6 > 5 \times 10^6 > 1 \times 10^8$ conidia/mL). Considering the difference in the termites' LT50, the concentrations of 1×10^8 conidia/mL (LT50 = 1.01 ± 1.00 d, mean ± standard error) and 1×10^6 conidia/mL (LT50 = 4.28 ± 0.14 d) were selected as 2 different susceptibility concentrations for subsequent experiments; while the concentration of 0 conidia/mL (LT50 = 13.34 ± 1.05 d) was used as the control concentration (Fig. 1).

Termite allogrooming behavior toward M. anisopliae-exposed termites

Frequencies of worker allogrooming behavior were significantly affected by fungal concentration ($\chi^2 = 28.07$, $P < 0.001$), worker treatment ($\chi^2 = 428.28$, $P < 0.001$), and their interaction ($\chi^2 = 21.10$, $P < 0.001$) (Fig. 2).
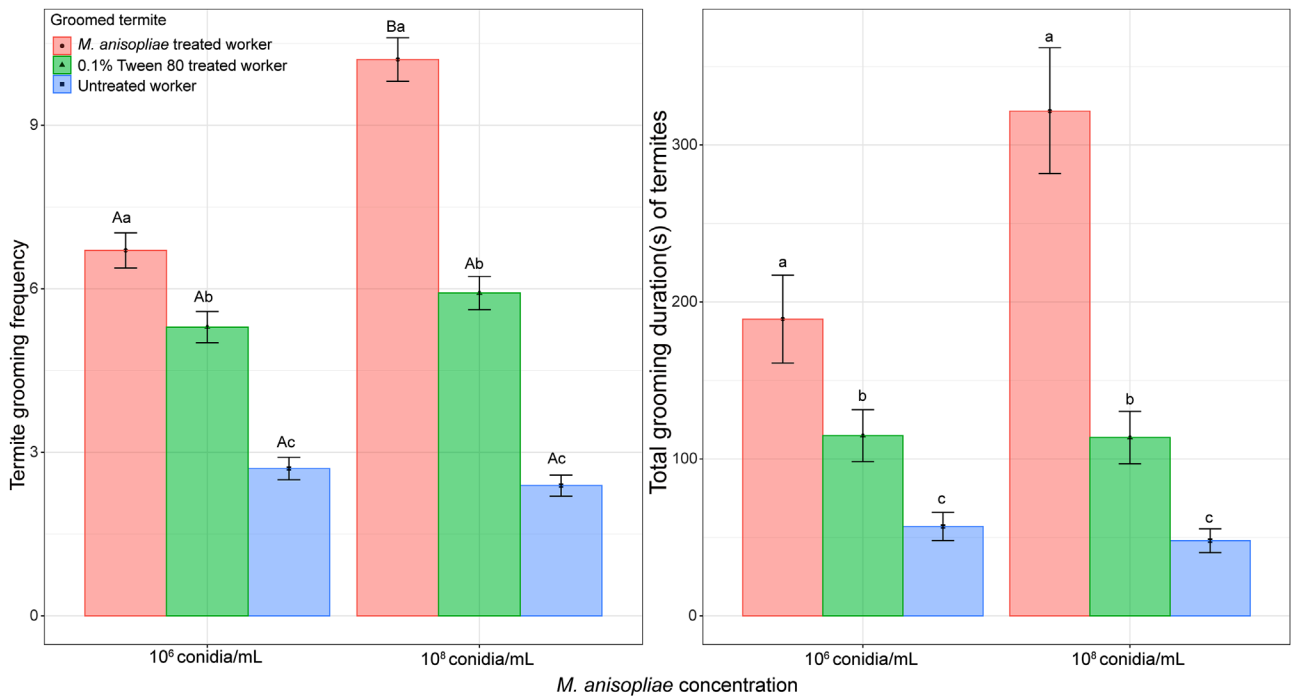
Fig. 2 Frequency and duration (mean ± SE) of termite (*Coptotermes formosanus*) allogrooming behavior at 2 concentrations of *Metarhizium anisopliae*. Different capital letters indicate significant differences between the 2 concentrations in the same group of groomed termites. Different lowercase letters indicate significant differences among 3 groups of groomed termites at the same concentration (Tukey's honestly significant difference [HSD] test, $P < 0.05$).

Workers treated with 1×10^6 and 1×10^8 conidia/mL were allogroomed at a significantly higher frequency than workers treated with 0.1% Tween 80 ($z = 3.24$, $P = 0.003; z = 8.43, P < 0.001$, respectively) or untreated workers ($z = 10.08, P < 0.001; z = 16.16, P < 0.001$, respectively).

The total grooming duration of workers was significantly affected by the workers' treatment ($F_{2,299} = 51.88$, $P < 0.001$), but not by the fungal concentration ($F_{1,299} = 1.12, P = 0.29$) or their interaction ($F_{2,299} = 3.01, P < 0.05$) (Fig. 2). The workers exposed to *M. anisopliae* conidia allogroomed for longer than workers treated with 0.1% Tween 80 ($t = 5.27, P < 0.001$), or untreated workers ($t = 10.16, P < 0.001$).

Identification of the volatiles of M. anisopliae-exposed termites

There was a significant difference in the composition of the volatiles in *M. anisopliae*-treated termites at 51.04 min in both the colonies: this difference was attributed to ergosterol, which was identified using a mass spectrometry system (Fig. 3).

Aggregation preference of the termites under different ergosterol treatments

Aggregation preference of the termites was significantly affected by the treatment ($\chi^2 = 268.40$, $P < 0.001$), concentration ($\chi^2 = 744.97, P < 0.001$), and their interaction ($\chi^2 = 314.94, P < 0.001$) (Fig. 4). At a concentration of 0 and 0.1 mg/mL, there was no significant difference between the number of termites that stayed on the filter paper treated with alcohol or ergosterol ($z = 0.32, P = 0.75; z = 1.88, P = 0.06$, respectively). However, a significantly higher number of termites preferred filter paper treated with ergosterol at the concentration of 0.05 ($z = 12.78, P < 0.001$), 0.10 ($z = 15.68, P < 0.001$), or 1.0 mg/mL ($z = 13.05$, $P < 0.001$) than filter paper treated with alcohol.

Termite allogrooming behavior in response to ergosterol

The frequencies of termite allogrooming behavior were found to be significantly affected by the treatment ($\chi^2 = 51.67, P < 0.001$) (Fig. 5). Termites treated with ergosterol followed by *M. anisopliae*
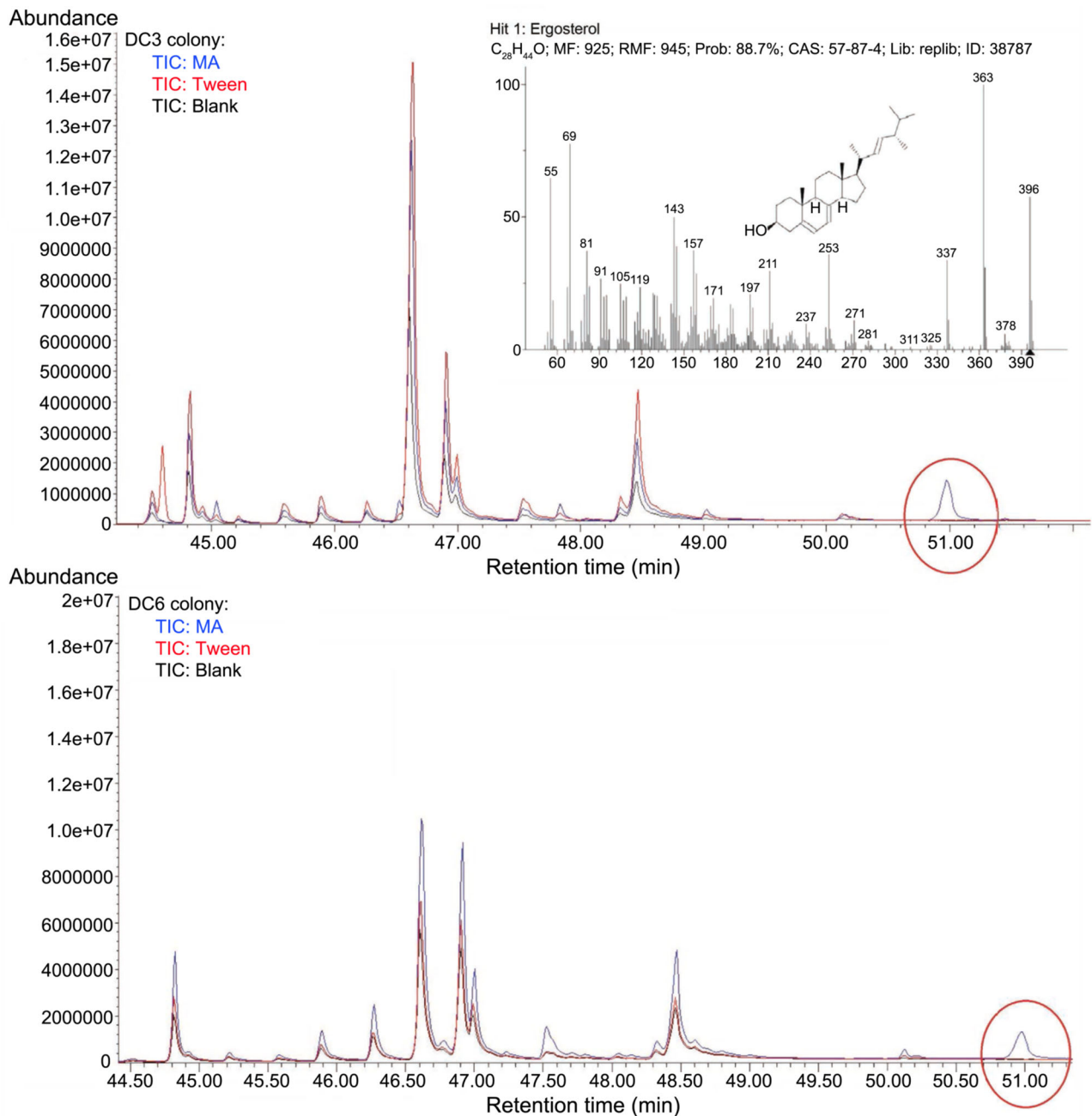
Fig. 3 Gas chromatogram of volatiles from the *Metarhizium anisopliae*-exposed termites (*Coptotermes formosanus*). MA: 1×10^8 conidia/mL *M. anisopliae*-treated workers; Tween: 0.1% Tween 80-treated workers; blank: untreated workers; the red circle shows the significantly different volatile (ergosterol).

conidia (E + M) were allogroomed at a significantly higher frequency than termites treated with alcohol followed by *M. anisopliae* conidia (A + M) ($z = 3.05$, $P = 0.013$), termites treated with alcohol followed by 0.1% Tween 80 (A + T) ($z = 5.21$, $P < 0.001$),

or untreated termites (CK) ($z = 5.51$, $P < 0.001$). Termites treated with alcohol followed by *M. anisopliae* conidia (A + M) were allogroomed at a significantly higher frequency than termites treated with alcohol followed by 0.1% Tween 80 (A + T) ($z = 2.41$,
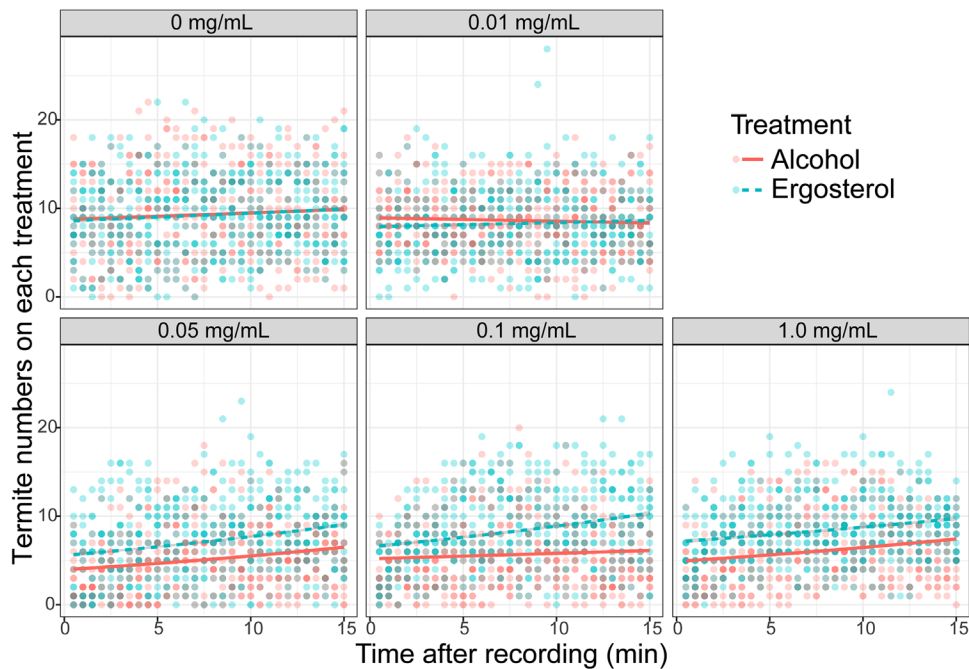
Fig. 4 Aggregation preference of termites (*Coptotermes formosanus*) under different ergosterol treatments. Dots represent raw data; deeper colors reflect overlapping data.

$P = 0.020$), or untreated termites (CK) ($z = 3.42$, $P = 0.004$).

The total grooming duration of termites was significantly affected by the treatment ($F_{3,55} = 30.87$, $P < 0.001$) (Fig. 5). Termites treated with ergosterol followed by *M. anisopliae* conidia (E + M) were allogroomed for a significantly longer duration than termites treated with alcohol followed by *M. anisopliae* conidia (A + M) ($t = 2.27$, $P = 0.027$), termites treated with alcohol followed by 0.1% Tween 80 (A + T) ($t = 5.93$, $P < 0.001$), or untreated termites (CK) ($z = 8.90$, $P < 0.001$). Termites treated with alcohol followed by *M. anisopliae* conidia (A + M) were allogroomed for a significantly longer duration than termites treated with alcohol followed by 0.1% Tween 80 (A + T) ($t = 3.66$, $P = 0.003$), or untreated termites (CK) ($t = 6.85$, $P < 0.001$).

Discussion

Termites are attracted to ergosterol (Mauldin & Rich, 1975), and the present study indicates this attraction enhances social immunity of termites carrying *M. anisopliae* conidia. Ergosterol could therefore be a special component that induces the termites to allogroom entomopathogenic fungi-exposed termites.

Sterols can perform various functions in several insect–fungus symbioses. The insects in these relationships are dependent on the sterols provided by the associated fungus (Maurer *et al.*, 1992; Mondy & Corio-Costet, 2000; Jing & Behmer, 2020). For instance, the fungus associated with the diet of the ambrosia beetle, *Xyleborus ferrugineus*, is required for oviposition, larval development, and pupation (Norris & Baker, 1967; French & Roeper, 1975). Some researchers have found that ergosterol can act as a pheromone; for example, female rock lizards (*Iberolacerta cyreni*) prefer the scent of males with more ergosterol, and male rock lizards may attract more females and mate more often by increasing the proportion of ergosterol in their scent when scent-marking their territories (Lopez *et al.*, 2006; Martin & Lopez, 2012). A study by Zhu *et al.* (2017) explored the metabolites secreted by the fungus beetle *Xylographus bostrichoides*, and stellasterol and ergosterol peroxide were found to act as pheromones or defensins in this species.

In termites, ergosterol has been identified as a feeding stimulant and has been patented as such for *C. formosanus* (Henderson *et al.*, 1999), where it is incorporated into a bait matrix for controlling the subterranean termites (Rojas & Morales-Ramos, 2001). In our study, the termites' aggregation preference was evaluated in response to a series of ergosterol concentrations. The results showed concentrations of 0.05, 0.10, and 1.0 mg/mL
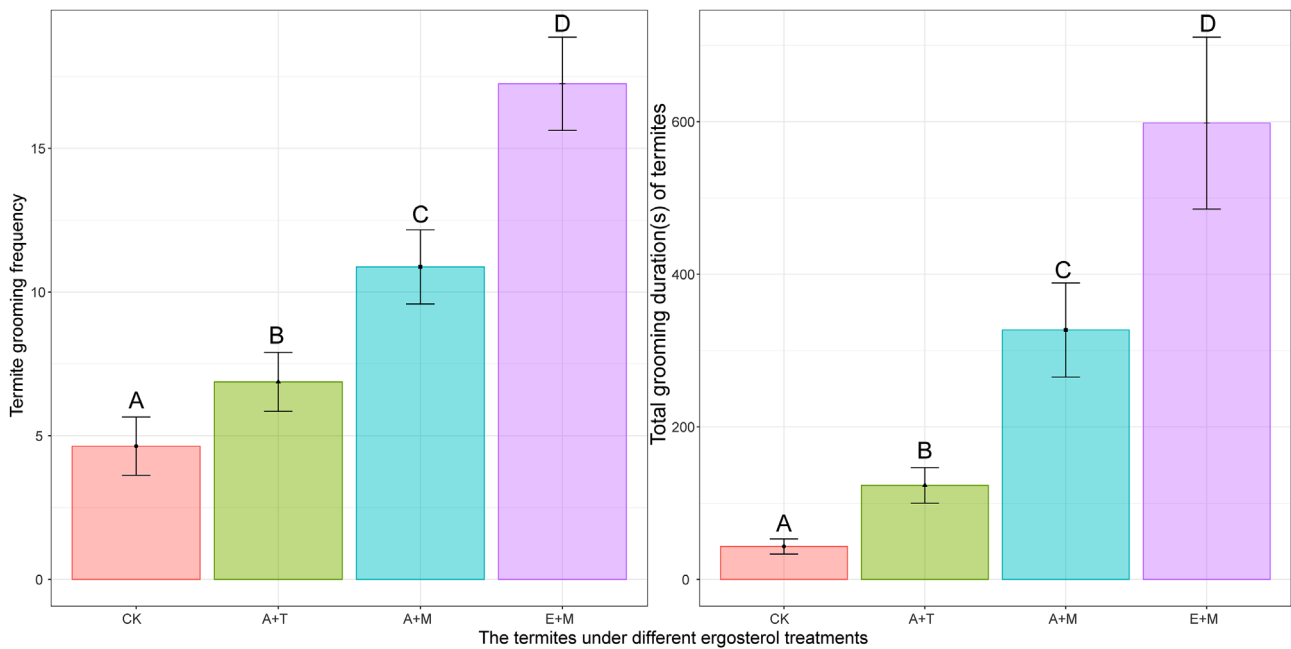
Fig. 5 Frequency and duration (mean ± SE) of termite (*Coptotermes formosanus*) allogrooming behavior under different ergosterol treatments. CK: untreated worker; A + T: alcohol + 0.1% Tween 80 treated worker; A + M: alcohol + *Metarhizium anisopliae* conidia treated worker; E + M: ergosterol + *M. anisopliae* conidia treated worker. Different capital letters above the bars indicate significant differences between the treatment groups (Tukey's honestly significant difference [HSD] test, *P* < 0.05).

of ergosterol to be attractive to the termites, which is similar to the results of a study by Cornelius (2003), where ergosterol was found to stimulate feeding at a concentration of 1 mg of ergosterol per gram of paper. Conversely, Cornelius *et al*. (2004) found that termites showed no preference for sawdust upon treatment with ergosterol, and the tunneling activity of the termites in the sawdust remained unaffected by ergosterol treatment. However, the response time (<1 h) may be too short for the termite to synthesize ergosterol ($C_{28}H_{44}O$). Of course, the presence of ergosterol precursors in termites cannot be ruled out. Nevertheless, in our research, we hypothesize that the most likely explanation is that the treated termites acquire this chemical by contacting the fungus. Hence, the termites would encounter ergosterol in virtually any soil type, and it is not surprising that the tunneling activity of termites was unaffected by adding ergosterol to the sawdust in Cornelius *et al*.'s (2004) study. Therefore, although termites can be significantly attracted by ergosterol, there must be additional reasons why ergosterol stimulates the termites.

In our research, the allogrooming frequency and duration for ergosterol + *M. anisopliae* conidia-treated termites were higher, compared to alcohol + *M. anisopliae* conidia-treated termites (Fig. 5). This indicates that the

termites increased their allogrooming behavior by recognizing the ergosterol in the presence of *M. anisopliae*. Social insects such as termites, ants, and bees are reported to exhibit increased susceptibility to the pathogens surrounding them (Traniello *et al*., 2002; Ugelvig & Cremer, 2007; Hamilton *et al*., 2011; Pusceddu *et al*., 2019). In termites, some studies have indicated that entomopathogenic fungi have a notably lethal effect (Zoberi, 1995; Jones *et al*., 1996; Rath, 2000). The present study also indicated the termites were susceptible to a high concentration of *M. anisopliae* (Fig. 1). However, efficient control in termite colonies via the use of *M. anisopliae* has not been realized (Shimizu & Yamaji, 2003; Yanagawa *et al*., 2009; Chouvenc & Su, 2010; Liu *et al*., 2019a). Therefore, the effect of individual innate immunity in termites may be limited. Colony-level disease defenses are important for resisting infection by pathogens and can quickly react to potential threats and benefit the fitness of the whole colony (Cremer *et al*., 2007, 2018; Rosengaus *et al*., 2011). Allogrooming behavior is an important component of colony-level hygienic behavior and can remove external pathogens from the termites' cuticles, thereby disinfecting them and inhibiting pathogen growth during the early stage of infection (Yanagawa & Shimizu, 2007; Hamilton & Bulmer, 2012; Konrad *et al*.,

2018; Liu *et al*., 2019a). Recent studies have revealed that termites respond sensitively to the musty odors of many species of entomopathogenic fungi (Yanagawa *et al*., 2009, 2010). While one study found that termites avoid the fungal odors (Yanagawa *et al*., 2012), another study showed that the termites had an aversion toward the fungal odor, but could groom termites that had been exposed to the fungal conidia (Yanagawa *et al*., 2012, 2015). Contrary to the odor components of previous studies, we found that ergosterol, which is a component of the cell wall in most types of fungi, appeared to be a novel odor via which the termites could recognize various entomopathogenic fungi and allogroom fungi-exposed termites. This serves to benefit the fitness of the whole colony by enabling the termites to recognize the ergosterol and increase their allogrooming behavior when individuals are infected by entomopathogenic fungi. Therefore, the results of this study indicate that ergosterol may serve as an important stimulant for the termites.

In conclusion, our study showed that termites can recognize *M. anisopliae* conidia-exposed termites, and ergosterol can enhance the allogrooming behavior of termites in the presence of the entomopathogenic fungus. Ergosterol may therefore be the cue that stimulates the termites to allogroom infected termites.

Acknowledgments

Disclosure

The authors declare that they have no conflict of interest.

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