Insect Science

Insect Science (2020) 0, 1–13, DOI 10.1111/1744-7917.12891

ORIGINAL ARTICLE

Red imported fire ants (Hymenoptera: Formicidae) cover inaccessible surfaces with particles to facilitate food search and transportation

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Abstract Eusocial insects have evolved diverse particle-use behaviors. A previous study reported that red imported fire ants, *Solenopsis invicta* Buren, deposited soil particles on substances treated with essential balm, a fire ant repellent. We hypothesized that *S. invicta* modifies inaccessible surfaces by covering them with soil particles to facilitate food search and transportation. Here, laboratory experiments were conducted to study the particle-covering behavior of *S. invicta* in response to viscose surfaces or surfaces treated with essential balm or liquid paraffin in the presence of real food (sausage) or non-food objects (acrylic plates). *S. invicta* workers deposited significantly more soil particles on these three types of treated surfaces than on untreated surfaces. In addition, significantly more particles were relocated on viscose and paraffin-smeared surfaces in the presence of food than in the presence of non-food objects. The particle-covering behavior on viscose surfaces was also observed in the field. Interestingly, when no soil particles were available, ants searched and transported food on viscose surfaces only if the surfaces were artificially covered with sufficient quantities of soil particles but could not do so on viscose surfaces without soil particles or with insufficient quantities of soil particles. In addition, ants actively relocated particles to cover viscose surfaces if the transportation distance was within 200 mm, whereas significantly fewer particles were relocated at longer transportation distances (400 mm). Our study provides a novel example of particle use by fire ants during foraging.

Key words eusocial insect; Fomicidae; foraging; particle-covering behavior; *Solenopsis invicta*

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Introduction

Ants have evolved diverse particle-use behaviors. One of the most common behaviors is the construction of underground and aboveground biostructures, such as nests, mounds, and tunnels (Kleineidam *et al.*, 2001; Robinson *et al.*, 2008; Cosarinsky & Roces, 2012). For example, the leaf-cutting ant *Atta vollenweideri* Forel not only excavates large underground nests containing thousands of fungal chambers but also constructs fine structures, such as turrets, that enhance wind-induced ventilation within their nests (Kleineidam *et al*., 2001). Pielström and Roces (2013) reported that the nest-construction tasks of *A. vollenweideri* were carried out by three functionally distinct groups of individuals, including excavators that excavated soil and formed small particles, short-distance carriers that collected soil particles and dropped them a few centimeters apart, and long-distance carriers that transported soil particles to their final deposition sites. Such processes of soil movement, namely bioturbation, can redistribute large amounts of soil from underground to the surface and significantly improve the water permeability and fertility of topsoil (Seal & Tschinkel, 2006; Sosa & Brazeiro, 2010; Tschinkel & Seal, 2016; Li *et al*., 2019a, 2019b).

Some ant species also use soil particles for many non-construction purposes. For example, ants such as *Aphaenogaster subterranea* Latreille, *Aphaenogaster senilis* Mayr, and *Pogonomyrmex badius* Latreille often place debris (e.g., soil particles and pieces of leaves and grasses) into liquid food and then transport the food-soaked debris back to their colonies (Morrill, 1972; Barber *et al*., 1989; Maák *et al*., 2017; Lőrinczi *et al*., 2018; Módra *et al*., 2020). Instead of only using debris as the liquid carrier, *Solenopsis richteri* Forel can use sand piles and paths as siphon tubes to acquire sugar water solutions, which significantly decreases the risk of drowning (Zhou *et al*., 2020). Some ant species such as *Myrmicaria natalensis* Smith, *Novomessor cockerelli* André, and *Messor capitatus* Latreille can move and throw soil particles or gravel into the entrances of the nests of enemies (e.g., ground-nesting halictid bees) or competing ant species (Samways, 1983; Gordon, 1988; Hölldobler & Wilson, 1990; Grasso *et al*., 2010). Although many termites cover/bury infected nestmates and corpses with particles to prevent disease transmission (Sun & Zhou, 2013; Wang *et al*., 2013), this type of particle use is uncommon in ants but has been documented in *Temnothorax lichtensteini* Bondroit (Hölldobler & Wilson, 1990).

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is a well-studied ant species because of its significance as an ecological and medical pest and its wide global distribution. However, some behaviors in *S. invicta* have only recently been observed. For example, Wang *et al*. (2018) reported that *S. invicta* can break the water tension of liquid food, which may adhere and trap feeding ants, by throwing particles into the edge of sugar water droplets. In addition, *S. invicta* and its closely related species, *Solenopsis saevissima* Smith, can bury food items when they are not able to immediately transport food (Hölldobler & Wilson, 1990; Xu *et al*., 2007; Maciel *et al*., 2015; Mendonça *et al*., 2019; Qin *et al*., 2019a). Unlike some birds and mammals that frequently bury their food (e.g., Henrich *et al*., 2017), such behavior is relatively rare in invertebrates (with the exception of some termite and beetle species).

In a recent study, Wen *et al*. (2016) reported that *S. invicta* deposited soil particles on a substance smeared with essential balm, a strong and long-lasting repellent against fire ants (Chen, 2009; Wen *et al*., 2016). By doing so, *S. invicta* foragers were able to walk on these particles and therefore avoid direct contact with essential balm and eventually access the food (Wen *et al*., 2016). Based on this observation, we hypothesize that: (i) *S. invicta* relocates particles onto inaccessible surfaces; (ii) the presence of food promotes particle-covering behavior in *S. invicta*; and (iii) covering the inaccessible surfaces with soil particles can facilitate food search and transportation. Here, laboratory and field studies were conducted to investigate the particle-covering behaviors of *S. invicta* in response to viscose surfaces or surfaces smeared with liquid paraffin or essential balm (our preliminary studies showed that *S. invicta* could not crawl on these surfaces). The biological significance of particle-covering behaviors for *S. invicta*, as well as the factors that affect these behaviors, were also investigated.

Materials and methods

Ant collection and maintenance

Twelve colony groups of *S. invicta* were collected between 25 May and 11 June 2018. Previous studies have shown that the polygyne type is the dominant social form of *S. invicta* in mainland China (Wang *et al*., 2020). In this study, all *S. invicta* colonies were considered polygyne because of the relatively high density of mounds observed at the collection site. Eight colony groups were collected from the Zengcheng Teaching and Internship Base (23°14′N, 113°37′E) of South China Agricultural University (SCAU), Guangzhou, China, and the remaining colony groups were collected from the green belt along a street (23°9′N, 113°21′E) near SCAU. Each colony group was collected from a single *S. invicta* mound (> 30 cm in diameter), and mounds were > 5 m apart from each other. Ants were collected using the method described by Wang *et al*. (2018) with some modifications. Briefly, nest materials of *S. invicta* with eggs, larvae, pupae, and adults were rapidly transferred using a shovel to a plastic container ($22 \times 15.5 \times 11.5$ cm [L×W×H], LONGSTAR®, Taizhou, China). Containers

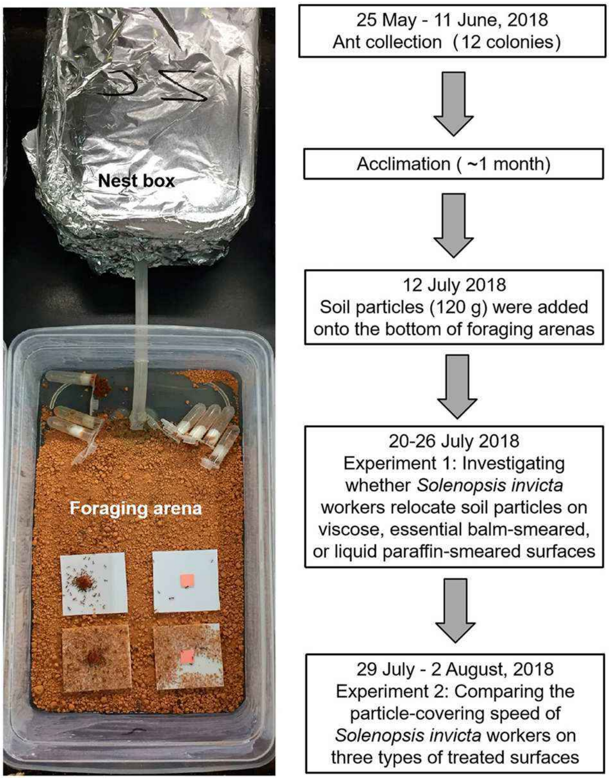

Fig. 1 Bioassay arena and time points of the laboratory experiments.

were brought to the lab within 2 h. A 20-cm silicone tube (inner diameter = 4 mm) was used to connect the plastic container (nest box) to a foraging arena (29×16×9.5 cm [L×W×H], DAISO®, Thailand) with walls coated with Teflon (Fig. 1). The nest box was then covered using aluminum foil to block light. Ants were allowed to acclimate for ~1 month (23–31 °C, 52%–93% relative humidity, and 14 : 10 h light : dark photoregimen). Eppendorf tubes (2 mL) containing 25% honey-water solution or tap water and frozen crickets were regularly provided.

Experiment 1 (laboratory study): Investigating whether Solenopsis invicta *workers relocate soil particles on viscose, essential balm-smeared, or liquid paraffin-smeared surfaces*

The topsoil used for the following experiments was collected from the arboretum of SCAU. A soil sample was sent to the Environmental Chemistry Laboratory (College of Natural Resources and Environment, SCAU) and identified as silty loam (39% sand, 56% silt, and 5% clay). Soil was sterilized at 80 °C for 5 d and ground with wooden mortars and pestles. Dried soil was sifted through a 3-mm sieve to remove plant materials and coarse particles, and 120 g of soil was thoroughly mixed with 12 mL of distilled water, which was then evenly placed on the bottom of each foraging arena. Ants were allowed to acclimate for 8 d after the soil was added. An earlier study showed that ants tend to bury the food with particles when they had been fed until satiation (Qin *et al*., 2019a). To suppress this burying behavior, solid food (frozen crickets) was not provided throughout the laboratory experiments.

First, the particle-covering behavior of *S. invicta* workers in response to viscose surfaces was investigated. There were 12 replicates (each colony group was tested once) for this experiment and subsequent experiments. The viscose surfaces were prepared by placing double-sided adhesive tape (5×5 cm, GUOXIN®, Dongguan, China) on a 5×5-cm square (white paper coated with a layer of plastic membrane). Squares without tape were used as untreated (i.e., control) surfaces. Before the experiment, the weight of each viscose square was measured using a 0.1-mg electronic balance. Two viscose squares and two untreated squares were randomly placed in the experimental arena (Fig. 1). To determine whether food could trigger particle-covering behaviors, either a piece of real food (a 10×10×1-mm sausage, Shuanghui®, Qingyuan, China) or a non-food object (a 10×10×1-mm acrylic plate) was placed on each viscose or untreated square. Each square was > 10 mm apart from adjacent ones. A high-resolution picture was taken for each square every 30 min for 4 h, and the area of squares covered by particles was measured using the regionprops function in MATLAB (MathWorks Inc., Natick, MA, USA). At the end of the experiment, the real food or non-food object as well as the ants were removed from each viscose square, and the weight of particles relocated by ants was calculated (weight change of the viscose square before and after the experiment). Only a few particles were relocated on the untreated squares, and these particles were collected and weighed.

Second, the particle-covering behavior of *S. invicta* workers in response to surfaces smeared with essential balm (Shuixian®, Zhangzhou, China) containing eucalyptol, camphor, menthol, methyl salicylate, and eugenol as the five main components was investigated. To prepare the treated surfaces, 50 μL of essential balm was evenly smeared on the squares. No essential balm was smeared on the untreated (control) squares. The experimental settings and procedures were the same as the viscose surface experiments except that pictures were taken every 60 min for 8 h. The duration of this experiment was longer because our preliminary observations revealed that particle relocation was slower on surfaces smeared with essential balm. All particles on each square were collected at the end of the experiment. The particles were completely dried at 50 °C for 2 d and weighed.

Lastly, the particle-covering behavior of *S. invicta* workers in response to surfaces smeared with liquid paraffin was investigated. Liquid paraffin was used because it creates a wet and sticky surface on which ants cannot easily forage. To prepare the treated surfaces, 50 μL of liquid paraffin with analytical reagent (DM®, Tianjin, China) was evenly smeared on the square. The experimental settings were the same as the viscose surface and essential balm experiments. Pictures for each surface were taken every 30 min for 4 h, and the weight of relocated particles was measured using the same method described in the essential balm experiment.

Experiment 2 (laboratory study): Comparing the particle-covering speed of Solenopsis invicta *workers on three types of treated surfaces*

The speed of particle relocation by *S. invicta* workers was compared among three types of treated surfaces as well as untreated ones in the presence and absence of food. For the experiments in which food was absent, four paper squares (either with double-sided adhesive tape, 50 μL of essential balm or liquid paraffin, or untreated) were placed on the foraging arenas in a random order. A high-resolution picture was taken for each square every 30 min for 4 h, and the area of squares covered by particles was measured using MATLAB. We did not measure the weight of relocated particles because different methods are required to weigh particles on different surfaces (e.g., oven-drying is needed before weighing for particles absorbing essential balm or liquid paraffin, whereas particles on viscose surfaces cannot be directly collected, oven-dried, and weighed), which may introduce bias into the results. The same procedures (except for the sausage) were used to compare the speed of particle relocation on the four surfaces when a piece of sausage ($10\times10\times1$ mm) was placed on the center of each square.

Experiment 3 (field study): Investigating whether Solenopsis invicta *workers relocate particles on viscose surfaces under field conditions*

Field studies were conducted to study particle-covering behaviors of *S. invicta* workers under natural conditions. Because laboratory studies suggested that all three types of surfaces triggered particle-covering behaviors by ants, only viscose surfaces were tested in the field. The experiments were conducted in the Zengcheng Teaching and Internship Base of SCAU from 11:00 to 16:00 hours. Ten *S. invicta* mounds were randomly selected. Only one experiment was conducted for each ant mound. Because the foraging arena was close to the nest box in the laboratory experiments (Fig. 1), it was unclear whether the distance from the nest could affect soil covering behaviors. The particle-covering behaviors of *S. invicta* workers were tested at the distances of 0.2 m and 2.0 m from their mound (Fig. S1A). Four squares (viscose or untreated surfaces with real food or a non-food object in the center) were prepared as described above and were placed at each distance in a random order (the distance between adjacent squares was the same) (Fig. S1B). A high-resolution picture was taken for each square at 3 h, and the area covered by particles was measured using MATLAB.

Experiment 4 (field study): Investigating whether particles placed on viscose surfaces facilitate food search and transportation by Solenopsis invicta *workers*

Experiments were conducted from 15 September to 6 October 2019. Eight trees (*Ficus concinna* Miq. (Moraceae)) with *S. invicta* activity on the trunk were randomly selected for experiments in the green belt along a street (23°9′ N, 113°21′ E) near SCAU. Five polyvinyl chloride (PVC) squares ($5\times5\times0.5$ cm) were fixed to the trunks of trees (height = 1 m) using packing needles (length = 10 cm, diameter = 0.2 cm) (Fig. S2). Each square was equally distanced from adjacent ones. Four PVC squares were randomly selected and covered with double-sided adhesive tape; the remaining square was not covered with adhesive tape. A piece of sausage ($3\times3\times1$ mm) was placed on the center of each square. On each viscose square, 2, 1, 0.25, or 0 g of soil particles (prepared as described in the laboratory studies) was then evenly added and spread in a random order (Fig. S2). Three phases of food transport by *S. invicta* workers—searching, preparation, and transportation—were identified as described by McCreery and Breed (2014) and Qin *et al*. (2019b). Here, we recorded the duration of the searching phase (between when a food item was placed and when the first ant located the food item) and prior-vertical-transportation phase (including the preparation and transportation phases on the horizontal surfaces, i.e., between when the food item was located by the first ant and when the food item was transported on the vertical surface of tree trunks). In addition, a picture was taken at the beginning of the transportation phase (when food had just been moved), and the number of ants involved in this process was determined (Fig. S2).

Experiment 5 (field study): Investigating whether transportation distance affects particle-covering behavior by Solenopsis invicta *workers*

Experiments were conducted in the Zengcheng Teaching and Internship Base of SCAU from 10:00 to 17:00 hours on 20 October 2019. Our previous studies revealed high levels of *S. invicta* activity in this location. Four days before the experiment, weeds and large stones in this area were removed. The PVC plates (YIRU®, Shanghai, China) were cut into circles with diameters of 50, 150, 250, 450, and 850 mm, and a circular adhesive tape (diameter = 50 mm) was placed on the center of each PVC plate (Fig. S3A–E). A piece of sausage (diameter = 10 mm, thickness = 1 mm) was placed on the center of each piece of adhesive tape. Therefore, ants had to transport particles in distances of 0, 50, 100, 200, or 400 mm on the PVC plates to reach the viscose surfaces. In total, 60 PVC plates (12 plates of each size) with adhesive tape were prepared. Fifteen PVC plates (three of each size) without adhesive tape (a piece of sausage was placed on the center of each PVC plate) were used as the control. All PVC plastic plates were randomly placed on the ground, and the distance between adjacent PVC plates was > 2 m from each other (Fig. S3F). High-resolution pictures of the circular adhesive tape of each PVC plate were taken at 3 h and 6 h, and the area of the viscose surface covered by particles was measured using MATLAB.

Data analyses

For each test of experiment 1, the areas of squares covered by soil particles were compared using repeated-measures analysis of variance (ANOVA, SAS 9.4, SAS Institute Inc., Cary, NC, USA) with time as the within-subjects factor and treatment (i.e., treated or untreated squares with real food or non-food object) as the between-subjects factor. In addition, the weights of relocated particles at the end of the test were compared among treatments using one-way ANOVA. For each test of experiment 2 (either in the presence or absence of food), the areas of squares covered by soil particles were compared using repeated-measures ANOVA (SAS 9.4) with time as the within-subjects factor and square type as the between-subjects factor. For experiment 3, data were transformed (transformed area = ln [1 + area of square covered by soil particles]) to meet the assumption of homogeneity of variances. We added 1 in the transformation to deal with 0s in the raw data. The transformed data were then compared using two-way ANOVA (SAS 9.4) with treatments and distance as fixed factors. For experiment 4, the duration of the searching phase was compared among treatments using one-way ANOVA (SAS 9.4). The duration of the prior-vertical-transportation phase was log-transformed to make the distribution normal, and the transformed data among treatments were compared using one-way ANOVA (SAS 9.4). Because the number of ant transporters at the beginning of the transportation phase had a Poisson distribution, data were compared using nonparametric Kruskal–Wallis tests (IBM SPSS Statistics version 20.0, Chicago, IL, USA). For experiment 5, the areas of squares covered by soil particles were compared among treatments using one-way ANOVA (SAS 9.4). Tukey's Honestly Significant Difference tests and Dunn's tests were conducted after each ANOVA and Kruskal–Wallis test, respectively, for *post hoc* comparisons. The significance level was $\alpha = 0.05$ for all tests.

Results

Experiment 1 (laboratory study): Investigating whether Solenopsis invicta *workers relocate soil particles on viscose, essential balm-smeared, or liquid paraffin-smeared surfaces*

The effects of time and treatment on the particle-covered area and the interaction between time and treatment were significant for each test (Table 1). In general, *S. invicta* workers deposited significantly more particles (measured as both particle-covered area and weight) on each of the three types of treated squares compared with untreated ones (Figs. 2 and 3; Figs. S4 and S5). The particles-covered area of viscose or paraffin-smeared squares and the weight of relocated particles were significantly higher in the presence of real food than in the presence of non-food objects (Fig. 3; Table S1). However, no significant difference was detected for squares smeared with essential balm when real food or non-food objects were provided (Fig. 3; Table S1).

Experiment 2 (laboratory study): Comparing the particle-covering speed of Solenopsis invicta *workers on three types of treated surfaces*

The effects of time and treatment and the interaction effect between time and treatment were significant in both the presence and absence of food (Table 1). In general, significantly more area of the viscose squares was covered by particles than squares smeared with liquid paraffin, and both viscose squares and squares with liquid paraffin had significantly more area covered by particles

Table 1 Statistical results of repeated-measures analyses of variance of experiments 1 and 2.

Experiment	Test	Treatment	Time	Treatment × time
1	Viscose	$F = 257.56$; df = 3, 44; $P < 0.0001$	$F = 17.97$; df = 7, 38; $P < 0.0001$	$F = 6.01$; df = 21, 73.4; $P < 0.0001$
	Paraffin-smeared	$F = 106.85$; df = 3, 44; $P < 0.0001$	$F = 11.72$; df = 7, 38; $P < 0.0001$	$F = 3.63$; df = 21, 73.4; $P < 0.0001$
	Essential balm-smeared	$F = 11.07$; df = 3, 44; $P < 0.0001$	$F = 13.63$; df = 7, 38; $P < 0.0001$	$F = 4.46$; df = 21, 73.4; $P < 0.0001$
2	With food	$F = 67.93$; df = 3, 44; $P < 0.0001$	$F = 30.53$; df = 7, 38; $P < 0.0001$	$F = 9.66$; df = 21, 73.4; $P < 0.0001$
	Without food	$F = 25.34$; df = 3, 44; $P < 0.0001$	$F = 10.30$; df = 7, 38; $P < 0.0001$	$F = 3.26$; df = 21, 73.4; $P = 0.0001$

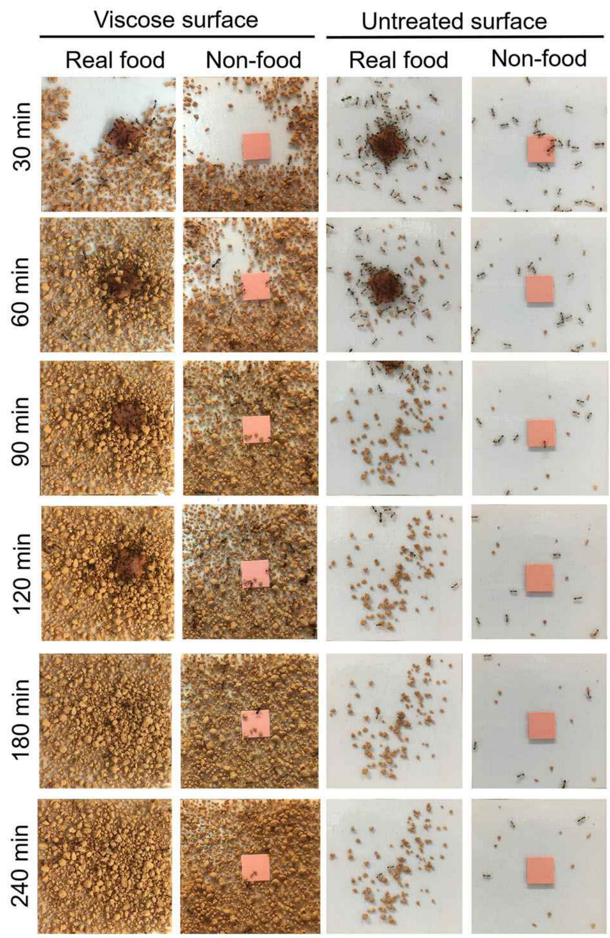

Fig. 2 *Solenopsis invicta* workers transported large amounts of soil particles onto viscose surfaces whenever the real food or non-food object was provided, whereas few particles were found on the untreated surfaces.

compared with squares smeared with essential balm or untreated squares, regardless of whether food was provided (Fig. 4; Table S2).

Experiment 3 (field study): Investigating whether Solenopsis invicta *workers relocate particles on viscose surfaces under field conditions*

On viscose surfaces, significantly more area was covered by particles on squares with real food compared with squares with non-food objects ($F = 99.35$, df = 3, 72, $P < 0.0001$; Table S3), and few particles were observed on untreated squares (Fig. 5A and B). The effect of distance ($F = 1.96$, df = 1, 72, $P = 0.1657$) and the interaction between treatment and distance ($F = 0.28$, df = 3, 72, $P = 0.8364$) were not significant.

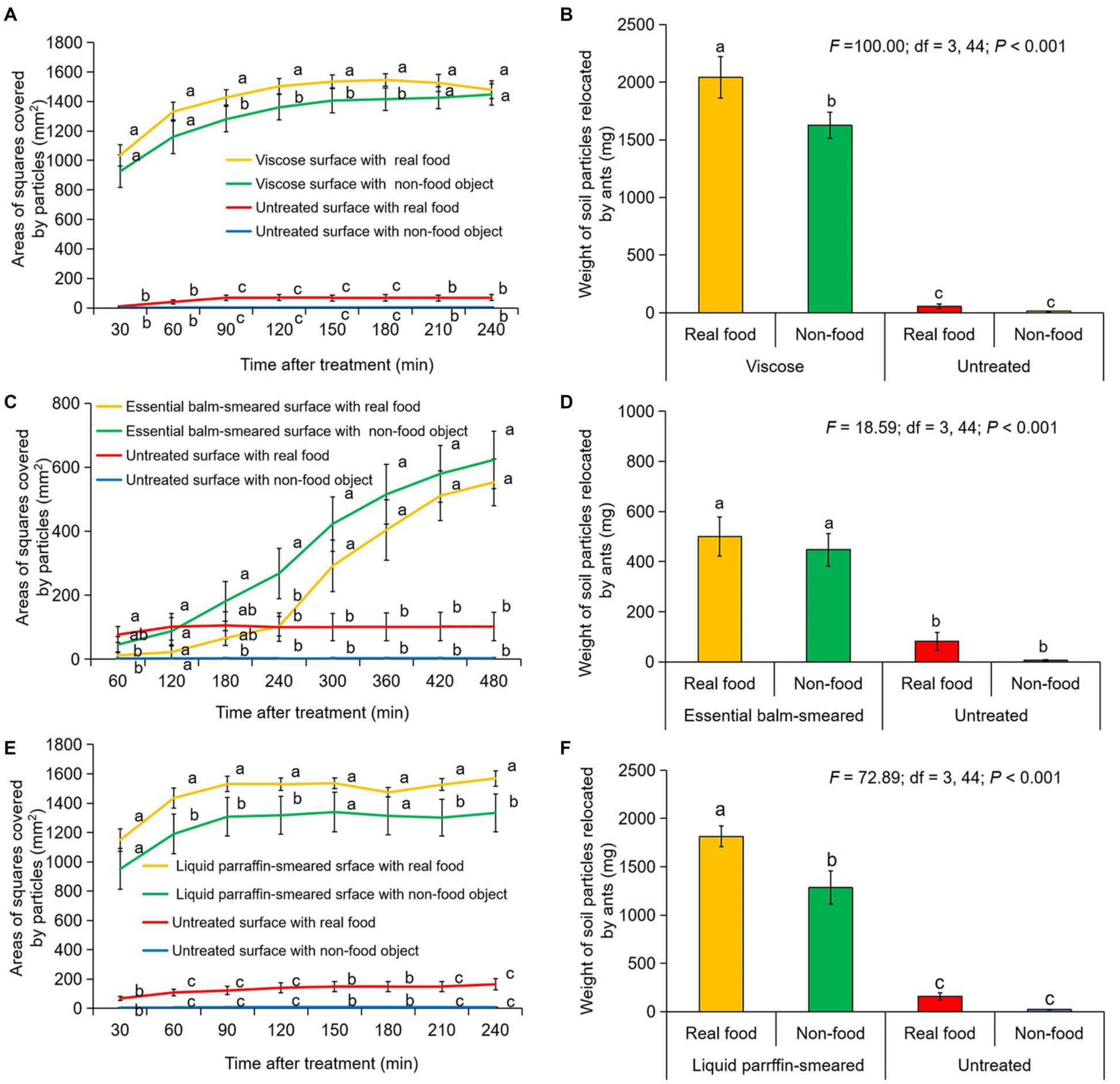

Fig. 3 *Solenopsis invicta* workers transported significantly more particles onto viscose surfaces (A: measured in particle-covered area; B: measured in weight of particles), essential balm-smeared surfaces (C: measured in particle-covered area; D: measured in weight of particles), or liquid paraffin-smeared surfaces (E: measured in particle-covered area; F: measured in weight of particles). Data are presented as mean ± SE. Different letters indicate significant differences ($P < 0.05$). Yellow lines and bars indicate treated surface with the real food; green lines and bars indicate treated surface with the non-food object; red lines and bars indicate untreated surface with the real food; and blue lines and bars indicate untreated surface with the non-food object.

Experiment 4 (field study): Investigating whether particles placed on viscose surfaces facilitate food search and transportation by Solenopsis invicta *workers*

All viscose squares with food covered by 1 or 2 g sand particles were successfully searched and the food was transported by ants, but they failed to do so on viscose surfaces without soil particles or with 0.25 g of soil particles. Ants spent significantly less time searching for food on untreated squares compared with viscose squares covered with 1 g of soil ($F = 5.52$, df $= 2, 21$, $P = 0.0118$, Fig. 6A; Table S4). However, there was no significant

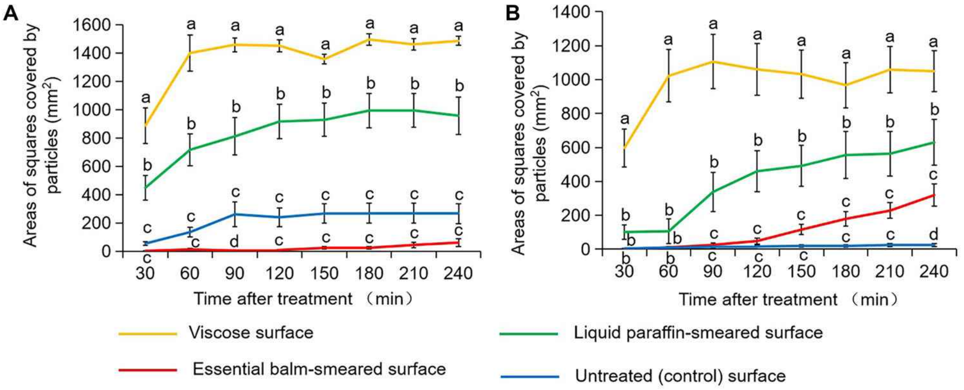

Fig. 4 Particle-covered areas of viscose squares, or squares smeared with liquid paraffin or essential balm, or untreated ones, when food was provided (A) or not (B). Data are presented as mean ± SE. Different letters indicate significant differences within each time period ($P < 0.05$). Yellow bars indicate the viscose surface; green bars indicate the liquid paraffin-smeared surface; red bars indicate the essential balm-smeared surface; and blue bars indicate the untreated surface.

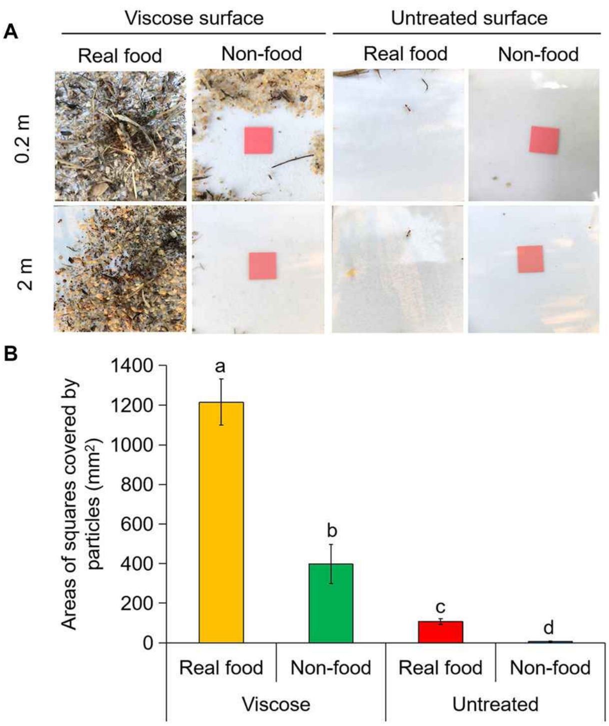

Fig. 5 *Solenopsis invicta* workers relocated particles onto viscose squares under field conditions (A). The particle-covered areas of viscose squares and untreated ones with real food or a non-food object are presented as mean ± SE (B). Different letters indicate significant differences ($P < 0.05$). Yellow bar indicates viscose surface with the real food; green bar indicates viscose surface with the non-food object; red bar indicates untreated surface with the real food; blue bar indicates untreated surface with the non-food object.

difference in the duration of the prior-vertical-transportation phase among untreated squares and viscose squares covered by 1 or 2 g of particles ($F = 0.35$, df = 2, 19, $P = 0.7075$, Fig. 6B; Table S4). At the beginning of the transportation phase (when food had just been moved), significantly more ant transporters were observed on untreated squares than on viscose squares artificially covered by 1 or 2 g of particles ($\chi^2 = 17.38$, df = 2, $P < 0.0001$; Fig. 6C; Table S4).

Experiment 5 (field study): Investigating whether transportation distance affects particle-covering behavior by Solenopsis invicta *workers*

Ants transported the food items on all PVC plates without the adhesive tape, indicating that they were able to walk and forage on the surface of PVC plates. At 3 h, significantly more particles were observed on the adhesive tape of 50-mm diameter PVC plates (i.e., no transport distance was required) compared with other treatments ($F = 17.34$, df = 4, 55, $P < 0.0001$; Fig. 7A; Supp. Material: Table S5). The fewest number of particles were observed on the adhesive tape of 850-mm diameter PVC plates (i.e., ants needed to transport particles a distance of 400 mm to reach the tape). At 6 h, there was no significant difference in covered areas of adhesive tape of PVC plates with diameters of 50, 150, and 250 mm (transport distance of 0, 50, and 100 mm, respectively); however, significantly fewer particles were observed on adhesive tape of PVC plates with a diameter of 850 mm (transport distance of 400 mm) ($F = 23.60$, df = 4, 55, $P < 0.0001$, Fig. 7B; Table S5).

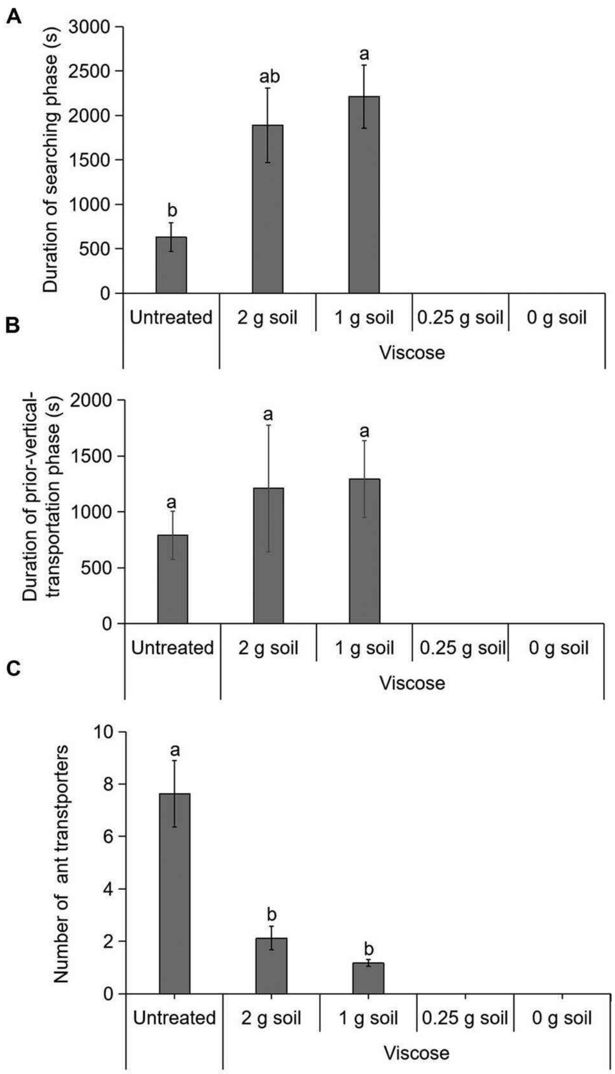

Fig. 6 Duration of searching phase (A) and prior-vertical-transportation phase (B), and number of ant transporters at the beginning of the transportation phase (C) on untreated surface, or viscose surfaces that was not covered, or viscose surfaces that was artificially covered by soil particles (0.5, 1, or 2 g). Data are presented as mean ± SE. Different letters indicate significant differences ($P < 0.05$).

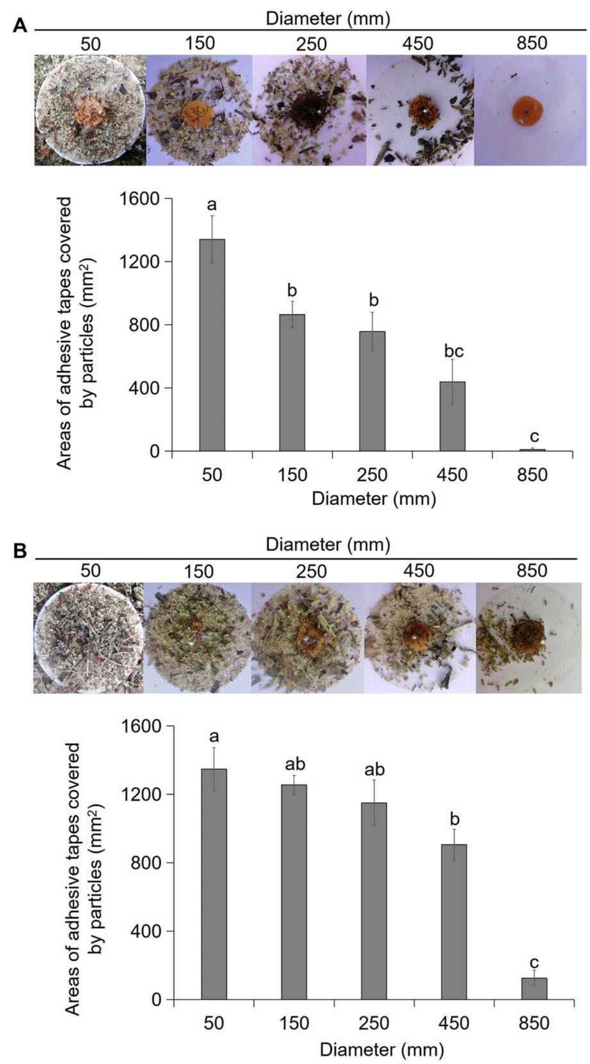

Fig. 7 Particle-covered area of adhesive tapes pasted on the polyvinyl chloride plate with the diameters of 50, 150, 250, 450, and 850 mm at 3 h (A) and 6 h (B). Data are presented as mean ± SE. Different letters indicate significant differences ($P < 0.05$).

Discussion

Our study showed that: (i) under laboratory conditions, *S. invicta* generally performed particle-covering behavior in response to inaccessible surfaces, and the presence of food was not necessary for triggering particle-covering behavior but promoted the expression of this behavior; (ii) *S. invicta* workers quickly covered viscose and paraffin-smeared surfaces, and the particle-covering speed on surfaces smeared with essential balm was much slower; (iii) similar particle-covering behaviors in response to viscose surfaces were observed in the field; (iv) *S. invicta* was able to search and transport food on viscose surfaces artificially covered with large amounts of soil particles, but they could not search and transport food on viscose surfaces without soil particles or lacking a sufficient amount of soil particles; and (v) long transport distances of particles negatively affected covering behavior.

As shown in this study, the covering of inaccessible surfaces with particles is a recently discovered behavior in *S. invicta*. This behavior is closely associated with foraging processes based on the following observations: (i) *S. invicta* searched and transported food items on viscose surfaces artificially covered with large amounts of particles but were not able to complete these activities on uncovered viscose surfaces or viscose surfaces that were covered with few particles; and (ii) under both laboratory and field conditions, *S. invicta* relocated significantly more particles on viscose or paraffin-smeared surfaces with real food than on surfaces with non-food objects. However, ants also covered treated surfaces without real food, indicating that the inaccessibility of treated surfaces, rather than food, triggered the particle-covering behavior in *S. invicta*. Wang and Henderson (2016) also observed that *S. invicta* relocated particles on filter paper soaked with distilled water. *S. invicta* originated in South America (Ascunce *et al*., 2011), where the ground is often too wet to walk on during the rainy season; consequently, they may have evolved particle-covering behaviors to modify wet or inaccessible surfaces in their foraging territories to search and transport food, which may have provided them with a competitive advantage over co-occurring ant species.

In this study, *S. invicta* spent much more time covering surfaces smeared with essential balm compared with viscose or paraffin-smeared surfaces, likely because essential balm is a strong ant repellent. However, some ants still relocated particles, albeit at a relatively low speed, and eventually accessed the food. Although this behavior may negatively affect the persistence of repellent effects, no previous studies have considered this factor during the screening and evaluation of fire ant repellents, as most of these studies have evaluated fire ant repellents under laboratory conditions where particles were not available to *S. invicta* colonies. Although some studies have studied fire ant repellents in the field (e.g., Wang & Henderson, 2016), these studies have generally examined repellent effects over short periods (i.e., < 1 h), yet longer periods may be needed to observe particle-covering behaviors triggered by surfaces covered with repellents. In a preliminary study, we found that many fire ant repellents generally triggered particle-covering behaviors, but a few repellents can suppress both food-foraging and particle-covering behaviors of *S. invicta*, which may have a more complete and long-lasting effect for repelling *S. invicta* (C. Wen and C. Wang, unpublished data).

Lőrinczi *et al*. (2018) reported that *A. subterranea* preferred certain types of particles to soak liquid food based on the availability and properties of particles that were "best matched to the particular foraging environment." Our field studies showed that *S. invicta* relocated various materials (e.g., soil particles and fragments of leaves and grass) to cover viscose surfaces, indicating that particle use by *S. invicta* was flexible. To increase covering efficiency and minimize energy costs, particles should be easy to transport, and each particle should have a large contact area. However, whether *S. invicta* prefers certain particles based on their weight, size, materials, and shape remains unclear. Distance and availability may also affect particle preference and use by ants (Lőrinczi *et al*., 2018). In this study, distance from the nest (mound) did not affect the covering behavior of *S. invicta*. However, significantly less area of viscose squares was covered when *S. invicta* needed to transport particles long distances (i.e., 400 mm), indicating that the availability of particles around or near inaccessible surfaces affects covering behaviors. Interestingly, Qin *et al*. (2019a) observed that a small proportion of *S. invicta* workers repeatedly transport soil particles to bury food, indicating that this task may be carried out by certain groups of workers. However, whether *S. invicta* workers show a division of labor for covering inaccessible surfaces with particles remains unclear. Methods such as video tracking and individual marking will prove useful for studying the behavioral patterns of *S. invicta* individuals during the particle-covering process.

S. invicta has become a significant problem in rural and urban areas because of its ability to sting and it poses a serious health threat (Wang *et al*., 2020). Several methods (observation of *S. invicta* activities and mounds, baiting, and pitfall trapping) have been developed to detect *S. invicta* (e.g., Bao *et al*., 2011; Lu *et al*., 2014; Lu *et al*., 2015; Wang & Lu, 2017), which are important for the successful management of this pest, especially during the early stage of invasion. However, all of these methods require identification of *S. invicta* specimens based on morphological characters (e.g., Zeng *et al*., 2005), and the public generally lacks knowledge of ant taxonomy. Some recent studies could successfully identify *S. invicta* based on DNA barcoding and immunoassays (e.g., Valles *et al*., 2016; Kim *et al*., 2019; Nakajima *et al*., 2019; Valles *et al*., 2020), but these methods require specific technologies and are costly. Our study may provide a simple method to detect *S. invicta* activities by placing a viscose square with food and observing the subsequent particle-covering behaviors. However, two requirements must be met to confirm the effectiveness of this detection method: (1) particle-covering behaviors should only be performed by *S. invicta* so that they can be reliably distinguished from non-target ant species; and (2) application of viscose surfaces should be as accurate as other methods, such as baiting and pitfall trapping, to detect *S. invicta*. A large-scale field study aiming to assess the efficacy of this detection method in different types of habitats and various regions in southern China is currently in progress.

Acknowledgments

We sincerely thank Dr. Gadi V. P. Reddy (Southern Insect Management Research Unit, Agricultural Research Service, U.S. Department of Agriculture) and Dr. Aaron Ellison (Harvard Forest, Harvard University) for valuable comments on our manuscript. We also thank Shiping Liang, Yinghao He, Zhengya Jin, Lang Zhang, and Wasim Javaid (College of Forestry and Landscape Architecture, SCAU) for assistance in the field studies. This work was supported by the Pearl River S&T Nova Program of Guangzhou (201806010182).

Disclosure

The authors declare no conflict of interest.

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Manuscript received July 20, 2020
Final version received October 16, 2020
Accepted November 16, 2020

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Field study to investigate whether *Solenopsis invicta* workers relocate particles on viscose surfaces under field conditions.

Fig. S2 Field study to investigate whether particles placed on viscose surfaces facilitate food search and transportation by *Solenopsis invicta* workers.

Fig. S3 Field study to investigate whether transportation distance affects particle-covering behavior by *Solenopsis invicta* workers.

Fig. S4 *Solenopsis invicta* workers transported large amounts of soil particles onto squares smeared with liquid paraffin, whereas few particles were found on the untreated surfaces.

Fig. S5 *Solenopsis invicta* workers transported large amounts of soil particles onto squares smeared with essential balm, whereas few particles were found on the untreated surfaces.

Table S1 Results of Tukey's Honestly Significant Difference (HSD) tests for *post hoc* comparisons of particles (measured as both particles-covered area and weight) transported by *Solenopsis invicta* workers onto treated or untreated surfaces when real food or a non-food object was placed.

Table S2 Results of Tukey's Honestly Significant Difference (HSD) tests after repeated measured analysis of variance for *post hoc* comparisons of particles-covered areas on three types of treated surfaces or untreated ones under food or non-food conditions.

Table S3 Results of Tukey's Honestly Significant Difference (HSD) tests after analysis of variance for *post hoc* comparisons of particles-covered areas on viscose or untreated surfaces when real food or a non-food object was placed under field conditions.

Table S4 Results of *post hoc* comparisons of duration of searching phase, duration of prior-vertical-transportation phase, and number of ant transporters.

Table S5 Results of Tukey's Honestly Significant Difference (HSD) tests after one-way analysis of variance for *post hoc* comparisons of particle-covered areas when the adhesive tapes were pasted on the polyvinyl chloride plate with the diameters of 50, 150, 250, 450, and 850 mm.