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A simple and efficient method for preventing ant escape (Hymenoptera: Formicidae) Dongdong Ning, Fan Yang, Qian Xiao, Hao Ran & Yijuan Xu

Abstract

During the process of insect rearing and research, reliable insect escape prevention is indispensable. However, methods for preventing the escape of small insects, such as ants, remain limited. We used a mixture of talc powder and ethanol to prepare an anti-escape solution and compared its effects on *Solenopsis invicta* BUREN, 1972 and *Monomorium intrudens* SMITH, 1874 with those of Fluon. Talc powder of six different finenesses mixed with ethanol at a concentration of 20% could prevent 100% of *S. invicta* from escaping, and the mixture of talc powder and ethanol could also prohibit 100% of *M. intrudens* from escaping when the particle diameter (D97) values were $2 \mu m$ and $3 \mu m$. Furthermore, the mixture could prohibit 100% of *M. intrudens* from escaping when the concentration of talc power (D97 = $2 \mu m$) was higher than 1%, and the anti-escape effect of this solution was maintained for more than three months and was stable for one year. However, Fluon could not prevent *M. intrudens* from escaping in one-day tests. Scanning electron microscopy showed that, at the same concentration, the gaps between particles decreased with decreasing talc powder particle size. The ratio of worker weight to adhesive-pad (arolium) area in *S. invicta* was approximately twice that in *M. intrudens*. The size and spacing of talc particles as well as the weight and adhesion ability of the ants are the main factors that determine anti-escape efficacy. The results suggest that the talc powder-ethanol mixture is more efficient than Fluon in the prevention of escape in small ant species.

Key words: Red imported fire ant, Solenopsis invicta, Monomorium intrudens, talc powder, ethanol, anti-escape liquid.

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Introduction

Ants are among the most common insects on the planet, encompassing a wide variety of species and a very large number of individuals. In addition, ants are the most widely distributed eusocial insects (WILSON 1971). Historically, the main techniques that were initially used to prevent ant escape in the laboratory were the use of water as a physical barrier or the spreading of Vaseline or mineral oil on the surface of a container (VOGT & KOZLOVAC 2006, CHEN & WEI 2007). However, these methods are either incredibly inconvenient or can easily kill the ants. Approximately 70 years ago, a dispersion of polytetrafluorethylene under the name of Fluon was demonstrated to be an effective barrier against insect pests (MERTON 1956). Because of the low coefficient of friction of Fluon (BOWDEN & TABOR 1950), a ring of this dispersion painted around the upper part of the inside of a container provides a completely impassable barrier to ants, earwigs and ladybirds (MERTON 1956). CHEN & WEI (2007) found that even using Fluon at a low concentration (also called Teflon and "Insect-a-Slip") can efficiently prevent *Solenopsis invicta* BUREN, 1972 from escaping from containers. However, Fluon is difficult to remove and does not work for all ant species, especially small ants.

Talc powder is a natural monoclinic hydrosilicate with the molecular formula $3MgO \cdot 4SiO_2 \cdot H_2O$ that is widely used in various industrial fields. Talc consists of layers of flaky sheets held together by relatively weak van der Waals forces, making the layers slippery and easily separated when external force is applied (ZHENG 1990). Talc powder has good lubricity and viscosity resistance, high temperature resistance and low price. Because of its excellent lubricity, talc powder is also often used to prevent insect escape (DREES 2002, KATAYAMA & SUZUKI 2010). However, we found that the adhesion of talc powder is so limited that it is not directly suitable for preventing ants from escaping. In addition, talc powder easily sticks to ants and affects their behavior, perhaps even causing ant death as a result of water loss.

The ant foot includes a sticky pad (arolium) (GLADUN & al. 2009), which helps ants to crawl vertically on glass, wood, gypsum board, cement, paraffin, and other surfaces (MERTON 1956). Ants use the arolium on the leg above their center of mass (CoM) and a setae array on the 3rd and 4th tarsal segments of the leg beneath the CoM to provide holding power, with the legs above the CoM bearing the majority of the weight (ENDLEIN & FEDERLE 2008, FEDERLE & al. 2001, ENDLEIN & FEDERLE 2015).

In light of the above problems, in this study, we propose a simple and efficient method of mixing talc powder with absolute ethanol to prevent ants from escaping. Workers of *Solenopsis invicta* and *Monomorium intrudens* SMITH, 1874, which are small and have strong climbing ability, were used to evaluate the effect of this mixture on escape prevention. The surface features of the talc and the pretarsi of the ants' forelegs were observed by scanning electron microscopy to further explore the escape mechanism.

Materials and methods

Chemicals: Six kinds of talc powder of different fineness were purchased from the market in Tianhe district, Guangzhou. The D97 (representing 97% of the cumulative undersize particle size distribution) values of the talc powder were $22 \,\mu$ m, $11 \,\mu$ m, $6 \,\mu$ m, $4 \,\mu$ m, $3 \,\mu$ m, and $2 \,\mu$ m. Fluon was produced by the Chemours Company (Wilmington, DE, USA) and contains 60% polytetrafluoroethylene (PTFE, size = $0.22 \,\mu$ m).

Ants: Red imported fire ants, Solenopsis invicta, were collected from Tianhe District Wisdom Park, Guangzhou, Guangdong Province, and Monomorium intrudens were collected from Fangchenggang, Guangxi Province. All ant colonies were identified to species using the ZHOU (2001) key. A total of ten colonies of each species were collected. The collected colonies were maintained in plastic nest boxes whose walls had been painted with Fluon or talc powder in a temperature controlled room at 26 ± 1 °C and 60 - 70% relative humidity and provided with sugar water (10% W/V) and locusts, Locusta migratoria manilensis (MEYEN, 1835), as food every day. Thirty workers of S. *invicta* (average body length \pm standard error: 3.35 \pm 0.07 mm) and 30 workers of M. intrudens (average body length \pm standard error: 2.01 \pm 0.02 mm) were selected to weigh with an electronic balance (BS224S, Sartorius, Germany). The weight of each worker ant was calculated, and this procedure was repeated 5 times.

Experiment 1. Effect of talc powder of different fineness on ant escape: Ten grams of talc powder of each fineness value (D97: $22 \mu m$, $11 \mu m$, $6 \mu m$, $4 \mu m$, $3 \mu m$ and $2 \mu m$) were mixed with absolute ethanol (analytically pure, Yongda Chemical Reagent Co., Ltd., Tianjin, China) and stirred well to obtain solutions of 20% (W/V). We coated the upper half of the inner wall of a 90 mm diameter glass Petri dish with the mixture using a brush and waited for the ethanol to evaporate. More than 10 workers of *Solenopsis invicta* or *Monomorium intrudens* were transferred to the Petri dish with a brush, and a 10% (W/V) sucrose solution was provided. After 24 hours, the number of ants that had escaped was counted. The experiment was repeated 10 times for each species.

Experiment 2. Effect of talc powder mixed with different concentrations of ethanol on ant escape: Absolute ethanol was diluted with water to produce solutions with concentrations of 20%, 40%, 60%, 80%, and 99.7%. Then, each ethanol solution was mixed evenly with 10 g of talc powder with a D97 of 2 μ m to obtain solutions with a concentration of 20% (W/V). We coated the upper half of the inner wall of a 90 mm diameter glass Petri dish with the mixture using a brush and waited for the ethanol to evaporate. More than 10 workers of *Solenopsis invicta* or *Monomorium intrudens* were transferred to the Petri dish with a brush, and a 10% (W/V) sucrose solution was provided. After 24 hours, the number of ants that had escaped was counted. The experiment was repeated 10 times for each species.

Experiment 3. Effect of talc powder mixed with different volumes of ethanol and Fluon on ant escape: Ten grams of talc powder with a D97 of 2 µm were mixed with absolute ethanol to obtain solutions with concentrations of 20%, 6.67%, 2%, 1%, 0.5%, and 0.2% (W/V). We coated the upper half of the inner wall of a 90 mm diameter glass Petri dish with the mixture using a brush and waited for the ethanol to evaporate. More than 10 workers of Solenopsis invicta or Monomorium intrudens were transferred to the Petri dish with a brush, and a 10% (W/V) sucrose solution was provided. Fluon dilutions served as controls. A series of dilutions were made by adding distilled water to the original Fluon product, resulting in solutions of 1/2, 1/3, 1/20, 1/40, 1/100, 1/250 and 1/500 of the original product (V/V). These solutions contained 30%, 20%, 3%, 1.5%, 0.6%, 0.24%, and 0.12% (W/W) PTFE, respectively. After 24 hours, the number of ants that had escaped was counted. The experiment was repeated 10 times for each species.

To confirm that the anti-escape efficiency of the talc powder mixture is better than that of Fluon in terms of ant escape, an additional experiment was performed using plastic boxes ($17 \text{ cm} \times 12 \text{ cm} \times 8 \text{ cm}$). Talc powder with a D97 of 2μ m was mixed with absolute ethanol to obtain solutions with a concentration of 20%, 6.67%, 2%, and 1% (W/V). We coated a 2-cm-width band onto the upper inner wall of a polycarbonate (PC) box with the mixture using cotton and waited for the ethanol to evaporate. More than 10 workers of *S. invicta* or *M. intrudens* were transferred to the box with a brush, and a 10% (W/V) sucrose solution was provided. Fluon solutions containing 30%, 20%, 3%, and 1.5% (W/W) PTFE served as controls. After 24 hours, the number of ants that had escaped was counted. The experiment was repeated for each species three times.

Experiment 4. Duration of the effect of talc powder mixed with ethanol on preventing ant escape: To test the duration of the effect of talc powder mixed with ethanol on preventing ant escape, 10g of talc powder with a D97 of 2 µm was mixed with absolute



Fig. 1: Anti-escape efficacy of mixtures of talc powder of different fineness values with ethanol (A), mixtures of ethanol and talc powder at different concentrations (B), mixtures of ethanol with talc powder (D97 = $2 \mu m$) at different concentrations applied to glass Petri dishes (C) and plastic boxes (E), and mixtures of water with Fluon at different concentrations applied to glass Petri dishes (D) and plastic boxes (F) for *Solenopsis invicta* and *Monomorium intrudens*. Data points with same capital (*S. invicta*) or lowercase (*M. intrudens*) letters indicate no significant differences at the 0.05 level (Nemenyi test).

ethanol to obtain a solution with a concentration of 20% (W/V). We coated the upper half of the inner wall of a round plastic bowl (height 5 cm, bottom diameter 7 cm, top diameter 7 cm) with the mixture using a brush and waited for the ethanol to evaporate. We placed the bowl in a plastic box ($17 \text{ cm} \times 12 \text{ cm} \times 8 \text{ cm}$) coated with the mixture. Thereafter, colony fragments (approximately 400 ~ 500 workers, as well as dozens of the brood) of *Solenopsis invicta* or *Monomorium intrudens* were placed in the small bowl and tested for three months. They were

given adult commercial *Tenebrio molitor* L. and a 10% solution of honey roughly every three days and provided with water ad libitum. Ten colony fragments were tested for each species. The number of ants that escaped from the small bowl was counted every 24 hours.

To test whether the coating can remain stable for months, 10g of talc powder with a D97 of $2\,\mu$ m was mixed with absolute ethanol to obtain solutions with a concentration of 20% (W/V). We coated the upper half of the inner wall of a plastic box (41 cm × 28 cm × 11 cm) with



Fig. 2: Scanning electron micrographs showing the surface morphology of mixed talc powder and ethanol coatings. (A) D97 = 2μ m, (B) D97 = 3μ m, (C) D97 = 4μ m, (D) D97 = 6μ m, (E) D97 = 11μ m, (F) D97 = 22μ m.

the mixture using a brush and waited for the ethanol to evaporate. We tested the anti-escape effect every 30 days. During testing, approximately 1000 workers of *Monomorium intrudens* were placed into the box, and the number of ants that were able to escape was counted 24 hours later. After counting, all the ants were removed and returned to their original colonies. Three colonies were tested.

Both the duration and stability experiments were conducted in the laboratory with the temperature maintained at 26 ± 1 °C and a relative humidity of 60 - 70%. **Experiment 5. Scanning electron microscopy:** To explore the mechanism of escape prevention by the talc powder-ethanol mixture and Fluon, we performed an electron microscope scan of talc powder-ethanol and Fluon-coated surfaces of PC boards. For this purpose, talc powder of each fineness value (D97: $22 \mu m$, $11 \mu m$, $6 \mu m$, $4 \mu m$, $3 \mu m$ and $2 \mu m$) was mixed with absolute ethanol and stirred well to obtain solutions with a concentration of 20% (W/V), and the original Fluon solution was mixed with water to obtain solutions with concentrations of 60%, 30%, 20%, 3% and 1.5% (W/W). We coated PC boards with



Fig. 3: Scanning electron micrographs showing the surface morphology of Fluon coatings at relatively high concentrations (A, B: 60% PTFE; C, D: 30% PTFE; E, F: 20% PTFE) at a scale of 10 µm (left panel) and 5 µm (right panel).

the solutions using a brush and waited for the ethanol or water to evaporate. The PC boards were fixed onto a sample table with electrically conductive adhesive. We also randomly selected 3 workers of *Solenopsis invicta* and 3 workers of *Monomorium intrudens* and fixed their forelegs onto the sample table with the assistance of a stereomicroscope (SteREO Discovery V20, Carl Zeiss AG of Germany). The ant foreleg and PC board samples were placed into a vacuum spraying instrument (Eiko IB5, Japan) for gold film plating. Finally, the surface features of the talc, Fluon and pretarsi of the ants' forelegs were observed and photographed under a scanning electron microscope (SEM, SU6600, Hitachi, Japan), and the observations were recorded. The accelerating voltage of the SEM was 20 kV.

Data analysis: We used SPSS 17.0 to analyze the data. Kruskal-Wallis (KW) nonparametric analysis of variance was used to compare the different treatments. The Nemenyi test for multiple comparisons among the different groups was used if the results of the Kruskal-Wallis test showed significant differences at the 0.05 significance level.



Fig. 4: Scanning electron micrographs showing the surface morphology of Fluon coatings at relatively low concentrations (A, B: 3% PTFE; C, D: 1.5% PTFE) at a scale of 10 µm (left panel) and 5 µm (right panel).

Results

The experimental results showed that the escape prevention rates associated with the mixtures of talc powder of different fineness and absolute ethanol, when used to treat glass Petri dishes, were 100% for *Solenopsis invicta* (Fig. 1A). However, significant differences were found in the effects of these mixtures on *Monomorium intrudens* escape prevention (Kruskal-Wallis test, χ^2 = 56.7, df = 5, P < 0.0001, Fig. 1A). For example, mixtures of fine (D97 = 2 µm and 3 µm) talc powder and absolute ethanol could prevent 100% of the *M. intrudens* from escaping, while mixtures of talc powder with D97 values of 4 µm or 6 µm with ethanol could prevent 0-100% of the *M. intrudens* from escaping. When coarse talc powder (D97 = 11 µm and 22 µm) was mixed with absolute ethanol, all the *M. intrudens* escaped (Fig. 1A).

The solutions of talc powder with a D97 of $2 \mu m$ mixed with ethanol at concentrations of 20%, 40%, 60%, 80%, and 99.7% could prevent 100% of the *S. invicta* and *M. intrudens* from escaping (Fig. 1B).

The solutions of talc powder (D97 = $2 \mu m$) mixed with absolute ethanol at concentrations of 20%, 6.67% and 2% could prevent 100% of the *S. invicta* from escaping from both the glass Petri dishes (Fig. 1C) and plastic boxes (Fig. 1E). The anti-escape effectiveness started to fall below 100% when the concentration was lower than 1%. In addition, the effectiveness significantly decreased with a decreasing concentration of talc powder (Kruskal-Wallis test, $\chi^2 = 57.326$, df = 5, P < 0.0001, Fig. 1C). The solutions of talc powder with a D97 of 2 µm mixed with absolute ethanol at concentrations of 20%, 6.67% and 2% could prevent 100% of the *M. intrudens* from escaping from both glass Petri dishes (Fig. 1C) and plastic boxes (Fig. 1E). However, the anti-escape effectiveness started to fall below 100% when the concentration was lower than 2%, and it significantly decreased with a decreasing concentration of talc powder (Kruskal-Wallis test, $\chi^2 = 55.113$, df = 5, P < 0.0001, Fig. 1C).

Fluon prevented 100% of the *Solenopsis invicta* from escaping when the concentration of PTFE was higher than 20% in both the glass Petri dish (Kruskal-Wallis test, $\chi^2 = 78.827$, df = 7, P < 0.0001, Fig. 1D) and plastic box test (Fig. 1F). However, Fluon could not prevent the *Monomorium intrudens* from escaping at any tested concentration (Kruskal-Wallis test, $\chi^2 = 75.61$, df = 7, P < 0.0001, Fig. 1D; $\chi^2 = 2.74$, df = 3, P = 00.433, Fig. 1F).

Furthermore, the treatment of a plastic bowl or box with the talc powder and ethanol mixture could completely prevent workers of both *Solenopsis invicta* and *Monomo*-



Fig. 5: Scanning electron micrographs of the pretarsi of *Solenopsis invicta* (A, B) and *M. intrudens* (C, D). Ar, arolium; Tc, tarsal claw; Aa, arolium area.

rium intrudens from escaping for three months. After the experiment was run for three months, more than 90% of the workers of both species survived. *M. intrudens* were still unable to escape one year after the box was painted with the solution.

We used scanning electron microscopy to observe the coatings composed of talc powder of six different finenesses and ethanol and found that, at the scale of $100 \,\mu\text{m}$, as the talc particle size decreases, the talc particle density of the coating gradually increases, the size of the gaps gradually decreases, and the degree of coalescence between the particles increases. No gaps were observed in the coating with D97 = $2 \,\mu\text{m}$, which formed a uniform and compact surface structure (Fig. 2). The average weight of *Solenopsis invicta* (body length: $3.35 \pm 0.07 \,\text{mm}$) was $0.5733 \,\text{mg} / \text{worker}$, and that of *M. intrudens* (body length: $2.01 \pm 0.02 \,\text{mm}$) was $0.1184 \,\text{mg} / \text{worker}$.

We also observed the Fluon coatings via scanning electron microscopy. The images showed that, at the scale of $10 \,\mu\text{m}$ and $5 \,\mu\text{m}$, gaps begin to appear on the surface when the concentration is lower than 30% (Figs. 3 & 4).

In addition, we also observed the foreleg pretarsi of *Solenopsis invicta* and *Monomorium intrudens* with scanning electron microscopy (Fig. 5) and found that the arolium area of *S. invicta* was approximately $28.7 \,\mu\text{m}^2$

(n = 10), while that of *M. intrudens* was approximately $11.1 \,\mu\text{m}^2$ (n = 10). The weight of an *S. invicta* worker is approximately 4.8 times that of *M. intrudens*.

Discussion

Talc particles adhere to the ant arolium, which makes climbing more difficult for ants (ANYON & al. 2012) by reducing their adhesion. Furthermore, smaller powder particles result in weaker adhesion of the arolium. This effect has little to do with the features of the particles. Ants retract the pretarsal side claw when climbing on the vertical plane (ENDLEIN & FEDERLE 2008), and they use the arolium to provide adhesion strength (ENDLEIN & FEDERLE 2015). Small particles of talc powder that stick to the arolium reduce contact between the arolium and the matrix, and thus talc powder reduces adhesion strength when ants are climbing vertically. In our study, significant differences were observed in the effects of these mixtures on Monomorium intrudens escape prevention. This result verifies that escape prevention is dependent on the fineness of the powder.

The anti-escape effect of talc powder occurs because the talc particles are easily detached, a mechanism that differs from that of Fluon. Fluon, an expensive polymer-based composite, has a very low surface friction

coefficient, which creates a smooth surface to prevent ants from escaping (YUAN & al. 2005). Diluting the Fluon solution can reduce its cost (CHEN & WEI 2007); however, the ability to prevent ants from escaping decreased as the concentration of PTFE decreased, which led to gaps in the Fluon coating. In addition, the escape of Monomorium intrudens from a glass Petri dish or plastic box could be completely prevented when the concentration of talc powder was higher than 1%. In contrast, Fluon could not prevent M. intrudens from escaping (Videos S1, S2; as digital supplementary material to this article, at the journal's web pages). This comparison shows that the talc powder and ethanol mixture can achieve a better anti-escape effect than Fluon while greatly reducing cost. In addition, the application of talc powder onto the inner wall of the plastic box also maintained good adhesion performance. These results further indicate that the talc powder and ethanol mixture could be used for long-term ant colony maintenance, in contrast to spreading talc powder on the inner wall of a container, which is a very limited and short-term method for preventing ant escape (VOGT & KOZLOVAC 2006).

Insects use their antennae to detect and orient to their surroundings (Blaesing & Cruse 2004, Okada & Toh 2006, ANYON & al. 2012), and they make use of this information to make decisions (KEVAN & LANE 1985, CAMHI & JOHNSON 1999, BERNADOU & FOURCASSIE 2008, CROOK & al. 2008, BERNADOU & al. 2009). Ants may also use their antennae to detect the mechanical brittleness of the substrate when crawling in a manner similar to that by which worms assess the size of gaps (BLAESING & CRUSE 2004) and may therefore back away from a powder particle barrier that reduces their adhesion (ANYON & al. 2012). In this experiment, ants were observed to constantly use their antennae to test the talc mixture coating. Furthermore, when the ants were placed in a Petri dish half coated with $2\mu m$ talc particles and half coated with $22\mu m$ talc particles, they were more likely to attempt escape across the $22\,\mu m$ talc coating rather than that with a D97 of $2\,\mu m$ (Video S3).

Our results show that the arolium area of S. invicta is approximately 4.8 times that of Monomorium intrudens. Therefore, Solenopsis invicta requires a stronger grip than M. intrudens when climbing vertically. The arolium area of S. invicta was 2.6 times that of M. intrudens. Because the workers of S. invicta are relatively heavy, the ratio of worker weight to adhesive-pad (arolium) area in S. invicta was approximately twice that in M. intrudens. This result indicates that S. invicta workers have a proportionally reduced arolium area, which may be one of the main reasons that S. invicta is less likely to escape than M. intrudens. In addition, the failure of S. invicta workers to escape from the containers with a talc coating may be related to their larger pretarsi. The electron microscopy results show that the width of the pretarsal claws (approximately 900 µm) was greater than that of the gaps in the talc powder coating (usually below 500 µm). Therefore, S. invicta workers could not effectively avoid stepping on the talc particles,

and they fell when attempting to cross the talc coating. In contrast, the width of the pretarsal claws in *M. intrudens* workers was found to be approximately $300 \,\mu\text{m}$, which may cause these ants to successfully escape from containers with talc coatings having larger intergranular spaces (e.g., D97 values of $13 \,\mu\text{m}$ and $25 \,\mu\text{m}$).

The ant arolium produces mucus to increase its adsorption capacity (Votsch & al. 2002, DRECHSLER & FE-DERLE 2006, DIRKS & al. 2010). The arolium can also maintain a high adsorption capacity when in contact with talc particles (DRECHSLER & FEDERLE 2006). Gaps occurred between talc particles with D97 values of $6 \,\mu$ m and $4 \,\mu$ m, although most gaps measured less than 300 μ m, and some workers of *Monomorium intrudens* could still escape. This result further suggests that *M. intrudens* may enhance their escape ability by secreting fluid from the arolium to increase friction.

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