Wasabi versus red imported fire ants: preliminary test of repellency of microencapsulated allyl isothiocyanate against Solenopsis invicta (Hymenoptera: Formicidae) using bait traps in Taiwan

Wasabi versus red imported fire ants; Preliminary test of repellency of microencapsulated allyl isothiocyanate against *Solenopsis invicta* Buren (Hymenoptera: Formicidae) using bait traps in Taiwan

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Shot title: Repellent effect of microencapsulated AITC against *S. invicta*

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Abstract

Sea container has been identified as a major pathway for the unintended entry and spread of alien ant species. In Japan, red imported fire ants, *Solenopsis invicta* Buren, which are among the most harmful alien ants, were first detected in a shipping container from China in May 2017, and the invasion into Japan via the trade pathway is still continuing. To prevent containers contaminated with *S. invicta* and its establishment in Japan, control measures, such as repellents, are urgently required. The present study is the first to evaluate repellency of microencapsulated allyl isothiocyanate (AITC) against *S. invicta*, as a preliminary step to use the innovative equipment for invasive species management in sea containers. In a field in Taiwan heavily infested with *S. invicta*, a repellent test of microencapsulated AITC using bait traps showed that the equipment completely prevents *S. invicta* from accessing the bait. Due to its volatility and irritancy, AITC, a safe natural repellant in wasabi (*Eutrema japonicum* (Miq.) Kiudz), has not been used for pest management in containerized cargo. However, the encapsulation of AITC solves this problem by allowing controlled vapor release. Microencapsulated AITC has

considerable potential as an effective measure to stop the spread of *S. invicta* through global trade.

Keywords: red imported fire ant, wasabi, allyl isothiocyanate, microcapsules, botanical repellent

INTRODUCTION

Sea container transportation has been identified as a major high-risk pathway for the unintended entry and spread of alien ant species (Bertelsmeier et al. 2018; Inoue and Goka 2009; Ward et al. 2006). International trade has reached unprecedented levels, and much of it is moved with sea containers (The world bank 2017). This situation presents an increasing global risk of incursions of alien ant species (Bertelsmeier et al. 2017).

One of the most harmful of these ants is the red imported fire ant, *Solenopsis invicta* Buren, which poses serious hazards to agriculture, natural environments, and public health (Lowe et al. 2000; Zhang et al. 2007). *S. invicta* has also successfully spread in shipped cargo from its native range in South American to the United States, Australia, New Zealand, China, and Taiwan (Ascunce et al. 2011). It is known that controlling introduced populations of *S. invicta* continuously is costly and its complete eradication is quite difficult. For example, the annual cost of *S. invicta* management is estimated to be about US$6 billion in the United States (Drees and Lard 2006; Gutrich et al. 2007). In Japan, *S. invicta* was discovered in late May 2017 at Amagasaki City and Kobe port,

Hyogo Pref. and the ants have been reported in 37 locations in 14 prefectures as of October 2018. Almost all *S. invicta* found in Japan entered the country in shipping containers imported from southern China. Although fortunately *S. invicta* colonization in Japan has not been confirmed, given this situation, there is an urgent need to minimize the risk of *S. invicta* contamination of containerized cargo.

Insect repellents are one of the major methods for preventing *S. invicta* from infesting stored products and containerized cargo, but the use of synthetic repellents has potential risks due to environmental pollution and health hazards. Especially, chemical treatment may be inappropriate for food, household goods and clothes, due to toxicity. Furthermore, treatment could leave residue in the container itself. In fact, between 10% and 20% of all containers arriving in European ports had harmful concentrations of toxic chemicals (Baur et al. 2015).

Accordingly, there has been an effort to find naturally occurring repellants from plants (Hu et al. 2017). Allyl isothiocyanate (AITC), which is extracted from plants such as wasabi (*Eutrema japonicum* (Miq.) Kiudz), is a well-recognized for strong repellent and activity against various arthropods, nematodes, and microorganisms (Dhingra et al.

2004; Park et al. 2000; Wu et al. 2014; Zanada and Ferris 2003). However, because of its strong volatility and irritancy, AITC has not been used as a repellent for invasive species, such as *S. invicta*, in containerized cargo. Recently, microencapsulation technology and applications of AITC, using spray-drying and polyethylene material, has been established (PATENT No. JP5033232B, WasaP" TM). This technology enables sustained-release of AITC through semi-permeable capsule membranes, which can decrease irritancy by preventing excessive release. Furthermore, the AITC encapsulated in polyethylene composites can be employed as applied as plastic packing-materials, such as plastic wrap, envelope-bags, and cargo cover. Therefore, in the present study, we conducted field studies to evaluate the repellency of AITC against *S. invicta*, using of polyethylene films containing the microencapsulated AITC.

MATERIALS and METHODS

Field studies of the repellency of microencapsulated AITC against *S. invicta* were conducted on October 22, 2018, in a construction site in Banqiao District, New Taipei City, Taiwan (25.034072N, 121.469637E), which was seriously infested with *S.*

invicta (Yang et al. 2009) (Fig.1 A). During the study, field temperatures and relative humidity were 28°C and 65%, which are ideal conditions for *S. invicta* workers to actively forage (Yue 2014).

For test material, we used a polyethylene film with 10 cm × 10 cm size and 0.08 mm thick, containing 6 mg of microencapsulated AITC, which was obtained from PRD Co., Ltd. (Osaka, Japan) (Fig.1 B). The microencapsulated AITC has a humidity-activated release mechanism and its release-rate increases with increasing atmospheric moisture (Ii et al. 2012). Although release kinetics of AITC from the film were not measured in this study, 1 mg of microencapsulated AITC develops a concentration of 250 ppm AITC gas in a 1 L enclosed space at 100% humidity (unpublished data).

To determine whether microencapsulated AITC can repel *S. invicta*, we used a bait-trap with a polyethylene film containing microencapsulated AITC on the inside (10 traps). The trap was a 50-mL centrifuge tube, with a 5-mm opening at the screw cap for *S. invicta* to enter and baited with a piece of oil-fried snack made from corn grits (Fig. 1 C and D). Oily corn grits have been used as one of the most attractive baits for *S. invicta* (Lofgren et al. 1975; Williams et al. 2001). In a control experiment, the same bait-trap

with an ordinary polyethylene film, which did not include microencapsulated AITC, was used (10 traps). Furthermore, to eliminate concerns that the workers do not avoid the AITC but only cannot smell the bait by AITC gas, we placed a bait outside of trap for 10 minutes, and then inserted the bait swarming with *S. invicta* into a centrifuge tube trap with the microencapsulated AITC film (10 traps). The three bait-traps (Non-AITC, AITC and AITC with ants) were placed about 30 cm from one nest-mound of *S. invicta* (total 10 mounds). The number of foraging *S. invicta* workers on the inside of the trap was counted 40 minutes after bait placement.

RESULTS and DISCUSSION

The results of this study showed clearly that polyethylene film containing the microencapsulated AITC can prevent *S. invicta* from reaching the baits (Fig. 2). In bait traps with the ordinary polyethylene film, an average of 157 ± 44.59 individuals of *S. invicta* were collected. In contrast, the average number of *S. invicta* trapping to baits with the microencapsulated AITC films was 0.00 ± 0.00, indicating that the film completely prevented *S. invicta* from reaching the baits. For the traps with baits swarmed by *S. invicta*,

we found 0 to 3 individuals (average 0.9 ± 0.56), all of which were dead. Furthermore, we could observe that foraging *S. invicta* avoided entering the tap with the microencapsulated AICT film. We video-recorded that, when an ant's antennae contacted the entrance hole of trap with the film, workers of *S. invicta* immediately retreated (see Supplementary material for movie).

The present study is the first to evaluate repellent potential of AITC against *S. invicta* and verified that the microencapsulated AITC repels the ants completely. AITC is a natural product considered harmless for human health and environment, and its safety for humans has been demonstrated (European Food Safety Authority 2010). However, due to its strong volatility, the excessive AITC vapor can irritate the human respiratory and eyes. AITC encapsulated in semi-permeable polyethylene composites can control the release rate of vapor, solving this problem. In addition to controlled vapor release, microencapsulated AITC has moisture sensitivity, which increases release rate of AITC accordingly with increasing humidity. Because the regions infested heavily by *S. invicta* in China are located in the humid subtropical zones, the moisture sensitive property of the microencapsulated AITC could be particularly useful as *S. invicta* repellent in

container cargos arriving from such regions. Considering these properties and the results from this study, microencapsulated AITC has the high potential as an extremely effective measure for stopping the spread of *S. invicta* through global trade. For the practical application of the microencapsulated AITC as *S. invicta* repellent, further experiments including the verification of persistence of AITC effect are urgently required in actual containerized cargo.

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CONFLICT OF INTEREST

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Conflict of interest: The authors declare that they have no conflict of interest.

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Figure Legends

Fig. 1 Study site and Bait-trap setting: (A) Repellent experiment site of microencapsulated allyl isothiocyanate (AITC) against red imported fire ants (*S. invicta*) at Banqiao District, New Taipei City, Taiwan. *S. invicta* nests were marked with yellow flags. (B) Polyethylene film containing microencapsulated AITC, and a bait-trap cap with a 5-mm diameter opening for *S. invicta* to enter. (C) Bait traps, made of 50-mL centrifuge tubes, used to test the repellent effect of AITC against *S. invicta*. The left trap with a polyethylene film containing microencapsulated AITC, and the right with an ordinary

polyethylene film. (D) An example of foraging behavior of *S. invicta* on the bait, which was placed at the bottom of a trap tube.

Fig. 2 Effect of microencapsulated allyl isothiocyanate (AITC) on foraging behavior of worker *S. invicta*: Box plots showing numbers of *S. invicta* captured by bait traps with an ordinary polyethylene film (Non-AITC), with a microencapsulated AITC film (AITC), and with a microencapsulated AITC film and bait swarmed by the foraging ants together (AITC with Ants), on the insides (N = 10 traps per treatment). Mean numbers of *S. invicta* captured are labeled on the box.

Supplementary information

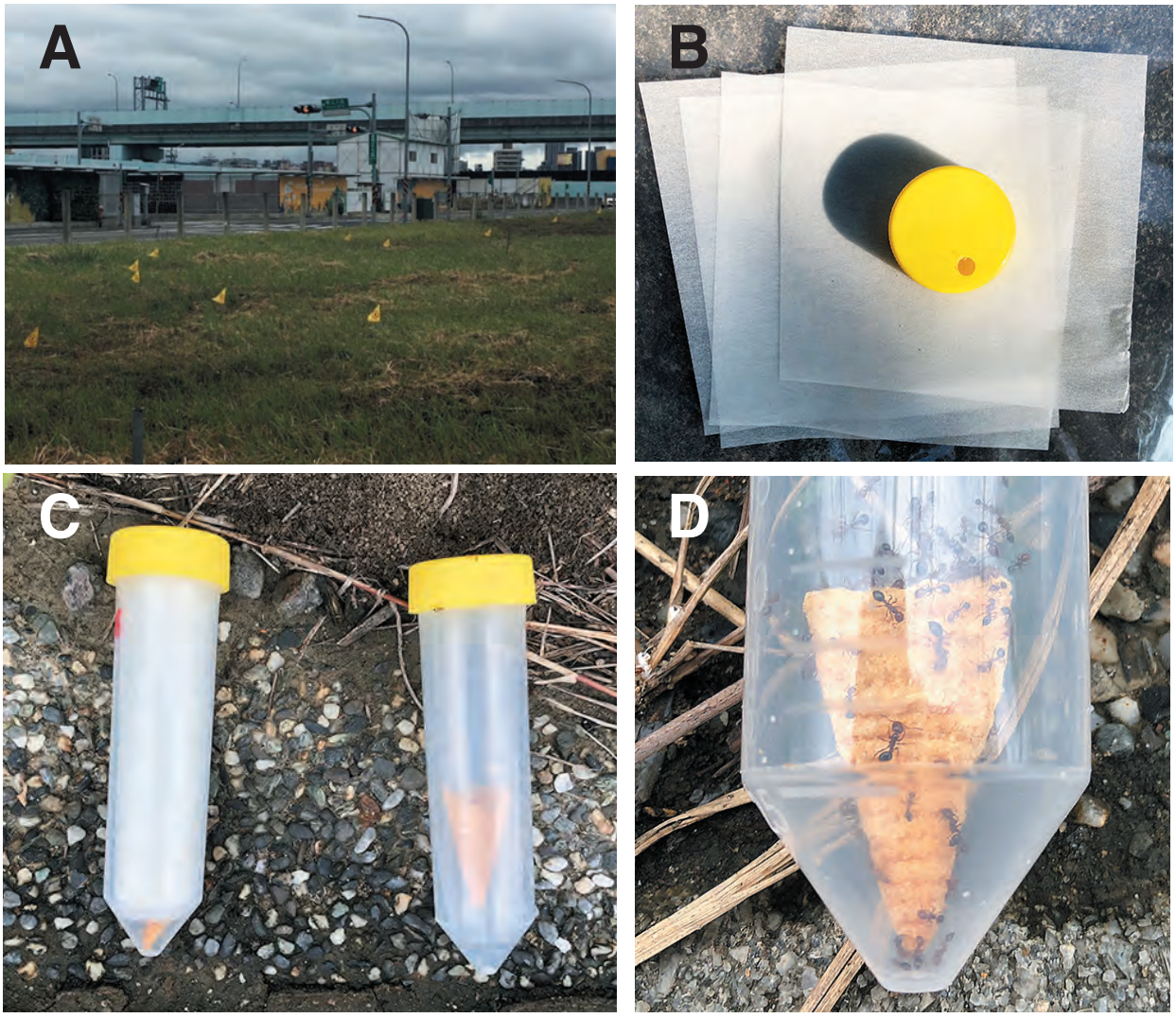
Supplementary move: Repellent behavior of *S. invicta* on bait trap with microencapsulated AITC film.

Supplementary movie from the paper "Wasabi versus red imported fire ants; Preliminary test of repellency of microencapsulated allyl isothiocyanate against *Solenopsis invicta*

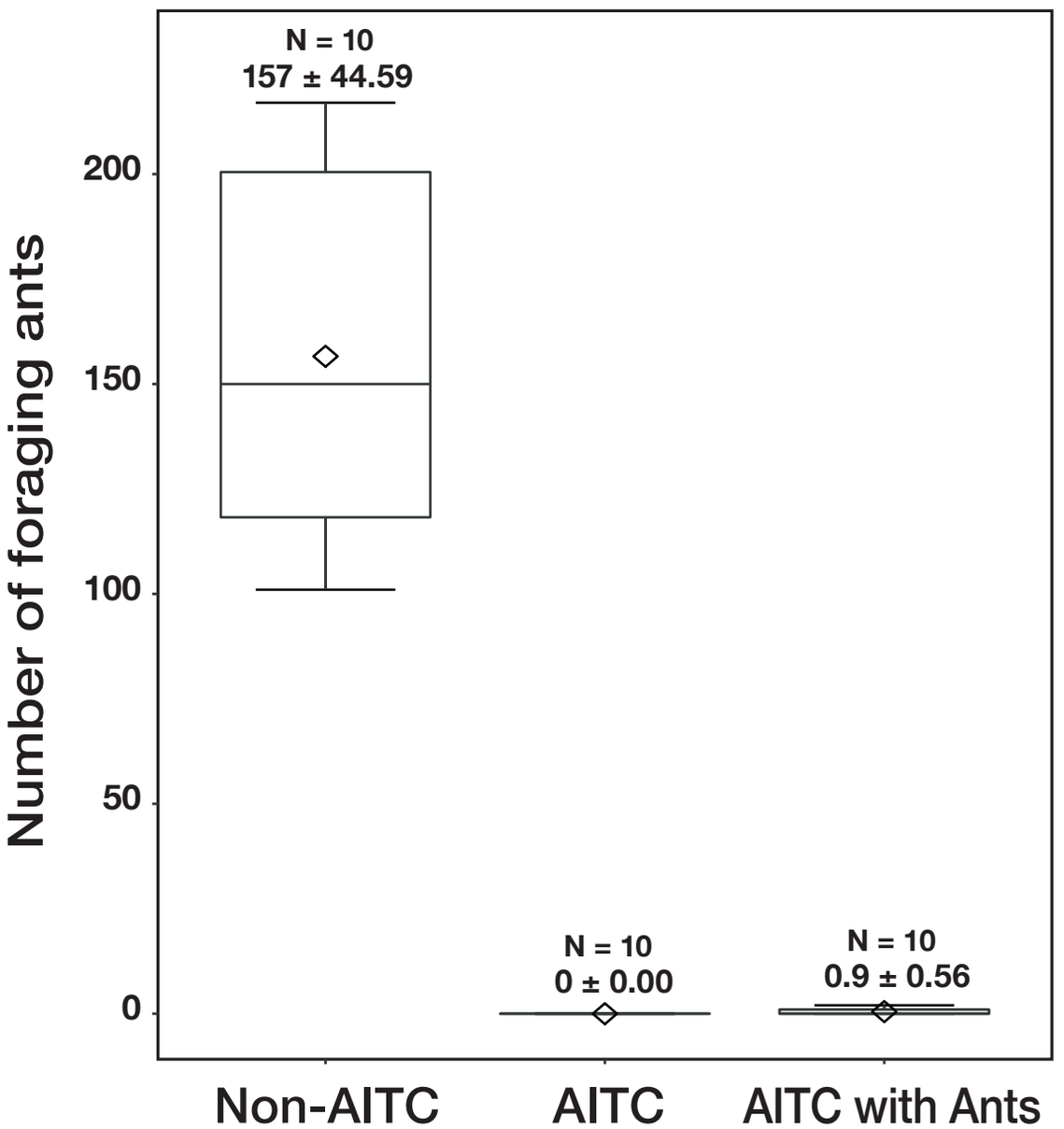
Buren (Hymenoptera: Formicidae) using bait traps in Taiwan" authored by Yoshiaki Hashimoto, Masashi Yoshimura and Rong-Nan Huang, published in XXXXXXX.

Fig1

(Fig. 1)



(Fig. 2)


N = 10
157 ± 44.59
N = 10
0 ± 0.00
N = 10
0.9 ± 0.56
200
150
100
50
0
Number of foraging ants
Non-AITC
AITC
AITC with Ants

Supplementary move