The effect of fumigation with microencapsulated allyl isothiocyanate in a gas-barrier bag against *Solenopsis invicta* (Hymenoptera: Formicidae)

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Abstract

Red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), which are among the most harmful alien ants, were first detected in Japan in a shipping container from China in May 2017, and continue to enter Japan via this trade pathway. Fumigation is one of the most effective ways to eliminate *S. invicta* from goods transported in containers. The present study is the first to evaluate the effect of fumigation with allyl isothiocyanate (AITC) against *S. invicta*. Fumigation using microencapsulated AITC pellets in a gas-barrier bag for 24 h completely killed *S. invicta*. Furthermore, our fumigation system was able to maintain a high concentration of AITC vapor for at least 13 days. AITC, a naturally occurring compound in wasabi [*Eutrema japonicum* (Miq.) Koidz], has been demonstrated to be safe for humans and the environment, but its use as a fumigant is limited by its strong volatility and pungency. However, its encapsulation solves this problem by allowing controlled vapor release. Thus, the use of microencapsulated AITC in combination with a gas-barrier bag has considerable potential to provide an effective and easy-to-use protocol for fumigation of *S. invicta*.

Keywords Red imported fire ant · Wasabi · Allyl isothiocyanate · Fumigant · Invasive alien species

Introduction

Red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is one of the most harmful invasive species and poses serious hazards to agriculture, natural

environments, and public health (Wylie and Janssen-May 2016). In Japan, *S. invicta* was first discovered in a shipping container from China in Amagasaki, Hyogo Prefecture, in May 2017, and the invasion into Japan via this trade pathway is continuing (Ministry of Environment Japan 2020). By the end of January 2020, 48 independent incursions of *S. invicta* had been detected in 15 prefectures of Japan, 28 of which were found in containerized cargo unloaded in ports or delivered to customers. Fortunately, these invasions were successfully intercepted and the ants were eradicated. However, the risk of *S. invicta* establishing in Japan rises with the increase in the invasion pressure associated with containerized cargo traffic (Wylie et al. 2020).

Fumigation is one of the quickest and most effective ways to eliminate pests from goods transported in containers (Rajendran 2004). Synthetic pesticides, such as methyl bromide and phosphine, are the most widely used chemicals to fumigate containerized cargo (Zettler and Arthur 2000). However, *S. invicta* is introduced into Japan with various transported goods, including stationery goods, tableware, blankets, and animal feedstuff (Ministry of Environment Japan 2020). Synthetic fumigants may be inappropriate for such non-mechanical products because of residual toxicity.

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Therefore, there is an urgent need to develop safe alternative fumigants for *S. invicta* to replace synthetic pesticides. Moreover, in Japan, imported goods are rapidly moved from ports of entry and delivered to customers within a few days. For example, in the first incursion of *S. invicta* in Japan, the cargo contaminated with the ants was delivered from Kobe port to a warehouse in an inland city, Amagasaki, within 4 days (Ministry of Environment Japan 2020). Safe use of synthetic fumigants requires special facilities and equipment, such as a vacuum pump and chamber, gas introduction and exhaust system, and protective clothing and gear, which are expensive and not readily available (Rajendran 2004). Therefore, to quickly eliminate *S. invicta* delivered to customers and to prevent its spread into inland Japan, the development of a safer and simpler fumigation method is urgently needed.

Allyl isothiocyanate (AITC) is a naturally occurring compound from plants, such as wasabi [*Eutrema japonicum* (Miq.) Koidz]. The vapor of AITC is well recognized as a strong fumigant for the control of insect pests, and its safety for humans and environment has been demonstrated (Tsao et al. 2002; Worfel et al. 1997; Wu et al. 2009). Especially, unlike synthetic fumigants, AITC is a gas that has a low water solubility and easy degradation in the atmosphere (Weerawatanakorn et al. 2015). Such properties make AITC an environmental and human safety fumigant without pesticide residue concerns (US EPA 2013). However, its use as a fumigant is limited by its strong volatility and pungency. In addition, AITC is usually sold in liquid form and requires the addition of a setting treatment to use it as a fumigant. Recently, microencapsulation technology and applications of AITC using spray-drying and polyethylene have been established (Patent No. JP5033232B). This technology enables the sustained release of AITC through semi-permeable capsule membranes, which can decrease irritancy by preventing the excessive release. Additionally, the microencapsulation can convert liquid AITC into pellet- or sheet-like polyethylene-based material, which allows easy handling of AITC when it is used as a fumigant. Therefore, although the microencapsulated AITC has not been used as a fumigant against *S. invicta*, it has a good potential as a safe and effective solution to sanitize cargo and eradicate the invading pest. Our previous study, which used microencapsulated AITC and bait traps, found that AITC not only completely prevented *S. invicta* from accessing the bait but also killed the workers that failed to escape from the traps (Hashimoto et al. 2019). Therefore, in the present study, we used microencapsulated AITC and a gas-barrier bag in fumigation experiments to evaluate the insecticidal activity of AITC against *S. invicta*. Furthermore, a fumigation only works properly if an adequate concentration can be

maintained for an adequate exposure period. Therefore, we measured the changes in AITC concentration in the bag to estimate the efficacy of our fumigation system. This study may provide a new and useful fumigation method to prevent *S. invicta* invasion to Japan.

Materials and methods

Insect materials

Solenopsis invicta workers were collected from a nest in Tucheng District, New Taipei City, Taiwan (23°24'58" N, 121°26'49.6" E). The ants were kept in a plastic tray containing a piece of damp cotton wool and reared on honey water (ca. 10%) in a room at around 25 °C and 70% RH in Taipei City, Taiwan.

Fumigation materials

As a fumigant, we used 50 g of microencapsulated AITC pellets containing in total 1 g of AITC (the weight of each pellet was 0.01 g, and contained 2% of AITC by weight); the pellets were obtained from PRD Co., Ltd. (Osaka, Japan). A transparent gas-barrier bag (91 cm width × 60 cm length × 40 cm depth, 80 L) used for fumigation consisted of a poly(ethylene terephthalate) film (12 µm thickness) deposited on a film of linear low-density polyethylene (80 µm thickness). The gas-barrier bags were developed by Moriya Industry Co., Ltd. (Osaka, Japan).

Fumigation treatments

For the ease of handling, 10 workers of *S. invicta* were placed in a polypropylene snap-cap vial (50 mL, 3.5 cm diameter, and 7.3 cm depth) with a piece of damp cotton wool. The vial had a cap with a mesh-covered 3 cm diameter opening, which prevented the ants from escaping and allowed air to flow into the inside (Fig. 1a). For fumigation treatment, we placed the vial in a cardboard box (15 cm width × 29 cm length × 13 cm depth) and then put the box in a gas-barrier bag with microencapsulated AITC pellets holding in a tea filter bag (Fig. 1b, c). In the control treatment, the bag contained no pellets. In both treatments, *S. invicta* were removed from the bag after 24 h of exposure, and mortality was determined by observation. Ants were considered dead if they did not respond to probing. They were considered moribund when locomotion was not possible and the movement of appendages was feeble and uncoordinated. We counted the number of moribund ants as alive but confirmed whether they showed any subsequent recovery

Fig. 1 Test device used to evaluate the effect of fumigation with microencapsulated AITC against *S. invicta*. **a** Polypropylene snap-cap vials with a mesh-covered 3-cm diameter opening. Ten workers of *S. invicta* were placed in each of the vial with a piece of damp cotton wool. **b** Cardboard box used for the fumigation test. A polypropylene snap-cap vial containing *S. invicta* workers was put in the box. **c** Gas-barrier bag used in the fumigation test. A microencapsulated AITC pellet (50 g) and a cardboard box with a vial containing *S. invicta* were placed in the bag. **GBB** gas-barrier bag, **MAP** microencapsulated AITC pellet. **d** Covered glass Petri dish used to observe the behavior of an *S. invicta* worker exposed to AITC. **MAS** microencapsulated AITC sheet, **SI** *S. invicta* worker

of activity during the next day. Using four gas-barrier bags, we performed two trials in each of the control and fumigation treatments daily and were repeated for 5 consecutive days (i.e., 10 trails per treatments).

Statistical analysis

For statistical analysis, to compare mortality rates between control and fumigation treatments, we used generalized linear mixed models (GLMM) with binomial distribution and a logistic regression link. To control for potential differences in gas-barrier properties among the bags and conditions of trial days, we included “bag differences” and “trial days” as random intercept effects in the GLMM. Analysis of the GLMM was performed using R package lme4 version 1.1-21 (Bates et al. 2015; R Core Team 2019).

AITC release kinetics in the gas-barrier bag

To evaluate the ability of the gas-barrier bag to maintain AITC vapor, we measured the kinetics of AITC release from the pellets in the bag. As in the fumigation treatment, we put 50 g of the microencapsulated AITC pellets together with a cardboard box containing a piece of damp cotton wool into a gas-barrier bag, but without *S. invicta*. Release kinetics were measured using the colorimetric gas

detector tube (No.149, Gastec Corp., Ayase, Japan; measuring range 5–500 ppm). The detector tubes are graduated glass tubes filled with chemical reagents that produce a color stain when exposed to the target gas. The length of the color change is proportional to the concentration of the gas being tested (Noda et al. 2001). Gas in the bag was pulled out by a gas sampling pump (Ap-1, Komyo Rikagaku Kogyo KK, Japan) and passed through a detector tube with a needle from a hole in the bag. The hole was tightly closed with sealing tape to avoid gas leakage during the experiment. Gas samples were taken at 1, 3, 6, and 24 h and 2, 5, and 13 days. The measurements had three replicates. We assessed AITC release kinetics in a laboratory of PRD Co., Ltd. in Osaka City, Japan, at around 25 °C and 70% RH.

Behavior observation

We observed ant behavior in responses to the vapor. A single worker of *S. invicta* and a microencapsulated AITC sheet (40 mm × 40 mm, 1 mm thick, AITC content 0.01 g) was placed in a covered glass Petri dish (64 mm diameter, 20 mm height) with a piece of damp cotton wool (Fig. 1d). The behavior of the worker within the arena was observed and

video-recorded on a smartphone camera (iPhone XS produced by Apple Inc, USA).

Results and discussion

Microencapsulated AITC pellets showed strong and rapid (after only 24 h exposure) insecticidal activity against *S. invicta* when used as a fumigant in a gas-barrier bag (Fig. 2). The mean mortality rate in the fumigation treatment was 0.89 (95% CI 0.81–0.94), which was significantly higher than the control treatment (mean 0.13, 95% CI 0.08–0.21) (GLMM, $\chi^2 = 83.61$, $df = 1$, $p < 0.001$, Table S1). All survivors in the fumigation treatment were in the moribund state and died within the next day, which indicated that the treatment completely killed *S. invicta*. We also directly observed that a worker of *S. invicta* was quickly paralyzed and knocked down for only 2-h exposure to AITC (see Supplementary Movies 1, 2, 3, and 4). These results indicated that the microencapsulated AITC is a good candidate for an effective fumigant against *S. invicta*. However, we tested the efficiency of AITC only against *S. invicta* workers, not against the queens. A fumigation test indicated that both of the queens and workers of *S. invicta* were effectively controlled by ethyl formate, but queens were more tolerant to the fumigant than the workers (Lee et al. 2019). Therefore, to verify the efficacy of our fumigation method against *S. invicta*, the insecticidal effect of AITC against the queens needs to be further explored.

The evaluation of volatiles released from the microencapsulated AITC pellets (50 g) in a gas-barrier bag showed a quick rise in the concentration within 6 h, and the value reached the range of 234–248 ppm ($n = 3$) at 24 h (Fig. 3). After 5 days, the concentration in the bag reached 500 ppm, which was the maximum that can be measured by the detector tube used, and the value was maintained for at least 13 days. Microencapsulated AITC has a high ability for a sustained release of the vapor through semi-permeable capsule membranes. For example, 5 g of the microencapsulated AITC pellets (AITC content 0.1 g) can maintain 10 ppm of the vapor in a cardboard box (10 cm × 10 cm × 8 cm) for 1 h, without any gas-barrier cover or bag (our unpublished data). Consequently, in combination with a gas-barrier, microencapsulated AITC can control and maintain a high concentration of the vapor for a long period, which is a prerequisite for efficient fumigation. Because our method allowed maintaining a high concentration of AITC vapor, it might be used as an effective and easy fumigation method against *S. invicta*.

Overall, the results show that the fumigation method with microencapsulated AITC and a gas-barrier bag could be a feasible solution for *S. invicta* disinfestation because AITC

Fig. 2 Probabilities of mortality rates predicted by the GLMM for *S. invicta* workers exposed to AITC for 24 h and the control. Vertical lines show 95% confidence intervals. Values shown in the graph are the mean $\pm$ standard error of the mean

has a strong insecticidal effect on the ants and the system is easy to use. In addition, high volatility and degradability of AITC, coupled with moderate release property of its microencapsulation, contributes to the low residue content after fumigation (Weerawatanakorn et al. 2015; Worfel et al. 1997). However, for the practical application of our fumigation method, future research is needed to evaluate the efficacy in scaled-up spatial circumstances and against greater numbers of ants, including queens and workers. Furthermore, more detailed research, such as determination of the median lethal concentration (LC_{50}) for *S. invicta*, is required to determine the recommended dose of microencapsulated AITC in our fumigation system. However, AITC is a naturally occurring chemical considered harmless to human health and has been shown to inhibit the growth of fungi, insects, and bacteria (Cardiet et al. 2012; Meira et al. 2017; Torrijos et al. 2019). Thus, the development of our fumigation system will be significant not only for preventing of *S. invicta* invasion in Japan but also for pest management in museums and the food industry.

Fig. 3 Concentration of AITC released from microencapsulated AITC pellets (50 g in total) in a gas-barrier bag. Symbols indicated each measured value (replicates = 3). The solid line connects the means

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Compliance with ethical standards

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

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