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Advancement on techniques for the separation and maintenance of the red imported fire ant colonies

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Abstract Advancement has recently been made on the techniques for separating and maintaining colonies of red imported fire ants, *Solenopsis invicta* Buren. A new brood rescue method significantly improved the efficiency in separating colony from mound soil. Furthermore, a new method was developed to separate brood from the colony using fire ant repellants. Finally, a cost-effective method was developed to coat containers with diluted Fluon® (AGC Chemicals America, Inc, Moorestown, NJ, USA), an aqueous polytetrafluoroethylene, to prevent housed ants from escaping a container. Usually the original Fluon® solution is directly applied to the wall of the containers. Reduced concentrations of Fluon® were found to be equally effective in preventing ant escape. The use of diluted Fluon® solutions to coat the containers was recommended because of environmental and cost-saving benefits. Application of these new techniques can significantly reduce labor, cost and environmental contamination. This review paper collates all the new techniques in one reference which readers can use as a manual.

Key words brood separation, Fluon® coating, *Solenopsis invicta* Buren
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Introduction

The red imported fire ant, *Solenopsis invicta* Buren is one of the most notorious invasive ant species, due to its significant impact on human health, agriculture, livestock and wildlife. Native to South America, *S. invicta* has invaded many countries and regions, such as the US, Puerto Rico (Buren, 1982) and West Indies (Davis *et al*., 2001). Red imported fire ants have also recently been found in China (Zeng *et al*., 2005), Australia (Nattrass & Vanderwoude, 2001; Solley *et al*., 2002), and New Zealand (Harris, 2001; Pascoe, 2001). Morrison *et al*. (2004) used a dynamic ecophysiological model of colony growth and predicted that large portions of Europe, Asia, Africa, Australia and numerous island nations are at risk for *S. invicta* invasion.

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Collecting and maintaining ant colonies are crucial for research on *S. invicta*. Tremendous effort has been made by numerous researchers to develop efficient measures to collect and maintain fire ant colonies in laboratories (Bishop *et al*., 1980; Khan *et al*., 1967; Markin, 1968; Williams *et al*., 1980). Banks *et al*. (1981) summarized all the developments into a single reference, which was frequently cited by other fire ant researchers.

Since fire ants are mound-building ant species, the mound soil has usually been collected together with ants by shoveling mound soil into a container, such as a bucket (Banks *et al*., 1981). In many research scenarios, ants have to be separated from soil. Markin (1968) described a method to separate ants from the soil, in which soil was dried in a tray and ants were forced to move into artificial nests. The water-drip method developed by Jouvenaz *et al*. (1977) has been more commonly used by ant researchers. In their method, water is slowly dripped into the container from medical intravenous fluid-drip tubing. Water saturates soil and forces ants to move upward to the soil surface and eventually accumulate on the water surface after mound soil is immersed in the water, and ants can then be

transferred to other containers with large spoons or ladles. Chen and Wei (2005) found that significant amounts of brood were left at the bottom of the containers after using this conventional method.

After being separated from the soil, ants have usually been housed in plastic trays. Adult ants can climb up almost all types of containers in the laboratory; therefore, measures must be in place to prevent ants from escaping. A common practice is to use some smooth materials to stop ants from climbing up the sides of the container. Although different materials can be used for this purpose, Fluon® has been a preferable product (Hölldobler & Wilson, 1990; Drees, 2002). Fluon® is an aqueous solution of polytetrafluoroethylene (PTFE). Fluon® AD1, which has been extensively used in my laboratory, contains 59%–62% PTFE (AGC Chemicals America, Inc., Moorestown, NJ, US). The inner wall of the ant-housing containers is usually coated with an aqueous Fluon® using a brush. After the coating has dried, it forms a fine layer of PTFE which effectively prevents ants from climbing up the wall. Typically, an undiluted Fluon® solution has been used to coat the container. Chen and Wei (2007) found that diluted Fluon® was equally effective as undiluted Fluon® in preventing ants from climbing the sides of a container. They recommended the use of diluted Fluon® considering environmental safety and the material cost.

Broods (eggs, larvae and pupae) are frequently needed to be separated from workers for research purposes. Such separation is usually a very labor-intensive process, due to worker intrinsic rescue behavior. Chen and Wei (2005) have developed an easy and quick method to separate brood from workers using ant repellants.

In this review, new technical innovations were collated in one reference. Readers can use this paper as a manual to implement these new techniques in their laboratories.

Colony separation with increased water dripping rate and new brood rescue method

An *S. invicta* colony was usually collected with mound soil in a container, such as a plastic bucket ($\approx$ 19 L). The wall of the bucket was coated with baby powder or corn starch to prevent ants from escaping. Water was then slowly dripped into the bucket. In response to increasing water level and saturation of soil, the ants moved out of the soil and workers rescued the brood by moving them out of the soil. After the soil is immersed in the water, adult ants and brood form an aggregation with queen and brood all floating on the water surface. The ants are then scooped out of the water and placed in a plastic tray. Banks *et al*. (1981) recommended the use of a speed of 20 to 40 drops per minute. This dripping process was time-consuming and often had to run overnight. Chen and Wei (2005) found that a significant amount of brood were left at the bottom of the bucket even with such a slow-drip speed.

Chen and Wei (2005) developed a new method to rescue brood after the dripping process. After the adult ants which floated on the water surface were removed, the soil in the bucket was manually stirred with a stick to cause the brood to float on the water surface. The brood and any debris were then scooped out and placed on a paper towel in the tray with workers. Worker ants separated brood from the debris by moving living brood to their nest and removed the dead into the refuse piles. This brood rescue method could collect 40%–200% more brood than the conventional method.

Since the brood left at the bottom of the container was rescued after the dripping process, the drip speed could be significantly increased to 300 drops per minute. This drip rate still allowed mature ants to move to the soil surface with some of the brood. A colony could be separated in 2–3 h at this increased drip rate versus an overnight process using the method of Banks *et al*. (1981).

The new procedure to separate the ant colony from mound soil which incorporates the new brood rescue method can be summarized in the following seven steps:

1. Shovel mound soil with ants into a bucket. The inner wall of the bucket is coated with baby powder.
2. Drip water into the bucket at the rate of 300 drops per minute.
3. After the soil is immersed in the water, scoop out the ant mass on the water surface into a plastic tray in which an artificial nest was placed at the center.
4. Stir the soil in the bucket with a wooden stick to cause brood to float on the water surface.
5. Scoop out the brood with debris on the water surface onto a paper towel in the plastic tray.
6. Allow worker ants to move living brood to the nest and remove the dead brood to the refuse piles.
7. Clean the debris and refuse piles using methods described by Banks *et al*. (1981).

Extraction of brood from the colony using fire ant repellants

Bank *et al*. (1981) described a method to separate brood from the colony. They used CO_2 to anesthetize the colony and then collected brood piles with a suction device or a spatula. Due to their intrinsic brood rescue behavior, some adult ants were likely included in the initial sample. These adult ants were then separated by spreading the anesthetized ants and brood on a large piece of tissue paper. Before

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workers were active enough to cling to the paper but not enough to exhibit rescue behavior, the brood was gently shaken onto a second piece of tissue. This process had to be repeated several times before most worker ants were separated from the brood. Chen and Wei (2005) found that fire ant repellants such as an over-the-counter essential oil product from China (product name: Essential Embrocation; brand name: Dragon & Tiger; ingredients: menthol, methyl salicylate, camphor, eucalyptus and eugenol; manufacturer: Zhonghua Pharmaceutical Company affiliated to Shanghai Pharmaceutical [Group] Co. Ltd., Shanghai, China) could be used to extract brood from colony. This product is a common insect repellant product in China. The detailed procedure is as follows:

1. The collected colony is separated using the water-drop method and the new brood rescue method.
2. Adult ants transfer all their brood into an artificial nest which may be a Petri dish with a lateral access hole or nest devices described by Banks *et al*. (1981).
3. After the ants with their brood become quiescent in the artificial nest, a small cotton ball treated with a few drops of fire ant repellent is placed at the center of the dish, such as ~50 μL of an over-the-counter essential oil product from China.
4. In response to the repellants, the adult ants will leave the artificial nest and the brood inside the artificial nest are left behind (Fig. 1). The brood are collected and transferred into another container after 30 min.

Fig. 1 The repellants on the cotton ball push adult ants away and leave the brood behind in the Petri dish.

Using diluted Fluon® to coat containers to prevent ant escape

Adult ants can escape almost all types of open containers. The escaped ant colony can be a disaster in the laboratory. Measures must be developed to prevent ant escape. The preferable method is to coat the inner walls of a container with Fluon® (Hölldobler & Wilson, 1990). Fluon® usually refers to Fluon® aqueous dispersion (AD) resin, which contains polytetrafluoroethylene (PTFE) and can be applied to different surfaces through impregnating or coating. PTFE is considered as the most slippery material (http://www.bbc.co.uk/dna/h2g2/A2953). The fume from overheated cookware coated with PTFE was found to be toxic to birds (http://www.ecavianassociation.com/healthsaftey/tef.asp). Release of Fluon® into the environment should be minimized. Fluon® is also an expensive material and efforts should be made to minimize its use in order to reduce the cost of coating. Chen and Wei (2007) found that fire ants were not able to climb up the wall of the plastic trays coated with Fluon® at a concentration as low as 1/128 of the original Fluon® solution for at least 2 weeks (original concentration: 59%−62% polytetrafluoroethylene in water). For aluminum pans, a lower concentration of 1/256 of the original solution effectively stopped the ants from climbing up the coated wall, while for both glass and disposable Petri dishes, concentrations ⩾ 1/16 of the original solution were needed. In general, the 1/32 dilution works well for plastic trays. However, if the colony is maintained for more than a year or the colony is too large, a more concentrated solution may be necessary. The conservative recommendation for dilution of Fluon® is listed in Table 1.

Table 1 Recommended dilutions of Fluon® solution (59%−62% polytetrafluoroethylene) in coating different types of containers to prevent ants from escaping.

Type of container	Recommended dilution	Concentration of PTFE† in the dilution
Plastic tray	1/25	2.36%−2.48%
Petri dish (plastic/glass)	1/16	3.69%−3.86%
Aluminum pan	1/50	1.18%−1.24%

†Polytetrafluoroethylene.

Summary

1. Combination of a higher water-dripping rate with a new brood rescue method can reduce the overall time of colony separation to 2−3 h and rescue from 40% to 200% more brood than the conventional colony separation method.
2. Repellants can be used to extract brood more rapidly from the colony.
3. Diluted Fluon® can be used to effectively prevent *S. invicta* from escaping.

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