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Simplified approaches to determine the attractant preference of *Solenopsis invicta* (Hymenoptera: Formicidae)

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Abstract

The red imported fire ant, *Solenopsis invicta*, is an invasive species that has been introduced into the United States and more recently into several countries in the Asian-Pacific region. The foraging dynamics of this pest ant is important in understanding its interactions with native ant species and in devising the most effective toxic bait control methods. In this study the effect of worker density, size of the foraging area, the number of food lures, duration of experiments, and the effect of the queen on foraging activity were investigated under laboratory conditions. The results showed that of the four ant densities investigated (very small, small, medium and high) the medium ant density most consistently had more workers at the food lures than the other ant density situations. The percentage of worker ants at the food lures was negatively correlated with an increase in foraging territory size for all four worker ant densities. Significantly fewer foragers were observed in the foraging areas when a queen was present, then when the queen was absent. In addition, when number of food lures was increased from 1 to 12, the mean number of workers found at the lures was significantly higher when four food lures were present. Furthermore, the number of worker ants observed on the food lures increased for the first 2.5 h and then decreased with time. The study suggests that observations of foraging activity could be restricted to 2.5 h. These data lay the ground work for further studies in optimizing the effectiveness of fire ant bait toxicants.

Key words: *Solenopsis invicta*; ant density; foraging area; hotdog; foragers

INTRODUCTION

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), has been an invasive pest in the southern United States since its accidental introduction in the 1930s (Williams et al., 2001). Its invasive potential has been demonstrated by its more recent introductions into California in 1997 (Jetter et al., 2002); Australia in 2001 (Henshaw et al., 2005); Taiwan in 2003 (Huang et al., 2004; Chen et al., 2006); Hong Kong and mainland China in 2004 and Mexico in 2005 (Oi and Oi, 2006).

Vogt et al. (2003) found that toxic bait products are the most effective means to control fire ant populations in larger areas. Several types of commercial baits are available around the world with different efficacies. Fire ant baits are typically composed of: 1) a toxicant that must exhibit delayed toxicity (e.g. pyriproxifen, an insect growth regulator), 2) a phagostimulant that also acts as a solvent for the delayed acting toxicant, usually a vegetable oil, e.g. soybean oil, and 3) a carrier for the vegetable oil/toxicant solution that allows distribution of the bait via commercially available spreaders. Fire ant food or bait preferences have been reported by many authors (Howard and Tschinkel, 1980; Glunn et al., 1981; Williams and Whelan, 1992; Hooper-Bui et al., 2002; Furman and Gold, 2006). The size of the foraging area and population of *S. invicta* or other ants used in previous studies varied. Extensive surveillance of infested areas and/or high-risk areas for fire ant invasion and outlier populations is critical for the success of control or eradication programs. The effectiveness of surveillance tools relies on the lure's

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attractiveness; therefore, a standard bioassay system is very important for both bait development and for development of surveillance tools. The objectives of this study were to optimize i) *S. invicta* worker density, ii) foraging area, iii) placement of lures/bait, and iv) data collection duration in order to measure attractant/phagostimulant preference, as well as determine the effect of the colony queen on the feeding activity of *S. invicta* workers.

MATERIALS AND METHODS

Source of *S. invicta*. *Solenopsis invicta* colonies were collected from Hsinchu county (Taiwan), a newly infested area in north Taiwan, separated from soil by a water drip out method (Chen, 2007), and reared under laboratory conditions at least one week before conducting experimental studies (Furman and Gold, 2006; Oi and Oi, 2006). After separation from the soil *S. invicta* colonies were placed into plastic boxes, whose inner sides were painted with Fluon® to prevent escape, and reared under ambient conditions (26±2°C and 55±5% RH). The colonies had water, live tenebrionid beetle larvae, and 30% sugar water available for hydration and food. Ants were starved 2 days prior to experiments to ensure that the ants were hungry (Furman and Gold, 2006).

Foraging area, nest preparation and food lure placement. To determine the appropriate size of the foraging area, circular artificial foraging areas were constructed: 20 cm×4.5 cm (very small); 30 cm×5.5 cm (small); 40 cm×17 cm (medium); 60 cm×17 cm (large); 80 cm×17 cm (very large); and 100 cm×17 cm (extra large) diameter and height, respectively (Fig. 1a). To prevent the ants from escaping from the foraging area, Fluon® was coated from the bottom to the top of the inner vertical surface of the container. An artificial nest was prepared using a Petri dish (6 cm dia.) that had dental plaster (1 cm thick) on the bottom (Fig. 1b). Two holes (3–5 mm dia.) were put in the side of the Petri dish and another hole (3–5 mm dia.) were put in the Petri dish cover. The artificial nest was placed at the center of the circular foraging area. Fresh hotdog (ca. 1 g) food lures were kept on the plastic weighing plate and placed at the four equidistant locations at the inner wall of the circular foraging area (Fig. 1c). The number and placement of the hotdog lures differed depending on the experiment. All experiments were conducted at 27±1°C and 50±3% RH with natural light and dark condition.

Determination of optimal foraging area and ant density. To determine optimal *S. invicta* worker density, four different worker levels were evaluated: 100 (very low density), 300 (low density), 600 (medium density) and 1,000 (high density). Brood (20% of the number of workers) was placed with the workers in the previously described artificial nest. The four worker/brood density situations were each setup in the very small, small, medium, large, very large and extra large sized foraging areas. Hotdog food lures were placed at four equidistant locations at the inner wall of the circular foraging areas. The number of fire ant workers feeding on the hotdog food lures was recorded every 30 min for 390 min. This experiment was replicated four times.

Effects of queen presence on the feeding ac-

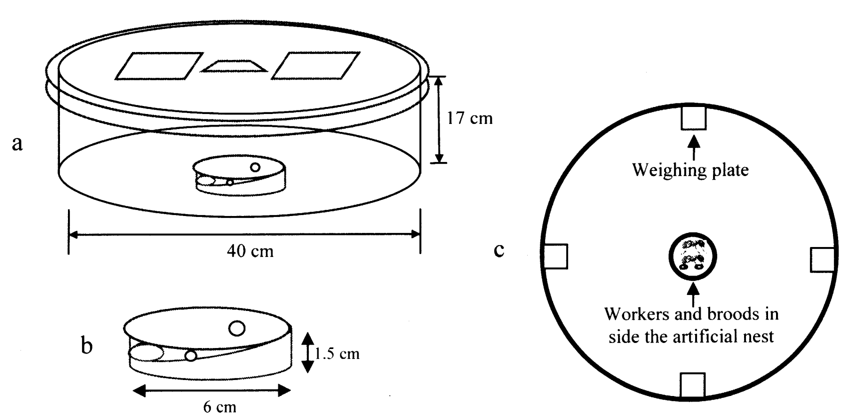

Fig. 1. Medium sized foraging area (a), artificial nest (b) and food lures placement design (c).

tivity of *S. invicta* workers. Based on the above study, the very small, small and medium sized ant density and medium sized foraging area were chosen for further study. To understand the effect of queen presence on the foraging activity of workers, 600 workers, 120 pieces of brood and one queen were placed together in the artificial nest of the very small, small and medium foraging arena. Hotdog (ca. 1 g) food lures were placed at four equidistant locations at the inner wall of the circular foraging areas. The number of fire ant workers visiting the hotdog food lures were recorded every 30 min for 390 min. This study was replicated four times and data were compared with the results from the optimal foraging area and ant density experiment.

Determination of the optimal number of food lures and their placement on the foraging area. To determine the optimal number of food lures in the foraging area 1, 2, 3, 4, 6, 8 and 12 food lures were placed on the medium artificial foraging area at equidistant locations at the inner wall of the circular foraging areas. Six hundred workers+120 pieces of brood (medium density) were placed together in the artificial nest and placed on the foraging area. The number of fire ant workers visiting each food lure was recorded every 30 min for 390 min. This study was replicated four times.

Determination of optimal duration needed to measure the feeding activity of *S. invicta* workers. To determine optimal duration of feeding activity, the *S. invicta* preference study and the foraging activity data from the above studies were grouped for each observation and compared. Means were compared using SNK test (SAS Institute, 2003).

RESULTS

Solenopsis invicta density and foraging area

When the very low ant densities (100 workers+20 brood) were placed in very small, small, medium, large, very large and extra large foraging areas with the food lure, significantly more and significantly less foraging ants were observed ($p=0.0001$, $F=17.12$) in the medium and extra large sized foraging area, respectively, however, the workers in the small, medium and large sized foraging area or workers in very large and extra large foraging areas were not significantly different (Table 1).

Similarly, when low ant densities (300 workers+60 brood) were placed in very small, small, medium, large, very large and extra large foraging areas with the food lure, significantly more and significantly less workers were observed ($p=0.0001$, $F=21.86$) in the medium sized and extra large foraging area, respectively, however, the number of workers in the very small, small, large or very large sized foraging area were not significantly different (Table 1).

In addition, when medium ant density category (600 workers+120 brood) was placed in very small, small, medium, large, very large and extra large foraging areas with the food lure, significantly more and significantly less workers were observed ($p=0.0001$, $F=25.34$) in the medium and very large or extra large foraging area, respectively, however, the number of workers in the very small and large or very large and extra large sized foraging area were not significantly different (Table 1).

Furthermore, when high ant density category (1,000 workers+200 brood) was placed in very

Table 1. Foraging activities of four *S. invicta* densities on the different sized foraging areas

Ant density[a]	Number of ants (mean±SE)[b] in foraging area[c]					
	Very small	Small	Medium	Large	Very large	Extra large
Very low	29.75±2.93 b	35.25±5.60 ab	50.00±8.10 a	44.75±3.75 ab	10.00±1.47 c	3.50±0.50 c
Low	108.00±9.89 b	132.50±10.97 b	170.50±9.61 a	96.75±5.36 b	98.25±12.50 b	40.75±3.47 c
Medium	210.50±14.31 c	291.00±28.04 b	352.00±22.43 a	224.25±11.99 c	136.25±7.52 d	110.50±16.57 d
High	358.75±29.95 bc	409.00±16.47 b	488.75±17.56 a	324.75±12.61 c	228.25±5.76 d	195.50±9.00 d

[a] Very low, 100 workers+20 broods; Low, 300 workers+60 broods; Medium, 600 workers+120 broods; and High, 1,000 workers+200 broods.

[b] Means within the same row followed by the same letter are not significantly different ($p<0.05$) using SNK test.

[c] Very small, 20 cm; Small, 30 cm; Medium, 40 cm; Large, 60 cm; Very large, 80 cm; and Extra large, 100 cm diameter.

small, small, medium, large, very large and extra large foraging areas with the food lure, significantly more and significantly less workers were observed ($p=0.0001$, $F=41.58$) in the medium sized and very large or extra large foraging area, respectively, however, the number of workers in the very small and small or very large and extra large sized foraging area were not significantly different (Table 1).

The numbers of workers on the food lure hotdogs, recorded during this study were changed to the percentage and compared according to the ant densities and foraging areas. The highest percentage (36.0%) of workers foraged on the hotdog food lure when the very small sized foraging area was used with the low worker density, followed by high (35.9%), medium (35.1%) and very low (29.8%) worker densities (Fig. 2a). In addition, when the small foraging area was used, the medium worker density had the highest percentage (48.5%) of workers on the hotdog lures, followed by low (44.2%), high (40.9%) and very low (35.3%) worker densities (Fig. 2a). Furthermore, when the medium sized foraging area was used, the medium worker density had the highest percentage (58.8%) of workers on the hotdog lures followed by low (58.7%), very low (50%) and high worker densities (48.6%) (Fig. 2a). Similarly, when the large sized foraging area was used, the very low worker density had the highest percentage (44.8%) of workers on the hotdog lures followed by medium (36.4%), low (32.8%) and high worker densities (32.5%) (Fig. 2a). Likewise, when the very large sized foraging area was used, the low worker density had the highest percentage (32.3%) of workers on the hotdog lures followed by high (22.8%), medium (22.7%) and very low worker densities (10.0%) (Fig. 2a). Moreover, when the extra large sized foraging area was used, the high worker density had the highest percentage (19.6%) of workers on the hotdog lures followed by medium (18.4%), low (13.6%) and very low worker densities (3.5%) (Fig. 2a).

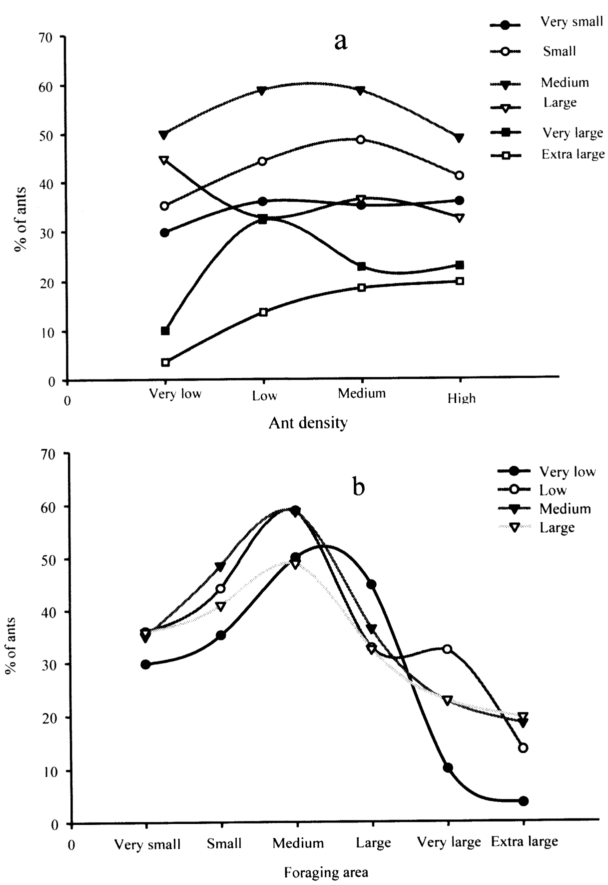

Fig. 2. Relationship between population size of fire ant workers and different sized foraging areas, ant density vs foraging area (a) foraging area vs ant density (b).

When the effects of ant densities and size of foraging areas were analyzed, the results of analysis revealed that ant densities and size of foraging areas, both factors significantly affected foraging activities (Table 2). Similarly, the interaction between two factor densities and foraging areas also significantly affected foraging activities (Table 2).

It is clear from Fig. 2 that regardless of the starting number of workers, very low to high, the percent of workers on the hotdog lures increased as the foraging area increased and reached the maximum at the medium sized foraging area then started to decrease and reached to the minimum at the extra large sized foraging area. The result of the study revealed that the size of the foraging area was negatively correlated with the number of workers at the food lures; however, the degree of decrease differed with worker density (Fig. 2b). Secondly, the most effective starting worker den-

Table 2. Effect of ant density and foraging area on the foraging activities

Source	F	p
Ant density	588.12	<0.0001
Foraging area	91.71	<0.0001
Ant density×Foraging area	11.08	<0.0001

Table 3. Comparison of foraging activity of *S. invicta* workers with and without queen placement conditions

Workers placement	Number of ants (mean±SE)[a] in foraging area[b]		
	Very small	Small	Medium
Without queen	210.50±14.31 a	291.00±28.04 a	352.00±22.43 a
With queen	142.00±23.74 b	197.50±12.33 b	251.25±16.45 b

[a] Means within the same column followed by the same letter are not significantly different ($p<0.05$) using SNK test.
[b] Very small, 20 cm; Small, 30 cm; and Medium, 40 cm diameter.

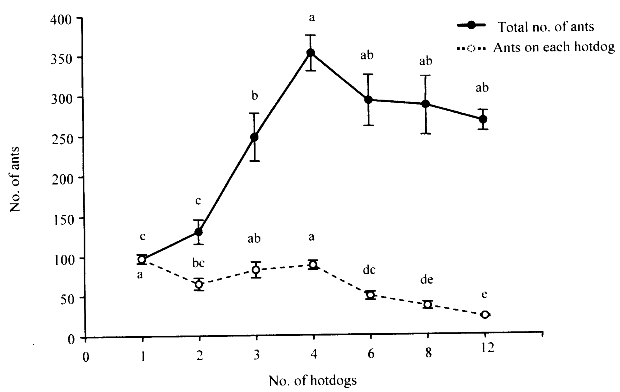

Fig. 3. Comparison of numbers of hotdogs placement on the foraging activity of *S. invicta* workers. The same line followed by the same letter are not significantly different ($p<0.05$) using SNK test.

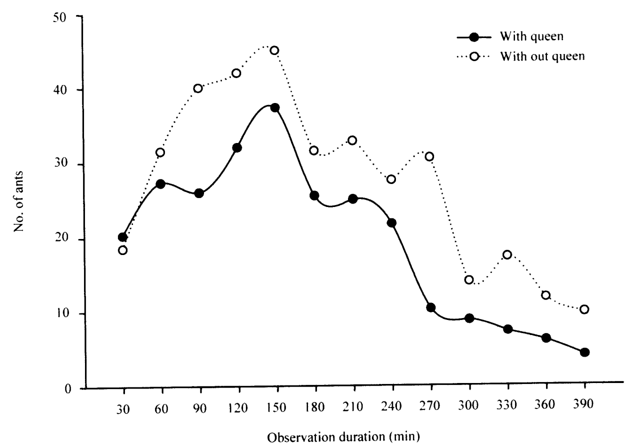

Fig. 4. Comparison of foraging activity of *S. invicta* workers with and without queen placement situations in the medium sized foraging area (40 cm diameter) with medium sized ant density (600 workers+120 broods).

sity was the medium density category, although the low category is almost as good except for the medium sized foraging area.

Effect of queen presence on the foraging activity of *S. invicta*

When the number of ants foraging on hotdog food lures with and without queen colony were compared, significantly higher numbers of workers at very small ($p=0.049$, $F=6.02$), small ($p=0.026$, $F=8.58$) and medium ($p=0.011$, $F=13.12$) sized foraging area respectively at the food lures without queen than at the lures when a queen was present (Table 3).

Optimal number of food lures placed on the foraging area

The study revealed that significantly higher numbers of ants foraged in the foraging area with four hotdog lures than foraging areas with less than four or more than four hotdog lures ($p=0.0001$, $F=14.14$) (Fig. 3). The least number of ants foraged in foraging areas with 1 and 2 hotdog lures (Fig. 3). The number of ants foraging increased as the number of hotdogs increased from 1 to 4 (Fig. 3). However, the number of foraging ants declined as the number of hotdogs were further increased from 6 to 12 (Fig. 3).

Number of ants foraged on the food lure hotdog collected during this study was further analyzed to determine the average number of ants on each hotdog. The study revealed that significantly more ants were observed on each hotdog when 1, 3 or 4 hotdogs were placed in the medium sized ant density in the medium sized foraging area ($p=0.0001$, $F=20.79$) and significantly less ants were observed when 12 hotdogs were placed on the foraging area (Fig. 3).

Optimal duration for the observation of *S. invicta* worker feeding activity

When the foraging trend was analyzed for colonies with and without a queen, the result revealed that, in either case, higher numbers of workers foraged at the 150 min observation time (Fig. 4). The mean number of workers foraging on hotdogs increased from 30 to 120 min for queen absent and queen present situations and the number of foraging ants generally decreased from 240 min onwards, with the fewest foraging ants observed at 390 min (Fig. 4). Out of the total number of foraging ants were observed in food lures, most of the foragers (~90%) were foraged within the 270 and 300 min in with and without queen situation, respectively (Fig. 4).

DISCUSSION

The foraging activity of ants depends upon the foraging territory and colony size (Tschinkel et al., 1995; Adams, 1998, 2003). In this study low and medium ant density colonies were more efficient followed by low ant density. Adams (2003) observed that small colonies more actively searched for food than the larger colony foraging workers. Furthermore, the activity of foragers from smaller ant colonies remained constant even in adverse environmental conditions (Tschinkel, 1993).

Results of this study revealed that in all four ant densities, when the foraging areas were increased, the number of foraging ants were also first increased and then start to decrease. Therefore, a positive relationship between the foraging area and the number of ants foraging on the food lure hotdogs was only observed when foraging areas were increased from very small to medium. However, when foraging areas were further increased, a negative relationship between the foraging area and the number of ants foraging on the hotdogs was clearly observed during the study. In overall observation, a negative relationship between ant's foraging activity and foraging area was observed. In smaller foraging areas, food lures were relatively nearer from the artificial nest than in the larger foraging areas. Therefore, foraging ants could detect food lures faster than in the larger foraging areas. That might be the reason that more forager ants were observed in smaller foraging areas than in the larger foraging areas. Since they could detect food lures faster in smaller foraging areas, they could transport, distribute and store food faster. That strategy was also explained by Heredia and Detrain (2005). The recruitment intensity of ants could also be adjusted according to the nest-source distance and can result in higher number of foragers mobilized to nearer food sources (Taylor, 1977; Heredia and Detrain, 2005). So that ants collected enough food quicker when foraging areas were smaller than from larger foraging areas. Similarly, because of the longer distance at the larger foraging areas, ants needed more time to detect, travel and collect enough food. Therefore, we observed fewer ants foraging on the food lures in larger foraging areas than in the smaller foraging areas. This result was similar with Thomas and Framenau (2005). Thomas and Framenau (2005) observed that the greenhead ant (*Rhytidoponera metallica*) workers from large colonies foraged for longer distances and spent more time outside the nest than foragers from small colonies spend.

The queen placement in the artificial nest affected negatively on the foraging activity of workers (Table 3). During the study, many workers were observed around the queen. In the fire ant colonies, several workers used to constantly take care of queen (Tschinkel, 2006). Therefore, less number of workers went out to forage from the nest with a queen situation than from the without queen situation.

When number of hotdogs were increased from 1 to 12, the mean number of ants attracted on hotdog were significantly higher at 4 hotdogs. When the less than 4 hotdogs were placed, foraging ants might spend more time to search and locate the food lures but when 4 food lures were placed on the 4 equidistant locations at the inner wall of the foraging area, ants could detect those food lures more easily. When hotdogs were further increased from 5 to 12, the foraging ants might be confused to locate the hotdogs that resulted in the disruption of the foraging activity of workers. That was because chemical cues from the different direction could interrupt the ants' normal ability to search and locate the food lures around them. There are some examples of natural behavior disruption of varieties of insects by applying many pheromones to confuse the targeted pest from locating their target (Welter et al., 2005). That might be the reason, that when hotdog numbers were increased from 5 to 12, the number of ants attracted toward them were not increased (Fig. 3).

Because ants adopt time and energy maximization strategies while foraging, numbers of ants foraged to food lures were increased for first 2.5 h and decreased with time (Fig. 4). Another possible reason might be, more ants shared foods and getting enough foods with time, so higher numbers of foragers mobilized for the first few hours of the food lures placement and decreased with time. That was a normal fire ants' foraging behavior because, the recruitment intensity of ants could be adjusted according to the situation (Taylor, 1977; Heredia and Detrain, 2005).

The previous studies by many authors (Howard and Tschinkel, 1980; Williams and Whelan, 1992; Hooper-Bui et al., 2002; Furman and Gold, 2006) used rectangular foraging areas without artificial nest for the food lures or baits preference studies. However, in the rectangular foraging areas, it is not possible to place more than two food lures or baits in equal distance from artificial nest. However, in the circular foraging areas, ants have equal distances from the artificial nest and equal chances to find, travel and bring food lures back to nest and share with nest mates. Therefore, based on the fire ants' preference and easier to set up and handle in the laboratory experienced during this study, the 40 cm diameter sized foraging area with 600 ant density and 120 broods without queen situation with 4 food lures could be considered as an effective fire ant bait, food/lure preference study bioassay set up. The observation duration of the foraging activity of *S. invicta* could minimize from 6.5 h to 2.5 h.

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