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Comparing different methods for trapping mated queens of weaver ants (*Oecophylla longinoda*; Hymenoptera: Formicidae)

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RESEARCH ARTICLE

Comparing different methods for trapping mated queens of weaver ants (*Oecophylla longinoda*; Hymenoptera: Formicidae)

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The predatory efficiency of African weaver ants *Oecophylla longinoda* and their utilisation in protein production is a function of ant abundance. Reliable control of insect pests in tropical crops is achieved when ant populations are constantly high. Transplanted populations of weaver ant colonies containing egg-laying queens are more stable than those without. Achieving such stability through collection of colonies established in the wild is usually difficult because of uncertainty in locating the nest containing the egg-laying queen. In this study, we investigated four methods that may be used to collect mated queens that subsequently can be used to stock ant nurseries. The catch efficiencies of (1) leaf traps, (2) paper traps (both types providing a refuge for founding queens), (3) random search for queens and (4) light trapping were compared. Light trapping was the most efficient way to collect queens followed by leaf traps, random search and, last, paper traps. Light trapping and random search, though, required the presence of a person throughout the ant's mating season (several months), whereas this was not required when using leaf and paper traps.

Keywords: *Oecophylla longinoda*; trapping; mated queen

1. Introduction

Weaver ants (*Oecophylla* spp. Latreille) live in trees and shrubs, where they nest in fresh plant leaves (Crozier, Newey, Schluns, & Robson, 2009). Being generalist predators, *Oecophylla* spp. can significantly reduce the abundance of several insect pests of tropical crops (Adandonon, Vayssières, Sinzogan, & Van Mele, 2009; Peng & Christian, 2004; Peng, Christian, & Gibb, 1995; Peng, Christian, & Reilly, 2011; Way & Khoo, 1989). Furthermore, *Oecophylla* ants are targeted as mini livestock for sustainable protein production, as they are used as food by humans and animals (Cèsard, 2004; FAO report, 2013; Offenberg, 2011; Sribandit, Wiwatwitaya, Suksard, & Offenberg, 2008; Van Itterbeeck, Sivongxay, Praxaysombath, & Van Huis, 2014). In both cases, a stable population of weaver ants is needed, and therefore, augmentation and introduction are often practised where weaver ants exist in low numbers or are completely absent. Transplanted colonies containing an egg-laying queen are more stable than those without queens (Renkang Peng, person

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communication, August, 2011). Hence, identification of the nest harbouring the egg-laying queen is usually an important component of augmentation or introductions to new areas. Each colony has one egg-laying queen but may consist of several hundred nests and cover a territory of up to one hectare (Crozier et al., 2009). This makes it difficult to locate nests with a mother queen.

Established weaver ant colonies produce thousands of winged queens and males each year, which during the rainy season leave their colonies for mating (Peeters & Andersen, 1989; Peng, Mogens, Offenberg, & Birkmose, 2013; Vanderplank, 1960; Van Mele & Vaysseres, 2007). According to Vanderplank (1960), the newly mated queens alight on plants, shed their wings and seek a suitable refuge and establish their colony. This can be on folded leaves or overlapping leaves of ant-free trees (Peng et al., 2013). Due to the difficulties in obtaining refuge, more than 99% of queens die within the first six months of initial colony establishment (Vanderplank, 1960). Consequently, developing efficient trapping techniques for mated queens and protecting them during colony establishment is important for practical use of *Oecophylla* spp. in biocontrol, and the captured mated queens could eventually be reared in nurseries in screen houses before being used in the field for pest management or protein production for human consumption.

Several insect trapping methods exist, some of which are applicable to mated weaver ant queens. For example, mated queens of *Oecophylla smaragdina* can be collected from artificial nests made of leaves (Peng et al., 2013). Sexualls of fire ants (*Solenopsis invicta* Buren) can be trapped using an airplane equipped with two nets (Marking, Diller, Hill, Blum, & Hermann, 1971) or nylon tulle netting (Gary, Ann, Robert, & Vander, 2011). Queens of *Curvispinosus* spp. are known to seek light after mating flights that occurred the previous night (Kennedy, 1948).

Due to difficulty in locating egg-laying queens in existing colonies, as well as securing refuge for newly mated queens, the need for developing methods for the collection of mated queens of *Oecophylla longinoda* after nuptial flights is imperative. Trapped mated queens can be used to stock ant nurseries and to spare users from the hustle of collecting them in the wilderness, where the certainty of getting an egg-laying queen usually is meagre. In the current study, we tested different methods that may be used to trap mated queens of weaver ants, thereby reducing their mortality after their nuptial flights. Four methods inspired by preliminary observations of the settling behaviour displayed by *O. smaragdina* in Thailand (Joachim Offenberg; unpublished data) were tested: the use of (1) rolled leaves where queens can seek refuge, (2) paper tubes where queens can seek refuge, (3) random searching for queens during the mating season and (4) light to attract queens after their nuptial flight. Here, we report on the effectiveness of the tested techniques.

2. Materials and methods

This study was conducted in a citrus orchard in the Tanga region of eastern Tanzania (S 06°47'12.3", E 37°39'01.7", 501 m.a.s.l.) from 2011 to 2013 and in a citrus plantation in the Morogoro region in eastern central Tanzania (S 06°53'27.6", E 037°36'44.5", 516 m.a.s.l.) from 2012 to 2014. Both regions are under a bimodal rainfall patterns with a long rainy season from mid-March to May and a short rainy season from November to December. The average rainfall is 1200 mm and 650 mm

for the two seasons, respectively, in the Tanga region, and 600 mm and 1800 mm for the two seasons, respectively, in the Morogoro region.

2.1. Queen trapping in Tanga

In an area of approximately one hectare of citrus inhabited by 15 weaver ant colonies, 20 citrus trees without resident weaver ant colonies or other ant species were randomly identified in the plantation. These trees were protected against established ant colonies via application of a sticky barrier around the tree trunks in order to avoid ant predation on founding weaver ant queens. On these trees, three different queen trapping methods were tested: (1) leaf traps, (2) paper traps and (3) hand collection of queens via random search. Leaf traps were made by rolling up living leaves and tying the rolled leaf with a rubber band at the middle. This produced a leaf tube open in both ends, where queens could enter to seek shelter and protection against predators. Paper traps were made of brown hard paper (Manila type) cut into small pieces (6 cm × 5 cm) and rolled to produce a tube of 2 cm in diameter. The tube was covered with nylon adhesive tape to prevent it from rolling up and to protect it against rain. As with the leaf traps, both sides were left open to allow water to pass through and to allow queens to enter from two directions. Fifty traps of each type were mounted on every tree with 10 traps in each of five directions. Thus, a total of 1000 traps of each type were used, with 100 traps per tree. In both years of this study, traps were mounted on trees in early January. After that, all traps were inspected to collect queens at the mid and at the end of each month from mid-January to the end of August and, additionally, during the dry season whenever intermittent showers occurred (as queens are known to disperse in association with rainy weather). During inspections, which took place between 8 am and 11 am, all queens hiding in the traps were collected, and the type and direction of the occupied traps were registered. During each inspection of traps, each of the 20 trees were also searched randomly to find naturally settled queens and queens still searching for a nesting site, i.e. queens found outside the traps (hereafter called search & catch). The time used to prepare and inspect the traps and the time spent on search & catch were recorded and held against the number of queens collected via each method. Two months after the mounting of the traps, the fate of each trap (intact, damaged or fallen from the tree) was observed to determine their durability. At the same time, all lost or damaged nests were replaced by new ones, and traps occupied by other arthropods (often spiders) were cleaned.

2.2. Queen trapping in Morogoro

A similar experiment was conducted in a citrus plantation in Morogoro in an area of approximately one hectare inhabited by 20 weaver ant colonies. In this case, however, paper traps were replaced by light trapping, as the initial data from Tanga showed low efficiency of the paper traps (see results) and since it was observed that founding queens dispersed from their maternal colonies at sunset and were attracted to light sources (Rosalia Rwegasira and Halfan Nene, unpublished data). Three trapping methods were compared: (1) leaf traps, (2) light trapping and (3) search & catch. Leaf trapping and search & catch were conducted as in Tanga, except that 100 leaf traps were mounted in each of 16 citrus trees, totalling 1600 leaf traps, and that traps were inspected once a week and on each day following a night where a nuptial

flight was observed. Traps were mounted on trees in mid-November in both years. Light trapping was conducted by placing a 60 watt light bulb near the middle of the plantation. The light was only switched on at sunset and only on nights where nuptial flights were observed (in 2012/2013, 10 mating flights were observed while only 3 were recorded in 2013/2014). On those nights, all queens attracted by the light bulb were collected by hand between 7 pm and 9 pm. As the queens mate during their nuptial flight, which takes place at sunset shortly before the light trap was turned on, we determined whether the queens attracted to the light had mated before being trapped. To test this, 22 of the queens collected in this way were randomly selected and allowed to rear brood in a screen house. Queens that were able to produce viable eggs (developing into larvae and older brood stages) were considered mated, as eggs produced by unmated weaver ant queens do not hatch (Joachim Offenberg, unpublished data). As in Tanga, the number of queens caught by each method was divided by the time spent collecting them, to compare each method's efficiency.

2.3. Data analysis

At each site and for each season, the average number of queens collected per tree by each method (except light trapping) was calculated and compared with Wilcoxon/Kruskal–Wallis tests performed in JMP 10. Also, the number of intact nests per tree after the two-month period was compared between leaf and paper nests with a Wilcoxon test. A *G*-test was performed to test if the queens collected in the traps showed any preference for cardinal direction. Based on the time expenditures described above, the average cost of obtaining one queen via the different methods was found by using a casual labour salary of 0.39 USD/h (=3.12 USD/day).

3. Results

3.1. Tanga

Mated queens were collected from the end of March throughout June in 2012, and from early April to the end of July in 2013 (Figure 1). No queens were collected during rain events in the dry season. A total of 34 and 27 queens were collected in Tanga in the first and second mating season, respectively. Of the 34 queens collected in first season, 73% were from leaf traps, 24% from paper traps and 3% from search & catch. A similar trend was observed in the second season, where 80% came from leaf traps, 16% from paper traps and only 4% from search & catch. The highest numbers of queens collected on one day was 17 in the leaf traps, 3 by paper traps and 1 by search & catch (Figure 1). In 2012, the seasonal average number of queens ($\pm$ SE) collected per tree was 1.2 ($\pm$ 0.32) in the leaf traps, 0.35 ($\pm$ 0.13) in the paper traps and 0.1 ($\pm$ 0.07) from the search & catch method. In 2013, the corresponding figures were 0.85 ($\pm$ 0.33), 0.45 ($\pm$ 0.20) and 0.05 ($\pm$ 0.05). In 2012, averaged over the season, the leaf trap method caught significantly more queens per tree than the two other methods, which were not significantly different from each other, whereas, in 2013, the leaf and paper traps were statistically equally efficient and significantly more efficient than the search & catch method (Table 1).

Figure 1. Number of queens caught by date using different techniques in Tanga during the ants' mating season in 2012 and 2013. Only dates where at least one queen was collected are shown; no queens were found during the dry season (after rainfall events).

The queens did not show any preference for the cardinal orientation of the traps they occupied ($G = 1.71$, $P = 0.19$). Thus, it does not seem to be important on what side of a tree the traps are positioned.

After two months in the field, paper traps were more durable than the leaf traps ($P < 0.0001$, $n = 20$). On average, there were $47.2 (\pm 1.58)$ intact paper traps per tree, whereas there were only $29.0 (\pm 1.58)$ leaf traps. Thus, 94% of the paper traps were functioning after two months compared to only 58% of the leaf traps (Table 3). The proportion of damaged traps was 0.6% and 13.2% for papers and leaf traps, respectively, while the corresponding proportions of traps fallen to the ground were 5% and 27%.

3.2. Morogoro

In the first and second season, 173 and 236 queens were collected, respectively. Of the 173 queens collected in 2012/2013, 48% were from light traps, 33% from leaf traps and 19% from search & catch (Figure 2A). In the second season, 91% of the collected queens were caught from the light trap, 8% from leaf traps and 1% by search & catch (Figure 2B). The highest number of queens collected in one day was 161 by the use of light and only 11 by leaf traps and 7 by search & catch (Figure 2). Similar to the results from Tanga, more queens were collected per tree from leaf traps than by search & catch ($P = 0.016$, $n = 16$ and $P = 0.017$, $n = 16$, in the first and second season, respectively). The mean number of queens collected in leaf traps and via the search & catch method in the first season was $1.89 (\pm 0.62)$ and

Table 1. Pairwise statistical comparisons of the seasonal trap efficiency (number of occupied traps per tree) of leaf and paper traps in Tanga.

Trap types	P-value (2012)	P-value (2013)
Search & catch versus paper traps	0.12	0.21
Paper traps versus leaf traps	0.001	0.09
Search & catch versus leaf traps	<0.0001	0.009

Figure 2. Number of queens caught by date using different trapping techniques in Morogoro during the ants' mating season in (A) 2012/2013 and (B) 2013/2014. Samplings were conducted weekly and whenever nuptial flights were observed (see methods). Only dates where at least one queen was collected are shown.

0.69 (± 0.35), respectively. In the following season, the corresponding figures were 1.19 (± 0.39) and 0.19 (± 0.14).

Of the 22 queens tested for queen fertility, 68% laid eggs that developed into either imagines or pupae, whereas 18% laid eggs that did not hatch and 14% did not lay eggs at all. Thus, a minimum of 68% of the queens caught at the light trap had mated before being caught via this method.

3.3. Time/cost efficiency

Time expenditure and related costs for the collection of queens by the various methods are summarised in Table 2. Based on the overall average, it took less than 5 minutes to collect a queen when using the light trap. Second most efficient were leaf nests, where an average investment of 0.8 h was needed to collect a queen, followed by a 2 h investment when searching randomly for queens. The time needed to prepare the paper traps combined with a low catch rate led to a high time investment per collected queen, as, in this case, almost 7 h were needed per caught queen. Thus, light was by far the most efficient method followed by the use of leaf nests.

Table 2. Time expenditures and costs of catching mated queens using different trapping techniques at the Tanga and Morogoro sites.

Location	Year	Trapping method	Total time spend (h)	Number of queens caught	Average time spend per queen (h)	Cost per queen (USD)
Tanga	2012	Leaf traps	25.2	25	1.0	0.4
		Paper traps	41.0	8	5.1	2.0
		Search & catch	10	1	10	3.9
	2013	Leaf traps	26.2	20	1.3	0.5
		Paper traps	42.3	4	10.6	4.1
		Search & catch	10	1	10	3.9
Morogoro	2013	Leaf traps	11	25	0.4	0.2
		Light trap	8	97	0.1	0.0
		Search & catch	12.8	11	1.3	0.5
	2014	Leaf traps	9.6	20	0.5	0.2
		Light trap	9	209	0.0	0.0
		Search & catch	1.4	4	0.9	0.4
Overall		Leaf traps	72.1	90	0.8	0.3
		Paper traps	83.3	12	6.9	2.7
		Search & catch	34.2	17	2.0	0.8
		Light trap	17.0	306	0.1	0.0

Note: A salary of 0.39 USD/h was used to convert time investments into costs.

4. Discussions

4.1. Efficiency of trapping methods

In the Tanga experiment, leaf traps were the best method for trapping mated queens followed by paper traps, and the number of mated queens collected by the search & catch method was very low in both years. This confirmed Vanderplank's (1960) proposition that, due to the difficulty in obtaining refuge, more than 99% of the mated queens of *Oecophylla* spp. die during the first six months of colony establishment. Shortage of refuge is one of the major mortality factors of mated queens. As they search for a safe refuge, *O. longinoda* may be attacked by birds, lizards, spiders and other species of ants, e.g. *Pheidole megacephala*. Similar observations were reported by Peng et al. (2013) on *O. smaragdina*. Many mated queens of *O. smaragdina* were collected from artificial leaf nests at Darwin in Australia after nuptial flights (Peng et al., 2013) and, unlike previous experience with a search & catch method, the use of artificial leaf nests increased the numbers of collected mated queens by 97%. Likewise, in the absence of artificial nests, the lives of mated queens were in danger.

The results from the Morogoro experiment indicated a great potential of saving the lives of mated queens if light traps were used. Collection of mated queens using light has never been tested with *Oecophylla* species before but has been reported for different species of ants, e.g. *Curvispinosus* spp. Kennedy (1948). Since the mating flight of *O. longinoda* occurs during sunset, there is great potential for collecting mated queens using light traps. Such an approach may be less appropriate for *O. smaragdina*, whose flights occur at sunrise, though a smaller fraction of queens are still looking for shelter in the evening and are attracted to light (Joachim

Table 3. The fate of traps used in Tanga two months after their mounting on the trees. Intact traps were functioning, undamaged traps and still mounted on trees.

Tree number	Intact traps		Damaged traps		Dropped traps	
	Paper traps	Leaf traps	Paper traps	Leaf traps	Paper traps	Leaf traps
Average no. of traps per tree ± SE	47.20 ± 0.55	28.95 ± 2.83	0.3 ± 0.18	6.6 ± 1.72	2.5 ± 0.57	13.45 ± 2.35
Percentage	94.4	57.9	0.6	13.2	5	26.9

Note: Damaged traps were non-functioning traps present on the trees, and dropped traps were traps that had disappeared from the trees.

Offenberg; unpublished data). Our results further show that the majority of the queens attracted to the light had already mated before 7 pm, as almost 70% of the queens collected at the light trap were able to produce viable offspring. During this study, no queens were observed around the light on days without mating flights. Thus, the light trapping technique requires a continued presence of an ant queen collector during the entire mating season. Light traps were not only the best method for large-scale collection of mated queens but also the most time and cost efficient method. Light trapping is time efficient, as it requires almost no preparation compared to preparing and mounting the leaf and paper traps. One challenge with light trapping was that it required a collector to be present and ready to collect queens whenever mating flights occurred. In contrast, the artificial nests did not require permanent attention during the mating season, as the traps could be checked whenever it is convenient. Thus, light traps can only be used in places that are reachable during the night-time and where electric current is accessible, but in places far from an electricity source and at less reachable places, the leaf trap method becomes the best alternative. Alternatively, both methods, light and leaf trap, could be used in the same place to optimise collections.

Having two different seasons in Tanzania is considered an added advantage, as it increases the yearly chances of collecting mated queens. In Morogoro, the mated queens were collected during the short rainy season, while in Tanga, they were collected during the long rainy season. Other studies (Peeters & Andersen, 1989; Peng et al., 2013; Rwegasira, Mwatawala, Rwegasira, & Offenberg, in press; Vanderplank, 1960; Van Mele & Vaysseres, 2007) also support that the collection of newly mated *Oecophylla* queens is restricted to rainy seasons.

4.2. Trap persistence

Although the preparation of paper traps was time consuming, these traps lasted longer in the field. Paper traps did not easily fall off the trees because they were tied up with a nylon adhesive tape on small branches, while leaf traps fell off easily due to senescence. Thus, paper traps may be used in places where the mating season of weaver ants is not well known, and more persistent traps therefore are desirable. In that case, high numbers of traps should be applied, as occupancy by other arthropods builds up over time, leaving fewer and fewer traps available for the ant queens. A higher trap density may also compensate for the relatively low catch rate

of the paper traps. Conversely, leaf nests could be used in places where the mating flight season is well known.

4.3. Trap orientation preference

The cardinal orientation of traps on the trees did not affect their catch rate, suggesting that it is of no importance where traps are placed in trap trees. Probably, queens are stressed to find safe shelter as fast as possible. Therefore, the first suitable shelter they find during their search will be accepted, regardless of its orientation.

In conclusion, artificial traps increased the number of mated queens collected. Light traps and leaf traps proved to be the most efficient methods of collecting mated queens because of less time investment. In Tanzania, the probability that a mated *O. longinoda* queen will find a safe refuge and subsequently survive to establish a new colony seems to be very low due to the presence of many competitors and predators, e.g. *P. megacephala*, birds, lizards and *O. longinoda* workers from other colonies (Peng et al., 2013). Therefore, trapping and artificial culturing of mated queens will inevitably improve their survival. The use of light in collecting mated queens may be optimised by developing a light-embedded device or facility, which will allow the entry of mated queens but not their exit, thereby avoiding the need for the presence of a person at the time of collection. Paper traps would make the best method of collection in places where mating flight is not well known, because of their longer persistence in the field.

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