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Sampling High-Altitude and Stratified Mating Flights of Red Imported Fire Ant

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ABSTRACT With the exception of an airplane equipped with nets, no method has been developed that successfully samples red imported fire ant, *Solenopsis invicta* Buren, sexuals in mating/dispersal flights throughout their potential altitudinal trajectories. We developed and tested a method for sampling queens and males during mating flights at altitudinal intervals reaching as high as ≈140 m. Our trapping system uses an electric winch and a 1.2-m spindle bolted to a swiveling platform. The winch dispenses up to 183 m of Kevlar-core, nylon rope and the spindle stores 10 panels (0.9 by 4.6 m each) of nylon tulle impregnated with Tangle-Trap. The panels can be attached to the rope at various intervals and hoisted into the air by using a 3-m-diameter, helium-filled balloon. Raising or lowering all 10 panels takes ≈15–20 min. This trap also should be useful for altitudinal sampling of other insects of medical importance.

KEY WORDS *Solenopsis invicta*, mating flights, aerial trapping, dispersal

The red imported fire ant, *Solenopsis invicta* Buren, is ubiquitous in the southern and southeastern United States (Glancey et al. 1987; Porter et al. 1991, 1992) and one of the most encountered insects of medical importance throughout its distribution (Williams et al. (2003). Every year an estimated 30–60% of people are stung by the red imported fire ant in infested areas and ≈1% experience anaphylaxis (deShazo et al. 1990, 1999; deShazo and Williams 1995). Williams et al. (2003) estimated that >200,000 persons per yr seek medical attention for fire ant stings.

The invasion success of this fire ant has been attributed to its weedy characteristics (Tschinkel 1986), particularly dispersal and reproductive potential (e.g., 462,000 female and male sexuals per ha per yr; Morrill 1974). Red imported fire ant mating occurs during mating flights that can occur any day of the year provided favorable meteorological conditions are met (see review by Tschinkel 2006). Although the red imported fire ant is one of the most intensively studied ant species in the world, the spatial distribution and dynamics of mating flights, and subsequent dispersal of sexuals is poorly understood. Arguably, the most important reason for the absence of these data are the high altitude of the mating flights (Markin et al. 1971) and the lack of a sampling technique that is cost effective and efficient at capturing sexuals in flight. The only study that examined mating swarms of the red imported fire ant in situ was by Markin et al. (1971). Using an airplane equipped with two nets, Markin et al. (1971) reported trapping sexuals primarily at heights of ≈61–107 m above ground.

Since the study by Markin et al. (1971), the red imported fire ant has been shown to consist of two social forms, monogyne (single queen) and polygyne (multiple queen) colonies (Glancey et al. 1973), differing in several important physiological and behavioral characters. Our present understanding of the mating dynamics and subsequent dispersal of both social forms has therefore been primarily through indirect methods using population genetics tools and laboratory studies, and by sampling preflight and postflight individuals (DeHeer et al. 1999, Goodisman et al. 2000, Shoemaker et al. 2006, Burns et al. 2007). Several studies, for example, suggest the dispersal potential of queens may be affected by their genotype at the *Gp-9* gene, which correlates with fat-body reserves (Keller and Ross 1993, 1999; DeHeer et al. 1999; Krieger and Ross 2002). Adequate fat body reserves of newly mated queens (NMQs) is crucial to successful colony foundation, because red imported fire ant NMQs found colonies claustrally, and the number of first workers produced is critical and dependent on fat reserves (Markin et al. 1972, DeHeer et al. 1999, DeHeer 2002, Goodisman et al. 2007). Population genetics studies indicate there is unidirectional gene flow from monogyne colonies to polygyne colonies (Shoemaker and Ross 1996, Mescher et al. 2003, Shoemaker et al. 2006) through the males of the former, although polygyne queens seem to mate disproportionately with males of their own social form (Fritz et al. 2006; G.N.F., unpublished data).

We describe the first stationary system that successfully traps large samples of red imported fire ant alates simultaneously throughout a broad range of

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elevations and will enable future studies aimed at resolving the mating dynamics of both social forms. Because previous studies on insect dispersal and migration patterns have commonly used marking (e.g., using fluorescent dyes to label individuals) or capture of individuals in various kinds of traps placed at limited altitudes (e.g., wind socks or ship masts, Yoshimoto and Gressitt 1964), our trapping system also should be useful for capturing other insects of medical importance that may disperse at relatively at high altitudes.

Materials and Methods

The trapping system we developed uses a helium-filled balloon attached to an electric winch, capable of dispensing up to 183 m of nylon rope. Multiple panels of nylon tulle netting impregnated with Tangle-Trap (Biconet, Biocontrol Network, Brentwood, TN) are spooled on a spindle and subsequently attached to the rope hoisted by the balloon to various elevations. We tested various spooling systems for releasing and gathering the rope and attached panels, and we tested different sizes and lengths of panels for their integrity, stability, and drag (drag reduces the altitude of the balloon in windy conditions). Our trap designs were tested in Florida during May and June 2009–2010. Because fire ant alates reportedly do not initiate mating flights in winds that exceed 8–16 km/h (Markin et al. 1971), we limited our designs to accommodate this range of wind speed. The components of the trapping system are described below.

Helium Balloon. The aerial trap consists of a 3-m-diameter, helium-filled balloon (Mobile Airships, Ontario, CA). The net lift of the balloon is ≈11 kg, and the suggested maximum payload is 3.6 kg. The balloon is attached to a 3-mm-diameter, Kevlar-core, braided, nylon rope that can be purchased at any length from the balloon supplier (Mobile Airships). The balloon should be colored or have flagging that make it visible up to 1.6 km. Beyond a height of 46 m, present Federal Aviation Administration rules indicate that tether ropes on balloons must have a marker visible up to 1.6 km away every 15 m.

Electric Winch. An electric winch (Golo power winch, Cordem Corp./Ballard Mullica Hill, NJ) and a spindle (see below) are attached to a plywood platform that is bolted to a swivel (Fig. 1). The platform holding the winch and spindle can be rotated in response to wind direction, which allows for the raising or lowering of the sampling panels (see below) without twisting. Our swivel was made from the base of a metal office chair and was attached, at its base, to a wooden platform (Fig. 1). A hole was made in the top wooden platform through which a metal pipe was inserted. This pipe nestled between bars of wood on the lower platform and could act as a brake preventing the swivel from turning. The winch we used dispensed up to 183 m of Kevlar-core nylon rope and can be powered in the field by a car battery and a DC to AC converter. The winch has a single drum and a variable speed control to moderate the ascent and descent of the balloon (Fig. 1).

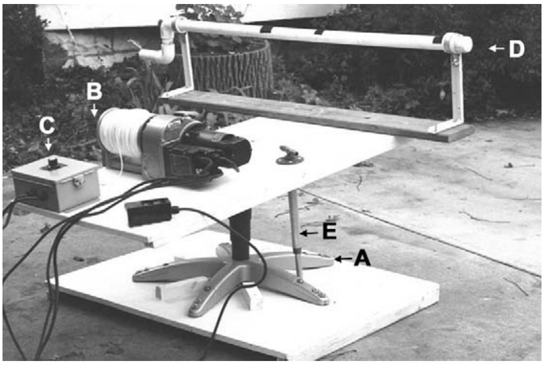

Fig. 1. Winch on swiveling platform showing swiveling base (A), electric winch (B), winch variable speed control (C), PVC spindle (D), and metal pipe stop (E).

Spindle. A spindle was made from a 1.2-m section of 8 cm PVC tubing. The ends of the PVC pipe were inserted into metal hoops bolted to a wooden plank (Fig. 1). Two short sections of PVC pipe were attached to one end of the tube to serve as a handle; the opposite end of the tube was capped (PVC cap) to prevent it from shifting out of the metal hoop. The spindle stored up to 10 ten panels (0.9 m × 4.6 m each) of nylon, tulle netting impregnated with Tangle-Trap. The spindle apparatus was bolted at one end of the winch platform and detachable (Fig. 1).

Tangle-Trap Impregnated Panels. We tested a variety of cloth and netting materials impregnated with two different formulations of Tangle-Trap for their ability to immobilize red imported fire ant alates throughout a 12-h period (plastic window screening, nylon tulle netting, army mosquito netting, cotton sheet, canvas sheet). Fifty female and male alates were hand-thrown onto vertical swaths of material impregnated with Tangle-Trap. The number of ants remaining after a 12-h period was recorded; nylon tulle netting was found to be the most suitable substrate (see Results).

Our trapping surfaces, then, were rectangular swaths of tulle impregnated with Tangle-Trap. Each of the tulle panels was 0.9 by 4.6 m and had a carbon-fiber tube (length, 90 cm; 5.0 mm o.d.; 2.5 mm i.d.) glued (Duco Cement, ITW Devcon, Danvers, MA) at each end of the panel and at 1.5-m intervals; thus, each panel had a total of four carbon-fiber tubes attached. Before gluing the carbon-fiber tubes onto the tulle, a 5- by 7.6-cm strip of Velcro (Velcro USA Inc., Manchester, NH) was attached to the midpoint of each tube. This step was accomplished by adhering the sticky-back side of two identical strips of Velcro around the shaft of a carbon-fiber tube. The surfaces of these two strips of Velcro should be identical and should not be the surface with the tiny hooks (because these hooks can catch on the tulle as it is rolled onto a spindle).

Once the carbon-fiber tubes are glued to the tulle, a little rectangle of the tulle is cut around the perimeter of each strip of Velcro so that its surface is exposed

Fig. 2. Raised balloon with 10 attached net panels. (Online figure in color.)

on both sides of the tulle panel (this cut enables the choice of attaching the carbon-fiber tubes to the nylon rope at either side of a panel).

Tangle-Trap Application. The tulle panels were impregnated with Tangle-Trap by stretching each panel onto the surface of a large, plywood table with a hardware cloth surface. The hardware cloth prevents the tulle surface from sticking to the plywood table. A plastic glue spreader was used to apply a thin coat of Tangle-Trap to the surface of the tulle. Ten panels of Tangle-Trap impregnated tulle were stored on a single polyvinyl chloride (PVC) spindle (described above). We used the Tropical Formula of Tangle-Trap, because the liquid formulation of Tangle-Trap (Ladd Research, Williston, VT) did not have sufficient adhesive properties to immobilize alates.

Panel Attachment. As the helium balloon is raised (Fig. 2), the panels on the PVC spindle are attached to the nylon rope by using strips (5 by 7.6 cm) of industrial Velcro. The topmost carbon-fiber tube is clipped at its centermost point into a loop in the nylon rope at a distance 1.5 m below the base of the balloon. The Velcro strip on the tube is then attached to a complimentary strip of equal size onto the rope to stabilize the panel and keep it attached to the rope. Every carbon-fiber tube thereafter is similarly attached to the rope by the Velcro strips. The bottommost carbon-fiber tube also is anchored to the rope by a clip inserted into a loop on the nylon rope. The function of the clips at both ends of each panel is to prevent the panels from collapsing on themselves and shifting their positions along the rope. The distance between each panel was ≈11 m; all 10 panels could be attached and the balloon raised to an altitude of 140 m in ≈15 min.

Panel Detachment. As the balloon descends (using the electric winch), panels are removed by rolling one end around the PVC spindle and turning the spindle handle. When the full length of the panel has been rolled onto the spindle, a sheet of heavy-duty plastic (≈1.2 by 0.9 m) is wrapped around the panel. The next panel can then be rolled onto the spindle without transferring ants from one panel to another. Each panel, then, represents a sample of ants from a different elevation. All 10 panels could be removed and stored onto the spindle in ≈20 min.

Removal of Ants from Panels. Once all panels are on the spindle, the spindle can be removed from the winch apparatus and taken to the laboratory for the removal of ants. Each panel can be unrolled onto a second spindle of equal size and at some distance away. We use a vertical structure made of wood that secures both spindles at a distance of ≈1.5 m. As each section of panel is unrolled, ants can be removed with forceps and placed into individual vials containing 100% ethanol. Tangle-Trap causes ants to adhere to each other, whether in or out of ethanol. Ants were cleansed of Tangle-Trap by transferring the contents of a vial into a metal, fine-mesh strainer that was subsequently dipped in and out of a bowl filled with mineral spirits. This step only takes a few seconds and is followed by dipping the ants into bowls filled with fresh, 100% ethanol. The ants are then transferred into fresh vials of ethanol for long-term storage. We found this method did not interfere with the recovery of DNA from specimens, did not interfere with polymerase chain reaction of this DNA and did not affect the removal of spermatozoa from spermathecae through dissection.

Results

As described above, various permutations of the trap were tested for stability, drag, and structural in-

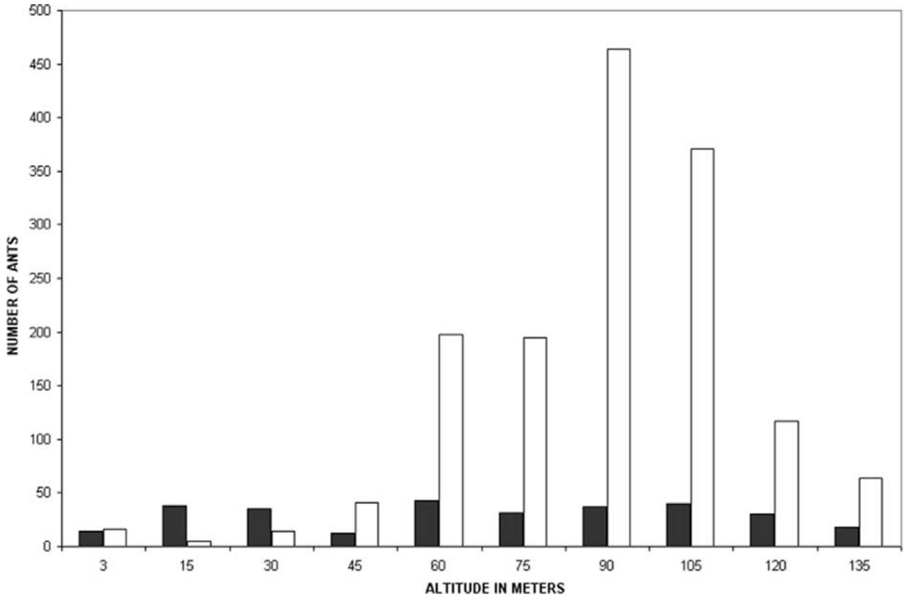

Fig. 3. Total number of female (black) and male (white) alates caught during three mating flights on 10 panels at different elevations and impregnated with Tangle-Trap.

tegrity. In addition, the adhesive properties of five different kinds of surfaces impregnated with Tangle-Trap were tested for their ability to hold alates of both sexes for 12 h (plastic window screening, nylon tulle netting, army mosquito netting, cotton sheet, canvas sheet). Only the nylon tulle was found to be effective in adhering all samples of 50 alates of each sex for as long as 12 h. The effectiveness of the nylon tulle netting was due, in part, to the relatively large pore size which prevented ants from gaining a good "foothold" across its surface. The relatively large pore size (but not large enough for ants to fly through) also reduces drag from wind while the trap is deployed. Furthermore, the large pore size may make the trap less visible to flying ants that might otherwise avoid obstructions perceived in the air.

Drag was a particular problem because wind can affect the altitude of the balloon and, therefore, of the trapping panels. Light-weight materials (carbon-fiber tubes and large-pore, nylon tulle) were used to minimize the payload of the balloon and reduce its list under windy conditions. The weight of 10 Tangle-Trap impregnated panels (0.9 by 4.6 m) including 140 m of nylon rope is just under 2.7 kg. Because the balloon has an 11-kg lift, the tethered balloon remains relatively vertical in 0–8 km/h winds. We estimated that our trap design (with 10 0.9- by 4.6-m panels) lost no more than ≈10–15 m of altitude under 8–16 km/h winds for a balloon at ≈140-m altitude (which was the maximum altitude we tested); 8–16 km/h winds, however, presumably inhibit fire ant mating flights (Markin et al. 1971).

The design outlined above was tested for 3–4 h during each of three mating flights in Gainesville, FL, in May–June 2010; in total, 1,783 ants were trapped (340, 389, and 1,054, respectively) at various altitudes (Fig. 3), indicating this sampling technique effective for examining the dynamics of mating flights in this species.

Discussion

Except for a study by Markin et al. (1971), previous studies examining mating flight dynamics and dispersal have either sampled individuals exiting colonies to initiate flights or sampled individuals on or near the ground, postmating. Our trapping system allows sampling of ants in mating flights at various elevations simultaneously. Because we now know that the genetic composition of fire ants is more complex than thought previously (e.g., two genetically distinct social forms, sterile and fertile diploid males, triploidy), genetic profiles in space and time can now be obtained during mating flights.

The trapping method described here can be easily managed in the field with two persons and set up or taken down quickly. In addition, the altitudes at which panels are placed can be varied as well as the trapping surface area of each panel or the number of panels, depending on the goals of a study. Wind will always be a consideration when employing this type of trap. For fire ants, however, mating flights do not normally occur in winds >16 km/h. Three or four tethered guy ropes would probably alleviate listing of the balloon in higher winds, but we did not test this option. Finally, our trap design should also be useful for sampling other flying insects, because our trap caught small beetles, flies, and various small heteropterans and bees; thus, the trap also should be useful for altitudinal sampling of insects of medical and veterinary importance that are within the size range of fire ant alates or smaller (such as mosquitoes, sandflies, black flies).

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