Mating Flight Activity as Dealation Factors for Red Imported Fire Ant (Hymenoptera: Formicidae) Female Alates

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ABSTRACT Queens of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), release a primer pheromone that inhibits dealation (wing removal) of nestmate female alates by presumably suppressing endogenous concentrations of juvenile hormone (JH). Alates cast their wings once separated from the queen; however, the point of initiating dealation varies upon conditions. Alates are stimulated to shed their wings after several days of the death or removal of the queen, whereas newly mated alates dealate within 1 h after the mating flight. We found no single premating behavior or combination of behaviors associated with the nuptial flight that induces dealation rates comparable with that of newly mated queens. Copulation by itself or in conjunction with other behavioral signals and environmental prompts seems to be critical in causing rapid dealation in newly inseminated alates. In addition, colonies containing alates treated with precosene would not initiate mating flights nor could they be induced to fly. We suggest that precosene treatment affects the corpora allata (CA), but CA products other than juvenile hormone (JH) or in combination with JH are responsible for rapid dealation after mating. Dealation in the two contexts, within the colony and after mating flights, seems to occur via separate mechanisms.

KEY WORDS dealation, juvenile hormone, nuptial flight, precocene

In the mid-1930s, the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), was unintentially introduced into the United States from Brazil (Lofgren et al. 1975) and swiftly dominated the native ant fauna in the south (Porter et al. 1988, Porter and Savignano 1990, Wojcik 1994). Several reasons have been identified to account for the fire ant's success. For example, upon its entrance into the United States, *S. invicta* has benefited from the absence of its natural enemies from South America (Porter et al. 1992). Other advantages include the presence of up to 250,000 highly aggressive workers in each mound (Blum 1992, Tschinkel 1993), with population densities reaching 150 mounds per hectare (monogyne social form, Porter et al. 1992). Each colony can generate up to 5,000 sexuals in a year (Markin et al. 1973), and the dispersal of reproductives is through nuptial flights, which may extend 300 m in the air (Markin et al. 1971). Mating flights can occur any time of the year if environmental conditions are right (Markin et al. 1971).

S. invicta queens produce an array of pheromones that suppress reproductive competition within the nest. For example, queens release primer pheromones that inhibit the development of sexual larvae and suppress reproductive activity in uninseminated female alates (Vargo 1998), and the distribution of these pheromones is aided by releaser pheromones that are attractive to workers (Vander Meer et al. 1980). Of particular importance is a primer pheromone, the dealation inhibitory primer pheromone that prevents female alates from shedding their wings (dealation; Fletcher and Blum, 1981a,b, 1983; Vargo 1998).

The dealation inhibitory primer pheromone is effective in preventing female reproductives from casting their wings (dealation), a behavior that normally occurs soon after mating and landing on the ground. Dealation precedes several physiological changes, including ovarian development (Fletcher and Blum 1981a,b,c), wing muscle histolysis (Toom et al. 1976), and pheromone production (Glancey et al. 1981, Vargo 1999). Previous investigations (Kearney et al. 1977; Barker 1978, 1979; Vargo and Laurel 1994) suggest that this primer pheromone suppresses natural juvenile hormone (JH) titers in female alates. Thus, when female alates are removed from their colony and the queen or if the colony queen dies, they will then shed their wings within a few days. These uninseminated dealates will eventually lay eggs that will develop into males, and although these males may par-

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ticipate in future mating flights, passing on maternal genes, they do not directly contribute to the workforce needed to support the colony. Hence, the release of the dealation inhibitory pheromone by the queen serves to eliminate reproductive and resource competition within the colony. The dealation inhibitory primer pheromone keeps female alates, flight ready, within the colony until they are stimulated by the right environmental conditions into mating flight activity (Obin and Vander Meer 1994, Alonso and Vander Meer 1997).

Two distinct dealation processes are evident. Dealation of female reproductives occurs normally after a mating flight and within the colony once the queen has died. However, the time at which dealation occurs is dramatically different within these two conditions. When the queen is removed from the colony, several days may follow before alates shed their wings (Fletcher and Blum 1981a,b,c), but dealation can occur within 1 h after a mating flight (Markin et al. 1971). It is important to understand the differences in the two dealation mechanisms, because dealation and the physiological/biochemical changes that occur with the process are critical to successful colony foundation. This article investigates various factors that contribute to the onset of dealation within the colony, and, in particular, those events that are characteristic of nuptial flight activities.

Materials and Methods

Dealation after Activities Associated with a Nuptial Flight. Once female alates leave the colony to engage in a nuptial flight, dealation normally occurs within 0.5 h of landing (Markin et al. 1971). The experiments described here attempt to determine what stage of the mating flight triggers rapid dealation. The rates of dealation were observed for alates displaying a variety of behaviors associated with a nuptial flight (Markin et al. 1972, Milio et al. 1988, Obin and Vander Meer 1994, Alonso and Vander Meer 1997). Five categories of nuptial flight behavior were examined: 1) excited alates running on the surface of the soil; 2) excited alates running on the surface of the soil and climbing onto tongue depressors; 3) tethered alates flying for 5 min under laboratory conditions, but not primed for a nuptial flight; and 4) alates displaying all of the identified premating behaviors (running, climbing, and flying); and 5) alates not displaying any of the identified premating behaviors (controls). Alates used in this investigation were acquired directly from monogyne colonies in the field and from colony fragments collected from the field and maintained in the laboratory.

Source Colonies. *S. invicta* colonies producing sexual brood were collected from the Gainesville, FL, area. Field collection sites and colonies were determined to be monogyne based upon the following characteristics: 1) the fire ant population had low mound density and large well-developed nests, 2) colony workers were morphometrically polymorphic (Greenberg et al. 1985), 3) collected colonies had a single physogastric inseminated queen, and 4) colony workers displayed a high degree of conspecific aggression (Morel et al. 1990).

Workers, sexuals, and brood from each queenright monogyne colony collected from the field were separated from the soil by slowly flooding the collection bucket and placed in a large tray (52 cm in length by 39 cm in width by 7.5 cm in depth) with inner sides coated with Fluon (ICI Americas, Inc., Exton, PA) to prevent ants from escaping. Each colony queen was separately brought back to the laboratory along with some workers and then reunited with her offspring. Nest cells consisted of a petri dish (14 cm in diameter) with a Castone (Dentsply Trubyte, York, PA) bottom and three equally spaced holes in the sides of the dish to permit movement of ants in and out of the cell. The Castone bottom was periodically moistened with water to increase humidity in the nest cell. Colonies were provided with nest cells covered with red cellophane to simulate dark nest conditions. Colonies were fed a copious diet of crickets, 10% sugar water absorbed on tissue wads, and tap water contained in test tubes (15 cm in length by 2.2 cm in diameter) plugged with cotton balls. Ants were maintained in the laboratory at 27°C and 47% humidity.

Sexually Mature Alates. Female sexual maturity has been reported as >7 d posteclosion (B. M. Glancey, unpublished data, cited in Lofgren et al. 1975; Fletcher et al. 1983); therefore, queenright colonies containing female alates (female alate pupae removed) were kept under laboratory conditions for at least 7 d before use of the colony female alates. Another measure of sexual maturity is alate weight (Fletcher et al. 1983, Burns et al. 2005).

Determination of Dealation Rates. Dealation was observed in test tube (70-ml) holding chambers. Each test tube chamber was made by filling half the tube with water and plugging the tube to the water with cotton balls. This left an area for the alate(s) to move within the tube. The alate(s) were placed in the tube and another cotton ball was placed in the mouth of the tube to keep the alate(s) inside. The portion of the holding chamber housing the alate(s) was wrapped with red cellophane to simulate darkness. Test alates were observed every 12 h to monitor dealation, defined as the removal of at least three of the four wings (Vargo and Laurel 1994). After the removal of wings, dealates commence production of the dealation inhibitory primer pheromone that prevents the casting of wings in cohabiting alates (Vargo 1999); therefore, in this study, upon wing removal, dealates were separated from alates to eliminate their pheromonal influence the remaining alates. The rates of dealation of isolated treatment alates were compared with those of isolated control.

Dealation Rate in Absence of Queen and Workers. This experiment measures alate dealation rates in the absence of workers and queen, as would occur after a mating flight. Sexually mature alates were isolated individually and dealation rates were compared with another set of sexually mature female alates that also were isolated individually, but with workers and

brood. This simulates within colony dealation where the queen is not present and inhibiting female alate dealation. Dealation was observed and recorded as described above. All ants were maintained under the specified laboratory conditions.

Nuptial Flight Categories 1 and 2. Outside the laboratory, alates were induced to display nuptial flight behaviors associated with mating flights by the following procedure. Buckets containing colony fragments were placed in direct sunlight (≈33°C and 52% humidity). Next, mists of water were sprayed on colony soil for ≈5 min, and excited workers were observed creating exit holes ≈30 min after spraying. Alates emerged from the soil 30–60 min after the onset of the formation of exit holes by excited workers. Alates displaying category 1 (excited alates running on the surface of the soil) or category 2 behaviors (running on the soil surface and climbing onto tongue depressors) were assembled into groups and placed in test tube holding chambers as described in the "influence of workers" section above. Alates were maintained outside of the laboratory for the duration of the experiment, and observed for dealation every 12 h. Field temperatures and humidity were recorded.

Nuptial Flight Category 3. Alates used for tethered flight (category 3) were collected directly from mounds in the field. Only those determined to be mature based on weight (>16 mg; Burns et al. 2005) were used. Each alate was prepared for tethered flight by attaching a tiny loop (0.05 cm in diameter) of fine silver wire (0.02 mm in diameter) onto the prothorax with glue (Pacer Technology, Rancho Cucamonga, CA) and extending the wire at an angle ≈5 cm from the loop. Next, the alate was warmed for 10 s by placing it ≈5–10 cm from a 40-W bulb. Flight induction was accomplished by waving the alate in the air several times by using forceps and blowing ≈5 s of light puffs of air underneath the wings (Vogt et al. 2000). Tethered alates are less likely to be induced to fly after being stimulated for >10 min. Therefore, attempts to stimulate flight did not last beyond 10 min for each alate. Once flight was induced, the wire extending from the alate was mounted onto a piece of modeling clay attached to a ring stand. The alate was positioned ≈5 cm from the light source. An alate that ceased to fly was restimulated by quickly blowing puffs of air underneath the wings (Vogt et al. 2000). For this study, a successful flight was defined as having a duration of at least 5 min, including time needed to restimulate alates that had temporarily ceased flying (note that alate flights have been reported up to 60 min in duration, Vogt et al. 2000). Once flight was terminated, two pairs of forceps were used to grip the wire attached on the top of the prothorax and then pulled in opposite directions to break the wire extension. Freed alates were placed in groups of three in test tube chambers. Dealation observations were taken every 12 h, as described above.

Nuptial Flight Category 4. We induced mature alates to display all identified premating behaviors (category 4: running, climbing, and flying). As described previously, colonies were taken outside of the laboratory to induce preflight behaviors in alates, and these excited alates were then stimulated for tethered flight under laboratory conditions. Alates displaying all of the premating behaviors were grouped (three alates per group) in test tube holding chambers. Alates were maintained outside of the laboratory for the duration of the investigation, and dealation was monitored every 12 h.

Nuptial Flight Category 5. Alate controls (category 5) were collected directly from mounds in the field. Only those determined to be mature based on weight were used (>16 mg; Burns et al. 2005). Controls were assembled in groups of three and placed in test tube holding chambers (70 ml) described above. Red cellophane to simulate darkness was wrapped around each tube, covering the portion of the tube housing the alates. Test tube chambers were placed outdoors, in a shaded area, for the duration of the experiment. Alates were observed every 12 h for dealation, and dealates were removed from tubes after each observation period.

Mating Flight Activities after Precocene II Treatment. Monogyne *S. invicta* colonies possessing female alates (>40) were collected and maintained as described above. Worker ants were separated from the soil, and sexual pupae were removed from each colony. To ensure sexual maturity of alates before topical treatments of precocene II (Sigma-Aldrich, St. Louis, MO), all colonies were left undisturbed under laboratory conditions for at least 9 d (Lofgren et al. 1975). Three Fluon-lined buckets (25 cm in depth by 25 cm in diameter), two thirds filled with moist soil were each prepared with a colony queen, 5 g of worker adults, 0.5 g of worker brood from the same colony, and 40 sexually mature female alates. Alates from one group were each treated topically with 1 μl of acetone containing 47 nmol of precocene and placed in a bucket. Precocene treatments decrease CA size, presumably affecting JH production (Burns et al. 2002). Alates from the second group were each treated topically with 1 μl of acetone (control 1) and placed in a different bucket. Untreated alates (control 2) were placed with their mother colony in a third bucket. The three colonies were left undisturbed for 4 d. After the 4-d acclimation period, the environmental conditions were altered to stimulate a mating flight (Markin et al. 1972, Milio et al. 1988, Obin and Vander Meer 1994). After ≈30 min, excited workers were observed forming exit holes. Alates that crawled onto tongue depressors ($n = 15$ for each category) were collected and placed in a Fluon-lined cup (265.5 ml). Precocene-treated alates did not exit the colony so they had to be collected from the soil by spreading a thin layer of colony soil into Fluon-lined trays. The collected alates were monitored for dealation every 12 h.

Precocene/JH III Recovery Experiments. We reported previously (Burns et al. 2002) that precosene II treatment prevented female alates from dealating outside the influence of the queen, but this could be reversed with subsequent treatment with JH III. The following experiments investigate the effect of pre-

Fig. 1. Rates of dealation of female alates out of the influence of their queen: isolated alate, $n = 15$; and single alate with workers and brood, $n = 15$.

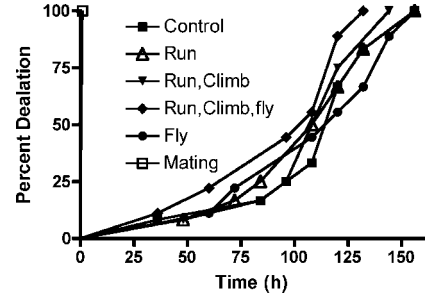

Fig. 2. Rates of dealation of female alates exhibiting different behaviors associated with the mating flight: alates running, $n = 12$; alates running and climbing, $n = 12$; alates flying (lab-induced), $n = 9$; alates running, climbing, and flying (lab-induced), $n = 9$; nonstimulated alates, control, $n = 12$; and mated alates (Markin et al. 1971).

cosene II on mating flight activity and possible recovery using JH III treatment.

Mating Flight Activities after Precocene II Treatment. Monogyne colonies possessing female alates (>40) were collected and maintained as described above. Ants were separated from the soil, and sexual pupae were removed from each colony. To ensure sexual maturity of alates before topical treatments of precocene II (Sigma-Aldrich), all colonies were left undisturbed under laboratory conditions for at least 9 d (Lofgren et al. 1975). Three Fluon-lined buckets (25 cm in depth by 25 cm in diameter), two thirds filled with moist soil, were each prepared with a colony queen, 5 g of worker adults, 0.5 g of worker brood from the same colony, and 40 sexually mature female alates. Alates from one group were each treated topically to the head with 1 μl of acetone containing 47 nmol of precocene and placed in a bucket. Precocene treatments decrease CA size, presumably affecting JH production (Burns et al. 2002). Alates from the second group were each treated topically with 1 μl acetone (control 1) and placed in a different bucket. Untreated alates (control 2) were placed in a third bucket with identified colony members. The three colonies were left undisturbed for 4 d. After the 4-d acclimation period, the environmental conditions were altered to stimulate a mating flight (Markin et al. 1972, Milio et al. 1988, Obin and Vander Meer 1994). After ≈30 min, excited workers were observed forming exit holes. Alates that crawled onto tongue depressors were collected and placed in one of three Fluon-lined cups (265.5 ml), each corresponding to a particular treatment. Precocene-treated alates did not exit the colony; therefore, they were collected from the soil by spreading a thin layer of colony dirt into Fluon-lined trays. The collected alates were monitored for dealation every 12 h, and in all cases sexuals were removed from cups upon dealation.

JH III Recovery Experiments. Twenty mature alates were treated, as described above, with 47 nmol of precocene II in 1 μl of acetone and placed in two buckets with colony ants (10 alates per bucket). After the 4-d acclimation period, 10 precocene II-treated alates were each topically applied with 75 pmol of JH III in 1 μl of acetone. The remaining 10 precocene II-treated alates were topically applied with 1 μl of acetone (control). Flight induction studies were conducted 3–6 h after applying JH III or acetone because preliminary experiments revealed that >60% dealation occurred within 12 h of topically applying 75 pmol of JH III onto precocene-treated alates (Burns et al. 2002). All collected alates (precocene/JH, precocene/acetone) were prepared for tethered flight in the manner described above. A successful flight was defined as having a duration of 5 min, including time needed to stimulate alates that had temporarily ceased flying.

Statistical Analyses. Dealation rates were compared using the logrank test which is equivalent to the Mantel–Haenszel test (GraphPad Software, Inc. 2005). Other standard statistical tests are indicated in the text.

Results

Dealation Rate in Absence of Queen and Workers. The dealation rate of alates decreased significantly when workers were not present ($n = 19$) compared with the dealation rate of alates in the presence of workers and brood ($n = 30$) (Logrank test: $\chi^2 = 11.28$, df = 1, $P < 0.01$, median survival ratio = 1.286, 95% CI ratio = 0.7228–1.849) (Fig. 1). One hundred percent of alates placed individually with workers and brood dealated within 108 h, whereas isolated alates did not all dealate until the 156-h observation period.

Dealation after Activities Associated with a Nuptial Flight. The experimental female alates collected in the field were considered mature as indicated by their weight (mean ± SEM, 14.6 ± 0.68 mg; $n = 54$). Age could not be used as a maturity criteria, because the females were collected from field colonies. All tested female alates shed their wings within 168 h of their particular treatment (Fig. 2). Comparison of dealation curves revealed no differences among dealation rates of alates in any of the four mating flight treatment categories and the control, e.g., control versus running + climbing + flying (shortest treatment time to 100% dealation) (Logrank test: $\chi^2 = 2.032$, df = 1, $P = 0.1540$, median survival ratio = 1.111, 95% CI ratio = 0.6898–

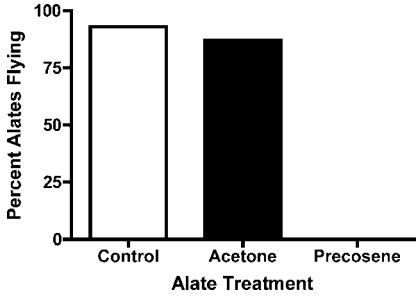

Fig. 3. Percentages of female alates flying for 5 min under three conditions: alates topically treated with 100 μg of precocene/μl acetone, (precocene), $n = 15$; alates topically treated with 1 μl of acetone (acetone), $n = 15$; and untreated alates (control), $n = 15$.

1.532) (Fig. 2). Mature female alates removed from the influence of their queen, but with workers, generally dealated within 108 h of isolation with workers present (Burns et al. 2005). However, for female alates in category 1 (running) only four of 12 (33%) dealated by 108 h. For category 2 (running and climbing) three of 12 (25%) dealated. In category 3 (flying) four of nine (44%) female alates dealated. For category 4 (running, climbing, and flying) five of nine (56%) female alates dealated within 108 h (Fig. 2). As expected, the female alate controls (no workers present; category 5) were similarly delayed in their time to dealation, only four of 12 (33%) dealated by 108 h (Fig. 2). Comparison of the shortest treatment time to 100% dealation (running + climbing + flying) with isolated alates with workers showed that the two were significantly different (Logrank test: $\chi^2 = 7.396$, df = 1, $P < 0.01$, median survival ratio = 1.111, 95% CI ratio = 0.8333–1.738), as were isolated alates with workers and isolated alates without workers (Figs. 1 and 2).

Effect of Precocene Treatments on Mating Flight Activity. Colonies in buckets containing treatment and control groups of alates were induced to initiate mating flights (Obin and Vander Meer 1994). Excited workers from the colony containing acetone-treated alates ($n = 15$), and those from the colony with untreated alates ($n = 15$) were observed creating exit holes. Alates from these two colonies were seen walking onto tongue depressors. However, none of the workers from the colony with precocene-treated alates ($n = 15$) were observed displaying typical mating flight activities, and no alates were observed walking onto tongue depressors.

Eighty-seven percent of 15 alates treated only with acetone and 93% of 15 untreated alates could be induced to fly for a minimum of 5 min (Fig. 3). There was no difference (two-sided Fisher exact test: $P = 1.0000$) in the proportion of acetone-treated alates stimulated to fly and that of untreated alates. However, none of the alates treated with precocene II could be stimulated to fly ($n = 15$; Fig. 3). Attempts to restore flight potential in precocene-treated alates by treatment with JH III were unsuccessful (JH III treatments, $n = 10$; or acetone controls, $n = 10$).

Discussion

We investigated dealation rates of *S. invicta* female sexuals under different conditions to identify potential physical factors influencing the time in which both uninseminated and newly mated females dealate (shed their wings). These two situations are characterized by having dramatically different rates of dealation. Alates that are pheromonally disinhibited within the context of the colony because of queen loss, dealate within 2–5d (Fletcher and Blum 1981a,b,c), whereas newly mated queens dealate almost immediately upon landing and ≈30 min after they leave their mother colony on a mating flight (Markin et al. 1971).

The dealation inhibitory primer pheromone has been postulated to slow but not stop JH buildup in nestmate alates (Fletcher and Blum 1983, Vargo and Laurel 1994). This is supported by the fact that the rate of dealation can be accelerated by topical JH concentrations (Kearney et al. 1977, Burns et al. 2002). Dealation rates for isolated immature and mature alates are identical (Burns et al. 2005), which implies that JH quantities in female alates do not increase with age enough to affect dealation rates. This also suggests that the dealation inhibitory primer pheromone is detected and acts on immature and mature female alates equally well (Burns et al. 2005). However, once released from pheromonal control, even immature alates are capable of producing JH or other factors that promote dealation.

When female alates leave the colony to engage in a nuptial flight, they leave behind both the colony queen and workers. Under these conditions, newly inseminated females shed their wings quickly after landing (Markin et al. 1971). We found that isolating alates from queen and workers in the laboratory significantly delayed the time to dealation. Thus, alates have the ability to remove their wings without worker assistance, because they readily dealate after mating flights. Under conditions of disinhibition within the colony context, workers apparently play an unknown role in female sexual dealation. Perhaps they provide alates with tactile or olfactory stimuli, which may induce dealation, as suggested by Fletcher et al. (1983). The apparent influence of workers in the dealation of disinhibited alates within the context of the colony is another feature that distinguishes this process from dealation after mating flights. Presumably, mating, behaviors associated with a nuptial flight, or a combination induce rapid dealation in newly inseminated females.

Dealation after Activities Associated with a Nuptial Flight. Results from previous studies (Kearney et al. 1977; Barker 1978, 1979; Vargo and Laurel 1994) with uninseminated female sexuals suggest that JH is critical in stimulating dealation. Though the role of JH in postmating dealation was not directly investigated in the current study, it may be possible that nuptial flight behaviors, along with environmental cues, induce dealation by elevating JH titers. Specific insect behaviors have been reported to play important roles in regulating JH. For example, in *Danaus plexippus*

plexippus (L.), JH-sensitive reproductive organs are less developed in migrating females and than in males (Urquhart 1960), and Lessman and Herman (1981) reported that flight is responsible for inactivating JH in females. They found that the hemolymph of monarchs that had flown for 40 min under laboratory conditions contained significantly higher levels of JH-specific esterases than those that had not flown. Lessman and Herman (1981) proposed that reduced reproductive development in migrating monarchs might be the result of flight-induced JH breakdown, which prevents inappropriately-timed reproduction. In addition, Huang and Robinson (1992) hypothesized that in *Apis mellifera* L., worker interaction influences JH titers and behavioral development. In an earlier study, Robinson (1987) reported that JH levels are lower in nurses than in older workers and foragers and that JH administered to nurses induced premature foraging. Huang and Robinson (1992) found that nurses isolated from foragers exhibited precociously high JH titers and engaged in foraging activities. It was proposed that under natural circumstances, foragers inhibit JH production in younger workers, and as the foragers die, younger workers receive less inhibition, thereby promoting behaviors associated with foragers. It has been shown that JH III titers of mature polygyne fire ant queens and alates (queenless but with workers) are twice the level of female alates (Brent and Vargo 2003; S.N.B. et al., in preparation), but the latter situation is far removed from the dealation event and is likely due to egg production. The former correlates JH III level with the onset of dealation for alates out of the influence of their queen. However, a previous study showed that topical treatment of alates with JH III could overcome the effects of the queen dealation inhibitory pheromone, but increasing JH III concentrations were insufficient to simulate the rapid dealation that occurs after mating.

We examined several premating behaviors/activities associated with nuptial flights of *S. invicta* to determine whether an individual premating behavior or a combination of behaviors is critical to dealation. We found that neither individual nor combined behaviors induced dealation rates similar to that of newly mated females. Less than 12% of alates exhibiting one or more premating behaviors shed their wings within 36 h, a significant delay compared with the rate of dealation in newly inseminated females (within ≈0.5 h of flight; Markin et al. 1971). Therefore, mating, alone or in combination with other behavioral and environmental cues, must be essential factors in stimulating rapid dealation. In *Rhodnius prolixus* Stål, for example, mating is responsible for activating and enhancing egg development by increasing endogenous levels of JH (Davey 1993). Mating may be responsible for stimulating the CA through signals transmitted by nerve connections. This is best illustrated in some species of Dictyoptera, such as *Diploptera punctata* (Eschscholtz) (Engelmann 1959, Stay and Tobe 1977) and *Periplaneta americana* (L.) (Pipa 1986), in which mating functions by removing inhibition from the nervi corporis allati, thereby allowing the CA to secrete JH. Mating also may involve the direct transfer of JH from the male to the female. *Hyalophora cecropia* (L.) males have been reported to transfer JH directly to females (Shirk et al. 1979). Along with the deposition of JH substances into the female, mating may have an allatotropic effect in females, thereby initiating JH biosynthesis (for review, see Ramaswamy et al. 1997). In *Heliothis virescens* (F.), allatotropic stimuli in the female may accompany male transfer of JH to enhance egg production (Park et al. 1998). All of the abovementioned examples support a role for copulation and JH in dealation. However, we found that increasing concentrations of topically applied JH III failed to induce dealation rates near those of newly mated alates (Burns et al. 2002).

The process of mating could not be assessed in this study. The difficulties of successful forced copulation of reproductives (Ball et al. 1983) prevented us from determining whether mating is a major stimulant. Environmental factors and tactile and/or olfactory cues from the male during mating also could be possible stimulants. Nonetheless, this investigation provides evidence as to the role of the most documented (Rhoades and Davis 1967, Markin et al. 1972, Milio et al. 1988, Alonso and Vander Meer 1997), and, to some degree, they readily induced premating behaviors on dealation. Further investigation may disclose important factors associated with mating that induce rapid dealation.

The mode of action of precocene involves the creation of unstable 3,4-epoxy derivatives in the CA, and these epoxide intermediates react with glandular proteins, causing necrosis in the CA (Wawrzenczyk 1997). The effects of precocenes on Hymenoptera are very limited (Goewie et al. 1978, Bowers 1983, Rembold et al. 1979). Goewie et al. (1978) reported that applications of precocene II to 90-h-old queen larvae of *A. mellifera* caused the development of worker-like intermediates and that the precocene treatments resulted in atrophy of the CA. However, Rembold et al. (1979) were not able to show that precocene II caused any anti-JH activity in the honey bee. We recently showed that precocene II treatments atrophy the corpora allata of *S. invicta* (Burns et al. 2002), which suggests a decrease in JH production (Bowers 1983).

Interestingly, we found that precocene II applications completely inhibited alates from initiating mating flights (Alonso and Vander Meer 1997); consequently, workers in these colonies did not engage in behaviors associated with mating flights by females (Markin et al. 1972, Milio et al. 1988, Obin and Vander Meer 1994). Interestingly, we found that application of 75 pmol of JH III was not sufficient to reverse flight inhibition in alates treated with precocene II, although this JH treatment level did reverse the negative dealation effects of precosene-treated queenless alates (Burns et al. 2002). The ineffectiveness of this JH dose in overcoming the inhibition to initiate mating flights may be due to insufficient time (3–6 h) allowed for the hormone to manifest an effect or the high dosage of precocene II may have had toxic effects on other endocrine glands as well as the CA.

This study has highlighted the dramatic difference in time to dealation in female alates that become disinhibited from the pheromonal influence of their queen within the context of the nest versus rapid dealation associated with mating flights. In nature, dealation via mating flights to produce reproductively viable potential foundress queens is the norm. Measurement of JH titers in alates that have undergone the mating flight behaviors investigated here and newly mated queens and/or successful development of artificial mating techniques will be necessary to definitively determine the behavioral and biochemical mechanisms involved in fire ant female sexual dealation after mating.

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