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Identification and action of juvenile hormone III from sexually mature alate females of the red imported fire ant, *Solenopsis invicta*

S.N. Burns [a], P.E.A. Teal [*,a], R.K. Vander Meer [a], J.L. Nation [b], J.T. Vogt [c]

[a] *Center for Medical, Agricultural and Veterinary Entomology (CMAVE), USDA–ARS, 1700 SW 23 Drive, PO Box 14565, Gainesville, FL 32604, USA*

[b] *Department of Entomology and Nematology, University of Florida, PO Box 110620, Gainesville, FL 32611, USA*

[c] *Biological Control and Mass Rearing Research Unit, USDA–ARS, Box 5367, 810 Highway 12E, Mississippi State, MS 39762, USA*

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Abstract

Analysis of extracts of hemolymph obtained from sexually mature alate females of *Solenopsis invicta* from monogyne colonies resulted in identification of juvenile hormone III (JH III). The average amount of JH III was 0.32 ± 0.04 pmol/μmolof hemolymph. Topical application of 0.038 pmol of JH III was sufficient to stimulate alates to shed their wings in the presence of the queen. The time in which alates were induced to dealate decreased linearly with increasing concentrations of JH III from 0.038 to 3.8 pmol. However, higher JH III concentrations deviated from linearity and did not reach dealation times comparable with those that occur after mating flights. Thus, it appears that the mechanism of dealation that occurs when female alates are out of the influence of their queen is different from the one associated with mating flights. Application of 0.42 μmol of precocene II inhibited dealation of alates in queenless colonies. However, this inhibition was reversed after applying 38 pmol JH III to precocene-treated alates. The sizes of corpora allata (CA) from sexuals treated with JH III did not differ from those of controls. However, the sizes of CA were reduced in alates treated with precocene II. The results indicated that JH was important to dealation. Published by Elsevier Science Ltd.

Keywords: Solenopsis invicta; Female alates; Flight; Dealation; Corpora allata; Juvenile hormone III; Precocene II

1. Introduction

Juvenile hormone (JH) is vitally important in the control of insect development and reproduction (Denlinger, 1985; Hoffmann and Lagueux, 1985; Pener, 1985; Raabe, 1989; Nijhout, 1994). Among the eusocial Hymenoptera, JH has been shown to, or is hypothesized to, regulate a number of different aspects of adult female life (Robinson and Vargo, 1997). For example, JH III has been identified in the European honey bee, *Apis mellifera*, and probably plays a role in age polyethism. Amounts of JH are lower in younger nurses than in older foragers (Fluri et al., 1982). However, nurses that are isolated from foragers possess unnaturally high amounts of JH and initiate foraging activities (Robinson et al., 1989). This suggests that the interaction between foragers and nurses prevents precocious behavioral development in nurses by limiting JH production (Huang and Robinson, 1992). The development of the hypopharyngeal glands in *A. mellifera* may also be under the control of JH (Fluri et al., 1982; Sasagawa et al., 1989). These exocrine glands are largest in nurses, and materials from the glands are fed to brood. The high degree of development of the hypopharyngeal glands has been shown to be associated with low JH biosynthesis, and the administration of synthetic JH to workers induces premature degeneration of the glands (Rutz et al., 1976; Huang et al., 1994). JH titers also change in queen honey bees. Egg-laying queens, with mature ovaries, have lower JH titers than young uninseminated queens, whose ovaries are less mature (Robinson et al., 1991; Fahrbach et al.,

* Corresponding author. Tel.: +1-352-374-5788; fax: +1-352-374-5707.

E-mail address: pteal@gainesville.usda.ufl.edu (P.E.A. Teal).

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1995). Conversely, in the bumble bee, *Bombus terrestris*, JH titer is positively correlated with ovarian development (Bloch et al., 2000) and studies have shown that topical application of JH I induces a dose-dependent increase in egg development (Röseler, 1977). Additionally, rates of JH biosynthesis are higher in dominant worker bumble bees than in subordinates (Larrere and Couillaud, 1993) and dominance in workers in queenless colonies can be induced by injection of JH I (van Doorn, 1989). Thus, in this species, JH acts as a gonadotropin and also affects division of labor. Studies have also indicated that JH plays these same roles in *Polistes* wasps (Bohm, 1972; Barth et al., 1975; Röseler et al., 1984). In a Malaysian *Diacamma* ant species the queen caste is absent, but an inseminated worker (gamergate) reproduces and establishes reproductive dominance by physically attacking newly eclosed workers and removing the gemmae, a pair of tiny bladder-like appendages attached to the thorax (Peeters and Crewe, 1984). No detectable amounts of JH were found in reproductives; however, JH III was identified in non-reproductives and JH titers were shown to increase with age (Sommer et al., 1993). Thus, in this species JH does not appear to have gonadotropic effects but may have effects on division of labor as in honey bees.

Although JH has not been identified in the red imported fire ant, *Solenopsis invicta*, an insect that possesses a similar degree of social complexity to honey bees (Michener, 1974; Fletcher and Ross, 1985), there is evidence supporting the action of JH in regulating adult physiology and behavior. Mating flights and subsequent dealation (casting of wings) by female sexuals are important events for colony establishment. Mature female and male sexuals leave the colony and engage in a mating flight during suitable weather conditions (Markin et al., 1972; Vinson and Sorenson, 1986; Milio et al., 1988). Before leaving the colony to mate, female alates are prevented from shedding their wings and developing their ovaries by a queen primer pheromone. However, when removed from queen pheromonal influences, female alates shed their wings and ovaries develop (Fletcher and Blum, 1981a,b, 1983; Vargo, 1999). Topical applications of synthetic JH I, II and III (Kearney et al., 1977) or methoprene (Vargo and Laurel, 1994), have been shown to induce dealation and ovary development, even in the presence of the queen. This suggests that the queen pheromone operates by suppressing JH titers in cohabiting alates (Vargo and Laurel, 1994).

We report here the results of studies aimed at the identification and quantification of juvenile hormone from the hemolymph of female sexuals. We also report results of studies using natural JH and precocene (a compound that destroys the source of JH, the corpora allata) to probe the relationship between JH and the dealation of female fire ant alates.

2. Methods and materials

2.1. Maintenance of colonies

Solenopsis invicta colonies containing sexual brood were collected from Gainesville, Florida, and were separated from the soil by flooding (Jouvenaz et al., 1977). Each colony was placed in a large tray (52 cm long by 39 cm wide by 7.5 cm deep) with inner sides coated with Fluon® to prevent ants from escaping. Colonies were provided with four nest cells consisting of a Petri dish (14 cm diameter) with a Castone® bottom and three holes drilled into the sides of the dish to permit movement of ants in and out of the cell; the plaster bottom was moistened with water to increase humidity in the nest cell (Drees and Ellison, 1998). Each Petri dish was covered with red cellophane to imitate natural, dark nest conditions. Colonies were fed a diet of crickets and 10% sugar water absorbed on tissue wads, and 10% sugar water in test tubes plugged with cotton balls (Obin, 1986). Ants were maintained in the laboratory at 27 °C and 47% humidity. Colonies were determined to be monogynous based upon the following characteristics: low mound density, large, well-developed nests, polymorphic colony workers (Greenberg et al., 1985), a single inseminated queen, and a high degree of conspecific aggression (Morel et al., 1990).

2.2. Collection of hemolymph

Hemolymph was collected from the thorax of sexually mature *S. invicta* female alates (14 or more days after eclosion). Each alate was pinned dorsally through the thorax at an angle, and pressure was applied with forceps to the base of the thorax, allowing hemolymph to exude from the wound. A fused silica needle (0.15 mm, o.d.) held in a 10 μl gas-tight syringe was used to withdraw hemolymph, which was then placed in a conical vial held on ice. Once 5 μl of hemolymph was collected (ca. 0.15 μl/alate), the sample was centrifuged at 8000***g*** for 2 min to ensure that the hemolymph was at the bottom of the tube. Forty-five microliters of methanol and 100 μl of hexane containing 10 pg/μl of farnesyl acetate (internal standard) were added to the vial of hemolymph. A crimp cap was placed on the vial, and the vial was vortexed. The vial was centrifuged at 18,000***g*** for 5 min and the organic layer was removed. The aqueous layer was extracted two additional times with hexane, and organic layers were combined for analysis.

2.3. Purification and analysis of JH from hemolymph

An aliquot of the hexane extract equivalent to 16 μl of hemolymph was concentrated to 20 μl under a fine stream of N_2 and subjected to purification by liquid chromatography. The sample was injected on to an Adsorb-

osphere silica column (15 cm × 2.1 mm, i.d.; 3 μm particles, Alltech Associates), with a Rheodyne 7125 injector (50 μl loop). The column was eluted at 0.3 ml/min with 1% ethanol in hexane, with a Spectroflow 400 pump. A Spectroflow 757 UV detector set at 210 nm was used to detect peaks. Under these conditions, JH III eluted at 4.8 min, JH II at 5.5 min, and JH I at 6.8 min. When the natural extract was chromatographed, fractions were collected at 0–4 min, 4–7 min (JH fraction) and 7–14 min. The fraction containing JH was concentrated to 50 μl, and a 3 μl aliquot was analyzed by gas chromatography–mass spectroscopy (GC–MS) (see below). Subsequently, we analyzed aliquots of the hexane extracts (ca. 0.25 μl equivalents of hemolymph) from each of 10 samples (5 μl of hemolymph/sample) by GC–MS directly without purification (see Teal et al., 2000).

2.4. Gas chromatography–chemical ionization mass spectroscopy

GC–MS analysis was performed with a Finnigan-MAT ITS 40 ion-trap mass spectrometer (MS) operated in chemical ionization (CI) mode (isobutane reagent gas) and interfaced to a Varian Star 3400 gas chromatograph (GC) equipped with a cool-on column injector. The analytical column in the GC, a DB5-MS capillary column (30 m × 0.25 mm, i.d.; 0.1 μl film thickness, J&W Scientific), was interfaced to a 10 m × 0.25 mm(i.d.) uncoated, deactivated fused silica retention gap and a 10 m × 0.5 mm (i.d.) length of uncoated, deactivated fused silica in the GC injector (Teal et al., 2000). The initial injector temperature for chromatography was 40 °C for 30 s, and temperature increased at 170 °C/min to 270 °C. The initial column temperature for chromatography was 40 °C for 5 min, and temperature increased at 5 °C/min to 210 °C. The linear flow velocity of the He carrier gas was 24 cm/s, and the GC/MS transfer line temperature was 230 °C. Under these conditions, farnesyl acetate (FA), JH III, JH II and JH I eluted at 32.3, 33.8, 35.4 and 37.3 min, respectively. Prior to analysis of natural products we conducted a concentration gradient study in which we analyzed amounts of synthetic JH I, JH II and JH III and farnesyl acetate (FA), the internal standard, ranging in concentration from 0.01 to 5.0 pmol. Linear regressions were calculated for each of the diagnostic ions used for routine quantitative purposes (JH I: m/e = 263, 245, 217, 161, 153 and 111; JH II: m/e = 249, 231, 203, 147, 139 and 111; JH III: m/e = 235, 217, 189, 147, 125 and 111; FA: m/e = 81, 109, 121, 135, 149 and 205—see Teal et al., 2000 for a description of cleavage patterns and use of the internal standard). We then analyzed natural product samples. Identification of JH in natural samples was based on the comparison of fragmentation patterns and retention indices of compounds eluting during analysis of natural product samples with those of synthetic standards. Quantification of amounts of JH was accomplished as described by Teal et al. (2000).

2.5. Timing of dealation with JH III treatments

Queenright colonies were comprised of 10 g of worker adults, 1.5 g of worker brood and 10 female alates. These colony units were housed in a porcelain tray (29 cm long by 18 cm wide by 5 cm deep), having sides coated with Fluon®, and were provided with one nest cell (10 cm diameter Petri dish). Ants were fed crickets, sugar water and water.

Juvenile hormone III was obtained from Sigma. Each alate in a queenright subunit was treated topically on the head with 1 μl of acetone or 1 μl of acetone containing either 0.038, 0.11, 0.38, 1.1 or 3.8 pmol JH III. Alates from queenright subunits were observed every 12 h to monitor dealation, defined as the removal of at least three of the four wings (Vargo and Laurel, 1994). Soon after dealation, virgin dealates begin to produce the primer pheromone that inhibits dealation by cohabiting female alates (Vargo, 1999). Therefore, in this study, dealates were removed immediately following wing casting.

A separate set of alates from queenright subunits was observed every hour to monitor dealation. Each of these alates was topically treated on the head with either 76 pmol JH III in 1 μl acetone or 1 μl acetone (control). In order to linearize the relationship between percentage dealation and time of dealation, percentages were converted to Probits. Data were then analyzed by Pearson Chi-Square Goodness-of-fit tests.

2.6. Timing of dealation with precocene II treatments

We examined the effects of precocene II (Aldrich) on dealation in female fire ant alates by applying either 280, 330, 380, 420 or 470 nmol precocene in 1 μl of acetone, or 1 μl acetone (control), to alates in queenless subunits composed of workers, brood and alates. Alates from the queenless subunits were observed for dealation every 12 h, as described above, for a total of 108 h.

2.7. Measurement of corpus allatum

Following dealation observations, the corpora allata (CA) of sexuals treated with 3.8 pmol JH III in 1 μl acetone, 470 nmol precocene II in 1 μl acetone, and 1 μl acetone were measured. The head was positioned dorsally in a wax-covered Petri dish, and minutien pins were placed through the compound eyes. Dissections were executed on the posterior portion of the head of the ant. The cuticle of the head was cut in a circular direction, clockwise from the right eye to the base of the head and counter-clockwise from the left eye to the base of the

head, and then removed with fine forceps. Anterior muscles and glands were excised to better observe the brain. The brain was then lifted to acquire access to the CA. Once the CA were located, the brain and CA were excised and placed on a microscope slide. The cross-sectional area of an individual coprora allatum was determined by measuring the diameter of the gland with an ocular micrometer.

2.8. Effect of JH III replacement therapy on dealation among precocene-treated alates

Precocene-treated (420 and 470 nmol) alates in queenless units that had not dealated after 108 h were combined in a single pool from which 45 were removed at random. Fifteen of these were treated by topically applying 1 μl of acetone. Two other sets of 15 were treated with 1 μl of acetone containing either 38 pmol or 76 pmol of JH III. These were then placed into queenless colony fragments with approximately equal numbers of workers and brood, and observed every 12 h to monitor dealation. Dealates were removed following wing casting.

3. Results

3.1. Isolation and identification of JH III

GC–MS analysis of fractions collected from the liquid chromatographic fractionation of the hexane extract of hemolymph resulted in identification of JH III (Fig. 1). No other homologs were detected. Similarly, only JH III was found in the 10 samples of hexane extracts of hemolymph analyzed directly by GC–MS. The mean [±standard error (SE)] amount of JH III per μl of hemolymph was 0.32 ± 0.04 pmol (n = 10).

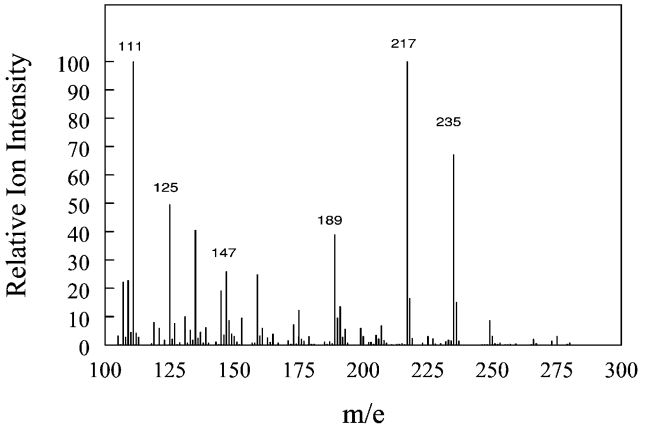

Fig. 1. Chemical ionization mass spectrum of naturally occurring JH III present in the hemolymph of sexually mature alates of *S. invicta*. The fragmentation pattern of synthetic JH III was identical to that of the natural product. Diagnostic ions used for quantification are indicated on the spectrum and are described fully in Teal et al. (2000).

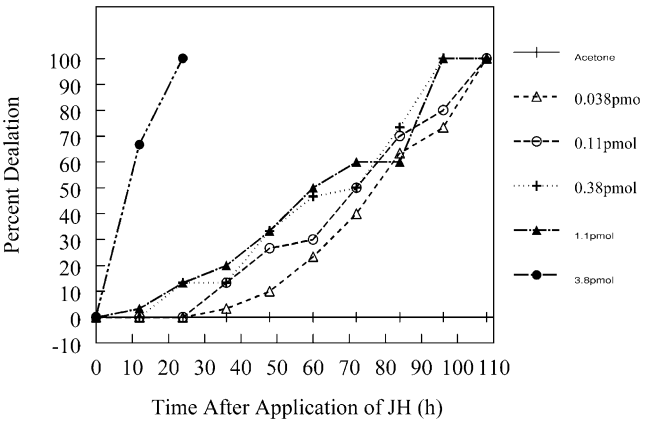

Fig. 2. Rates of dealation of sexually mature *S. invicta* female alates in queenright colonies after topical application of either 1 μl of acetone or 1 μl of acetone containing 0.038, 0.11, 0.38, 1.1 or 3.8 pmol of JH III. n = 30 per treatment.

3.2. Time of dealation with JH III treatments

Results showing the effect of JH III on the rate of dealation are presented in Fig. 2. All alates treated with JH III dealated within 108 h of applying hormone treatments. Comparison of Probit slopes showed that alates treated with 3.8 pmol JH III shed their wings at a significantly higher rate (χ^2 = 57.9, df = 4, $P < 0.05$) than alates given lower doses. One hundred per cent dealation occurred within 24 h in alates treated with 3.8 pmol JH III (n = 30). Alates treated with 1 μl acetone (n = 30) did not shed their wings during the 108 h observational period. There were no significant differences (χ^2 = 6.49, df = 3, $P > 0.05$) in rates of dealation among alates given JH III treatments ranging from 0.038 to 1.13 pmol JH.

Fig. 3 shows the results of applying JH III to alates

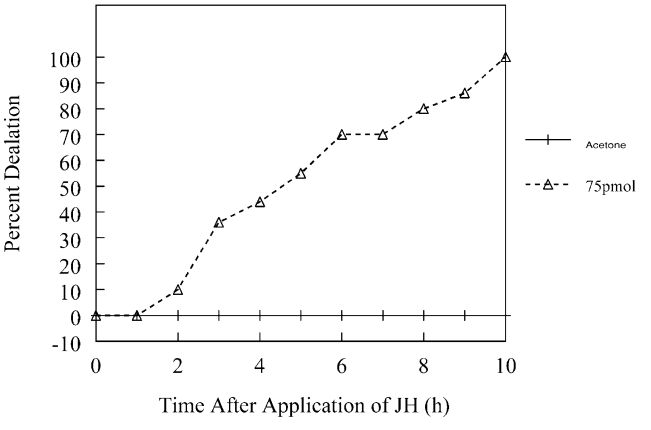

Fig. 3. Rates of dealation of sexually mature *S. invicta* female alates in queenright colonies after topical application of either 1 μl of acetone (n = 10) or 1 μl of acetone containing 75 pmol of JH III (n = 20).

in queenright colonies at a concentration 20 times higher than in the previous experiment. Observations taken every hour revealed that 100% ($n = 20$) dealation occurred within 10 h of applying 76 pmol JH III. Fifty-five per cent dealation occurred within 5 h of JH III application. Alates administered 1 μl acetone ($n = 10$) did not shed their wings during the 10 h observational period (Fig. 3).

A plot of the time to 50% alate dealation, DT_{50}, for all JH concentrations except the highest concentration (76 pmol) showed a very high linear correlation (Fig. 4, insert, $y = -18.4x + 79.1$; $r^2 = 0.98$). However, when the data for the 76 pmol JH III application are added to the data set, the linear regression degenerates to $y = -0.810x + 62.607$; $r^2 = 0.484$ (Fig. 4). Therefore, it appears that concentrations of JH III greater than 3.8 pmol do not follow the downward trend in time to dealation of the lower JH III concentrations and have little effect on the time of dealation (Fig. 4).

3.3. Time of dealation with precocene treatments

The results of experiments to determine the effect of the topical application of precocene on dealation are shown in Fig. 5. Alates in queenless colonies were used because queens produce a primer pheromone that inhibits alates from shedding their wings. Normally 100% dealation occurs within 108 h when alates are removed from the influence of their queen (see acetone control, Fig. 5). There were no significant differences ($\chi^2 = 5.53$, df = 3, $P > 0.05$) in the dealation rates of alates given 280–380 nmol precocene II ($n = 30$/treatment) and 1 μl acetone ($n = 30$). However, significantly more alates treated with 420 nmol ($n = 30$) or 470 nmol ($n = 30$) precocene II retained their wings [analysis of variance (ANOVA), $F_{1,13} = 56.0$, $P < 0.05$] than those given lower doses. Only 17 and 3% of alates treated with 420 or 470 nmol precocene II, respectively, dealated within 108 h ($n = 30$/dose).

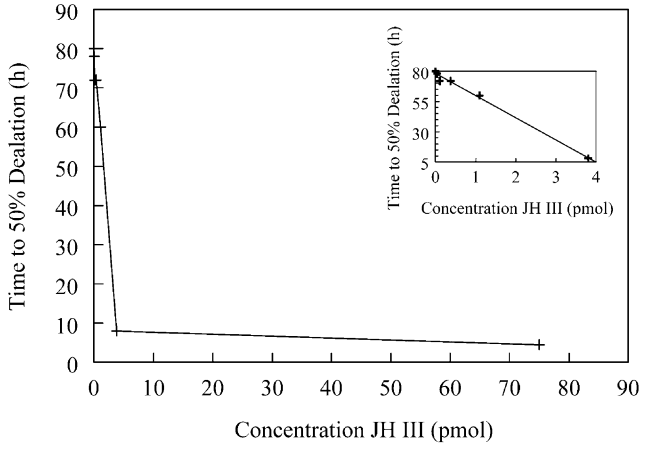

Fig. 4. Plot of the time to 50% dealation (DT_{50}) for the various JH III concentrations applied to fire ant female alates. Insert shows the linear relationship between DT_{50} and JH III concentrations ranging from 0.038 to 3.8 pmol.

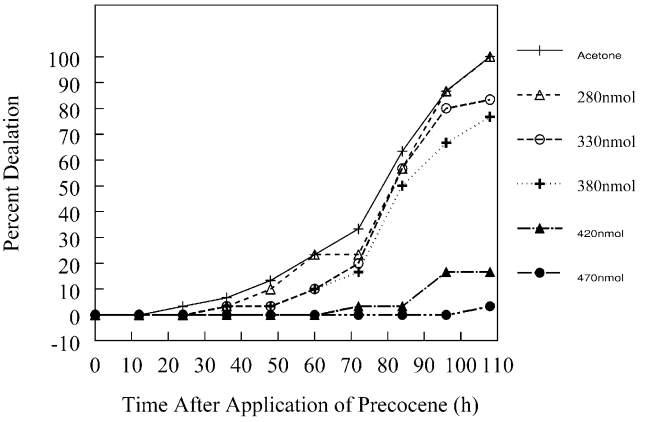

Fig. 5. Rates of dealation of sexually mature *S. invicta* female alates in queenless colonies after topical application of either 1 μl acetone or 1 μl of acetone containing 280, 330, 380, 420 or 470 nmol of precocene II. $n = 30$ per treatment.

3.4. Time of dealation with JH replacement treatment

Attempts at reversing the effects of precocene II, shown in Fig. 5, were carried out by applying 38 ($n = 15$) and 76 pmol ($n = 15$) JH III to individual alates from a pooled group of alates previously treated with either 420 or 470 nmol precocene. Within 24 h after JH III treatment, 73 and 80% of alates, respectively, shed their wings (Fig. 6). By 48 h, 93% of alates treated with 38 pmol JH III shed their wings, and 100% of alates treated with 76 pmol JH III dealated. Precocene-treated alates treated with 1 μl acetone ($n = 15$) did not shed

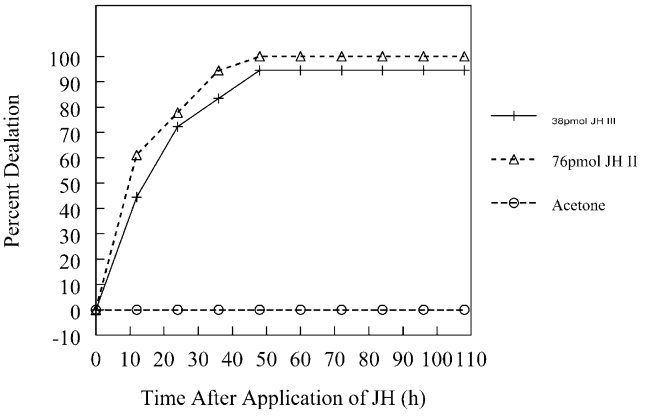

Fig. 6. Rates of dealation of precocene-treated sexually mature *S. invicta* female alates in queenless colonies following topical application of either 1 μl acetone or 38 or 76 pmol of JH III in 1 μl of acetone. Females treated initially with 420 or 470 nmol of precocene II and that did not dealate over the 108 h observation period were combined and an equal number of females ($n = 15$ per treatment) were treated with either 1 μl of acetone or 1 μl of acetone containing either 38 or 76 pmol of JH III and observed for an additional 108 h.

their wings during the 108 h observational period (Fig. 6).

3.5. Measurement of corpus allatum

The effect of JH III and precocene II treatments on the corpora allata was assessed by gland dissection and direct measurement. The difficulty of the dissection procedure precluded the measurement of both corpora allata from a single individual, thus each replicate measurement is from a different individual. A one-way ANOVA test revealed that there was no significant difference ($F_{1,8} = 0.509$, $P > 0.05$) in area measurements of CA between alates that had been treated with 3.8 pmol JH III and had dealated (40.1 ± 2.3 μm^2, $n = 5$) and alates treated with 1 μl acetone (39.9 ± 1.0 μm^2, $n = 5$). However, there was a significant difference ($F_{1,13} = 208$, $P < 0.05$) between the combined alate and dealate data above and CA measurements of alates treated with 470 nmol precocene II (35.4 ± 1.8 μm^2, $n = 5$).

4. Discussion

Despite the fact that JH is known, or has been hypothesized, to regulate a large number of physiological and behavioral events in adult Hymenoptera (Robinson and Vargo, 1997), it has been positively identified from surprisingly few species. For example, although JH had not been identified from *S. invicta*, numerous studies have provided evidence for JH regulation of ovarian development and dealation (Barker, 1978, 1979; Kearney et al., 1977; Vargo, 1992; Vargo and Fletcher, 1989; Vargo and Laurel, 1994). In our study we identified only JH III from hemolymph of sexually mature alate females. It is possible that other homologs of JH are produced because Kearney et al. (1977) found that synthetic JH I, II and III induced dealation when applied topically. However, JH III is the only homolog that has been identified from hemolymph or body homogenates of other Hymenoptera including the honey bee (Trautmann et al., 1974; Robinson et al., 1987; 1989), bumble bees (Strambi et al., 1984; Bloch et al., 2000), and in a *Diacamma* species of ant (Sommer et al., 1993).

Kearney et al. (1977) were unable to induce dealation by topical application of 380 fmol of JH I, JH II or JH III to the abdomen. In contrast, topical application of as little as 38 fmol of JH III to the head was sufficient to stimulate alates to shed their wings. There are at least four possible explanations for this contrast: (1) there may be differences in the purity of the JH III available in 1977 versus 2000; (2) Kearney et al. (1977) narcotized their alates with carbon dioxide prior to topical application of JH III, whereas our method did not use carbon dioxide (CO_2 appears to negatively affect the physiology of ants, Weir, 1957; Wardlaw, 1995); (3) our topical application of JH III to the head of the alate may be more effective than abdomen applications at getting the compound through the cuticle and achieving a critical concentration of JH III; or (4) topical application of JH III to the head of the alate rather than the abdomen may be more effective at getting JH III to a site that activates dealation.

The fact that topical application of only 38 fmol of JH III was sufficient to induce dealation indicates that the amount of JH III needed to trigger dealation, or overcome the effects of the queen-produced dealation inhibitory primer pheromone (presumably acting through control of JH levels; Vargo and Laurel, 1994), is quite small. Indeed, effective dealation was induced by application of an order of magnitude less JH than that present per μl of hemolymph. However, each alate probably contains less than 1 μl of hemolymph, and the critical level of JH III required to stimulate dealation may not be much higher than that present in hemolymph. Thus, the sudden burst of JH provided by a single topical application of 38 fmol may be all that is required to induce dealation, particularly if a sudden perturbation in the JH level is more important than hemolymph JH III concentration. The point of JH III application (head vs. abdomen) may dictate what exogenous JH III concentration will trigger dealation.

The rates of dealation for alates treated with JH III from 0.038 to 1.1 pmol, a 29-fold concentration range, at first appear to be indistinguishable and very similar to the rate of dealation found for alates simply removed from the influence of their queen (see acetone control, Fig. 5). However, a plot of the time to 50% dealation versus JH III concentration gave a linear relationship through a 100-fold concentration range. These data support the causative role JH plays in the dealation process associated with alates out of the influence of their queen.

In nature there are two unique routes to dealation for fire ant female alates. The first is when a colony containing alates loses its queen. This eliminates the queen primer pheromone responsible for maintaining the alate condition in female sexuals and results in dealation. However, dealation under this circumstance is a slow process sometimes requiring days. The second route to dealation is when female alates go on mating flights, mate, land on the ground, and very soon thereafter dealate. The entire process from beginning of the mating flight to dealation may take only 30 min; therefore, the two dealation events, loss of queen and mating flight, appear to be triggered by different events and are perhaps governed by different mechanisms.

If the dealation process associated with mating flight activity were an extension of the mechanism identified with primer pheromone disinhibition, then we would expect that the linear relationship between JH concentration and the time for 50% of the alates to dealate would continue to the 0.5 h observed for female alates

participating in mating flights. However, a further 20-fold increase in the JH III concentration applied to the head of alates only reduced the DT_{50} to 4 h, still not comparable to dealation times from mating flights, and the linear relationship is lost. This strongly suggests that dealation after mating is governed by a different mechanism than that for dealation after disinhibition from queen-produced primer pheromones.

Previous studies that have addressed the influence of precocenes in 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 *Apis mellifera* caused the development of worker-like intermediates and that 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 have shown here for the first time that precocene has anti-JH activity in the Formicidae: specifically we have demonstrated that precocene II treatments atrophy the corpora allata of *S. invicta*. Allatal atrophy suggests a decrease in JH production (Bowers, 1983). This was demonstrated to the extent that JH levels are involved in dealation, since precocene applications prevented alates from shedding their wings when they were out of the influence of their queen.

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, thereby causing necrosis in the CA (Wawrzenczyk, 1997). It is possible that the high concentrations of precocene II needed to alter the dealation process caused general toxicity that affected the ability of the alates to dealate. Two pieces of information mitigate against this possibility. The first is that corpora allata were significantly smaller in precocene-treated alates, indicative of atrophy. Secondly, JH III replacement therapy succeeded in overcoming the effects of the precocene, thus supporting JH involvement and suggesting that the effect of the precocene was limited to the suppression of JH production.

In classic experiments by Bowers et al. (1976), precocene II and JH III recovery applications were instrumental in examining JH regulation of development and reproduction in Hemiptera. Bowers and his colleagues (1976) found that exogenous treatments of JH III reversed precocious maturation and sterility in precocene-treated Hemiptera. Barker (1978) found that applications of a synthetic mixture of JH I isomers (3.8–38.0 nmol) were sufficient to override the inhibition to dealate in surgically allatectomized *S. invicta* alates. In the present study, precocene-II-treated alates, out of the pheromonal influence of their queen, generally dealated following JH III applications. These JH "recovery" treatments gave further support of JH's role in dealation (Kearney et al., 1977; Barker, 1978) and of the queen's influence in suppressing endogenous JH titers and wing casting in cohabiting alates (Fletcher and Blum, 1983; Vargo, 1992; Vargo and Laurel, 1994).

In summary, the results of our study have provided the first direct evidence that JH III is produced by sexually mature alates of *S. invicta* and that topical application of physiologically relevant amounts of this homolog alone will stimulate females to shed their wings while under the influence of the queen-produced dealation inhibitory primer pheromone. The fact that precocene inhibited wing casting and that effects of this compound could be overcome by application of JH supports the importance of JH III titer for the induction of dealation in disinhibited *S. invicta* female alates. Interestingly, it appears that the very rapid dealation process that occurs after mating is probably governed by a different mechanism than the much slower dealation after female alates are out of the influence of their queen.

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