Field and Laboratory Evaluation of Female Sex Pheromone for Detection, Monitoring, and Management of Brownbanded Cockroaches (Dictyoptera: Blattellidae)

DANGSHENG LIANG,[1] AIJUN ZHANG,[2] ROBERT J. KOPANIC, JR., WENDELL L. ROELOFS,[2] AND COBY SCHAL

Department of Entomology, North Carolina State University, Raleigh, NC 27695

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ABSTRACT The synthetic sex pheromone of the female brownbanded cockroach, *Supella longipalpa* (F.), was highly attractive to males in the field. Supellapyrone dispensers used in our experiments showed nearly constant pheromone release rates up to 30 d. Trapping efficacy in apartments was positively correlated with the amount of pheromone used in traps. Glue traps without pheromone captured more nymphs than did jar traps, but the latter were superior in trapping adult males using pheromone. Moreover, jar traps with pheromone caught more total cockroaches than the other 2 trap types. Two commercial baits that presumably contain attractants were compared with the female sex pheromone by using jar traps. Both attracted all life stages and both sexes of the brownbanded cockroach; they increased trap catch 8-fold relative to unbaited jars. Jar traps baited with 1 μg of synthetic pheromone in a slow-release formulation captured twice as many cockroaches as traps with either of the commercial baits. Combination of the food attractant and pheromone resulted in further increases in trap catch. The pheromone increased the number of adult males in all treatments by 6–28 times relative to the respective controls. Supellapyrone thus offers a powerful monitoring and pest management tool.

KEY WORDS *Supella longipalpa*, supellapyrone, sex pheromone, trapping, pest detection, monitoring

VOLATILE FEMALE SEX pheromones have been identified in several pest cockroach species, including the American cockroach, *Periplaneta americana* (L.) (Persoons et al. 1979), *P. fuliginosa* (Serville) (Takahashi et al. 1995), *P. japonica* Karny (Takegawa and Takahashi 1989), and the brownbanded cockroach, *Supella longipalpa* (F.) (Charlton et al. 1993). The female sex pheromone of the brownbanded cockroach is 5-(2,4-dimethylheptanyl)-3-methyl-2*H*-pyran-2-one, or supellapyrone (Charlton et al. 1993). Only the (2′R, 4′R)-isomer is produced by females (Mori and Takeuchi 1994, Leal et al. 1995). Females release the pheromone during a calling behavior that is exhibited only during the scotophase (Smith and Schal 1990). In laboratory olfactometer tests, this pheromone was highly attractive to brownbanded cockroach males (Liang and Schal 1990a, b) that responded to as little as 0.0001 ng of a synthetic racemic blend on filter paper (Charlton et al. 1993).

With heightening popular awareness of the potential negative effects of pesticides, there is an increasing need to practice environmentally sound insect pest management. Sex pheromones are highly species specific, nontoxic compounds that have great potential in cockroach control. They may be used in detection, monitoring, mass trapping, mating disruption, and in attract-and-kill strategies for controlling economically important pest species. The sex pheromone of the American cockroach was found to increase the number of males captured in traps both in laboratory (Bell et al. 1977) and field tests (Chow et al. 1976, 1981; Bell et al. 1984). Using synthetic periplanone-B, a component of the pheromone, Bell et al. (1984) also showed that the pheromone could be used effectively to reduce the repellency of insecticides and thus result in better control by attracting males to insecticide-treated areas. Our recent identification and synthesis of supellapyrone, the female sex pheromone of the brownbanded cockroach, has led to the current study. The objective of this study was to evaluate supellapyrone as a potential tool for detection and monitoring of populations of the brownbanded cockroach. We propose that combining pheromone with food attractants can further enhance overall trap efficacy, and we conclude that supellapyrone should be integrated into *S. longipalpa* pest management programs.

Materials and Methods

Pheromone. Supellapyrone was synthesized as described by Zhang (1992) as a mixture of 4 isomers and formulated by Hercon (Emigsville, PA) into laminated slow-release dispensers. Each dispenser (2.5 by

[1] Current address: The Clorox Service Company, 7200 Johnson Drive, Pleasanton, CA 94588.

[2] Department of Entomology, New York Agricultural Experimental Station, Cornell University, Geneva, NY 14456.

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2.5 cm) contained 1 μg of racemic supellapyrone. Of the 4 stereoisomers, only the (2'R,4'R)-isomer is behaviorally active (D.L., unpublished data).

Electroantennogram (EAG). The EAG apparatus was similar to that described in Schal et al. (1992). A detached head from an adult male was immersed in saline that was in contact with the reference electrode. The tip of an antenna was cut and inserted into a silver-silver chloride Pasteur pipette recording electrode filled with insect saline. The antenna was exposed to a stream of purified, humidified air. The pheromone dispenser, which was cut into smaller sections when necessary, was placed inside a Pasteur pipette and 2 ml of air was puffed over it into the airstream. There were 3 replications for each treatment. The EAG reponse of each replication was an average reading of a total of 12 puffs, 3 each repeated 4 times. The EAG responses to control puffs of air were subtracted from experimental results.

Field Sites and Insects. Field trapping experiments were conducted during the Summer of 1995 in several locations in Raleigh, NC, including laboratories in the Department of Entomology, North Carolina State University, and in vacant apartments of the Raleigh Housing Authority. We released several hundred nymphs and adults of unknown ages into each of 6 recently vacated apartments. Cockroaches were given at least 3 d to redistribute within the apartments before the start of trapping experiments.

Effect of Pheromone Concentration on Trap Catch. Three different pheromone release rates were achieved by cutting pheromone dispensers into pieces of predetermined sizes (1, 0.25, 0.06 units of the dispenser). One piece of pheromone dispenser was placed into each glass jar (0.5-liter mason jars), the inner walls of which were coated with petroleum jelly to prevent trapped cockroaches from escaping. The jars were wrapped in a paper towel held in place with rubber bands to facilitate cockroach climbing. An empty jar served as control. A randomized complete block design was used. Within each replication, a 4-d trapping session was conducted. All 4 treatments were placed in a room together and trapped insects were counted and removed daily. The placement of each pheromone dosage was rotated based on a predetermined scheme; thus, each trapping location experienced all 4 possible treatments. A total of 3 replications was conducted.

Comparison of Traps. Three types of traps were tested: Jar trap, Mr. Sticky trap, and Raid Roach trap. Mr. Sticky (Leon Wright Enterprises, Yonkers, NY) is a triangular glue trap with a flat sticky floor. Raid trap (S.C. Johnson, Racine, WI) is rectangular with 2 inclined ramp entrances leading to sticky surfaces on all 4 inner walls. Both glue traps presumably contained proprietary cockroach attractants. One slow-release dispenser containing 1 μg of supellapyrone was used in each treated trap. Traps without pheromone dispenser served as control. The placement of the 6 treatments (3 trap types × 2 pheromone levels) in the field was based on a randomized complete block design so that each treatment was tested once at each trapping location during the test. Trapped cockroaches were counted and removed daily.

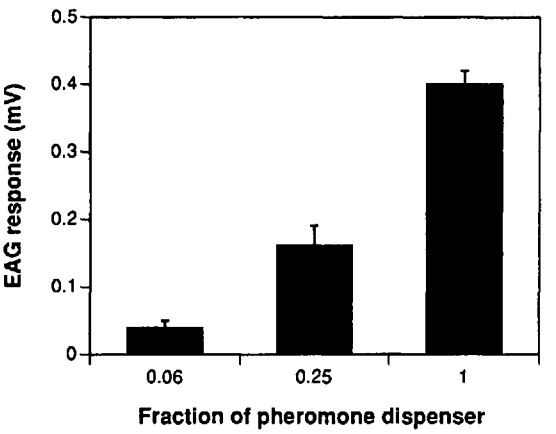

Fig. 1. Electroantennogram responses of *S. longipalpa* male antennae to slow-release pheromone dispensers of 3 different sizes. Responses to blank controls were subtracted from the individual responses to supellapyrone. Bars represent SEM (n = 3).

Comparison with Food Attractants. Effectiveness of commercial attractants and supellapyrone were tested with jar traps. Two types of food attractants were tested: Avert 310 powder bait and Biosys GP-2 attractant tablets (AgriSense, Palo Alto, CA). Avert 310 insecticidal bait (Whitmire Micro-Gen, St. Louis, MO) was found to be highly attractive to brownbanded cockroaches (D.L., unpublished data). Five hundred milligrams of fresh Avert bait in a 20-ml scintillation vial cap (1.5 by 1.0 cm i.d.) was used in each jar. Biosys GP-2 attractant tablets (Biosys, Decatur, IL) are normally packaged with AgriSense Lo-Line traps (AgriSense) and also presumably contain food attractants. One pellet (700 mg) was used in each jar. These pellets were compared with 1 dispenser unit of supellapyrone and a blank control. There were 6 treatments: (1) empty jar; (2) Avert 310 only; (3) Biosys GP-2 only; (4) supellapyrone only; (5) Avert 310 and supellapyrone; and (6) Biosys GP-2 and supellapyrone. The design was a randomized complete block with 6 treatments over 6 consecutive nights so that each treatment was placed at each location once.

Statistical Analysis. Other than as specifically mentioned, analysis of variance (ANOVA) of the number of trapped cockroaches was conducted with the general linear model (GLM) procedure (SAS Institute 1990). Mean separation was achieved using the Ryan multiple range test.

Results

Pheromone Dispensers. The relative amount of supellapyrone released from dispensers was examined with the EAG technique. As air was puffed through the dispenser, the relative EAG response represented the amount of pheromone released by the dispenser. As expected, EAG responses were positively correlated with the surface area of dispenser used (Fig. 1).

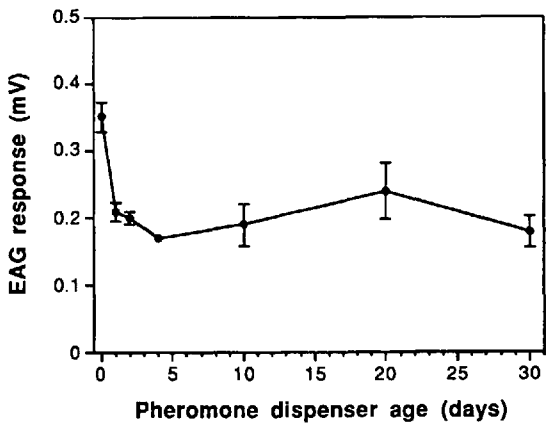

Fig. 2. Electroantennogram responses of *S. longipalpa* male antennae to one unit of slow-release pheromone dispenser (containing 1 μg of synthetic supellapyrone) aged in an open container at room temperature for various time periods. Bars represent SEM (n = 3).

One dispenser unit (containing 1 μg of supellapyrone) elicited an ≈0.4 mV EAG response and even 0.06 unit of dispenser elicited significant EAG responses.

Aging of pheromone dispensers was examined using EAG during a 1-mo period (Fig. 2). The EAG responses to a previously frozen fresh dispenser were significantly higher than to all aged dispensers (ANOVA, F = 6.58, df = 6, P = 0.0018). From day 1 on, the pheromone release rate remained fairly constant through the 30-d testing period. This release rate suggested that the dispensers emitted constantly over time and could be used in long-term monitoring and detection of cockroach populations.

The mean number of trapped adult male cockroaches was dose dependent (Fig. 3). When a series of dosages of pheromone were compared in the field, the number of trapped males positively correlated with the amount of pheromone placed in traps. One

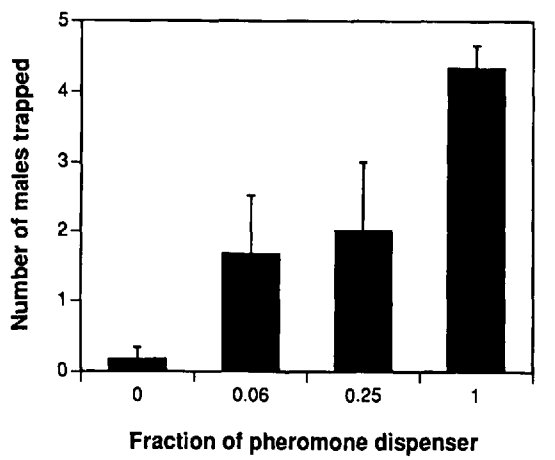

Fig. 3. Number of *S. longipalpa* males captured in jar traps baited with various amounts of slow-release pheromone dispensers. Bars represent SEM (n = 6).

dispenser unit trapped an average of 4.33 ± 0.33 (mean ± SEM) adult males, significantly more than other doses (F = 7.27, df = 3, P = 0.003). However, even the smallest amount tested (0.06 dispenser unit) attracted 10 times more males than the control.

Trap Types and Supellapyrone. The trapping efficacies of 3 trap types (glass jar, Raid, Mr. Sticky) were compared in field studies (Table 1). The mean numbers of males captured in unbaited traps were similar and low for all 3 types of traps (F = 0.20, df = 2, P = 0.82), ranging from 0.7 to 1.0 males per trap. Thus, attractants that were formulated into the glue traps were relatively ineffective for adult male brownbanded cockroaches. Addition of the female sex pheromone increased the number of trapped males. However, statistical analysis revealed that this pheromone-induced increase in trapped males was highly dependent on trap type (F = 27.54, df = 2, P < 0.0001). The glass jar trap had the most dramatic pheromone-induced increase in number of males trapped with a mean trap catch of 14.7 males per trap compared with 0.7 males per trap in the unbaited control, a more than 20-fold increase. In contrast, only 3- to 4-fold increases were observed for glue traps (Table 1).

The number of trapped adult females and nymphs was not affected significantly by presence of the female sex pheromone (Table 1). Without the pheromone, more nymphs were trapped than either males or females, probably because nymphs accounted for >80% of the total released population. More nymphs were caught in Raid traps than in the other 2 trap types. However, statistical analysis showed a significant effect of trap location on nymph catch (F = 4.42, df = 5, P = 0.0071), indicating that trap placement could affect the number of trapped nymphs. These data suggest a limited foraging range for nymphs of the brownbanded cockroach. In contrast, the number of trapped males or females was not affected by trap location (P = 0.24 and P = 0.60, respectively). When the total number of trapped cockroaches was analyzed, Raid traps had the highest trap catch without pheromone because of the large number of nymphs they caught. However, supellapyrone significantly increased the total number of cockroaches trapped in jar traps because of its greater efficacy of trapping adult males. As a consequence, jar traps baited with pheromone caught the highest total number of cockroaches (Table 1).

Food Attractants and Supellapyrone. When all stages and sexes were considered together, addition of supellapyrone resulted in a 16-fold increase in the total number of cockroaches captured in otherwise blank jars (Table 2). Commercial food baits (Avert 310 bait and GP-2 Roach Attractant tablet) also significantly increased the total number of insects in jar traps, but only by 8- to 9-fold relative to controls (F = 4.49, df = 2, P = 0.030) (Table 2). No differences were observed between the 2 baits. When pheromone was used in combination with food baits, a further 2.5-fold increase in the total number of trapped insects was observed relative to food baits alone (F = 4.46, df =

Table 1. Captures (mean ± SEM) of *S. longipalpa* using 3 different trap designs with or without female sex pheromone

Stage/Sex	Trap type	Effect of trap and pheromone		
		(−) Pheromone	(+) Pheromone	Pheromone
Males	Jar trap	0.7 ± 0.3a	14.7 ± 2.0b	***
	Mr. Sticky	1.0 ± 0.4a	3.7 ± 0.8a	*
	Raid trap	0.7 ± 0.5a	2.0 ± 0.8a	NS
Females	Jar trap	0.3 ± 0.3a	0.0 ± 0.0a	NS
	Mr. Sticky	0.2 ± 0.2a	0.3 ± 0.2a	NS
	Raid trap	0.2 ± 0.2a	0.3 ± 0.3a	NS
Nymphs	Jar trap	2.8 ± 0.6a	1.7 ± 0.6a	NS
	Mr. Sticky	3.8 ± 1.4a	4.7 ± 1.7a	NS
	Raid trap	10.3 ± 2.8b	12.0 ± 2.3b	
All stages	Jar trap	3.8 ± 0.8a	16.3 ± 2.4b	***
	Mr. Sticky	5.0 ± 1.4a	8.7 ± 2.3a	NS
	Raid trap	11.2 ± 2.9b	14.3 ± 2.1ab	NS

Means followed by the same letter within a column for the same stage/sex are not significantly different (ANOVA and Ryan's multiple range test, $P < 0.05$). Statistical significance between treatments with and without pheromone (t-test): ***, $P < 0.01$; **, $P < 0.05$; *, $P < 0.1$; and NS, not significant, $P > 0.1$.

1, $P = 0.061$ for GP-2 Tablet; $F = 3.41$, df = 1, $P = 0.0945$ for Avert bait).

Analyses indicated that the number of adult males trapped was affected only by the pheromone (Table 2). Although it appeared that more males were trapped in jars baited with either Avert 310 or GP-2 than in blank control jars (without pheromone), this difference was not statistically significant ($F = 0.98$, df = 2, $P = 0.3987$). However, supellapyrone significantly increased the number of males trapped by 6–28 times relative to the respective controls (Table 2) ($F = 49.21$, df = 1, $P < 0.0001$).

The commercial baits significantly increased the number of trapped females and nymphs compared with unbaited jars (Table 2) ($F = 4.91$, df = 2, $P = 0.0185$; and $F = 5.87$, df = 2, $P = 0.0099$, respectively). Supellapyrone failed to increase female trap catches, but nymphal trap catch increased marginally by addition of Avert 310 bait and supellapyrone ($P = 0.1$) (Table 2).

Discussion

The female sex pheromone of the brownbanded cockroach is highly active in behavioral assays in the laboratory. In response to the pheromone, males wave their antennae, then initiate locomotory behavior followed with upwind orientation, and finally arrive at the pheromone source (Liang and Schal 1990b). It was estimated that the threshold pheromone concentration could be as low as 0.001 female-equivalents of a whole-body hexane extract of virgin females (Liang and Schal 1990a) or ≈0.00001 ng (Charlton et al. 1993). The pheromone was identified as a 3,5-dialkyl-substituted α-pyrone and was confirmed by synthesis (Zhang 1992, Charlton et al. 1993). Although our synthetic pheromone (supellapyrone) contains a mixture of all 4 possible isomers, only 1 (2′R, 4′R configuration) is naturally produced by females (Mori and Takeuchi 1994, Leal et al. 1995). It appears that the presence of other isomers neither increases nor inhibits male responses to the natural isomer (Charlton et al. 1993; D.L., unpublished data). Our trapping experiments with natural populations in research laboratories and with seeded populations in vacant apartments demonstrated that supellapyrone also was attractive to males in the field. This highly active and highly specific compound could have great potential for the management of brownbanded cockroach populations.

Table 2. Captures (mean ± SEM) of *S. longipalpa* in jar traps baited with food attractants, female sex pheromone, or both

Stage/Sex	Bait type	Effect of bait and pheromone		
		(−) Pheromone	(+) Pheromone	Pheromone
Males	Blank	0.5 ± 0.5a	14.2 ± 3.5a	***
	GP-2 tablet	1.0 ± 0.4a	12.7 ± 3.5a	***
	Avert bait	1.5 ± 0.6a	9.8 ± 2.9a	**
Females	Blank	0.0 ± 0.0a	1.2 ± 1.2a	NS
	GP-2 tablet	1.2 ± 0.5ab	1.2 ± 0.5a	NS
	Avert bait	2.2 ± 0.9b	3.7 ± 1.8a	NS
Nymphs	Blank	0.7 ± 0.3a	3.8 ± 2.5a	NS
	GP-2 tablet	7.5 ± 1.7b	10.2 ± 4.8ab	NS
	Avert bait	7.5 ± 2.7b	16.2 ± 5.9b	*
All stages	Blank	1.2 ± 0.5a	19.2 ± 6.0a	**
	GP-2 tablet	9.7 ± 2.3b	24.0 ± 6.4a	*
	Avert bait	11.2 ± 3.7b	29.7 ± 9.3a	*

Means followed by the same letter within a column for the same stage/sex are not significantly different (ANOVA and Ryan's multiple range test, $P < 0.05$). Statistical significance between treatments with and without pheromone (t-test): ***, $P < 0.01$; **, $P < 0.05$; *, $P < 0.1$; and NS, not significantly, $P > 0.1$.

Several important parameters need to be tested and validated before pheromones can be integrated into pest management programs. First, the pheromone must be efficacious under field conditions and competitive with existing attractant technology. Our field trapping tests demonstrated that traps with pheromone captured 6–28 times more males than blank traps or traps with commercial food baits (Table 2). Because adult males are highly mobile, they constitute an important target for detection and monitoring programs.

Second, the pheromone must be formulated for a slow but effective release to minimize the frequency of servicing traps. Supellapyrone was formulated into laminated plastic dispensers (Hercon), similar to the periplanone-B formulation (Bell et al. 1984). Our results show that such formulations elicit EAG responses (Fig. 1), and exhibit slow release (Fig. 2) and dose-response (Figs. 1 and 3) characteristics. The EAG responses to a 6.5-cm^2 dispenser is ≈0.35–0.4 mV (Figs. 1 and 2) when the dispenser is fresh and 0.2 mV after 24 h. These values are on the low end of the sigmoid dose-EAG response curve (see Figure 4B in Charlton et al. 1993). More than 100-fold higher concentrations can be applied before the EAG response is saturated. Therefore, the already highly effective pheromone trap can be made more effective by increasing the amount of supellapyrone used. Although an optimal formulation will need to await commercial-scale synthesis of the proper enantiomer of supellapyrone and analytical studies of release rates, it is clear that the current formulation constitutes an effective tool for detection and for monitoring cockroach populations.

Third, trap design must be optimized. Other than the report of Philips and Wyatt (1992) on the effect of tilting angle of the ramp on the trapping efficiency of glue traps, no analysis of trap design and the efficacy of trapping has been reported. Typically, new attractants are added to available traps whose deployment is based more on economy and convenience (e.g., folding pattern) than efficacy. Our experimental data showed that unbaited jar traps caught fewer cockroaches than 2 types of glue traps. However, with supellapyrone, jar traps caught more cockroaches than glue traps baited with pheromone (Table 1). We also found that jar traps were superior in trapping adult males. Addition of pheromone to glue traps increased male catch by only ≈3–4 times whereas 20 times more males were trapped in jar traps baited with supellapyrone (Table 1). Two important conclusions stem from these data. It appears that attractants formulated into the glue traps are relatively ineffective, based on low trap-catch of males and females. Although the Raid trap caught relatively more cockroaches than Mr. Sticky and jar traps without pheromone (see also Moore and Granovsky 1983), it was mainly due to the large number of trapped nymphs, which could be affected significantly by trap placement. However, jar traps baited with pheromone were a more specific and less variable tool. They trapped the largest number of cockroaches and most of the captured insects were males, whose trapping was unaffected by trap placement. It is not known why pheromone-baited jar traps performed better than glue traps baited with pheromone. It is plausible that adult males could sense either the glue or other features of the glue trap with their long antennae. Also, males readily jump into pheromone-baited jars whose inner rim had been treated with petroleum jelly. Using white bread as bait, Owens and Bennett (1983) also found that jar traps were the most representative of all available sampling techniques for *Blattella germanica* (L.).

It is important to demonstrate the interactive effects of pheromone with currently deployed technology. Because food baits are common tools in cockroach control programs, we sought to determine whether supellapyrone might detract or enhance their attractiveness to cockroaches. Our results showed that pheromone alone attracted twice as many cockroaches as food attractants even when all stages of both sexes were taken into account (Table 2). This finding indicates that pheromone is more effective than either of the 2 food attractants tested. One of the attractants (Avert 310) was formulated as an insecticidal bait rather than for deployment in traps, and both attractants target primarily the German cockroach. Nonetheless, distiller's grain, the presumed bait base for Avert bait, was found to be highly attractive to the brownbanded cockroach (Adler 1985; D.L., unpublished data). Moreover, we demonstrated that pheromone and food baits can be combined to achieve a highly effective attractant mixture for the brownbanded cockroach. Our field trapping results showed that a combination of pheromone and food attractants was more effective than either one alone (Table 2). It is likely that this additive effect is due to the use of different receptor systems by the 2 attractants. Adult males respond to female sex pheromone whereas all cockroaches are attracted to food. Therefore, the combined efficacy of the 2 kinds of attractants is greater because they sample a larger fraction of the population.

It has been reported that, in addition to attracting males, sex pheromone also significantly increased trapping of nymphs of the American cockroach (Chow et al. 1976, Chow and Wang 1981, Bell et al. 1984). Similar results were observed in some of our trapping studies with brownbanded cockroaches (Table 2). Because female sex pheromone attracts only adult males, it is unlikely that nymphs are attracted to it. Bell et al. (1984) suggested that this may be the result of intraspecific interactions between trapped males and foraging nymphs.

Pheromones can be integrated with other cockroach management tactics, especially in detection and monitoring, and also in mass trapping, mating disruption, and reducing repellency of insecticides. For example, a crude extract containing aggregation pheromone of German cockroaches was used to mask the intrinsic repellency of some insecticides (Rust and Reierson 1976, 1977). Schal and Hamilton (1990) concluded that "lack of efficient trapping methods for

cockroaches is probably the most significant single factor contributing to a heavy reliance on scheduled applications of insecticides." Supellapyrone is highly species specific and more attractive than food-based attractants. In addition, it can be integrated with food attractants to further increase trapping efficacy. It, therefore, should serve as an important tool for detection and monitoring of the presence and location of infestations. Other control measures can then be implemented, including judicious use of insecticides. It is not known whether supellapyrone can be used in mass trapping. However, because in some preliminary tests we were able to retrap most adult males that were released into a room, it is possible that deployment of a large number of pheromone-baited traps may remove enough males to effect a decline in birth rate. It any case, intensive trapping may be efficient in removing most of the resident males and intercepting immigrant males, the most mobile and therefore annoying stage, and thereby reducing health risks associated with human exposure to cockroach populations. In combination with food attractants, which attract females and nymphs, supellapyrone shows promise in an attract-kill control strategy for the brownbanded cockroach.

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