A STRAIN DIFFERENCE AND ABLATION EXPERIMENTS INVOLVING A DISPERSAL PHEROMONE IN THE GERMAN COCKROACH, *BLATTELLA GERMANICA* (DICTYOPTERA: BLATTELLIDAE)

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ABSTRACT

The results of bioassays involving a dispersal pheromone that is secreted by adult female German cockroaches are described. Males with excised antennae were unable to sense the dispersant. Repellency to males with excised maxillary palps did not differ significantly from that in the controls. Males of an insecticide - resistant field strain responded more strongly to the dispersant than males of a susceptible laboratory strain. Filter papers conditioned by crowded field strain females were more repellent than papers conditioned similarly by laboratory strain females.

Key Words: Cockroach, pheromone, dispersal, behavior, strain - difference.

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INTRODUCTION

Evidence that the German cockroach, *Blattela germanica* (L.), emits a dispersant when crowded was first reported by Suto and Kumada (1981). It was detected in salivary gland secretions (Nakayama et al. 1984). Differences in the response of nymphs, adult males, and gravid (ootheca - bearing) and non - gravid females were found using filter papers conditioned by crowded females (Ross and Tignor 1985). Also, papers conditioned by crowded non - gravid females (6 - 10 days old) were more repellent than those similarly conditioned by gravid females. A subsequent study showed that papers conditioned by crowded adult males or late instars were not repellent (Ross and Tignor 1986). It was concluded that the dispersal pheromone is secreted by adult females only, at least in our standard laboratory strain (VPI strain).

The above studies raised two further questions that were explored recently. One was whether secretion of the dispersant varies during the female reproductive cycle. Studies on secretion during the first week postecdysis showed little or no response to papers conditioned by 1 - 5 day - old - females whereas papers conditioned by like numbers of 6 and 7 -day - old females were strongly repellent (Ross and Tignor 1988). The other question addressed the effects of starvation. Since the female reproductive cycle is in some measure controlled by food availability (Adiyodi and Adiyodi 1974; Durbin and Cochran 1985; Kunkel 1976) and secretion of the pheromone was related to the reproductive cycle, it seemed likely that starvation would inhibit secretion. Bioassays supported this hypothese. Filter papers conditioned by crowded starved females were not repellent (Tignor and Ross 1987).

Reported here are the results of additional experiments on the dispersal pheromone. They include ablation experiments and a comparison of secretion and response in two strains, the VPI strain and a recently-collected field strain (Fairbanks).

MATERIALS AND METHODS

The VPI wild-type strain was used in ablation experiments. Tests for strain differences in response to or secretion of the dispersal pheromone used both the VPI and the Fairbanks strain. The VPI strain is an insecticide-susceptible laboratory strain that has been in culture for approximately 160 generations. The Fairbanks is a susceptible strain that was collected in Fairbanks, Alaska, in 1983. The stocks were maintained in a laboratory on a 14D/10L photocycle, temperature range 24-27°C, and ambient humidity, with water and food supplied *ad lib.*

Bioassay Procedures

Bioassays were similar to those conducted previously in that they utilized strips of Whatman no. 1 filter papers (3.8 × 7.0 cm), folded in four, and randomly positioned near the margins of a circle on a sheet of non-glare glass (Ross and Tignor 1985). The circle was enclosed in a glass chimney (14.5 diam. × 19.5 cm in height). A light coat of vaseline around the base of the chimney prevented cockroaches from climbing the sides when released on the glass sheet. One paper was "conditioned"; the other served as a control. The distribution of test groups of cockroaches was recorded by time lapse photography at 10 min intervals for 180 min after release into the system. All experiments were begun between 8:00 and 9:00 a.m. Each experiment was replicated 12 times.

Papers used in ablations experiments and in comparisons of the response of VPI and Fairbanks strain males were conditioned by an aqueous solution obtained from crowding 60 7-10 day-old non-gravid females into small plastic vials (2.9 by 7 mm). Each vial contained a strip of Whatman no. 1 filter paper (3.8 × 7 cm). A total of 360 VPI strain females were used, i.e., 60/each of 6 vials. Females were held in the vials for 1 h. Papers were then removed, cut in squares, and rinsed in 15 ml of distilled water for 10 min. This was repeated two times using the same sample of distilled water (the dispersant is water solable; Nakayama et al. 1984). The agueous solution was extracted three times in 10 ml of ethyl ether to remove aggregation pheromone. After centrifugation at 3000 rpm to remove any bits of filter paper remaining in the solution, strips of filter paper were inoculated with 0.25 ml of the solution. Papers conditioned in this manner were allowed to dry for 30 min before use in the bioassays.

Experiments testing for strain differences in secretion used filter papers conditioned directly by the females, as described previously (Ross and Tignor 1985). Procedures were like those described above in that females were crowded into small plastic vials, each of which contained a strip of filter paper (3.8 × 7 cm) folded in four. After 1 h, the papers were removed form the vials and used directly for bioassays.

Ablation Experiments.

Antennae were surgically removed from 2 to 3 wk-old adult males. Males were allowed to recover for 4 da before bioassays were begun. Males were tested in groups of 12. Controls used groups of 12 2 to 3 wk-old males with intact antennae. Equal numbers of males with ablated and intact antennae were tested

against papers conditioned by the same aqueous solution. The latter tests were carried out either on the same day or within 24 h of one another. New solutions were prepared several times in the course of the experiments.

Experiments on ablation of maxillary palps followed procedures like those of antennal ablation.

Strain Differences

Two experiments were conducted. One tested for strain differences in response to the dispersant. Procedures were like those of the antennal ablation experiments, except that bioassays used groups of 12 2 wk - old males of either the VPI or the Fairbanks strain rather than males with ablated or non - ablated antennae.

A second experiment tested for strain differences in secretion. Papers were conditioned directly by either 30 non - gravid 7 day - old Fairbanks strain or VPI strain females. Test insects were again groups of 12 two - wk - old VPI strain males.

Analysis

Aggregation indices (A.I.s) were calculated for each experiment following the formula of Roth and Cohen (1973):

A.I. = no. of males on conditioned paper minus the no. on the control paper divided by the total number on both papers

A.I.s range from +1 to -1. Positive values indicate attractancy; negative ones, repellency. In the present experiments, mean A.I.s were calculated from the 12 replicates used in each experiment. They were based on data collected from 80 to 180 min, i.e., after the cockroaches had settled down and distribution stabilized. Nearly all of the cockroaches were on the filter papers. Those few that were on the jar bottom were not included in the analyses. A.I.s were tested for significance by analysis of covariance, using Tukey's Studentized Range Test (Sokal and Rohlf 1969). Also, A.I.s were tested for significant differences from 0 (no response) using the Student's t - test.

RESULTS AND DISCUSSION

Numerous bioassays using conditioned filter paper have been used in experiments on *B. germanica*. They have one characteristic in common - settling of most or all of the cockroaches on vertical surfaces provided by folded strips of filter paper. Thus, the experiments test for choice between the two papers. When dealing with papers contaminated by uncrowded insects, i.e., experiments on aggregation pheromone, cockroaches prefer the conditioned paper. As shown here and in other studies (Suto and Kumada 1981; Nakayama et al. 1984; Ross and Tignor 1985, 1986), the opposite results are found when papers are conditioned by crowded females or aqueous solutions of their secretions.

Ablation Experiments

The results of the ablation experiments are shown in Table 1. The mean A.I. (- 0.07) in experiments on males with ablated antennae did not differ significantly from 0, that is, no response. It differed significantly from the A.I. in the control data (- 0.43). Significant repellency also occurred in the experiments

Table 1. Effects of excision of antennae and of maxillary palps on the response of adult male German cockroaches to a dispersal pheromone.

Treatment	Mean aggregation index ± s.e. (80 - 180 min)*
Control†	- 0.43 ± 0.247a
Antennal ablation	- 0.07 ± 0.021 b(*)
Control‡	- 0.39 ± 0.159a
Maxillary palp excision	- 0.37 ± 0.058a

* Indices calculated from 144 ♂♂/experiment. Means with the same letter are not significantly different ($P < 0.05$); (*) mean is not significantly different form 0, i.e., no response to the conditioned paper.
† Controls for antennal ablation experiments.
‡ Controls for experiment on maxillary palp excision.

on males with ablated maxillary palps. The mean A.I. did not differ significantly from that in the controls or from the A.I. in controls of the antennal ablation experiment.

The above results leave little doubt that perception of the dispersant is mainly by receptor cells located on the antennae. The antennae are also the sensory organs for perception of the aggregation and sex pheromones, at least in the American cockroach (Block and Bell 1974). Bell et al. (1973) reported an olfactory response to aggregation pheromone, although other experiments indicated that contact chemoreception is involved in maintaining aggregations (Burk and Bell 1973). No conclusive information is yet available on whether response to the dispersal pheromone is olfactory or by contact chemoreception. Nevertheless, certain volatile fatty acids that are repellent to *B. germanica* (McFarlane 1984) were found in "frass" collected from larvae and adults of *B. germanica* (McFarlane and Alli, 1985). The fatty acids were referred to as being in "excreta" (McFarlane 1984). Probably "frass" refers to fecal pellets. Conceivably, it could have been contaminated with salivary gland secretions, the apparent source of the dispersal pheromone (Nakayama et al. 1984). If volatile fatty acids are indeed responsible for repellent effects of the dispersal pheromone, perception may involve olfactory rather than tactile cues.

Strain differences

The results of experiments on response to and secretion of the dispersant by VPI and Fairbanks strain cockroaches are shown in Table 2. Fairbanks strain males were more strongly repelled by papers conditioned with the aqueous solution than VPI males. Apparently, the Fairbanks males are more sensitive than the VPI males to the dispersal pheromone. A second strain difference was indicated by the greater repellency to VPI strain males of secretions by Fairbanks strain females than by those of VPI females. This may have been due to variation in either the amount of the pheromone secreted or its chemical composition.

Little is known concerning strain differences in behavior in *B. germanica*. Because it seemed likely that modifications of behavior have accompanied the development of varying levels and types of insecticide resistance, studies on strain differences in insecticide - induced behavior were initiated recently in our laboratory. Differences were found between the VPI strain and a resistant field strain (Bret and Ross 1985, 1986; Harmon and Ross 1987). Also, the rate of dispersal caused by insecticied vapors varys among field strains (Wooster and Ross, unpubl.). It is

Table 2. Results of experiments on response and production of the dispersal pheromone in two strains of the German cockroach.

Experiment	Paper conditioned by:	Test group* (♂♂)	Mean aggregation index ± s.e. (80 - 180 min)
Response	aqueous solution	VPI	- 0.49 ± 0.033
		Fairbanks	- 0.68 ± 0.028†
Secretion	direct contact by VPI♀♀	VPI	- 0.47 ± 0.038
	direct contact by Fairbanks♀♀	VPI	- 0.97 ± 0.008‡

* 144 ♂♂ tested/experiment (12/replicate).

† Significantly different from the response of VPI ♂♂ to the aqueous solution.

‡ Response of VPI ♂♂ to secretions of Fairbanks ♀♀ significantly different from that to VPI ♀♀ secretions.

probable that field strains also vary in their response and secretion of pheromones. However, the only comparisons made thus far were between laboratory and field strains. Wileyto et al. (1984) found a stronger attractancy of aggregation pheromone to nymphs of a resistant field strain than to those of a susceptible laboratory strain. Since the stronger response to the dispersal pheromone was also by a field strain, it appears that selection either lowers the frequency of individuals in laboratory cultures that respond strongly to pheromones or selects for such individuals in field populations. However, other selective forces acting on field populations could alter this picture. In aphids, Dawson et al. (1983) found an apparent association between resistance to organophosphorus insecticides and a decreased response to an alarm pheromone.

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