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Intra and inter-specific attraction of cockroach faecal extracts: studies for improving bait activity

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Abstract

The attraction behaviour of four synanthropic species of Blattaria (*Blattella germanica*, *Supella longipalpa*, *Blatta orientalis* and *Periplaneta americana*) was investigated. Methanol faecal extracts always showed a higher intraspecific attractivity than aqueous extracts in "Y" olfactometer bioassays. A gel with resistance to dehydration was prepared, and then methanol faecal extracts were added. Arena bioassays showed that the new gel containing faecal extracts was always more attractive than commercial gel formulations.

Key words: Blattaria, aggregation pheromone, pest control, gel bait, faecal extract.

Introduction

The management of cockroach infestations in urban environment has undergone some changes in recent years, moving from the predominant use of sprays to an increasing use of bait, consisting of an insecticide added to a food. The efficacy of bait is determined by the overall performance of its components, including active and inert ingredients. The most recent types of bait are gel-formulated.

Gel-formulated bait has an important advantage compared to conventional treatments: it is safer because the insecticide is not released directly in the environment, but some drops are put in specific critical points such as cracks, crevices, or other sites where the roaches likely nest; the possibility of contact with humans and pets is minimized. This means that they can be used safely in dwellings, even in the presence of children. Gel formulations may also be used where food is processed: kitchens, restaurants and food industries, without the need of stopping production processes.

Studies carried out so far and some tests that we conducted showed that commercial gel bait has some drawbacks: it is effective only if the drops are applied very close (about 10 cm) to the shelters of cockroaches (Appel, 1992; Ajjan *et al*., 1997; Durier and Rivault, 2002); moreover, as it is not particularly attractive or appealing, its efficiency is strongly influenced by the presence of alternative food and water sources (Appel and Tanley, 2000; Nalyanya *et al*., 2001) and by some behavioural aspects of the cockroach species or stage of development (Kaakeh and Bennett, 1999; Appel and Tanley, 2000; Durier and Rivault, 2000a; 2000b). Many gel formulations are also subject to rapid drying that affects their consistency and durability (Appel and Benson, 1995).

Studies on cockroach aggregation have been conducted since 60s (Roth and Willis, 1960); Ishii and Kuwahara (1967; 1968) showed that the aggregation pheromone of *Blattella germanica* (L.) is produced in rectal pad cells and excreted with frass. Roth and Cohen (1973) proved that young nymphs of several species of cockroaches aggregate on paper contaminated by their own or faeces of other cockroach species. Studies also investigated the species specificity of the aggregation pheromones of some Blattids and Blaberids (Bell *et al*., 1972) and the interspecific aggregation of Blattellidae on contaminated papers (Rust and Appel, 1985). According to Sakuma and Fukami (1990) the major components of the *excreta* are ammonia, methylamine, dimethylamine and trimethylamine. Only little research has been carried out till now, and no practical use of aggregation pheromone is known.

The purpose of this study was to improve knowledge on cockroach semiochemicals, focusing on the aggregation pheromone and its role in inducing attraction and aggregation of individuals of the same species, and that of closely related species.

Materials and methods

Insects

The insects used in the bioassays came from colonies maintained at 26 °C and 65% R.H. under a 12:12 (L:D) photoperiod with access to cat food pellets and water. Plastic boxes (20 × 30 × 40 cm) were used as rearing containers. Corrugated cardboard was placed in the containers for shelter. Test insects were adult of both sexes and nymphs of different instars, from the second on; cockroaches were collected from rearing containers removing harborages and shaking them after anaesthesia with CO_2.

Faecal extracts preparation

Faecal material was collected weekly from the bottom of the containers used for mass-rearing of *B. germanica, Supella longipalpa* (F.), *Blatta orientalis* L. and *Periplaneta americana* (L.) respectively. It was separated from extraneous material (dead insects, exuviae, oothecae, waste food, etc.) through a fine mesh sieve (mesh No. 10); collected faeces were placed in a freezer (−20 °C) until the amount of material was suitable for the extraction of soluble components.

As it is thought that the main attractive components of aggregation pheromones of cockroaches are mostly mixtures of alkylamines, perhaps present as salts (Sakuma *et al.*, 1997a; 1997b), extraction was performed with two different solvents: with methanol and, as recommended by some authors (e.g. Miller *et al.*, 2000), with water.

For both extractions the same procedure was adopted: 10 g of faeces were added to 30 g of solvent in a flask (V = 50 ml), mixed using a magnetic stirrer and settled for 60 minutes. The mixture was centrifuged for 5 minutes at 4 °C at about 130x *g* to separate the liquid phase from the solid one. The supernatant was collected and stored at 4 °C in a glass vial until the use in olfactometer bioassays.

Olfactometer bioassays

A "Y-tube" Plexiglas olfactometer was used (internal diameter 28 mm). The airflow was set to 250 ml min^{-1} for each arm, for a total of 500 ml min^{-1} in the main arm. Bioassays were carried out with faecal extracts of the four species listed above, obtained with water and methanol (separately). The trial assessed the attractivity of each extract against insects (10 adult females + 10 adult males + 20 nymphs of 2^{nd} and following instars) of each species. Each insect was used only once.

At one end of the olfactometer, randomly chosen, a piece of filter paper (about 24 cm^2) soaked with 500 µl of faecal extract at 1:10 dilution was placed, while at the other end a filter paper soaked with the same amount of distilled water was placed as a control.

Each insect was given 5 minutes to make a choice. The faecal extract was considered attractive if the cockroach, after reaching the attractant, spent at least 1 minute in the arm (to avoid false-positive). The insect was discarded if it spent more than 1 minute in the main arm or in the case it had not moved from the starting cage. The time spent to reach the extract was also recorded.

New gel formulations and bioassay in arenas

Some gels were prepared using the following ingredients in different proportions (not all the ingredients have been used for each gel): wheat protein, modified corn starch, guar gum, locust bean gum, carboxymethylcellulose, alginate, carrageenan, gelatin, agar agar, pectin, xanthan gum, emulsifiers.

Twelve gels were prepared, and they were separated in four groups depending on the main ingredient: modified corn starch, pectin, carrageenan and gelatin. Subsequently three of these gels were selected, considering the most suitable consistency obtained and according to the results of olfactometer bioassays carried out to evaluate the palatability of the gel. In these assays 20 individuals of all ages of *B. germanica* were given the choice among gels belonging to different groups, and the time spent to reach the gel and feeding on the gel itself was recorded. Peach jam and distilled water were used as control.

The three selected gels (all belonging to the group having carrageenan as main ingredient) were tested for resistance to desiccation by placing drops of gel on a glass and leaving them for 6 days at 21.5 ± 1.5 °C and 28 ± 4% RH. The weight loss was recorded every 24 hours and 3 replications were carried out.

The gel that showed the best physical characteristics was used in subsequent tests mixed with faecal extracts. Since it was estimated that the optimal concentration of faecal extract (*i.e.* the one that showed the best attraction power) is that obtained at 1:10 dilution, the concentration of the extract added to the gel was carried out homogenizing 1 g of faecal extract (extracted from faeces with methanol) and 9 g of gel.

PVC arenas (1 × 1 m) with walls treated to avoid insects climbing were used to estimate the attractivity of this new gel and to compare it to commercial alternatives: Solfac Gel (Imidacloprid 2.15% - Bayer), Goliath Gel (Fipronil 0.05% - BASF), Maxforce Roach Bait Gel (Hydramethylnon 2.15% - Bayer) and Avert Cockroach Bait Gel (Abamectin 0.05% - BASF).

Since the exact content of commercial gel formulations is not known, with the exception of the active ingredient, it was not possible to evaluate their ingredients separately; for this reason we decided to evaluate the new prepared gel in its complexity (base and faecal extracts together).

Two shelters made of corrugated cardboard were placed in two opposite corners inside each arena, and insects were left 24 hours in the arena to adapt, before the bioassay was carried out.

To verify the attractivity of the gel formulation, a drop (~0.2 g) was placed in the middle of a cardboard sticky trap (Agrisense Lo-line Cockroach Trap, approx. 210 × 100 × 25 mm) located in the centre of the arena. An empty sticky trap was used as control in separated replicates (in each bioassay only one sticky trap was put in the arena). The assessment of the catches was carried out after 1, 2, 3, 4 and 5 days, and caught insects were left inside the trap.

Three replicates were carried out for each product (21.5 ± 1 °C, RH 29 ± 3.5%, L:D = 12:12). These assays were carried out with *B. germanica* (20 individuals/arena) and *S. longipalpa* (10 individuals/arena) separately, using gel added with the respective faecal extract.

Repellency of active ingredients

As the base of the commercial gel products without active ingredients was not available, it was impossible to assess their attractivity and repellency separately. Some tests were carried out with the method of "Ebeling choice boxes", as described by Ebeling *et al.* (1966; 1967) and Busvine (1971), to assess a possible repellency of commercial formulations caused by the presence of the insecticide.

Statistical analysis

Olfactometer bioassays: statistical analysis was performed as the chi-square test (SPSS 15); insects that did not respond (nr) were not considered in statistical analysis, according to previous similar studies (Schal *et al.*, 1992); values of $P < 0.05$ were considered significant.

Arena bioassays: mean differences in total trap catch between each product and the control were analyzed with GLM model (IBM SPSS Statistic 19); the mean difference was considered significant at the 0.05 level.

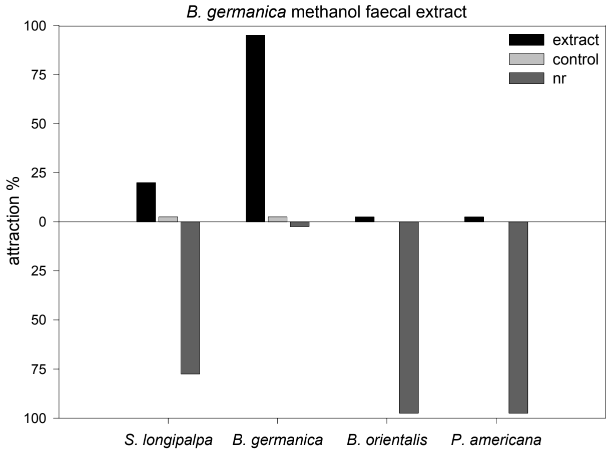

Figure 1. Response to *B. germanica* methanol faecal extracts (*B. germanica*: $\chi^2 = 35.103$, $P \approx 0$; *S. longipalpa*: $\chi^2 = 5.444$, $P = 0.02$).

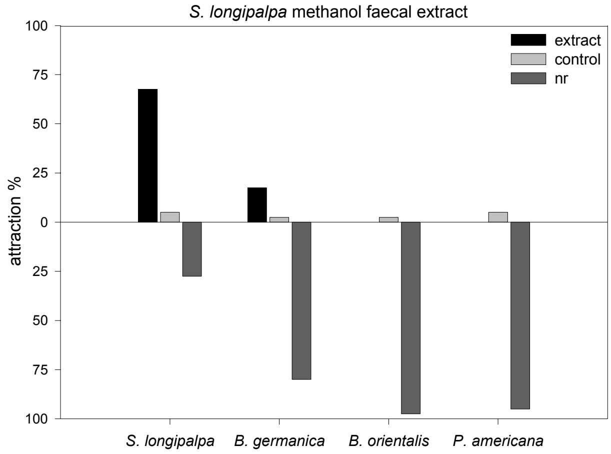

Figure 2. Response to *S. longipalpa* methanol faecal extracts (*S. longipalpa*: $\chi^2 = 20.571$, $P \approx 0$; *B. germanica*: $\chi^2 = 4.5$, $P = 0.034$).

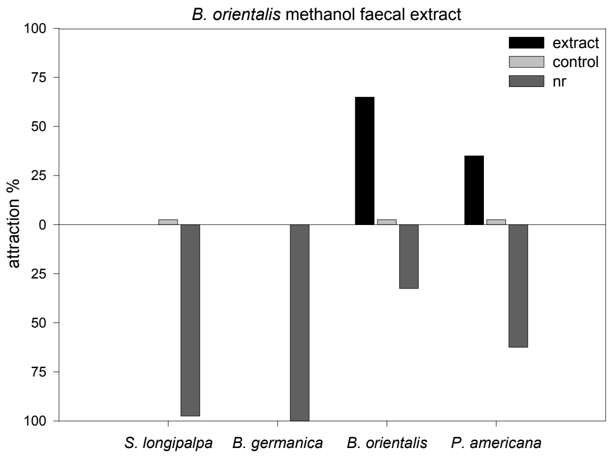

Figure 3. Response to *B. orientalis* methanol faecal extracts (*B. orientalis*: $\chi^2 = 23.248$, $P \approx 0$; *P. americana*: $\chi^2 = 11.267$, $P = 0.01$).

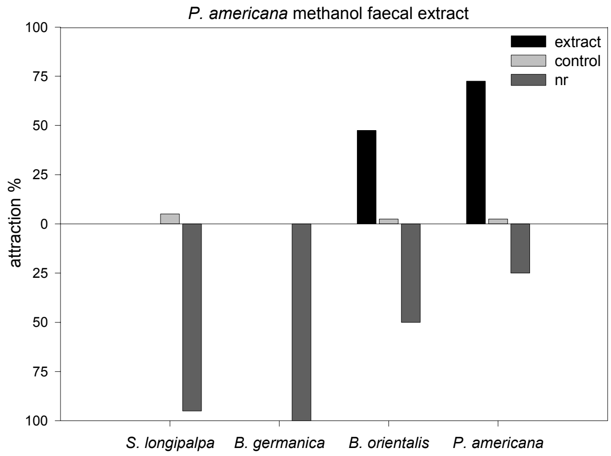

Figure 4. Response to *P. americana* methanol faecal extracts (*P. americana*: $\chi^2 = 26.133$, $P \approx 0$; *B. orientalis*: $\chi^2 = 16.2$, $P \approx 0$).

Results and discussion

Intra and inter-specific attraction of faecal extracts

The intraspecific attractivity of aqueous faecal extracts was very low for all the tested species, and the interspecific attractivity was negligible. Given the high number of insects that gave no response, no statistical significance was calculated ($P > 0.05$).

Faecal extracts obtained with methanol always showed a good intraspecific attractivity, above all with immature instars.

Interspecific attraction was recorded with faecal extracts of *S. longipalpa* in the presence of *B. germanica* and *vice-versa* as well as with *B. orientalis* and *P. americana* and *vice-versa*. The intraspecific attractivity was always higher than any interspecific. Detailed results are shown in figures 1-4.

Excluding insects that gave no response (nr), in all cases of intraspecific attraction there was statistical significance of the responses ($P < 0.05$), while in cases of interspecific attraction there was significance in the methanol extracts of *S. longipalpa* against *B. germanica* and *vice-versa*, and in those of *B. orientalis* against *P. americana* and *vice-versa*.

Considering all the 40 insects used in each test in statistical analysis to calculate χ^2 and P, and aggregating those who gave no response to those who have been attracted to the control, we would have seen $P < 0.05$ for the intraspecific attractivity of *S. longipalpa*, *B. germanica* and *P. americana* extracts, while $P = 0.058$ for *B. orientalis*.

New gel formulations

The best gel out of the twelve preparations was chosen for arena bioassays; results of preliminary tests are shown in table 1. In the tests for assessing resistance to desiccation this gel only showed a 20% weight loss in 6 days, much less than most of the commercial gel baits (40-60%). The new gel showed a good preservability if kept refrigerated, maintaining the original features for 8-12 months; however, this is an experimental product, not intended for commercial use, thus further improvable.

Table 1. Percentage of positive responses, average time to reach gel and average time spent feeding on it, in preliminary olfactometer bioassays with *B. germanica*.

Gel	% olfactometer positive responses	Average time to reach the gel (sec.) (max 300 sec.)	Average time spent on the gel (sec.) (max 60 sec.)
AM01	10	212	11
AM02	5	190	7
P01	25	250	21
P02	20	168	18
P03	35	109	33
F60	35	144	31
F61	75	115	49
F62	50	187	40
F63	65	99	51
F64	65	120	44
F65	40	234	38
G44	40	177	18
Control 1 (peach jam)	85	93	55
Control 2 (distilled water)	5	219	4

Table 2. Average differences in trap catch between control (empty trap) and each gel product in *B. germanica* (number of insects out of 20) and statistical significance.

Product	Average trap catch ± SE	Average difference in trap catch ± SE	Significance
New Gel + *B. germanica* faecal extract	20	17.333 ± 0.667	≈ 0
Solfac Gel	9.333 ± 0.667	6.667 ± 0.942	0.002
Goliath Gel	12.333 ± 1.667	9.667 ± 1.795	0.000
Maxforce Roach Bait Gel	4.333 ± 0.882	1.667 ± 1.106	0.777
Avert Cockroach Bait Gel	7.333 ± 0.667	4.667 ± 0.943	0.030
Control	2.667 ± 0.7		

Table 3. Average differences in trap catch between control (empty trap) and each gel product in *S. longipalpa* (number of insects out of 10) and statistical significance.

Product	Average trap catch ± SE	Average difference in trap catch ± SE	Significance
New Gel + *S. longipalpa* faecal extract	9.667 ± 0.333	9 ± 0.471	≈ 0
Solfac Gel	2.333 ± 0.333	1.667 ± 0.471	0.348
Goliath Gel	4.333 ± 0.882	3.667 ± 0.943	0.006
Maxforce Roach Bait Gel	1.667 ± 0.882	1 ± 0.942	0.800
Avert Cockroach Bait Gel	2	1.333 ± 0.333	0.567
Control	0.667 ± 0.333		

The results of the arena assays, to evaluate the attractivity of a gel drop placed inside a sticky trap (tables 2 and 3; figures 5 and 6), showed that the gel mixed with faecal extract has always been more attractive than other formulated gels, used as comparison. Regarding *B. germanica*, the new gel was the only product able to attract into the sticky trap 100% of insects placed in the arenas within 5 days. All the products, except for Maxforce, were statistically different from the control.

Regarding *S. longipalpa*, the attractivity of the new gel containing faecal extracts is more than twice higher than commercial products, and it has led to the capture of 96.7% of insects in 5 days. In these bioassays the new gel and Goliath were statistically different from control.

Repellency of active ingredient

The average repellency ± standard deviation, ranged from 1.25 ± 1.37% in control (no product), to 3.33 ± 1.29% in Solfac Gel; this indicated that the repellency of commercial gel bait was very low. Values of repellency were not statistically different from the repellency of the control. This is probably due to the very low amount of a.i. present in bait. For this reasons we can assume that the discrepancy caused by the absence of insecticide in the new gel formulation was insignificant. Also Durier and Rivault (2000b) showed that the presence or absence of fipronil did not influence the level of attractivity of Goliath gel.

In this study we measured and improved two important factors influencing the efficiency of gel bait: the

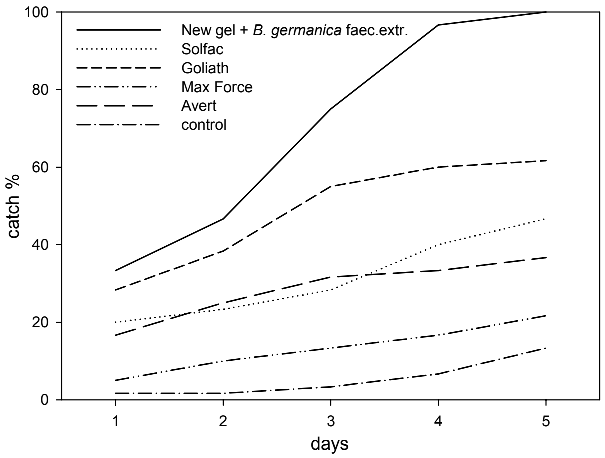

Figure 5. Intraspecific attractivity of the new gel with *B. germanica* faecal extracts.

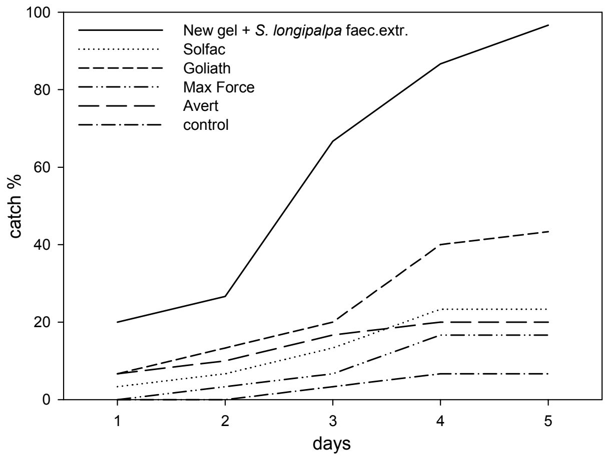

Figure 6. Intraspecific attractivity of the new gel with *S. longipalpa* faecal extracts.

food base in term of bait stability and feeding stimulation, and the attractivity using faecal extracts.

In conclusion, the cockroach faecal extracts obtained with methanol can effectively increase the attractivity of bait, especially if combined with a gel with good texture and durability.

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