

SECONDARY TRANSFER OF TOXICITY FOLLOWING CONSUMPTION OF FIPRONIL GEL BAIT BY ORIENTAL COCKROACHES (DICTYOPTERA: BLATTIDAE)

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Abstract - Necrophagy was shown to provide efficient transfer of fipronil toxicity from poisoned oriental cockroach (*Blatta orientalis*(L.)) cadavers to groups of untreated cockroaches in laboratory experiments. Adult females poisoned with Goliath gel deposits of 0.1 - 40 mg 0.05% w/w a.i. formulation were individually caged with 5 adult females following death. Mortality in the secondary populations increased with increasing weight of gel consumed by the primary cockroach and reached a higher level over 5 days when no alternative food was provided than when alternative food was available. Third instar nymphs (10) or adult females (5) were caged for 5 days in the jars in which adult females had consumed gel bait, died, and been removed. Mortality in the secondary populations again increased with weight of bait consumed by the cockroach. Under laboratory conditions, adult oriental cockroaches consumed 24.6 ± 0.9 and 24.7 ± 15.3 mg gel bait respectively within a 1 h period. The 5 day LD_{50} and LD_{95} of the gel bait for adult females were estimated to be 0.12 mg and 0.21 mg, respectively. A field trial was carried out in which two treatments of gel bait were applied with a 14 day interval to control oriental cockroaches. One area of the site was treated with gel deposits dispensed onto card squares (30 mg/deposit and 2.9 deposits/m²) which were removed after 48 h. 31% of these deposits were consumed within 48 h, with 100% consumed in or near harbourages. Females killed by freezing were placed in the monitored area following removal of the gel deposits, and 11% of these cadavers showed signs of necrophagy during the subsequent 3 days. The baiting operation reduced 5-day sticky trap catches to 16% of the pre-application catch by 14 days and to 1.4% of the pre-application catch by 30 days after the first treatment. Females caged with cadavers freshly recovered from the trial site after the first treatment were all killed within 48 h.

Key words - Fipronil, gel bait, necrophagy, *Blatta orientalis*

INTRODUCTION

The Goliath gel formulation of fipronil is approved for use in a number of countries as a bait to control cockroach infestations. It is dispensed into harbourages and in foraging areas as small deposits (*ca.* 30 mg) with deposit density dependent on the level of infestation and the target species. Studies on the effect of deposit density on efficacy against Oriental cockroaches in laboratory arenas showed that the bait is highly attractive to this species. In some circumstances deposits were rapidly and entirely consumed by a few adult insects as they emerged from the harbourage, so that only a small proportion of the population was exposed to the primary effect of the toxicant. However, mortality in the rest of the population continued to rise over the following 2 weeks even though no bait deposit remained (le Patourel, unpublished results). Possible mechanisms of secondary transfer of the toxicant from cockroaches which consumed the gel bait to other members of a colony were therefore investigated.

Secondary transmission of fipronil toxicity within cockroach colonies has not been reported, although the effect has been shown to be significant in the control of German cockroach infestations using hydramethylnon bait (Kopanic and Schal, 1997; Kaakeh *et al.*, 1996). Transmission of hydramethylnon toxicity within a colony is thought to be mediated by coprophagy, and to be most significant in the early, non-foraging stadia which do not ingest bait formulations directly. However, the toxic action of hydramethylnon normally takes a number of days to take effect and cockroaches can excrete a proportion of the compound in their feces prior to death, whereas fipronil has a rapid action following ingestion (Kaakeh *et al.*, 1997). In the present study additional mechanisms of secondary transfer of fipronil toxicity from poisoned cockroaches were investigated under laboratory conditions and their contribution to the effect of fipronil action in field use assessed.

MATERIALS AND METHODS

Laboratory culture and bait formulation. *Blatta orientalis* was from a culture maintained at Silwood Park for more than 8 years at $28\pm 1^\circ\text{C}$ on a diet of laboratory mouse food ((RME1 pellets, SDS Ltd.). Goliath gel (0.05% w/w fipronil) and a blank gel containing the same formulants but no active ingredient were supplied by Rhone-Poulenc Rhodic, Ongar, Essex. The Goliath gel was used either as supplied or diluted 1 : 10 w/w with blank gel; all weights of gel consumed by cockroaches are expressed in terms of weight of the Goliath formulation. Toxicity tests were carried out at $28\pm 1^\circ\text{C}$.

Bait consumption. Adult females (22) and males (23) were taken from the culture 8 h into photophase, held for 24 h without food (water provided), and then transferred to individual 300 ml screw-top jars and allowed to acclimate for 2 h. Goliath gel (*ca.* 50 mg) was accurately weighed onto glass cover slips which were attached to filter paper strips (3 x 7 cm). The strips were introduced into the jars so that they formed a slant harbourage with the bait at the top. Cockroaches fed on the gel bait with minimal contact of other body parts with the deposit. Consumption of the gel normally started within a few seconds. The strips were removed after 1 h, the cover slips detached and the weight of gel consumed by each cockroach determined.

Transfer of toxicity between cockroaches under laboratory conditions. Adult females were held individually for 24 h in 300 ml jars without food (water provided). Gel bait was accurately weighed onto glass cover slips with 10 replicates each of Goliath gel weights in the range 0.1 – 30 mg. Controls comprised 5 replicates each of 5 and 15 mg blank bait. The cover slips were attached to filter paper strips and introduced into the jars as above. Consumption of gel normally started within a few seconds. Dead insects (one per jar) were transferred to 300 ml jars containing 5 live adult females, a water source, a slant paper harbourage and either no alternative food source or a food pellet. The insects were transferred after 24 h (> 5 mg gel consumed) or 2 – 5 days (< 5 mg gel consumed). Mortality in secondary populations of cockroaches was determined 5 days after introducing the cadaver.

Following transfer, either third instar nymphs (10, 5 replicates) or adult females (5, 5 replicates) were added to the treatment jars containing the paper harbourage and any feces or other elimination products produced during poisoning. Both nymphs and adults were provided with water but no alternative food source. Mortality was determined after 5 days.

Field trial. A trial site with an infestation of oriental cockroaches was located in the basement of a post-office sorting office and consisted of an underground service corridor (92 x 1.5 m, Site A) and an electricity sub-station (floor area 287 m², Site B). Harbourages were identified in both areas in floor ducting which extended beyond the trial area and either carried electrical cables or were disused and partially filled with rubble. Site A also had a number of drains, some with partially broken covers. No treatment in either area had been carried out for 4 years prior to the trial. Five days prior to treatment 1 both areas were surveyed by installing 10 sticky traps in Site A and 11 traps in Site B. Traps were collected for analysis on the day of treatment and Site A swept to remove any old cadavers. There was evidence of mouse activity in Site B and some cockroaches were removed from traps leaving only legs remaining in the glue.

Treatment 1 was applied with a gel applicator gun in Site B according to label recommendations (approximately 2 x 30 mg deposits/m²). In Site A treatment 1 was applied using 394 bait deposits (2.9 deposits/m²) individually dispensed onto card squares (c. 15 x 15 mm) for subsequent recovery. After 2 days the bait deposits at site A were inspected for bait take and then removed. All fresh cadavers were collected and 35 newly freezer-killed adult female cockroach cadavers (sentinels) distributed at floor/wall junctions and around drain and ducting covers in Site A. Five days after treatment 1 (3 days after removal of the bait deposits) the sentinels were inspected for indications of necrophagy and removed, together with any additional cadavers (assumed secondary kill).

Cadavers collected after removal of the bait deposits were transferred to the laboratory and caged individually with an adult female cockroach. Mortality was assessed after 48 h. Both sites were re-surveyed with sticky traps set 9 days after treatment 1 and analysed 14 days after this treatment. Treatment

2 was then applied to site A at 3 deposits/m² (to compensate for early removal of the bait in the first treatment) and at 2 deposits/m² where there was evidence of persistence of infestation in site B. A further sticky trap survey was carried out 30 days after treatment 1.

RESULTS

Bait consumption. Mean gel bait consumption over a 1 h period was 24.6 ± 10.9 mg (range 8.7 - 54.6 mg) for adult males and 24.7 ± 15.3 mg (range 3.4 - 50.0 mg) for adult females. This corresponded to a mean consumption of 1230 ng fipronil (males) and 1235 ng fipronil (females). There was no significant difference ($p > 0.05$) between the weight of gel consumed by the two sexes. During the exposure period the insects normally located and consumed the gel within 2 min, remaining at rest on the harbourage or floor of the jar for the remainder of the time.

Transfer of toxicity from poisoned adult females. Mortality in the primary and secondary populations of cockroaches is shown in Table 1. Cockroaches were scored as dead if immobilised and on their backs; limb tremors were observed up to 2 days following this stage but such cockroaches were never observed to recover and were vulnerable to necrophagy by other cockroaches. Death normally occurred in 6 – 12 h following consumption of more than 2 mg Goliath gel but took up to 5 days for cockroaches consuming lower weights. The LD_{50} and LD_{95} of the formulated bait to adult females were estimated by probit analysis as 0.122 ± 0.007 and 0.23 ± 0.03 mg Goliath gel respectively. The LD_{50} is equivalent to 61 ng fipronil/insect or $0.081 \mu\text{g/g}$ and the LD_{95} to 115 ng fipronil/insect or $0.152 \mu\text{g/g}$, respectively.

Table 1. Mean mortality (5 day) in secondary populations of adult females (5) allowed to feed on an adult female poisoned with fipronil.

Wt of Goliath gel eaten by 1 ^o cockroaches (mg)	Mean % mortality of 1 ^o cockroaches (n = 10)	Mean % mortality of 2 ^o cockroaches with alternative food (n = 5)	Mean % mortality of 2 ^o cockroaches without alternative food cockroaches (n = 5)
0	0	0 ^a	0 ^a
0.1	30	0	0
0.2	90	8	25
0.5	100	4	4
1.0	100	0	56
2.0	100	16	76
5.0	100	14 ^a	92 ^a
10	100	12	96
20	100	32	92
30	100	64	96

^a n = 10

Timing and extent of consumption of poisoned primary adult females following transfer to jars containing 5 untreated cockroaches was variable between replicates. Complete consumption of the cadaver could occur within 24 h, but in a few cases the dead insect appeared desiccated when necrophagy first became obvious, particularly when alternative food was available.

Only a few or no frass pellets were observed in the jars containing females poisoned with the gel bait, but cockroaches consuming bait weights above 5 mg frequently left a yellowish-brown liquid (presumed to be regurgitate) either on the paper harbourage or the base of the jar. Mean 5 day mortality of 10 third instar nymphs or 5 adult females caged in these jars (corrected for control mortality) following removal of the poisoned adult female are shown in Table 2.

Table 2. Mean mortality (5 day) of 3rd instar nymphs (10) or adult females (5) caged in jars used to contain adult females feeding on fipronil gel bait following removal of cadaver.

Wt of Goliath gel eaten by 1 ^o cockroach (mg)	Mean % mortality of 10 third instar nymphs caged in treatment jar (n = 5)	Mean % mortality of 5 adult females caged in treatment jar (n = 5)
0	8	0
5	-	28
10	36	16
15	58	-
20	66	40
30	70	96

Toxicity of retrieved cadavers to secondary adult females and necrophagy of sentinels in field trial. Two days following the first treatment 31% of the bait deposits on card squares in the corridor had been consumed; in all cases the bait deposit was either completely consumed or apparently untouched. Areas of complete consumption were concentrated around drain and ducting covers, areas where large numbers of cockroaches were caught during the pre-treatment sticky trap assessment.

Eighteen adult or late instar nymph cadavers were collected and removed from site A 2 days after first treatment, at the same time as the bait deposits were removed. These were each caged with a single adult female cockroaches with no alternative food source; there was 100% mortality in these secondary cockroaches over the following 48 h. 11% of the sentinels distributed in Site A 2 days after removal of the bait and left for the following 3 days showed signs of necrophagy due to other cockroaches. This consisted of a characteristic excavation of the thorax or abdomen with partial removal of the contents (distinct from mouse disturbance). A single additional cadaver was located in Site A when the sentinels were collected, possibly killed by secondary transfer of toxicity.

Reduction in sticky trap catches following gel application at trial site. Sticky trap count analyses for the trial are shown in Tables 3 and 4. Total trap count was reduced to 13% of the initial number by 14 days after first treatment (DAT 1) and 0.5% of the initial number by 14 DAT 2 in trial area A. The corresponding figures for trial area B were 19% and 2.3%.

DISCUSSION

Like other comensal species of cockroach *Blatta orientalis* is a generalist feeder which can include virtually any digestible source of protein in its diet. Under laboratory culture conditions (and particularly when deprived of food) it is cannibalistic, attacking and eating conspecifics especially when they are moulting and relatively defenceless. It is also necrophagic, including cadavers of cockroaches in its diet if available and encountered. The extent to which the species exhibits these traits under field conditions has not been investigated. Fox (1975) considered cannibalism to be more frequent than commonly supposed in natural populations.

Table 3. Analysis of sticky traps from area A of trial site.

sticky trap placement	sticky trap collection	adult females	adult males	late nymphs	early nymphs	total
- 5 DAT ¹ ^a	0 DAT 1	5	23	128	30	186
				treatment 1		
9 DAT1	14 DAT 1	1	5	15	3	24
14 DAT1				treatment 2		
29 DAT1	34 DAT 1	0	0	1	0	1

^a days after treatment 1

Table 4. Analysis of sticky traps from area B of trial site.

sticky trap placement	sticky trap collection	adult females	adult males	late nymphs	early nymphs	total
-5 DAT 1	0 DAT 1	1	2	38	16	86 ^a
				treatment 1		
9 DAT 1	14 DAT 1	0	0	16	0	16 ^a
14 DAT 1				treatment 2		
29 DAT 1	34 DAT 1	0	0	2	0	2

^a including remains of cockroaches apparently removed by mice

The necrophagic trait in this species provides a potential mechanism for transfer of toxicity following consumption of fipronil bait. When adult cockroaches (male or female) were allowed to feed freely on the Goliath gel they consumed a mean weight corresponding to approximately 200 times the LD₅₀ dose for adult females. The mortality of cockroaches feeding on fipronil-poisoned cadavers was shown to increase with the weight of fipronil consumed and occurred to a greater extent when the secondary cockroaches were deprived of an alternative food source. The efficiency of necrophagy as a mechanism of secondary transfer of toxicity depends in part on the extent to which the fipronil is metabolised to non-toxic products between initial consumption of bait and necrophagy by other cockroaches and in part on an even distribution of the cadaver between them. Although not quantified in this study, observations on the manner in which adult females fed on the cadavers suggested that this distribution was generally uneven, with one female breaking open and taking most of the contents of the abdomen and thorax from a corpse, and other females browsing within it thereafter. The rate of fipronil breakdown in the gut of *B. orientalis* and its distribution within the body at death has not been established.

That necrophagy can occur under field conditions was demonstrated in the field trial, where approximately 12% of adult females killed by freezing and left in the treated area were partially consumed over a 3 day period. Following field application of Goliath gel bait, cadavers of cockroaches which consumed sufficient gel could provide an alternative food source for surviving cockroaches, particularly if they died in or close to a harbourage. In view of the relatively small number of cadavers located in the treated areas, it seems likely that most of the population returned to harbourages following bait consumption and prior to death. Kopanic and Schal (1997) suggested that in the case of German cockroaches, secondary transmission of hydramethylnon toxicity via coprophagy is most likely to affect first instar nymphs which forage infrequently. Sticky trap catches from the trial site (Tables 3 and 4) suggested that

the most active foraging stadia of the Oriental cockroach are the late instar nymphs (fourth instars up to the pre-adult stage). It remains to be determined for how long the dried cadavers of fipronil-poisoned cockroaches remain toxic to other cockroaches; if the residue is substantially stable in dried cadavers within harbourages the accumulating corpses could provide a toxic bait for non-foraging stages, immigrants or survivors of treatment, contributing to a prolonged treatment effect following initial consumption of the gel.

An additional mechanism of transfer of fipronil toxicity was observed when adult females or third instar nymphs were held in the jar in which the poisoned female had died. This mortality was interpreted as involving consumption of regurgitate produced by cockroaches feeding on more than 5 mg of gel bait and visible on the glass base of the treatment jar or on the paper harbourage.. Although the effect was observed in the laboratory experiment its relevance to practical control of infestations is doubtful since a liquid regurgitate is likely to be absorbed by the substrate and to be unavailable for consumption by other cockroaches. Transfer of fipronil toxicity by this mechanism was only observed in cases where the primary cockroach had fed on 5 mg or more of the bait.

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