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A NOVEL IMIDACLOPRID BAIT FOR CONTROL OF *MONOMORIUM PHARAONIS* (HYMENOPTERA: FORMICIDAE)

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Abstract A novel semi-liquid ant bait matrix containing imidacloprid between 0.001 and 0.1% destroyed all laboratory colonies of Pharaoh's ant in a dose related time span. Best performance was observed with concentrations ranging from 0.01 to 0.1% imidacloprid.
Two concentrations (0.01 and 0.05%) were field tested in a town in Czech Republic. All flats treated with the higher concentration of 0.05% were free of ants within two weeks after treatment. The lower rate of 0.01% also eliminated all ant infestations but needed a prolonged period of time of 3 month and some re-treatment bait application. A further trial treating 147 apartments with a 0.03% imidacloprid bait resulted in complete eradication of ant infestations. In this trial, bait was applied by bait gun in distinct bait droplets at a rate of 80 and 250 mg of bait per m² treated area. At the first monitoring time point 21 days after application; all treated flats were free of ants. A small number of ants were again seen 40 days after treatment but consequently disappeared again without the need of re-treatment.
Key Words Pharaoh's ant, imidacloprid, baits.

INTRODUCTION

Pharaoh's ant *(Monomorium pharaonis)* is an invasive ant species with cosmopolitan distribution, mostly as indoor pest in urban of multifamily apartment buildings and commercial buildings such as hospitals, hotels, warehouses and other places where food and water are abundant. Apart from being a nuisance pest it also can be a hygiene problem carrying many species of potentially pathogenic germs (Beatson, 1972) and be source of indoor aeroallergens (Kim et al., 2006). Successful control of Pharaoh's ant is usually based on attractive baits containing orally active ingredients such as methoprene, hydramethylnon, sulfluramid, fipronil, fenoxycarb, pyriproxyfen (Boase and Rupes, 1996; Rupes et al., 1997)

An active ingredient missing from the above list but known to be a highly efficacious insecticide is imidacloprid (Leicht, 1993). Like all members of this class of chloronicotinyl insecticides it inhibits the nicotinergic acetylcholine receptor. This receptor is more abundant in the insect nervous system than in warm-blooded animals, making the chemical selectively more toxic to insects. It has been introduced to practical use in 1991 and is the active ingredient of many commercial products in agriculture and professional pest control including bait products (Miller, 1999; Pospischil, 2005; Nishiwaki, 2006).

The objective of the research reported here was to determine the effective dose range of imidacloprid in a semi-liquid bait formulation matrix on laboratory colonies of Pharaoh's ant and evaluation of their field efficacy.

METHODS AND MATERIALS

Laboratory Colonies and Test Methods

Small laboratory colonies of Pharaoh's ant were used for the laboratory tests. The colonies were placed in whole glass Petri dishes 15 cm in diameter and each included on the beginning 3-6 queens, 200-600 workers, larvae III and other brood. To prevent the escape of ants the rims of dishes were coated with non toxic synthetic paste, normally used for protection of fruit trees against crawling insects. The wooden nests with an inner space of 30 x 15 x 2 mm were covered with microscopic slides and which in turn were covered with metal strips to keep the interior dark. The food (hard boiled hen eggs yolk, crushed imagines of laboratory

reared house fly and water) was provided in abundance as competition to the baits tested. The untreated control colonies were supplied with the standard diet only. The food has been changed twice weekly. The impact of the treatment was assessed by temporarily removing the metal cover to each nest and examining the nests through the microscopic slide using binocular stereo-microscope, without disturbing the ants in the nest. The number of living females, workers and presence of the brood in the nests boxes were estimated. The test colonies were reared in standard conditions and temperature 24-26°C (Rupes et al., 1997).

In total, four populations of Pharaoh's ant have been used in this study. Two populations have been collected by laboratory of National Institute of Public Health, Prague in the field at different towns and different time in Czech Republic: Population "U" in a zoo garden in 1983 and population "O" in an apartment blocks at 1997 in Olomouc, the town in which the study has been performed. "Germany" was collected originally in the field in Germany and first reared in a German research laboratory and transferred to our laboratory in 2003. Population "USA" was originally collected in the field at Wisconsin, USA and first reared in a research laboratory in the US, acquired by our laboratory in 1994. The colonies of the different populations of ants were reared in the laboratory under standard conditions and temperature 24-26°C in whole glass container of the size 30 x 20 x 20 cm, without any contacts to toxicants, and fed as the test colonies in Petri dishes.

Aging of Samples

In one part of the study we compared fresh and aged bait samples. To achieve this, bait samples of 250 mg were stored under ambient conditions in the laboratory for 7 days. After aging, these samples were directly compared against fresh samples.

Insecticides

Baits were manufactured and provided by Bayer Environmental Science, Monheim, Germany. Baits formulations are transparent viscous liquids and were supplied in standard 30 g cartridges. For comparison bait boxes of commercial formulation Faratox Turbo (manufactured by ICB Pharma, Poland), containing granulated bait with 0.1% of double walled microencapsulated trichlorfon, has been used.

Field Trials Methods

The field tests were performed at a town in Czech Republic. All apartments in the apartment blocks in which the most apartments has been infested by Pharaoh's ant were monitored for Pharaoh's ant and later treated. The apartment blocks were of higher quality, having been erected in the last 20-30 years. The level of hygiene was mostly high or standard. The apartments were centrally heated, and typically consisted of a kitchen, living room, 1-3 bedrooms, bathroom and toilet, covering an area of 40 to 90 m^2. The field trials were performed in winter heating seasons 2005/06 and 2006/07 as heating provides uniform warmth in the building encouraging greater movement and foraging of the ants. The sites were infested by ants for at least 3 years without any treatments against them. Two sites were left untreated as control. The monitoring and treatments has been performed by authors of this paper and two co-workers named in acknowledgments. The dates of trappings at treated and at untreated control sites were identical and are indicated in Table 6.

To monitor ants before and after treatment, yolk traps (glass tubes, 8 x 1 cm, containing pieces of freshly hard-boiled hen egg yolk in amount which was not completely depleted in any exposed trap) were used. The traps were exposed overnight, at places frequented by the ants (mostly on the desk of kitchen unit near sink), always two traps to one apartment, at intervals shown in tables. At all trappings in all intervals and sites about 60-80% randomly selected apartments were monitored. The total numbers of caught ants in each apartment were recorded. The density of ant populations and efficacy of treatments were expressed by percentage of positive traps (= trap with at least 1 worker in) and by average numbers of workers per evaluated and positive trap (Boase and Rupes, 1996; Rupes et al., 1997).

The experimental viscous liquid baits were applied in the form of small portions, using a Piecemaker Bait gun, with setting 2 for the smaller droplet size of about 100 mg and setting 5 for the larger droplet size of 200 mg. Bait spots, in the day 0 of the trials, were placed in horizontal positions in kitchens, bathrooms and toilets which, across all flats, which had an average size of 20 m^2. We chose places where the foraging

workers were expected and where the baits spots were unlikely to be tampered with during the period of the trial. In exceptional cases, bait was placed in other locations such as sitting rooms or bedrooms if the inhabitants reported occurrence of ants around the flowerpots, aquariums or cages with small pets.

RESULTS AND DISCUSSION

Laboratory Tests

The candidate bait matrix was tested in two variations differing in composition and viscosity, herein named version A and B. Both versions were tested at imidacloprid concentrations of 0.001, 0.01 and 0.1%. Version A bait at the two higher concentrations (0.01 and 0.1%) of imidacloprid and version B bait, at all three concentrations (0.001, 0.01 and 0.1%) of imidacloprid had very high efficacy against laboratory colonies of Pharaoh's ant (Table 1). Estimates of ant mortality indicated that consumption of baits of both versions was high and the synchronised mortality of workers, females and larvae indicated the extent to which the baits had been distributed amongst individuals within colonies. Most dead ants were found in the nest cells. At the end of the tests no living larvae of any instars were found. The progressive increasing of concentrations of imidacloprid in both bait versions abridged the time necessary for destroying the exposed colonies. However, only the versions containing 0.1 and 0.01 % imidacloprid were able to eliminate the colonies within 1-2 weeks. In our experience field situations frequently require a higher active ingredient dose to control ants than is needed under laboratory conditions.

In this stage of development we recommended the use of imidacloprid concentrations between 0.01 and 0.1 % for field trial development. Some liquid baits have the tendency to loose their palatability shortly after application as they dry out, causing their active ingredient concentration to rise and becoming repellent, or in the worst case become solid. To test the palatability and efficacy of the novel bait formulation we allowed version B containing 0.05% imidacloprid to age under room temperature for 7 days. This aging process had no detrimental effect on palatability and efficacy of the bait (Table 2). Both fresh and aged samples of version B bait at 0.05% imidacloprid had killed the whole colonies within 2 and 1 weeks in average. This feature may be beneficial under field conditions as under some circumstances, ants may be recruited late to the bait spots or re-invasion may occur.

Table 1. Efficacy of the novel bait of versions A and B with different concentrations of imidacloprid and commercial formulation Faratox Turbo on the laboratory colonies of Pharaoh´s ant of population U.

Bait version - conc. of Imidacloprid in %	Number of exposed			Number of		Colonies destroyed in week		End of test surviving		
	colonies	females	workers	colonies destroyed	females killed	range	average	colonies	females	workers
A — 0.1	5	16	1800	5	16	1	1.0	0	0	14
A — 0.01	5	18	2100	5	18	1-2	1.2	0	0	1
A — 0.001	5	17	2000	3	17	8-9	8.3	2	3	700
B — 0.1	5	17	2050	5	17	1	1.0	0	0	1
B — 0.01	5	15	2000	5	13	1-2	1.2	0	2	14
B — 0.001	5	16	1800	5	16	3-7	4.6	0	0	15
Faratox Turbo	5	17	1900	5	17	1-2	1.2	0	0	7
Untreated control	4	14	1550	0	0	-	-	4	14	2700

Table 2. Efficacy of the fresh and aged novel bait of version B with 0.05% imidacloprid on the laboratory colonies of Pharaoh´s ant of population U.

Bait version - % of imidacloprid age of bait	Number of exposed			Number of		Colonies destroyed in week		End of test surviving		
	colonies	females	workers	colonies destroyed	females killed	range	average	colonies	females	workers
B — 0.05 fresh	7	21	3000	7	21	1-5	2.0	0	0	0
B — 0.05 aged 7 days	3	9	1250	3	9	1	1.0	0	0	0
Untreated control	5	15	1650	0	0	-	-	5	15	2350

Field Test

Version B bait containing 0.01 and 0.05% imidacloprid was field tested in the city of Olomouc in the winter of 2005/2006 (Table 3). Using a conventional bait gun, each apartment received on average 10.3 bait spots having an average weight of 330 mg in kitchens and bathrooms having a combined size of 20 m². Consequently, bait was applied at a rate of 170 mg per m² treated area. Two apartment blocks, with a total of 64 apartments have been treated. Efficacy results per apartment block were as follows: **Site 1**: This apartment block contained 24 apartments of which 23 where treated with version B bait with 0.05% imidacloprid. 1 apartment was indicated as inaccessible at a later stage and was therefore treated with the control product Faratox Turbo and excluded from monitoring.

2 weeks after treatment all traps were empty of ants, and remained free of ants until the end of the trial. **Site 2**: This apartment block contained 40 units of which 36 were treated with version B bait containing 0.01% imidacloprid. The remaining 4 units were indicated as inaccessible at a later stage and were therefore treated with the control product Faratox Turbo and excluded from monitoring. 2 weeks after treatment, ants had disappeared from most apartments except 3. These three apartments were still infested by ants at 36 days after treatment (DAT), and were re-treated 49 DAT after treatment with a double dose. 2 weeks later, a further 2 apartments were ant free and only 1 unit showed a small number of ants trapped. At 99 DAT, all apartments were free of ants.

Version B bait with concentrations of 0.01 and 0.05% imidacloprid, applied at a rate of 170 mg bait / m² treated area, had a very high efficacy in apartment blocks infested by Pharaoh's ant. However, in the block treated with the low dose, re-treatment was necessary. Based on these finding, an active ingredient concentration higher than 0.01 up to 0.05% was recommended. In winter 2006/2007 the final formulation intended to be marketed was field tested. The final formulation is based on version B bait containing 0.03% imidacloprid. All sites were treated with imidacloprid 0.03% and approximately 15 spots of bait per apartment were applied. Some apartments received a dose rate of approximately 80 mg per m² of treated area (site 3 and floor 1-5 of site 5), and the others 240 mg per m^2 of the product (site 4 and floor 9-13 of site 5). 3 buildings with 116 apartments were treated in total.

Efficacy results per apartment block were as follows: **Site 3**: The 16 apartments of this block were treated with imidacloprid 0.03% at 80 mg per m² treated area. Ant pressure was low with an average number of 12 — 17 ants per trap and about 30% of flats were positive for ants (Table 4). At the first monitoring time point at 14 DAT, all flats were free of ants, and remained free of ants until the end of the monitoring period at day 118 of the trial. **Site 4**: These apartments were treated with imidacloprid 0.03% at 250 mg per m² treated area. Ant pressure was high with around 800 ants trapped in each pre-treatment monitoring and about 50% of flats were infested with ants. Ants had totally disappeared at the first monitoring time point 21 days after application. At the next monitoring time point at day 40, 2 traps in one apartment showed occurrence of ants. However, since day 70 all apartments were clean of ants, and remained free until the end of the trial (Table 4). **Site 5**: This high rise building housing 77 apartments on 13 stories was divided into two treatment zones. Stories 1-5 were treated at the rate 80 mg product per m² of treated area and stories 9-13 received 250 mg per treated m² of the same product (Table 5). The stories 6-8 were also treated by the rate 250 mg

per m^2 but not monitored afterwards as they represented a buffer zone between two treatment regimes. Ant pressure was moderate with around 400 ants trapped in each trapping and 40 to 50% of apartments were infested with ants.

All treatments, both with standard and low dose, resulted in a total disappearance of ants at the first monitoring time point at day 27 after treatment. All apartments remained free of ants until the end of the monitoring period at day 127 after application of the bait (Table 5).

These results demonstrated that bait of version B with 0.03% imidacloprid performed very well under field conditions eliminating ant populations at two dose rates of 80 and 250 mg/m² treated area.

Table 3. Efficacy of the novel bait of version B with two concentration of imidacloprid on Pharaoh's ant in field trials at two apartment blocks in 2006.

Day of test	Site 1, 8 stories, 24 apartments			Site 2 8 stories, 40 apartments		
	Bait version B 0.05% imidacloprid			Bait version B 0.01% imidacloprid		
	% of positive traps	Average number of ants per trap		% of positive traps	Average number of ants per trap	
		evaluated	positive		evaluated	positive
-57	37.5	7.0	18.7	55.3	38.9	74.4
-1	42.9	10.1	23.7	34.6	24.5	70.8
0 treatment 15. and 17.02.	170 mg/m² of bait in treated area			170 mg/m² of bait in treated area		
14	0	0	0	8.6	4.0	47.0
36	0	0	0	9.4[1]	5.4	57.5
65	0	0	0	2.9	0.5	17.5
99	0	0	0	0	0	0

[1] 3 apartments (1/1, 6/5 and 7/5 = floor level/No. of apartment) of the site 2 with surviving ants has been retreated in day 49 of the test.

Table 4. Efficacy of the novel bait of version B with 0.03% imidacloprid on Pharaoh's ant in field trials at two apartment blocks in 2007.

Day of test	Site 3 8 stories, 16 apartments			Site 4 8 stories, 23 apartments		
	% of positive traps	Average number of ants per trap		% of positive traps	Average number of ants per trap	
		evaluated	positive		evaluated	positive
-57	36.4	4.6	12.6	57.1	56.4	98.7
-29	25.0	4.3	17.0	40.9	37.0	90.5
0 treatment 03. and 08. 03.	80 mg/m² of bait in treated area			250 mg/m² of bait in treated area		
21	0	0	0	0	0	0
40	0	0	0	0	3.7	45.0
70	0	0	0	0	0	0
118	0	0	0	0	0	0

The untreated control of both 2006 and 2007 trials are depicted in Table 6. The variation of the proportion of positive traps and numbers of workers per trap were relatively low in untreated control sites over both winter periods of the trials. It can be concluded that changes in ant populations in all apartments of all treated sites could be attributed to the tests baits applied. At the request of tenants, the commercial bait Faratox Turbo was applied on 05. 05. 2006 and the ants were subsequently eliminated.

In a last experiment, the final formulation imidacloprid 0.03% was checked in the laboratory against *M. pharaonis* strains from multiple locations. Imidacloprid 0.03% had very similar efficacy on 4 different populations of Pharaoh´s ant from two continents. All exposed laboratory colonies had been completely destroyed within 1 or 2 weeks (Table 7) but in about 50% of colonies nearly all workers and females were killed and colonies were destroyed only within 4 days. These results demonstrate that the efficacy of imidacloprid 0.03% is not population-specific and can be expected independent of geographic location.

Table 5. Efficacy of the novel bait of version B with 0.03% imidacloprid on Pharaoh's ant in field trials in 2007. Site 6 was divided in two treatment zones (stories 1-5 and 9-13), treated with the different rate doses. Stories 6-8 were also treated but excluded from the analysis.

Site 5 13 stories, 77 apartments						
Day of test	**% of positive traps**	**Average number of ants per trap**		**% of positive traps**	**Average number of ants per trap**	
		evaluated	**positive**		**evaluated**	**positive**
-22	13.0[1]	10.9[1]	25.2[1]	-	-	-
-1	19.0[2]	9.2[2]	23.2[2]	51.9[3]	12.1[3]	23.3[3]
0 treatment 02. 03.	stories 1 - 5 80 mg/m^2 of bait in treated area			stories 9-13 250 mg/m^2 of bait in treated area		
27	0	0	0	0	0	0
42	0	0	0	0	0	0
72	0	0	0	0	0	0
120	0	0	0	0	0	0

[1] average of stories 1 – 13; [2] average of stories 1-5; [3] average of stories 9 – 13

Table 6. Pharaoh´s ant populations in the control untreated sites of the field trials in 2006 and 2007

Site 6 8 stories, 24 apartments				**Site 7 7 stories, 46 apartments**			
Date of trapping in 2006	**% of positive traps**	**Average number of ants per**		**Date of trapping in 2007**	**% of positive traps**	**Average number of ants per**	
		evaluated	**positive**			**evaluated**	**positive**
19.01.	42.9	11.7	27.3	11.01.	21.8	3.8	18.0
15.02.	22.2	6.1	31.3	02.03.	14.6	4.9	34.3
01.03.	39.1	16.3	41.8	29.03.	20.6	7.1	34.6
23.03.	56.0	15.9	28.4	12.04.	25.0	8.0	32.1
21.04.	64.3	15.5	24.2	17.05.	27.6	7.7	26.9
05.05.	Treatment: Faratox Turbo 5 bait stations per apartment			04.07.	35.7	8.8	24.7
25.05.	3.3	0.03	1.0	-	-	-	-

Table 7. Efficacy of the novel bait of version B with 0.03% imidacloprid on the laboratory colonies of Pharaoh´s ant of four different populations.

Population	Number of exposed			Number of		Colonies destroyed in week		End of test surviving		
	Colonies	Females	Workers	Colonies destroyed	Females killed	Range	Average	Colonies	Females	Workers
U	21	74	7600	21	74	1-2	1.2	0	0	36
O	14	45	5700	14	45	1-2	1.5	0	0	37
Germany	14	44	4800	14	44	1	1.0	0	0	27
USA	14	51	5600	14	51	1-2	1.3	0	0	20
Untreated control	8[1]	27	3050	0	0	-	-	8	27	3300

[1] two untreated colonies of each population

CONCLUSIONS

Imidacloprid has been shown to be efficacious active ingredient for the control of in baits. The conducted studies demonstrate that concentrations of 0.01 and 0.05% do control Pharaoh's ants in the field (Table 3). The need for some re-treatments with the low concentration baits led to the recommendation of a higher concentration than 0.01 for the final product. Indeed, imidacloprid 0.03% performed well in field trials conducted in winter 2006/2007 (Table 4, 5). Even at lower dose rates of around 80 mg/m² treated area excellent control was achieved. The result s obtained with dose rates of around 250 mg/m² were equally good. With respect to robustness and safe guard against rebounding colonies, the higher use rate is recommended. The bait of version B with 0.03% imidacloprid could be applied by a conventional bait gun and allowed easy and safe application.

Additional laboratory studies indicate that the bait matrix is very palatable to Pharaoh's ants, and remains palatable for at least 7 days after application (Table 2). This feature ensures that ants that find the bait not immediately still feed sufficient amounts to lethally intoxicate the colony. Further tests documented that bait of version B with 0.03% imidacloprid is efficacious against various strains of regardless their geographic origin (Table 7). Imidacloprid has also been shown to successfully control other species of ants, and (Brooks, 2007, personal communication).

The bait of version B with 0.03% imidacloprid is marketed as ant bait for the professional pest control operator under the brand name Maxforce® Quantum.

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