Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

EXPERIMENTAL DESIGN FOR EFFICACY TESTING OF BAITS AGAINST *MONOMORIUM PHARAONIS* (HYMENOPTERA: FORMICIDAE)

ANNE KRÜGER, JUTTA KLASEN, AND ERIK SCHMOLZ
Federal Environment Agency, Sect. IV 1.4 Health Pests and their Control,
Boetticher Str. 2, Building 23, 14195 Berlin

Dedicated to the memory of our friend and colleague, Gabi Schrader

Abstract In the European Union, baits against pharaoh ants (*Monomorium pharaonis*) have to be authorized as biocidal products. The dossier to be submitted for authorization of the product has to include a proof of product efficacy. No consistent test standards have been established by the European Commission. A test method for efficacy testing of baits against Pharaoh ants was published by the Federal Environment Agency Germany in 1998 and is in use for efficacy tests according to the German Infectious Diseases Protection Act. The test system is a test arena of interconnected test boxes (each 25 cm x 25 cm). In our study, this test system was compared with a dish (diameter 23 cm) as test arena. Test duration was 50 days for both arena types (boxes and dish) for choice and 20 days for no-choice tests. The results for choice tests revealed that in the box system more colonies were eradicated (boxes: 75%, dishes: 0%) within 50 d, and more queens died (average number of surviving queens was 0.5 in boxes and 29.7 in dishes).
Key words Pest control, insecticides, Pharaoh ant, proof of efficacy

INTRODUCTION

Monomorium pharaonis is an ant species of major importance as a health pest organism, since it is a mechanical vector for human pathogens such as salmonella (*Salmonella* sp.), *Pseudomonas* sp., *Klebsiella pneumoniae*, *Streptococcus* sp., *Proteus mirabilis*, *Enterobacter* sp. (Beatson, 1972). Moreover, pharaoh ants can also cause respiratory allergies (Kim et al., 2007). Due to global transport, pharaoh ants are distributed worldwide and can be found on all continents except for Antarctica. The ability to distribute is facilitated by their small size, as they can nest in numerous small transportable objects. Pharaoh ants are polygynous with many queens and hence, new colonies containing only a few workers with queens and brood (Peacock et al., 1955; Petersen and Buschinger, 1971) can be founded easily through fission or budding (Vail and Williams, 1994). Pharaoh ants prefer humid and warm conditions and occur mainly indoors. They infest apartment and public buildings, zoos, restaurants and hospitals.

For control of *Monomorium* the best method is the use of oral baits. Not all of the ants in a colony forage outside of the nest, and thus insecticides sprays or dust hardly achieve eradication. Oral baits are distributed to queens, workers and brood in the nest through trophallaxis (Edwards and Abraham, 1990).

In the European Union, biocidal products and active substances are regulated under the EU Biocides Regulation (EU BPR) 528/2012. Biocide products have to be authorized before being used or sold on the EU market. Since the pharaoh ant is a health pest, insecticidal baits for their control are categorized as biocidal products. For authorization of bait products, the applicants have to submit a dossier which has to include a proof of efficacy. The Technical Notes for Guidance (PT 18) give only general information about testing conditions for baits controlling pharaoh ants. Up to now, valid international standards are lacking, and test duration, size of test arena or size of colony used for testing are not standardized.

A German test guideline (Iglisch, 1998) for efficacy tests has been published by the Federal Environment Agency Germany for efficacy tests according to the Infectious Diseases Protection Act (Infektionsschutzgesetz § 18). The test system prescribed by the guideline is a multi-compartment system which tries to simulate the situation of infestation and control of pharaoh ants. In case of an infestation the bait would be placed on an ant trail somewhere between the nest and a food source. Thus, the test system consists of three compartments, a nest compartment connected to a bait compartment which again is connected to a compartment containing the food source, and the ants have to pass the bait to reach the food source. The assumption of this test system is that if the bait is sufficiently attractive, ant foragers prefer it over the non-toxic food source since it can be acquired with lower foraging costs.

Although this test system simulates the practical situation in which a bait product is used, it is expensive to build and consumes larger areas of laboratory space, thus limiting the number of replicates in an experiment. Small ready-made test arenas made from laboratory material already available for other purposes may allow a larger number of replicates with lower costs. Without valid standards for test designs it is challenging to evaluate efficacy tests for authorities. There is little information about the influence of test design (e.g. position of the bait in relation to the food source, distance between nest and bait) on test outcome, which makes an objective assessment of test data difficult.

In this study the test system used for testing the efficacy of baits against pharaoh ants by the German Infectious Diseases Protection Act was compared with a single-compartment system to get information about the of the test design´s impact on the test outcome.

MATERIALS AND METHODS

Study Animals. Pharaoh ants were taken from the laboratory colony in the Federal Environment Agency. Ants were kept in cylindrical glasses (300 mm high) coated with polytetrafluoroethylene (PTFE) at the sidewalls to prevent escape. Small plastic containers with cellulose tissues served as nest boxes. Ants were fed dead *Periplaneta americana* and a drinking trough consisting of a small petri dish with cotton ball soaked with water (66%), honey (30%) and multivitamin syrup (Sanostol®) (4%). Climate conditions were 25 ± 1.5°C and 60 ± 10 % humidity.

Transfer of Ants and Nesting Boxes. Small glass tubes were placed in an empty cylindrical rearing glass and nest boxes containing queens, workers and brood from the laboratory colony were poured out into the glass. The ants used the tubes as nest shelters and moved into the tubes with brood and queens. The tubes were used as a means of assembling and transferring the ants into the test arenas. Before placing the tubes in the test systems the number of queens was visually estimated and about 30 queens were introduced in each arena. In the arena wood cubes (30 mm x 30 mm x 13 mm) with holes (diameter 25 mm, height 6 mm) served as nesting boxes. At each of the four sides a 1 mm diameter hole was drilled as a nest entrance. Nest boxes were covered with glass slides and the slides were covered with grey plastic to keep the interior dark. In every test arena two nesting boxes were placed.

Bait. The insecticide agent of the bait used in the choice and no-choice tests was borax. Borax and boric acid baits are used for the control of pest ant species (Vobrázková et al. 1976, Klotz and Moss 1996; Klotz et al. 2000, Rust et al. 2004, Sola et al. 2013). Borax has not been notified in the EU as a biocidal insecticide (PT 18). We used this active ingredient to avoid possible conflicts with biocide authorisation, as the aim of our study was the evaluation of the test system rather than the insecticide.

We used a bait composition similar to that described by Klunker et al. (1990): whole egg powder (63 %), honey (23 %) and borax (14 %). Bait was offered in small petri dishes (diameter 3 cm).

Test Systems. All of the test arenas were placed on a plate surrounded by double walls filled with paraffin oil between the two walls in order to prevent ant escapes. Two types of test arenas were investigated: two or three boxes connected with tubes (multi-compartment system) and crystallizing dishes (single-compartment system) (Figure 1).

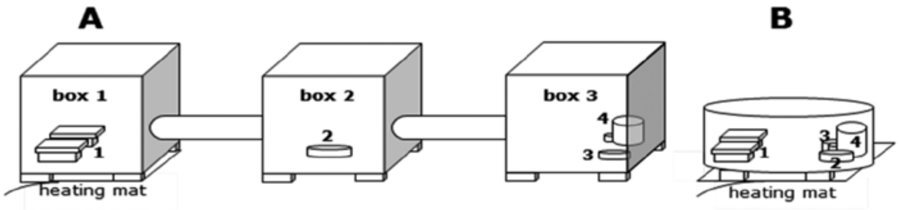

Figure 1. Test design of the multi-compartment system (A) and single-compartment system (B). 1 = nest boxes, 2 = bait, 3 = non-tox food, 4 = trough

Multi-compartment system. The system consisted of three connected test boxes for choice trials and two connected test boxes for controls and no-choice tests, respectively. The test boxes were connected with tubes. Boxes as well as tubes were made of acrylic glass. Each box had a size of 25 cm x 25 cm x 20 cm, the tubes were 25 cm long, and had an inner diameter of 4 cm. To avoid ants from escaping, the upper part of the walls was covered with insect glue. One test box/compartment was used as nesting compartment in which the nesting boxes were placed. The bottom of this box was heated to 30 °C with a heating mat. The nesting compartment was directly connected to a box which either contained bait and drinking trough in no-choice tests or food and a drinking trough for the control group. For choice tests, the box next to the nest box contained bait and was connected to a third box which contained non-tox food (dead oriental cockroaches, *Blatta orientalis*) and a drinking trough. The ants started foraging in box 1 (nest compartment), had to pass the ant bait in box 2 (bait compartment) before they reached non-poisoned food and the drinking trough in box 3 (food compartment).

Single-compartment system. The single-compartment system consisted of a crystallizing dish as a test arena. The dishes were made of glass and had a diameter of 23 cm and a height of 15 cm. The walls were coated with polytetrafluoroethylene (PTFE) to prevent ants from escaping. In choice tests the nesting boxes were placed on one side of the crystallizing dish, and the drinking trough and the bait were placed side by side on the opposite side of the dish. In no-choice tests only the bait and water and in the control only nontoxic food and water were offered. Heating mats were placed under the crystallizing dishes to achieve a temperature of 30°C like in the nesting compartment of the multi-box system. In multi-compartment systems the distance between nest and food source was about 120 cm, and from nest to bait about 50 cm. In single-compartment systems the distance to both food and bait was about 15 cm.

Test Protocol. The ant colonies were acclimatized in the respective test arena for one week prior to the introduction of poison baits. During this period, they were fed with dead oriental cockroaches and a mix of water and honey (for exact composition, see above).

In the multi-compartment system, at the beginning of the experiments the bait was placed in the bait compartment (box 2) in choice tests. In no-choice tests the non-toxic food (cockroaches) was replaced by the bait. The mix of water with honey was replaced with water. Water and non-toxic food replenished when needed. The baiting period was scheduled after Iglisch (1998) with 50 days in choice tests and 20 days in no-choice tests in experiments with both types of test arenas (multi-compartment as well as single-compartment). A trial ended either at the end of the baiting period or when a colony in an arena was eradicated. Once a week a photograph was made of every test arena and ants were counted. In the nests the number of queens was estimated by visual inspection and the test arenas were inspected for dead queens. At the end of all trials, test arenas with surviving ants were frozen and queens and workers were counted.

RESULTS AND DISCUSSION

To evaluate the two test designs the parameters for comparison were the number of workers counted on the photographs and the number of dead queens collected during the experiment, the number of the survived workers and queens after the trial period and if an eradication (100% mortality) was achieved. In none of the tests systems was eradication of all colonies achieved (Table 1). This was due to the limited duration of the tests (50 and 20 days). Results indicate that longer test durations are more realistic for bait efficacy tests. Eradication was achieved in 75 % of the colonies after 50 days of bait exposure in the multi-compartment system in choice tests. 50 % of the colonies were eradicated after 20 days in no-choice tests. In the single-compartment system no eradication was achieved in choice tests and 25 % of the colonies were eradicated in no-choice tests (Table 1).

The number of surviving queens during the experiments differed markedly between the arena designs. In multi-compartment systems, nearly no queens survived (0.5, SD 1) in choice as well as no choice tests. In the single-compartment system queen survival was highest in choice tests (29.7, SD 9). 20 days after the beginning of the experiment the number of queens was approximately half in no-choice tests in the single compartment system (Table 2). In multi-compartment systems also almost no surviving workers were found in contrast to the single-compartment system (Table 1, Figure 2).

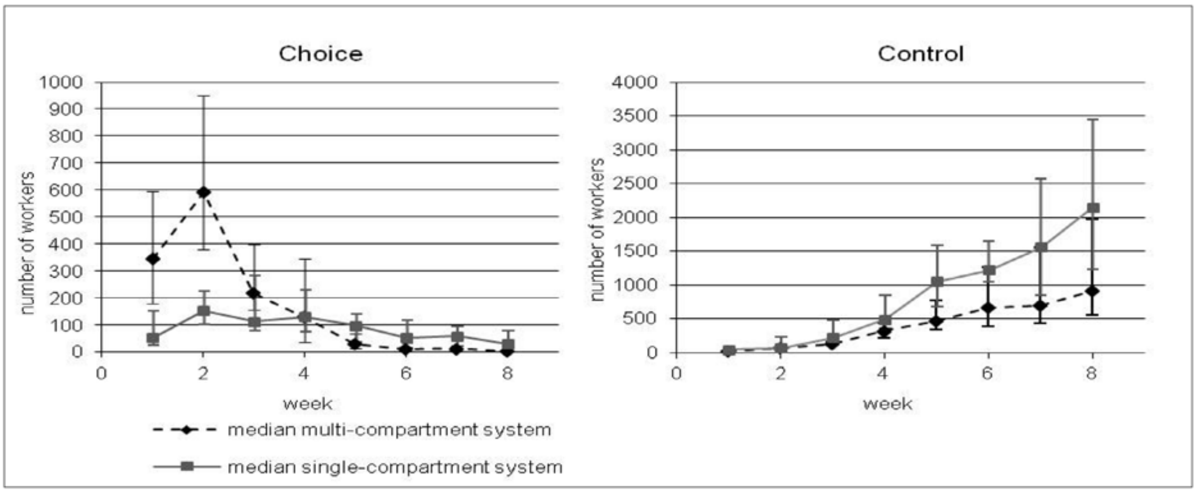

Figure 2. Number of workers in multi-compartment systems (n = 4) and single-compartment systems (n = 12) counted weekly. Squares denote the median of number of workers, bars denote the 1. and 3. quartile.

More workers foraged in the multi-compartment system than in the single-compartment system at the beginning, but this changed in week 4 (Figure 2). An explanation for this would be that the longer distance between nest and food sources in the multi-compartment system increased time and energy expenditure for foraging. Hence, in the multi-compartment system, ant workers had to be more active to meet the food and energy demands of the colonies, which were of equal size in both test systems. The difference in colony development between the multi- and single-compartment system could also be observed in the development of queen numbers in the controls: although the number of queens was approximately identical in both test systems at the beginning of the experiment, it increased about 3.5 fold in the multi-compartment system and about 7.5 fold in the single compartment system (Table 2).

We hypothesize that the loss of workers due to the action of the insecticide had a more profound effect on colonies with a higher energy demand due to greater foraging distances. After approximately 4 weeks we observed that workers in multi-compartment systems stayed in the food and bait boxes and did not return to the nest to feed the queens and brood. This could be another reason for the differences in the number of surviving queens between both test systems, as the queens in the multi-compartment system probably died due to starvation. In single-compartment systems the workers took care of the queens until the end of the test period.

A second factor which could explain the differences in mortality and queen survival in choice tests is the arrangement of food and bait. In the single-compartment system, food and bait were placed side by side. In the multi-compartment system the bait was placed at half the way to food and trough. The decision to take food or bait can be influenced by their distance to the nest or their arrangement. Fewell (1988) could show that in the harvester ant *Pogonomyrmex occidentalis,* saving time is more important than direct energy costs in the choice of the food source. Hence, in the multi-compartment systems the shorter time needed for foraging of the bait compared to the non-toxic food biased the ant decisions towards the bait. This decision had not to be made in the single-compartment system, since food and bait were placed side by side at an equal distance to the nest. Energy or time costs were not crucial factors for foraging decisions. The bait has to be much more attractive than the non-toxic food. In our experiments cockroaches were the non-toxic food. Cockroaches are attractive to pharaoh ants and are preferred over otherwise very attractive and effective baits (Sy, 1987; Adams et al., 1999). Although different bait choice behavior related to bait position may has an influence on test outcome, this holds only good for choice tests. The differences in queen survival and time for colony eradication were observed in no-choice tests in the absence of a non-toxic food source, when ant foragers obviously could not make a decision which bait to prefer.

Table 1. Control and colony survival in efficacy tests of an insecticide in two different types of test arenas.

Type of test	n	Bait exposure time (d)	Minimum time for eradication (d)	Eradicated colonies (%)	Number of surviving workers		
					Median	1. quartile	2. quartile
Choice							
Multi-compartment	4	50	28	75	0	0	1
Single-compartment	12	50	-	0	25.5	14.3	55.3
No-choice							
Multi-compartment	4	20	16	50	1	0	8.3
Single-compartment	4	20	19	25	10	3.8	16

Table 2. Queen survival in efficacy tests of an insecticide in two different types of test arenas.

Type of test	**Test design**	**n**	**Queens inserted (estimated)** Mean (Standard deviation)	**Dead queens (counted)** Mean (Standard deviation)	**Surviving queens (counted)** Mean (Standard deviation)
Choice	Multi-compartment	4	28.3 (3.3)	33.8 (2.8)	0.5 (1)
	Single-compartment	12	25.6 (5.1)	2.5 (4.1)	29.7 (9)
No-choice	Multi-compartment	4	27.8 (7.2)	31.5 (11.4)	0.5 (1)
	Single-compartment	4	25 (5.4)	1 (17.9)	13.3 (16.3)
Control	Multi-compartment	4	23.3 (4.8)	1 (1.2)	82.5 78.4
	Single-compartment	4	20 (4.1)	0.25 (0.5)	151.3 163.5

In conclusion, the absolute distance between bait and nest had an influence on test outcome in all tests, and the relative distance of bait and non-toxic food may have had an influence on the test outcome in choice tests. Our study shows that the test design, size of test arenas, and test duration have to be taken into account for evaluation of efficacy studies. In contrast to our initial expectation, it may be more challenging to achieve an eradication of an ant colony in small test arenas than in large test systems. Further studies are needed to identify the impact of parameters like bait position and distance to the nest.

REFERENCES CITED

Adams, A., Kunkel, S., Dodd, G., and S. Höbel. 1999. Method and procedure for evaluating biological performance of Pharaoh ant, *Monomorium pharaonis* (Hymenoptera: Formicidae), baits. *In*: Robinson W. H., Rettich F. und Rambo G. W. (Hrsg.): Proc. of the 3rd International Conference on Urban Pests: 203-209.

Beatson, S.H. 1972. Pharaoh´s ants as pathogen vectors in hospitals. Lancet 299: 425-427.

Edwards, J. P. and L. Abraham. 1990. Changes in food selection by workers of the Pharaoh's ant, *Monomorium pharaonis*. Medical and Veterinary Entomology 4: 205-211.

Fewell, J.H. 1988. Energetic and time costs of foraging in harvester ants, *Pogonomyrmex occidentalis*. Behavioral Ecology and Sociobiology 22: 401-408.

Iglisch, I. 1998. Richtlinien für die amtliche Prüfung von Mitteln und Verfahren auf Wirksamkeit zur Bekämpfung tierischer Schädlinge gemäß §10 c Bundes-Seuchengesetz. [In German; *Guidelines for official testing of pesticides and techniques for control of health pests according to § 10c of the Federal Act on Epidemics*]. Bundesgesundheitsblatt 4: 184- 189.

Kim, C.W., J.S. Song, S.Y. Choi, J.W. Park, and C.S. Hong. 2007. Detection and Quantification of Pharaoh Ant Antigens in Household Dust Samples as Newly Identified Aeroallergens. Internat. Arch. Allergy and Immunology 144: 247–253.

Klotz, J.H. and J.I. Moss. 1996. Oral toxicity of a boric acid - sucrose water bait to Florida carpenter ants (Hymenoptera: Formicidae). Journal of Entomological Science. 31: 9-12.

Klotz, J.H., Greenberg, L., Amrhein, C., and M. K. Rust. 2000. Toxicity and Repellency of Borate- Sucrose Water Baits to Argentine Ants (Hymenoptera: Formicidae). Journal of Economic Entomology 93:1256-1258

Klunker, R., S. Scheurer, and T. Neumann. 1990. Zur Bekämpfung der Pharaoameise mit Borax-Köderpräparaten [In German; *Control of the Pharaoh's ant with borax bait formulations*]. Zeitschrift für die gesamte Hygiene und ihre Grenzgebiete 36: 664-667

Peacock, A.D., J.H. Sudd, and A.T. Baxter. 1955. Studies in Pharaoh´s ant, *Monomorium pharaonis* (L.). 11. Colony foundation. Entomologist's Monthly Magazine 91: 125-129.

Petersen, M. and A. Buschinger. 1971. Untersuchungen zur Koloniegründung der Pharaoameise *Monomorium pharaonis* (L.). [Article in German; *Investigations on colonyfounding of the pharaoh ant Monomorium pharaonis* (L.)]. Anzeiger für Schädlingskunde und Pflanzenschutz 44: 121-127.

Rust, M. K., Reierson, D.A., and J.H. Klotz. 2004. Delayed Toxicity as a Critical Factor in the Efficacy of Aqueous Baits for Controlling Argentine Ants (Hymenoptera: Formicidae). Journal of Economic Entomology 97:1017-1024.

Sola, F., Falibene, A. and R. Josens. 2013. Asymmetrical behavioral response towards two boron toxicants depends on the ant species (Hymenoptera: Formicidae). Journal of Economic Entomology 106:929-938.

Sy, M. 1987. Über einige Probleme bei der Bekämpfung der Pharaoameise (*Monomorium pharaonis* L.) mit dem Rinal Pharaoameisenköder. [Article in German; *Problems in control of the pharaoh ant Monomorium pharaonis L. with Rinal ant bait*] Anzeiger für Schädlingskunde und Pflanzenschutz 60: 51-55

Vail, K.M. and D.F. Williams. 1994. Foraging of the Pharaoh ant, *Monomorium pharaonis*: An exotic in the urban environment. *In*: Williams D.F. (eds.) Exotic ants: biology, impact and control of introduced species. California: Westview Press: 228-239.

Vobrázková, E., Vanková, J. and K. Samsinák. 1976. Application of Bathurin and borax in the biological control of *Monomorium pharaonis* in housing estates. Angewandte Parasitologie 17: 94-99.