Proceedings of the Eleventh International Conference on Urban Pests 2025
AF-Borgen, Academic Society, Lund, Sweden
William H Robinson, editor

FIGHTING AGAINST A SUPERORGANISM: HOW TO CONTROL PHARAOH ANT COLONIES IN URBAN AREAS

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Abstract Pharaoh ant (*Monomorium pharaonis*) is a polygynous ant species that successfully colonizes the human environment and is capable of nesting within buildings and even in the smallest artificial structures allowing easy spread of colonies by transportation under different ecological conditions. It can be found in households, hospitals, and storage facilities worldwide, posing a threat to human health and goods. This is the most widely distributed and most successful invasive species in the family *Formicidae*. The formation of large colonies with several queens is characteristic of this species, helping these ants to be present in high densities over larger areas. This poses significant challenges even for professional pest control operators. Treating the whole colony, including hidden queens, is essential for successful pharaoh ant eradication. Due to their specific nesting habits, conventional, fast acting insecticide active substances and non-chemical methods can not effectively control large, established colonies of this ant species. The combination of a palatable bait formulation with a specific insect growth regulator-type active substance of indirect, delayed action, S-methoprene can serve as a potent tool for solving this problem based on simulated-use laboratory studies and field efficacy tests performed in many countries. This ready-to-use, easy applicable product is also recognized as a tested and recommended tool for the control of health pests in accordance with § 18 of the Infection Protection Act by the German Environment Agency (Umweltbundesamt) providing complete pharaoh ant colony eradication within 10-18 weeks after the beginning of treatment depending on colony size and abiotic conditions.

Key words pharaoh ant, IGR, S-methoprene, colony control

INTRODUCTION

Five of the world´s top 20 most expensive invasive invertebrates threatening agricultural production, goods, properties, and human health, as well as harmful to local ecosystems belong to unicolonial ant species (Pedersen et al., 2006; Tsutsui and Suarez, 2003; Arim et al., 2006). The pharaoh ant (*Monomorium pharaonis*, Linnaeus, 1758) is one of the true unicolonial ant species among these species, which can have more than one (even hundreds) queens in multiple nests in their supercolonies living in larger areas, and ant workers move freely among nests (Bourke and Franks, 1995). Actually, their supercolonies are close associations of some mother colonies and larger and smaller daughter colonies (buds) with lower overall relatedness within the supercolony than in the case of a monogynous ant colony, but without antagonism between workers. This seems to contradict the principle of kin selection theory, but due to the absence of intra-specific aggression toward more (but not too much) distant relatives, these ants can use their energy for more efficient exploitation of local resources and successful inter-specific competition. This is beneficial for invasive species by helping their populations to spread faster and easier in new habitats (Holway and Suarez, 2004; Tsutsui et al., 2000). Another positive

aspect of unicoloniality is the lower parasite load because of lower relatedness and higher genetic variability within the supercolony (Schmid-Hempel and Crozier, 1999).
This very effective social structure is the leading cause of why getting rid of these ant species is so difficult. Pharaoh ant control can also be challenging because as a tropical, fast-developing ant species (the whole process from egg to adult can take place within five weeks), they breed continuously throughout the year in heated buildings, and mating occurs hidden in their nests (seasonal, harmonized nuptial flights are not required). Thus, they successfully colonize the human environment and can nest within buildings and even in the smallest artificial structures, allowing easy spread of colonies by transportation under different ecological conditions (Peacock et al., 1955; Petersen and Buschinger, 1971; Vail and Williams, 1994).

The formation of large colonies with several queens is characteristic of this species, helping these ants to be present in high densities over larger areas. Therefore, treating the whole supercolony, including hidden queens, is essential for successful pharaoh ant eradication. However, due to their specific nesting habits, conventional, fast-acting insecticide active substances and non-chemical methods (e.g., trapping) can not effectively control large, established colonies of this ant species. Moreover, disturbing the colonies can make the eradication process even more difficult or sometimes impossible, resulting in the formation of migrant subcolonies spreading into neighboring buildings and properties.

Therefore, oral baits (gels or granules) are the best method for controlling pharaoh ant colonies (Edwards and Abraham, 1990). Alternatives to fast-acting active substances are insect growth regulators, such as S-methoprene, which is especially suitable for use in baits due to its specific mode of action. In response to S-methoprene treatment, the development of the insects is interrupted. However, an instant killing effect does not occur following bait consumption, resulting in a longer baiting period required, but ensuring that the effect can extend to the entire colony by trophallaxis, including the brood and queens hiding in the core of their nests, resulting in total colony control. Because of the delayed effect of S-methoprene, the ants can also form stocks from the bait in their nests.

MATERIALS AND METHODS

The aim of this free-choice, simulated-use study was to evaluate the palatability and efficacy of BIOPREN® Pharaoh Ant Colony Eliminator bait in two formulations, one without the active substance and one with 0.5 w/w% S-methoprene in the same bait matrix to show colony control efficiency of this potent tool against pharaoh ants.

Laboratory-reared pharaoh ant colonies were provided with artificial nests containing proper hiding material placed in larger containers. Water, vitamins, macro- and micronutrients, sugar and protein source (non-toxic food) were provided *ad libitum* in the arena. The ants were kept at room temperature (25±5 °C), at 50±10% relative humidity (RH), under natural light conditions. The bioassays were conducted using whole ant colonies showing similar activity, with a similar number (estimated) of worker ants, queens, brood, and males if contained in the nests. Test colonies were removed from the rearing boxes within their nests and released in the test arenas prior to treatment. Each parallel colony (replicate) was kept in a separate test arena. 5-5 parallel treatments were carried out to test each bait variant (with or without S-methoprene) and for the untreated control, respectively. Control colonies were kept in the same test room, under the same abiotic conditions as treated colonies during the experiment.

The baits were offered in transparent, plastic bait stations of the commercial product, BIOPREN® Pharaoh Ant Colony Eliminator, and alternative (non-toxic) food sources, as well as

water were also provided *ad libitum* to treated colonies in a free-choice test design. After transferring the nests into the test arenas, the colonies acclimatized with non-toxic food and water for 1 week. During the following starving period, the ants were provided with only water for 4 days. After that, non-toxic food and the respective bait were offered.

Palatability, mortality, and colony state evaluation were performed 0, 60, and 120 min after bait and food offering and during the experiment twice a week. The baiting period took 16 weeks, followed by a 2-week post-baiting observation period. For the palatability evaluation, worker ants at the bait station and non-toxic food were counted for 1 min at each evaluation point until mortality reached 100%, or up to the end of the study. Furthermore, dead ants in the arena were counted for efficacy evaluation and removed. Bait stations, non-toxic food, and water were replaced if empty. In controls, the number of ants at the non-toxic food and the number of dead ants were documented the same way as in the treatments.

At least once a week, the plastic cover of the nest was opened to document colony activity, as well as to examine whether the queens and brood were alive using an ordinal scale to evaluate the colony state (0-4: from score 0 for no vital brood in the colony to score 4 for a blooming colony with many large progeny conglomerates). At the end of the experiments, all dead ants were counted and removed, and the test arenas with alive ants were frozen to count the number of alive queens and worker ants.

RESULTS AND DISCUSSION

Palatability and foraging activity After the 4-day starving period and directly after food and bait offering, high foraging activity was observed in all three treatments and their replicates. On day 0, no difference in preference between the non-toxic food and the baits was observed regardless of whether S-methoprene was included in the bait. Ants carried the non-toxic food and the bait equally from the containers into the arena, especially near the ant trails, at the nest, and into the nest. The number of ants counted on the baits was lower from day 3 than observed on the non-toxic food, mainly because the protein source (pre-killed cockroaches) could not be carried into the nest or in the arena, and the ants had to consume it directly at the feeding site. Foraging activity over the whole test period was comparable in different treatment groups (Figure 1). The activity in the treatments using baits containing S-methoprene decreased after 73 days of baiting as a consequence of a massive reduction in the number of ants in the colonies.

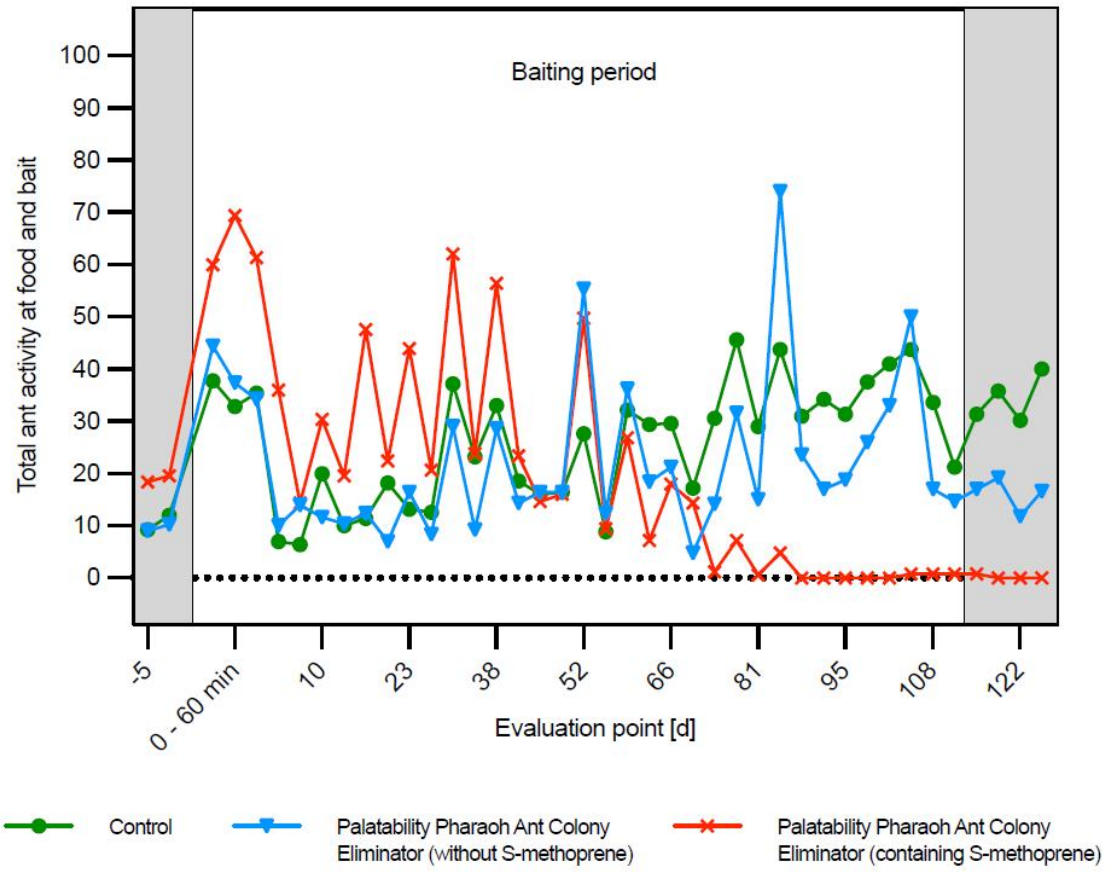

Figure 1. Activity of worker ants in the acclimatization and starving period (grey), baiting period (white), and post-baiting period (grey) in simulated-use tests. (The mean sum of worker ants at the non-toxic food and the bait stations in the 5 replicates within 1 min are shown.)

Efficacy In untreated controls, 990 worker ants were counted dead in the five replicates up to the end of the test period. At the end of the study, 5652 worker ants were alive in all replicates. Queen mortality in controls was low, with only 8 dead queens within the test period and 102 alive queens in total. In treatments using the bait without S-methoprene, 705 worker ants were documented as dead within the test period, and 2929 worker ants were alive at the end of the study. Queen mortality was low. Only 6 queens were found dead within the test period. At the end of the study, 205 queens (189 mated, 16 unmated) were still alive. In treatments using the S-methoprene containing bait, 1271 worker ants were found dead until day 95 post application (p.a.) No alive worker ant was found from day 95 p.a. in the parallel colonies. After all the worker ants were dead, queens were still alive in the replicates. The last queen was found dead on day 122 p.a. (1 queen was in knocked-down state on day 126 only in one replicate). In total, 83 queens were found dead, and no alive queens were found in the 5 replicates. In treatments using S-methoprene containing bait, 256 immature queens were found dead, showing malformed wings between day 42 and day 73 p.a. Queen survival rate in the untreated controls and the two

treatments was analyzed using the Kaplan-Meier method (Figure 2) resulting in statistically significant differences ($p < 0.0001$) between the treatments.

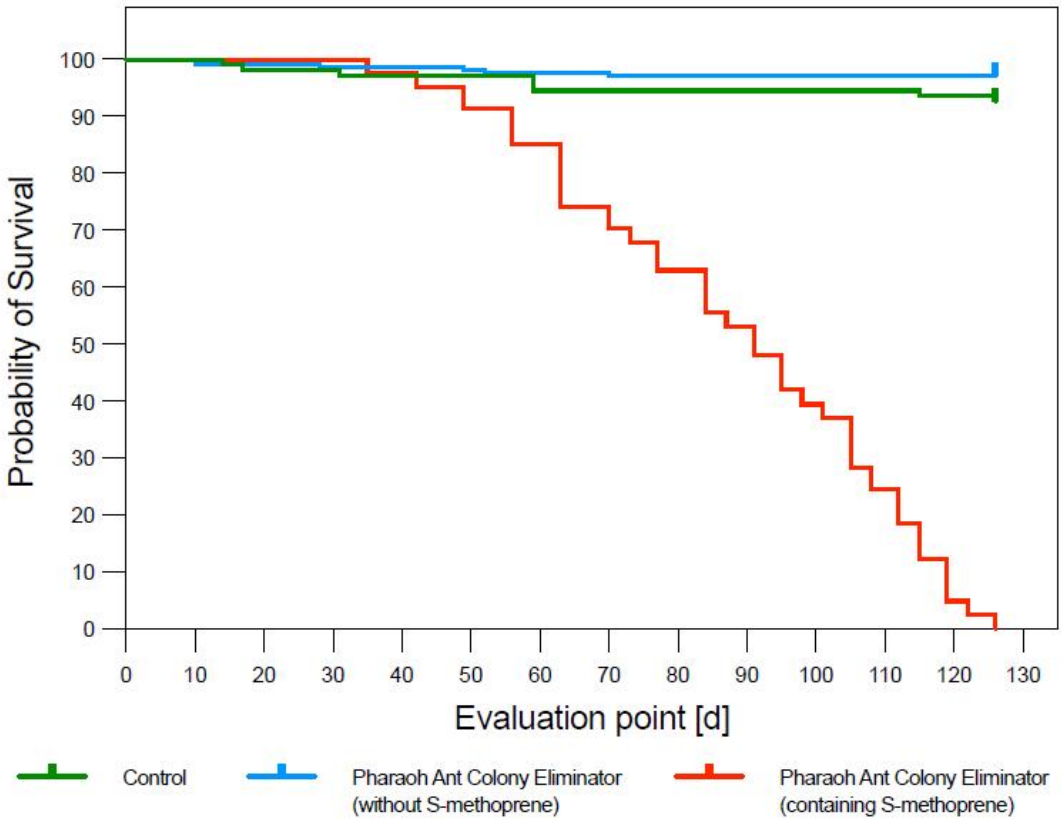

Figure 2. Queen survival (Kaplan-Meier survival curve) in simulated-use tests.

Colony state All colonies in untreated controls and treatments started with several queens, worker ants, and brood in all stages. Colony state counts at the beginning of the trial ranged between 2 and 3 in all replicates. Colony state counts increased to 3-4 within the test period in controls and S-methoprene-free bait treatments. In treatments using S-methoprene containing bait, a decrease in the number and size of brood was detectable from day 31 p.a., and no other freshly hatched offspring, such as workers or males, were present in these colonies (Figure 3). This indicates the successful development-inhibiting effect of S-methoprene. Treatments using S-methoprene containing bait were successful, all the treated colonies were 100% eradicated. On the other hand, replicates treated with the S-methoprene-free bait variant showed similar colony growth tendencies as observed in control colonies, resulting in growing, active colonies with multiple healthy queens and lots of brood and workers. Thus, the only colony growth disrupting factor in the bait seems to be the S-methoprene active substance.

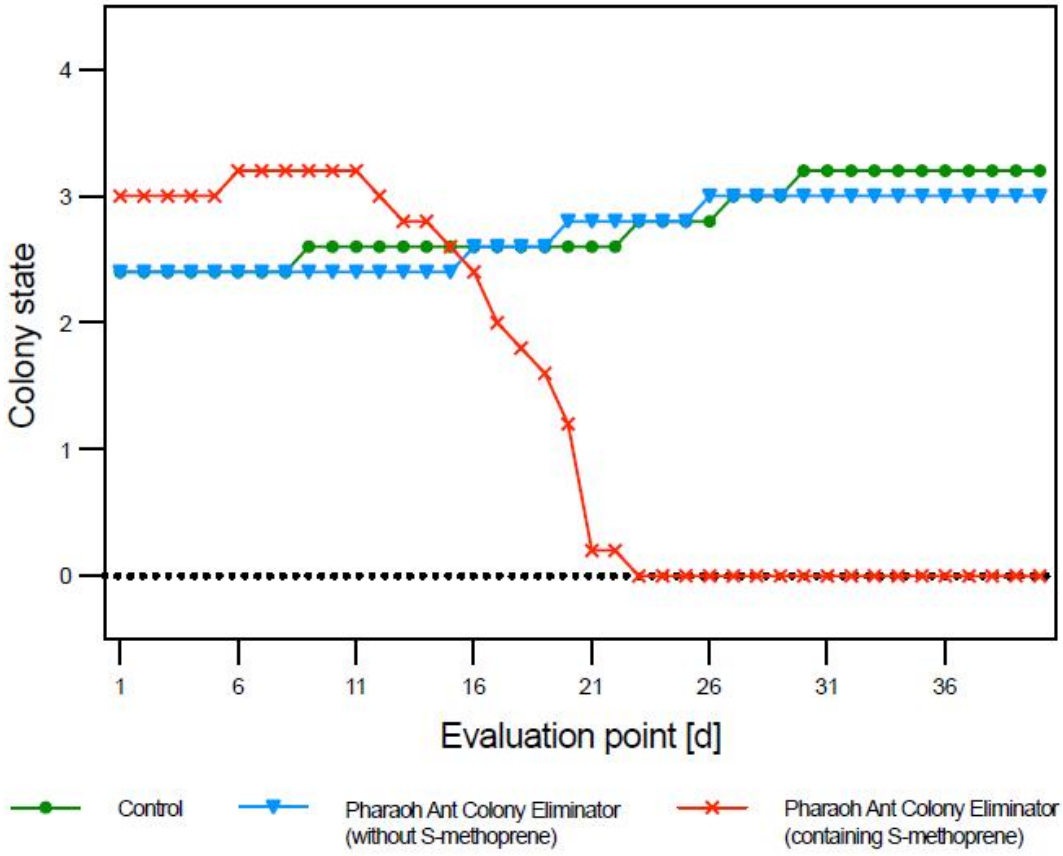

Figure 3. Mean colony state of the five replicates of treatments during the test period (colony state criteria: 0 – no vital brood; 1 – no eggs; some larvae/pupae present; 2 - eggs and larvae/pupae present in small quantities; 3 – more eggs; larvae and pupae present continuously; 4 – blooming colony with many large progeny conglomerates)

CONCLUSIONS

The BIOPREN® Pharaoh Ant Colony Eliminator bait palatability was comparable to the alternative food sources directly after offering. There was no observable difference in palatability between the S-methoprene-containing and S-methoprene-free bait versions. Due to the ant behavior of taking sugar and bait into their nests to form food stocks, the bait collecting activity decreased during the experiment, which is a natural phenomenon. However, the efficacy evaluation showed that treatment using the S-methoprene containing bait achieved a massive colony breakdown, and no brood was observed after 9 weeks of baiting. Furthermore, queens could not complete their development, and at the end of the trials, no worker ant, brood, or queen was alive in S-methoprene treated colonies.

Based on our previous laboratory results and field experiences, S-methoprene has an apparent effect on pharaoh ant queens' fecundity within 1-2 weeks of exposure, but it takes 3-4 weeks to see a remarkable decrease in the number of queens and size of progeny conglomerates in the nests because of the specific mode of action of S-methoprene having no direct killing effect as

being an IGR-type active substance, but a delayed, cascading influence on development and physiological processes resulting in impairment of vital functions. Moreover, because the eggs that are already laid are not affected by the treatment, it allows the ants to produce new workers for a while (considering the life cycle of these ants, about 1 month after treatment start) even if all the queens are still affected and infertile. Therefore, complete eradication of an established, active pharaoh ant colony can be achieved after an application period of ca. 10-18 weeks, depending on original size of the colony and the actual number of fertile queens to be controlled.

Results indicate that BIOPREN® Pharaoh Ant Colony Eliminator bait containing S-methoprene is a very effective tool to control pharaoh ant colonies by successfully interrupting insect development and by causing developmental abnormalities and infertility in queens, resulting in the irreversible collapse of the colonies. This ready-to-use, easily applicable product is also recognized as a tested and recommended tool for controlling health pests following § 18 of the Infection Protection Act by the German Environment Agency (Umweltbundesamt).

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Fighting against a superorganism

Proof of efficacy based on simulated-use colony studies

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BIOPREN Pharaoh Ant Colony Eliminator bait with S-methoprene causes a stop of colony development followed by colony eradication.

Incomplete queen development: Immature dead queens with malformed wing approach (red arrow).

Introduction

- Pharaoh ant control can be difficult [1, 2, 3]. Oral baits are the best method for controlling the pharaoh ant *Monomorium pharaonis* [4]. An alternative to toxic baits are insect growth regulators such as S-methroprene. Due to the mode of action inhibiting the ants' development and not immediately killing them, eradication needs more time. However, the effect can extend to the entire colony, including the queens allowing total control.
- For the efficacy testing of the S-methoprene (0.5%) containing BIOPREN Pharaoh Ant Colony Eliminator, simulated-use tests (choice tests) using this oral bait were conducted under laboratory conditions. The study aimed to provide information about the palatability and efficacy of the bait against workers and queens of the pharaoh ant *M. pharaonis*.

Material and Methods

- Whole colonies in 5 replicates: worker ants, brood, and several queens
- Test arena (size: 30 cm x 20 cm, 12 cm height): with nest and water
- Alternative food: sugar, pre-killed cockroaches
- Bait stations: commercial product (transparent blister)
- Acclimatization period: 1 week; starving period: 4 d
- Test period: 16 weeks baiting, 2 weeks post-baiting
- Evaluation points during test period: 0, 60, and 120 min post application and twice a week.

Results

- No preference between alternative food and bait observable directly after bait and food offering in treatments (Figure 1).
- Ants carried non-toxic food and bait from dishes into arena and nest
  → Number of ants at baits lower than at non-toxic food from day 3 on.
- No queen (126 d), worker ant (95 d), and brood (66 d) alive at the end of the test period in treated colonies.
- Differences in queen survival between the controls and treatments statistically significant ($p < 0.0001$, Figure 2).
- S-methoprene treatment: observation of 256 incompletely developed, dead queens with malformed wings between 42 d and 73 d.
- 100% colony eradication in BIOPREN Pharaoh Ant Colony Eliminator treatments.

Discussion

- Proof of successful development-inhibiting effect of S-methoprene.
  → No brood in nests and all worker ants, males, and queens are dead at the end of the trial.
- BIOPREN Pharaoh Ant Colony Eliminator bait palatability is comparable to alternative food source directly after offering.
  → Decrease of bait collecting activity during experiment due to food and bait stocks in their nests.

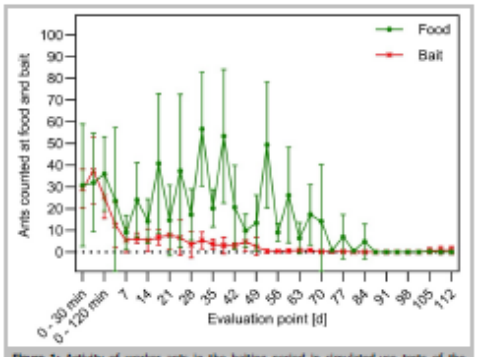

Figure 1: Activity of worker ants in the baiting period in simulated-use tests of the BIOPREN Pharaoh Ant Colony Eliminator. The mean and the SD of the number of worker ants at the non-toxic food and the bait stations in the 5 replicates within 1 min are shown.

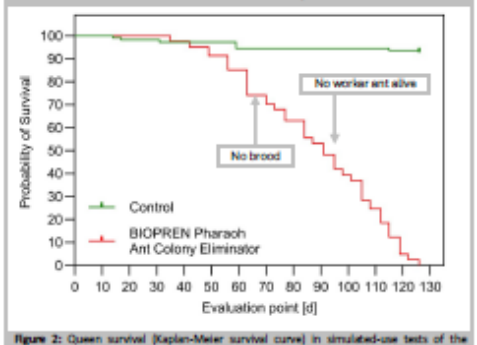

Figure 2: Queen survival (Kaplan-Meier survival curve) in simulated-use tests of the BIOPREN Pharaoh Ant Colony Eliminator and the control.

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