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Effects of a juvenile hormone analogue pyriproxyfen on monogynous and polygynous colonies of the Pharaoh ant *Monomorium pharaonis* (Hymenoptera: Formicidae)

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Abstract. To evaluate the effects of the juvenile hormone analogue pyriproxyfen on colonies of the Pharaoh ant *Monomorium pharaonis* (L.), peanut oil containing different concentrations (0.3, 0.6, or 0.9%) of pyriproxyfen was fed to monogynous (1 queen, 500 workers, and 0.1 g of brood) and polygynous (8 queens, 50 workers, and 0.1 g of brood) laboratory colonies of *M. pharaonis*. Due to its delayed activity, pyriproxyfen at all concentrations resulted in colony elimination. Significant reductions in brood volume were recorded at weeks 3 – 6, and complete brood mortality was observed at week 8 in all treated colonies. Brood mortality was attributed to the disruption of brood development and cessation of egg production by queens. All polygynous colonies exhibited significant reduction in the number of queens present at week 10 compared to week 1. Number of workers was significantly lower in all treated colonies compared to control colonies at week 8 due to old-age attrition of the workers without replacement. At least 98.67 ± 1.33% of workers were dead at week 10 in all treated colonies. Thus, treatment with slow acting pyriproxyfen at concentrations of 0.3 – 0.9% is an effective strategy for eliminating Pharaoh ant colonies.

INTRODUCTION

The Pharaoh ant, *Monomorium pharaonis* (L.), is well known as the most widely distributed and prevalent pest ant in the world (Yap & Lee, 1994; Wetterer, 2010). These ants are easily spread by human commerce and have adapted themselves to urban habitats (Lee, 2002). They are considered to be tramp ants, which are characterized by being highly polygynous (have multiple queens) and polydomous (multiple interconnected nests), forming unicolonial population, and being able to exchange workers after budding (Passera, 1994). Pharaoh ants also are important disease-transmitting vectors that pose serious threats to public health (Beatson, 1972; Edwards, 1986). Pharaoh ant infestations have frequently been reported in hospitals, health care facilities, and food preparation outlets (Yap & Lee, 1994; Wetterer, 2010).

Juvenile hormone analogues (JHAs) are substances with chemical compositions that differ from naturally secreted juvenile hormone. Pyriproxyfen (2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy] pyridine) is a JHA that has been used successfully to control a broad spectrum of urban pests, including mosquitoes (Nayar *et al.*, 2002), houseflies (Kawada *et al.*, 1987), ants (Vail & Williams, 1995), and cockroaches (Koehler & Patterson, 1991). In addition to pyriproxyfen, methoprene and fenoxycarb are among the JHAs that have been widely used to control pest ant infestations (Vail & Williams, 1995). Pyriproxyfen mimics the action of juvenile hormone and competes for juvenile hormone binding site receptors in insects, thereby interfering with normal growth and

development of immature stages of insects; however, it does not kill the adult stages. It suppresses metamorphosis and embryogenesis in larvae, and inhibits adult emergence in insects (Ishaaya *et al.*, 1994; Sullivan & Goh, 2008). Besides disrupting the brood development process, pyriproxyfen also has been shown to reduce the fecundity in queens of *M. pharaonis* (Kao & Su, 1995; Vail & Williams, 1995; Tay & Lee, 2014) and *Pheidole megacephala* (F.) (Reimer *et al.*, 1991). Pyriproxyfen has been used successfully to control various species of pest ants, including *Solenopsis invicta* Buren (Banks & Lofgren, 1991), *M. pharaonis* (Oi *et al.*, 2000), and *P. megacephala* (Reimer *et al.*, 1991). In addition, Hwang (2009) reported that pyriproxyfen successfully controlled an infestation of a field population of fire ants in Taiwan.

Slow acting pyriproxyfen is effective against *M. pharaonis*, which is characterized by large population size and multiple nest sites. Because pyriproxyfen has no effect on the workers, workers can spread it throughout the entire colony, including to the brood and queens, via trophallaxis (Oi *et al.*, 2000; Lim & Lee, 2005). In this way, colony elimination can be achieved. Use of pyriproxyfen also prevents the unnecessary killing of other beneficial vertebrate and invertebrate species in and around the treated environment, making it a generally accepted and popular treatment option for pest management control (Ishaaya & Horowitz, 1992).

Pharaoh ants are highly polygynous species. However, under special situation such as after a budding event, Pharah ant has the ability to establish a new colony rapidly with only single queen (monogynous). Lim & Lee (2005) reported morphological abnormalities in Pharaoh ant queens after application of pyriproxyfen at very low concentrations (1.0–5.0 µg/ml). However, colony growth was not affected. Hence, this study was conducted using higher concentrations of pyriproxyfen to evaluate the effects of pyriproxyfen on colony growth in monogynous and polygynous laboratory colonies of *M. pharaonis*.

MATERIALS AND METHODS

Ant Colonies

The *M. pharaonis* colonies were obtained from established colonies cultured at the Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia.

Test Chemical

Analytical grade (99%) pyriproxyfen (Sumitomo Chemical Co., Ltd., Takarazuka, Japan) was used in all experiments.

Experiment Design

Each colony was reared in a polyethylene container (13 x 11.5 x 6 cm); the inner surface was coated with Fluon® (polytetrafluoroethylene suspension) (BioQuip, Rancho Dominguez, CA) to prevent the ants from escaping. A small Petri dish (5.5 cm in diameter and 1.7 cm in depth) with entry holes and a piece of corrugated paper was provided in each container to serve as the harborage. Water and 10% sucrose solution were provided *ad libitum* in vials (5.0 cm in diameter and 1.2 cm in depth). One queen, 500 workers, and 0.1 g of brood of mixed stages (for monogynous colonies) and 8 queens, 50 workers, and 0.1 g of brood of mixed stages (for polygynous colonies) were separated and introduced into the harborage of each container. Pyriproxyfen was diluted into an ethanol:peanut oil mixture yielding three final concentrations (0.3, 0.6, or 0.9%). Each colony was fed with 0.1 ml of peanut oil (Sime Darby Edible Products Ltd., Jurong Town, Singapore) impregnated with pyriproxyfen on a Whatman No. 1 filter paper (2 cm x 2 cm) weekly. Control colonies were fed with peanut oil in ethanol without pyriproxyfen. All colonies were acclimatized for 3 days before pyriproxyfen was introduced. Three replicates were performed for each concentration. Based on photographs, the number of live queens, workers, and the relative volumes of brood were recorded at weeks 1, 2, 3, 4, 6, and 8.

Statistical Analysis

Values for the percentage reduction of brood volume and number of queens and workers were arcsine square root transformed. The differences for brood, queens, and workers among the concentrations and time (week) were subjected to one-way ANOVA. Means were then compared with Tukey's honestly significant difference (HSD) test. The analyses were performed using SPSS version 11.5 at $\alpha = 0.05$ (SPSS Inc, 2002).

RESULTS

Brood

Complete brood mortality was achieved in all treated colonies at week 8 in both monogynous and polygynous colonies. Monogynous colonies treated with 0.3% pyriproxyfen showed significant reduction in brood volume compared to that of the control starting from week 3 ($F = 4.987$; $df = 3, 8$; $P = 0.031$). Significant reduction in brood volume was recorded at all pyriproxyfen concentrations starting from week 4 ($F = 13.215$; $df = 3, 8$; $P = 0.002$) (Table 1). In polygynous colonies, all treated colonies showed significant reduction in brood volume compared to that of control colonies at week 6 ($F = 16.000$; $df = 3, 8$; $P = 0.001$) (Table 2).

Queens

Statistical analysis showed that pyriproxyfen did not kill the queen in all of the monogynous colonies (Table 3), and only the polygynous colonies subjected to 0.3 and 0.6% pyriproxyfen showed a significant reduction in the number of queens compared to that of control at week 10 and week 8, respectively (0.3%; $F = 6.975$; $df = 3, 8$; $P = 0.013$, 0.6%; $F = 4.348$; $df = 3, 8$; $P = 0.043$). However, all treated colonies exhibited a significant reduction in queen number at week 10 compared to week 1 (0.3%; $F = 5.745$; $df = 6, 14$; $P = 0.003$, 0.6%; $F = 5.128$; $df = 6, 14$; $P = 0.006$, 0.9%; $F = 5.646$; $df = 6, 14$; $P = 0.004$). No significant reduction in queen number was observed in the control colonies throughout the experiment ($F = 0.286$; $df = 6, 14$; $P = 0.934$). The average reduction in queen number was 4.17 ± 4.17% to 8.33 ± 4.17% in the control colonies (Table 4).

Workers

In the monogynous colonies, the number of workers was significantly lower in all treated colonies compared to that of control colonies starting at week 8 ($F = 60.324$; $df = 3, 8$; $P < 0.001$). Colonies treated with 0.3% pyriproxyfen showed a significant reduction in worker numbers compared to week 1 starting at week 4 ($F = 20.917$; $df = 6, 14$; $P < 0.001$) (Table 5). In the polygynous colonies, colonies subjected to 0.3% pyriproxyfen exhibited a significant reduction in worker numbers compared to the control colonies at week 4 ($F = 3.997$; $df = 3, 8$; $P = 0.049$). The number of workers was significantly reduced in all treated colonies compared to that of control colonies starting at week 8 ($F = 34.607$; $df = 3, 8$; $P < 0.001$). Colonies treated with 0.3% pyriproxyfen exhibited a significant reduction in worker numbers at week 8 compared to week 1 ($F = 10.562$; $df = 6, 14$; $P < 0.001$), whereas colonies subjected to 0.6% pyriproxyfen showed a significant reduction in worker numbers at week 10 compared to week 1 ($F = 7.648$; $df = 6, 14$; $P = 0.001$). Complete worker mortality occurred at week 10 for all treated colonies. In contrast, worker number continued to increase in the control colonies throughout the experiment (Table 6).

DISCUSSION

Brood

The reduction in brood observed in pyriproxyfen-treated colonies in our study was similar to that reported for *P. megacephala*, which exhibited a significant brood reduction at week 3 (Reimer *et al.*, 1991). Slow acting baits containing pyriproxyfen spread throughout the entire colony, including the brood and queens, via trophallaxis (Oi *et al.*, 2000). Larvae are the only members of the colony that can break down solid food into nutrients needed by all castes (Børgesen & Jensen, 1995).

Table 1. Effects of different concentrations of pyriproxyfen on the percentage reduction of brood volume (mean ± SEM) in monogynous colonies over a 10-week period[a,b]

Concentration (%)	Time (week)						
	1	2	3	4	6	8	10
Control	–33.33 ± 33.33 a(a)	–66.67 ± 66.67 a(a)	–20.00 ± 30.55 a(a)	3.33 ± 8.82 a(a)	–53.33 ± 24.04 a(a)	–56.67 ± 23.33 a(a)	–93.33 ± 6.67 a(a)
0.3	50.00 ± 0.00 a(a)	93.33 ± 3.33 a(b)	93.33 ± 3.33 b(b)	93.33 ± 3.33 b(b)	96.67 ± 3.33 b(b)	100.00 ± 0.00 b(b)	100.00 ± 0.00 b(b)
0.6	60.00 ± 5.77 a(a)	76.67 ± 13.33 a(a)	76.67 ± 13.33 ab(a)	76.67 ± 13.33 b(a)	93.33 ± 3.33 b(a)	100.00 ± 0.00 b(a)	100.00 ± 0.00 b(a)
0.9	–3.33 ± 26.03 a(a)	53.33 ± 27.28 a(ab)	60.00 ± 30.00 ab(ab)	80.00 ± 15.28 b(ab)	100.00 ± 0.00 b(b)	100.00 ± 0.00 b(b)	100.00 ± 0.00 b(b)

[a] Means followed by different letters within the same column are significantly different ($p < 0.05$).
[b] Means followed by different letters (in parenthesis) within the same row are significantly different ($p < 0.05$).

Table 2. Effects of different concentrations of pyriproxyfen on the percentage reduction of brood volume (mean ± SEM) in polygynous colonies over a 10-week period[a,b]

Concentration (%)	Time (week)						
	1	2	3	4	6	8	10
Control	53.33 ± 3.33 a(a)	80.00 ± 5.77 a(b)	76.67 ± 3.33 a(ab)	63.33 ± 8.82 a(ab)	66.67 ± 3.33 a(ab)	70.00 ± 5.77 a(ab)	66.67 ± 3.33 a(ab)
0.3	53.33 ± 8.82 a(a)	86.67 ± 3.33 a(ab)	76.67 ± 3.33 a(b)	90.00 ± 0.00 ab(ab)	93.33 ± 3.33 b(ab)	100.00 ± 0.00 b(b)	100.00 ± 0.00 b(b)
0.6	46.67 ± 12.02 a(a)	86.67 ± 3.33 a(b)	80.00 ± 5.77 ab(b)	86.67 ± 8.82 ab(b)	93.33 ± 3.33 b(b)	100.00 ± 0.00 b(b)	100.00 ± 0.00 b(b)
0.9	50.00 ± 5.77 a(a)	86.67 ± 3.33 a(b)	96.67 ± 3.33 b(b)	93.33 ± 3.33 b(b)	93.33 ± 3.33 b(b)	100.00 ± 0.00 b(b)	100.00 ± 0.00 b(b)

[a] Means followed by different letters within the same column are significantly different ($p < 0.05$).
[b] Means followed by different letters (in parenthesis) within the same row are significantly different ($p < 0.05$).

Table 3. Effects of different concentrations of pyriproxyfen on the percentage reduction of queen ants (mean ± SEM) in monogynous colonies over a 10-week period[a,b]

Concentration (%)	Time (week)						
	1	2	3	4	6	8	10
Control	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)
0.3	0.00 ± 0.00 a(a)	33.33 ± 33.33 a(a)	33.33 ± 33.33 a(a)	33.33 ± 33.33 a(a)	33.33 ± 33.33 a(a)	66.67 ± 33.33 a(a)	66.67 ± 33.33 a(a)
0.6	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	66.67 ± 33.33 a(b)
0.9	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	33.33 ± 33.33 a(a)	66.67 ± 33.33 a(a)

[a] Means followed by different letters within the same column are significantly different ($p < 0.05$).
[b] Means followed by different letters (in parenthesis) within the same row are significantly different ($p < 0.05$).

Table 4. Effects of different concentrations of pyriproxyfen on the percentage reduction of queen ants (mean ± SEM) in polygynous colonies over a 10-week period[a,b]

Concentration (%)	Time (week)						
	1	2	3	4	6	8	10
Control	4.17 ± 4.17 a(a)	4.17 ± 4.17 a(a)	4.17 ± 4.17 a(a)	8.33 ± 4.17 a(a)	8.33 ± 4.17 a(a)	8.33 ± 4.17 a(a)	8.33 ± 4.17 a(a)
0.3	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	12.50 ± 21.65 a(a)	25.00 ± 7.22 a(ab)	54.17 ± 23.20 ab(ab)	79.17 ± 20.83 b(b)
0.6	0.00 ± 0.00 a(a)	4.17 ± 4.17 a(a)	20.83 ± 20.83 a(ab)	45.83 ± 25.34 a(ab)	62.50 ± 26.02 a(ab)	79.17 ± 15.02 b(ab)	100.00 ± 0.00 b(b)
0.9	0.00 ± 0.00 a(a)	0.00 ± 0.00 a(a)	8.33 ± 4.17 a(a)	16.67 ± 4.17 a(ab)	29.17 ± 8.33 a(ab)	41.67 ± 4.17 ab(ab)	58.33 ± 20.83 ab(b)

[a] Means followed by different letters within the same column are significantly different ($p < 0.05$).
[b] Means followed by different letters (in parenthesis) within the same row are significantly different ($p < 0.05$).

Table 5. Effects of different concentrations of pyriproxyfen on the percentage reduction of worker ant numbers (mean ± SEM) in monogynous colonies over a 10-week period[a,b]

Concentration (%)	Time (week)						
	1	2	3	4	6	8	10
Control	3.07 ± 5.27 a(a)	5.07 ± 8.50 a(a)	5.07 ± 10.45 a(a)	17.47 ± 9.15 a(a)	27.53 ± 5.28 a(a)	33.20 ± 6.36 a(a)	28.33 ± 5.80 a(a)
0.3	29.20 ± 5.43 a(a)	38.40 ± 11.02 a(a)	56.93 ± 5.78 a(ab)	73.20 ± 8.39 a(bc)	88.80 ± 4.61 ab(c)	99.33 ± 0.67 b(c)	100.00 ± 0.00 b(c)
0.6	21.93 ± 18.07 a(a)	18.20 ± 25.22 a(a)	37.47 ± 21.39 a(a)	49.33 ± 21.20 a(a)	66.67 ± 16.53 b(a)	92.87 ± 5.17 b(a)	98.67 ± 1.33 b(a)
0.9	45.00 ± 23.53 a(a)	36.53 ± 33.78 a(a)	47.47 ± 26.50 a(a)	71.27 ± 15.75 a(a)	89.73 ± 4.63 b(a)	98.47 ± 0.41 b(a)	100.00 ± 0.00 b(a)

[a] Means followed by different letters within the same column are significantly different ($p < 0.05$).
[b] Means followed by different letters (in parenthesis) within the same row are significantly different ($p < 0.05$).

Table 6. Effects of different concentrations of pyriproxyfen on the percentage reduction of worker ant numbers (mean ± SEM) in polygynous colonies over a 10-week period[a,b]

Concentration (%)	Time (week)						
	1	2	3	4	6	8	10
Control	−603.33 ± 56.74 a(ab)	−838.67 ± 73.77 a(a)	−720.67 ± 45.35 a(ab)	−657.33 ± 15.76 a(ab)	−558.00 ± 21.57 a(b)	−498.67 ± 69.80 a(a)	−636.67 ± 48.50 a(ab)
0.3	−236.67 ± 37.12 b(ab)	−369.33 ± 54.36 a(a)	−246.00 ± 74.47 a(ab)	−225.33 ± 76.44 b(ab)	−82.00 ± 73.73 b(bc)	84.00 ± 2.31 b(c)	100.00 ± 0.00 b(c)
0.6	−422.67 ± 61.13 ab(ab)	−600.67 ± 80.02 a(a)	−576.67 ± 152.47 a(a)	−409.33 ± 84.78 ab(ab)	−173.33 ± 166.06 ab(abc)	19.33 ± 61.76 b(bc)	100.00 ± 0.00 b(c)
0.9	−385.67 ± 49.95 b(a)	−413.33 ± 175.58 a(a)	−468.00 ± 206.17 a(a)	−304.67 ± 148.49 ab(a)	−166.67 ± 103.49 ab(a)	46.00 ± 9.02 b(a)	100.00 ± 0.00 b(a)

[a] Means followed by different letters within the same column are significantly different ($p < 0.05$).
[b] Means followed by different letters (in parenthesis) within the same row are significantly different ($p < 0.05$).

Pyriproxyfen is harmful to brood because it prolongs the larval stage, causing larval and pupal death (Vail & Williams, 1995). Moreover, upon exposure to pyriproxyfen, larvae are not able to undergo normal metamorphosis. Unsuccessful molting or molting that occurs before the suitable time can result in larvae with a thinner exoskeleton, which can pose problems such as death from dehydration (Lim & Lee, 2005). As a result, the larvae die before reaching the adult stage. In our study, total mortality of larvae was observed in all treated colonies, and dead larvae eventually dried up in the harborage and fungus began to grow on the carcasses. Complete brood mortality occurred by week 8.

According to Peacock *et al.* (1955), Pharaoh ants can raise a new queen from the existing brood stages. However, Williams & Vail (1993) reported delays in the production of queens in Pharaoh ant colonies after exposure to 0.1–0.5% fenoxycarb. Previous studies also showed that *P. megacephala* (Reimer *et al.*, 1991) and *S. invicta* (Banks & Lofgren, 1991) colonies can shift caste differentiation from worker larvae to sexual castes upon exposure to 2.0% pyriproxyfen. However, no new queen was found in the treated colonies throughout the experiment in our study because the entire brood was killed by week 8 (i.e., before a sexual brood was successfully produced). The death of the brood also prevented the emergence of the worker population; the workers eventually reached the end of their life span, which resulted in a significant reduction in number of workers in all treated colonies at week 8.

Queens

In the current study, the number of queens was reduced in most of the treated polygynous colonies. However, there was no evidence that this reduction was due to pyriproxyfen. Instead, the queens eventually died because the worker caste, which is crucial for colony maintenance (i.e., to serve the queen and take care of the brood), was diminished (Vail & Williams, 1995; Vail *et al.*, 1996). This situation subsequently led to eradication of the colony. Vail and Williams (1995) reported that complete queen mortality likely would occur over a longer duration of observation.

Queens with no supply of larvae exhibit reduced fertility (Børgesen, 1989). Pyriproxyfen may affect the reproductive capabilities of the queen, as low amounts of brood were observed in both monogynous and polygynous treated colonies. Pyriproxyfen acts on the endocrine system in insects and may be stored in the queen's reproductive system and eggs and inhibit reproductive capabilities (Schneider *et al.*, 2008; Sullivan & Goh, 2008). Previous studies showed that after being subjected to pyriproxyfen, queens ceased egg production at week 8 in *M. pharaonis* (Tay & Lee, 2014) and at week 6 in *P. megacephala* (Reimer *et al.*, 1991). As a result, the worker population in the colonies declined gradually when egg production ceased.

Workers

The experiment was terminated at week 10 when few survivors of the brood and worker castes were detected in all treated colonies. The distribution of the bait throughout the colony is the key factor for a successful eradication program, especially in polydomous species such as *M. pharaonis* (Oi *et al.*, 2000). According to Oi *et al.* (2000), use of a fast acting toxic bait can quicken the rate of worker caste mortality, thereby limiting distribution of the bait to the entire colony. Because pyriproxyfen is a slow acting toxin that does not affect or kill insects at the adult stage, it has a higher chance of being distributed extensively to other colony members; however, it may take several weeks before the reduction in the ant population is clearly visible (Oi *et al.*, 2000).

In the current study, the brood in all treated colonies decreased significantly within 3 – 6 weeks, whereas the number of workers took a few weeks longer to decline. This means that the workers had sufficient time to distribute pyriproxyfen to all colony members. Pyriproxyfen treatment results in a gradual reduction of worker numbers, and it was suggested that older workers died from natural causes (Reimer *et al.*, 1991). The

reduced size of the worker population starting at week 8 in all treated colonies was due to brood mortality, as a healthy brood is necessary for worker replacement. Oi *et al.* (2000) reported a similar result (73% decline at week 8) for Pharaoh ant workers subjected to pyriproxyfen. In comparison, Edwards (1975) described complete worker mortality between weeks 9 and 20 in Pharaoh ant colonies subjected to methoprene.

Pyriproxyfen can cause complete mortality of the brood population and decreased egg productions by queens. In our study, no brood was observed by week 8 in the monogynous and polygynous colonies, which indicates that the remaining surviving queens in the treated colonies had ceased egg production. This in turn likely prevented worker replacement. At least 98.67 ± 1.33% of workers had died in all treated colonies by week 10. The remaining queens and workers in the colonies likely would have gradually died off when they reached the end of their lifespan.

Our results indicate that pyriproxyfen affected all castes of *M. pharaonis* either directly or indirectly, resulting in colony elimination. However, 0.9% pyriproxyfen was not as effective as the other concentrations tested, probably due to the repellency of the chemical. Similar observations were reported in Pharaoh ant (Williams & Vail, 1993) and *S. invicta* (Banks *et al.*, 1988) colonies treated with a high concentration of fenoxycarb.

In summary, we conclude that pyriproxyfen is a highly effective and slow acting treatment for controlling *M. pharaonis*, which is characterized by a large population size and multiple nest sites. Use of food impregnated with pyriproxyfen may prove to be the most effective and environmentally friendly solution for the control of *M. pharaonis* infestation in hospitals, nursery centers, food establishments, apartment buildings, and field populations covering large areas.

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