EVALUATION OF ORTHENE® FIRE ANT KILLER (75% ACEPHATE) AS A DUST APPLICATION FOR THE CONTROL OF RED IMPORTED FIRE ANTS

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Acephate, formulated as a 75 percent wettable powder (Orthene® Fire Ant Killer and Orthene® Turf, Tree and Ornamental Spray) is a widely-used individual mound treatment for control of the red imported fire ant, Buren. Application as a dust treatment, using one to two teaspoons of product per mound, provides elimination of ant activity in treated mounds within days. Its fast-acting and low-labor properties make it an attractive method for controlling ant colonies in urban environments.

The penetration of the acephate dust into a fire ant mound is poorly understood. Possible routes of ant exposure to acephate applied as a dry powder include:

1) fumigation or vapor action
2) direct contact by:
    a) ants tracking the material into interior mound surfaces
    b) dust sifting into the mound's galleries

The odor produced by acephate formulations results from the mercaptans (thiols), sulfur-containing organic chemicals. These compounds are not known to be toxic (or repellent) to ants. However, the active ingredient, acephate (an organophosphate cholinesterase inhibitor), is known to be highly toxic to ants even at very low exposure rates. Thus, the idea that this product is distributed through fire ant mounds by worker ants "tracking" the toxicant from the treated surface through the rest of the mound, or by contact with the fine dust "sifting" through the highly structured soil particles comprising the mounds surface are likely possible routes into the mound. How the product reaches the queen ants and whether the dust treatment kills queen ants were questions addressed by a series of trials reported here.

This report documents trials conducted with Orthene® Fire Ant Killer (75 percent wettable powder) applied to individual red imported fire ant mounds or ants under laboratory conditions:

I. **Field trial 1.** Montgomery County 1990 (Drees et al. 1991).
II. **Field trial 2.** Brazos County 1991 (Drees and Barr 1992).
III. **Laboratory trial 1.** Possible effects of acephate vapor on confined ants, 1990.
IV. **Laboratory trial 2.** Dye-labeled acephate "antarium" experiments, 1992.
V. **Field trial 3.** Dye-labeled acephate field experiments, Brazos County, 1992.
VI. **Laboratory trial 3.** Queen confinement/isolation experiments, 1992.
VII. **Field trial 4.** Queen confinement mound experiments, Brazos County, 1992-1993.

I. Field trial 1. Montgomery County 1990 (Drees et al. 1991).

This trial was established on 11 June 1990 to evaluate several individual fire ant mound treatments, particularly the bait formulated products, Amdro® and Logic®. Orthene® Fire Ant Killer, applied as a dry wettable powder application (2 teaspoons per mound) was included. In this trial, all mounds within plot areas were marked and treated. This method allowed for documentation both of the reduction of treated ant mounds and the reduction of ant mounds in the areas. This method is capable of detecting "satellite" mound formation when the number of treated mounds is reduced after treatment but the number of mounds within the treated plot areas is not.

In summary, active fire ant mounds treated with dry acephate wettable powder were totally eliminated within one week. Only 2 "new" mounds were found within the 0.25 acre plot on the July 9 and August 1 evaluations, indicating that satellite mound formation as a result of treatment likely did not occur within this 8 week trial.

Materials and Methods

On June 11, 1990, 8 circular plots, 60 feet in radius, were surveyed for fire ant mound activity. All mounds were marked with Kerr® canning jar lids and numbered sequentially. Mound activity was determined by light disturbance of the mound. The rating scale below was used to document the level of ant activity within each mound:

0 = fewer than 10 ants, very slow reaction to disturbance
1 = 10 to 100 ants, slow reaction to disturbance
2 = 100 to 1000 ants, and/or vigorous reaction to disturbance
3 = more than 1000 ants, very vigorous reaction to disturbance

The following day, all marked mounds within each plot were treated as indicated below:

Plot	Active ingredient	Formulation	Rate	No mounds treated
1	untreated	none	none	34
2	**acephate**	**Orthene® Fire Ant Killer**	**3 tsp/mound**	**47**
3	fenoxycarb	Logic®	3 Tbsp/mound	32
4	"	Amdro® "89"	"	50
4	hydramethylnon	Amdro "90"	5 Tbsp/mound	51
5	hydramethylnon	Amdro "A"	5 Tbsp/mound	37
6	"	Amdro "B"	"	39
7	"	Amdro "C"	"	36

Post-treatment evaluations were made after one-week (20 June 1990), two-week (27 June), four-week (9 July), and eight-weeks (1 August). Evaluations were made in the evening after ant activity had resumed.

Analysis of data were performed in two ways: 1) the mean mound ratings were calculated for the first sequentially numbered 30 mounds within each plot and separated using ANOVA and the least significant difference (LSD) test at $P \leq 0.05$; 2) the number of active mounds within each quadrant of each plot (1/16 acre) were determined for each evaluation date and these values were used to determine the mean number of active mounds per unit area per evaluation. These data were statistically analyzed as above.

Results and Discussion

Extreme heat and high humidity were persistent for the duration of the experiment. Daily heat indexes were 105-110○F. Soil moisture conditions were very poor (dry) until after the first evaluation. Some rainfall occurred during this period resulting in the increased activity noted in the ratings, particularly in the control plot.

Fire ant foragers were attracted to the bait formulations almost immediately upon application. No residual granules were noted the following week. At that time, large numbers of dead ants in "bone piles" were noted near the Amdro-treated mounds. No such accumulations were seen in the Orthene, Logic, or control plots.

All formulations of Amdro and Orthene performed similarly, eliminating all ant activity within the first week of treatment (Table 1 and 2). In plot 7, treated with Amdro C, activity in a few treated mounds resumed 4 and 8 weeks following treatment, and several new mounds were detected in the plot. These mounds were a result of either control failure, or migration into the plot from untreated areas. This determination can not be made with the available data. Logic-treated colonies declined more slowly, requiring 4 weeks before statistically-equivalent suppression to other treatments was achieved and 8 weeks before elimination of mound activity was realized.

Table 1. Mean red imported fire ant mound rating[a/] prior to and following treatment of 30 individual marked mounds within 0.25-acre circular plots, Montgomery County, Texas 1990.

	----------------------Mean mound rating[a/]-----------------------				
Treatment	11 June Pre-count	20 June 1-week	27 June 2-week	9 July 4-week	1 Aug. 8-week
Untreated	1.57ab.	1.23a..	1.63a..	1.13a.	0.83a.
Orthene®	**1.50abc**	**0.00..c**	**0.00..c**	**0.00.b**	**0.00.b**
Logic®	1.47abc	0.73.b.	0.80.b.	0.03.b	0.00.b
Amdro® 89	1.33abc	0.00..c	0.00..c	0.00.b	0.00.b
Amdro 90	1.20..c	0.00..c	0.00..c	0.00.b	0.00.b
Amdro A	1.27abc	0.00..c	0.00..c	0.00.b	0.00.b
Amdro B	1.30abc	0.00..c	0.00..c	0.00.b	0.00.b
Amdro C	1.57a..	0.00..c	0.00..c	0.17.b	0.13.b
LSD 5%	0.303	0.359	0.287	0.322	0.230

[a/] Means followed by the same letter(s) are not significantly different according to ANOVA and the least significant difference (LSD) test ($\leq$ 0.05).

Table 2. Mean number of active red imported fire ants per 0.13-acre quadrant of a 0.25-acre circular plot, Montgomery County, Texas, 1990.

	----------------------Mean mound rating[a/]-----------------------				
Treatment	11 June Pre-count	20 June 1-week	27 June 2-week	9 July 4-week	1 Aug. 8-week
Untreated	8.50a	8.00a.	7.25a.	7.00a.	5.25a.
Orthene®	**11.50a**	**0.00.b**	**0.00.b**	**0.00.b**	**0.00.b**
Logic®	8.00a	5.50a.	4.75a.	0.25.b	0.00.b
Amdro® 89	12.50a	0.00.b	0.00.b	0.00.b	0.00.b
Amdro 90	12.75a	0.00.b	0.00.b	0.00.b	0.00.b
Amdro A	9.25a	0.00.b	0.00.b	0.50.b	0.00.b
Amdro B	9.75a	0.00.b	0.00.b	0.50.b	0.00.b
Amdro C	9.00a	0.00.b	0.00.b	1.50.b	0.50.b

LSD 5%	5.613	3.161	2.999	3.383	2.668

[a/] Means followed by the same letter(s) are not significantly different according to ANOVA and the least significant difference (LSD) test (≤ 0.05).

II. Field trial 2. Brazos County 1991 (Drees and Barr 1992).

This trial was initiated on 12 June 1991, to evaluate several individual fire ant mound treatments. Methods were changed from the previous year, using plots of variable size to containing 5 active fire ant mounds each. This technique allowed for a replication in plots in a complete randomized block design. The plots were replicated four times for each treatment. Again the number of marked and treated mounds as well as the number of mounds in treated areas were monitored. Orthene® Fire Ant Killer was applied at a rate of 2 teaspoons dry formulation per mound.

This trial documented a 90 to 100 percent reduction in active ant mounds over this 8 week trial. Re-infestation/satellite mound formation averaged 2.0 mounds per plot by the end of the test, compared to 3.0 mounds per plot for the untreated plots. This rate of re-infestation is most likely because of the relatively small sizes of plots established in this trial.

In addition to the small-plot test, another test using one-acre circles was established adjacent to the first. All mounds within the circles were marked. Four clusters of five mounds each within the plot were marked with coded canning jar lids and evaluated on the dates listed above. Orthene® Fire Ant Killer treatments resulted in 100% control and no satellite mound formation within the entire one-acre circle for the duration of the test.

Materials and Methods

On 12 June, 1990 on mowed turf areas adjacent to the airport runway of Coulter Field, Brazos County, Texas, circular plots were established to encompass 5 active red imported fire ant mounds. Four replications were established for each of seven treatments:

Treatment	Rate
sulfuramid	5 tbsp./mound
Amdro® (hydramethylnon)	5 tbsp./mound
Amdro® lot-91	5 tbsp./mound
Orthene® Fire Ant Killer (acephate)	**2 tsp./mound**
Logic® (fenoxycarb)	3 tbsp./mound
Spectracide® (granular diazinon)	0.5 cup + 1 gal. water/mound

The location of the colonies within each plot was mapped, marked with numbered Kerr® canning jar lids and rated for degree of activity. Activity was determined by observing the defensive movement of the ants following mound disturbance. This was done by slightly probing the mound with a thin metal wire and estimating the number of ants that came to the surface on a rating scale from 0 to 3: 0 = 0 ants; 1 = 1-100 ants; 2 = 100 - 1000 ants; and 3 > 1000 ants. Colonies were rated prior to treatment and 2 days, 1, 2, 4 and 8 weeks post treatment.

Results and Discussion

All individual mound treatments except for Logic® produced statistically similar reductions in ant activity ratings (Table 1). The 2-day post-treatment decline in ant activity in Amdro and sulfuramid treated mounds was surprisingly rapid for bait-formulated materials. Significant reduction in ant activity in Logic-treated mounds relative to untreated mounds did not occur until 8 weeks following treatment. Numerically, mounds treated with Spectracide and drenched with water resulted in the greatest reduction of ant activity.

Evaluation of treatment efficacy using the number of occupied ("active") mounds per plot (not the rating scale) for analysis provides similar results (Table 2). However, this analysis indicates that there is a numerical trend for the 1990 lot of Amdro to reduce mound activity more slowly that did the 1991 lot. Furthermore, the Spectracide treatment produced reductions significantly more quickly than did the 1990 lot of Amdro.

The turf area on which this test was conducted was mowed routinely by city services and was not watered. Drainage was excellent and resulted in a rather dry terrain even after heavy rains. The density of fire ant mounds on this site was rather low for this area, averaging 115 mounds per acre (ranging from 147 to 97) with an average mound diameter of 25.1 cm. Low mound density and dry conditions resulted in little migration of colonies and little detectable re-infestation of treated plots. The average plot size, containing 5 fire ant mounds, was 0.044 acre (24.8 ft. radius).

Table 1. Efficacy of individual mound treatments for the red imported fire ant, Coulter Field, Bryan, Texas 1991.

	Pre-treat.	Mean level of fire ant activity* Post-treatment				
	12 June 0 day	14 June 2 day	19 June 1 week	26 June 2 weeks	11 July 4 weeks	8 Aug. 8 weeks
untreated	2.4a	2.2a	2.1a	1.3a	1.2a	1.1a
Orthene®	**2.3a**	**0.2b**	**0.2b**	**0.0b**	**0.1b**	**0.0b**
Spectracide®	2.3a	0.2b	0.0b	0.0b	0.0b	0.0b
Logic®	2.3a	2.0a	2.1a	0.9a	1.1a	0.4b
Amdro® 91	2.2a	0.5b	0.2b	0.2b	0.1b	0.2b
Amdro® 90	2.3a	0.6b	0.3b	0.5b	0.1b	0.1b
sulfuramid	2.2a	0.4b	0.0b	0.5b	0.0b	0.3b

0.3411 36.769 56.763 8.670 7.969
3.680
0.9059 0.0000 0.0000 0.00020.0003
0.0146

* Mean values followed by the same letters are not significantly different according to ANOVA and the Duncan's Multiple Range Test ($\leq$ 0.05).

Table 2. Efficacy of individual mound treatments for the red imported fire ant, Coulter Field, Bryan, Texas 1991.

	Pre-treat.	Mean number of active ant mounds per plot* Post-treatment				
	12 June 0 day	14 June 2 day	19 June 1 week	26 June 2 weeks	11 July 4 weeks	8 Aug. 8 weeks
untreated	5.0a	5.0a	4.8a	3.3a	3.3a	2.5a
Orthene®	**5.0a**	**1.0bc**	**0.5bc**	**0.0b**	**0.5b**	**0.0b**
Spectracide®	5.0a	0.5c	0.0c	0.0b	0.0b	0.0b
Logic®	5.0a	5.0a	4.8a	2.5a	2.8a	1.0b
Amdro® 91	5.0a	2.0bc	0.5bc	0.5b	0.3b	0.5b
Amdro® 90	5.0a	2.3b	1.3b	0.3b	0.3b	0.3b
sulfuramid	5.0a	2.0bc	0.0c	0.3b	0.0b	0.5b

--- 14.653 69.909 8.2290 9.660

			4.213	
---	0.0000	0.0000	0.0002	0.0001
			0.0080	

* Mean values followed by the same letters are not significantly different according to ANOVA and the Duncan's Multiple Range Test (≤ 0.05).

III. Laboratory trial 1. Possible effects of acephate vapor on confined ants, 1990.

In October, 1990 tests were undertaken in an attempt to determine if the "vapor" from Orthene® was toxic to fire ants. Although inconclusive, results indicate that acephate is highly toxic to fire ants in very low concentrations.

Materials and Methods

Tall Petri dishes were coated with Fluon® to prevent ant escape. A piece of filter paper was placed in the bottom each dish. Small, clear plastic containers, approximately 1 inch deep and 1-1/2 inches in diameter were dipped in Fluon to prevent ant access to the interior and contact with the treatment. Ten ants were added to each tall petri dish. A small piece of plastic with two drops of honey-water was provided to the ants to help prevent desiccation. One eighth teaspoon of Orthene® Fire Ant Killer was placed into each of six of the small containers. Empty ones were placed in the remaining six Petri dishes. The dishes were covered with lids, in which two holes had been melted to allow some air circulation. After 5 and 24 hours, ant mortality was evaluated. Results were analyzed using the Student's t test ($P \leq 0.05$).

Results and Discussion

Results (Table 1) indicate that either acephate "vapor" or very low concentrations of airborne wettable powder is toxic to red imported fire ants. After 24 hours, there was slight mortality in the untreated dishes, and 100% mortality in all Orthene treatments. Mercaptan, the odor-causing chemical in 75 percent acephate is not known to be toxic to ants. It is possible then, that the airborne dust moved out from the weighing dishes into the tall petri dish and that low concentrations of the dust caused ant death.

Table 1. Effect of acephate applied in containers within tall Petri dishes to prevent fire ant contact, on fire ant workers.

	Average number of live ants/10 per dish			
	5 hours post-treatment		24 hours post-treatment	
Dish	Orthene®	untreated	Orthene®	untreated
mean	8.0	9.2	0.0	4.5
S.D.	± 1.4	± 0.8	± 0.0	± 2.6
	-1.7838		-4.2584	
	0.0524		0.0008	

d.f. = 10

IV. Laboratory trial 2. Dye-labeled acephate "antarium" experiments, 1992.

In order to visually trace movement of the acephate, a series of experiments was conducted using a fluorescent dye-labeled acephate wettable powder formulation supplied by the manufacturer. These studies determined that the fluorescence of the dye-formulation was eliminated when in contact with organic matter in soil or media. Because of this, the true "soil profile" of the dry wettable powder formulation could not be documented. Furthermore, ant activity in the "antarium" continued in some trials deep in the soil for many days following application of the dry-formulated treatment.

Materials and Methods

A 10 gallon glass aquarium was partially reconstructed so that it stood on end with the top open. Two plexiglass panels were cemented inside to form two compartments measuring 12 inches wide, 24 inches tall, and 2 inches deep. Wood frames were cemented around the top edge and ½ inch wide electrical heating strips were clipped to the frames. The heating strips were maintained at 140○F by means of a rheostat. Previous tests indicate that fire ants will not cross a surface of this temperature or higher.

Several methods of getting the ants and the soil into the narrow compartment openings were tried with varying degrees of difficulty. However, all resulted in approximately 18 inches of soil and a colony of 10-20,000 ants in both compartments. Opaque covers, a heating pad, and various arrangements of lights were used to encourage the ants to build galleries against the glass so their movement could be more easily seen. The ants were maintained with frozen crickets and honey water for several days while they built a mound structure within the compartments.

Both regular Orthene® Fire Ant Killer and dye-labeled Orthene® Fire Ant Killer were applied at a rate of 2 teaspoons per compartment. The material was gently sprinkled through the opening so that the entire soil surface was covered. Treatment combinations varied over the course of the tests.

Visualization was accomplished by ceiling light in the test room. A twin-tube fluorescent ultra-violet light was then shone on the antarium so that the dye fluoresced vividly. (Previous tests indicated that the aquarium glass allowed the UV light to penetrate, though it did alter the fluorescent color from bright pink to orange.) A tripod mounted camera (35mm Pentax ME with close up lenses) was used to photograph both the whole "antarium" and small areas of interest within the mound structure. Due to the extremely low light conditions, shutter speeds of 2 to 8 seconds were required for proper exposure. Photographs were made immediately after Orthene application and periodically thereafter for up to 24 hours. After test completion, the soil was washed out and all surfaces washed with Alconox® detergent to remove any traces of Orthene. The antarium was then dried and reused. A total of six trials were conducted with varying degrees of success in viewing the dye-treated acephate dust.

Results and Discussion

At best, the dye acephate wettable powder formulation penetrated to a depth of 5 inches in very small, isolated spots - most likely by being carried on the backs of ants that were on the surface at the time of application. Dye penetration was uniform to a depth of 1-1/2 inches. Excessive soil moisture seemed to inhibit dye penetration. Complete ant death occurred within 48 hours in all trials with sufficient soil moisture. However, dry soil was used in one test and ants survived for 10 days at a depth of over 6 inches. When drenched, the ants died within one day.

These inconsistent results suggested that moisture was having an effect on the dye and possibly the acephate formulation itself. To test the interaction of the dye, water and soil, glass jars were filled with various media including water, soil similar to that used in the tests, coarse sand, fine sand, gravel, and a heavily organic artificial potting media. Four teaspoons of dye-marked Orthene were placed in each jar. Water was added so that it covered the media and the entire mixture was shaken thoroughly and allowed to settle. Water was found not to have an effect on dye fluorescence. However, the presence of organic matter in the media greatly reduced or eliminated fluorescence.

To try to eliminate the organic matter, the antarium was filled with coarse sand. The ants died within 24 hours, probably due to desiccation or contamination of the sand. The antarium was then filled with a mixture of coarse and fine grade vermiculite. The ants built galleries normally and Orthene® Fire Ant Killer (non-dye formulation) was applied. Surface ant activity was stopped but ant activity continued at a depth below 6-8 inches and persisted for over one month. At this time, the compartment was drenched with water and the ants died within 48 hours indicating that the Orthene was still active.

Ant behavior in this "antarium" was probably not natural. In dry wettable acephate powder treatment trials, the ants moved deep in the soil column and remained active for extended periods of time following treatment. In the natural environment, fire ant colonies are known to "thermo-regulate", moving to locations within the mound with optimum moisture and temperature conditions. In cool early morning hours, for instance, most of the members of a fire ant colony are located just underneath the surface of the eastern slope of the mound being warmed by sunlight. If the surface of the mound had been treated, this movement to the surface could result in the ants' contact with the toxicant.

V. Field trial 3. Dye-labeled acephate field experiments, Brazos County, 1992.

This trial was conducted in an attempt to document the distribution of fluorescent dye formulated acephate wettable powder after dry application to fire ant mounds under field conditions. Ant activity in 4 of six mounds treated was eliminated 3 days after treatment. However, no penetration of acephate into the mound profile was documented due to the deactivation of the fluorescence upon contact with organic matter into the soil.

Materials and Methods

On 10 April 1992, 6 active fire ant mounds were marked with flags. The mounds were located next to curbs to facilitate observation. U-shaped wooden frames were constructed to hold 2 pieces of plexiglass, 6 inches high x 16 inches long. The plexiglass panes were clipped together, inserted in the frames then the entire apparatus was placed in a marked mound so that the plexiglass cut through the middle of the mound. The ants were allowed to rebuild the mound structure around the plexiglass for several days.

On 13 April, the mounds were treated with 2 teaspoons of dye-labeled Orthene each. On April 15, 8:30 p.m., UV lights were set up by the treated mounds so that the dye fluoresced. The clips were carefully removed from the panes of plexiglass and the outer one gently pulled away carrying that half of the mound with it. The goal was to obtain an unobstructed view of the mound galleries through the remaining piece of plexiglass.

Results and Discussion

Unfortunately, the plexiglass blocked the UV light and prevented fluorescence. Previous tests had indicated that plexiglass allowed UV light to penetrate, but the plastic used for these panes was apparently of a different type. As a result, the pane was gently removed to expose the galleries and mound profile.

The dye was vividly fluorescent on the surface of treated mound No. 1, but no fluorescence appeared below the surface or within the mound structure. Photographs were taken and then the mound was excavated to depth of 9 inches where a few live worker ants were observed. In treated mound No. 2, similar results were observed though more active worker ants appeared around the edges of the mound. Observations from treated mound no. 3-5 were similar, although no active fire ants were present. On one small surface section of treated mound No. 3, fluorescent dye appeared mixed with the soil to a depth of 1/4 inch. Apparently worker ants had continued to deposit new soil on top of the mound after treatment. This finding supports the observation that Orthene® Fire Ant Killer is not repellent to fire ants. Various combinations of UV and incandescent lighting were used to obtain suitable photographs. The treated mound no.6 had not been rebuilt after insertion of the frame and was not examined. The finding that organic matter decomposed the fluorescent dye ended further field investigation.

VI. Laboratory trial 3. Queen confinement/isolation experiments, 1992.

In order to evaluate whether Orthene® Fire Ant Killer applied as a dry wettable powder application to individual red imported fire ant mounds actually kills fire ant queens, a method was developed to isolate and confine queen ants within ant colonies.

Materials and Methods

Two methods of queen ant confinement were developed and evaluated in the laboratory: The first used a 20 ml. glass scintillation vial with a mesh-covered hole in the lid. The mesh size allowed worker ant access to the queen, but prevented queen escape. The second method used a capsule constructed of a very fine brass mesh which confined the queen and prevented worker access.

In the first experiment, one queen was confined to a scintillation vial container within each of 5 small laboratory fire ant colonies containing worker ants and brood (larvae and pupae). After 24 hours, the vials were emptied of all brood and worker ants. The queen was then re-inserted and moved into a new small colony box adjacent to the original box with a cardboard bridge to allow ant movement between the two colony boxes. Migration of the ant colonies (workers and brood) to the colony box housing the confined queen was observed for 7 days. Confined queens, along with some worker ants were then introduced into their original laboratory colonies. Queen survival was monitored for three weeks.

The two types of confinement containers were compared in a trial whereby individual queen ants were placed into each type and then introduced into unrelated laboratory colonies. The test was replicated five times. Queen survival was monitored for two weeks.

Results and Discussion

Queen ants confined to scintillation vials survived through the duration of this trial. Within 48 hours of separating queen ants from the remainder of the colony, two of the colonies had migrated to the boxes housing their queens. By 7 days, four of the five colonies had moved all brood into the boxes containing the queens. This experiment supported the idea that a colony, with a known number of confined queens, could be "planted" in the field.

Confined queen ants were reintroduced into their original field-collected colony. The five confined queens survived for 2 days, 4 for 8 days, and 2 for three weeks. This test supported the idea that an introduced queen could be accepted into a colony for at least a short period.

In the comparison of the two types of confinement containers, all queens in the mesh capsules lived at least three days. The queens in the glass vials were alive after two weeks and appeared able to live normally and indefinitely. These results indicated that field trials with these containers were feasible.

VII. Field trial 4. Queen confinement mound experiments, Brazos County, 1992-1993.

In order to evaluate whether Orthene® Fire Ant Killer applied as a dry wettable powder application to individual red imported fire ant mounds actually kills fire ant queens, methods developed to isolate and confine queen ants within ant colonies were used in the field to evaluate the effect of treatment.

Materials and Methods

<u>Trial 1</u>. The first test involved inserting both types of queen containers (20 ml. glass scintillation vial with a mesh-covered hole in the lid and fine brass mesh capsule) into active fire ant mounds. Due to a limited number of containers, only one mound was used per treatment. The control mound received 1 vial and 2 screens, the others: 1 vial + 4 screens and 2 vials + four screens. On 10 April 1992, the queen containment containers were inserted and the ants allowed to rebuild the mound for 48 hours. Two mounds were then treated with 2 teaspoons of Orthene® Fire Ant Killer each, 12 April. The third was left as an untreated control to confirm queen survival. The mounds were dug open after 48 hours (14 April) and the queen containers examined.

<u>Trial 2</u>. This experiment involved separating several laboratory ant colonies into "colonoids" that were allowed to live for several weeks in separate containers to stabilize. The colonies were then carefully examined and all queens placed into vial-type containers that allowed worker access, but prevented queen escape. The containers used in this trial were 7 ml. plastic vials in which the bottom had been filled with plaster to reduce volume and provide a moisture reservoir. The screw-on plastic cap had several holes melted into the top using a medium-sized paper clip wire to create the melted holes of appropriate size.

The eight "colonoids" were then taken to an area with little grass and few nearby fire ant colonies. The ground was loosened, and the queen container placed at approximately ground level in November 1992. The containers were then covered with approximately an inch of loose soil and the workers placed on top of the pile. Frequent rains and cold weather prevented any further action with the mounds until weeks later. Once mound-building activity was noted, four of the mounds were treated with 2 teaspoons of Orthene® Fire Ant Killer on January 25, 1993. Two days later (January 27, 1993), the queen containers were removed from all mounds and examined for living queens.

Results and Discussion

<u>Trial 1</u>. Results were inconsistent. All queens in the vials in treated mounds were dead, but some were missing. All queens in the treated mesh capsules were dead, but some were missing and others appeared to have been broken apart by workers before treatment. Queen survival in the untreated mound was also inconsistent with some queens in the mesh capsules appearing to have been killed by workers. The mesh capsules did not appear to exclude workers adequately, though why is unknown, and their use was discontinued.

Trail 2. Results were, again, inconsistent. Surprisingly, all the mounds had worker ant activity, and all but one contained either a live queen or their bodies. Of the four treated colonies, 2 contained live queens, one dead, and one missing. Of the untreated colonies, three of the four had at least one live queen in a container. The fourth contained a queen that appeared to have been dead for some time.

Because of cool weather during the time this trial was conducted, ants did not build up the field established mounds well and the soil on the tops of the mounds was crusted over. This crust may have prevented "sifting" of the acephate wettable powder into the mound as would be more likely on recently "worked" mounds. In this trial, queen ants were enclosed in vials that protected the queen from exposure to all sources of toxicant transmission but contact with other ants. If the treatment acts solely as a tracking powder, queen ants would be affected using this method. Conversely, should the treatment provide coverage within the colony through sifting, the queens so protected would be spared.

Conclusions

The objectives of these studies were not resolved conclusively. Field trials document excellent elimination of live ants in treated mounds with little or no "satellite" mound formation in treated areas. This is probably the best indirect data indicating that entire colonies, including queen ants are killed by the dust treatments. Experiments using fluorescent dye formulated acephate 75 percent wettable powder failed to demonstrate whether the dust treatment was a tracking powder or whether ant mortality resulted from a sifting action into the mound substrate. Failure resulted from the loss of fluorescence when the labeled Orthene® Fire Ant Killer contacted moist organic matter. In some of these trials, ants remained active below the treated surface for extended periods of time in the laboratory "antarium".

Attempts to cage queens were successful and this method allowed the establishment and maintenance of queens and colonies in the field. However, due to low numbers of replications of this labor-intensive procedure, results from Orthene® Fire Ant Killer treatments to caged-queen-colonies in the field were not consistent. Evidently, the dust treatment does not act solely as a tracking powder. This treatment more than likely acts to some extent as a sifting powder, as well. Since the Orthene® Fire Ant Killer is apparently not repellent to fire ants, ants contacting surfaces (soil or other ants) treated with even low concentrations of the insecticide are eliminated.

Literature Cited

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