

**Evaluation of Acceptance and Toxicity of Cacodylic Acid Formulations
to Laboratory Colonies of the Red Imported Fire Ant
(Formicidae: Hymenoptera)**

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Cacodylic acid, or hydroxydimethylarsine oxide, is one of the organic arsenical herbicides. It is a white, crystalline solid, soluble in water. Like other arsenical herbicides it has a much lower toxicity than elemental arsenic. Its acute oral LD50 is 830 mg/kg. Cacodylic acid, (CH₃)₂AsOOH and its sodium salt are used as general contact sprays. They desiccate and defoliate a wide variety of plant species. Cacodylic acid is not translocated in plants. It is rapidly inactivated in soils, but continuous use over long periods of time may cause phytotoxic levels of elemental arsenic to accumulate. Cacodylic acid may act by interfering with phosphorous metabolism, complexing with sulfhydryl-containing enzymes, uncoupling oxidative phosphorylation, or both (D. E. Stephenson, 1992, Pecos Valley Pest Management News, TAEX, 5(4):8-10).

Cacodylic acid (99.9% technical material), two bait formulations, 3 % and 11 %, as well as a non-toxic bait check material were tested as attractants for the red imported fire ant, Solenopsis invicta Buren. Since little or no information was available regarding cacodylic acid and fire ants, a series of test were performed to determine the most suitable formulation for ant acceptability, followed by a toxicity test based on the preference test results.

I. Formulation and evaluation of technical cacodylic acid:

Materials and Methods

Test #1 - Since fire ants are largely grease and oil feeders, an initial trial was performed to determine the acceptance of cacodylic acid in soybean oil, the most common toxicant carrier in fire ant bait-formulated insecticides. A sample of technical material was weighed and dissolved in soybean oil in serial dilutions. Oil solutions were drawn into 0.5 mm capillary tubes and mounted on a glass slide placed on a Petri dish lid. This arrangement allowed free and uniform access while eliminating dripping or other loss of material. The length of the oil column was recorded for each sample. One tube of each sample was placed in each of six active ant colonies. When the oil in one tube of each set was fully consumed, all of the tubes were removed from that colony, frozen to kill remaining ants, then the columns were measured to determine the amount of oil consumed.

Test #2 - Due to the questionable solubility of technical cacodylic acid in soybean oil, the preference procedure was repeated using an aqueous solution of commercial honey dissolved in distilled water at a rate of 1 part honey to 4 parts distilled water. The technical cacodylic acid

dissolved readily. A stock solution of 0.1 gram cacodylic acid in 10 ml honey water was made followed by serial dilutions, resulting in 10,000 ppm, 1.0 ppm and 0.1 ppb concentrations of cacodylic acid. These solutions were evaluated in the manner described for Test #1.

Test #3 - In an effort to detect an upper concentration limit and try to differentiate between soybean and honey-water solution attractiveness, 1.0 g of the technical material was dissolved in 1.0 ml of each carrier solution and placed in glass scintillation vials. Vials of both carriers alone and technical material alone were included as checks. The vials were then placed in active fire ant colonies and removed when one of the vials in each colony had been emptied.

Test #4 - Based on the information from the previous tests, an additional preference test was conducted using higher concentrations than in Test #2. The method was the same as in the first two tests.

Results and discussion

Test #1. All soybean oil formulations were attractive to the ants and differences between cacodylic acid concentrations were not significant (Table 1). The main problem encountered was the lack of solubility of technical cacodylic acid in soybean oil. During mixing, the solution had to be heated in boiling water to dissolve the material. Cacodylic acid may not have dissolved into the oil solution. Thus, the intended concentration of the dilutions were questionable.

Test #2. As shown in Table 2, all concentrations of cacodylic acid formulated in honey-water tested proved to be attractive to foraging worker ants, with no apparent upper limit of technical material reducing attractiveness.

Test #3. During this test, observations were made as to numbers of ants in each vial. Again, the honey-water check was the most attractive, followed closely by the oil check (Table3). The technical material in oil had 10-20 ants per vial while the technical + honey-water had 5-10 ants. The technical material alone even had two or three ants per vial, indicating that it had no repellent properties.

Test #4. All of the cacodylic acid concentrations were relatively attractive (Table 3). Differences were observed, however, between acceptability of 100,000 ppm and 500,000 ppm cacodylic acid solutions. Therefore, a concentration of 100,000 ppm cacodylic acid in honey-water (1 g toxin/10 ml honey-water) was determined to be the most effective concentration for use in evaluating the affect of cacodylic acid on fire and laboratory colonies.

Table 1. Evaluation of acceptability of cacodylic acid formulated in soybean oil to red

imported fire ants (Test #1).

Amount consumed - mm before/mm after

Colony

| <u>Sample</u> | <u>1</u> | <u>2</u> | <u>3</u> | <u>4</u> | <u>5</u> | <u>6</u> | <u>Mean</u> |
|---------------|------------|-----------|----------------|----------|----------|----------|-------------|
| Oil Check | 34/3 | 33/9 | 33/0 | 33/8 | 33/3 | 36/9 | 33.7/5.3 |
| Saturated | 31/11 | 34/12 | 33/5 | 35/11 | 34/5 | 35/6 | 33.7/8.3 |
| 1.0 ppm | 30/5 | 34/12 | 32/3 | 30/0 | 30/0 | 36/0 | 32.0/3.3 |
| 0.1 ppb | 32/0 | 30/0 | 33/3 | 35/11 | 33/4 | 34/0 | 32.8/3.0 |
| | | | | | | | |
| | Av. Before | Av. After | Av. % consumed | | | | |
| Oil Check | 33.7 | 5.3 | 84.3% | | | | |
| Saturated | 33.7 | 8.3 | 75.4% | | | | |
| 1.0 ppm | 32.0 | 3.3 | 89.7% | | | | |
| 0.1 ppb | 32.8 | 3.0 | 90.9% | | | | |

Table 2. Acceptability of cacodylic acid solutions in honey-water to red imported fire ants (Test #2).

Amount consumed - mm before/mm after

Colony

| <u>Sample</u> | <u>1</u> | <u>2</u> | <u>3</u> | <u>4</u> | <u>5</u> | <u>6</u> |
|---------------|------------|-----------|----------------|----------|----------|----------|
| Honey-water | 33/1 | 31/0 | 36/4 | 35/0 | 35/0 | 35/0 |
| 10,000 ppm | 36/11 | 35/0 | 36/4 | 37/9 | 35/3 | 37/1 |
| 1.0 ppm | 37/1 | 37/1 | 34/3 | 36/8 | 37/0 | 33/0 |
| 0.1 ppb | 34/0 | 35/1 | 35/4 | 37/5 | 34/0 | 38/0 |
| | | | | | | |
| | Av. Before | Av. After | Av. % consumed | | | |
| Honey-water | 34.2 | 0.8 | 98% | | | |
| 10,000 ppm | 36.0 | 3.0 | 92% | | | |
| 1.0 ppm | 35.7 | 2.2 | 94% | | | |
| 0.1 ppb | 35.5 | 1.7 | 95% | | | |

Table 3. Evaluation of the attractiveness of cacodylic acid formulations to red imported fire ants (Test#3).

| Weight of material removed (grams) | | | | | | |
|--|------------------------------------|----------|----------|----------|----------|----------|
| Sample | Colony | | | | | |
| | <u>1</u> | <u>2</u> | <u>3</u> | <u>4</u> | <u>5</u> | <u>6</u> |
| Dry technical | 0.01 | 0.01 | --- | 0.05 | --- | 0.03 |
| Tech. + Oil | --- | 0.06 | 0.02 | --- | 0.04 | 0.07 |
| Tech. + Honey-water | 0.03 | 0.09 | 0.04 | 0.01 | 0.09 | 0.08 |
| Oil Check | --- | 0.03 | 0.05 | --- | 0.05 | 0.01 |
| Honey-water Check | 1.01* | 0.17 | 1.02* | 1.06* | 0.75 | 0.64 |
| | | | | | | |
| Sample | Average weight removed(g) | | | | | |
| Dry Technical | 0.017 | | | | | |
| 1.0 g Tech. + 1.0 ml Oil | 0.031 | | | | | |
| 1.0 g Tech. + 1.0 ml Honey-Water (1:4) | 0.057 | | | | | |
| Oil Check | 0.024 (large numbers of dead ants) | | | | | |
| Honey-Water (1:4) Check | 0.775 | | | | | |

--- = greater ending weight than initial due to ants dying in the vials

* = all material removed from vial

Table 4. Evaluation of acceptability of cacodylic acid concentrations to red imported fire

ants (Test #4).

Amount consumed - mm before/mm after

Colony

| <u>Sample</u> | <u>1</u> | <u>2</u> | <u>3</u> | <u>4</u> | <u>5</u> | <u>6</u> |
|----------------|-------------------------------------|----------|----------|----------|----------|----------|
| 1,000,000 ppm | 37/6 | 40/19 | 35/28 | 35/28 | 37/28 | 38/8 |
| 500,000 ppm | 35/21 | 36/22 | 36/13 | 36/1 | 37/23 | 36/17 |
| 100,000 ppm | 37/13 | 39/15 | 40/8 | 33/5 | 37/7 | 37/8 |
| 50,000 ppm | 35/15 | 35/12 | 34/3 | 39/3 | 36/6 | 35/9 |
| 10,000 ppm | 35/3 | 35/5 | 37/2 | 36/4 | 35/3 | 35/6 |
| Honey-water Ck | 36/2 | 38/3 | 38/3 | 38/2 | 39/5 | 38/4 |
| | | | | | | |
| <u>Sample</u> | <u>Average amount consumed (mm)</u> | | | | | |
| 1,000,000 ppm | 17.5 | | | | | |
| 500,000 ppm | 19.5 | | | | | |
| 100,000 ppm | 27.8 | | | | | |
| 50,000 ppm | 26.7 | | | | | |
| 10,000 ppm | 31.7 | | | | | |
| Honey-water Ck | 34.7 | | | | | |

II. Colony Mortality Test

Materials and Methods

Twelve fresh fire ant colonies were dug from the field, extracted from the soil, and placed in Fluon[®]-coated plastic boxes. Based on the results of Preference Test #4, a solution of 10% (100,000 ppm weight/volume) cacodylic acid in honey-water (1 part honey:4 parts distilled water) was made and offered to the ants in 20 ml test tubes plugged with cotton. One tube of the solution was placed in each of six colonies, providing them with a continuous supply of the toxin.

Six identical tubes, containing only honey-water, were placed in the remaining six colonies. A standard diet of frozen crickets was maintained as were the normal water tubes in all colonies. The normal daily additions of honey-water were discontinued due to the presence of the honey-water in test tubes. The colonies were observed periodically and rated as to approximate number of worker ants, relative amount of brood, and presence of queen ants.

Results and Discussion

Red imported fire ant colonies given unlimited access to the 100,000 ppm cacodylic acid 1:4 honey-water solution declined steadily during the 62 day trial period (Table 5). Total estimated number of ant averages declined steadily until only a few workers were left in two of the original six treated colonies. Queen ant decline was first noticed after 20 days of treatment initiation. Brood and queens were present in all untreated colonies throughout this trial.

Shortly following initiation of this trial (27 September), all cacodylic acid-treated colonies were observed to have ants evenly scattered across the floor of the colony box while ants in untreated colonies were concentrated in Petri dishes together with brood and queens as is normal for laboratory colonies. It appeared as if worker ants consuming cacodylic acid were avoiding returning to the colony.

Removal of untreated honey-water occurred at a higher rate than cacodylic acid-treated honey-water. By 30 September an average of 1.5 ml. cacodylic acid honey-water had been consumed. By 25 November, an average of 11.2 ml. had disappeared. Ants were seen to visit the cacodylic acid-containing tubes for only the first three days of the test. The remaining decrease in volume is believed to be due to evaporation.

By 30 September, small piles of dead ants were beginning to form in untreated colonies as is usual. In cacodylic acid-treated colonies, however, dead ants were observed to be scattered on the colony floors. Treated colonies were found to have an unusual acrid odor as well. Very small amounts of cacodylic acid-treated honey-water had been consumed (1-2 ml) while treated colonies also drank large amounts of water. Brood in treated colonies began to appear yellowish-brown and were noticeably fewer in numbers beginning 11 October, and had virtually disappeared by 22 October.

Summary

Cacodylic acid appeared no to be repellent to fire ants and was readily accepted when combined

with the proper attractant. Soybean oil formulations proved to be attractive to the ants, but the toxin's solubility in oil was questionable. Honey-water formulations proved to be quite attractive to the ants, even at extremely high cacodylic acid concentrations. The baits, as formulated, were not attractive to the ants. [Other products formulated on this type of bait (Orthene[®] and Sevin[®]) have also been unattractive in past experiments.

The colony mortality experiment demonstrated that a very small amount of cacodylic acid caused extensive and continuing worker mortality beginning within a few days of ingestion, the cessation of brood production within three weeks, and complete colony death (including queens) within eight weeks. The treated colonies were, for practical purposes, non-functional after one month.

Cacodylic acid consumption quickly altered worker ant behavior. Affected ants were evenly scattered on the colony tray floor, displayed noticeable loss of appetite and increased water consumption. Later observations showed that the few remaining ants moved to the water tubes which provided a higher humidity environment and continuous access to water. These behaviors are indicative of ants under water stress.

Our experience has shown that it is unusual for most toxicants to cause the death of every ant in a laboratory colony. Though the cacodylic acid was somewhat slow in causing the complete death of the colony, it showed rather rapid and persistent worker kill with only a small dose. From these results, cacodylic acid appears to have potential as a fire ant insecticide, though its method of delivery needs additional investigation.

Table 5. Estimated number of ants, presence of queen and condition of brood in laboratory colonies of red imported fire ants treated with 100,000 ppm cacodylic acid + honey-water (1:4 honey:water), 1991.

No. 1000 ants (\pm S.D.)/colony* Percent untreated colonies with

| Date | Day | Treated | Untreated | brood | queens |
|----------|-----|----------------|-----------------|-------|--------|
| Sept. 25 | 1 | 17.5 \pm 5.2 | 32.5 \pm 16.1 | 100 | 100 |
| Sept. 30 | 6 | 11.3 \pm 2.9 | 32.5 \pm 16.1 | 100 | 100 |
| Oct. 4 | 10 | 7.2 \pm 3.1 | 32.5 \pm 16.1 | 100 | 100 |
| Oct. 7 | 13 | 3.7 \pm 2.0 | 32.5 \pm 16.1 | 100 | 100 |
| Oct. 11 | 17 | 2.7 \pm 0.5 | 32.5 \pm 16.1 | 100 | 100 |
| Oct. 14 | 20 | 2.3 \pm 0.5 | 32.5 \pm 16.1 | 83 | 100 |
| Oct. 22 | 28 | 1.3 \pm 0.5 | 33.3 \pm 16.3 | 17 | 100 |
| Oct. 28 | 34 | 0.7 \pm 0.3 | 33.3 \pm 16.3 | 0 | 100 |
| Nov. 4 | 41 | 0.3 \pm 0.2 | 32.5 \pm 15.1 | 0 | 100 |
| Nov. 13 | 50 | 0.1 \pm 0.1 | 29.2 \pm 12.4 | 0 | 83 |
| Nov. 25 | 62 | 0.0 \pm 0.0 | 29.2 \pm 12.4 | 0 | 33 |

* All means (treated versus untreated) are significantly different ($P \neq 0.05$) according to the Student's t test (d.f. = 10; $t = 2.1764, 3.1780, 3.7986, 4.3686, 4.5516, 4.6025, 4.7976, 4.8994, 5.2343, 5.7397, 5.7500$).