

EVALUATION OF D-LIMONINE AS AN INDIVIDUAL RED IMPORTED FIRE ANT MOUND TREATMENT

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D-limonine, a terpene extracted from citrus peel oils, is known to have insecticidal properties causing spontaneous activity of sensory and motor nerves, resulting in twitching, lack of coordination, convulsions and knockdown paralysis of target insect pests. Several insecticide products containing D-limonine are currently registered by the Environmental Protection Agency for control of insects such as fleas. However, none is currently registered for control of red imported fire ants. This trial was conducted to determine if a D-limonine formulation developed by the Environmental Pesticides Group is effective when applied as an individual fire ant mound drench.

Materials and Methods

On August 4, 1992 a 40-foot wide strip was marked on the earthen dam impounding Lake Somerville, Burlison County, Texas. The area within the strip was carefully surveyed and each active mound marked with a surveying flag. A plot marker flag was placed after locating 10 active mounds. Therefore, each plot was of an equal width (40 feet) and contained 10 mounds, but varied in length. This design allowed for similar re-infestation pressure on all plots. The plots were then divided into 3 blocks (replications) of 6 plots each and treatments were then randomly assigned within the blocks. Mound density was approximately 380 mounds per acre. Mound activity was determined by lightly disturbing the mound with a pointed wooden tool handle. A mound was considered active if ants came to the surface in some numbers within 15 seconds of disturbance.

On August 5, 1992, the treatments were applied beginning at 9:00 a.m. and ending at 4:00 p.m. The material was mixed in a large drum then dispensed into 2-gallon garden sprinkler cans. The breaker nozzle was removed from the end of the can spout to give a solid stream that penetrated the mound structure better. The method of application was similar for all plots. The following treatments were applied:

- 1) 5% D-limonine + 2% Mazclean; 1.5 gallons per mound
- 2) 4% D-limonine + 1% Mazclean; 1.5 gallons per mound
- 3) 3% D-limonine + 1% Mazclean; 1.5 gallons per mound
- 4) Emulsifier only: 4% Mazclean; 1.5 gallons per mound
- 5) Standard treatment: 2 teaspoons Orthene®/gallon water; 1 gallon per mound
- 6) Untreated control: 1.5 gallons water per mound

Post-treatment evaluations were made beginning at 9:00 a.m. on 6, 7 and 12 August, and 11 September (1, 2, 7 and 37 days post-treatment, respectively). The 7 day evaluation of the treated mounds was accompanied by a survey of the entire plot area to locate any satellite mounds or re-infestation from outside the plot. Also noted was the number of application sites showing

phytotoxicity and the relative severity of the damage. Damage was rated on a scale of 1 to 3, with 1 being yellowing of the leaves and 3 being considerable browning and necrosis. Results were analyzed using Analysis of Variance (ANOVA) and Least Significant Difference (LSD) test ($P \leq 0.05$).

Results and Discussion

Due to varying temperature, moisture, sunlight, and the application of the large volumes of water to the mounds themselves, ant activity in mounds varied greatly. The activity of the treated mounds had to be compared to those that served as untreated controls. Generally, a mound was considered "active" if, upon disturbance, enough ants rose to the surface within 15-30 seconds to create what is considered to be a functional, nuisance mound. Rarely was 100% kill encountered in any of the six treatments, nor were some of those mounds rated as active as vigorous and healthy as they had been prior to treatment.

One day after application, the 5% D-limonine treatment had produced numerically the best control, while all active ingredient treatments produced significantly different reductions of treated active ant mounds compared to the Mazclean and water drench treatments (Table 1). By 2 days after application, the number of active mounds increased in all the D-limonine plots, although these treatments performed statistically the same. Orthene® Fire Ant Killer performed best, numerically, but statistically the same as 5% D-limonine. Mazclean, the emulsifier for the D-limonine solutions, showed a significant level of active ant mound suppression over the water-drenched plots. By the 7-day evaluation, the most noticeable change was the unexpected reduction in activity of the Mazclean-treated plots, similar to active mound suppression resulting from D-limonine treatments. Other treatments remained both statistically and numerically similar to 2 day post treatment results. The re-infestation survey showed considerable new mound formation in some plots, although there was no statistically significant difference between them. This data indicates that treatments did not cause mound movement that would have resulted in the appearance of "new" (satellite) mounds, or that ant colonies migrating into treated plots from adjacent areas.

Results of the phytotoxicity survey provides some reason for concern. Of the 90 mounds treated with the D-limonine formulations, 80% of them showed some phytotoxicity to the surrounding vegetation:

| <u>Treatment</u> | <u>Mean no. affected mounds/Average rating</u> |
|--|--|
| D-limonine 5% + 2% Mazclean/1.5 gal. | 9.33 / 2.43 |
| D-limonine 4% + 1% Mazclean/1.5 gal. | 8.67 / 1.35 |
| D-limonine 3% + 1% Mazclean/1.5 gal. | 6.00 / 1.61 |
| Emulsifier only: Mazclean 4%/1.5 gal. | 0.67 / 1.00 |
| standard treatment: Orthene® 2 tsp./gal. | 0.00 / 0.00 |
| untreated control: 1.5 gal. water | 0.00 / 0.00 |

The severity of the damage varied with the formulation, the 5% D-limonine treatment being considerably worse, having an average of 2.43 on a 3-point scale and causing considerable yellowing with some browning and death of foliage. The primary vegetation at this site is Bahia grass, a rugged "invader" used for hay and erosion control, as well as numerous other native species. It is unlikely that this level of damage would be considered acceptable on ornamental turf.

Table 1. Mean number of ten active red imported fire ant mounds following individual mound drenches with D-limonine, Burleson Co., Texas, treated 5 August 1992.

| <u>Treatment and rate</u> | Mean no. of active fire ant mounds/10 per treatment* | | | |
|--|--|---------------|---------------|----------------|
| | <u>1 day</u> | <u>2 days</u> | <u>7 days</u> | <u>37 days</u> |
| D-limonine 5% + 2% Mazclean/1.5 gal. | 1.3 a | 2.0 cde | 0.7 c | 0.7 b |
| D-limonine 4% + 1% Mazclean/1.5 gal. | 2.7 a | 3.3 cd | 3.3 b | 0.7 b |
| D-limonine 3% + 1% Mazclean/1.5 gal. | 3.3 a | 3.7 bc | 2.7 bc | 0.7 b |
| Emulsifier only Mazclean 4%/1.5 gal. | 9.0 b | 6.0 b | 1.7 bc | 2.0 b |
| standard treatment Orthene® 2 tsp./gal. | 2.7 a | 0.3 e | 0.7 c | 1.0 b |
| untreated control 1.5 gal. water | 9.7 b | 9.3 a | 8.0 a. | 9.0 a |
| <i>F</i> -ratio | 18.254 | 16.287 | 17.410 | 15.619 |
| <i>P</i> | 0.0001 | 0.0002 | 0.0001 | 0.0002 |

* Means in columns followed by the same letter are not significantly different using Analysis of Variance (ANOVA) and Least Significant Difference (LSD) test ($P \leq 0.05$).