

EVALUATION OF WORKS WELL SHORT-CHAINED ALIPHATIC HYDROCARBONS AS A RED IMPORTED FIRE ANT MOUND TREATMENT

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Works Well®, a 100% volatile (flash point of 135°F) liquid mixture of short-chain aliphatic petroleum hydrocarbons, was initially evaluated in 1995. The product provided 60-80% control of individually treated red imported fire ant, *Solenopsis invicta* Buren, mounds over the course of the one month test. The number of active mounds were statistically similar to those of a standard treatment, Orthene® Turf, Tree and Ornamental Spray (acephate 75% dust), and significantly lower than those in untreated control plots.

Results were forwarded by the manufacturer to the Environmental Protection Agency (EPA) as part of the registration process. The EPA responded with three specific experimental protocols to address efficacy concerns: 1) the effect of high ambient temperatures, 2) the effect of sandy versus clay soil type and, 3) the effects of disturbance (in addition to disturbance caused by treatment) at time of treatment. The result of negotiations with the manufacturer was a protocol for a single test designed to “address” all three concerns. The test was conducted in relatively hot, dry conditions on a site with sandy soil and mounds were probed before treatment. It must be emphasized that, all three factors differed from the first test. With different treatment conditions, results obtained from the two trials can not be directly compared.

MATERIALS AND METHODS

The 1997 trial was conducted in an area below the earthen dam of Lake Conroe, Montgomery County, Texas. The soil on the test site was a deep, moderately coarse sand or loamy sand. The test site was mowed to a height of 3.5 inches during the last week of May. The test was established on 3 June in the following manner: Two strips, 40 feet wide and approximately 200 feet long each, were measured and marked using six-foot long pieces of 3/8-inch diameter reinforcing rod at all corners. The strips were surveyed for active fire ant mounds and mound activity assessed using the minimal disturbance technique. Beginning at one end of a strip, 10 active mounds were marked with red surveyor’s flags. The next 10 mounds were marked with orange flags and so on, alternating colors, until 12 sets of 10 mounds (plots) had been marked. The length of each plot was then measured, arrayed lowest to highest and divided into four blocks of three plots each (replications). Treatments were assigned within blocks so that the total lengths for each treatment were approximately the same. Treatments included the following:

- 1) Untreated Control
- 2) Works Well liquid formulation - amount applied according to mound size
- 3) Spectracide® Dursban®, 6% chlorpyrifos, 2 oz. concentrate in one gallon water per mound.

Treatments were applied 5 June, beginning at approximately 10:30 a.m. The weather was partly cloudy and humid, with a slight breeze. Temperature at the end of the test was approximately 85

degrees F and the soil was dry to slightly moist.

Dr. Warren Hardwick, manufacturer of the Works Well formulation, was present for the test and applied all the Works Well treatments personally. Dr. Hardwick located the site of greatest ant activity in each mound and probed to a depth where resistance was met using a pointed metal rod approximately 3/8-inch in diameter. The material was applied directly from a half-gallon metal can with a single-hole squirt cap. He applied the desired amount of Works Well into the hole then sprayed a band of fluid around the perimeter of the mound and on top of the mound. The time of each application was recorded on a stopwatch. After all mounds were treated, the remaining fluid in each can was measured in a graduated cylinder and subtracted from the stated full volume. In this way, the amount applied per mound could be calculated later. Standard chlorpyrifos drenches were applied during the same time period using a plastic sprinkler can with a breaker nozzle. Before applications began, the plots were re-surveyed for any moved or missed mounds. These mounds were marked with blue flags and treated with the appropriate chemical after marked-mound treatments. For the remainder of the trial, these "blue flagged" ant mounds were ignored.

The first evaluations was conducted on 6 June at 9:00 a.m.. Weather conditions were similar to those of the treatment date. Each marked mound was disturbed with a pointed tool handle until ants rose to the surface in a defensive action ("active") or failed to appear after 10 - 20 seconds ("inactive"). Dr. Hardwick was present for all evaluations and agreed with the evaluator's assessment of mound activity at the time. The number of active mounds was recorded for each plot. During the first evaluation, active mounds in Works Well formulation treated and standard-treated plots were marked with large, yellow flags for later re-treatment. All plots were surveyed and any new active unmarked ("satellite") mounds were also marked with large, yellow flags.

The second evaluation was conducted on 9 June, beginning at 8:00 a.m. The weather was overcast, with sprinkling rain, temperature 70-75 degrees F. Evaluations were conducted in a similar manner. Active Works Well formulation-treated mounds were re-treated by Dr. Hardwick at this time and the application times recorded. Active unmarked new, "satellite" mounds were also treated. No chlorpyrifos-treated mounds were active, so only unmarked ones were treated.

The one-week post-treatment evaluation was conducted 12 June 1997, beginning at 9:00 a.m. The weather was partly cloudy, temperature 75 - 80 degrees F and the soil was slightly moist. Minimal disturbance evaluations were conducted on all originally flagged mounds and given "active" or "non-active" ratings. The plots were also surveyed for new, "satellite" mound formation. Flags were removed from previously marked satellite mounds if they showed no activity.

The final, two-week evaluation was conducted on 19 June. Marked mounds were evaluated using the method of mound activity rating as defined by Harlan *et al.* (1981) and modified by Lofgren and Williams (1982). Plots were also surveyed again for new, "satellite" mound formation. Conditions at the time of evaluation (beginning 9:00 a.m.) were partly cloudy, 80 - 85 degrees F, with high humidity and calm winds. The soil was moist. Dr. Hardwick was present for all evaluations. Resulting active mound data were analyzed using analysis of variance (PC-SAS ANOVA) ($P \leq 0.05$) and means separated using Tukey's Studentized Range test.

RESULTS AND DISCUSSION

Works Well Application Volume. The application volume for the initial application of Works Well formulation was 80.63 " 26.80 ml. (St. Dev.) per mound (2.84 oz. \pm 0.94 oz.). The marked mound density of Works Well formulation-treated plots was 335 mounds per acre. If "blue flagged" mounds are included, the density rises to 469 mounds per acre. This area is considered to be infested by the multiple queen (polygyne) form of the red imported fire ant. If one were to extrapolate per acre treatment volume requirement from these small plot treatments, the application rate would have been 7.43 " 2.46 gallons formulation/acre of the Works Well formulation. Moreover, had all mounds (including "blue-flagged" mounds) had been treated with the calculated rate, the total volume per acre would rise to 8.79 " 3.44 gallons/acre. Re-treatments on the 19 still-active mounds were made with considerably more volume: 153.16 " 41.06 ml. (St. Dev.) per mound (5.39 oz. " 1.45 oz.). Had this rate been used for the initial treatment of marked mounds the per acre rate would have been 14.11 " 3.79 gallons/acre, and for all mounds, the rate would have risen to nearly 20 gallons per acre. In contrast, 56.2 fl. oz. chlorpyrifos active ingredient per acre would have been required (calculated from the 6% chlorpyrifos solution contained in Spectracide Dursban Indoor & Outdoor Insect Control product). At a retail cost of \$11.00 per quart, the cost of treating all mounds in an acre of land at this level of infestation would have been \$322 (or \$0.69 per mound) with this chlorpyrifos product. According to the manufacturer, the Works Well formulation is anticipated to cost \$12.00/0.5 gallon and an approximate treatment cost of \$0.30/mound.

Efficacy. Application of the Works Well formulation to individual mounds resulted in significant ($P < 0.05$) elimination of ant activity as compared to those mounds receiving no treatment at all evaluation dates (**Table 1**). However, Works Well formulation-treated mounds were significantly more active than those receiving a standard chlorpyrifos drench treatment at one, four and seven days post-treatment. In one EPA report to the manufacturer, a requirement for 90% control was mentioned as the required level of efficacy to claim "control" of treated fire ant mounds. The following table lists "Percent control" for each post-treatment evaluation date in this test as calculated from pre-treatment levels of treated active mounds.

<u>Treatment</u>	Percent control (% inactive mounds of 40 treated)			
	<u>1 day</u>	<u>4 days*</u>	<u>7 days</u>	<u>14 days</u>
Untreated control	0.0	5.0	17.5	22.5
Works Well formulation	22.5	52.5	77.5	100.0
Standard	97.5	100.0	100.0	100.0

* all active mounds retreated

At 14 days after the initiation of the treatment regime, 100 percent of the mounds in Works Well treated plots showed no ant activity. Also, plots also contained no more new, "satellite" mounds than those found in the chlorpyrifos-treated standard plots. Only at that time, however, did the mean total number of both treated and new, "satellite" mounds combined in Works Well formulation-treated plots differ significantly from that of untreated control plots (see "Total" columns, **Table 1**).

Mound activity ratings as defined by Harlan *et al.* (1981) and modified by Lofgren and Williams (1982) at day 14 showed complete, 100 percent *index of control* of mounds treated with both the Works Well formulation and the chlorpyrifos standard.

Although almost immediate elimination of ant activity in Works Well formulation-treated mounds was anticipated by the formulator, data indicate a slower decline. Factors that could explain these documented results include soil type, mound structure and temperature. It is suspected that the combination of higher temperatures and/or sandy, porous soil was responsible for the slower-than-expected results. Some of the ant mounds in treated plots were constructed at the base of clump grasses. These were observed to be more difficult to treat effectively with the Works Well formulation, as expected, and were re-treated as instructed on the product's experimental label..

Harlan, D. P., W. A. Banks, H. L. Collins and C. E. Stringer. 1981. Large area tests of AC-217,300 bait for control of imported fire ants in Alabama, Louisiana, and Texas. *Southwest Entomol.* 6:150-157.

Lofgren, C. S. and D. F. Williams. 1982. Avermectin B_{1a}: A highly potent inhibitor of reproduction by queens of the red imported fire ant. *J. Econ. Entomol.* 75: 798-803.

Table 1. Efficacy of individual red imported fire ant mound treatments, Lake Conroe Dam, Montgomery County, Texas, treated, 5 June 1997.

<u>Treatment</u>	Mean no. active mounds*					
	1-day-----			4-days-----		
	<u>Marked</u>	<u>New</u>	<u>Total</u>	<u>Marked</u>	<u>New</u>	<u>Total</u>
Untreated control	10.0a	1.0a	11.0a	9.5a	0.8a	10.3a
Works Well formulation	8.3b	2.5a	10.8a	4.8b	2.3a	7.0a
Chlorpyrifos "standard"	0.3c	0.8a	1.0b	0.0c	1.3a	1.3b
<i>F</i>	104.24	1.08	17.97	32.56	0.72	5.96
<i>P</i>	0.0001	0.455	0.002	0.0003	0.633	0.025
MSD	1.4004	3.334	3.740	2.3153	2.8945	5.302
Crit. value = 4.339						
d.f. = 6						

Continued:

<u>Treatment</u>	7-days-----			14-days-----		
	<u>Marked</u>	<u>New</u>	<u>Total</u>	<u>Marked</u>	<u>New</u>	<u>Total</u>
Untreated control	8.3a	1.8a	10.0a	7.8a	1.8a	9.5a
Works Well formulation	2.3b	1.0a	3.3a	0.0b	1.0a	1.0b
Chlorpyrifos "standard"	0.0c	1.5a	1.5b	0.0b	1.3a	1.3b
<i>F</i>	30.69	2.12	15.95	61.29	1.45	24.18
<i>P</i>	0.0003	0.193	0.002	0.0001	0.330	0.001
MSD	2.1392	2.315	3.254	1.5761	1.947	2.730
Crit. value = 4.339						
d.f. = 6						

* Means in columns followed by the same letter are not significantly different using analysis of variance (PC SAS - ANOVA) ($P \leq 0.05$) and means separated using Tukey's Studentized Range test.