

**Final Report on Testing of TB131-1, a *Beauveria bassiana* Product
Manufactured by Troy Biosciences for the Control of Individual Colonies of
Red Imported Fire Ants**

Charles L. Barr, Extension Program Specialist - Fire Ant Project
Rody L. Best, Extension Assistant - Fire Ant Project
Lisa Lennon - Fire Ant IPM Agent, Travis/Williamson Counties
Dale Mott - EA-IPM, Williamson/Milam Counties
Scott Russell, Fire Ant IPM Agent, Dallas/Tarrant Counties

In 1998, Troy Biosciences contracted with the Texas Agricultural Extension Service’s Fire Ant Applied Research Laboratory to conduct a series of tests on their TB131-1 product, a formulation of spores from the fungus *Beauveria bassiana*, for the control of red imported fire ant (*Solenopsis invicta* Buren) colonies. Five trials were conducted to test the product’s effectiveness at five different sites, at different times of the year and in different soil types. Due to persistent, widespread drought conditions from the spring of 1999 through the following fall, finding suitable test sites was difficult. Since it was felt that the fungus would perform best in warm, moist conditions, three of the four tests were completed in the spring of 2000. **Table 1** summarizes the test dates, locations, site characteristics and participants.

Table 1. Summary of tests.

Test	Dates	Location	Site and Test Characteristics	Participants
#1	11/9/98 - 12/7/98	Taylor	Cool weather; heavy, calcareous, black clay soil; excess moisture	Mott, Barr, Best, Lennon
#2	7/30/99 - 8/27/99	Navasota	Very hot weather; clay loam soil; moist changing to very dry	Barr, Best
#3	3/20/00 - 4/17/00	D/FW	Warm weather; heavy, calcareous, black, clay soil; adequate moisture	Russell, Merchant
#4	3/31/00 - 5/3/00	Bryan	Warm weather; sandy soil over claypan; adequate moisture	Barr, Best
#5	5/24/00 - 6/21/00	Burleson County	Warm weather, bottomland silty loam soil; adequate moisture	Barr, Begnaud

Materials and Methods

The standard “railroad track” design described below was used for all the tests. This method provides a means of tracking not only the status of treated mounds, but also “new” mound-building activity within a defined area. In this way, the appearance of new mounds can be detected and inferences made as to whether these result from colony relocation, “shattering”

(splitting into more than one colony) after treatment or from re-invasion by colonies from outside the test area.

The first step in establishing field trials using the “railroad track” design involves marking off a strip of land 30 to 40 feet wide (depending on mound density) and of indeterminate length. Starting at one end, every active fire ant mound found progressing along the strip is marked with a wire surveyor’s flag until 10 are marked. Flag color is then switched for the next set of 10 and so on, alternating flag colors. Then, a third color flag is placed along the edge of the strip halfway between the last mound of one plot and the first mound of the next plot. Each set of 10 mounds and the encompassing area is considered a plot and each plot is numbered sequentially.

Plot lengths are then measured, recorded and plot numbers are arrayed from longest to shortest to give a range of plot mound density (length being inverse to density). The lengths are then divided into four equal groups so that there is a set or block of low-density plots, two medium density sets, and a high-density set. These sets represent replications. Next, treatments are randomly assigned within replications. The total length (sum of four replications) for each treatment is then calculated. If needed, treatments are then swapped within replications to try to equalize the treatment totals. In this way, all treatment plot sets have the same number of active mounds prior to treatment, within roughly the same total area. This eliminates pre-treatment differences between treatment plots and helps to equalize the chances of a colony re-locating within a plot or colonies re-invading from outside the plot following treatment.

Though the test protocols remained substantially the same, some modifications were made by the manufacturer to help increase product effectiveness. Comparison products or “standard treatments” varied by test. Exact treatments used in each test are listed in individual test result tables. Nevertheless, treatments included the following:

- 1) untreated control
- 2) TB131-1, *Beauveria bassiana*, ½ oz. per mound (¼ oz. per mound in Test #1)
- 3) TB131-1, *Beauveria bassiana*, ½ oz. per mound applied at test initiation and again in two weeks to still-active mounds (or ½ oz. per mound in Test #1)
- 4) Standard treatment (see individual test results for specific product used)

TB131-1 was applied by first punching a hole at least ½-inch in diameter in the top of a mound in the area of highest brood concentration. The product was then simply poured down the hole where it was quickly absorbed onto the soil.

Ant activity was evaluated using the minimal disturbance technique. Criteria used to consider a mound as “active” varied with weather, time of day, test site, activity of untreated mounds and the best judgement of the evaluator. Generally, a mound was considered active if 10 to 20 ants rose to the surface in a defensive manner within 15 seconds of disturbance. Evaluations were made at 7, 14 and 28-30 days post-treatment, weather permitting. Plots were surveyed at least once during the test and at the end of the test for “new” mound formation. The “total” number of mounds reported in tables are the number of active treated mounds plus “new” mounds.

All data were analyzed using PC SAS analysis of variance procedures with means separated using Tukey’s studentized range test, $P < 0.05$.

Note: an identical test was conducted in Robertson County, November - December 1998.

Due probably to the cool, wet conditions, virtually all the untreated mounds were naturally abandoned within two weeks of test initiation. Given this very unusual occurrence, the Robertson County test results are not reported here and another test was conducted later to complete the five tests needed.

Results

Test #1 - Taylor, Williamson County, Texas. Since this was the first test in the series, two rates of TB131-1 (*Beauveria bassiana*), ½ and ¼ fluid ounce per mound were used.

This test was conducted rather late in the season. Though the ants were building mounds quite actively, they did not appear to be foraging well. Consequently, Orthene® Fire Ant Killer (75% acephate applied at 2 teaspoons per mound) was used as the standard treatment.

Weather during the test period was cool and wet, preventing the evaluation of the test more than twice. During treatment and evaluations, active mounds were built-up with brood visible near the surface. Ants were active when disturbed.

Results do not show any significant ($P < 0.05$) differences between the number of active treated mounds in fungus-treated and untreated plots. Orthene-treated plots had significantly fewer treated mounds throughout the test, though they had numerically more “new” mounds appear after treatment. Consequently, there were no significant differences in the total number of active mounds between any of the treatments at either seven days or one month post-treatment.

Table 2. Mean number of active mounds of 10 treated or as indicated, 4 replications; Taylor, Texas

<u>Treatment</u>	<u>1 week treated</u>	<u>1 week new</u>	<u>1 week total</u>	<u>1 month treated</u>	<u>1 month new</u>	<u>1 month total</u>
untreated	9.00 a	2.50 a	11.50 a	5.25 a	2.50 a	7.75 a
TB131-1 ½ oz	7.50 a	3.00 a	10.50 a	5.75 a	2.50 a	8.25 a
TB131-1 ¼ oz	9.25 a	2.25 a	11.50 a	5.25 a	1.75 a	7.00 a
Orthene	1.25 b	4.00 a	5.25 a	1.50 b	3.75 a	5.25 a
F	40.85	0.26	3.08	8.79	0.93	1.09
P	0.0001	0.8514	0.0682	0.0023	0.4563	0.3925
R ²	0.9108	0.06146	0.4352	0.6872	0.1886	0.2134
MSD*	2.4616	6.3486	7.1639	2.7935	3.6107	5.2917

Means in the same column followed by different letters are significantly different ($P < 0.05$) using PC SAS analysis of variance procedure with means separated using Tukey’s studentized range test.

* Minimum significant difference

Test #2 - Navasota Airport, Grimes County, Texas. The protocol for this second trial was modified from that of the first in two ways. First, rather than two rates of TB131-1, the ½ oz. rate was used for both treatments. Both were treated at test initiation, then, in one treatment, all mounds that were still active after two-weeks were re-treated with ½ oz. of material. The second change was the use of Organic Resources (0.01% pyrethrin, 83.3% diatomaceous earth, 0.1% piperonyl butoxide applied at 4 tablespoons per gallon of water per mound) as a standard because of its “organic” nature and potential as a market competitor to TB131-1. Organic Resources is a very fast acting mound drench.

Results indicate that Organic Resources reached 100 percent control of treated mounds within three days and stayed at that level of control throughout the test. By the end of the test, TB131-1, both as a single and double application resulted in significantly ($P < 0.05$) fewer active, treated mounds than the untreated control. There was no indication that any of the products resulted in colony movement or shattering. At one month, the total active mounds (treatment plus new) for the TB131-1 ½ oz. x 1 was significantly less than in untreated plots.

The test was conducted during the hottest time of the year. At treatment, however, mounds were small, but freshly built-up with brood near the surface. The test site received rain a few days before test initiation, but was then subject to very high temperatures and no rain for the duration of the test, resulting in drought conditions by that time.

Table 3. Mean number of active mounds of 10 treated or as indicated, 4 replications; Navasota Airport, Grimes County, Texas

<u>Treatment</u>	<u>day 3 treated</u>	<u>1 week treated</u>	<u>1 week new</u>	<u>1 week total</u>	<u>2 weeks treated</u>	<u>1 month treated</u>	<u>1 month new</u>	<u>1 month total</u>
untreated	8.25 a	8.25 a	1.25 a	9.50 a	8.00 a	8.25 a	1.00 a	9.25 a
TB131-1 ½ oz x 1	7.25 a	6.75 a	0.50 a	7.25 a	5.00 a	4.25 b	0.75 a	5.00 b
TB131-1 ½ oz. x 2	8.00 a	8.50 a	1.00 a	9.50 a	6.50 a	5.50 b	1.25 a	6.75 ab
Org. Res.	0.00 b	0.00 b	1.00 a	1.00 b	0.00 b	0.00 c	0.50 a	0.50 c
F	27.09	31.22	0.49	18.12	14.12	30.59	0.48	29.02
P	0.0001	0.0001	0.6975	0.0001	0.0003	0.0001	0.7047	0.0001
R ²	0.8713	0.8864	0.1086	0.8192	0.7793	0.8844	0.1638	0.8787
MSD*	3.1779	2.9996	1.8922	3.9623	3.8803	2.6065	1.9637	2.8745

Means in the same column followed by different letters are significantly different ($P < 0.05$) using PC SAS analysis of variance procedure with means separated using Tukey’s studentized range test.

* Minimum significant difference

Test #3 - DFW International Airport, Texas. The trial was conducted in mid-spring with adequate moisture and moderate temperatures. TB131-1 was applied in the same manner as Test #2 with initial application and follow-up application on active mounds in one treatment. Amdro® (0.73% hydramethylnon bait applied at 5 tablespoons per mound) was used at the request of Troy BioSciences personnel as the standard comparison product for this and subsequent tests. Conditions were such that ants could forage well enough to pick up the bait.

Results indicate that only Amdro® resulted in significantly ($P < 0.05$) fewer active mounds versus the untreated control at two weeks and one month post-treatment. There was a substantial amount of natural mound abandonment and formation of “new” mounds in all treatments during this same period, probably the result of heavy rains.

This test was conducted with the assistance of Dr. Michael Merchant - Extension Specialist, District 4 (Dallas).

Table 4. Mean number of active mounds of 10 treated or as indicated, 4 replications; DFW International Airport, Texas

Treatment	day 3 treated	1 week treated	1 week new	1 week total	2 weeks treated	3 week treated	1 month treated	1 month new	1 month total
untreated	9.00 a	8.50 a	0.50 a	9.00 a	7.50 a	5.00 a	3.00 ab	5.25 a	8.25 a
TB131-1 ½ oz x 1	9.50 a	7.00 a	1.25 a	8.25 a	6.50 ab	5.25 a	4.25 a	4.00 a	8.25 a
TB131-1 ½ oz. x 2	9.25 a	8.50 a	1.00 a	9.50 a	7.75 a	5.75 a	4.25 a	4.00 a	8.25 a
Amdro®	9.75 a	7.25 a	2.50 a	9.75 a	3.50 b	3.50 a	1.00 b	3.75 a	4.75 a
F	0.77	0.75	0.78	0.27	5.11	0.68	9.83	0.23	1.40
P	0.5330	0.5406	0.5293	0.8476	0.0166	0.5828	0.0015	0.8767	0.2906
R ²	0.1613	0.1587	0.1628	0.627	0.5610	0.1447	0.7107	0.0534	0.2593
MSD*	1.5445	3.8685	4.0539	5.3692	3.6234	4.9418	2.0551	5.9838	6.2097

Means in the same column followed by different letters are significantly different ($P < 0.05$) using PC SAS analysis of variance procedure with means separated using Tukey’s studentized range test.

* Minimum significant difference

Test #4 - Coulter Field, Brazos County, Texas. This test site was chosen for its high mound density, approximately 600 mounds per acre, and sandy, claypan soil type. Weather during the test was moderate with several rains, so soil moisture was adequate throughout. Fire ant mounds were small to medium in size, but well-formed with freshly worked soil. Ants were very active with brood near the surface at the time of treatment.

Results show significantly ($P < 0.05$) fewer active treated mounds in Amdro®-treated

plots at each evaluation date and significantly ($P < 0.05$) fewer total mounds (treated + new) at four weeks. There were no significant difference between TB131-1-treated plots and untreated controls, though there was some numerical reduction in the number of treated mounds at two and four weeks. There was some natural abandonment of mounds and a fairly high number of “new” mounds formed within the plots. This is probably again due to several heavy rains. New mound formation was numerically less in Amdro-treated plots than in the others at one-month post-treatment. This phenomenon was observed in the abandoned test conducted in Robertson County, where there were no new mounds found in Amdro-treated plots and a substantial number found in others.

Table 5. Mean number of active mounds of 10 treated or as indicated, 4 replications; Coulter Field, Brazos County, Texas

Treatment	day 3 treated	1 week treated	1 week new	1 week total	2 weeks treated	1 month treated	1 month new	1 month total
untreated	8.75 ab	8.50 a	3.00 a	11.50 ab	8.75 a	8.25 a	3.00 a	11.25 a
TB131-1 ½ oz x 1	10.00 a	9.75 a	5.25 a	15.00 a	7.75 a	6.75 a	3.25 a	10.00 a
TB131-1 ½ oz. x 2	9.75 ab	7.50 a	4.75 a	12.25 ab	5.75 ab	4.75 ab	5.00 a	9.75 ab
Amdro®	7.50 b	6.00 b	2.00 a	8.00 b	3.00 b	2.00 b	1.75 a	3.75 b
F	4.28	2.38	1.32	4.76	5.49	7.93	1.31	5.50
P	0.0286	0.1208	0.3143	0.0207	0.0131	0.0035	0.3156	0.0130
R ²	0.5167	0.3730	0.2477	0.5434	0.5784	0.6646	0.2471	0.5791
MSD*	2.3076	4.3171	5.5376	5.5459	4.5450	4.0312	4.9045	6.0068

Means in the same column followed by different letters are significantly different ($P < 0.05$) using PC SAS analysis of variance procedure with means separated using Tukey’s studentized range test.

* Minimum significant difference

Test #5 - Burleson County, Texas. The pecan orchard land used for this final trial was chosen for its silty, bottomland soil type which represents some of the best cropland in the state. Weather during the test was hot and humid. Soil moisture was adequate with the site experiencing at least one moderate rain over the course of the test. Fire ant mounds were generally very small, but freshly built and with brood near the surface of most.

Results show that only Amdro® significantly ($P < 0.05$) reduced active mound numbers versus untreated controls at any point during the test. Because of the heat, even untreated mound numbers dropped almost 50 percent, but there were virtually no differences between TB131-1-treated and untreated mound numbers. “New” mound formation was very slight during the test

because of high temperatures.

This test was conducted with the assistance of Jennifer Begnaud, Student Worker, Fire Ant Project.

Table 6. Mean number of active mounds of 10 treated or as indicated, 4 replications; Burleson County, Texas

<u>Treatment</u>	<u>1 week treated</u>	<u>2 week treated</u>	<u>2 week new</u>	<u>2 week total</u>	<u>1 month treated</u>	<u>1 month new</u>	<u>1 month total</u>
untreated	8.50 a	7.00 a	0.75 a	7.75 a	5.50 a	1.25 a	6.75 a
TB131-1 ½ oz x 1	8.00 ab	6.75 ab	0.25 a	7.00 a	5.25 a	1.50 a	6.75 a
TB131-1 ½ oz. x 2	7.75 ab	7.25 a	1.00 a	8.25 ab	5.25 a	1.00 a	6.25 a
Amdro®	4.25 b	3.00 b	0.50 a	3.50 b	2.25 a	0.50 a	2.75 a
F	4.26	4.46	0.77	6.80	2.78	0.81	3.81
P	0.0289	0.0252	0.5330	0.0063	0.0866	0.5105	0.0396
R ²	0.5157	0.5272	0.1613	0.6296	0.4102	0.1691	0.4877
MSD*	3.9507	3.9969	1.5450	3.4548	3.8921	1.9869	4.1546

Means in the same column followed by different letters are significantly different ($P < 0.05$) using PC SAS analysis of variance procedure with means separated using Tukey's studentized range test.

* Minimum significant difference

Summary Discussion

These five trials represented a cross-section of weather and soil conditions across the state of Texas. One was performed in December, another during the hottest, driest time of year, two in nearly ideal "fire ant conditions" in the spring, and one in the late spring/early summer with adequate moisture. Soil types included heavy black calcareous clay, sand and loam over claypan and deep bottomland soil. Fire ant colonies all appeared to be of the small, but high-density polygyne type most common in the state.

The test design, informally known as the "railroad track" method after its linear string of plots, has been used for years by the Texas Agricultural Extension Service's Fire Ant Applied Research Laboratory to test a variety of individual mound treatment products. The method not only tracks the condition of treated mounds, but also helps detect colony movement, shattering and re-invasion from outside the plots in a manner that equalizes these parameters between treatments.

Results appeared to be similar among the tests, but a final statistical analysis was performed on the "bottom line" evaluation, that conducted at one month post-treatment. The first

test (Taylor) had two different rates of TB131-1 product and a unique standard product, so it could not reasonably be included. Standard products also varied between the remaining tests so those treatments were eliminated, as well. Therefore, the table below includes results from the last four tests, so that each treatment represents a total of 160 treated fire ant mounds.

Table 7. Summary of TB131-1 treatment results. Mean number of active mounds, 4 tests, 4 replications per test, 10 active mounds treated per replication.

<u>Treatment</u>	1 month, of 10 <u>treated</u>	1 month <u>new</u>	1 month <u>total</u>
untreated	6.250 a	2.625 a	8.875 a
TB131-1, ½ oz x 1	5.125 a	2.375 a	7.750 a
TB131-1, ½ oz. x 2	4.938 a	2.813 a	7.500 a
F	3.61	5.48	4.06
P	0.0083	0.0006	0.0043
R ²	0.30065	0.3950	0.3259
MSD*	1.6508	1.7173	2.0816

Means in the same column followed by different letters are significantly different ($P < 0.05$) using PC SAS analysis of variance procedure with means separated using Tukey's studentized range test.

* Minimum significant difference

As shown, neither TB131-1 treatment reduced active mound numbers significantly ($P < 0.05$) versus an untreated control one month after initial treatment. Numerical differences were not substantial, either. Overall, TB131-1 gave about 50 percent control of treated mounds, but there was substantial natural mound abandonment. Field observations suggest a decline in vigor of some treated colonies, though some brood was still observable in most active colonies.

The most successful test in terms of TB131-1s performance was the one conducted at the Navasota airport during August 1999. Despite high temperatures and very dry conditions, the product gave roughly 50 percent control of treated mounds with the untreated plots maintaining over 80 percent activity.

Control by standard products ranged from a high of 100 percent by Organic Solutions in the Navasota test to a low of about 75 percent by Amdro® (hydramethylnon) in the Burleson County test. Standard treatments covered the range of product activity with Organic Solutions (pyrethrins, piperonyl butoxide and diatomaceous earth) being one of the fastest-acting and Orthene® (acephate) being one of the relatively slower of the available contact insecticide-type products. Both typically give better than 90% control when properly applied. Amdro, applied as an individual mound treatment, is the fastest acting of the bait products and usually takes a week or so to work.

In summary, we feel that this series of five trials gave TB131-1 a very thorough testing across a wide array of weather, seasonal and edaphic conditions. Despite the product's ease of use and "organic" nature that would appeal to many environmentally-conscious consumers, a control rate of only 50 percent over a month is somewhat disappointing.