

Laboratory evaluations of Esteem® (pyriproxyfen) fire ant bait blended with dry fertilizer for attractiveness to red imported fire ant foragers

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The concept of applying dry fertilizer with a broadcast-applied bait product for the suppression of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) promises to reduce application cost by eliminating a trip across the field. Earlier work (Barr 1991) indicated that blending a bait-formulated fire ant insecticide with dry fertilizer quickly reduces the acceptability of the bait product compared to not blending the bait with fertilizer. However, ants were not repelled by the fertilizer and retrieved bait when applied simultaneously but without prior blending. This concept was demonstrated using a “tandem” treatment in a field trial by mounting the Herd GT-77 model seeder on top of a dry fertilizer spreader (Drees et al. 1994). Assessment of an ant bait product formulation blended with encapsulated dry fertilizer in a field trial resulted in spotty performance of the bait, in part, because the bait separated from the fertilizer in the hopper due to the different weights of the particles, and because the broadcast of heavier fertilizer particles with the light weight bait likely resulted in differential swath widths between the two components. These laboratory trials were conducted using Esteem® (pyriproxyfen) fire ant bait blended with commercially provided dry fertilizer formulations to better document the effect of time and humidity on bait acceptance to foraging fire ants.

Materials and Methods

Eight laboratory colonies were used for the first three trials. Colonies had been field collected, March 10, 2007, from the USDA Pecan Genetics Laboratory Grounds in Brazos Co, TX. Ants were separated from soil by drip-flotation on March 10 and 11, 2007. Ants were removed and placed in plastic trays measuring 27 by 37 cm and 9 cm tall. One Petri dish (14 cm diameter and 2.5 cm tall) containing set Castone® moistened with water and with lids in which holes were melted to allow ants to enter and exit was placed in each colony tray to house the queen, brood and worker ants. Each colony was provided with distilled water, and maintained under laboratory conditions. Colonies used in these trials were assessed for queen(s), winged sexual male and female reproductive ants (alates), estimated numbers of worker ants, worker brood (larvae and pupae) and sexual brood.

Trial 1, March 21-31, 2007. Esteem (1/4 cup) plus 1 cup fertilizer (24-6-16) was aged in lab (starting March 20, 2007 at 12:06 p.m. at 78 degrees F and 73% relative humidity) in a Ziploc® container (2.5 cup, 20 oz, or 591 ml size) and mixed thoroughly. Fresh bait was placed into a freezer. After 5, 24, 48 and 96 hrs of aging at room temperature under laboratory conditions, four replicate sets of 12 particles of bait were removed from the bait-fertilizer blend into small plastic trays, weighed, and placed in a freezer. On March 26, one set of each exposed bait series was placed in each of four laboratory *S. invicta* colonies. Periodically, removal of bait particles was observed and documented by

recording the number of bait particles remaining in the VWR International weighing dish after 10 days of exposure. Data were analyzed using Analysis of Variance (ANOVA) with means separated using the Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$

Trials 2a & b, April 6, 2007: Comparison of fertilizers. On April 2, 2007 (4:30 p.m.), 0.25 cup Esteem® Fire Ant Bait (pyriproxyfen) was mixed with 1 cup each of two dry fertilizer formulations (#1 = 24-6-16; #2 = 24-6-18) provided by Valent U.S.A. for evaluation. They were placed outdoors and temperature and humidity conditions were documented using a data logger (Hobo® Pro Series, HOB-032-02 #543716 RH, Temp.). Periodically (16, 24, 48, 72 hrs) thereafter, four groups of 12 randomly selected bait particles were removed from the bait-fertilizer blend and placed into small plastic trays (VWR International hexagonal 4.5 cm polystyrene weighing dishes). After bait particles were removed from the bait-fertilizer blend, they were placed in trays and were immediately placed in a freezer (22 degrees F). Extinguish® Plus (hydramethylnon plus methoprene) was used as a standard treatment for comparison to fresh Esteem. On April 6, 2007, at 9:30 a.m. (76 degrees F and 63% relative humidity), one set of each of six treatment trays was placed in each of four laboratory *S. invicta* colonies. Number of bait particles remaining in weigh dishes were assessed 0, 2, 6, 24, 48 and 72 hrs following initial exposure. Results were analyzed using Analysis of Variance (ANOVA) with means separated using the Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ ($F = 3.215$, $P = 0.021$, Mean Square = 31.155, d.f. = 6) (SPSS 14.0).

Trials 3a & b, April 12, 2007: Outdoor versus indoor aging. Eight new imported fire ant colonies were collected from the Pecan Genetics Laboratory grounds, March 10, 2007 and assessed as described above. Esteem Fire Ant Bait blended with 24-6-16 fertilizer was aged outside under ambient conditions and bait particles collected 0, 8, 24, 48, 72 hours after exposure. Bait collected was placed in a freezer prior to the trial initiated 9:40 am, April 12, 2007. Extinguish® (methoprene) was used as a standard treatment. Numbers of bait particles remaining of 12 following 8, 24, 48 and 72 hrs of exposure to laboratory colonies were recorded . A separate trial using fertilizer and bait blend aged indoors was also conducted, with both trials replicated using four laboratory colonies. Results were analyzed as described above.

Trial 4. Dry 24-6-18 fertilizer was finely ground in a coffee grinder. Esteem® Fire Ant bait was blended with either finely ground or non-ground, course fertilizer particles and aged as described above. Bait particles were removed after 0, 1, 18 and 26 hours. April 20, 2007, ant colonies were exposed to the bait in weigh dishes. Bait particles remaining after 0, 1, 18,26 and 72 hours were recorded and data were analyzed as described above.

Trial 5. Colony decline, April 12 - July 10

Results

Trial 1. The 12 bait particles weighed $0.0307 \text{ g} \pm 0.00454 \text{ g}$ ($n = 20$). Thus, average individual bait particles used weighed 0.003 grams. The trial was conducted from March 21 through 31, 2007. Ants were not observed picking many Esteem® (pyriproxyfen) fire

ant bait part within the first 24 hrs. of exposure to red imported fire ant colonies. Even after 10 days, bait particles remained in trays (**Table 1**). This trial was considered preliminary. Significantly more bait particles had been removed from the fresh bait compared to 5 and 96 hr fertilizer blended and aged bait. Although raw data show a trend towards aged fertilizer-bait being less attractive to foraging worker red imported fire ants, there was no definitive trend possibly due to the conditions under which bait was aged, or because colonies used in this trial did not appear to be “hungry” for the bait.

Trials 2a & b. Colonies used in these trials were assumed to be polygyne as half contained more than one queen ant. Absence of observed queens in three colonies did not necessarily indicate that these colonies had no queens as they are difficult to find in ant masses (**Table 2**). Colonies selected for the two trials were “matched” at least for worker numbers. Outdoor “aging” of the fertilizer-bait blend under more extreme (warmer and cooler), more humid conditions (**Appendix 1**), quickly resulted in bait adhering to fertilizer particles causing clumping within 24 hours. In the trial using bait aged in 24-6-16 #1 fertilizer (Trial 2a), no significant differences were detected between treatments through the duration of the trial, although colonies tended to continue to remove bait particles from weighing dishes over time (**Table 3**). However, colonies exposed to blends of bait aged in the 24-6-16 #2 (Trial 2b) fertilizer formulation removed significantly more bait particles from “fresh” bait not previously blended with fertilizer (Treatment 1 Esteem 0 hrs) than fertilizer blended with bait for 24 hrs (Treatment 3) 6 through 48 hrs following initial exposure, and bait aged in fertilized for 72 hrs (Treatment 5) 2 through 6 hrs after exposure (**Table 4**). Thus, the concept of developing directions for mixing and using the bait fertilizer blend “within a day” would need to be within less than 24 hours. Worker ants showed a trend in both trials to remove fresh bait, but removed even fertilizer blended and aged bait over exposure time. This trial documented possible differences in ant attractiveness to different types of fertilizer. However, these two trials were conducted with two “matched” sets of four replicated laboratory red imported fire ant colonies. Thus, statistical analysis between the two trials (2b and 2B) was not conducted.

Trials 3a & b. Colonies used in these trials were larger than those used in Trials 2a & b and were also assumed to be polygyne even though few queen ants were observed (**Table 5**). However, the two sets of four colonies were again “matched” for size. In Trial 3a, Esteem® Fire Ant Bait (pyriproxyfen) aged outdoors (**Appendix 1**) in 24-6-16 fertilizer for 72 or more hours were significantly less attractive to foraging fire ants removing bait particles from weigh dishes after 24 hours of exposure or longer (**Table 6**). Bait aged indoors appeared to be more attractive and only that aged for 72 hours was significantly less attractive to ants after 24 hrs of exposure. Beyond that time, no significant differences were observed. These trials document possible differences in attractiveness of bait aged in fertilizer under different environmental conditions, with outdoor conditions being more hot and humid than indoor conditions. However, bait aged in fertilizer either indoors or outdoors for 24 hours or less was equally attractive to ant foragers.

Trial 4. This trial evaluated the effect of “dusty” formulation of fertilizers. Dry fertilizers contain particles of various sizes and the smallest particles sift to the bottom of

containers. Such “fine” particles were simulated by grinding fertilizer in a coffee grinder to create a powder. Bait particles blended with the powder immediately became covered and less recognizable as a bait particle. Esteem® Fire Ant Bait (pyriproxyfen) aged in the fine powder for 18 and 26 hours was significantly less attractive to ants removing particles from the weighing dishes 1 hour or more (until 72 hrs after exposure when there remained no treatment differences) after exposure. Bait aged in powdered fertilizer for 1 hr was significantly less attractive 18 hrs or more after exposure to ant foraging. Only bait blended with fertilizer powder for 1 hr was not significantly less attractive than fresh bait particles. This trial documented reduced attractiveness due to fertilizer particle size. No significant differences in attractiveness of non-powdered or coarse fertilizer aged bait was documented compared to attractiveness or removal of fresh (0 hr aged) bait in this trial although numerically fewer fertilizer blended and aged bait particles were removed over time.

Discussion

One observation made during the process of aging bait outdoors for this trial was that the fertilizer particles absorbed moisture from the highly humid conditions prevalent at that time. The fertilizer particles appeared to “sweat” and formed clusters of bait particles stuck to fertilized granules. In some cases, bait needed to be pried off to collect them for these laboratory assays. If these conditions existed during actual application of the fertilizer plus bait blend, treatment could be adversely affected.

These trials showed that blending a fire ant bait product such as Esteem® with dry fertilizer reduces attractiveness of bait particles for foraging red imported fire ants. However, no significant differences in bait acceptability were documented in bait blended with fertilizers tested and aged for less than 24 hrs, although there was a numerical trend toward reduced attractiveness. Factors affecting reduced attractiveness of bait particles blended with fertilizer include: 1) type of fertilizer (Trials 2a & b); 2) aging conditions including heat and humidity (Trials 3 a & b); and 3) fertilizer particle size with dusty or powdered fertilizer reducing attractiveness more quickly (Trial 4).

These trials did not assess effectiveness of bait removed by the ant on the colonies. Conceivably, lower amounts of the dose provided by a bait aged in fertilizer may still provide sufficient active ingredient to affect the colony.

Literature Cited

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Table 1. Number of Esteem® Fire Ant Bait (pyriproxyfen) particles of 12 (fresh, unexposed or aged in a blend of 24-6-16-25 fertilizer) remaining in VWR International weighing dishes after 10 days of exposure to laboratory red imported fire ant colonies (March 21 - 31, 2007).

Replicate	Fresh	0 hrs	5 hrs	24 hrs	48 hrs	72 hrs	96 hrs
1	0	0	5	3	2	7	8
2	3	1	2	10	0	1	9
3	0	4	12	0	0	3	11
4	0	6	8	4	0	0	3
Mean*	0.75a	2.75ab	6.75b	4.25ab	0.5a	2.75ab	7.75b

* Means in row followed by the same letter(s) are not significantly different using Analysis of Variance (ANOVA) with means separated using the Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ ($F = 3.215$, $P = 0.021$, Mean Square = 31.155, d.f. = 6).

Table 2. Assessment of laboratory red imported fire ant colonies, collected March 21, 2007, used in Trial 2 evaluating attractiveness of fertilizer and ant bait blends, Brazos Co., TX, 2007.

Trial 2a: 24-6-16 #1	Queens	Alates	Workers	Worker Brood	Sexual Brood
Red 1	0	0	12,000	200 pupae	0
Green 2	0	0	10,000	25 pupae	72 larvae
Blue 3	4	100 female	8,000	20 pupae	0
Orange 4	2	0	15,000	50 pupae	0
Trial 2b: 24-6-18 #2					
Purple 1	0	0	15,000	20 pupae	30 larvae
Yellow 2	3	21 female	15,000	0	45 larvae
Gray 3	1	0	10,000	6 pupae	8 larvae
Pink 4	4	27 female	5,000	20 pupae	0

Table 3. Number of Esteem® Fire Ant Bait (pyriproxyfen) particles of 12 (fresh, unexposed or aged in a blend of 24-6-16 #1 fertilizer aged outdoors) remaining in VWR International weighing dishes after 0, 2, 6, 24 48 and 72 hrs. exposure to laboratory red imported fire ant colonies; Trial 2a initiated April 6 - 9, 2007 beginning at 76 degrees F and 63% relative humidity and ending at 78 degrees F and 59% relative humidity in the laboratory, Brazos Co., TX.

			No. bait particles remaining of 12 particles*					
			<u>0 hr</u>	<u>2 hrs</u>	<u>6 hrs</u>	<u>24 hrs</u>	<u>48 hrs</u>	<u>72 hrs</u>
<u>Treatment/Aging Time</u>	<u>Rep</u>	<u>Rep</u>						
1	Esteem® 0 hrs	1	12	8	7	6	0	0
		2	12	9	9	5	5	5
		3	12	0	0	0	0	0
		4	12	12	11	11	8	2
		Mean	12	7.3	6.8	5.5	3.3	1.8
2	Esteem 16 hrs	1	12	7	4	0	0	0
		2	12	9	7	4	4	4
		3	12	0	0	0	0	0
		4	12	10	9	5	5	5
		Mean	12	6.5	5.0	2.3	2.3	2.3
3	Esteem 24 hrs	1	12	12	12	8	0	0
		2	12	7	7	5	5	5
		3	12	0	0	0	0	0
		4	12	12	12	3	0	0
		Mean	12	7.8	7.8	4.0	1.3	1.3
4	Esteem 48 hrs	1	12	10	10	10	8	4
		2	12	11	11	9	8	7
		3	12	0	0	0	0	0
		4	12	12	12	11	10	9
		Mean	12	8.3	8.3	7.5	6.5	5.0
5	Esteem 72 hrs	1	12	10	10	10	7	0
		2	12	12	12	11	10	7
		3	12	12	7	6	4	4
		4	12	12	11	1	1	1
		Mean	12	11.5	10.0	7.0	5.5	3.0
6	Extinguish Plus 0 hrs	1	12	12	12	12	11	10
		2	12	11	3	3	2	2
		3	12	7	6	4	4	4
		4	12	11	10	5	5	5
		Mean	12	10.3	7.8	6.0	5.5	5.3
d.f. = 5								
F				0.756	0.538	0.908	1.342	1.225
P				0.593	0.745	0.497	0.292	0.338
Mean Square				19.278	10.967	15.375	13.069	11.367

* Means in columns followed by the same letter(s) are not significantly different using Analysis of Variance (ANOVA) with means separated using the Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ ($F = 3.215$, $P = 0.021$, Mean Square = 31.155, d.f. = 6).

Table 4. Number of Esteem® Fire Ant Bait (pyriproxyfen) particles of 12 (fresh, unexposed or aged in a blend of 24-6-18 #2 fertilizer aged outdoors) remaining in VWR International weighing dishes after 0, 2, 6, 24 48 and 72 hrs. of exposure to laboratory red imported fire ant colonies; Trial 2b initiated April 6 - 9, 2007 beginning at 76 degrees F and 63% relative humidity and ending at 78 degrees F and 59% relative humidity in the laboratory, Brazos Co., TX.

			No. bait particles remaining of 12 particles*					
			0 hr	2 hrs	6 hrs	24 hrs	48 hrs	72 hrs
Treatment/Aging time		Rep						
1	Esteem® 0 hrs	1	12	0	0	0	0	0
		2	12	1	0	0	0	0
		3	12	6	1	0	0	0
		4	12	5	0	0	0	0
		Mean	12	3a	0.3a	0a	0a	0
2	Esteem 16 hrs	1	12	9	5	4	4	4
		2	12	0	0	0	0	0
		3	12	7	8	0	0	0
		4	12	6	5	5	5	5
		Mean	12	5.5ab	4.5ab	2.3ab	2.3ab	2.3
3	Esteem 24 hrs	1	12	7	6	6	6	4
		2	12	0	0	0	0	0
		3	12	12	12	12	12	11
		4	12	12	12	12	12	11
		Mean	12	7.8ab	7.5b	7.5b	7.5b	6.5
4	Esteem 48 hrs	1	12	11	2	2	2	2
		2	12	1	0	0	0	0
		3	12	10	9	9	9	0
		4	12	9	9	8	8	8
		Mean	12	7.8ab	5ab	4.8ab	4.8ab	2.5
5	Esteem 72 hrs	1	12	11	5	5	5	3
		2	12	11	11	7	7	6
		3	12	12	12	8	8	4
		4	12	9	7	0	0	0
		Mean	12	10.8b	8.8b	5.0ab	5.0ab	3.25
6	Extinguish Plus 0 hrs	1	12	9	8	2	0	0
		2	12	11	10	10	3	0
		3	12	7	4	0	0	0
		4	12	12	12	12	12	12
		Mean	12	9.8b	8.5b	6ab	3.8ab	3.0
d.f. = 5								
F				2.302	2.810	1.640	1.510	1.138
P				0.088	0.048	0.200	0.236	0.377
Mean Square				31.889	41.600	29.200	26.275	17.667

* Means in columns followed by the same letter(s) are not significantly different using Analysis of Variance (ANOVA) with means separated using the Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ ($F = 3.215$, $P = 0.021$, Mean Square = 31.155, d.f. = 6).

Table 5. Assessment of laboratory red imported fire ant colonies, collected March 10, 2007, used in Trial 3 evaluating attractiveness of fertilizer and ant bait blends starting April 12, 2007, Brazos Co., TX, 2007.

Trial 3a: Aged outdoors	Queens	Alates	Workers	Worker Brood	Sexual Brood
Red 1	1	0	35,000	larvae, pupae ½ tsp (2.46 ml)	0
Green 2	1	0	20,000	larvae, pupae ½ tsp (2.46 ml)	0
Blue 3	0	1 female	35,000	larvae, pupae 1/8 tsp (0.61 ml)	0
Orange 4	1	1 female	35,000	larvae, pupae 1/8 tsp (0.61 ml)	0
Trial 3b: Aged indoors					
Purple 1	0	0	35,000	larvae, pupae ½ tsp (2.46 ml)	pupae 1/8 tsp (0.61 ml)
Yellow 2	0	1 female	35,000	larvae, pupae ½ tsp (2.46 ml)	0
Gray 3	0	5 females, 4 males	20,000	larvae, pupae ½ tsp (2.46 ml)	larvae 1/8 tsp
Pink 4	1 physogastric	3 females, 16 males	35,000	larvae, pupae ½ tsp (2.46 ml)	0

Table 6. Number of Esteem® Fire Ant Bait (pyriproxyfen) particles aged outdoors in 24-6-16 fertilizer of 12 remaining in VWR International weighing dishes after 0, 2, 6, 24 48 and 72 hrs. of exposure to laboratory red imported fire ant colonies; Trial 3a initiated, April 12, 2007 beginning at 76 degrees F and 61% relative humidity and ending at 78 degrees F and 59% relative humidity in the laboratory, Brazos Co., TX.

			No. bait particles remaining of 12 particles*				
			0 hr	8 hrs	24 hrs	48 hrs	72 hrs
			9:40am	5:40pm	9:40am	9:40am	11:37am
<u>Treatment</u>	<u>Actual time:</u>	<u>Rep</u>					
1	Esteem® 0 hrs	1	12	8	8	2	2
		2	12	9	6	6	6
		3	12	12	0	0	0
		4	12	12	2	0	0
		Mean	12.0	10.3	4.0a	2.0a	2.0a
2	Esteem 8 hours	1	12	11	11	11	10
		2	12	12	11	11	11
		3	12	12	10	0	0
		4	12	12	7	0	0
		Mean	12.0	11.8	9.8ab	5.5ab	5.3ab
3	Esteem 24 hrs	1	12	12	12	12	12
		2	12	12	5	5	5
		3	12	11	10	7	0
		4	12	10	0	0	0
		Mean	12.0	11.3	6.8ab	6.0ab	4.3ab
4	Esteem 48 hrs	1	12	9	9	9	9
		2	12	11	10	10	10
		3	12	12	12	11	11
		4	12	12	12	12	12
		Mean	12.0	11.0	10.8b	10.5b	10.5b
5	Esteem 72 hrs	1	12	12	12	12	12
		2	12	12	12	12	7
		3	12	12	11	8	7
		4	12	11	7	7	7
		Mean	12.0	11.8	10.5b	9.8b	8.3ab
6	Extinguish Plus 0 hrs	1	12	12	12	12	12
		2	12	11	11	10	10
		3	12	12	0	0	0
		4	12	12	1	0	0
		Mean	12.0	11.8	6.0ab	5.5ab	5.5ab
df = 5							
F			--	1.093	1.954	1.921	1.720
P			--	0.398	0.135	0.141	0.181
Mean Sq			--	1.442	30.742	39.242	36.142

* Means in columns followed by the same letter(s) are not significantly different using Analysis of Variance (ANOVA) with means separated using the Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ ($F = 3.215$, $P = 0.021$, Mean Square = 31.155, d.f. = 6).

Table 7. Number of Esteem® Fire Ant Bait (pyriproxyfen) particles aged indoors in 24-6-16 fertilizer of 12 remaining in VWR International weighing dishes after 0, 2, 6, 24 48 and 72 hrs. of exposure to laboratory red imported fire ant colonies; Trial 3b initiated, April 12, 2007 beginning at 76 degrees F and 61% relative humidity and ending at 78 degrees F and 59% relative humidity in the laboratory, Brazos Co., TX.

			No. bait particles remaining of 12 particles*				
			0 hr	8 hrs	24 hrs	48 hrs	72 hrs
			9:40am	5:40pm	9:40am	9:40am	11:37am
Treatment	Actual time:	Rep					
1	Esteem® 0 hrs	1	12	12	11	10	9
		2	12	10	6	6	6
		3	12	12	12	4	4
		4	12	6	5	0	0
		Mean	12.0	10.0	8.5ab	5.0	4.8
2	Esteem 8 hours	1	12	12	7	5	5
		2	12	12	8	7	7
		3	12	12	10	8	8
		4	12	12	8	3	3
		Mean	12.0	12.0	8.3ab	5.8	5.8
3	Esteem 24 hrs	1	12	11	10	7	6
		2	12	7	0	0	0
		3	12	11	9	0	0
		4	12	12	11	9	6
		Mean	12.0	10.3	7.5ab	4.0	3.0
4	Esteem 48 hrs	1	12	12	11	11	0
		2	12	7	7	7	7
		3	12	12	9	5	0
		4	12	11	9	3	3
		Mean	12.0	10.5	9.0ab	6.5	2.5
5	Esteem 72 hrs	1	12	12	10	0	0
		2	12	12	11	11	11
		3	12	12	12	10	7
		4	12	12	10	7	7
		Mean	12.0	12.0	10.8b	7.0	6.3
6	Extinguish Plus 0 hrs	1	12	12	5	5	5
		2	12	11	6	6	6
		3	12	11	2	0	0
		4	12	9	5	5	4
		Mean	12.0	10.8	4.5a	4.0	3.8
d.f. = 5							
F			--	0.912	2.218	0.435	0.777
P			--	0.495	0.097	0.818	0.579
Mean Sq			--	3.067	17.067	6.375	9.067

* Means in columns followed by the same letter(s) are not significantly different using Analysis of Variance (ANOVA) with means separated using the Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ ($F = 3.215$, $P = 0.021$, Mean Square = 31.155, d.f. = 6).

Table 8. Number of Esteem® Fire Ant Bait (pyriproxyfen) particles aged in finely ground or non-ground (course) 24-6-16 fertilizer of 12 remaining in weighing dishes after 0, 1, 18, 26 and 72 hrs. of exposure to laboratory red imported fire ant colonies; Trial 4 initiated, April 20, 2007 beginning at 78 degrees F and 68% relative humidity and ending at 78 degrees F and 68% relative humidity in the laboratory, Brazos Co., TX.

		No. bait particles remaining of 12 particles*					
<u>Treatme</u>		<u>Rep</u>	0 hr	1 hr	18 hrs	26 hrs	72 hrs
1	Esteem 0 hrs	1	12	0	0	0	0
		2	12	5	4	4	0
		3	12	10	7	0	0
		4	12	1	0	0	0
		Mean	12.0	4.0a	2.8a	1.0a	0.0
2	Esteem Course 1 hour	1	12	0	0	0	0
		2	12	12	12	12	5
		3	12	12	12	12	0
		4	12	4	3	3	0
		Mean	12.0	7.0ab	6.8ab	6.8abc	1.3
3	Esteem Fine 1 hour	1	12	9	9	9	0
		2	12	11	11	10	0
		3	12	9	9	9	0
		4	12	9	9	7	2
		Mean	12.0	9.5ab	9.5b	8.8bc	0.5
4	Esteem Course 18 hours	1	12	5	0	0	0
		2	12	12	12	12	6
		3	12	11	7	3	0
		4	12	2	1	0	0
		Mean	12.0	7.5ab	5.0ab	3.8ab	1.5
5	Esteem Fine 18 hours	1	12	12	12	12	2
		2	12	12	12	12	0
		3	12	12	12	12	0
		4	12	9	9	9	0
		Mean	12.0	11.3b	11.3b	11.3c	0.5
6	Esteem Course 26 hours	1	12	12	6	6	0
		2	12	12	12	12	9
		3	12	10	7	7	2
		4	12	3	0	0	0
		Mean	12.0	9.3ab	6.3ab	6.3abc	2.8
7	Esteem Fine 26 hours	1	12	12	12	12	0
		2	12	12	12	12	0
		3	12	12	12	12	0
		4	12	9	9	9	0
		Mean	12.0	11.3b	11.3b	11.3c	0.0
	d.f. = 5		--				
	F		--	1.813	2.653	3.845	0.768
	P		--	0.145	0.045	0.010	0.604
	Mean		--	26.786	42.036	57.583	3.893

Appendix 1. Data logger (Hobo® Pro Series, HOB-032-02 #543716 RH, Temp.) temperature and relative humidity conditions for Trials 2a&b and 3a&b from April 2 through 13.

Date/Time	Logger 1		Logger 2	
	Temperature (*F)	RH (%)	Temperature (*F)	RH (%)
Laboratory Conditions				
04/02/07 04:00:00.0	78.01	49	78.71	50
04/02/07 08:00:00.0	77.31	53.1	77.31	54.7
04/02/07 12:00:00.0	77.31	54.2	78.01	53.6
04/02/07 16:00:00.0	74.53	43.8	74.53	45.4
Aging Trial 2a&b				
	Outdoors		Indoors	
04/02/07 20:00:00.0	75.92	75.5	70.39	46.4
04/03/07 00:00:00.0	71.77	88	71.08	53.6
04/03/07 04:00:00.0	69.71	90.1	71.77	57.8
04/03/07 08:00:00.0	70.39	88.4	71.77	56.2
04/03/07 12:00:00.0	81.53	57.8	74.53	47.4
04/03/07 16:00:00.0	90.22	44.8	71.77	47.4
04/03/07 20:00:00.0	79.41	69.1	71.77	50.5
04/04/07 00:00:00.0	73.84	87.5	71.08	51.6
04/04/07 04:00:00.0	69.71	68.1	71.77	56.2
04/04/07 08:00:00.0	66.28	79.3	72.46	58.8
04/04/07 12:00:00.0	73.15	54.2	75.22	46.9
04/04/07 16:00:00.0	76.62	34.3	73.84	40.8
04/04/07 20:00:00.0	64.91	44.3	73.15	40.8
04/05/07 00:00:00.0	56.66	62.4	71.08	43.8
04/05/07 04:00:00.0	52.49	73.5	69.02	44.3
04/05/07 08:00:00.0	55.28	50.5	67.65	43.3
04/05/07 12:00:00.0	70.39	27.6	75.92	36.8
04/05/07 16:00:00.0	85.1	19.1	71.77	35.3
04/05/07 20:00:00.0	66.28	38.7	71.08	36.8
Mean	71.03	60.69	71.95	47.09
Standard Deviation	9.92	21.10	1.99	7.32
Laboratory Conditions				
04/06/07 00:00:00.0	59.42	58.3	71.08	39.8
04/06/07 04:00:00.0	56.66	61.4	69.71	41.8
04/06/07 08:00:00.0	55.28	59.9	68.33	43.3
04/06/07 12:00:00.0	77.31	37.2	78.71	37.8
04/06/07 16:00:00.0	74.53	34.3	73.84	35.8
04/06/07 20:00:00.0	72.46	35.3	72.46	36.3
04/07/07 00:00:00.0	69.71	35.7	69.71	36.8
04/07/07 04:00:00.0	66.96	34.3	66.96	34.8
04/07/07 08:00:00.0	64.22	32.8	64.91	33.3
04/07/07 12:00:00.0	71.08	29	71.08	29.5
04/07/07 16:00:00.0	72.46	26.6	72.46	27.6
04/07/07 20:00:00.0	71.77	26.6	71.77	27.6
04/08/07 00:00:00.0	71.08	27.1	71.08	27.6
04/08/07 04:00:00.0	71.08	27.1	71.08	28.5
04/08/07 08:00:00.0	71.77	27.1	71.08	28.5

04/08/07 12:00:00.0	77.31	26.2	76.62	27.6
04/08/07 16:00:00.0	75.92	27.1	75.92	28
04/08/07 20:00:00.0	76.62	25.7	76.62	26.6
04/09/07 00:00:00.0	77.31	25.3	77.31	26.2
04/09/07 04:00:00.0	77.31	25.7	77.31	26.6
04/09/07 08:00:00.0	76.62	27.6	76.62	28.5

Trials 3a & b Aging

Indoors

Outdoors

04/09/07 12:00:00.0	73.84	28	65.59	39.3
04/09/07 16:00:00.0	73.15	31.8	66.28	46.9
04/09/07 20:00:00.0	75.92	34.8	61.48	60.9
04/10/07 00:00:00.0	75.92	34.8	60.11	68.6
04/10/07 04:00:00.0	75.92	35.7	59.42	76
04/10/07 08:00:00.0	75.92	37.7	58.04	90.9
04/10/07 12:00:00.0	80.12	40.3	72.46	65
04/10/07 16:00:00.0	75.92	37.2	82.24	46.4
04/10/07 20:00:00.0	69.71	40.3	72.46	67.6
04/11/07 00:00:00.0	71.77	44.8	65.59	85.8
04/11/07 04:00:00.0	71.77	49	62.85	91.3
04/11/07 08:00:00.0	71.08	47.9	59.42	82.2
04/11/07 12:00:00.0	73.15	36.2	78.71	29.9
04/11/07 16:00:00.0	71.77	34.8	88.74	19.1
04/11/07 20:00:00.0	75.92	38.7	71.08	38.3
04/12/07 00:00:00.0	76.62	33.3	61.48	51.1
Mean	74.28	37.83	67.87	59.96
Standard Deviation	2.69	5.64	9.06	22.24

04/12/07 04:00:00.0	75.92	32.3	56.66	63
04/12/07 08:00:00.0	76.62	31.8	55.97	61.9
04/12/07 12:00:00.0	76.62	31.4	78.71	24.4
04/12/07 16:00:00.0	72.46	34.3	85.83	20.8
04/12/07 20:00:00.0	71.08	33.8	71.77	33.8
04/13/07 00:00:00.0	72.46	36.2	66.28	54.2
04/13/07 04:00:00.0	71.77	39.2	66.96	68.6
04/13/07 08:00:00.0	70.39	42.8	69.02	85.3
04/13/07 12:00:00.0	73.84	42.8	73.84	45.4
04/13/07 16:00:00.0	73.84	43.8	73.84	45.4