

Evaluation of Long-Term Residual Insecticidal Property of ARINIX™ Permethrin-Impregnated Nylon Cable Wrap as a Barrier Application for Protection of a Food Lure Target from Red Imported Fire Ant Foraging

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In 2005, Nix, Inc. (2-3-3, Minatomirai, Nishi-ku, Yokohama 220-6108, Japan) obtained registration of ARINIX™ by the U. S. Environmental Protection Agency (EPA): EPA Reg. No. 73745-2; EPA Est. No. 73745. This product consists of 8.56% permethrin-impregnated nylon plastic which has been manufactured in a wide variety of shapes for different uses (grommet, spiral wrap, flat strip, L-shaped strip, tackroll). Data submitted for supporting product claims associated with product efficacy as a barrier treatment for elimination of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) was developed by Drees and Summerlin (2005, posted on <http://fireant.tamu.edu>).

The approved label directions state: “Apply enough product to provide a continuous barrier around the perimeter or circumference of the application surface and in such a manner that no space exists between the product and the surface to which it is being applied.” In addition, the label states: “Laboratory tests conducted with ARINIX™, under accelerated aging conditions, showed that ARINIX™ is effective for five years. Sunlight, dirt and grime may reduce residual efficacy during use.”

As a condition for registration, Nix of America (Metro Plaza, 181 Metro Dr., Suite 590, San Jose, CA 95110; 408/971-3115; Fax: 408/971-3305; nikkonix@ix.netcom.com), has agreed to conduct further trials to demonstrate long-term residual effectiveness of ARINIX™ parts. Earlier studies were conducted with cylindrical parts (measuring 22 mm in diameter and 15 mm long) aged by Nix of America using a heating process. The higher the temperature, the faster a given chemical reaction will proceed. By using the Arrhenius Equation, $k=A*\exp(-E_a/R*T)$, the following aging procedure was calculated: 5 years at 30°C corresponds to 57.03 days at 80°C. The oldest existing prototype part evaluated was produced in 1999 (3 cm long, 25 mm outside diam, 2 mm thick) (Drees and Summerlin 2005b).

Trials reported here describe first year efforts to assess field and laboratory aging of the Spiral Wrap 20 (22.5 mm wide, 20 mm diameter and 1.25 mm thick produced in 2 inch lengths). Bioassay methods were planned to assess efficacy against *S. invicta* as a barrier periodically for 5 years, and assess the potential of using ARINIX™ parts to protect a food lure “target” as a barrier treatment. Use of this product was intended to include sensitive electronic components such as switching mechanisms, but a bioassay “target” using peanut butter as a food lure was employed in these trials.

Materials and Methods

ARINIX™ (8.56% permethrin-impregnated nylon) Spiral Wrap 20 (NSSG-20) parts, including ARINIX I (P’06.10.30, produced Oct. 30, 2006) and ARINIX II containing etofenprox (Agrochemicals Div., Mitsui Chemicals, Inc., Shiodome City Center, 1-5-2, Higashi-Shimbashi, Minato-ku, Tokyo 105-7117, Japan, +81-3-6253-3780, fax: +81-3-6253- 4233) parts labeled E’07.06.29, produced June 29, 2007 and an

untreated (non-pesticide containing) part (N'07.05.30, produced May 30, 2007), were provided for evaluation by Nix of America. ARINIX II is a second generation part of ARINIX that uses a safer insecticide that is registered with EPA that is also UV protected. Over 100 parts of each treatment were provided for each treatment and aging regime. Aging treatments will include:

- 1) Untreated control parts kept in the laboratory for the 5 year duration of the study.
- 2) Laboratory aged parts kept in a storage container at room temperature (75-78 degrees F).
- 3) Field/outdoor aged parts kept in an opened storage container in a sun-exposed location on the grounds of the Center for Urban & Structural Entomology on the Texas A&M University campus grounds and aged under ambient conditions for College Station (Drees and Best 2000).
- 4) Field aged parts were to be kept in a storage container such as a utility housing which shields parts from sunlight and dirt but provides higher heat.

In future years, Nix of America will supply: 1) recently manufactured spiral wraps annually as “factory fresh” Spiral Wraps, and provide manufacturing dates for all parts submitted for evaluation throughout this trial, and 2) artificially aged (via Arrhenius method) parts provided by Nix of America.

At the end of each years' bioassay, Spiral Wraps used in trials will be sent to Nix of America for chemical analysis to document decay of insecticide (permethrin and etofenprox) concentration in the nylon matrix.

Bioassay for Aged Treatments

Laboratory evaluation. Methods used were similar to those used by Drees and Summerlin (2005a):

Establishment of laboratory colonies. To obtain ant colonies, each mound of red imported fire ants was shoveled into 5 gal. plastic bucket with inner surfaces dusted with talcum powder to prevent ant escape. Water was then dripped into the buckets slowly, causing ant colonies to float on the surface. 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 with holes melted to allow ants to enter and exit was placed in each colony tray to house the queen, brood (eggs, larvae and pupae) and worker ants. Each colony was provided with distilled water and a standard laboratory diet of dead insects (crickets or mealworms) and diluted honey water daily.

Foraging structure. In each laboratory ant colony, a rectangular base, constructed of 3 by 3 cm pine and measuring 38 by 24 cm. that could support up to eight 19 mm (3/4 inch) diameter and 39.5 cm tall dowel rods were placed in holes drilled into the base (**Fig. 1**). Each rod had a nail, 9.5 cm from the top, so that age-treated Spiral Wrap parts could be affixed 7 cm from the top of the rod. The 5 treatments above were compared within each of 6 replicate laboratory colonies.

Assay technique. On top of each dowel rod housing an age-treated or untreated

Spiral Wrap part (see treatments listed above), a 7 mm (1/4 inch) long, 4 mm diameter “bead” of Jif® Creamy Peanut Butter (The J. M. Smucker Co., Orrville, OH 44667 containing roasted peanuts, sugar, 2% or less of molasses, partly hydrogenated vegetable oil (soybean oil), fully hydrogenated vegetable oil [rapeseed and soybean], mono- and diglycerides and salt) weighing approximately 0.3 g was applied using a plastic squeeze bottle. After 6 and 24 hours of exposure to foraging ants, the amount of peanut butter remaining (estimated as percent remaining and converted to grams) and number of ants associated with the peanut butter was estimated. This procedure was replicated 3 times over a 2 week period using the same colonies. Results were analyzed for each trial using analysis of variance (ANOVA) and means were separated using Duncan’s Multiple Range Test at $P < 0.05$ to determine which treatments had significantly less mean weight of peanut butter remaining or higher mean foraging ants associated with the food lure target than dowel rods with no ARINIX™ Spiral Wrap.

Field evaluation. (Highly preferred by ESA’s Mark Suarez, Entomologist - 1B). Prior to aging and periodically thereafter (once per year), Spiral Wrap parts were aged under each different regime or treatment (above) were moved from the location where they were being aged in order to perform a comparative bioassay. Each Spiral Wrap was affixed to a dowel rod of appropriate diameter which allowed the part to fit snugly around the rod part, if not already housed in such a manner. The dowel was affixed to a frame around a red imported fire ant mound with the ARINIX™ parts affixed 3-4 inches from the top of the rod. A food lure, as a 4 mm diameter 0.3 g “bead” of Jif® Creamy Peanut Butter, was placed on top of the dowel to document if foraging ants would be prevented from removing it periodically (over a 24 and 96 hour period) compared to untreated control dowel rods with a no permethrin-impregnated nylon barriers. Rods containing treatments (above) were placed around single ant colonies or mounds. This regime was replicated 6 times using different *S. invicta* colonies in the field and replicated over a two-week period. Data obtained (amount peanut butter remaining and number of foraging worker ants associated with the food lure target 24 and 48 h after exposure was statistically analyzed using Analysis of Variance (ANOVA) with means separated using Duncan’s Multiple Range Test at $P < 0.05$.

Bioassays were and again in future years will be conducted both in the field or in the laboratory. Results of outdoor assays may be influenced by temperature and other environmental conditions that may prevent direct comparison of treatments. Laboratory bioassays would be a more reliable method for making direct comparisons.

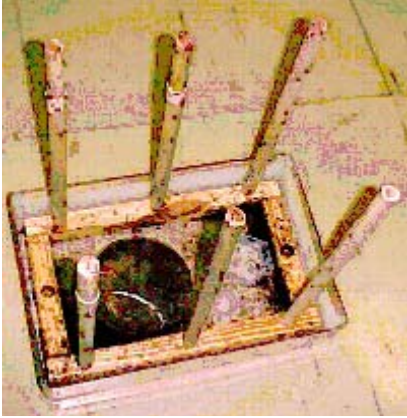


Fig. 1. Foraging structure housed in a red imported fire ant laboratory colony tray used to assess prevention of ant foraging on peanut butter placed on top of dowel rods with permethrin-impregnated nylon Spiral Wraps or cylinders placed part way to the top of the rods.

Storage and aging

Nix of America provided three plastic boxes (18 3/4 by 17 by 5 1/2 inch bottom with 19 by 18 3/4 by 2 1/2 inch tall lid). ARINIX I and II parts were equally divided among the storage boxes and each box was supplied with a Hobo Pro Series Data Logger to monitor temperature and relative humidity. One storage unit was placed in the laboratory, one outdoor with lid closed and one outdoors with lid open to expose parts to sunlight in addition to temperature.

Timeline. Bioassays will be performed annually for 4 or more years. Bioassays will involve removing parts from aging sites/location. Thereafter, the rods will be replaced in their aging “treatment” sites until the next scheduled bioassay. New colonies will be collected from the field for each annual or semi-annual bioassay.

2007 Bioassays

Two types of ARINIX (I & II) and a similar number of “untreated” Spiral Wrap parts were provided for the 2007 assessment. Also provided were housing units for aging the parts over the coming years and three HOBOTM H8 Pro Series Data Loggers (Onset Computer Corp., 470 MacArthur Blvd., Bourne, MA 02532, www.onsetcomp.com). Colonies were assessed for queen, alate (winged male and female sexual reproductive ant stages), estimated numbers of worker ants, brood (presence of and estimated volume of worker and sexual larvae and pupae). Six colonies were selected for conducting the first trial.

Laboratory trials

Trial 1. On Aug. 23, 2007, 12 red imported fire ant colonies were collected at the USDA Pecan Genetics Field Laboratory (Hwy 50, Brazos Co., TX) between 9:00 and

9:45 a.m. and brought to the laboratory at the Center for Urban and Structural Entomology. Ant colonies were extracted from the soil and placed in plastic trays (27 x 37 cm and 9 cm tall) containing a Petri dish nesting cell to house the queen, brood and worker ants. These colonies were used in Trials 1 and 2 (below). At initiation of each trial, 0.18 g peanut butter was placed at the top of each rod. Estimated amount of peanut butter remaining was documented periodically thereafter. Data were analyzed using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test at $P < 05$ (SPSS 14.0).

The first laboratory trial was initiated at 10:45 a.m., Aug. 30, 2007. Temperature and humidity were recorded hourly on Data Logger #1 (Hobo® Pro Series, HOB-032-02 #543716 RH, Temp.). Two parts (replicates) of each of three treatments (ARINIX I, ARINIX II and untreated control) were applied to recently purchased and cut dowel rods. Furthermore, the parts were fixed closer to the base of the rods (3 inches from the bottom) so that the ants would need to crawl a longer distance after crossing the barrier to reach the food lure target. Amount of peanut butter remaining and worker ants associated with the food lure at the tops of each rod was assessed 5 hrs. (5:00 p.m.) after establishment. Thereafter, one replicate of each treatment was removed from each colony so only one part of each treatment remained for the duration of this trial. In addition, the rods were turned upside-down so that the spirals were 3-4 inches from the top rather than 3-4 inches from the bottom of each dowel rod. At 24 hrs, colonies were again assessed for worker numbers and other parameters. Amount of peanut butter remaining and foraging worker ants associated with bait was monitored 24, 48, 72 and 96 hrs after initiation.

Trial 2. Due to extensive worker ant mortality and low food lure consumption in Trial 1, the trial was repeated. The second set of colonies collected on Aug. 23 were used in this trial. The same treatments on the same rods from Trial 1 were used. Temperature and humidity were recorded hourly on Data Logger #1. The trial was initiated Sept. 3, 2007 at 12:00 noon and evaluated as described for Trial 1 after 5, 24, 48, 72, 96, 120, 144 and 168 hours. This trial was repeated beginning Sept. 11, 2007 after colonies from Trial 2 were combined with colonies from Trial 1 to develop increase colony vigor of these suspected polygyne ants. It was monitored for 65 hrs.

Trial 3. Six new red imported fire ant colonies were collected from the field, Sept. 10, 2007, from the USDA Pecan Genetics Field Laboratory and extracted from soil as previously described. The trial was initiated Sept. 17, 2007, 9:53 a.m., using fresh peanut butter and the same dowel rods from Trials 1 & 2. In addition, the "old" poles used in previous trials were added containing the untreated Spiral Wrap and no Spiral Wrap as additional treatments, making 5 treatments for this trial. "Old" dowel rod treatments were monitored at 6, 24, 46 and 72 hrs following exposure, and all dowel rods were monitored after 96 hrs.

Trial 4. The final laboratory trial was initiated Sept. 24, 2007 at 9:12 a.m. using the same ant colonies from Trial 3, but using fresh peanut butter and the "old" dowel rods. The trial was monitored at 6, 24, and 48 hrs following initiation. Thereafter, colony positions were rearranged in the laboratory to determine whether or not the air flow from the air conditioning system might be affecting foraging behavior of ants. The trial continued to be monitored at 72, 120, 144 and 168 hrs following exposure.

Field Trials

Trial 5. On Sept. 10, 2007 six frames supporting three dowel rods were placed around six red imported fire ant colonies in the orchard of the USDA Pecan Genetics Field Laboratory (Hwy 50, Brazos Co., TX). The next day at 9:10 a.m., an 0.18 g peanut butter was placed on the tops of each of the rods. Each frame contained three treatments, placed in frames at random. Numbers of foraging ants and quantity of peanut butter remaining were recorded at 24, 48, 72 and 144 hrs thereafter. Temperature and humidity were recorded on Data Logger (#2) placed outside at the station headquarters.

Trial 6. Trial 5 was repeated using the same colonies in this field location. However, the “new” dowel rods were replaced with “old” dowel rods that showed less repellent to laboratory colonies in trials conducted there. In addition, fresh peanut butter was used to replace the “old” peanut butter half way through this trial (Sept. 19 at 72 hrs) in hopes of increasing foraging intensity on untreated dowel rods. This trial was initiated Sept. 18, 2007, at 10:30 a.m. and was monitored at 24, 48, 72 and 96 hrs. thereafter.

Results and Discussion

Laboratory trials. Temperature and relative humidity data recorded during these trials is provided in **Appendix 1**.

Trial 1. Within the 5-hr monitoring period, massive death of worker ants was observed in all colonies. Worker ant numbers were reduced from an average of 63,333 (+ 6,055 Standard Deviation) prior to exposure to ARINIX parts, to 22,119 + 11,917 SD following 24 hrs of exposure (**Tables 1 and 2**). After one duplicate set of each treatment were removed and rods were re-positioned to distance the ARINIX parts from the colony tray, monitoring of ants associated with food lures showed a significant difference in numbers of ants associated with the untreated control versus ARINIX treatments at 24 hrs of trial initiation (**Table 3**). However, no differences occurred between ants associated with ARINIX I or II. Thereafter, the number of foraging ants associated with the food lures declined, and no significant differences were documented between treatments. Peanut butter was not consumed by ants until 72 to 94 hrs after initiation, but by the end of the trial a significant amount of peanut butter was consumed from the tops of the untreated treatment rods, with no difference between ARINIX I and II. However, only half (3 of 6) of the colonies consumed peanut butter from the untreated treatment dowel rod. No peanut butter was removed from any dowel rods with either ARINIX barrier treatment. Colonies surviving this trial did not respond to any food provided and evidently most foraging worker ants, a stage attained through worker ant age rather than other specialized “caste”, had been eliminated.

ARINIX I and II parts, recently removed from the shipping container, evidently had considerable vapor activity and many more ants were rendered moribund or killed by close proximity. Interestingly, a number of winged and wingless (queen) female ants were seen on top of dowel rods as the trial progressed, perhaps indicating that these stages were attempting to leave the colony.

Trial 2. The second set of colonies collected on Aug. 23 that were used in this trial had an average of 50,000 + 11,832 worker ants (**Table 4**). Foraging ant activity was low

throughout this trial (**Table 5**) and no differences were observed between treatments. Only two colonies began to consume peanut butter from untreated control dowel rods beginning 72 hrs after trial initiation, and they consumed the entire amount by 168 hrs. However, this was not a significant reduction among the 6 replicates. No peanut butter was removed from ARINIX I or II rods throughout the trial. Reasons for poor foraging and food lure consumption in this trial were not well understood, with possible factors influencing outcome being: 1) poor colonies, perhaps satiated with peanut butter-like resources from the pecan orchard where they had been collected; 2) peanut butter may have been old and less attractive; 3) something toxic may have been covering the new dowel rods used in Trials 1 and 2.

The duplication of this trial using colonies combined from Trials 1 and 2 was unsuccessful because few ants foraged and only one colony consumed peanut butter from the untreated control dowel rod.

Trial 3. Colony status is presented in **Table 6**. Foraging fire ant workers consumed peanut butter bait on “old” dowel rods, although more slowly on the “old” dowel rods with the untreated Spiral Wrap. Thus, these untreated Spiral Wraps evidently reduce foraging to some extent either because of texture or some chemical deterrent (**Table 7**). After 96 hours of exposure, amount of peanut butter remaining on the untreated spiral “new” dowel rods was significantly greater than on old rods, and significantly less had been removed from rods affixed with ARINIX I and II spirals. No peanut butter had been removed from the ARINIX I (permethrin) Spiral Wrap treatment rods.

Trial 4. Although numbers of foraging ants on untreated Spiral Wrap dowel rods was numerically higher initially, no significant differences were documented among treatments (**Table 8**). Peanut butter on ARINIX I and II treatment rods was not removed and by 48 hrs of trial initiation, the amount of this food lure on the untreated Spiral Wrap dowel rods was significantly reduced. However, only half (3 of 6) of the colonies totally consumed the peanut butter from untreated treatment rods. One possible explanation may have been air circulation in the laboratory room, with the three unresponsive colonies being located close to the windows. Cooling system air may have been suppressing ant foraging activity. Colony position was changed for the subsequent data collection intervals. Regardless, the number of foraging ants associated with the peanut butter food lure was not significantly different between treatments for the remainder of the trial, although numerically more ants were associated with ARINIX II than ARINIX I during most observations. Peanut butter removal continued on untreated dowel rods and was significantly greater than the ARINIX I treatment. At 120 hrs, ARINIX II food lure removal was not significantly different from that for the untreated control. Thus, there appeared to be a trend indicating ARINIX I may be slightly more effective than ARINIX II.

Field Trials. Temperature and relative humidity data recorded during these trials is provided in **Appendix 2**.

Trial 5. Conditions were cloudy, overcast. Temperatures fluctuated but were generally 80- 81 degrees F and 76 to 83 percent relative humidity. After the 72 hr monitoring event, two frames were moved to new locations because the ant colonies on

which they had been placed at the beginning of the trial had abandoned their nest sites and these mounds were no longer active. Although minimal foraging ant activity was observed during visits over the days following initiation of this trial, two native ant species (*Pheidole* species, *Monomorium minimum*) were found to be feeding on peanut butter on top of dowel rods on and around red imported fire ant mounds (**Table 9**). This was rather surprising as this territory was thought to be dominated by the fire ant. Texture of peanut butter removed by these native ants was much finer and distinctly different from texture of the peanut butter being fed upon by the fire ant. No statistical analysis was performed on ant number data. We suspect that ant foraging intensity increases at times of the day such as evening or morning hours relative to the mid-morning monitoring conducted during these efforts.

By 78 hours after establishing this trial, a significantly lower average amount of peanut butter remained on dowel rods with the untreated Spiral Wraps (**Table 9**). Five of six colonies fed to some extent on peanut butter affixed to untreated dowel rods. No peanut butter was removed from ARINIX I or II treatment rods during the 144 hr duration of this trial.

Trial 6. During this trial, daytime temperatures in the shade of the orchard ranged from 86 to 92 degrees F and relative humidity from 70 to 75 percent. Conditions were clear, sunny, bright and calm. Results of this trial were similar to those of Trial 5, with native ants as well as imported fire ants feeding on peanut butter on top of dowel rods with the untreated Spiral Wrap (**Table 10**). In only one case was one ant found on top of a rod with an ARINIX I Spiral Wrap. Peanut butter removal occurred over time and, after 48 hrs, was significantly less on the untreated spiral rods. In only one case, was peanut butter completely removed from one ARINIX II treatment rod (72 hrs). However, this did not result in that treatment performing significantly different from the ARINIX I treatment.

Summary

In this series of six laboratory and field trials, ARINIX I and ARINIX II Spiral Wrap barrier treatments were found to protect a target of 0.18 g peanut butter food lure for the duration of the assays compared to an untreated Spiral Wrap treatment. Although no significant differences were documented between the performance of ARINIX I (permethrin) and ARINIX II (undisclosed active ingredient), observations were made to suggest that perhaps ARINIX II is somewhat less active against red imported fire ant workers than ARINIX I. How these treatments will hold up over time under various aging regimes will be documented in similar efforts to be conducted annually during the coming years.

Table 1. Red imported fire ant colony assessment prior to Trial 1 using ARINIX I and II parts, Aug. 24, 2007.

Colony	Queens	Alates	Workers	Worker Brood	Sexual Brood
1	1 Physogastric	1 female	60,000	1 tsp larvae, pupae	1/4 tsp larvae, pupae
2	5 slim gaster/ 10 physogastric	6 physogastric female/ 20 female/ 15 male	60,000	2 TBS larvae, pupae	0
3	8 physogastric	20 female/ 30 male	75,000	3 TBS larvae, pupae	1/8 tsp larvae, pupae
4	100 Physogastric	80 female/ 20 male	60,000	2 TBS larvae, pupae	0
5	1 Physogastric	100 female/ 25 male	60,000	3 TBS larvae, pupae	1/8 tsp larvae, pupae
6	2 slim gaster/ 6 physogastric	75 female/ 30 male	65,000	3 TBS larvae, pupae	0

Table 2. Red imported fire ant colony assessment, following Trial 1 using ARINIX I and II parts, Sept. 4, 2007.

Colony	Queens	Alates	Workers	Worker Brood	Sexual Brood
1	0	3 female	57,000	1 tsp larvae, pupae	0
2	3 slim gaster/ 15 physogastric	20 female/ 30 male	2,000	2 TBS larvae, pupae	0
3	8 physogastric	2 female/ 100 male	3,000	3 TBS larvae, pupae	0
4	100 physogastric	100 female/ 4 male	1,500	1 tsp eggs, larvae, pupae	0
5	1 physogastric	60 female/ 18 male	3,000	3 TBS larvae, pupae	0
6	1 physogastric	4 female/ 150 male	5,000	3 TBS larvae, pupae	0

Table 3. Mean number of red imported fire ant workers associated with peanut butter food lure bait following exposure to ARINIX I, ARINIX II or untreated barriers, Trial 1, initiated Aug. 30, 2007 at 10:45 a.m. Brazos Co., TX.

	Time following initiation of trial				
	5 h	24 h	48 h	72 h	96 h
No. ants associated with food lure					
Untreated Control	3.8	1.8a	0.3	0.8	0.0
ARINIX I (Permethrin)	0.2	0.2b	0.0	0.0	0.0
ARINIX II (etofenprox)	1.2	0.5ab	0.3	0.2	0.0
d.f.=2					
F	2.874	5.316	0.714	1.280	-
P	0.088	0.018	0.505	0.307	-
Mean Square	21.556	4.667	0.222	1.167	-
Peanut butter remaining of 0.18 g					
Untreated Control	0.18	0.18	0.18	0.12	0.09a
ARINIX I (Permethrin)	0.18	0.18	0.18	0.18	0.18b
ARINIX II (etofenprox)	0.18	0.18	0.18	0.18	0.18b
d.f.=2					
F	-	-	-	2.5	5.0
P	-	-	-	0.116	0.22
Mean Square		-	-	0.007	0.003

Means in columns followed by the same letter are not significantly different using Analysis of Variance with means separated using Duncan's Multiple Range Test at $P < 0.05$ (SPSS 14.0).

Table 4. Red imported fire ant colony assessment prior to Trial 1 using ARINIX I and II parts, Aug. 24, 2007.

Colony	Queens	Alates	Workers	Worker Brood	Sexual Brood
1	20 physogastric	150 male/ 40 female/ 9 physogastric female	60,000	2 tsp. larvae, pupae	0
2	6 physogastric	50 female/ 20 male	60,000	1 tsp larvae, pupae	0
3	0	50 female/ 20 male	55,000	1 tsp larvae, pupae	0
4	0	150 female/ 75 male	55,000	1 tsp larvae, pupae	0
5	1 physogastric	3 female/ 100 male	35,000	1/8 tsp larvae, pupae	0
6	0	100 male	35,000	1 TBS larvae, pupae	0

Table 5. Mean number of red imported fire ant workers associated with peanut butter food lure bait following exposure to ARINIX I, ARINIX II or untreated barriers, Trial 2, initiated Sept. 30, 2007 at 12:00 noon. Brazos Co., TX.

	Time following initiation of trial			
	5 h	24 h	48 h	72 h
No. ants associated with food lure				
Untreated Control	2.2	1.3	0.5	1.2
ARINIX I (permethrin)	2.0	1.2	0.0	1.0
ARINIX II (etofenprox)	1.8	0.7	1.2	0.2
d.f.=2				
F	0.24	0.261	1.281	0.542
P	0.976	0.774	0.282	0.593
Mean Square	0.167	0.722	2.056	1.722
Peanut butter remaining of 0.18 g				
Untreated Control	0.18	0.18	0.18	0.177
ARINIX I (permethrin)	0.18	0.18	0.18	0.18
ARINIX II (etofenprox)	0.18	0.18	0.18	0.18
d.f.=2				
F	-	-	-	2.50
P	-	-	-	0.116
Mean Square		-	-	2.22E-006

Means in columns followed by the same letter are not significantly different using Analysis of Variance with means separated using Duncan's Multiple Range Test at $P < 0.05$ (SPSS 14.0).

Table 5, cont. Mean number of red imported fire ant workers associated with peanut butter food lure bait following exposure to ARINIX I, ARINIX II or untreated barriers, Trial 2, initiated Sept. 30, 2007 at 12:00 noon. Brazos Co., TX.

	Time following initiation of trial			
	96 h	120 h	144 h	168 h
No. ants associated with food lure				
Untreated Control	0.8	0.2	0.3	0.0
ARINIX I (permethrin)	0.7	0.0	0.0	0.2
ARINIX II (etofenprox)	0.0	0.3	0.3	0.0
d.f.=2				
F	1.438	1.154	0.714	1.000
P	0.268	0.342	0.505	0.391
Mean Square	1.167	0.167	0.222	0.056
Peanut butter remaining of 0.18 g				
Untreated Control	0.14	0.13	0.12	0.12
ARINIX I (permethrin)	0.18	0.18	0.18	0.18
ARINIX II (etofenprox)	0.18	0.18	0.18	0.18
d.f.=2				
F	2.143	2.299	2.50	2.50
P	0.152	0.135	0.166	0.116
Mean Square	0.002	0.005	0.007	0.007

Means in columns followed by the same letter are not significantly different using Analysis of Variance with means separated using Duncan's Multiple Range Test at $P < 0.05$ (SPSS 14.0).

Table 6. Red imported fire ant colony assessment prior to Trial 3 using ARINIX I and II parts, Sept. 24, 2007.

Colony	Queens	Alates	Workers	Worker Brood	Sexual Brood
1	0	0	30,000	1 tsp. larvae, pupae	0
2	8 physogastric	7 female/ 25 male	20,000	2 TBS pupae	0
3	1 physogastric	31 females	15,000	3 tsp pupae	0
4	9 physogastric	50 males	15,000	200 pupae	0
5	0	75 males	15,000	2 tsp pupae	0
6	1 slim and 1 physogastric	50 female/20 male	20,000	1 tsp pupae	0

Table 7. Mean number of red imported fire ant workers associated with peanut butter food lure bait following exposure to ARINIX I, ARINIX II, untreated spiral barriers, plus untreated and no spiral wraps on “old” dowel rods, Trial 3, initiated Sept. 17, 2007 at 9:53 a.m. Brazos Co., TX.

	Time following initiation of trial				
	6 h	24 h	46 h	72 h	96 h
No. ants associated with food lure					
Untreated Control	-	-	-	-	-
ARINIX I (permethrin)	-	-	-	-	-
ARINIX II (etofenprox)	-	-	-	-	-
“old” dowel untreated control	9.0±20.1	12.0±20.9	1.5±2.3	8.7±9.6	-
“old” dowel no spiral wrap	8.2±8.5	3.0±2.8	1.2±2.4	5.3±6.9	-
d.f.=2					
F	-	-	-	-	-
P	-	-	-	-	-
Mean Square	-	-	-	-	-
Peanut butter remaining of 0.18 g					
Untreated Control	-	-	-	-	0.06b
ARINIX I (permethrin)	-	-	-	-	0.18a
ARINIX II (etofenprox)	-	-	-	-	0.14a
“old” dowel untreated control	0.18	0.16±0.04	0.09±0.10	0.03±0.07	0.00c
“old” dowel no spiral wrap	0.18	0.06±0.09	0.04±0.07	0.00±0.00	0.00c
d.f.=4					
F	-	-	-	-	26.841
P	-	-	-	-	0.00
Mean Square	-	-	-	-	0.040

Means in columns followed by the same letter are not significantly different using Analysis of Variance with means separated using Duncan’s Multiple Range Test at $P < 0.05$ (SPSS 14.0).

Table 8. Mean number of red imported fire ant workers associated with peanut butter food lure bait following exposure to ARINIX I, ARINIX II or untreated barriers, Trial 4, initiated Sept. 24, 2007 at 9:12 a.m. Brazos Co., TX.

	Time following initiation of trial*							
	6 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h
No. ants associated with food lure								
Untreated Control	15.8	5.0	0.2	3.0	1.3	0.67	1.2	0.7
ARINIX I (permethrin)	0.7	0.2	0.0	0.2	0.0	1.83	0.8	1.0
ARINIX II (etofenprox)	2.0	1.7	0.0	0.8	17.67	1.5	1.8	1.8
d.f.=2								
F	2.073	1.480	1.000	1.394	1.066	0.326	0.305	0.477
P	0.160	0.259	0.391	0.278	0.369	0.727	0.742	0.630
Mean Square	423.167	36.722	0.056	13.167	580.667	2.167	1.556	2.167
Peanut butter remaining of 0.18 g								
Untreated Control	0.17	0.12	0.09a	0.09a	0.09a	0.060a	0.04a	0.03a
ARINIX I (permethrin)	0.18	0.18	0.18b	0.18b	0.18b	0.18b	0.176b	0.17b
ARINIX II (etofenprox)	0.18	0.18	0.18b	0.18b	0.17ab	0.15ab	0.15b	0.14b
d.f.=2								
F	2.500	2.711	5.000	5.271	4.656	5.204	8.436	8.645
P	0.116	0.099	0.02	0.018	0.027	0.019	0.004	0.003
Mean Square	8.89E-005	0.008	0.016	0.017	0.015	0.023	0.029	0.032

* Means in columns followed by the same letter are not significantly different using Analysis of Variance with means separated using Duncan's Multiple Range Test at $P < 0.05$ (SPSS 14.0).

Table 9. Mean number of red imported fire ant workers associated with peanut butter food lure bait following exposure to ARINIX I, ARINIX II or untreated barriers, Trial 5, initiated Sept. 11, 2007 at 9:10 a.m. in the orchard of USDA Pecan Genetics Field Laboratory, Hwy 50, Brazos Co., TX.

	Time following initiation of trial ¹			
	24 h	48 h	72 h	144 h
No. ants associated with food lure				
Untreated Control	0.0	6.5 ²	3.5 ³	1.2 ⁴
ARINIX I (permethrin)	0.0	0.0	0.0	0.0
ARINIX II (etofenprox)	0.0	0.0	0.0	0.0
Peanut butter remaining of 0.18 g				
Untreated Control	0.18	0.15	0.07a	0.07a
ARINIX I (permethrin)	0.18	0.18	0.18a	0.18b
ARINIX II (etofenprox)	0.18	0.18	0.18b	0.18b
d.f.=				
F	-	1.276	9.979	11.660
P	-	0.308	0.002	0.001
Mean Square	-	0.002	0.023	0.025

¹Means in columns followed by the same letter are not significantly different using Analysis of Variance with means separated using Duncan's Multiple Range Test at P < 0.05 (SPSS 14.0).

²50 *Pheidole* on one rod, 39 red imported fire ants on another

³1 Little black ant (*Monomorium minimum*) on one rod, 20 red imported fire ants on another

⁴6 little black ants, 1 red imported fire ant

Table 10. Mean number of red imported fire ant workers associated with peanut butter food lure bait following exposure to ARINIX I, ARINIX II or untreated barriers, Trial 6, initiated Sept. 18, 2007 at 10:15 a.m. in the orchard of USDA Pecan Genetics Field Laboratory, Hwy 50, Brazos Co., TX.

	Time following initiation of trial ¹			
	24 h	48 h	72 h	96 h
No. ants associated with food lure				
Untreated Control	18.7 ²	2.8 ⁴	1.8 ⁵	0.2 ⁶
ARINIX I (permethrin)	0.0 ³	0.0	0.0	0.0
ARINIX II (etofenprox)	0.0	0.0	0.0	0.0
Peanut butter remaining of 0.18 g				
Untreated Control	0.12	0.09a	0.03a	0.03a
ARINIX I (permethrin)	0.18	0.18b	0.18b	0.18b
ARINIX II (etofenprox)	0.18	0.18b	0.15b	0.18b
d.f.=				
F	2.500	5.150	10.500	25.00
P	0.116	0.020	0.001	0.000
Mean Square	0.007	0.016	0.038	0.045

¹Means in columns followed by the same letter are not significantly different using Analysis of Variance with means separated using Duncan's Multiple Range Test at P < 0.05 (SPSS 14.0).

²12 little black ants (*Monomorium minimum*) on one dowel rod, 100 on another

³8 *Pheidole* on one rod

⁴23 little black ants on one rod

⁵4, 7 red imported fire ants on 2 rods, respectively

⁶1 red imported fire ant on one rod

Appendix 1. Temperature and relative humidity data recorded during laboratory Trials 1-4 (Data Logger #1, Hobo® Pro Series, HOB-032-02 #543716 RH, Temp.).

Date/Time	Temperature (*F)	RH (%)
ARINIX Lab Trial 1		
08/30/07 08:00:00.0	71.77	54.2
08/30/07 12:00:00.0	71.08	50.5
08/30/07 16:00:00.0	70.39	48.4
08/30/07 20:00:00.0	71.77	49.5
08/31/07 00:00:00.0	69.71	47.9
08/31/07 04:00:00.0	70.39	50
08/31/07 08:00:00.0	71.77	57.8
08/31/07 12:00:00.0	73.15	49
08/31/07 16:00:00.0	71.08	46.4
08/31/07 20:00:00.0	71.77	52.1
09/01/07 00:00:00.0	70.39	53.1
09/01/07 04:00:00.0	71.08	57.3
09/01/07 08:00:00.0	71.08	57.3
09/01/07 12:00:00.0	70.39	51.6
09/01/07 16:00:00.0	71.08	48.4
09/01/07 20:00:00.0	71.08	46.4
09/02/07 00:00:00.0	69.71	45.9
09/02/07 04:00:00.0	70.39	48.4
09/02/07 08:00:00.0	71.08	50
09/02/07 12:00:00.0	71.08	51.6
09/02/07 16:00:00.0	70.39	49.5
09/02/07 20:00:00.0	71.08	50
09/03/07 00:00:00.0	71.77	52.1
09/03/07 04:00:00.0	71.08	52.6
09/03/07 08:00:00.0	69.71	52.1
ARINIX Lab Trial 2		
09/03/07 12:00:00.0	71.77	46.9
09/03/07 16:00:00.0	71.08	44.8
09/03/07 20:00:00.0	71.77	49.5
09/04/07 00:00:00.0	71.08	51.6
09/04/07 04:00:00.0	71.08	53.1
09/04/07 08:00:00.0	70.39	52.6
09/04/07 12:00:00.0	73.84	50.5
09/04/07 16:00:00.0	73.15	49
09/04/07 20:00:00.0	73.15	47.4
09/05/07 00:00:00.0	72.46	48.4
09/05/07 04:00:00.0	73.15	57.3
09/05/07 08:00:00.0	73.15	59.3
09/05/07 12:00:00.0	73.15	50.5
09/05/07 16:00:00.0	73.84	49.5
09/05/07 20:00:00.0	73.15	51
09/06/07 00:00:00.0	72.46	53.1
09/06/07 04:00:00.0	72.46	56.2
09/06/07 08:00:00.0	73.15	59.9
09/06/07 12:00:00.0	73.84	51
09/06/07 16:00:00.0	74.53	47.4
09/06/07 20:00:00.0	73.84	47.9
09/07/07 00:00:00.0	72.46	49
09/07/07 04:00:00.0	73.15	54.7
09/07/07 08:00:00.0	72.46	52.1
09/07/07 12:00:00.0	73.15	50.5
09/07/07 16:00:00.0	72.46	46.4
09/07/07 20:00:00.0	71.77	45.9
09/08/07 00:00:00.0	71.08	49
09/08/07 04:00:00.0	71.08	51
09/08/07 08:00:00.0	71.77	54.2

09/08/07 12:00:00.0	71.77	57.3
09/08/07 16:00:00.0	71.77	52.6
09/08/07 20:00:00.0	71.08	49
09/09/07 00:00:00.0	71.08	52.1
09/09/07 04:00:00.0	71.77	54.2
09/09/07 08:00:00.0	71.08	52.1
09/09/07 12:00:00.0	71.08	50.5
09/09/07 16:00:00.0	71.77	51
09/09/07 20:00:00.0	71.77	51.6
09/10/07 00:00:00.0	70.39	50
09/10/07 04:00:00.0	71.08	53.1
09/10/07 08:00:00.0	71.08	54.2
09/10/07 12:00:00.0	72.46	47.9
ARINIX Lab Trial 3		
09/17/07 08:00:00.0	71.77	50
09/17/07 12:00:00.0	71.77	45.4
09/17/07 16:00:00.0	71.77	43.3
09/17/07 20:00:00.0	70.39	43.3
09/18/07 00:00:00.0	71.08	47.9
09/18/07 04:00:00.0	70.39	44.8
09/18/07 08:00:00.0	71.08	47.4
09/18/07 12:00:00.0	72.46	49.5
09/18/07 16:00:00.0	71.77	45.9
09/18/07 20:00:00.0	70.39	43.8
09/19/07 00:00:00.0	70.39	46.4
09/19/07 04:00:00.0	71.08	50
09/19/07 08:00:00.0	70.39	48.4
09/19/07 12:00:00.0	71.77	48.4
09/19/07 16:00:00.0	71.08	45.4
09/19/07 20:00:00.0	71.08	48.4
09/20/07 00:00:00.0	71.77	49.5
09/20/07 04:00:00.0	70.39	47.9
ARINIX Lab Trial 4		
09/24/07 08:00:00.0	71.77	53.6
09/24/07 12:00:00.0	71.77	45.4
09/24/07 16:00:00.0	71.77	47.9
09/24/07 20:00:00.0	71.77	44.8
09/25/07 00:00:00.0	70.39	45.9
09/25/07 04:00:00.0	71.77	50
09/25/07 08:00:00.0	70.39	49.5
09/25/07 12:00:00.0	72.46	47.4
09/25/07 16:00:00.0	71.08	45.4
09/25/07 20:00:00.0	71.08	44.8
09/26/07 00:00:00.0	71.08	47.9
09/26/07 04:00:00.0	71.77	51.6
09/26/07 08:00:00.0	71.08	52.6
09/26/07 12:00:00.0	73.84	46.9
09/26/07 16:00:00.0	72.46	43.8
09/26/07 20:00:00.0	71.77	45.4
09/27/07 00:00:00.0	70.39	45.4
09/27/07 04:00:00.0	71.08	47.9
09/27/07 08:00:00.0	70.39	50.5
09/27/07 04:00:00.0	71.08	47.9
09/27/07 08:00:00.0	70.39	50.5
09/27/07 12:00:00.0	71.08	46.9
09/27/07 16:00:00.0	71.08	44.8
09/27/07 20:00:00.0	71.08	46.9
09/28/07 00:00:00.0	70.39	46.4
09/28/07 04:00:00.0	71.77	50
09/28/07 08:00:00.0	71.77	53.6
09/28/07 12:00:00.0	71.77	48.4
09/28/07 16:00:00.0	71.77	44.3

09/28/07 20:00:00.0	71.77	44.8
09/29/07 00:00:00.0	71.08	44.8
09/29/07 04:00:00.0	71.08	47.4
09/29/07 08:00:00.0	72.46	53.1
09/29/07 12:00:00.0	71.08	47.4
09/29/07 16:00:00.0	70.39	45.9
09/29/07 20:00:00.0	71.77	49
09/30/07 00:00:00.0	71.08	48.4
09/30/07 04:00:00.0	71.77	51.6
09/30/07 08:00:00.0	71.08	50
09/30/07 12:00:00.0	71.08	51
09/30/07 16:00:00.0	71.77	49.5
09/30/07 20:00:00.0	71.77	49.5
10/01/07 00:00:00.0	71.08	52.1
10/01/07 04:00:00.0	71.08	50.5
10/01/07 08:00:00.0	70.39	51.6
10/01/07 12:00:00.0	73.84	49

Appendix 2. Temperature and relative humidity data recorded during field Trials 5 & 6 (Data Logger #1, Hobo® Pro Series, HOB-032-02 #543716 RH, Temp.).

Date/Time	Temperature (*F)	RH (%)
ARINIX Field Trial 5		
09/10/07 08:00:00.0	71.77	53.1
09/10/07 12:00:00.0	74.53	43.3
09/10/07 16:00:00.0	100.98	35.3
09/10/07 20:00:00.0	83.67	72.6
09/11/07 00:00:00.0	78.71	86.7
09/11/07 04:00:00.0	75.22	92.6
09/11/07 08:00:00.0	75.92	91.3
09/11/07 12:00:00.0	82.95	74.5
09/11/07 16:00:00.0	81.53	71.1
09/11/07 20:00:00.0	74.53	87.1
09/12/07 00:00:00.0	73.15	86.7
09/12/07 04:00:00.0	71.08	86.7
09/12/07 08:00:00.0	71.08	87.1
09/12/07 12:00:00.0	80.82	65.5
09/12/07 16:00:00.0	84.38	60.4
09/12/07 20:00:00.0	79.41	74.1
09/13/07 00:00:00.0	73.84	88
09/13/07 04:00:00.0	71.77	92.1
09/13/07 08:00:00.0	73.84	91.7
09/13/07 12:00:00.0	79.41	75.5
09/13/07 16:00:00.0	88.01	53.6
09/13/07 20:00:00.0	82.24	64.5
09/14/07 00:00:00.0	74.53	88.4
09/14/07 04:00:00.0	71.08	90.5
09/14/07 08:00:00.0	70.39	95.3
09/14/07 12:00:00.0	88.01	52.6
09/14/07 16:00:00.0	93.97	40.8
09/14/07 20:00:00.0	85.1	62.5
09/15/07 00:00:00.0	78.01	83.1
09/15/07 04:00:00.0	74.53	88.4
09/15/07 08:00:00.0	73.84	93.7
09/15/07 12:00:00.0	88.01	60.4
09/15/07 16:00:00.0	91.71	44.9
09/15/07 20:00:00.0	81.53	64
09/16/07 00:00:00.0	73.15	84.4
09/16/07 04:00:00.0	69.02	90.9
09/16/07 08:00:00.0	69.02	93.3
ARINIX Field Trial 6		
09/18/07 08:00:00.0	68.33	92.1
09/18/07 12:00:00.0	86.55	56.2
09/18/07 16:00:00.0	92.46	45.9
09/18/07 20:00:00.0	82.95	63
09/19/07 00:00:00.0	77.31	81.2
09/19/07 04:00:00.0	73.84	91.7
09/19/07 08:00:00.0	72.46	94.9
09/19/07 12:00:00.0	85.83	71.6
09/19/07 16:00:00.0	88.74	60.4
09/19/07 20:00:00.0	74.53	98.2
09/20/07 00:00:00.0	75.22	96.4
09/20/07 04:00:00.0	72.46	95.3
09/20/07 08:00:00.0	71.08	95.7
09/20/07 12:00:00.0	84.38	58.3
09/20/07 16:00:00.0	89.48	40.8
09/20/07 20:00:00.0	80.82	64
09/21/07 00:00:00.0	71.77	77.9
09/21/07 04:00:00.0	68.33	91.7

09/21/07 08:00:00.0	66.96	94.9
09/21/07 12:00:00.0	83.67	55.7
09/21/07 16:00:00.0	90.22	40.3
09/21/07 20:00:00.0	80.82	62.5
09/22/07 00:00:00.0	73.15	81.2
09/22/07 04:00:00.0	68.33	89.3
09/22/07 08:00:00.0	68.33	91.3
09/22/07 12:00:00.0	85.83	53.6
09/22/07 16:00:00.0	92.46	35.3
09/22/07 20:00:00.0	81.53	58.3
09/23/07 00:00:00.0	73.84	70.6
09/23/07 04:00:00.0	73.15	77.9
09/23/07 08:00:00.0	71.08	86.7
09/23/07 12:00:00.0	85.83	50.5
09/23/07 16:00:00.0	88.01	47.4
09/23/07 20:00:00.0	80.82	64
09/24/07 00:00:00.0	73.84	84.4
09/24/07 04:00:00.0	69.02	92.9
09/24/07 08:00:00.0	67.65	96
09/24/07 12:00:00.0	84.38	57.3