

## **Evaluation of Piperonyl Butoxide (PBO) as a Potential Insect Growth Regulator for Red Imported Fire Ants**

Charles L. Barr, Extension Associate and  
Bastiaan M. Drees, Professor and Extension Entomologist

Piperonyl butoxide (PBO) is frequently used as a synergist in combination with other pesticides, particularly pyrethrins. Recent studies by Satoh and Plapp (1992) indicate that PBO may also act as a juvenoid type of insect growth regulator (IGR). These trials were conducted to determine if PBO had an effect on the development of the red imported fire ant, *Solenopsis invicta* Buren. Fenoxycarb, the active ingredient in Logic® and Award® fire ant baits, acts as a juvenoid IGR. Colonies treated with fenoxycarb in soybean oil fail to produce worker ant larvae and pupae and larvae present at the time of treatment develop only into larger reproductive larvae and pupae. These effects are observable within several weeks of treatment.

The first trials were conducted to determine the most acceptable concentration of PBO in soybean oil to fire ants. Thereafter, trials were conducted to determine if PBO produced insecticidal or IGR effects in laboratory colonies of fire ants.

### **Materials and Methods**

#### **I. Preference Trials**

Trial 1. Three red imported fire ant colonies were field collected, extracted from the soil, and placed in Fluon®-coated plastic boxes. On 12 January 1994 a set of capillary tubes were filled with varying concentrations of PBO in soybean oil and placed together in the ant colonies. Concentrations exposed to the ants were:

- 1) Technical (100%) in soybean oil
- 2) 10,000 ppm (1%) in soybean oil
- 3) 100 ppm (0.01%) in soybean oil
- 4) 1 ppm in soybean oil
- 5) 0.01 ppm in soybean oil
- 6) 0 ppm, soybean oil alone

The capillary tubes were filled with the solutions to approximately 35 mm of the tubes' length. Exact distances (corresponding to volume) were recorded for each tube. The tubes were then exposed to ant colonies until one tube in each colony was emptied by the ants' feeding activities. The entire set of tubes was then removed and frozen (to kill any ants) before the amount of PBO-soybean oil solution was measured (in mm) and recorded to determine solution consumed. The entire test was repeated (Run 2).

Trial 2. Results from the first set of trials were used to formulate a narrower range of PBO concentrations in soybean oil (PBO was acceptable to ants at concentrations between 1 and 100 percent). The procedure described above was repeated, 13 January, 1994 using the following

concentrations:

- 1) 50% PBO in soybean oil
- 2) 25% PBO in soybean oil
- 3) 10% PBO in soybean oil
- 4) 1% PBO in soybean oil

## **II. Efficacy Trials**

Twelve freshly collected fire ant colonies were separated into a treatment and a control group based on the approximate number of workers and quantity of brood present. The colonies of the two groups were paired according to colony size so that each group had an approximately equal number of large and small colonies.

Trial 3. On 7 February 1994, a capillary tube with a known volume of solution was placed in each of the six treatment colonies. In order to deliver as much PBO as possible to the colonies, 25 percent PBO was chosen. A capillary tube of soybean oil alone was placed in the control colonies.

Ants were not apparently attracted to the 25 percent solution of PBO within an hour. The capillary tubes were removed and replaced with ones containing a known volume of 10% PBO. These tubes were removed later in the day and the volume of oil or solution consumed was recorded for all tubes. Results indicated very erratic acceptance of the PBO solution. The procedure was repeated with a 1 percent solution of PBO with tube removal at the end of the day. Consumption was again erratic.

On 8 February 1994 the above procedure was repeated using a 0.1 percent PBO solution. This concentration appeared to be acceptable to all colonies and the procedure was repeated again that day when the tubes were emptied and again on 11 February. In an effort to introduce as much PBO as possible into the colonies, two tubes were used on 11 February and three on 13 February.

The colonies were then examined weekly. Estimated numbers of worker ants and presence of worker brood (ant larvae and pupae) was recorded for each colony through 17 March 1994.

Trial 4. Because of poor results of Trial 3, another trial was conducted to attempt to deliver more PBO to treatment colonies. Colonies from Trial 3 were cleaned of debris and moved to new boxes as necessary. The procedure used in Trial 3 was repeated except that the PBO concentration was increased to 5 percent and was delivered in a disposable 1 ml pipette plugged with cotton to slow leakage. Colonies were observed periodically through 21 April. Worker ant numbers were estimated and presence of worker brood (larvae and pupae) was noted for each colony.

## **Results and Discussion**

### **I. Preference Trials**

Trial 1. No clear correlation occurred between amount of piperonyl butoxide concentrations in

soybean oil and amount of solution consumed by foraging red imported fire ant activity. The debris piled up by foraging worker ants around the end of the tubes containing technical material indicates that the material was not repellent, but not attractive, at least not compared to the other food sources available (**Table 1**). The 1 percent solution was as "attractive" as any other formulation or oil alone.

Trial 2. Based on the results of Trial 1, the concentrations of this test were arrayed between 1 and 100 percent. The debris piling phenomenon was observed again on several tubes making the results difficult to interpret. The technical material was clearly not attractive as was the 50% concentration (**Table 2**). The lower concentrations yielded highly variable results, but the 25% solution appeared to be attractive enough to use in the delivery of PBO to the Efficacy Test colonies.

## **II. Efficacy Trials**

Trial 3. As indicated by the data in **Tables 3 and 4**, ants or brood (worker ant larvae and pupae) were not eliminated by the addition of piperonyl to soybean oil. Contrarily, most colonies were observed to increase brood production and some in worker numbers at some point during the duration of this trial. These observations indicate that either PBO is ineffective as a juvenoid insect growth regulator or not enough active ingredient was administered to colonies to cause an effect.

Trial 4. In this trial, the capillary tube method that delivered at most 6.2 mg PBO over a week was abandoned in favor of disposable pipettes containing 0.4 ml of 5 percent PBO that could deliver a maximum of 14 mg PBO within 24 hours. Though large amounts of debris were piled around the mouths of the tubes and some leaked out onto their mounting surface, all material was gone within 24 hours. In the larger colonies, ants were seen feeding on the puddles of material that leaked out.

As indicated in **Table 5**, the piperonyl butoxide administered to fire ant colonies in soybean oil again had no visible effect on either brood production or worker mortality as compared to the control. All colonies experienced some decline in brood and worker numbers over the course of the course of the trial.

### **Conclusion**

The results obtained from these trials failed to document piperonyl butoxide formulated as a bait in soybean oil as an effective insect growth regulator for the red imported fire ant.

### **Literature cited**

Satoh, G.T. and F.W. Plapp, Jr. 1992. Use of juvenoid insect growth regulators for management of cotton aphid and sweet potato whitefly populations. Proc. Beltwide Cotton Conf. 2:751-757.

**Table 1.** Amount (mm) of piperonyl butoxide (PBO) in soybean oil removed from capillary tubes by red imported fire ant workers in laboratory colonies, Trial 1, 12 January 1994.

<u>PBO Concentration</u>	Consumption of PBO:soybean oil (mm)			<u>Total/Avg.</u>
	<u>Colony 1</u>	<u>Colony 2</u>	<u>Colony 3</u>	
<b>Run #1</b>				
Technical	38*	3	34*	75 / 15.0
1.0%	19	32	32	83 / 27.7
0.01%	23	20	12	55 / 18.3
1.0 ppm	30	11	28	69 / 23.0
0.01 ppm	30	18	34	82 / 27.3
soybean oil	32	19	15	66 / 22.0
<b>Run #2</b>				
Technical	35*	37*	34*	76 / *
1.0%	15	18	32*	65 / 21.7
0.01%	24	20	27	71 / 23.7
1 ppm	17	17	32*	66 / 22.0
0.01 ppm	25	26	8	59 / 19.7
soybean oil	18	13	17	48 / 16.0

\* Indicates that ants piled debris around the end of capillary tubes causing the oil solution to soak out of the tube. Consumption cannot be confirmed, so these values should be treated as much less than indicated.

**Table 2.** Amount (mm) of piperonyl butoxide (PBO) in soybean oil removed from capillary tubes by red imported fire ant workers in laboratory colonies, Trial 2, 13 January 1994.

<u>PBO Concentration</u>	Consumption of PBO:soybean oil (mm)			<u>Total/Avg.</u>
	<u>Colony 1</u>	<u>Colony 2</u>	<u>Colony 3</u>	
Technical	0	3	2	5 / 1.7
50%	2	3	10	15 / 5.0
25%	11	32*	34*	77 / *
10%	20	35*	33*	88 / *
1%	22	31*	32*	86 / *

\* Indicates that ants piled debris around the end of capillary tubes causing the oil solution to soak out of the tube. Consumption cannot be confirmed, so these values should be treated as much less than indicated.

**Table 3.** Amount of soybean oil or piperonyl butoxide in soybean oil consumed by untreated control (CK) or PBO treated (TRT) colonies during efficacy Trial 3, February 1994.

Consumption of soybean oil (mm)									
Date of exposure and PBO concentration used									
	2/7	2/7	2/8	2/9	2/9	2/11	2/11	2/13	
<u>Colony</u>	<u>0.0%</u>	<u>0.0%</u>	<u>0.0%</u>	<u>0.0%</u>	<u>0.0%</u>	<u>0.0%</u>	<u>0.0%</u>	<u>0.0%</u>	<u>Total PBO</u>
CK1	60	43	70	70	70	70	140	210	0
CK2	27	62	59	63	70	70	140	210	0
CK3	50	35	70	70	70	70	140	210	0
CK4	13	68	70	68	70	70	140	210	0
CK5	44	60	18	65	70	70	140	210	0
CK6	30	48	70	38	70	70	140	210	0

	<u>10%</u>	<u>1.0%</u>	<u>1.0%</u>	<u>0.1%</u>	<u>0.1%</u>	<u>0.1%</u>	<u>0.1%</u>	<u>0.1%</u>	<u>Total PBO</u> <u>(mg**)</u>
TRT1	70*	35*	70*	10	70	70	140	210	6.2 / .37
TRT2	70*	10*	70*	70*	70	70	140	210	6.0 / .36
TRT3	70*	25*	70*	65	70	70	140	210	6.2 / .36
TRT4	0*	4	68*	65	70	70	140	210	0.9 / .43
TRT5	2*	5	20*	58*	70*	70*	140	210	0.7 / .29
TRT6	0*	5	40*	25*	70*	70*	140	210	0.7 / .29

\* Indicates debris piled around end of capillary tube. The amount of debris and amount of oil soaked out of the tube varied widely.

\*\* The first number indicates amount of PBO removed from tube from all factors. The second number indicates amount of PBO "guaranteed consumed", e.g. not including the amounts from those tubes with debris piles. (The weight of PBO was approximated using 1 ml = 1 gram X specific gravity factor of 0.7 for soy bean oil and PBO. Capillary tube volume =  $\pi(.575\text{mm})^2(\text{mm})$ ).

**Table 4.** Vigor and brood status of red imported fire ant laboratory colonies fed either soybean oil (CK) or piperonyl butoxide concentrations in soybean oil (TRT), February through March 1994.

Treatment	No. workers x 1,000, brood quantity*					
<u>Colony</u>	<u>2/7</u>	<u>2/16</u>	<u>2/23</u>	<u>3/2</u>	<u>3/9</u>	<u>3/17</u>
CK1	50, 4SB	50, 5SB	50, 5SB	50, 5SB	50, 5SB+	50, 5SB
CK2	45, 4SB	45, 4SB	45, 4SB	45, 4SB+	40, 4SB+	40,
4SB+						
CK3	40, 3SB	45, 4SB	40, 4SB	40, 4SB+	40, 4SB+	40,
4SB+						
CK4	25, 1LB+	25, 1LB+	25, 1LB+	25, 1LB+	20, 1LB+	20, 1LB
CK5	20, 1LB	20, 1LB	25, 1LB++	20, 1LB+	20, 1LB	20, 1LB
CK6	20, .5LB	20, 1LB+	20, 1LB+	15, 1LB	15, 1LB	15, 1LB
Avg.	33.3	34.1	34.2	32.5	30.8	30.8
TRT1	60, 2LB	60, 2LB+	50, 2+2	50, 2+2	45, 2+2	40, 2+2
TRT2	40, 3SB	40, 4SB	40, 4SB	40, 4SB	40, 4SB	40,
4SB+						
TRT3	40, 2LB	40, 2LB	40, 2LB	35, 2LB	35, 2LB	30, 2LB
TRT4	25, 1LB	25, 1LB	30, 1+1	30, 1+1	30, 1+1	35, 1+1
TRT5	20, 1LB	20, .75LB	20, .75LB	20, 1LB+	20, 1LB	20, 1LB
TRT6	15, .5LB	20, 1LB	20, 1LB+	20, 1LB+	20, 1LB	20, 1LB
Avg.	33.3	34.1	33.3	32.5	31.7	

\* Worker numbers are estimated on the basis of 20,000 in a "full" large petri plate and 10,000 in a "full" small petri plate. Brood quantity was evaluated on the basis of the amount in an average petri plate. LB indicates a "full" large petri plate, SB a "full" small petri plate. The number indicates the number of plates. The addition of + or ++ indicates an unusually large amount of brood. ### indicates number of petri plates of both sizes with brood.

**Table 5.** Vigor and brood status of red imported fire ant laboratory colonies fed either soybean oil (CK) or piperonyl butoxide concentrations in soybean oil (TRT), March through April 1994.

Treatment	# workers x 1,000, brood quantity*				
<u>Colony</u>	<u>3/24</u>	<u>3/31</u>	<u>4/7</u>	<u>4/14</u>	<u>4/21</u>
CK1	50, 5SB	45, 5SB	40, 4SB	30, 3SB	25, 1SB
CK2	40, 4SB	40, 4SB	40, 4SB	35, 4SB0	30, 2SB
CK3	40, 4SB	40, 4SB	40, 4SB	35, 4SB	30, 2SB
CK4	20, 1LB	20, 1LB	20, 1LB	20, 1LB	15, 1LB
CK5	20, 1LB	20, 1LB	20, 1LB	20, 1LB	15, 1LB
CK6	15, 1LB	20, 1LB	20, 1LB	10, .5LB	10, .5LB
Avg.	30.8	30.8	30.0	25.0	20.8
TRT1	40, 2LB	30, 2LB	30, 2LB	30, 2LB	30, 1LB
TRT2	45, 4SB	40, 4SB	40, 4SB	40, 4SB	40, 4SB
TRT3	30, 2LB	25, 2LB	25, 1.5LB	25, 1LB	25, 1LB
TRT4	25, 1LB	25, 1LB	20, 1LB	20, 1LB	20, 1LB
TRT5	20, 1LB	20, 1LB	20, 1LB	20, 1LB	20, 1LB
TRT6	20, 1LB	20, 1LB	20, 1LB	20, 1LB	20, 1LB
Avg.	30.0	26.7	25.8	25.8	25.8

\* Worker numbers are estimated on the basis of 20,000 in a "full" large petri plate and 10,000 in a "full" small petri plate. Brood quantity was evaluated on the basis of the amount in an average petri plate. LB indicates a "full" large petri plate, SB a "full" small petri plate. The number indicates the number of plates. The addition of + or ++ indicates an unusually large amount of brood. ## indicates number of petri plates of both sizes with brood.