

selves are responsible for the toxic effects observed. However, the importance of chronic low-level exposure to the bromonaphthalenes remains to be assessed.

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## Toxicity of an Imported Fire Ant Bait Based on Phloxin B (D + C Red 27)

Robert M. David and James R. Heitz\*

Laboratory tests have shown that incorporation of Phloxin B (D + C Red 27) into a soybean oil-corn cob grit bait yielded an efficient toxicant for the imported fire ant. Phloxin B induces two lethal mechanisms within the imported fire ant: the first is light dependent and is effective in terms of hours; the second is light independent and is effective in terms of days. The relatively rapid light-dependent reaction is observed after a 1-day pretreatment which indicates that the reaction will not interfere with field collection of the bait by worker ants. Both toxic mechanisms were more pronounced in insects subjected to a slight energy stress caused by food deprivation.

The imported fire ants, *Solenopsis richteri* and *Solenopsis invicta*, are major pests of a great portion of the southern United States. Current control measures are centered on the use of mirex as the active toxicant. Historically, the most used formulation has been the "4X-mirex bait" consisting of 850 parts corn cob grits, 147 parts soybean oil, and 3 parts mirex. This formulation has been cancelled, and other mirex-containing formulations are due to be cancelled effective December 31, 1977 (Federal Register, 1976). There is an urgent need for an improved method for controlling the imported fire ants.

Recently, certain dye molecules have been shown to be toxic to the imported fire ant (Broome et al., 1975a,b). The dyes were fed to the ants in the dark in an aqueous sucrose solution; and when the ants were subsequently exposed to visible light, mortality was observed in a matter of hours. If the ants were left with the dye in the dark for a longer period of time, never being exposed to light, mortality was observed in a matter of days.

In dye screening studies where inhibition of enzyme activity was the experimentally determined variable for dye-sensitized photooxidation, rose bengal was the most effective in every test (Callahan et al., 1975, 1977). However, when toxicity studies with the imported fire ant were performed, phloxin B (Figure 1) was very nearly as effective as rose bengal (Broome et al., 1975a). At first,

this was attributed to experimental anomaly; but now, it is felt that phloxin B may simply be as toxic to fire ants as rose bengal for unknown reasons other than the efficiency of dye sensitization.

These above observations, coupled with the knowledge that some mammalian toxicological studies had been performed on phloxin B prior to its registration by the Food and Drug Administration as a drug and cosmetic (D + C) dye, led to the use of phloxin B as the basis for the new fire ant bait. Rose bengal is not currently registered as a D + C dye. It is felt that since some mammalian toxicological studies have been done with phloxin B, the registration of phloxin B as a pesticide may not take as long as for rose bengal. At the present time, two forms of phloxin B are registered with the Food and Drug Administration. The protonated form is D + C Red 27 and the disodium salt is D + C Red 28. D + C Red 27 was chosen for this application due to the fact that it is oil soluble while D + C Red 28 is not. The bait tested consists of corn cob grits, soybean oil, and phloxin B and is similar to the "4X-mirex bait". The results of both the light-dependent and the light-independent lethal reactions are presented here.

#### MATERIALS AND METHODS

Mounds of *S. richteri* were field collected and maintained in the mound soil in all-glass aquaria in the laboratory. A 5-cm strip of Fluon GP-1 (Northeast Chemical Co., Woonsocket, R.I.) was applied around the top of the inside walls of the aquaria for containment purposes. Water, but no food, was added daily to the mounds. After

\*Department of Biochemistry, Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Mississippi State, Mississippi 39762.

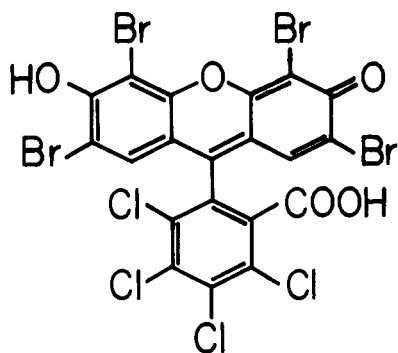


Figure 1. Chemical structure of phloxin B (D + C Red 27).

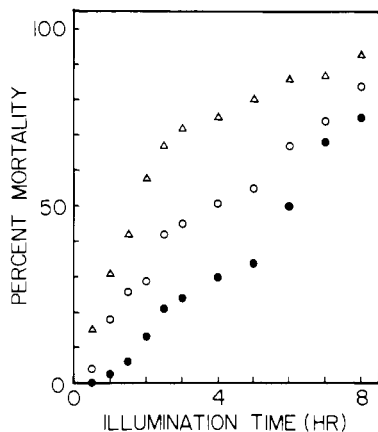


Figure 2. Mortality as a function of illumination time for the imported fire ant. Populations were acclimatized for 7 days prior to feeding in the dark for 22 h on a corn cob grit-soybean oil bait containing 0.15% (●), 0.30% (○), and 0.60% (△) phloxin B. Illumination ( $3800 \mu\text{W}/\text{cm}^2$ ) was from fluorescent light sources.

a period of 1 to 10 days, specimens were selected for study by allowing 20–50 ants to swarm over wet cotton wicking before placing it in the bottom of a glass petri dish. A coating of Fluon GP-1 on the inside wall of the petri dish retarded insect escapes. Experimental baits consisted of 850 parts corn cob grits, 147 parts soybean oil, and 1.5–6.0 parts phloxin B. The dye was a gift of the Hilton-Davis Chemical Co., Cincinnati, Ohio. This bait was sprinkled on the bottom of the petri dish containing the ants. Investigations of the light-catalyzed reaction were made on populations of ants incubated in the dark for 22 h prior to illumination by fluorescent light with a measured intensity of  $3800 \mu\text{W}/\text{cm}^2$  over the 380–750-nm wavelength range using an ISCO Model SR spectroradiometer. Investigations of the light-independent reaction were made on populations of insects held in the dark over the entire time course of the experiment except for the brief periods when mortality was scored in a dimly lit room.

#### RESULTS AND DISCUSSION

Populations of imported fire ants, held in the laboratory for 1 week, were exposed to the bait for 22 h in the dark prior to light exposure. Observation of the ants when presented with the bait, as well as inspection of the mortality data, indicates that the insects are immediately drawn to and readily accept this bait material. The acclimatization period of 1 week, where water was given to the ants but food was withheld, was used to both dissipate any shock effects due to field collection as well as to insure a more uniform feeding response. This energy stress due to food deprivation should not be considered too severe since mounds have been kept alive in the laboratory under these conditions for 6 weeks and longer.

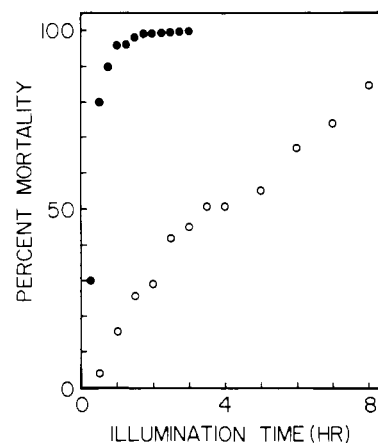


Figure 3. Comparison of length of acclimatization period on toxicity of the (phloxin B)-containing bait material. Imported fire ants were fed for 22 h in the dark on 0.30% (phloxin B)-containing bait after acclimatization of 7 days (○) and 14 days (●). Illumination ( $3800 \mu\text{W}/\text{cm}^2$ ) was from fluorescent light sources.

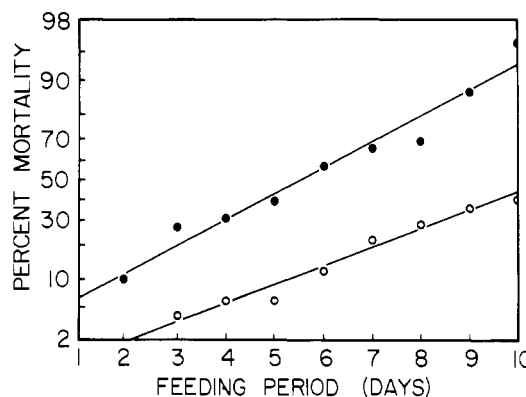


Figure 4. Light-independent mortality as a function of dark feeding period. Imported fire ants were fed continuously on a bait containing 0.30% phloxin B after an acclimatization of 1 day (○) and 6 days (●).

Figure 2 shows the resulting mortality as a function of light exposure time for three dye concentrations: 0.15, 0.30, and 0.60%. The efficiency of the bait increased as the concentrations of the phloxin B increased. Control mortality was insignificant for all illumination times and dye concentrations.  $LT_{50}$  values (defined as the time required to kill 50% of the insects) were calculated for the various baits: 0.15% phloxin B, 6.22 h; 0.30% phloxin B, 4.46 h; and 0.60% phloxin B, 2.30 h.

The efficiency of the bait was also studied as a function of the energy level due to food deprivation of the ants. Figure 3 depicts the observed mortality for ants after 1 and 2 weeks in the laboratory. The hungrier the insects are, or the lower the relative energy level, the more efficient the light-catalyzed lethal reaction. The increased efficiency is probably due to two specific factors: increased consumption of bait by hungrier insects and faster movement of the consumed dye across the gut membrane due to an energy stress in the insect. Dye levels in the ants could not be quantitated according to a published procedure (Broome et al., 1975b) due to the development of heterogeneous extracts. Evidently, the presence of soybean oil in the bait was responsible for this biphasic condition. Figure 4 shows the observed mortality as a function of dark feeding period for ants collected 1 day and 6 days prior to the initiation of the feeding period. These linear probit plots show that the  $LT_{50}$  values decrease with increasing acclimatization period i.e., decreasing energy level, as

**Table I. Mortality Observed in Imported Fire Ants Fed and Incubated in the Dark with a Phloxin B Impregnated Bait Formulation<sup>a</sup>**

Days after mound collection	Total insects	Percent mortality <sup>b</sup>		
		Feeding period, days		
		1	2	10
1	50	1	0	40
2	130	0	0	50
3	59	0	2	71
4	32	0	14	88
5	57	0	4	72
6	67	1	10	96
8	62	11	13	58
9	137	0	1	45
10	74	0	3	66

<sup>a</sup> Bait consisted of 850 parts corn cob grits, 147 parts soybean oil, and 3 parts phloxin B. <sup>b</sup> Uncorrected mortality was less than 10% after 10 days treatment for each group.

would be expected from the data of Broome et al. (1975b).

The light-independent lethal reaction was likewise investigated as a function of the energy level of the fire ants. Populations were removed from the mounds during the first 10 days after the insects were field collected and brought into the laboratory. Table I shows the observed mortality for fire ants exposed to the 0.30% phloxin B impregnated bait for 1, 2, and 10 days. For each population, there was less than 15% mortality after 48-h exposure to the bait. At 10 days exposure, there was a gradual increase in mortality over the first 6 days after mound collection after which a decrease was observed. The reason for the decrease in toxicity after the sixth day in the laboratory is unknown. A similar response was noted on ants fed dye on a sugar water medium (Broome et al., 1975b). The delayed toxicity which this represents satisfies one of the three criteria proposed for an effective fire ant bait (Stringer et al., 1964). This is the mechanism which would be depended upon to eliminate the queen(s) who lives in the deep recesses of the mound where light is minimal. The second toxicity mechanism (light de-

pendent) is much quicker acting. Mortalities are observed in terms of hours of light exposure. This will not affect the foraging of the worker ants until at least the second day. The observed mortality occurred in fire ants preincubated with the bait for 22 h in the dark. This allows the dye to be ingested in quantity and to be transported to critical tissues prior to the light exposure. In reality, the light-induced mortality occurred on the second day of exposure to the bait. Although this light-dependent mechanism will be effective in lowering the number of foraging worker ants in the colony, adequate foraging should be accomplished by those workers on their first day of contact with the bait.

These laboratory studies show that one of the xanthene dye sensitizers, phloxin B, can be incorporated into an acceptable bait for the control of the imported fire ant. The dye is ingested from this bait, penetrates to critical tissue, and causes mortality based on both the light-dependent and the light-independent mechanisms.

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## Sorption-Desorption of Parathion in Soils

P. A. Wahid and N. Sethunathan\*

Sorption of parathion by soils differing widely in their physicochemical characteristics was investigated employing radiotracer technique. Organic matter was the most important single factor affecting parathion sorption in soils. The relationship between parathion sorption and organic matter content of soils was logarithmic. In soils oxidized with H<sub>2</sub>O<sub>2</sub>, parathion sorption was correlated with clay and free Fe oxides. Similarly, inorganic soil constituents influenced parathion sorption in soils with <2% organic matter, but their role was apparently masked by organic matter at levels above 2%. Desorption studies revealed that parathion sorption in soils high in organic matter content such as Kari and Pokkali soils of Kerala, South India, was almost irreversible. An equation,  $\log x/m = \log [10.899 + 3.14 (\% \text{ organic matter})^2] + 1.05 \log c$ , developed from the relationship between Freundlich constant,  $k$ , and soil organic matter would assist in predicting parathion sorption in soils with known organic matter content.

Parathion (*O,O*-diethyl *O-p*-nitrophenyl phosphorothioate) is an important broad-spectrum organophosphorus insecticide. Persistence of this insecticide in soils was

reported to vary from a few weeks to several years (Sethunathan et al., 1977). Degradation of parathion in soil is mediated by both chemical (Adamson and Inch, 1973; Yaron, 1975) and biological (Sethunathan et al., 1975, 1977) reactions. The sorption-desorption pattern of a pesticide in relation to soil physicochemical characteristics would largely determine the amount of the chemical

\* Central Rice Research Institute, Cuttack-753006, Orissa, India.