

# Insecticidal Activity of Photoactive Dyes to American and Migratory Grasshoppers (Orthoptera: Acrididae)

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**ABSTRACT** Many photoactive dyes are relatively nontoxic to vertebrates despite their insecticidal properties. Several photoactive dyes known to be toxic to some groups of insects were evaluated at various concentrations for toxicity to American and migratory grasshoppers in laboratory and field studies. Rose bengal and phloxine B were effective at inducing mortality of grasshoppers when applied at 2 and 5% to bran bait, though erythrosin B and uranine were ineffective. Partial replacement of phloxine with uranine in dye mixtures resulted in no significant loss of efficacy. Some indication of feeding inhibition was observed at high dye concentrations, so minimum effective dosages, probably 2%, are optimal. Phloxine B and rose bengal appeared to be stable upon exposure to sunlight, and able to withstand at least 24 h of sunlight without significant degradation. Dyes such as phloxine B could be a viable grasshopper control agent for small or medium-sized grasshopper species because mortality can be induced by consumption of a single flake dusted with 5% dye, and yet pose little hazard to vertebrates. Large species such as American grasshopper must consume several flakes before mortality is induced.

**KEY WORDS** bait formulation, halogenated xanthene dyes, grasshoppers

HALOGENATED XANTHENE DYES, when ingested by insects, can rapidly become toxic to insects exposed to light. Light catalyzed chemical reactions affect enzymes, membrane lipids, and nucleic acids of insects, disrupting their metabolism. Even in the absence of light, some toxicity occurs. Interestingly, photoactive dyes are considered relatively safe to humans, and are commonly used in cosmetics and food products (Heitz 1995). Thus, photoactive dyes have the potential to form the basis for selective, low-toxicity insect control agents. Because they must be ingested to be effective, the selectivity afforded by bait formulations provides an additional measure of safety. Heitz (1987, 1995, 1997) provides recent reviews of the insecticidal properties of photoactive dyes.

Dyes such as sodium fluorescein, eosin yellow, erythrosin B, phloxine B, methylene blue chloride, rhodamine, and rose bengal have long been known to be toxic to insects. Beginning in 1928 with the observation that mosquito larvae are susceptible to this class of chemicals, researchers have demonstrated susceptibility by ants, caterpillars, flies, and cockroaches. Dyes differ greatly in their efficacy, and susceptibility varies considerably among insects. As part of a continuing effort to identify materials that can be used to suppress grasshopper numbers without harming humans and wildlife, we evaluated several dyes for their insecticidal properties to grasshoppers. Grasshoppers consume bran readily, and this affords a low cost, convenient means of applying toxicant-treated bait to grasshoppers.

## Materials and Methods

Bioassays were conducted using cultures of American grasshopper, *Schistocerca americana* (Drury), and migratory grasshopper, *Melanoplus sanguinipes* (F.). Laboratory cultures of these species have been maintained for many years on a diet of romaine lettuce and dry diet (soybean meal, wheat flour, and flaky wheat bran in a ratio of 1:1:2 by weight) as described by Henry (1985). The *S. americana* colony was started from field-collected grasshoppers 8 yr ago; the *M. sanguinipes* colony is a diapause-free strain obtained from the USDA Rangeland Insects Laboratory in Bozeman, MT, in 1982, and apparently in culture for >40 yr.

Relative toxicity of several xanthene dyes that had previously been shown to have potential for insect control were assessed under laboratory and field cage conditions. We tested erythrosin B, phloxine B, rose bengal, and uranine (Fisher) at various dye concentrations applied to flaky wheat bran and fed *ad libitum* under laboratory conditions to both species of grasshoppers. Mortality of adult grasshoppers and consumption of diet were the response parameters measured. The effective dyes, phloxine B and rose bengal, were similarly tested against American grasshoppers in field cages. We also assessed the degradation potential of sunlight on dye toxicity for up to 24 h for these same two dyes. Lastly, we assessed the ability of grasshoppers to discern between treated and untreated bran flakes, and determined the number of treated flakes that would cause mortality when consumed.

**Relative Susceptibility in the Laboratory.** Powdered phloxine B, rose bengal, and uranine were added to wheat bran flakes at concentrations of 5, 2, 0.5, 0.1, and 0% by weight for evaluation with *S. americana*, and all except the highest dosage for the smaller *M. sanguinipes*. The dry dye dust was mixed with the bran flakes until the dust was uniformly distributed over the flakes; no adjuvants were used to enhance adhesion or other characteristics of the toxicant. Erythrosin B was less toxic in preliminary tests, and therefore was evaluated at 15, 10, 5, and 2%. Unsexed adult grasshoppers were caged in 0.3 cubic meter cages for these tests, cultured at 30°C, and exposed to light from fluorescent and incandescent light bulbs for 14 h per day. We evaluated three replicates of 10 grasshoppers per cage, with different starting times (days) serving as the basis for replication, and all dye treatments tested on the same day. Relative toxicity was determined by making available to grasshoppers 3 g bran flakes to *M. sanguinipes* or 7 g to *S. americana* with one of the dye concentrations for 48 h, at which time mortality and bran consumption were determined. The grasshoppers were provided with no other food during the tests. Data were analyzed with one-way analysis of variance (ANOVA) and significant differences among treatments tested for with the Tukey–Kramer multiple comparison test ( $P = 0.05$ ) using InStat statistical analysis software (InStat 1993).

We also investigated the potential for mixing various proportions of the relatively expensive dye phloxine B, with the less expensive dye uranine. Some research has suggested that uranine synergizes phloxine B (Heitz 1995, Walthall and Stark 1999), allowing reduction in phloxine concentrations on bait with no loss in efficacy. Thus, we mixed several proportions of phloxine and uranine at ratios of 100:0, 87.5:12.5, 75:25, 50:50, 25:75, and 0:0 (untreated control), and applied the dye or dye mixture at 2% by weight to bran flakes. The bran flakes were fed to adult migratory grasshoppers in four replicate groups of 10 grasshoppers. The grasshoppers were housed under the aforementioned rearing conditions. Effects of the dye concentrations were assessed by determining mortality and bran consumption after 48 h. Data were analyzed with one-way ANOVA and significant differences among treatments tested for with the Tukey–Kramer multiple comparison test ( $P = 0.05$ ).

**Susceptibility in Field Cages.** The American grasshopper was tested for susceptibility under field conditions by caging grasshoppers outdoors in 1-m<sup>3</sup> hardware cloth cages where grasshoppers had a choice of feeding on dye-treated bran and lawn grass. The bran was presented in petri dishes on the floor of the cage, though the grasshoppers could also feed on leaf blades of bahiagrass, *Paspalum notatum*, which protruded into the cage. There were four replicate cages of 10 adult grasshoppers fed bran with 5, 2, or 0% phloxine B, or the same concentrations of rose bengal, with different starting time (days) serving as replicates. The tests were conducted during August 1998, and the number of dead grasshoppers was tabulated after 48 h. Data were analyzed with one-way ANOVA and sig-

nificant differences determined among treatments with the Tukey–Kramer multiple comparison test ( $P = 0.05$ ).

**Degradation of Dyes in Relation to Sunlight.** Degradation was assessed by evaluating the toxicity to, and bran consumption by, grasshoppers fed dye-treated bran which had been exposed to sunlight previously. Three g aliquots of bran flakes treated with 2% phloxine B or rose bengal were placed in a thin layer in petri dishes and exposed to outdoor direct sunlight conditions during July and August 1998 to generate light-exposed, dye-treated bran. Solar radiation was measured with an IL 1700 Research Radiometer/Photometer (International Light, Newburyport, MA). Exposure periods were 6, 12, and 24 h, after which the exposed bran flakes were fed to grasshoppers for 48 h. The 24-h exposure period was accomplished by exposing the bran to two consecutive 12-h periods of daylight, with the bran returned to the lab during the intervening evening to prevent consumption by scavengers. Grasshoppers were housed in 0.3 cubic meter cages, cultured at 30°C, and exposed to light from fluorescent and incandescent light bulbs for 14 h per day. Percent mortality and bran consumption were recorded for each of three replicate groups of 10 adult American grasshoppers fed bran exposed to sunlight, and an equivalent number of grasshoppers fed dye-free bran. We determined significant differences in mortality for each dye and time period with corresponding controls using Students *t*-test.

**Feeding Deterrent Effects of Dyes.** The ability of grasshoppers to discern various dye concentrations, and to avoid consumption of treated bran flakes, was assessed with dual choice tests because there have been reports of feeding inhibition associated with high concentrations (Heitz 1995). American grasshoppers were provided with two petri dishes, each containing 3 g of bran, and consisting of dye-treated bran and its corresponding untreated control. Phloxine B and rose bengal were tested at concentrations of 5, 2, 0.5, and 0.1%. Grasshoppers were housed in 0.3 cubic meter cages, cultured at 30°C, and exposed to light from fluorescent and incandescent light bulbs for 14 h per day during these tests. Each cage contained 10 unsexed American grasshoppers, and there were 10 replicate cages for each dye concentration and its corresponding untreated control. Replicates were based on different starting times (dates), and because the large number of tests were spread over a long period of time we used paired controls. Paired Students *t*-tests were run to determine if significant differences in treated and untreated bran consumption occurred for each dye and dye concentration.

**Effect of Bait Consumption Level on Mortality.** To determine how many flakes of dye-treated bran should be consumed to cause mortality, adult American and migratory grasshoppers were offered various numbers of treated bran flakes. Phloxine at 5% concentration was used for all treatments except the untreated control, and 48 h of exposure was allowed to elapse before mortality was tabulated. The grasshoppers were caged individually in 500-ml vented plastic

**Table 1.** Effect of ingested photoactive dyes on *S. americana* and *M. sanguinipes* grasshopper mortality and diet consumption during 48 h of exposure

Dye	Concn. (%) / ratio	Mortality (% ± SE)		Consumption (g ± SE)	
		<i>M. sanguinipes</i>	<i>S. americana</i>	<i>M. sanguinipes</i>	<i>S. americana</i>
Phloxine B	0	10 ± 6a	0 ± 0a	0.36 ± 0.05a	1.81 ± 0.54a
	0.1	13 ± 13ab	20 ± 11a	0.34 ± 0.10a	1.15 ± 0.09ab
	0.5	53 ± 24ab	60 ± 0b	0.25 ± 0.05a	0.81 ± 0.08ab
	2.0	90 ± 6b	90 ± 6b	0.25 ± 0.05a	0.59 ± 0.17ab
	5.0	—	87 ± 9b	—	0.53 ± 0.13b
Rose bengal	0	13 ± 7a	7 ± 7a	0.60 ± 0.05a	1.27 ± 0.28a
	0.1	66 ± 12b	53 ± 7b	0.38 ± 0.09a	1.10 ± 0.03b
	0.5	100 ± 0c	87 ± 9c	0.41 ± 0.06a	0.76 ± 0.03b
	2.0	96 ± 57bc	97 ± 3c	0.29 ± 0.07a	0.71 ± 0.08b
	5.0	—	100 ± 0c	—	0.57 ± 0.04b
Phloxine: uranine	100:0	75 ± 3a	—	0.38 ± 0.07ab	—
	87.5:12.5	57 ± 14ab	—	0.27 ± 0.02b	—
	75:25	47 ± 6ab	—	0.30 ± 0.07ab	—
	50:50	62 ± 11ab	—	0.28 ± 0.07ab	—
	25:75	37 ± 11ab	—	0.33 ± 0.07ab	—
	Untreated	10 ± 6b	—	0.59 ± 0.09a	—

Means ± SD followed by the same letter are not significantly different ( $P = 0.05$ ) by the Tukey-Kramer multiple comparison test. ANOVA statistics for *M. sanguinipes* fed phloxine B are  $F = 6.94$ ;  $df = 3, 8$ ;  $P = 0.013$  and  $F = 0.872$ ;  $df = 3, 8$ ;  $P = 0.495$ , respectively, for mortality and consumption. Statistics for *S. americana* fed phloxine B are  $F = 32.91$ ;  $df = 4, 10$ ;  $P < 0.001$  and  $F = 3.81$ ;  $df = 4, 10$ ;  $P = 0.039$ , respectively, for mortality and consumption. ANOVA statistics for *M. sanguinipes* fed rose bengal are  $F = 32.20$ ;  $df = 3, 8$ ;  $P < 0.001$  and  $F = 3.24$ ;  $df = 3, 8$ ;  $P = 0.081$ , respectively, for mortality and consumption. Statistics for *S. americana* fed rose bengal are  $F = 43.37$ ;  $df = 4, 10$ ;  $P < 0.001$  and  $F = 4.52$ ;  $df = 4, 10$ ;  $P = 0.020$ , respectively, for mortality and consumption. ANOVA statistics for *M. sanguinipes* fed various phloxine B/uranine mixtures are  $F = 5.63$ ;  $df = 5, 18$ ;  $P < 0.003$  and  $F = 2.84$ ;  $df = 5, 18$ ;  $P = 0.046$ , respectively, for mortality and consumption.

cups and held at 30°C in a room with fluorescent and incandescent light and 14 h of photoperiod daily. American grasshoppers were offered 0, 2, 4, 5, 6, 10, or 15 treated bran flakes. Migratory grasshoppers were offered 0, 1, 2, 3, 4, 5, 6, 10 or 15 treated flakes. Replicate treatment groups consisted of five individual grasshoppers exposed to the same treatment and started on the same day. Five to 10 replicates were completed for each bran flake treatment level. Grasshopper mortality and bran flake number data were analyzed by linear regression analysis.

## Results and Discussion

**Relative Susceptibility in the Laboratory.** Grasshopper mortality was positively related to dose of phloxine B and rose bengal (Table 1). In the case of *M. sanguinipes*, 90% mortality was attained after consumption of bran treated with 2% phloxine B, and 100 and 96% mortality was attained with 0.5 and 2% rose bengal, respectively. The larger *S. americana* grasshoppers also succumbed to the dyes, displaying 87–100% mortality at 2 and 5%. The wet and dry weights of *M. sanguinipes* were ≈0.30 and 0.10 g for males, respectively, with corresponding weights for females of ≈0.45 and 0.14 g. In contrast, wet and dry weights of male *S. americana* were ≈1.4 and 0.5 g, and female weights ≈2.5 and 0.7 g, respectively. The size of many pest grasshoppers is equivalent to or somewhat larger than *M. sanguinipes*, but rarely as large as *S. americana*. Thus, photoactive dye concentrations of 2–5% appear to be adequate for induction of mortality among most pest grasshoppers.

Bran consumption levels were inversely related to dye concentration for both species of grasshoppers, and for both phloxine B and rose bengal (Table 1).

However, the differences in bran consumption by *M. sanguinipes* were not significant. The differences in bran consumption appeared to be correlated with grasshopper mortality, but could also be caused by avoidance of treated bran if the dyes were detected by the grasshoppers. For this reason, we assessed the ability of American grasshoppers to differentiate between dye-treated and untreated bran (see below).

The effects of the other dyes tested were variable, but generally were not effective (data not shown). Uranine and erythrosin B alone failed to induce significant levels of mortality. Consumption of bran flakes was similarly unaffected by these dyes. When uranine and phloxine B were mixed in various proportions, there was no evidence of a reduction in grasshopper mortality as increasing amounts of uranine were substituted for phloxine B. Similarly, bran consumption was not greatly affected by the dye mixtures other than for diminution of consumption when phloxine B was present. Thus, one could make the case that lower cost uranine could be substituted for phloxine, as has been reported previously. However, more comprehensive studies should be conducted to verify the effectiveness of substituting uranine for phloxine B, because there was weak indication of a trend toward loss of efficacy as increasing proportions of uranine were incorporated. Research reportedly is under way to use mixtures of phloxine B and uranine in a bait formulation as a substitute for malathion in tephritid fruit fly suppression programs (Heitz 1995, Moreno and Mangan 1995).

**Susceptibility in Field Cages and Degradation of Dyes in Relation to Sunlight.** Phloxine B and rose bengal were effective at causing American grasshopper mortality ( $F = 22.89$ ;  $df = 2, 9$ ;  $P < 0.001$  and  $F = 24.55$ ;  $df = 2, 9$ ;  $P < 0.001$ , respectively) and at re-

**Table 2.** Percent mortality (mean  $\pm$  SE) of *S. americana* fed photoactive dyes in field cages

Dye concn. (%)	Rose bengal	Phloxine B
0	2.5 $\pm$ 2.5a	2.5 $\pm$ 2.5a
2	62.5 $\pm$ 8.5b	52.5 $\pm$ 8.5b
5	57.5 $\pm$ 7.5b	62.5 $\pm$ 7.5b

Means  $\pm$  SD followed by the same letter are not significantly different ( $P = 0.05$ ).

ducing consumption under field cage conditions (Table 2). As was observed in the laboratory, the 2 and 5% formulations gave equivalent levels of mortality, suggesting that the 2% level was adequate to cause significant levels of population reduction. The level of mortality was slightly lower under field conditions than observed in the laboratory. Light intensity outdoors was  $\approx$ 113,000 lux in full sunlight whereas in the laboratory it was 1,500–4,800 lux, depending on location within the cage. Because mortality rate tends to be proportional to light intensity (Yoho et al. 1973, Clement et al. 1980), we might have expected higher levels of mortality in the field cages than in the laboratory. However, maximum absorbance of these dyes is in the 540–560 nm range, and cool white fluorescent lights emit considerable light in this range. Thus, fluorescent lights actually are more efficient than sunlight in inducing toxicity (Heitz 1995). Also, the grasshoppers had to locate the dishes of bran in a much larger environment, and could elect to feed on grass growing through the bottom of the cage rather than on the bran flakes.

Photoactive dyes are reported to degrade after exposure to fluorescent light or sunlight (Carpenter and Heitz 1980). However, in this study there was no significant indication of dye degradation after exposure to natural sunlight for 6, 12, or 24 h ( $F = 2.26$ ;  $df = 2, 6$ ;  $P = 0.186$  for phloxine B;  $F = 0.40$ ;  $df = 2, 6$ ;  $P = 0.687$  for rose bengal). A loss of color intensity was noted as the red-tinted bran became pink. Mortality of grasshoppers fed 2% dye-treated bran displayed 70–100% mortality within the 48-h test period irrespective of light exposure period whereas their corresponding controls displayed negligible mortality (data not shown). Similarly, consumption of bait was lower in treated-bait replicates relative to untreated controls. These observations are consistent with the results of the field cage mortality study and indicate that the phloxine B and rose bengal dyes will persist for reasonable lengths of time under field conditions. Under grasshopper suppression program conditions, bait is administered at low rates and rarely persists for >24–48 h before it is consumed by grasshoppers or other scavengers. Therefore, although long-term persistence of the dyes apparently is not necessary, it is evident that the dye toxicant is relatively stable in sunlight. Rainfall was not evaluated, but we would expect some loss of efficacy under heavy rainfall conditions caused by damage to the bran bait as well as leaching of the dye.

**Feeding Deterrent Effects of Dyes.** We tested phloxine B and rose bengal to determine whether

**Table 3.** Consumption by *S. americana* of dye-treated and untreated wheat bran in dual choice tests

Dye	Dye concn. (%)	Consumption (g $\pm$ SE)		Statistics		
		Treated	Untreated	<i>t</i>	<i>df</i>	<i>P</i>
Rose bengal	0.1	0.46	0.64	0.96	4	0.390
	0.5	0.42	0.54	0.08	4	0.419
	2	0.38	0.49	6.29	4	0.003
	5	0.36	0.41	0.82	4	0.983
Phloxine B	0.1	0.74	0.63	0.02	4	0.983
	0.5	0.54	0.61	0.45	4	0.674
	2	0.44	0.58	1.16	4	0.309
	5	0.42	0.67	3.57	4	0.023

American grasshoppers would detect the presence of the powdered dye and avoid consumption. Although there was a trend for reduced consumption of treated bran (Table 3), only at higher dye concentrations were significant differences found. Thus, it is desirable to minimize the dose of toxicant both to reduce the cost of preparation and to reduce the likelihood of aversion by grasshoppers.

**Effect of Bait Consumption Level on Mortality.** If bran bait is scattered widely and at a low density, or if grasshoppers are extremely numerous and competing for the bran resource, the individual grasshopper may have opportunity to consume only a few particles of bran. Thus, it is important to know if such a small dose of toxicant is adequate to kill a high proportion of individual grasshoppers.

In the case of migratory grasshopper, mortality among grasshoppers fed untreated bran was  $\approx$ 5.7%, but even consumption of a single flake of treated bran caused mortality to increase to 60.0%. Consumption of two or four flakes increased mortality slightly, producing mean values of 68.9 and 70.0%, respectively. Exposure to greater numbers of flakes did not increase mortality further, and these small grasshoppers often did not consume >5 flakes within the 48 h of the tests. Regression analysis produced a positive linear relationship between percent mortality and number of bran flakes consumed ( $y = 16.2 + 16.7x$ ;  $r = 0.75$ ;  $P < 0.001$ ), but inclusion of data above four flakes resulted in a decreasingly significant relationship because, as noted above, mortality failed to increase.

For the larger American grasshopper, mortality was 11.4% in the population fed untreated bran, but was 35.0, 52.5, 66.7, 64.0, 76.7, and 83.0% when fed 2, 4, 5, 6, 10, and 15 dye-treated bran flakes, respectively. Thus, a greater number of bran flakes were required to cause high levels of mortality, but as in the case of migratory grasshopper, incomplete mortality was obtained. Regression analysis produced a significant linear relationship ( $y = 18.1 + 7.1x$ ;  $r = 0.74$ ;  $P < 0.0001$ ) between percent mortality and number of bran flakes when only data for 2–10 bran flakes was considered. Inclusion of the 15-flake data diminished the correlation coefficient and *P* values, suggesting nonlinearity of the data.

Many grasshoppers feed readily on bran flakes; Onsager et al. (1980a), for example, reported that migratory grasshopper consumed 15 flakes per hour over a

2-h feeding period. However, field and laboratory studies commonly report less than complete mortality among grasshoppers fed bait treated with toxicant. Onsager et al. (1980b) reported 63–90% mortality of grasshoppers treated with 1.96% carbaryl bait, and Mukerji et al. (1981) observed 45–91% mortality with dimethoate-treated bran. Some variation is because of significant differences among grasshopper species in their propensity to accept bait. Onsager et al. (1980b), for example, reported that among western species, members of the subfamilies Acridinae and Gomphocerinae (the slant-faced grasshoppers) consumed bait readily; members of the subfamily Melanoplinae (Cantopinae or spur-throated grasshoppers) consumed bait less readily; and members of subfamily Oedipodinae (banded-wing grasshoppers) generally accepted bait poorly. However, among grasshoppers that consumed insecticide-treated bait, mean mortality ranged from ≈60–90%, further suggesting that complete elimination of grasshoppers is unlikely by using bait treatments. The bran flakes used in these studies weighed ≈0.7 mg (range, 0.5–0.9 mg) so the amount of toxicant ingested by grasshoppers is small. Nevertheless, even ingestion of several flakes failed, in some cases, to induce mortality. There are several possible explanations for incomplete mortality, with individual variation in susceptibility or uneven treatment of bait with the toxicant among the most plausible for the studies reported herein. Under large-scale field conditions, however, unavailability of bait as a result of competition by grasshoppers for the relatively scarce bran resource is a major factor in causing less than complete mortality.

Photoactive dyes have long been known to affect insects, with rose bengal, erythrosin B, and phloxine B generally considered to be the most effective insecticidal agents, and rose bengal more effective than the other two compounds (Heitz 1995). Here we confirm that rose bengal and phloxine B are effective agents for suppression of grasshoppers when applied at 2 and 5% to bran bait, although with grasshoppers erythrosin B seems to be ineffective. The 2% concentration is probably optimal because of some avoidance of bait at the higher concentrations. We focused research mostly on phloxine B because it seems most likely to be registered because of interest in tephritid fruit fly suppression and because of its well-documented low level of toxicity to vertebrate nontarget organisms; there is much less environmental data on rose bengal. Phloxine B appeared to be stable upon exposure to sunlight, and to survive at least 24 h of sunlight without loss of insecticidal activity. Phloxine B could be a viable grasshopper control agent for small or medium-sized grasshopper species, although the requirement by such large species as American grasshopper that several flakes of bran be ingested for induction of mortality calls into question the practicality of using photoactive dyes for large species. Also, the dyes are somewhat soluble in water, tending to stain porous surfaces.

Thus, use of dye-treated bait might be limited to agricultural or waste areas, where clothing, homes and other items would not be discolored.

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