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Pesticidal Applications of Halogenated Xanthene Dyes

As the twentieth century comes to an end, a determined battle is being waged by those producing food and fiber, as well as those charged with protecting man and other animals from disease, to enhance the capability of humans to control insect, weed, fungal, bacterial, nematode and rodent pests. On the negative side, the pest targets are, in many cases, developing resistance to the chemical control methods employed against them. At the same time, peripheral environmental issues are restricting the application of other control measures. On the positive side, chemists and toxicologists are continuing to develop new chemical structures and strategies to replace those chemistries rendered ineffective by either resistance or environmental pressures.

This editorial focuses specifically on halogenated xanthene dyes as insecticides. These dyes have been known to possess light-dependent insecticidal properties since 1928 when Barbieri (2) first reported on the toxicity of members of this class of chemicals against mosquito larvae. Later, Schildmacher (12), using mosquito larvae, and Yoho *et al.* (13), employing house fly adults, confirmed these findings. These studies showed that insects which ingested the dyes, died when later illuminated by visible light. The work of Yoho *et al.* was really the watershed for developing sustained interest in research in this area. This is documented in a review (4). Two books (5,6) which include research reported at symposia on light-activated pesticides, held at two annual meetings of the American Chemical Society, are compilations of these efforts.

The structure of some xanthene dyes

Substitutions at positions A and B are as follows: A = I, B = C1, rose bengal; A = I, B = H, erythrosin B; A = Br, B = Cl, phloxine B; A = Br, B = H, eosin yellowish; A = H, B = H, fluorescein.

The primary toxic mechanism of action of the halogenated xanthene dyes involves the absorption of light by the photoactive agent. The energy of the absorbed photon of light causes an electron to rise to a higher orbital, thereby raising the dye to the first excited singlet state. For a xanthene dye such as fluorescein, which contains no large halogen atoms, the dye must then release the excess energy as heat or as a photon of light, which is referred to as fluorescence. The presence of the large bromine atoms in phloxine B allows for spin-orbit coupling, which enables the dye to move to the first excited triplet state by inverting the spin on the excited electron. Only when the dye is in the first excited triplet state can the energy of the absorbed photon be transferred to a ground state (triplet) oxygen. The oxygen molecule is thus raised to the first excited singlet state, whereupon it is called singlet oxygen, and the dye drops back to the ground singlet state. Singlet oxygen is an excellent oxidizing agent and attacks several targets in the cell (3). These can include amino acids at the active site of enzymes, unsaturated lipids in membranes, and/or nucleic acids.

A secondary mechanism of action for the halogenated xanthene dyes is light-independent (4). These dyes are somewhat toxic to insects even when the insects are held in the dark. The mechanism of this toxic reaction is not known, but it is definitely much less efficient than the light-dependent mechanism. Insects, having ingested certain levels of the dyes, can be killed within minutes in visible light but may not die for days in total darkness.

These dye molecules are very large, with molecular weights ranging between 400 and 1000 daltons. They also contain two negative ions at neutral pH. This makes it very difficult for these molecules to penetrate the insect cuticle and requires that the dyes be ingested by the target insect before the toxic mechanism can become effective. It can be well stated that the effectiveness of these dyes as insecticides is no better than the bait technology used to deliver the dyes to the insect.

There are three major positive features associated with the use of the photoactive xanthene dyes as insecticides. First, they are safe for humans. Phloxine B is registered by the U.S. Food and Drug Administration as D&C Red 27 and 28 (1); (D&C Red 28 is the disodium salt of D&C Red 27). It is used as a colorant in lipsticks and in drugs and has been consumed safely by humans in the United States for decades. The U.S. Food and Drug Administration has determined an acceptable daily intake of 1.25 mg/kg/day for phloxine B. By comparison, the Food and Agriculture Organization/World Health Organization allows an acceptable daily intake of 0.02 mg/kg/day for malathion. Second, the method of administration to insects is gustatory. Whereas many of the current insecticides are contact toxicants, the dyes must be consumed by the target insect for toxicity to occur (4). This provides a measure of protection for nontarget beneficial and predatory insects if the bait is designed to be nonattractive to such insects. Third, the halogenated xanthene dyes are photolabile when exposed to sunlight. Phloxine B in dilute aqueous solutions exposed to sunlight has been shown to have a half-life of hours or less, rather than days, weeks or months (7).

Within the last 4 years increased interest has been expressed by entomologists and toxicologists in the development of the halogenated xanthene dyes as new candidate insecticides. Much of this interest has coincided with the formation of PhotoDye International, Inc., which was founded with the express purpose of commercializing this technology. The efficacy of the dyes against more than two dozen insect species indicates that the xanthene dyes are serious candidates for development as commercial insecticides. At the present time there are two major products under development against various Tephritid flies and the imported fire ants (*Solenopsis richteri* and *S. invicta*). Beyond that, there are several other insect applications being investigated for future commercial utilization.

The Mediterranean fruit fly (*Ceratitis capitata*) is a serious pest of fruits over a large part of the world. In the United States, control of this insect utilizing malathion in aerially applied protein baits has been controversial. There is great interest in developing a replacement for malathion in this area. Research by Liquido *et al.* (10) in the U.S. Department of Agriculture at Hilo, Hawaii, has shown that phloxine B in similar protein baits is very promising as a control agent. Concomitantly, work by Mangan and Moreno (10) in the USDA laboratory at Weslaco, Texas, demonstrated similar promise for phloxine B against the Mexican fruit fly (*Anastrepha ludens*). The U.S. Environmental Protection Agency has awarded an Experimental Use Permit to the USDA for large-scale spraying against the Mediterranean and Mexican fruit flies. Initial large-scale field tests have been completed in Hawaii, California, Mexico and Guatemala. The efficacy of phloxine B, under the tradename SureDye^{TM}, in a protein bait was very promising. SureDye^{TM} was as good as, or better than, malathion in those tests where both active ingredients were compared (11). More field tests are planned for Mexico, Brazil, Texas and Hawaii.

In one of the recently completed field tests in Guatemala, aerial applications of phloxine B in a protein bait were used to control the Mediterranean fruit fly. As part of the test, it was found that SureDye^{TM} had no effect on honey bee (*Apis mellifera*) mortality (9). Subsequently, it was demonstrated that SureDye^{TM}-containing protein baits are harmless to the parasitic wasp *Diachasmimorpha longicaudata* and the honey bee in laboratory experiments (M.K. Hennessey and J.R. King).

Hilton Head Laboratories of Bluffton, South Carolina, is developing a bait for use against both imported fire ant species using phloxine B as the active ingredient. The bait contains vegetable oil on a corn cob grit base as the attractant for the foraging ants. The ants in the mound die within 4–6 days. It is quite possible that this product will be on the market by the summer of 1997 (W.H. Ball, personal communication, 1996).

As more laboratories have reported positive results with the xanthene dyes, interest has grown. Currently there are research projects conducted and/or interest expressed by scientists in the United States, Canada, Mexico, Guatemala, Australia, New Zealand, Austria, India, and Israel. Although interest in the xanthene dyes as commercial pesticides has arisen after a rather long latency period, it has, nonetheless, finally come about. The efficacy has been proven in many insect systems, the gustatory mechanism of action insures a product more protective of nontarget beneficial insects than the conventional insecticides, and the photolability promises a product with a limited life span in the environment. The photoactive xanthene dyes appear ready to take their place in the arsenal of control materials employed by those responsible for the management of certain pests around the world.

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