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# THE PHOTODYNAMIC EFFECT IN ROSE BENGAL-FED ADULTS OF DIFFERENTLY PIGMENTED STRAINS OF *DROSOPHILA MELANOGASTER*

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## ABSTRACT

The deteriorating effect of chemicals interacting with visible light (which in itself is normally harmless) on biological systems is called photodynamic action. Insect species susceptible to such phototoxic compounds include, ***inter alia,*** the house fly, ***Musca domestica,*** the face fly, ***Musca autumnalis,*** several ***Culex, Aedes*** and ***Anopheles*** mosquito spp., the boll weevil, ***Anthomonus grandis,*** the black imported fire ant, ***Solenopsis richteri,*** and several species of cockroaches and of Lepidoptera. Many of these photosensitizers are dyes; among those, the ones most frequently investigated were xanthenes, such as rose bengal, phloxin B, erythrosin B, eosin yellowish, fluorescein, rhodamine B and rhodamine 6G. Orally ingested rose bengal induced a severe light-dependent toxicity in ***Drosophila melanogaster*** adults. The wild strain G and two light coloured strains, ***y*** (yellow) and ***stw*** (straw), were much more susceptible to the photodynamic effect than the heavily pigmented strains ***b*** (black) and ***e*** (ebony), the k.d.-$T5_0$'s being 45 min for G, 50 min for ***y*** and ***stw,*** 1.5 h for ***b,*** and 2 h for ***e.***

KEY WORDS: Photodynamic action, phototoxins, photosensitizers, xanthenes, ***Drosophila melanogaster*** adults, ***Drosophila*** mutants differently pigmented, response to rose bengal.

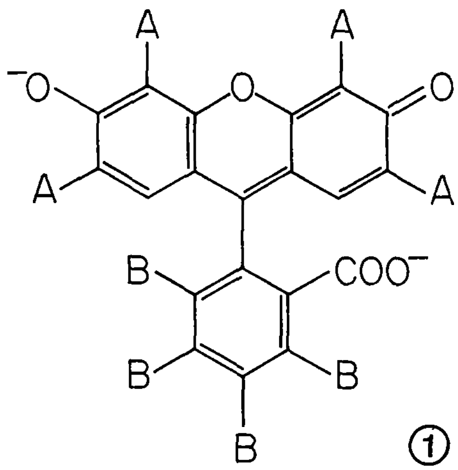
## INTRODUCTION

Apparently, the first findings of sunlight enhancing the toxic effect of chemicals were reported by Marcacci (1888) with alkaloids, against seeds, plants, on fermentation, and against amphibian eggs. In 1897/1898, Raab (1900; see also v. Tappeiner, 1900, 1901) discovered the poisoning of paramecia by the combination of a dye (acridine) and light. Subsequently, the term photodynamic action was suggested by v. Tappeiner and Jodlbauer (1904) for this phenomenon. During the five years following Raab's first publication, the deteriorating effect of a dye or a pigment interacting with visible light (330-770 nm, which in itself is normally innocuous) on various biological systems was studied by several groups (Jacobson, 1901; v. Tappeiner, 1900, 1901, 1904; Ledoux- Lebard, 1902; Dreyer, 1903; Raab, 1903; v. Tappeiner and Jesionek, 1903; Halberstaedter, 1904; Jodlbauer and v. Tappeiner, 1904; Neisser and Halberstaedter, 1904; v. Tappeiner and Jodlbauer, 1904). An early book on the phenomenon of phototoxicity was written by Blum (1941).

A structurally diverse group of compounds consisting of phytochemicals has biocidal activity in the presence of sunlight or long wavelength UV (UVA) radiation (320-400 nm) exclusively.

It is accepted today that photodynamic substances, in the presence of oxygen and light of appropriate wavelength and intensity, bring about the formation of either toxic free radicals (type I mechanism) or of 'singlet oxygen' (type II mechanism), a highly activated and toxic form of oxygen, or both.

The first insects studied with phototoxic dyes were mosquito larvae (Barbieri, 1928; Sch ldmacher, 1950), followed from the beginning of the seventies by further insect species

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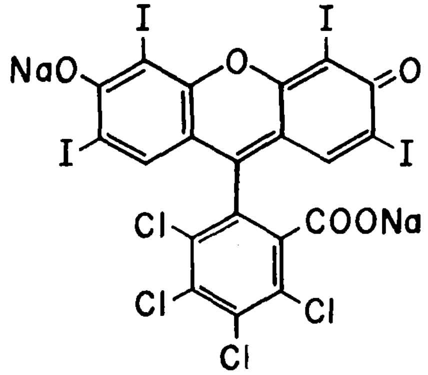


ROSE BENGAL ②

Fig. 1. The structure of some xanthene dyes. Substitutions at positions A and B are as follows: A = I, B = Cl is rose bengal; A = I, B = H is erythrosin B; A = Br, B = Cl is phloxin B; A = Br, B = H is eosin yellowish; A = H, B = H is fluorescein.
Fig. 2. The photodynamic dye rose bengal.

susceptible to such lethal dye-sensitized photooxidation reactions. The dyes employed most frequently in these studies belonged to the halogenated fluorescein family of the xanthenes (Fig. 1), such as rose bengal (Fig. 2), the subject of the present paper; phloxin B; erythrosin B (registered as 'Synerid' or 'Intercept' house fly larvicide in caged layer facilities; a combination of 70% erythrosin B and 30% sodium fluorescein was labelled 'Synerid 100'); eosin yellowish; fluorescein; tetrachlorofluorescein; and rhodamine B or rhodamine 6G. The majority of the studies with xanthenes were conducted with Diptera:

*Musca domestica*, the house fly (*vide* Yoho et al., 1971, 1973, 1976; Yoho, 1972; Fondren et al., 1978; Fondren and Heitz, 1978b, 1979; Heitz, 1979; Pimprikar et al., 1980a,b; Jordan and Smith, 1981; Respicio and Heitz, 1981, 1983, 1986; Crounse and Heitz, 1982; Sakurai and Heitz, 1982; Carpenter et al., 1984a; Pimprikar and Heitz, 1984; Meyer et al., 1985, 1986; Robinson and Bentson, 1985; Wages, 1985; Meyer and Bradley, 1986; Koehler and Patterson, 1986; Wages and Heitz, 1987).

*Musca autumnalis*, the face fly (Fairbrother, 1978; Fondren and Heitz, 1978a; Fondren et al., 1978; Heitz, 1979; Fairbrother et al., 1981, 1982).

Erythrosin B did not reduce populations of the beneficial insect *Hermetica illucens*, the black soldier fly, in chicken manure (Pimprikar et al., 1980a).

Among mosquitoes, studies with photodynamic dyes were conducted on *Anopheles* and *Culex* spp. (Barbieri, 1928, see above); *Aedes aegypti* (Schildmacher, 1950, see above; Barbosa and Peters, 1970a,b; Bridges et al., 1977); *Aedes triseriatus* (Pimprikar et al., 1979, 1984; Carpenter et al., 1981); *Anopheles superpictus* (Schildmacher, 1950, see above); *Culex pipiens quinquefasciatus* (Pimprikar, 1979; Carpenter and Heitz, 1980, 1981; Carpenter et al., 1984b, 1985; Pimprikar et al., 1984; Respicio et al., 1985).

The following further orders were studied as to the photoxic effects of xanthene dyes:

The Hymenoptera were represented by the black imported fire ant, *Solenopsis richteri* (Broome et al., 1975a,b; David and Heitz, 1978) and the red imported fire ant, *S. invicta* (Lemke, 1986; Lemke et al., 1987).

The Coleoptera were represented by the boll weevil, *Anthonomus grandis* (Callaham et al., 1975a,b, 1977a,b; Broome et al., 1976; Fondren et al., 1978).

Among the Orthoptera several cockroach species were investigated: *Blatta orientalis* (Weaver et al., 1976); *Periplaneta americana* (Weaver et al., 1976, 1982); *Blattella germanica* (Ballard et al., 1988); *Supella longipalpis* (Ballard et al., 1988).

Among the Lepidoptera studied were *Agrotis ipsilon* (Clement et al., 1980), *Diphania nitida, Helicoverpa (Heliothis) zea,* and *Trichoplusia ni* (Creighton et al., 1980).

There are several hundred compounds that have been investigated as photosensitizers. They consist of a large number of dyes apart from the xanthenes: acridines, thiazines (such as methylene blue), azines, anthraquinones, synthetic porphyrines, and phthalocyanines. The second large group consists of natural products, especially phytochemicals, such as thiophenes, polyacetylenes, furanocoumarins, alkaloids, iron-free porphyrins, flavins, quinones, etc.

Among the dyes, apart from the xanthenes, the phototoxic properties of methylene blue were studied relatively often. Methylene blue, as just stated, is a quinone-imine of the thiazine class and can be considered a derivative of phenothiazine. Early studies on its toxic effects against insects, before the role of light as a factor influencing toxicity was recognized, were reviewed by Barbosa and Peters (1971). Its photodynamic effects on insects in conjunction with light were demonstrated, *inter alia,* with the Lepidoptera *Laspeyresia (Cydia) pomonella* (Hayes and Schechter, 1970), *Pieris brassicae* (Lavialle and Dumortier, 1978; Lavialle, 1983), the coleopteran *Tenebrio molitor* (Graham et al., 1972), the dipteran *Musca domestica* (Yoho et al., 1973) and a homopteran, the aphid *Myzus persicae* (Lavialle, 1983).

The potential use of photosensitizers as insecticides was first discussed by Graham (1963, 1972). The development of dyes, especially halogenated xanthenes, as phototoxic insecticides was reviewed progressively by Heitz and Wilson (1978), Heitz (1982), Robinson (1983), Heitz (1987), Pimprikar and Coign (1987), Weaver (1987), and by Lemke et al. (1987) as regards field experimentation and development of the photopesticides.

As to reviews on photoactivated insecticidal phytochemicals, especially the biosynthetically-produced, naturally occurring polyacetylenes (polyines) and their thiophene derivatives (as well as some of their synthetic analogues), the reader is referred to Arnason et al. (1987, 1988, 1989) and to the recent book edited by Lam et al. (1988). Photoactivated biocides from higher plants in general were discussed, *inter alia,* by Arnason et al. (1983), and the role of natural photosensitizers in plant resistance to insects by Downum (1986). Different phototoxins, all of them phytochemicals, were reviewed in a symposium edited by Berenbaum (1986). Finally, the lectures of a comprehensive symposium on light-activated pesticides in general, both dyes and phytochemicals, with the main emphasis on insecticides, followed by phototoxic herbicides and fungicides, were published in a book edited by Heitz and Downum (1987).

During the last 15 years, the common fruit fly, *Drosophila melanogaster,* has become quite a problem as a pest of grapes in Israeli vineyards. Sprays with conventional insecticides, e.g., with chlorpyrifos ('Dursban'), have lately failed to control it. In a search for alternative control methods, the photodynamic effect of one of the most active xanthene dyes, rose bengal, on adults of this insect was investigated. Preliminary results on this study were reported in a lecture at St. Gallen, Switzerland (Ascher, 1981).

## MATERIALS AND METHODS

### Rearing of *Drosophila melanogaster* strains

*Drosophila* was reared on an artificial diet which was prepared in the following way: 50 g 'Difco Bacto Agar' or simple bacteriological agar is dissolved in 800 ml water by heating and 800 g of fresh baker's yeast is introduced with constant stirring; the mixture is boiled and removed from the hotplate. Then 40 ml glacial acetic acid is stirred into the mixture, which is finally poured into half-liter Erlenmeyer flasks, about 200 ml per container. After cooling, the flasks, into which a fluted filter paper is stood upon the medium to absorb surplus moisture, are infested with *Drosophila* adults which are allowed to lay eggs for two days before being removed from the flasks.

The following strains were grown:

(i) A field-collected strain from vineyards in the coastal plain of Israel (originally collected by the late E. Gurevitz; henceforth called strain G) and thence reared for many generations in the laboratory as described above.

The following four mutants were obtained through the courtesy of Prof. R. Falk, Department of Genetics, The Hebrew University of Jerusalem, Jerusalem, Israel.

(ii) *y*: yellow (*vide* Lindsley and Grell, 1944, p. 281). "Phenotype: body colour yellow; hairs and bristles brown with yellow tips. Wing veins and hairs yellow. Tyrosinase formed in adults (Horowitz). Larval setae and mouth parts yellow to brown; hence distinguishable from the dark brown wild type. RK1."

(iii) *stw*: straw (*loc. cit.*, p. 240). "Phenotype: hair colour yellowish, especially on legs. Bristles pale at tips. Heterozygous for *stw* produces paling of body colour. RK2."

(iv) *b*: black (*loc. cit.*, p. 22). "Phenotype: black pigment on body and tarsi and along wing veins, darkening with age. Heterozygote shows somewhat darker trident, but it is never confused with homozygote. Puparium usually somewhat lighter than wild type and newly emerged flies not clearly distinguishable from wild type. Tyrosinase formed in adult (Horowitz). RK1 in aged flies."

(v) *e*: ebony (*loc. cit.*, p. 82). "Phenotype: body colour shining black. Puparia much lighter than in wild type. Classifiable throughout larval period by darkened colour of spiracle sheaths. Viability lowered to about 80 percent wild type. Heterozygote has slightly lighter colour than normal."

Strain G could be cultured well at 27°C, whereas cultures of strains *stw*, *y*, *e* and *b* succumbed after one or two generations at this temperature and, therefore, had to be grown at a lower temperature; 24°C was found to be adequate.

### Feeding the dye to the flies

Adults of *Drosophila*, 1–2 days old, were transferred, through shaking, from the culture flasks to glass jars which were then cooled in crushed ice. The immobilized insects were introduced with the help of a fine-hair brush into 175-ml foamed polystyrene (Polybid Co., Mishmar haNegev, Israel) drinking cups. These were covered with pieces of transparent nylon sheets which were fastened with rubber bands and perforated with a pin to ensure ventilation.

Drinking solutions were offered in the following way: a 2.5-cm-diam plastic screw cap (of scintillation vials) was pressed on the lower half of the outer wall (near the bottom) of the drinking cup and with a suitable needle the cap's circumference on the cup was perforated; thus a hole was formed into which the screw cap fitted tightly. Cotton wads were soaked in the various test and control solutions and were pushed into the screw caps. The latter were then inserted into the holes in the cups and thus drinking fluids were made available to the insects and could be renewed or exchanged easily without previously immobilizing the insects in the cup.

Solutions of rose bengal (C.I. 45440; C.I. acid red 94) [Bengal rose (Fluka)] in distilled water (we had found earlier that high concentrations of rose bengal, such as 5 g/l, did not dissolve completely in our tap water) and containing 5% sucrose were offered for 18 h to flies in darkness in an incubator kept at 27°C.

### Transfer of flies and their exposure to artificial light

The cups with the flies were taken out of the incubator in a room in which the lights had been turned off and the blinds had been closed. The screw caps were exchanged for other ones containing cotton wads soaked in 5% aqueous sucrose only and the cups were placed in a black closed box. These precautions to exclude light during the transfer of the flies from darkness to artificial light had to be taken because rose bengal-fed *Drosophila* adults turned out to be extremely susceptible to light after they had been fed high dye concentrations and in an undarkened room knock down occurred soon after the flies had been removed from the incubator. The flies were then exposed to artificial light from two 60-cm-long 20-W fluorescent tubes (General Electric, cool white), placed 18 cm above the experimental table, for 5 h or more. Light intensity, measured with a Trilux photometer (Gossen & Co. GmbH, Erlangen, Federal Republic of Germany) at the height of the rim of the plastic cups, was 4500 lx (=418 fc) at the center of the illuminated area and 3500 lx (=325 fc) at its circumference. Temperature was maintained constant at 25°C.

Counts of knock down of the flies were taken at 5-min intervals from the start of exposure to the artificial light for 15 to 20 min, and later on, according to the progressive knock down of the flies, at 10-, 15- and 30-min intervals.

The accompanying control experiments consisted of flies fed 5% sucrose solution only (without dye) and then exposed to light, and of flies fed the dye solutions and left in the dark incubator. In the rare cases in which there was a low mortality in these controls exposed to light, it was corrected for by Abbott's (1925) formula.

The standard strain G was assayed at different concentrations of rose bengal, whereas the response of the light coloured (*y*; *stw*) and dark (*b*; *e*) strains was compared at the 0.1 g/l concentration of the dye.

Each experiment consisted of four replications per concentration, with 25 flies per replication. The experiments with strain G at different dye concentrations were repeated up to eight times and the comparative experiments with the different strains at 0.1 g dye/l were repeated up to 11 times.

## RESULTS

Results obtained with the wild strain G fed different concentrations of rose bengal for 18 h and subsequently exposed to artificial light for 5 h or more are given in Table 1, which shows that

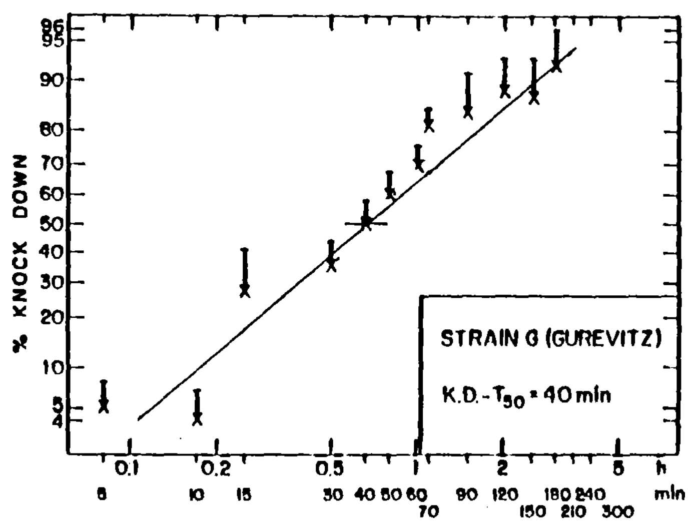


Fig. 3. Knock down caused by exposure to artificial light of strain G adults of *Drosophila melanogaster* fed 0.1 g/l rose bengal in 5% aqueous sucrose solution in the dark for 18 h. Data presented are means ± S.E.

TABLE 1

Knock down caused by exposure to artificial light of *Drosophila melanogaster* adults (strain G) fed various concentrations (ranging from 0.01 to 5.0 g/l) of rose bengal in 5% aqueous sucrose in the dark for 18 h. Data presented as means ± S.E.

| Dye concentration in aqueous 5% sucrose solution, g/l | Mean % knock down, in min of exposure to light after feeding | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 30 | 40 | 50 | 60 | 70 |
| 5.0 | 100% knock down immediately on exposure to light | | | | | | | | |
| 1.0 | 79.6 ± 5.2 | 89.3 ± 3.0 | 91.9 ± 2.8 | | 97.0 ± 2.9 | | | 97.2 ± 1.2 | |
| 0.5 | 15.4 | 38.0 | 63.3 | 83.6 ± 10.4 | 100 | 100 | | 98.5 | |
| 0.25 | | 16.0 | | 52.0 | 88.0 | 94.6 | 97.3 | 98.6 | 98.6 |
| 0.1 | 5.3 ± 2.7 | 4.0 ± 2.8 | 27.2 ± 14.5 | | 35.1 ± 9.6 | 50.7 ± 8.3 | 60.8 ± 8.2 | 70.0 ± 5.8 | 81.4 ± 4.2 |
| 0.05 | 0 | | | 0 | | 0 | | 11.3 ± 3.3 | |
| 0.01 | no kill at all after 450 minutes | | | | | | | | |
| | 90 | 120 | 150 | 180 | 210 | 220 | 240 | 300 | 350 |
| 1.0 | | 98.4 ± 0.9 | | 97.8 ± 0.9 | | | 98.4 ± 0.7 | 98.7 ± 0.8 | |
| 0.5 | | 98.5 | | 100 | | | | | |
| 0.25 | 100 | 100 | | | | | | | |
| 0.1 | 84.0 ± 7.0 | 87.9 ± 4.8 | 87.5 ± 6.1 | 97.7 ± 5.2 | 100 | 99.0 | 94.8 ± 3.6 | | |
| 0.05 | | 37.9 ± 6.5 | | 49.6 ± 5.7 | | | 68.0 ± 3.4 | | 68.2 ± 0.2 |

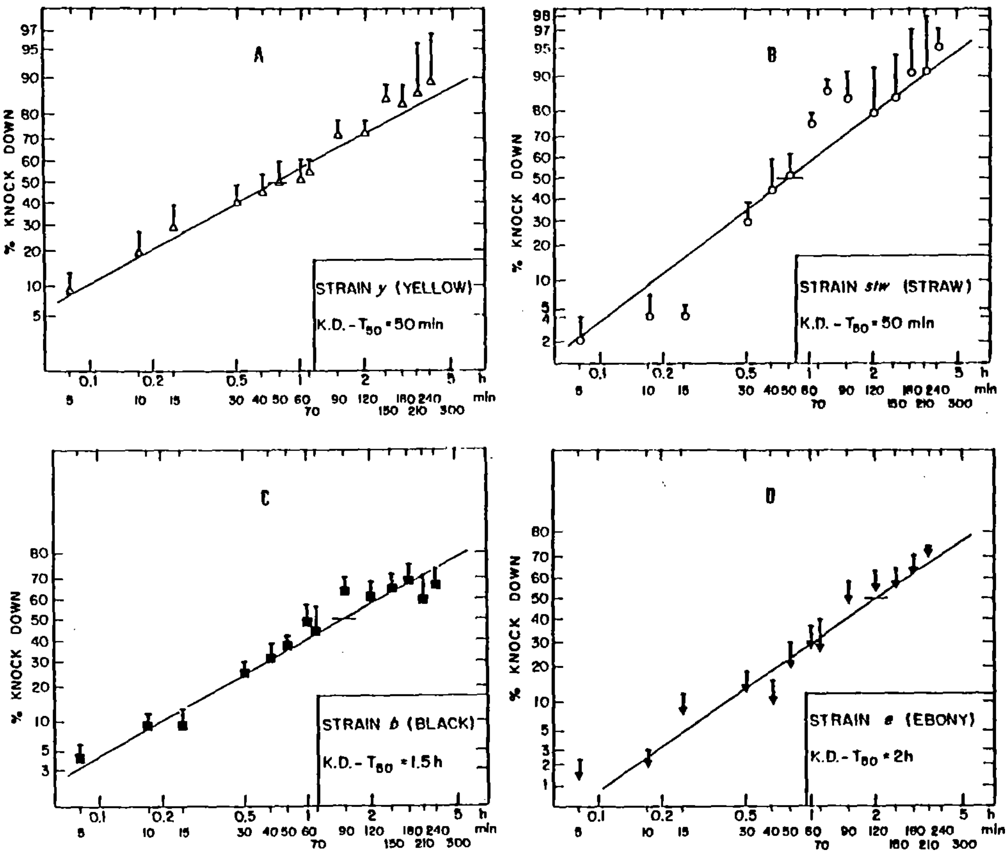


Fig. 4. Knock down caused by exposure to artificial light of adults of various strains of *Drosophila melanogaster* fed 0.1 g/l rose bengal in 5% aqueous sucrose in the dark for 18 h. A = strain *y* (yellow); B = strain *stw* (straw); C = strain *b* (black); D = strain *e* (ebony). Data presented are means ± S.E.

exposure to light, after the flies had fed on 5 g/l rose bengal, was immediately lethal. With 1.0 g/l and 0.5 g/l full knock down was already reached after 0.5 h. With 0.1 g/l full knock down set in after 3.5 h, whereas 0.05 g/l was much less active, with no complete kill being reached within 6 h; with 0.01 g/l no kill was obtained even after 7.5 h. It thus seems that the concentration at which knock down occurred after a satisfactory length of time as regards observations, was 0.1 g/l; it served, therefore, as the comparative concentration for all strains tested.

The data for knock down with 0.1 g/l in strain G plotted in Fig. 3 indicate a k.d.–$T_{50}$ of 40 min. Analogous experiments with the two light-coloured strains, strain *y* (Fig. 4A) and strain *stw* (Fig. 4B), yielded a k.d.–$T_{50}$ of 50 min in both strains. On the other hand, the two heavily pigmented, dark strains *b* (Fig. 4C) and *e* (Fig. 4D) gave k.d.–$T_{50}$'s of 1.5 and 2 h, respectively.

In the controls accompanying every experiment, which consisted of flies fed only a 5% sucrose solution and then exposed to light and of flies fed the dye solution in the dark and not exposed to light, mortality was nearly always zero or negligible; for the few exceptions, Abbott's correction (see above) was used.

## DISCUSSION

There is comparatively little work with *Drosophila* and phototoxic compounds. Matoltsy and Fábián (1946, 1947) reared *D. melanogaster* larvae in the dark from the egg stage on diets into which the cancerogenic substances 3,4-benzpyrene, methylcholanthrene and 1,2,5,6-dibenzanthracene, all of which show fluorescence in UV light, had been incorporated. Treated and control 3rd-instar larvae were irradiated with UV light from a quartz lamp. All three compounds exhibited marked phototoxicity: benzpyrene-treated larvae perished with a sixth of the UV radiation dose required to kill the controls, the methylcholanthrene-treated with a third, and the dibenzanthracene-treated with a half of the control dose. Kagan and Chan (1983) and Kagan et al. (1983b) showed that both the polyacetylene phenylheptatriyne and alpha-terthienyl (alpha-T) were ovicidal for *D. melanogaster* in the dark. Irradiation by UVA-light enhanced their ovitoxicity by 37- and 4333-fold, respectively. 8-Methoxypsoralen (8-MOP) had no ovicidal effect in the dark, but with UVA-light it became active at high concentrations. The ovicidal effect against eggs of *D. melanogaster* of a further polyacetylenic compound, *cis*-dehydromatricaria ester (Kagan et al., 1984), was enhanced by UVA-light. Kagan et al. (1983a) investigated the phototoxicity of some 1,3-butadiynes and related thiophenes also against larvae of *D. melanogaster*; 8-MOP was not photoactivated against this stage.

Regarding photosensitizing effects of dyes on *D. melanogaster*, David (1955) found that the photodynamically active methylene blue (see above) affected gametogenesis in this insect. Later David (1963) observed that this dye retarded growth dramatically (from 17 to 400 h) and reduced to 25% the fecundity of *D. melanogaster* females. However, it is difficult to evaluate these two early papers in the present context, since light intensity was not monitored or controlled. Yoho et al. (1971) stated that "in 1966, C.K. Dorsey of West Virginia University (unpublished data) observed high mortality in *Drosophila* that were fed fluorescent dyes and exposed to light." Apart from this short notice we did not find any reference as to the photodynamic effect of xanthenes on *Drosophila*. There is little doubt from the present work that *Drosophila melanogaster* adults are more susceptible to the photodynamic effect of rose bengal than other insects, e.g., the house fly (Yoho et al. 1973, 1976: Ascher and Nemny, unpublished data). The immediate or nearly immediate knock down on exposure to light of *Drosophila* adults fed higher concentrations of rose bengal serves also as evidence of the extremely high susceptibility of this insect to rose bengal.

Heitz (1982) stated that when comparisons could be made, adults of the imported fire ant, the boll weevil, the house fly and the face fly showed the same general range of susceptibility to the series of xanthene dyes. Although the adult stage of the boll weevil was much less suceptible than the larval stages of mosquitoes, he conjectured that these results were probably due to factors other than pigmentation. The higher susceptibility of light coloured strains of *Drosophila* points, however, towards the possibility of employing the photodynamic effect with profit mainly in transparent or lightly pigmented insect species, though not all of these are bound to be susceptible.

Deposition of melanin is an important, though not the exclusive, cause of dark coloration in many insects; the other one is the tanning process, the protein–quinone crosslinkage involved in sclerotization. Analogously with our findings, Berenbaum (1987) showed that black-mutant last-instar larvae of the tobacco hornworm, *Manduca sexta*, were much more susceptible to the phototoxic effect with UV light of topically applied xanthotoxin (8-methoxypsoralen; 8-MOP), than normally pigmented larvae of this insect. It is of considerable interest, as argued by Berenbaum, that *M. sexta* feeds on the foliage of the tomato, *Lycopersicon esculentum*, which contains (Méndez and Brown, 1971) the phototoxic furanocoumarin bergapten (5-methoxypsoralen; 5-MOP). Berenbaum (1987) infers that "melanin, then, appears to confer protection against the photoactivation of furanocoumarins by UV light and such protection may account for the persistent presence of melanic individuals in some insect populations."

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