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The photoovicidal activity of plant components towards *Drosophila melanogaster*[1]

J. Kagan and G. Chan

Department of Chemistry, University of Illinois at Chicago, P.O. Box 4348, Chicago (Illinois 60680, USA), June 18, 1982

Summary. Phenylheptatriyne, alpha-terthienyl, and 8-methoxypsoralen are 3 natural products which, in combination with long wavelength ultraviolet light (UVA), prevent the eggs of *Drosophila melanogaster* from hatching. Both phenylheptatriyne and α-terthienyl display ovicidal activity in the dark as well, but the irradiation step increased it 37- and 4333-fold respectively. The photoovicidal activity of 8-MOP was $\frac{1}{10}$ that of PHT. This new type of activity for natural products perhaps contributes to the natural protection of plants from insects.

A simple 'Drosophila test' was reported as a bioassay for insecticide activity in plant extracts, which disclosed that several naturally occurring polyacetylenic molecules had ovicidal activity toward *Drosophila melanogaster*[2-4]. The use of this test has now been extended to the determination of photoovicidal activity, and the feasibility of this method of insect control is illustrated with 3 naturally occurring molecules, phenylheptatriyne (PHT), alpha-terthienyl (αT, 2,2′; 5′, 2″-terthiophene), and 8-methoxypsoralen (8-MOP).

We are not aware of any previous reports on the photosensitized inhibition of the development of eggs in insect species, although short wavelength UV light alone can have this effect[5]. In our experiments, the light source had little emission in the short wavelength range, and most of it was removed by the pyrex filter used. The ovicidal activity of PHT had already been reported, but without the UV light treatment[4]. Both PHT and αT are found in plants of the family Compositae, while 8-MOP and other furanocoumarins are widespread in the families Umbelliferae, Rutaceae, Leguminosae, and Moraceae. All 3 compounds are well known sensitizers[6], particularly 8-MOP, for which a number of medical applications have been suggested[7]. This last compound has also been the topic of studies concerning plant-insect relationships, but the problem of specific toxicity to eggs was not discussed[8,9].

Materials and methods. An alcohol solution of sensitizer (20 µl) was added to a Whatman No. 1 filter paper disc, 1.5 cm in diameter. The solvent was evaporated, and the disc was saturated with distilled water. On a disc, a concentration of 11.5 µg/cm^2 corresponded to a sensitizer concentration of 1 g/l. Eggs less than 4 h old were planted on the discs in a darkroom dimly lit through amber Kodak OC Safelight filters. The eggs were incubated in total darkness, and the results provided the dark controls for the experiments in which the eggs were handled identically, except for a 45-min irradiation with long wavelength UV light (UVA) starting 1 h after the beginning of the incubation. The discs containing 10–40 eggs were in a petri dish covered with pyrex during the irradiations with a bank of 8 low-pressure tubes which had maximum emission at 350 nm (No. RPR-3500A, Southern New England Co, Hamden, Conn.). They were mounted horizontally 5 cm apart, 8.9 cm above the paper discs. At the surface of the discs, the light intensity was 13 J/m^2 · sec, as measured with a Yellow Springs Instruments radiometer Model 65A. The viability of the eggs decreased by less than 10% upon irradiation under the same conditions in the absence of added chemicals. No increased ovicidal activity was observed when the eggs were incubated in the dark over paper discs irradiated after addition of the chemicals. All the experiments were repeated at least 10 times with each compound at each concentration.

8-MOP was purchased from Sigma Chemical Co, PHT and αT were synthesized by Drs J.-P. Beny and S.N. Dhawan in our laboratories.

Results. The results illustrated in the figure indicate that all 3 compounds tested possessed photoovicidal activity toward *D. melanogaster*. In the dark, both PHT and αT displayed ovicidal activity with LD_{50} values corresponding to sensitizer concentrations of 0.13 and 1.26 g/l respectively. The value for PHT corresponds to 1.5 µg/cm^2, and is in good agreement with the published number of 2 µg/cm^2 (Nakajima and Kawazu[4]). In contrast, 8-MOP did not show any ovicidal activity in the dark at concentrations up to 10 times greater.

The increase in activity observed when the eggs were exposed to UV light for a short fraction of their incubation period was dramatic. Taking the concentration of sensitizer solution required for killing 50% of the eggs as the criterium, the activity of PHT was increased 37 times by the exposure to the UV light, from 0.13 to 0.0035 g/l (or from 1.5 to 0.4 µg/cm^2). With αT, the LD_{50} corresponded to a change from 2.6 to 0.0006 g/l (or from 30 to 0.007 µg/cm^2), or an increase in sensitivity greater than 3 orders of magnitude.

Finally, the photoovicidal activity of 8-MOP under the same conditions had a LD_{50} corresponding to a sensitizer concentration of 0.035 g/l (0.4 µg/cm^2). Although 10 times greater than that of PHT, this value is lower than found with either PHT or αT in the dark.

The relationship between the age of the eggs and the timing, duration, wavelength, and intensity of the UVA treatment remains to be determined. While most of the useful phototoxic properties of 8-MOP have been based on its ability to modify DNA, exceptions are known, in which singlet oxygen sensitization is the predominant pathway[10].

Further work will ascertain whether all 3 compounds operate through the same mechanism, and whether DNA is the target of the photosensitized reactions described in this report.

The ovicidal properties of natural products, both in dark reactions and in the presence of light, may be an important part of the plant-insect relationship. The results described here with *D. melanogaster* suggest the need for experiments in which egg survival should be scrutinized, particularly during the interaction between herbivorous insects and plants and their constituents in a natural environment.

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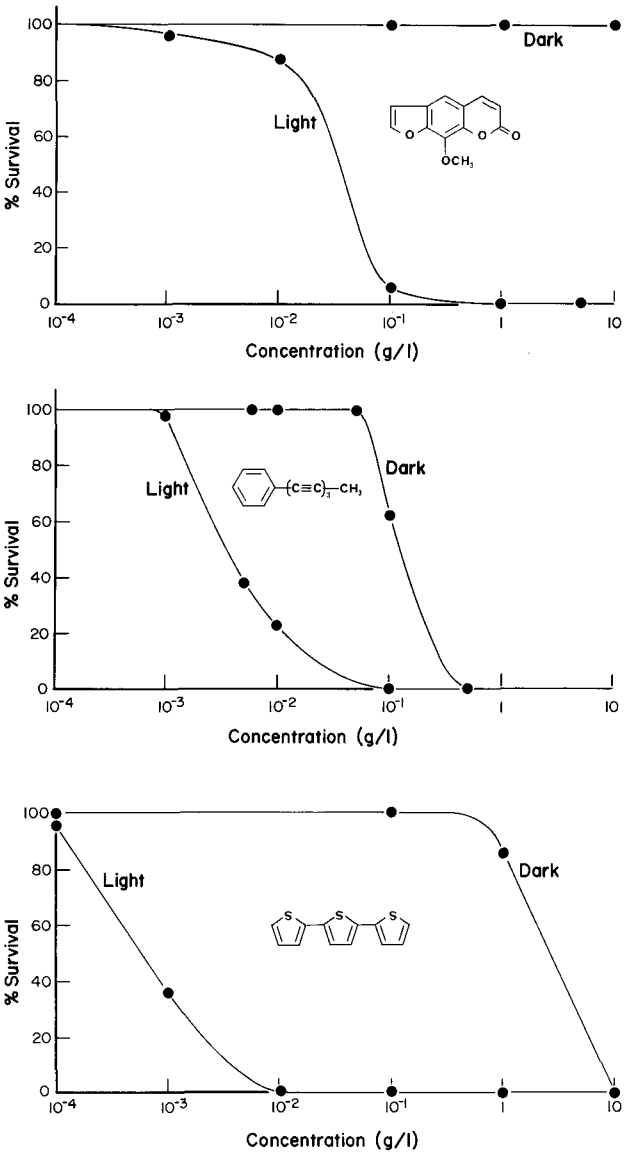

The ovicidal activity of PHT, αT, and 8-MOP. The survival of *D. melanogaster* is expressed as a function of the concentration of sensitizer used, plotted on a logarithmic scale. The concentrations in $\mu g/cm^2$ can be obtained by multiplying the values shown by 11.5.

Antifeedant activity of some ajugarin derivatives in three lepidopterous species

R.B.M. Geuskens, J.M. Luteijn and L.M. Schoonhoven[1]

Department of Animal Physiology and Department of Organic Chemistry, Agricultural University, Haarweg 10, NL-6709 PJ Wageningen (The Netherlands), July 26, 1982

Summary. The trans decalin unit of clerodendrin has antifeedant activity in larvae of *Pieris brassicae,* but not in larvae of *Spodoptera littoralis* and *S. exigua.* It is suggested that antifeedant activity of clerodanes for *Spodoptera* spp. is based on the combination of a furofuran ring and epoxy diacetate groups in the decalin unit.

Naturally occurring antifeedants may occasionally be rendered much more deterrent to insects by minor modifications of their molecular structure. Thus, the antifeedant activity of clerodendrin (fig., A), a bitter principle isolated from the Indian bhat tree *(Clerodendron infortunatum),* was raised 15-fold by transforming it into its methanol adduct[2]