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Photosensitization and feeding deterrence of *Euxoa messoria* (Lepidoptera:Noctuiidae) by α-terthienyl, a naturally occurring thiophene from the Asteraceae[1]

D.E. Champagne, J.Th. Arnason[2], B.J.R. Philogène, G. Campbell and D.G. McLachlan

Department of Biology, University of Ottawa, Ottawa (Ontario, Canada K1N6N5), 21 June 1983

Summary. Alpha-terthienyl, a phototoxic thiophene derivative from species in the Asteraceae reduced feeding and growth of the phytophagous lepidopteran, *Euxoa messoria,* when incorporated into artificial diets at a concentration of 100 ppm. The effects of this substance were substantially enhanced by including photosensitizing near-UV radiation in the trials. The results suggest that the phototoxic properties of this secondary substance provide significant protection to the plants containing it.

Our recent investigations[3,4] have documented some of the effects of naturally occurring polyacetylenes and thiophenes on insects. A remarkable property of these secondary metabolites of the plant family Asteraceae is their greatly enhanced activity in the presence of sunlight or near-UV. 9 of 14 substances tested had photosensitizing lethal activity on mosquito and blackfly larvae at 0.5 ppm. One of these substances, α-terthienyl (α-T) (fig. 1) which forms the basis of the current investigation, was found to be more toxic (LC_{50} = 19 ppb) to *Aedes aegypti* larvae in the presence of near-UV, than DDT[3].
This substance was also toxic, without near-UV activation, at 100 times this concentration. Other workers have also demonstrated the non-photosensitizing effects of these substances. For example, cis-dehydromatricaria ester and tridec-1-ene-3,5,7,9,11-pentayne were found to be ovicidal to the fruit fly *Drosophila melanogaster* and the housefly *Musca domestica*[5]. Although these studies have suggested that the presence of polyacetylenes and thiophenes may protect the host plant from insect attacks, little work has been undertaken with truly phytophagous species. In the only quantitative study to date, we reported that the polyacetylene phenylheptatriyne was a feeding deterrent to larvae of the cutworm *Euxoa messoria,* a highly polyphagous lepidopteran whose diet exposes it to a wide range of secondary substances[6]. These trials were also conducted without irradiation.
In the present study, we have investigated the feeding deterrence of the thiophene (α-terthienyl, α-T) to *Euxoa messoria* with and without near-UV exposure. In a subsequent experiment, the effects of chronic exposure of larvae to α-T and near-UV were examined.
Materials and methods. a) *Growth studies.* All larvae were reared according to the procedure described by Devitt et al.[7] in individual 2.5 × 4 cm polystyrene vials capped with UV-transparent Stretch'n Seal perforated to prevent condensation. 4 groups of 15 larvae were used: 1 group exposed to 100 μg/g α-T prepared as described previously[8] and near-UV light, 1 group exposed to 100 μg/g α-T without UV, and 2 other groups exposed to untreated diet with and without UV. Larvae were weighed, and diets were changed, every 2 days until all of the +α-T+UV-group had died. Illumination was provided by a bank of 12 Vita-Lights (Duro-Test No.48T12) producing 10 W/m^2 including 1 W/m^2 near-UV. Control groups without UV were shielded by a Kodak Wratten 2-B filter (400 mm cut-off), and so were exposed to visible light and the same photoperiod (16 L:8 D) as insects in the +UV groups.
b) *Feeding studies.* Groups of 10 sixth instar cutworm larvae (200–400 mg), reared under visible + near-UV light and not previously exposed to α-T, were individually weighed and offered a weighed appropriately treated diet cube. Remaining diet, frass, and the larvae were weighed after 24, 48, and 72 h. The 4 combinations of α-T and UV as described for the growth experiments were examined.
Results and discussion. In a feeding deterrence trial, groups of sixth-instar larvae that had not previously been exposed to α-T were presented with diets containing 100 ppm α-T and divided into groups treated with and without near-UV (table). In the absence of near-UV, 100 ppm α-T reduced feeding and weight gain of larvae. These effects were also observed in trials with a feeding specialist, *Manduca sexta* (Champagne et al., unpublished), and are similar to results obtained previously with the polyacetylene phenylheptatriyne[6]. Thus, despite their very different structures, thiophenes and polyacetylenes have comparable feeding deterrent activities towards insects.
The presence of near-UV alone did not significantly change feeding rates compared to the non-UV treated group; however, exposure to both α-T and near-UV decreased feeding activity to a level well below that resulting from exposure to α-T alone. The interaction of α-T and UV has been observed in every replicate of this experiment. Hence, it appears that photosensitization can affect feeding behavior. Weight gain was also decreased by exposure to both α-T and UV, to a larger extent than was expected if α-T and UV did not interact.
The efficiency of diet conversion to insect biomass, expressed as the ratio of larval weight gain to diet consumption, was markedly decreased by exposure to α-T. The presence of near-UV did not enhance this expression of toxicity. These results suggest that non-specialized insects feeding on plants containing α-T not only consume less, but are less well able to utilize what is consumed.
In a 2nd experiment, larvae were exposed to α-T (100 ppm) during their entire development period (fig. 2). Larvae exposed to α-T without near-UV initially showed poor growth relative to controls, but growth performance improved after 2 weeks. A similar pattern was observed in insects exposed only to near-

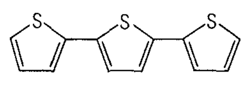

Figure 1. Alpha terthienyl.

UV without α-T. In both cases, the initial growth rate is the result of decreased initial feeding rates. Rapid growth began when the larvae began feeding at a normal rate.

Larvae exposed to both α-T and near-UV also showed initial poor growth which may be attributed to poor feeding rates. However, when these insects started feeding normally, they did not enter a phase of rapid growth. The larvae continued to show poor relative weight gain and all larvae died by the end of the 4th week, before molting to the fifth instar. These results are indicative of the photosensitizing effect of α-T. Results similar to these have been observed with the feeding specialist *Manduca sexta*[9]. In our experiments, larvae were exposed to only 1 W/m^2 near-UV and 100 ppm α-T. In full sunlight in summer at Ottawa, Canada (45° N), the near-UV component may exceed 50 W/m^2. Concentrations of α-T in plant material often exceed 1000 ppm. Our results then strongly suggest that α-T has an important function in protecting the plants that contain it from insect herbivores.

The ability of α-T to photosensitize insects is comparable to other known naturally occurring photosensitizers, including furanocoumarins[10]. The latter interfere with gene product synthesis by forming monofunctional or difunctional adducts with DNA. In contrast, α-terthienyl is a photodynamic sensitizer which produces activated species of O_2 that oxidize target molecules[11,12]. The site of action of α-T appears to be membranes, but another photodynamic secondary plant substance, berberine, may intercalate and photooxidize DNA[13]. Thus, a wide variety of mechanisms is possible in photosensitization reactions with plant secondary metabolites. A particular selective advantage may be conferred on plants containing these substances; this advantage may occur in the access to excited state chemistry in which phototoxins can initiate many more damaging processes than occur in the ground state.

Variations in larval weight gain, diet consumption, and assimilation efficiencies, with exposure to combinations of α-T and near-UV

Treatment	Diet consumption (g/unit wt/day)	Larval weight gain (%/day)	Efficiency of conversion (%)[a]
Control	205.8 ± 27.1	49.8 ± 9.7	65.8 ± 8.5
+ UV	212.1 ± 31.0	47.6 ± 7.0	76.7 ± 5.5
α-T − UV	170.0 ± 29.1	22.5 ± 7.0	34.9 ± 7.6
α-T + UV	125.6 ± 19.1	15.9 ± 7.4	35.7 ± 10.7

[a] Larval weight gain (g/insect/day) divided by diet consumption (g/insect/day), × 100.

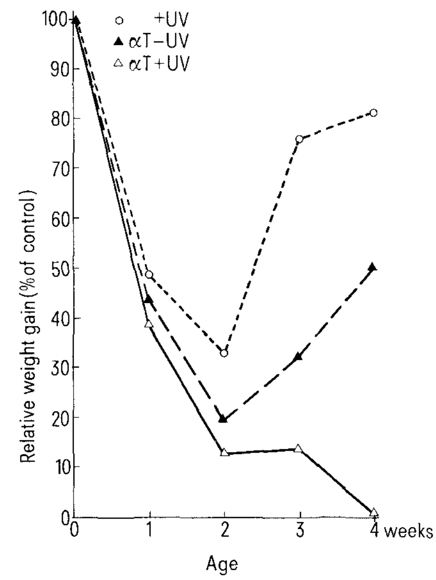

Figure 2. Effect of α-T and near-UV on larval growth of *Euxoa messoria*.

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A fossil entomogenous fungus from Dominican amber

G.O. Poinar, Jr, and G.M. Thomas

Division of Entomology and Parasitology, University of California, Berkeley (California 94720, USA), 2 June 1983

Summary. A worker ant (Formicidae: Hymenoptera) embedded in amber (25 million years old) from the Dominican Republic was covered with an entomogenous fungus containing characters very similar to present day strains of *Beauveria bassiana.* This represents the first report of a fossil insect-pathogenic fungus belonging to the class Deuteromycetes.

While examining fossilized resin for evidence of invertebrate diseases, a piece of clear, yellow amber from the Dominican Republic was found to contain a worker ant (Formicidae: Hymenoptera) covered with a white, powdery fungus (fig. A, B). When viewed under the compound microscope, conidiophores, conidiogenous cells and conidia were observed (fig. C, D, E). The conidia were 1-celled, hyaline, smooth and varied in shape from globose to broadly ellipsoidal. Their greatest diameter ranged from 1.2–2.2 μm (N = 25). The conidia were born on a geniculate rachis (fig. C, D) and the basal portions