TOXICITY AND RESIDUAL ACTION OF THE PHOTOACTIVATED COMPOUND, CYANO-ALPHA-TERTHIENYL, AND ITS EFFICACY FOR REDUCING PRE-IMAGINAL POPULATIONS OF MOSQUITOES

L. M. DOSDALL,[1] M. M. GALLOWAY[2] AND J. T. ARNASON[3]

ABSTRACT. The photoactivated compound, cyano-alpha-terthienyl (cyano-α-T), was highly toxic to pre-imagines of the mosquitoes *Culex restuans*, *Cx. tarsalis* and *Culiseta inornata* when synergized with piperonyl butoxide (PBO). Lethal concentrations for 50% mortality, determined during an outdoor trial using caged fourth-instar *Culex* spp. larvae, were 19.4, 15.4 and 12.9 g/ha at 24, 48 and 72 h after treatment, respectively. No residual activity of cyano-α-T was observed beyond 24 h following treatment. In artificial pool tests, greatest population reductions were achieved using dosages of 20 and 40 g/ha; statistically significant reductions were not observed following applications of 5 g/ha. Cyano-α-T plus PBO was more effective for reducing mosquito populations than alpha-terthienyl (α-T) plus PBO at comparable dosages, although it exhibited slightly lower insecticidal activity at a dosage of 20 g/ha than a formulation of *Bacillus thuringiensis* var. *israelensis* (Vectobac® 12 AS, 0.12 ml/m^2). Greatest effectiveness of cyano-α-T plus PBO was observed in pools with low organic content relative to pools high in organic content.

INTRODUCTION

The use of phototoxic compounds that exhibit toxicity to organisms in the presence of light is a rapidly developing new technology in agriculture and medicine (Heitz and Downum 1987). Several phototoxins are derived from plants (Towers 1984), and a few of these are promising alternatives to conventional insecticides because they are highly active, relatively nonpersistent, and have a novel mode of action with demonstrated potential for controlling insects that are resistant to standard insecticides (Arnason et al. 1989). Alpha-terthienyl (α-T), a naturally occurring thiophene of the plant family Asteraceae, is known to have exceptional phototoxic activity to mosquito larvae (Arnason et al. 1981a, Philogène et al. 1985, Borovsky et al. 1987, Hasspieler et al. 1988) and pupae (Kagan et al. 1987).

The phototoxicity of α-T and related molecules results from irradiation with near-ultraviolet wavelengths (300–400 nm), leading to the generation of singlet oxygen by energy transfer (Arnason et al. 1981b, Scaiano et al. 1987). Singlet oxygen is damaging to several classes of biological molecules, especially lipids and amino acids (Larson and Berenbaum 1988). In mosquito larvae, direct evidence of lipid peroxidation and oxidation of glutathione by α-T has been obtained (Hasspieler et al. 1990). At the tissue level, photooxidative damage may occur to the midgut epithelium and the lumen of Malpighian tubules (Hasspieler et al. 1988, 1990), and anal gill membranes may undergo increased halide leakage (Arnason et al. 1987).

Quantitative structure activity relationship of 20 or more derivatives of α-T indicated that observed toxicity can be predicted accurately ($r^2 > 0.8$) on the basis of the rate of singlet oxygen production and lipophilicity (Marles et al. 1991a, 1991b). Cyano-alpha-terthienyl (cyano-α-T) was found to be near the optimum lipophilicity for activity to mosquito larvae and an efficient singlet oxygen generator. In addition, its lipophilicity was below the optimum for activity to a crustacean (*Artemia salina* Leach), suggesting that it may have some target organism specificity. These observations prompted the present field trials to evaluate the phototoxicity of cyano-α-T in relation to other phototoxins and microbial toxins. The objectives of this study were: 1) to evaluate the relative effectiveness of cyano-α-T and cyano-α-T plus the synergist, piperonyl butoxide (PBO), for reducing populations of pre-imaginal mosquitoes in artificial pools, and to compare these with α-T and a standard formulation of *Bacillus thuringiensis* Berliner var. *israelensis* de Barjac (*B.t.* H-14), 2) to compare the efficacy of cyano-α-T plus PBO in breeding sites with high and low organic content, 3) to estimate the LC_{50} of cyano-α-T for *Culex* spp., and 4) to assess the residual activity of cyano-α-T plus PBO under field conditions.

METHODS AND MATERIALS

Cyano-α-T (20 g) was synthesized according to the method described previously (Scaiano et al. 1987, Soucy-Breau et al. 1991), by treating α-T with chlorosulfonyl isocyanate in methyl chloride, and then adding dimethylformamide to the mixture.

[1] Alberta Environmental Centre, Bag 4000, Vegreville, Alberta, Canada, T9C 1T4.
[2] Department of Entomology, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2.
[3] Department of Biology, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5.

Tests were conducted in 24 small (1 m^2) and 12 large (9 m^2) artificial pools at the University of Manitoba Glenlea Research Station, located approximately 30 km south of Winnipeg, Manitoba. Pools were constructed with wooden frames, double-lined with heavy black plastic (6 mil), and filled with approximately 80 liters (m^2 pools) or 2,000 liters (9 m^2 pools) of river water. All 1 m^2 pools were lined with landscaping sod; half the 9 m^2 pools were sod-lined and half were sod-free. Pools were located along the southern edge of a poplar woodlot and received full sunlight for approximately 6 (9 m^2 pools) or 8 (1 m^2 pools) h per day. Pools were constructed, filled, and left standing for mosquito colonization to occur for 11 (Trial 1) and 7 days (Trial 2) before treatment. Mosquito oviposition in the sod-free pools was supplemented by adding egg rafts from sodded pools. Water temperatures were recorded with Taylor® maximum-minimum thermometers.

The first of 2 similar field trials commenced July 26, 1988. Samples were collected approximately 1 h before treatment, and at post-treatment intervals of 24, 48 and 72 h. At each sampling time, 5–450 ml dipper samples were taken from each 1 m^2 pool (one from each pool corner and one from the center), and 8 dipper samples were collected from each 9 m^2 pool (2 from each pool corner). Dead individuals were removed from post-treatment samples. Specimens were preserved in 95% ethanol.

The second trial commenced August 9, 1988. The same sampling protocol was used, except that 10 dipper samples were collected from each 9 m^2 pool (2 from each corner and 2 from the center).

For both trials, treatments were applied at 1130 h in full sunlight (800 W/m^2). During each trial, three 1 m^2 pools were untreated controls and three 1 m^2 pools were selected randomly for each of the 7 treatments: 1) cyano-α-T plus PBO, 5 g/ha (0.5 mg cyano-α-T + 0.25 mg PBO + 0.0005 ml Atlox 1087 + Shell TS22 added to make 1 ml); 2) cyano-α-T plus PBO, 10 g/ha (1.0 mg cyano-α-T + 5.0 mg PBO + 0.0010 ml Atlox 1087 + Shell TS22 added to make 1 ml); 3) cyano-α-T plus PBO, 20 g/ha (2.0 mg cyano-α-T + 10.0 mg PBO + 0.0020 ml Atlox 1087 + Shell TS22 added to make 1 ml); 4) α-T plus PBO, 50 g/ha (5.0 mg α-T + 25.0 mg PBO + 0.005 ml Atlox 1087 + Shell TS22 added to make 1 ml); 5) cyano-α-T, 50 g/ha (5.0 mg cyano-α-T + 0.005 ml Atlox 1087 + Shell TS22 added to make 1 ml); 6) *B.t.* H-14, 0.12 ml/m^2 Vectobac® 12 AS; 7) solvent (0.01 ml Atlox 1087 + 0.99 ml Shell TS22). Atlox 1087 (polyethylene glycol sorbitan ether monooleate) was used as a spreading agent in the formulations; the solvent, Shell TS22, contains xylene.

The importance of pool organic content on effectiveness of cyano-α-T plus PBO was tested in the large pools. Three sod-lined and 3 sod-free 9 m^2 pools (selected randomly) were treated with 40 g/ha cyano-α-T plus PBO (36.0 mg cyano-α-T + 180.0 mg PBO + 0.036 ml Atlox 1087 + Shell TS22 added to make 9 ml); 3 large pools of each type were untreated.

To estimate the LC_{50} of cyano-α-T plus PBO, fourth-instar larvae of *Culex* spp. were placed in screened bioassay cages (25 larvae per cage and 1 cage per pool) within the control pools and those subjected to treatment of cyano-α-T plus PBO, according to the method of Philogène et al. (1985). Numbers of living and dead larvae in the bioassay cages were counted and recorded at intervals of 24, 48 and 72 h after treatment.

The residual activity of cyano-α-T plus PBO at 40 g/ha was examined in the 9 m^2 pools in both the July and August trials. Bioassay cages, each containing 25 fourth-instar larvae of *Culex* spp., were placed in each large pool approximately 1 h before treatment and at 24 and 48 h after treatment. Numbers of living and dead larvae in the bioassay cages were recorded at intervals of 24, 48 and 72 h after treatment.

Percent reductions of individuals in treated pools were calculated using pre- and post-treatment sample numbers, corrected for control mortality using the formula of Mulla et al. (1971). Data were subjected to analysis of variance (ANOVA) using a General Linear Models procedure and Duncan's Multiple Range Test (SAS Institute Inc. 1985), after performing $\log_{10}(x + 1)$ transformations on counts of larvae and pupae comprising each sample. Recruitment of new individuals by hatching of eggs laid after treatment was corrected for by excluding first-instar larvae from the analysis of 48 h post-treatment samples, and first- and second-instar larvae from analysis of 72 h post-treatment samples. Recruitment corrections were based on development times for *Culex tarsalis* Coq. of 1.5 and 1.0 d for first- and second-instar larvae, respectively, when reared under similar environmental conditions (Reisen et al. 1989). Mortality data from the bioassay cages were subjected to probit analysis (SAS Institute Inc. 1985) to determine the LC_{50} and 95% fiducial limits for cyano-α-T plus PBO. Mortality of caged larvae exposed to different dosages of cyano-α-T plus PBO was compared before and after treatment using ANOVA and Tukey's Studentized Range Test (SAS Institute Inc. 1985).

Table 1. Mean numbers ± SE of *Culex restuans*, *Cx. tarsalis* and *Culiseta inornata* pre-imagines per 450 ml dipper sample, and percent population reductions (*in italics*) in 1 m^2 artificial pools receiving various treatments on July 26, 1988 (Trial 1). Means in the same row followed by the same letter are not significantly different ($P > 0.05$).

Treatment	Mean no. individuals/sample, pre- and post-treatment (h)			
	Pre-treatment	24	48	72
Controls	15.1 ± 3.2 a	16.0 ± 3.8 a	17.4 ± 1.3 a	15.7 ± 1.7 a
Bacillus thuringiensis H-14 (0.12 ml/m^2)	11.3 ± 3.0 a	0.9 ± 0.4 b *92.8*	0.2 ± 0.2 b *98.3*	0.1 ± 0 b *99.6*
Cyano-α-T (50 g/ha)	15.3 ± 2.9 a	2.4 ± 0.9 b *85.1*	9.7 ± 4.9 a *44.9*	8.2 ± 1.1 a *48.2*
α-T + PBO (50 g/ha)	12.5 ± 1.6 a	2.8 ± 2.0 b *78.9*	6.9 ± 3.3 a b *52.4*	10.9 ± 4.5 a b *16.8*
Solvent	12.6 ± 3.1 a	7.7 ± 0.9 a *42.7*	10.9 ± 2.9 a *24.9*	9.1 ± 3.3 a *30.5*
Cyano-α-T + PBO (5 g/ha)	10.5 ± 2.8 a	5.2 ± 0.9 a *53.3*	7.4 ± 0.3 a *39.0*	6.4 ± 1.8 a *41.2*
Cyano-α-T + PBO (10 g/ha)	11.6 ± 0.1 a	12.1 ± 10.2 a *2.2*	6.6 ± 1.2 a *50.6*	5.5 ± 2.5 a *54.1*
Cyano-α-T + PBO (20 g/ha)	10.4 ± 1.6 a	0.8 ± 0.7 b *93.2*	7.6 ± 5.6 a b *37.1*	8.0 ± 3.3 a b *26.3*

RESULTS

In Trial 1, most pre-imaginal mosquitoes inhabiting the pools were identified as *Culex restuans* Theobald (73.3%) and *Cx. tarsalis* (23.8%). Small numbers of *Culiseta inornata* (Williston) (2.9%) also were collected. In Trial 2, *Cx. restuans*, *Cx. tarsalis* and *Cs. inornata* comprised 62.7, 26.0 and 11.3% of individuals, respectively. A greater proportion of mosquito larvae inhabiting the small pools were first and second instars in Trial 2 (61.6%), compared with Trial 1 (44.2%). Consequently, a larger proportion of individuals in Trial 1 were third and fourth instars (55.8%), compared with Trial 2 (38.4%). In the large pools, first-instar larvae comprised a smaller percentage of pre-imagines in Trial 1 (7.0%) than in Trial 2 (30.5%). However, percentages of second-, third-, and fourth-instar larvae were similar in both trials. In Trial 1, 23.3, 24.6 and 28.9% of larvae were second, third and fourth instars, respectively, compared with Trial 2 where these percentages were 25.8, 18.3 and 24.3. A larger proportion of pupae were observed in the large pools in Trial 1 (16.2%) compared with Trial 2 (1.1%) and the small pools (0%). The mean water temperature of the 1 m^2 pools during the 4-day sampling period of Trial 1 was 26°C (daily mean temperature range = 24–28°C); the mean temperature of the 9 m^2 pools was 21°C (range = 18–23°C). In Trial 2, the mean water temperature of the 1 m^2 pools during the study period was 24°C (range = 22–27°C); the mean temperature of the 9 m^2 pools was 20°C (range = 18–22°C).

No significant changes in mean numbers of individuals per sample were observed in the 1 m^2 untreated pools during either of Trials 1 or 2 (Tables 1 and 2). In both trials, 24 h post-treatment populations in pools treated with *B.t.* H-14 declined significantly ($P < 0.05$) relative to pre-treatment populations, and remained significantly lower than pre-treatment densities for the remainder of the study period (Tables 1 and 2). Population reductions greater than 98% were observed 48 h post-treatment, and these reductions were maintained for the remainder of both study periods.

Pre-imaginal mosquito populations in pools treated with α-T plus PBO declined significantly ($P < 0.05$) after treatment in both trials (Tables 1 and 2). In Trial 2, mean number of individuals per sample remained significantly lower during all post-treatment sampling dates compared with pre-treatment densities. In Trial 1, however, a significant population decline was observed only 24 h after treatment.

Application of cyano-α-T also caused a population decline that was significant ($P < 0.05$) 24 h after treatment in both trials (Tables 1 and 2). In Trial 2, populations observed 72 h after treatment declined significantly relative to pre-treatment densities ($P < 0.05$), but in Trial 1, no significant decrease occurred between these dates.

In both trials, mean numbers of individuals per sample did not differ significantly between sampling dates for pools treated with the solvent alone, or for pools treated with 5 g/ha cyano-α-T plus PBO ($P > 0.05$) (Tables 1 and 2). How-

Table 2. Mean numbers ± SE of *Culex restuans*, *Cx. tarsalis* and *Culiseta inornata* pre-imagines per 450 ml dipper sample, and percent population reductions (*in italics*) in 1 m^2 artificial pools receiving various treatments on August 9, 1988 (Trial 2). Means in the same row followed by the same letter are not significantly different ($P > 0.05$).

Treatment	Mean no. individuals/sample, pre- and post-treatment (h)			
	Pre-treatment	24	48	72
Controls	72.9 ± 45.3 a	35.6 ± 4.0 a	30.0 ± 12.5 a	53.6 ± 8.5 a
Bacillus thuringiensis H-14 (0.12 ml/m^2)	29.3 ± 20.7 a	1.3 ± 1.2 b *90.9*	0.2 ± 0.1 b *98.4*	0.4 ± 0.3 b *98.3*
Cyano-α-T (50 g/ha)	22.3 ± 8.7 a	1.0 ± 0.6 b *90.5*	11.8 ± 6.1 a b *−28.0*[1]	2.0 ± 0.3 b *87.6*
α-T + PBO (50 g/ha)	12.9 ± 2.5 a	2.4 ± 1.8 b *62.3*	1.1 ± 0.7 b *79.2*	3.6 ± 2.1 b *62.0*
Solvent	24.1 ± 9.9 a	11.0 ± 5.8 a *6.5*	29.2 ± 11.8 a *−193.7*[1]	30.8 ± 13.1 a *−73.7*[1]
Cyano-α-T + PBO (5 g/ha)	31.5 ± 26.3 a	5.8 ± 5.1 a *62.4*	6.0 ± 5.1 a *53.4*	10.1 ± 8.3 a *56.3*
Cyano-α-T + PBO (10 g/ha)	21.5 ± 12.2 a	0.7 ± 0.4 b *93.4*	5.7 ± 4.5 a b *36.0*	2.0 ± 0.7 b *87.3*
Cyano-α-T + PBO (20 g/ha)	31.6 ± 15.9 a	0.5 ± 0.2 b *96.5*	1.0 ± 0 b *92.6*	1.3 ± 0.1 b *94.4*

[1] Negative values indicate that relative population densities were greater in the treated than the untreated pools.

Table 3. Mean numbers ± SE of *Culex restuans*, *Cx. tarsalis* and *Culiseta inornata* pre-imagines per 450 ml dipper sample, and percent population reductions (*in italics*) in 9 m^2 artificial pools treated with 40 g/ha cyano-α-T plus PBO on July 26 (Trial 1) and August 9, 1988 (Trial 2). Means in the same row followed by the same letter are not significantly different ($P > 0.05$).

Treatment	Mean no. individuals/sample, pre- and post-treatment (h)			
	Pre-treatment	24	48	72
Trial 1				
Control, sod-lined pools	36.9 ± 5.8 a	18.1 ± 7.0 a	23.9 ± 4.3 a	21.3 ± 5.0 a
Control, sod-free pools	4.9 ± 1.5 a	2.8 ± 1.2 a	5.1 ± 3.0 a	1.4 ± 1.2 a
Sod-lined pools, cyano-α-T + PBO	22.4 ± 10.2 a	2.8 ± 0.8 b *74.9*	1.8 ± 0.7 b *87.7*	2.4 ± 1.8 b *81.8*
Sod-free pools, cyano-α-T + PBO	7.1 ± 2.0 a	0.2 ± 0.2 b *95.2*	0 ± 0 b *100*	0 ± 0 b *100*
Trial 2				
Control, sod-lined pools	8.4 ± 3.2 a	3.8 ± 1.9 a	3.7 ± 1.8 a	3.2 ± 2.0 a
Control, sod-free pools	1.5 ± 0.3 a	1.4 ± 0.4 a	0.8 ± 0.3 a	1.0 ± 0.9 a
Sod-lined pools, cyano-α-T + PBO	1.9 ± 0.9 a	0.3 ± 0.1 a b *66.7*	0 ± 0 b *98.8*	0.1 ± 0.1 b *88.5*
Sod-free pools, cyano-α-T + PBO	2.4 ± 1.1 a	0.1 ± 0 b *96.4*	0 ± 0 b *99.2*	0 ± 0 b *100*

ever, in pools treated with 10 g/ha cyano-α-T plus PBO, post-treatment populations declined significantly ($P < 0.05$) in Trial 2 relative to pre-treatment densities. By 72 h post-treatment, mosquito populations were 87.3% lower than those recorded before treatment. In Trial 1, however, populations did not differ significantly from pre-treatment densities on any post-treatment sampling date ($P > 0.05$).

Significant population reductions occurred in both trials following application of 20 g/ha cyano-α-T plus PBO (Tables 1 and 2). In Trial 1, a 93.2% reduction was observed 24 h after treatment; however, mean numbers of individuals per sample 48 and 72 h after treatment did not differ significantly from pre-treatment densities. In Trial 2, population reductions greater than 92% were observed 24, 48 and 72 h after treatment, and all were significantly lower than pre-treatment densities ($P < 0.05$).

In both Trials 1 and 2, no significant changes in densities of pre-imaginal mosquitoes were observed between any of the sampling dates in the 9 m^2 control pools ($P > 0.05$) (Table 3). In both trials, however, significant reductions occurred in the sod-lined and sod-free pools

treated with 40 g/ha cyano-α-T plus PBO ($P < 0.05$). Mosquito populations were reduced to a greater degree in the sod-free pools than in the sod-lined pools. By 72 h post-treatment, 81.8 and 88.5% reductions were observed in sod-lined pools, but 100% reductions occurred in sod-free pools.

Mortality of *Culex* spp. larvae in the bioassay cages was affected by dosage and time after treatment. For example, in the July trial, no significant differences in mortality were observed in the control pools during the study period ($P > 0.05$); however, by 24 h after treatment 20 g/ha of cyano-α-T plus PBO caused a significant increase in mortality ($P < 0.05$) that exceeded the mortality caused by dosages of 5 or 10 g/ha (Table 4). By 72 h after treatment, greatest mortality was still evident in pools treated with 20 g/ha. In the July trial, LC_{50} values (fiducial limits) of cyano-α-T plus PBO were 19.4 (15.7–27.7), 15.4 (12.7–20.6) and 12.9 (10.6–16.8) g/ha at 24, 48 and 72 h after treatment, respectively. In the August trial, LC_{50} values (fiducial limits) were 35.0 (23.4–86.3), 19.8 (9.9–42.5) and 15.6 (6.1–31.3) g/ha at 24, 48 and 72 h after treatment.

Application of cyano-α-T plus PBO at 40 g/ha to the 9 m^2 pools induced high mortality to larvae introduced just prior to treatment, but mortality to larvae introduced 24 or 48 h after treatment was very similar to that of controls (Table 5). The residual activity of cyano-α-T plus PBO at 40 g/ha in pools with and without sod was similar or slightly greater for pools without sod (Table 6).

DISCUSSION

Cyano-α-T plus PBO was highly phototoxic to pre-imagines of *Cx. restuans*, *Cx. tarsalis* and *Cs. inornata*. The phototoxic activity of cyano-α-T plus PBO exceeded that of α-T plus PBO.

Table 4. Mean percent mortalities of fourth-instar *Culex* spp. larvae in bioassay cages subjected to different dosages of cyano-α-T plus PBO in 1 m^2 pools for Trial 1 (July 26–29, 1988). Means in a column followed by the same letter are not significantly different ($P > 0.05$); means in a row followed by the same number are not significantly different ($P > 0.05$).

Rate (g/ha)	Mean mortality (%) at various times (h) following treatment			
	0	24	48	72
0	0 a 1	1.3 a 1	4.0 a 1	20.0 a 1
5	0 a 1	9.3 a 1,2	13.3 ab 1,2	20.2 a 2
10	0 a 1	24.0 a 2	36.0 bc 2	42.7 ab 3
20	0 a 1	52.0 b 2	58.6 c 2	62.0 ab 2

Although both caused statistically significant population reductions, a greater dosage (50 g/ha) of α-T plus PBO achieved a lower mean reduction (58.6%) during the 2 studies than did a lower dosage (40 g/ha) of cyano-α-T plus PBO (83.1%).

Cyano-α-T plus PBO, at the highest dosage tested in the m^2 pools (20 g/ha), had slightly lower insecticidal activity against pre-imaginal mosquitoes than did the Vectobac® 12 AS formulation of *B.t.* H-14. (The *B.t.* H-14 dosage used here was mid-range according to the product label guide.) Comparable toxic activity in organically rich habitats may require dosages of 45 or 50 g/ha cyano-α-T plus PBO. Dosages of 30 g/ha would probably provide acceptable population reductions in organically poor habitats.

The toxicity of cyano-α-T plus PBO was greatest in organically poor habitats. By the end of the study period, population reductions were approximately 15% greater in the pools with low organic content treated with 40 g/ha than in the highly organic pools. Adsorption of phototoxin molecules onto organic particles may have caused its decreased toxicity in the enriched pools.

The solvent did not cause significant population reductions of pre-imaginal mosquitoes; in fact, population increases during the study period were observed in Trial 2. Addition of PBO enhanced the phototoxic activity of cyano-α-T, although this was more evident in Trial 1 compared to Trial 2. The experimental design did not accommodate a treatment of PBO alone, and therefore it should be noted that the impact of α-T and cyano-α-T on pre-imaginal mosquito populations may not have resulted exclusively from the photoactivated molecules, but may have been caused partly by PBO. PBO is known to affect aquatic organisms adversely (Mayer and Ellersieck 1986).

There is no evidence from this study that the species studied differed in their sensitivities to cyano-α-T plus PBO. For example, *Cx. restuans*, *Cx. tarsalis* and *Cs. inornata* comprised 65.5, 28.3 and 6.2%, respectively, of individuals from Trial 1 pre-treatment samples inhabiting 9 m^2 pools treated with 40 g/ha cyano-α-T plus PBO. By 48 h post-treatment, the species composition of survivors was similar: 73.8% *Cx. restuans*, 26.2% *Cx. tarsalis* and 0% *Cs. inornata*. It is probable, however, that interspecific (and perhaps interstrain) differences in susceptibility to larvicidal effects of cyano-α-T exist, because such differences were observed for α-T (Hasspieler et al. 1988). Laboratory toxicity tests are needed to investigate this question.

Mortality data from the bioassay cages demonstrate that this photoactivated material ex-

Table 5. Mean percent mortalities ± SD of fourth-instar *Culex* spp. larvae placed in bioassay cages on consecutive days before and after treatment of the 9 m^2 artificial pools with cyano-α-T plus PBO in Trial 2 (August 9–12, 1988)

Rate (g/ha)	Time of cage introduction (h after treatment)	Mean mortality (%) at various times (h) following treatment			
		0	24	48	72
40	0	0 ± 0	42.7 ± 6.1	86.6 ± 14.0	96.0 ± 5.6
	24	—	0 ± 0	1.3 ± 2.3	8.0 ± 4.0
	48	—	—	0 ± 0	4.0 ± 4.0
0	0	0 ± 0	4.0 ± 4.0	5.3 ± 6.1	6.6 ± 2.0
	24	—	0 ± 0	0 ± 0	5.3 ± 2.3
	48	—	—	0 ± 0	2.6 ± 2.3

Table 6. Mean percent mortalities ± SD of fourth-instar *Culex* spp. larvae placed in bioassay cages on consecutive days before and after treatment with 40 g/ha cyano-α-T plus PBO in Trial 1 (July 26–29, 1988) in sod-lined and sod-free 9 m^2 artificial pools.

Pool type	Time of cage introduction (h after treatment)	Mean mortality (%) at various times (h) following treatment			
		0	24	48	72
Sod	0	0 ± 0	92.0 ± 4.0	96.6 ± 4.0	98.0 ± 2.8
	1	—	0 ± 0	21.3 ± 10.0	20.0*
	2	—	—	0 ± 0	12.0 ± 16.0
No sod	0	0 ± 0	96.0 ± 4.0	100 ± 0	100 ± 0
	1	—	0 ± 0	48.0 ± 24.3	56.1 ± 28.0
	2	—	—	0 ± 0	9.3 ± 12.8

* Single cage.

hibits potent efficacy under field conditions, and low residual action suggesting very little persistence of the parent material. The efficacy results are comparable to those found for spring *Aedes* spp. by Fields et al. (1991) where LC_{50} values (fiducial limits) of cyano-α-T were 16.4 (14.1–18.7) and 10.8 (7.8–12.5) g/ha at 24 and 48 h after treatment, respectively.

It is possible to evaluate the persistence of cyano-α-T not by measuring toxicity to caged mosquito larvae, but by chemical analysis of extracted water samples. The procedure, however, is complex and was not undertaken in this study. Research by Kagan et al. (1991) has shown that phototoxic compounds readily become adsorbed onto plastic. Adsorption onto the plastic liner of the mosquito pools, therefore, may also partly explain the reduced toxicity of cyano-α-T over time.

Results of this study clearly indicate that cyano-α-T has considerable potential for reducing pre-imaginal populations of mosquitoes under field conditions of full sunlight. The lifetime of the electronically excited species of cyano-α-T is even shorter than that of α-T (Scaiano et al. 1987). Further research is required, however, to determine the impact of this photoinsecticide on non-target organisms before consideration can be given to its implementation in mosquito abatement programs.

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REFERENCES CITED

Arnason, J. T., T. Swain, C. K. Wat, E. A. Graham, S. Partington, J. Lam and G. H. N. Towers. 1981a. Mosquito larvicides from polyacetylenes occurring naturally in Asteraceae. Biochem. Syst. Ecol. 9:63–68.

Arnason, J. T., C. F. Q. Swain, C. K. Wat, K. Downum and G. H. N. Towers. 1981b. Oxygen requirement of near-UV mediated cytotoxicity of α-terthienyl to *Escherichia coli* and *Saccharomyces cerevisiae*. Photochem. Photobiol. 33:821–824.

Arnason, J. T., B. J. R. Philogène, P. Morand, J. C. Scaiano, N. Werstiuk and J. Lam. 1987. Thiophenes and acetylenes: phototoxic agents to herbivorous and blood-feeding insects, pp. 255–264. *In:* J. R. Heitz and K. B. Downum (eds.), Light activated pesticides. Am. Chem. Soc. Symp. Ser. 339.

Arnason, J. T., B. J. R. Philogène, P. Morand, K. Imrie, B. Hasspieler and A. E. R. Downe. 1989. Naturally occurring and synthetic thiophenes as insecticides. Am. Chem. Soc. Symp. Ser. 387:164–172.

Borovsky, D., J. R. Linley and J. Kagan. 1987. Polycyclic aromatic compounds as phototoxic mosquito larvicides. J. Am. Mosq. Control Assoc. 3:246–250.

Fields, P. G., J. T. Arnason, B. J. R. Philogène, R. R. Aucoin, P. Morand and C. Soucy-Breau. 1991. Phototoxins as insecticides and natural plant defences. Mem. Entomol. Soc. Can. 159:29–38.

Hasspieler, B. M., J. T. Arnason and A. E. R. Downe. 1988. Toxicity, localization and elimination of the phototoxin, alpha-terthienyl, in mosquito larvae. J. Am. Mosq. Control Assoc. 4:479–484.

Hasspieler, B. M., J. T. Arnason and A. E. R. Downe. 1990. Modes of action of the plant-derived phototoxin alpha-terthienyl in mosquito larvae. Pestic. Biochem. Physiol. 38:41–47.

Heitz, J. R. and K. R. Downum (eds.). 1987. Light activated pesticides. Am. Chem. Soc. Symp. Ser. 339:1–340.

Kagan, J., E. D. Kagan, S. Patel, D. Perrine and V. Bindokas. 1987. The phototoxicity of alpha-terthienyl in eggs, larvae and pupae of the mosquito *Aedes aegypti*. J. Chem. Ecol. 13:593–604.

Kagan, J., N. Stephens, A. T. Kulakkattolickal and T. P. Wang. 1991. Variables affecting the phototoxicity of α-terthienyl toward fish in the laboratory. Chemosphere 22:699–703.

Larson, R. A. and M. R. Berenbaum. 1988. Environmental phototoxicity. Environ. Sci. Technol. 22:354–360.

Marles, R. J., J. T. Arnason, L. Compadre, C. Compadre, B. Mehta, P. Morand, R. W. Redmond and J. C. Scaiano. 1991a. Quantitative structure activity relationship analysis of natural products: phototoxic thiophenes, pp. 371–396. *In:* N. H. Fisher (ed.), Modern phytochemical methods. Plenum Press, New York.

Marles, R. J., R. L. Compadre, C. M. Compadre, C. Soucy-Breau, R. W. Redmond, F. Duval, B. Mehta, P. Morand, J. C. Scaiano and J. T. Arnason. 1991b. Thiophenes as mosquito larvicides: structure-toxicity relationship analysis. Pestic. Biochem. Physiol. 41:89–100.

Mayer, F. L., Jr. and M. R. Ellersieck. 1986. Manual of aquatic toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. U.S. Dep. Interior, Fish Wildl. Serv. Res. Publ. 160.

Mulla, M. S., R. L. Norland, D. M. Fanara, H. A. Darwazeh and D. W. McKean. 1971. Control of chironomid midges in recreational lakes. J. Econ. Entomol. 64:300–307.

Philogène, B. J. R., J. T. Arnason, C. W. Berg, F. Duval, D. Champagne, R. G. Taylor, L. C. Leitch and P. Morand. 1985. Synthesis and evaluation of the naturally occurring phototoxin, alpha-terthienyl, as a control agent for larvae of *Aedes intrudens*, *Aedes atropalpus* (Diptera: Culicidae) and *Simulium verecundum* (Diptera: Simuliidae). J. Econ. Entomol. 78:121–126.

Reisen, W. K., R. P. Meyer, J. Shields and C. Arbolante. 1989. Population ecology of preimaginal *Culex tarsalis* (Diptera: Culicidae) in Kern County, California. J. Med. Entomol. 26:10–22.

SAS Institute Inc. 1985. SAS user's guide: statistics, version 5 edition. SAS Institute Inc., Cary, NC.

Scaiano, J. C., A. MacEachern, J. T. Arnason, P. Morand and D. Weir. 1987. Singlet oxygen generating efficiency of α-terthienyl and some of its synthetic analogues. Photochem. Photobiol. 46:193–199.

Soucy-Breau, C., A. MacEachern, L. C. Leitch, J. T. Arnason and P. Morand. 1991. Synthesis and characterization of alkyl, acyl, and azo substituted derivatives of the potent phototoxin, alpha-terthienyl. J. Heterocycles 28:411–416.

Towers, G. H. N. 1984. Interaction of light with phytochemicals in some natural and novel systems. Can. J. Bot. 62:2900–2910.