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ALLELOPATHIC PROPERTIES OF α-TERTHIENYL AND PHENYLHEPTATRIYNE, NATURALLY OCCURRING COMPOUNDS FROM SPECIES OF ASTERACEAE

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Abstract—Alpha-terthienyl (α-T), a naturally occurring polyacetylene derivative from roots of *Tagetes erecta* L., and phenylheptatriyne (PHT), from leaves of *Bidens pilosa* L., were tested as possible allelopathic agents against four seedling species (*Asclepias syriaca* L., *Chenopodium album* L., *Phleum pratense* L., *Trifolium pratense* L.). *Asclepias* was the most sensitive of the species. Allelopathic activity was enhanced in the presence of sunlight or sources of near-UV, with LC_{50}s for *A. syriaca* of 0.15 ppm and 0.66 ppm with α-T and PHT, respectively; 0.27 and 0.85 for *C. album*; 0.79 and 1.43 for *P. pratense*, and 1.93 and 1.82 for *T. pratense*. Near-UV exposure was saturating but never more than found in summer sunlight at Ottawa, Canada. Growth inhibition was observed with seedlings treated with α-T and PHT but without near-UV irradiation. Germination of seedlings was also sensitive to α-T and PHT with or without near-UV treatment. α-T was extracted from soil surrounding the roots of *Tagetes*. Concentrations calculated for the soil (0.4 ppm) indicate that seedling growth could be significantly hindered. The activity and specificity of α-T was sufficiently high to warrant future field trials to assess its potential as a natural weed-control agent.

Key Words—Allelopathy, α-terthienyl, phenylheptatriyne, *Asclepias syriaca* L., *Chenopodium album* L., *Phleum pratense* L., *Trifolium pratense* L., *Tagetes erecta* L., *Bidens pilosa* L., Asteraceae, soil compounds.

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INTRODUCTION

Species of the Asteraceae are well known for their aggressive character and broad ecological amplitude. The family contains many species that are prone to become weedy, given a disturbed habitat. While the family is not noted for its importance as a food source, it is well known for its ability to compete successfully with agricultural crops (Frankton, 1971). Many species contain physiologically active compounds that have been utilized in folk medicine for their curative properties (Wat et al., 1980), while many of the substances synthesized by the family are toxic or show other significant physiological activity (Heywood et al., 1977).

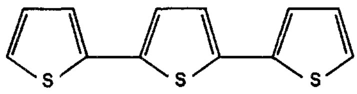
During the past two decades many new compounds have been identified in the Asteraceae (Heywood et al., 1977), including, for example, polyacetylenes and their thiophene derivatives which find their greatest diversity in the family (Bohlmann et al., 1973). While a number of reports indicate that polyacetylenes are physiologically very active against other organisms, the function of such compounds in the plant has not been seriously questioned until very recently (Towers and Wat, 1978).

Recently, we have begun a systematic study of the biological activity of polyacetylenes which has revealed for the first time that many polyacetylenes show high cytotoxic activity under natural sunlight or near-UV light (Towers et al., 1977). The photosensitizing effect of the pure polyacetylenes has been demonstrated with nematodes (Gommers and Geerligs, 1973), bacteria and fungi (Towers et al., 1977), mosquito and black fly larvae (Arnason et al., 1981b), and selected marine and freshwater algae (Arnason et al., 1981a).

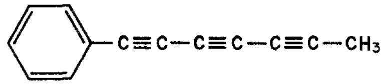
Only two reports in the literature were found that describe the effects of polyacetylenes on higher plants (Ichihara et al., 1976; Kobayashi et al., 1980). These workers found that C-10 polyacetylenes of composites, especially *cis*-dehydromatricariaester and *cis*-lachnophylumester were inhibitory to plant growth. The role of near-UV in this process was not investigated by these workers.

Many secondary metabolites are thought to have been selected for in plant evolution because they provide a protective advantage against pathogens, insects, and competing plants, a fact that seems to have been ignored in this technical age of synthetic biocides. By contrast, in many parts of the Third World the toxic effect of certain plants is used in folk medicine to control infections and in agriculture to control insect pests (Towers and Wat, 1978). Because synthetic biocides are coming under increasing scrutiny regarding their possible long-term effect in both natural and agroecosystems, we extended our study of the allelopathic properties of polyacetylenes and derivatives to their herbicidal effects on two selected forage plants and two weeds.

The compounds used in the study (Figure 1) were α-terthienyl (α-T), a



ALPHA TERTHIENYL



PHENYLHEPTATRIYNE

FIG. 1. The structure of alpha-terthienyl (α-T) and phenylheptatriyne (PHT).

thiophene derivative naturally occurring in roots of the common marigold (*Tagetes* spp.), and phenylheptatriyne (PHT), from the leaves of *Bidens pilosa* L. (Bohlmann et al., 1973). *B. pilosa* is a pantropical weed that is a serious problem in agriculture in the humid tropics in the Central American lowlands. The compounds were tested against seedlings of *Asclepias syriaca* L. and *Chenopodium album* L., two common weeds, and *Phleum pratense* L. and *Trifolium pratense* L., two common forage species of eastern North America.

METHODS AND MATERIALS

Plant Sources. Dehiscing pods of *Asclepias syriaca* were collected in Ottawa, Ontario, Canada. The seeds were stratified in distilled water at 3° C for three weeks to induce a high level of germination. *Chenopodium album* seeds were supplied by Agriculture Canada from sources in southern Ontario. *Phleum pratense*, *Trifolium pratense*, and *Tagetes erecta* seeds were obtained from a local farm seed supplier. *Tagetes* was grown in a greenhouse from seed, while *Bidens pilosa* was harvested from wild populations growing in vacant lots in Miami, Florida.

Chemicals. The α-terthienyl was isolated and purified from *Tagetes erecta* root tissue (Chan et al., 1975), and phenylheptatriyne was obtained from leaf tissue of *Bidens pilosa* (Towers et al., 1977).

Survival Curves. To determine the toxic effects of α-T and PHT on seedlings of the four plant species, germinated seedling of identical length (10 cm) were placed in Petri dishes lined with Whatman No. 1 filter paper. Since the activity of α-T and PHT is enhanced by near-UV, one trial consisted of polyacetylene and near-UV exposure. The UV exposure was omitted in one trial to observe dark effects of the pure compounds. The toxic effect of near-UV exposure was monitored by running trials without α-T or PHT treatment. Controls were run without UV exposure and without polyacetylene treatment. EtOH was added to the controls to monitor its possible toxic effects.

Solutions of the polyacetylenes were made from stock diluted in Hoagland's solution to achieve the following concentrations: 10, 5, 1, 0.1, 0.8, 0.5, and 0.01 ppm. Five milliliters were sprayed directly on the seedlings in each plate. There were 100 seedlings per trial.

The near-UV exposure was made using four Westinghouse blacklight blue tubes (Westinghouse F20T12/BLB). After a 16-hr exposure of the uncovered seedlings, all plates were then placed in a growth chamber with a temperature regime of 22° C–30° C and a 16-hr photoperiod (fluorescent and incandescent). Seedling death was assayed each day for five days. The experiments were carried out in quintuplicate. From the data collected, probit plots were obtained from which LC_{50} and LC_{90} values were determined for all plant species.

Using a YSI 65-A radiometer and a Kodak Wratten filter #2A, incident UV radiation of less than 400 nm was estimated to be $3 W/m^2$. Similar measurement on solar radiation indicated that the UV component of summer sunlight at Ottawa (45° N latitude) was the same order of magnitude ($8 W/m^2$, measured at midday on a clear day in June).

Germination Studies. The effect of α-T and PHT on germination were tested as follows. Seeds for each plant were placed in Petri dishes lined with Whatman No. 1 filter paper. A total of 800 seeds were separated into four sets of plates. The plates were moistened with 5 ml of the appropriate polyacetylene solutions. Three concentrations were used: 10, 1, and 0.1 ppm, with or without near-UV. The near-UV exposure was 8 hr, after which the seeds were covered in foil and placed in the growth chamber under conditions identical to the seedling trial. Germination was assayed over a 2-week period. The experiment was carried out in triplicate.

Light Saturation curve. To test the effect of varying near-UV exposure on the toxicity of the polyacetylenes, *Asclepias syriaca* was chosen since it was the most sensitive of all seedlings tested. One hundred seedlings of identical length were chosen and 0.3 ppm α-T was applied as stated previously for the survival curves. Plates were exposed to near-UV light for periods varying from 2 to 24 hr. Seedling death was assayed each day for five days. The toxicity levels of α-T were considered to have reached saturation when little increase in toxicity could be observed with increased near-UV exposure. The experiment was carried out in triplicate.

Growth Curves. Seedlings of the four species were placed in Petri plates containing Hoagland's solution with α-T and PHT concentrations of 100, 30, 10, 3, 1, and 0.3 ppm. Treatments and controls were established as previously described for the survival curve. The near-UV treatment was given for 6 hr each day for 14 days. The fresh weight was taken at the end of the fourteenth day. Graphed results represent percent mean growth of the test seedlings versus the controls. Ten seedlings were measured for each data point.

Extraction of Active Compounds from Soil. One hundred grams of soil were obtained from soil surrounding the roots of *Tagetes* and run through a 2-mm sieve to remove root particles. The resulting soil was suspended in 200 ml of ethanol and mixed in a shaker for 24 hr. After filtration the ethanolic solution was extracted with 500 ml of petroleum ether which was subsequently brought down in vacuo to a 5-ml-thick residue. Phototoxic activity was assayed by a standard assay method using yeast (Chan et al., 1975).

The α-T was separated by thin-layer chromatography of 100 μl of soil extract. A reference α-T solution was developed with the extract chromatograms. All plates were developed in 1:9 anhydrous ethyl ether and petroleum ether on silica gel G-25 UV254. A second solvent system (1:1 hexane and chloroform) was used to confirm the presence of α-T in the extract. Plates were analyzed under UV light to detect the blue fluorescing compound α-T. To quantitate the relative amounts of α-T in the soil, the TLC fluorescent spots were scraped from plates and eluted with ethanol and determined spectrophotometrically. The complete procedure was repeated three times.

RESULTS

The probit plots (Figure 2) represent data points that lie between 10 and 90% seedling survival for seedlings treated with α-T or PHT in the presence of near-UV radiation. At least three points of toxicity were found for α-T and PHT for the four species tested. The symptoms of α-T and PHT damage were remarkably similar in all four species. *Asclepias syriaca* showed the most acute signs of damage, characterized by necrosis of the roots and chlorosis of the leaves. The leaf response of *Trifolium pratense*, *Chenopodium album*, and *Phleum pratense* was similar. However, no root damage as seen in *Asclepias syriaca* was observed. The final criterion for seedling death was a complete chlorosis and wilting of leaf and stem tissue. No "dark effect" was observed at the concentrations used and no toxicity was observed with the near-UV controls. In all cases, it was difficult to obtain data points in the intermediate toxicity range and many replicates were needed.

LC_{50} and LC_{90} values (Table 1) show that *A. syriaca* was the most sensitive species followed by *C. album*, *T. pratense*, and the monocot *P. pratense*.

Figure 3 represents the effects of varying the exposure time to near-UV of *Asclepias syriaca* seedlings treated with 0.3 ppm α-T. Minimal increases in seedling death were observed after 16 hr of near-UV exposure (2.5 W/m^2). Saturation was assumed to occur at this point, and all experiments involving seedling mortality had this minimum level of UV exposure.

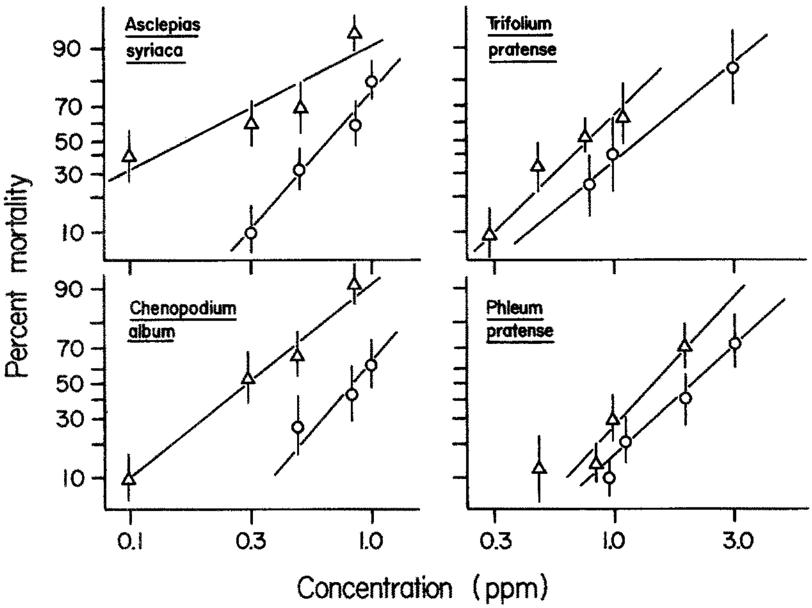


FIG. 2. The log of seedling mortality over seedling survival (probit plot) for four plant species exposed to varying concentrations of α-T (triangles) and PHT (circles). Near-UV exposure was 16 hr at 3 W/m^2. Data points represent intermediate toxicity between 100% seedling survival and 100% seedling mortality. Standard error is included.

TABLE 1. LETHAL CONCENTRATION LEVELS FOR SEEDLINGS TREATED WITH α-T AND PHT EXPOSED TO NEAR-UV.

Test species	α-T	PHT	Lethal conc.
Asclepias	0.15	0.66	LC^{50}
syriaca	0.79	1.51	LC^{90}
Chenopodium	0.27	0.83	LC^{50}
album	0.83	1.57	LC^{90}
Phleum	1.82	2.88	LC^{50}
pratense	3.63	5.08	LC^{90}
Trifolium	0.79	1.43	LC^{50}
pratense	1.99	3.63	LC^{90}

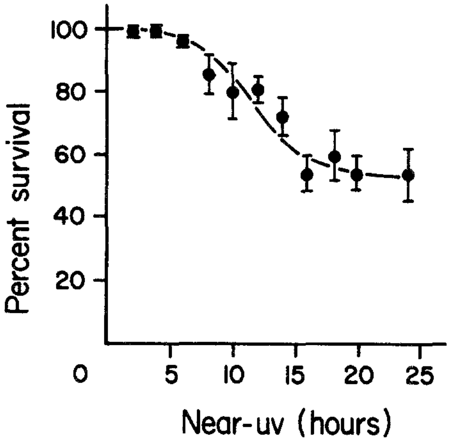


FIG. 3. The effect of near-UV exposure on seedling survival of *Asclepias syriaca* treated with 0.3 ppm α-T. Intensity was 3 W/m^2.

Even under conditions which are nonlethal, α-T and PHT possess demonstrable allelopathic potential (Figures 4 and 5). Growth inhibition is shown for α-T and PHT treatment without near-UV irradiation or with daily near-UV fluences that are approximately one-half saturation (8 hr) for the phototoxic effect (Figure 3). Without near-UV treatment, α-T and PHT inhibit the growth of all species at concentrations of approximately 10 ppm and higher, with α-T showing somewhat greater activity. With near-UV treatment, a similar pattern of growth inhibition was observed at lower concentrations.

Germination of the four test species was also sensitive to α-T or PHT treatment (Table 2). As with the growth experiments, significant inhibition of germination was observed even without near-UV treatment. Surprisingly, *C. album*, the smallest seed, was the least sensitive.

The extracts of soil taken from pure stands of *Tagetes* were found to have phototoxic activity in assays using yeast. Thin-layer chromatography of the crude extract revealed the presence of two blue fluorescent spots. Cochromatography with authentic α-T revealed that the lower spot was α-T ($R_f = 0.66$ with ethyl ether–petroleum ether, 1:9; and $R_f = 0.80$ for chloroform–hexane, 1:1; UV absorption bands at 253 and 350 nm). The upper spot ($R_f = 0.81$ with ethyl ether–petroleum ether, 1:9; and $R_f = 0.89$ with chloroform–hexane 1:1; UV absorption bands at 253 and 346 nm) was tentatively identified as the bithienyl. By using published values for the extinction coefficient (Bohlmann et al., 1973), the concentration of α-T in the soil was estimated to be 0.48 ± 0.21 ppm. A recovery estimate of α-T placed in soil and extracted by an identical procedure gave a value of 81%.

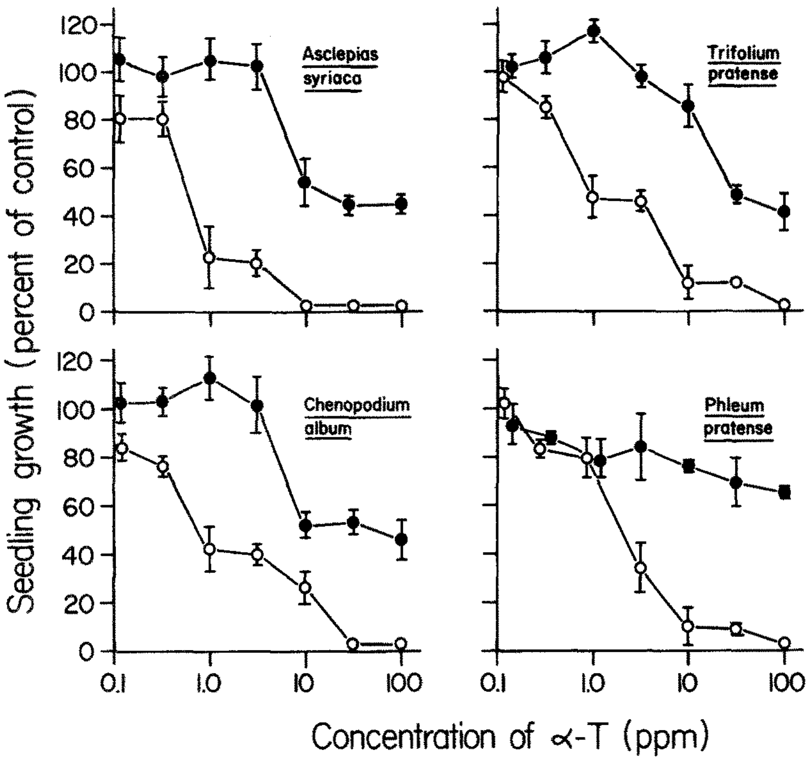


FIG. 4. The growth of four seedling species (fresh weight) as a percent of control growth is plotted against the concentration of α-T in the growth medium. Near-UV exposure (open symbols) was for 8 hr/day for 14 days. Plants given no near-UV treatment are represented by closed circles. Growth was monitored after 14 days. Each data point represents combined results of ten seedlings.

DISCUSSION

To demonstrate that a naturally occurring plant compound is allelopathic, it must first be shown that it is toxic towards competing plants. The probit plots indicate that both α-T and PHT are toxic towards four species of seedlings when irradiated with near-UV. Although probably not the natural competitors of *Bidens* or *Tagetes*, the species are in widely separated families and demonstrate the broad spectrum of activity of these compounds. In addition, the growth curves and germination study indicate that these compounds are inhibitory with or without near-UV exposure.

The role of near-UV in the action of allelopathic substances requires clarification. These compounds could be absorbed from the soil by newly emergent competing seedlings to a degree where the sun's near-UV rays could

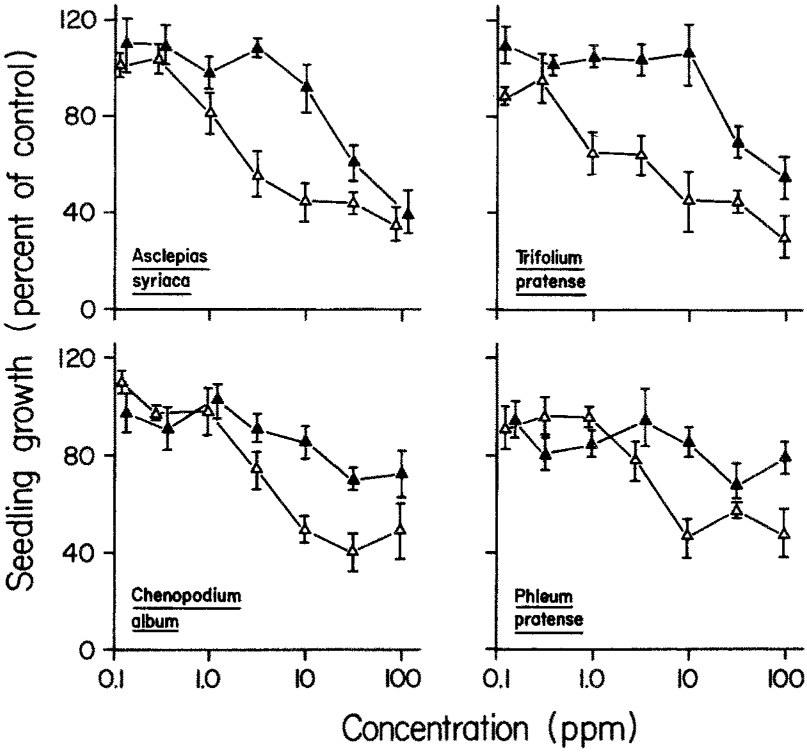


FIG. 5. As in Figure 4 using PHT.

TABLE 2. EFFECT OF α-T AND PHT ON SEED GERMINATION[a]

Species	Concentration (ppm)	α-T +UV	α-T −UV	PHT +UV	PHT −UV
Ascelepias syriaca	10	19.4 ± 4.9	46.8 ± 6.3	42.1 ± 3.8	54.4 ± 8.4
	1	48.7 ± 8.1	63.2 ± 9.4	83.1 ± 3.4	79.6 ± 4.5
	0.1	96.2 ± 1.8	101.1 ± 2.0	98.2 ± 7.3	104.7 ± 0.9
Chenopodium album	10	49.2 ± 3.4	100.1 ± 8.8	106.0 ± 1.7	101.1 ± 0.6
	1	81.6 ± 8.1	97.6 ± 1.3	99.1 ± 2.9	87.8 ± 1.1
	0.1	100.4 ± 7.4	102.6 ± 2.1	100.1 ± 0.9	97.4 ± 4.8
Phleum pratense	10	27.4 ± 4.6	48.8 ± 6.1	61.4 ± 6.4	69.1 ± 8.8
	1	81.3 ± 6.2	100.1 ± 1.1	100.0 ± 0.7	97.9 ± 2.2
	0.1	100.9 ± 8.4	99.7 ± 9.1	102.0 ± 1.2	96.1 ± 0.9
Trifolium pratense	10	22.1 ± 4.4	59.1 ± 7.7	59.0 ± 8.8	71.0 ± 10.1
	1	76.4 ± 8.3	92.9 ± 3.4	84.0 ± 10.1	97 ± 4.4
	0.1	97.4 ± 5.1	103.8 ± 1.7	99.0 ± 1.2	101.0 ± 0.3

[a]Germination expressed as percent of control ± SE.

invoke photosensitized damage. Growth inhibition shown by the dark activity of α-T and PHT also indicates that significant effects on competing seedlings could occur even without the presence of UV light. However, the near-UV radiation levels from the lamps are not as high as the near-UV irradiation from the sun incident at the earth surface, an important consideration when one looks at the natural functions of these compounds. Saturation occurred at much higher doses with plants than previously found for other organisms such as mosquito larvae (1 hr) (Arnason et al., 1981b). This effect may be due to the presence of UV screening compounds such as flavonoids in the plants.

The second stage in identifying an allelopathic compound is to demonstrate that the chemical is present in the soil in sufficient quantitities to inhibit growth after being released from the plant body. There are four known methods by which plants can release these compounds into the soil (Tukey, 1969). They are: (1) the decomposition of leaf litter through mechanical or biological means; (2) highly volatile compounds could be vaporized and exert their effects on other plants; (3) the roots could release the compounds by direct exudation or by mechanical or biological decomposition; and (4) leaching of the leaves by rain to the soil. The most likely possibility for *Tagetes* spp. is (3), since α-T is found in such high concentrations in the root tissue. For *Bidens pilosa*, (1) and (4) are the two most likely since PHT is found at high concentrations in the leaf tissue. PHT is also found in the roots of other Asteraceae (Bohlmann et al., 1973).

In the case of *Tagetes*, α-T was found in the soil of pure stands in concentrations as high as 0.4 ppm, which would be more than enough to inhibit competing seedlings if one considers the role of near-UV. One could also consider the possibility that competing seedlings could absorb these lipophilic compounds and concentrate them in membranes to highly inhibitory levels. No pure stands of *Bidens* were available, so soil assays of PHT were not attempted.

The next step in assessing the allelopathy of α-T is to show that it is active not only in controlled in vitro experiments but also under natural conditions. We are currently investigating this problem and attempting to determine how the host plant protects itself from autotoxic effects.

We are also investigating the toxic mechanism for both α-T and PHT. Alpha-T was found to be more phytotoxic in near-UV to the plant seedling than PHT, which agrees with previously published data on polyacetylene phototoxicology (Arnason et al., 1980). Also, work on the mechanism of action of α-T and related polyacetylenes has revealed that the action spectrum for photosensitization resembles the absorption spectrum with the photoreceptor molecule being the polyacetylene or thiophene compound. The research to date has related known photosensitizers to the activity of these compounds. There are two known groups of photosensitizers, the largest being the photodynamic compounds which produce toxic species of oxygen like OH· (hydroxyl radical) and $^1O_2$ (singlet oxygen). The second group does not require O_2 to exert damage, like the furanocoumarins whose toxicity is caused by a UV-induced cross-linking of DNA. Alpha-T has been shown to be photodynamic, as anaerobic conditions inhibit its activity and quenchers of activated species of O_2 such as SOD and NaN_3 lower toxicity (Arnason et al., 1980). PHT photosensitization studies indicated survival of *E. coli* was enhanced in aerobic conditions. In addition, SOD and NaN_3 did not effect the survival curves. Thus activated species of oxygen do not appear to be involved in the toxic process, and PHT is nonphotodynamic in vivo. Studies indicate, however, that there is no cross-linking of DNA as in photosensitization with the furanocoumarins (Towers et al., 1977). It is thus believed that PHT represents a new class of photosensitizer, possibly producing toxic free radicals.

Asclepias syriaca was the most sensitive of the seedlings tested, while the monocot *P. pratense* was the least sensitive of all seedlings exposed to α-T and PHT in the presence of near UV. The ratio in LC_{50}'s for these two species is 12:1 for α-T. This selectivity is potentially useful since milkweed is a common problem of overgrown pastures in eastern North America. Herbicides such as 2,4-D are not very effective against this species and cutting leads to regeneration of plants from the adventitious root systems.

Alpha-terthienyl was found to have such high toxicity that additional experiments are now being conducted to ascertain its potential as a natural weed control agent through potted plant and weed plot field trials. The use of allelopathic agents poses an alternative in weed control where the potential for biodegradation and reduced environmental impact are attractive aspects of using natural products in pest control.

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