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THE ALLELOPATHIC AND HERBICIDAL POTENTIAL OF
ALPHA-TERTHIENYL AND
PHENYLHEPTATRIENE, NATURALLY OCCURRING COMPOUNDS FROM
SPECIES OF THE ASTERACEAE

by

© GLENN HAROLD CAMPBELL, B. Sc.

A THESIS
PRESENTED TO CARLETON UNIVERSITY
in partial fulfillment of the
requirements for the degree of
Master of Science
in
the Department of Biology

The undersigned recommend to the Faculty of Graduate Studies acceptance of the thesis "The Herbicidal and Allelopathic Potential of Alpha-Terthienyl and Phenylheptatriyne, two Naturally Occurring from Species of the Asteraceae" submitted by Glenn Campbell in partial fulfillment of the requirements for the degree of Master of Science.

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SEPTEMBER 22, 1983.

ABSTRACT

Two naturally occurring photosensitizers alpha-terthienyl, a thiophene compound from Tagetes spp., and phenylheptatriyne, a polyacetylene from Bidens pilosa, were tested for potential allelopathic activity against two selected forage species (Phleum pratense L. and Trifolium pratense L.) and two common pasture weeds (Asclepias syriaca L. and Chenopodium album L.) in Petri dish seedling trials. Seedling survival and growth were examined with and without photosensitizing near UV irradiation. Asclepias syriaca was the most sensitive (LC_{50} - 0.15 ppm for -T, 0.66 for PHT), and the monocot Phleum pratense was the least sensitive (LC_{50} - 1.82 ppm for -T, 2.88 ppm for PHT), which indicated significant species selectivity. The herbicidal effect of alpha-terthienyl against a standard corn-broadleaf weed system (Amaranthus retroflexus L. and Chenopodium album L.) was assayed in controlled environmental chamber and field trials. Efficacy to weeds (ED_{50} - 29.9 kg/ha) was lower than atrazine and corn was damaged at similar concentrations. A more hydrophilic analog of alpha-terthienyl is proposed as a method of increasing its activity.

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CONTENTS

ABSTRACT	ii
ACKNOWLEDGMENTS	iv

<u>Chapter</u>	<u>page</u>
I. INTRODUCTION	1
II. MATERIALS AND METHODS	13
III. RESULTS	25
IV. DISCUSSION	70
REFERENCES CITED	83

LIST OF TABLES

<u>Table</u>	<u>page</u>
1. NATURAL SOURCES OF POLYACETYLENES	6
2. NATURAL SOURCES OF ALPHA-TERTHIENYL AND PHENYLHEPTATRIYNE	6
3. CHEMICAL DATA OF EXTRACTED THIOPHENES FROM TAGETES ERECTA	26
4. THE CHEMICAL CHARACTERIZATION OF THE ULLMANN SYNTHESIS PRODUCTS	27
5. LETHAL CONCENTRATIONS FOR SEEDLINGS EXPOSED TO α - T, PHT, AND NEAR UV	45
6. ED ₀₁ VALUES FOR ATRAZINE AND α -T IN GROWTH CHAMBER AND FIELD TRIALS	66

LIST OF FIGURES

<u>Figure</u>	<u>page</u>
1. The structure of alpha-terthienyl and phenylheptatriyne.	4
2. The UV spectrum of alpha-terthienyl.	30
3. The UV spectrum of phenylheptatriyne.	32
4. The mass spectrum of synthesized alpha-terthienyl.	34
5. Probit plots of seedling mortality of seedlings exposed	37
6. The effect of near UV exposure on seedling survival	40
7. The effects of α -T on seedling growth in Petri plate hydroponics.	42
8. The effects of PHT on seedling growth in Petri plate hydroponics.	44
9. The light and dark activated components of α -T sublethal effects	49
10. The inhibitory effects of Citowett Plus	52
11. The herbicidal efficacy of α -T at the 2, 4, and 6 leaf stages	54
12. Growth chamber trials of α -T against corn and <u>Chenopodium album</u>	56
13. Herbicidal efficacy of atrazine against weeds at the 4 leaf stage	59
14. The herbicidal efficacy of α -T against weeds in field trials.	61
15. Field trial herbicidal efficacy of atrazine against weeds	63

16. The persistence of α -T in the soil.	65
17. The persistence of α -T residues on the plant surface	69

Chapter I

INTRODUCTION

Species of the plant family Asteraceae are well known for their aggressive character and broad ecological amplitude. While the family is not noted for its importance as a food source, many members are well known for their ability to compete successfully with agricultural crops and have become serious weed problems (Frankton, 1977). Cirsium arvense L. (Canada thistle) and Centaurea repens L. (knapweed) are two of the most important representative examples of the pest problems posed by some species of the Asteraceae in Canada. This competitive advantage in an altered or disturbed habitat could be due to a variety of factors such as reproductive efficiency, efficient utilization of resources and nutrients available to the plant, or chemical inhibition of competing plant species (allelopathy).

This family is also unusual in its vast and distinct secondary chemistry which includes sesquiterpene lactones (Rodriguez, 1977), phenolics, diterpenes, triterpenes, steroids, alkaloids, pyrethrins, and a class of compounds known as polyacetylenes and thiophene derivatives (Mabry and Bohlman, 1977) which forms the focus of this study. Secondary metabolites in general have not been understood in terms of

their physiological importance and it has only recently been discovered that many of the compounds may play an ecological role such as insect attractants, repellants, or as chemical defense agents against damaging plant pathogens (phytoalexins) and competing plants (allelopathic agents).

Polyacetylenes attain their greatest numbers and diversity in the Asteraceae but are also found in several other plant families, algae, and certain species of marine invertebrates (Bohlmann et al, 1973). These compounds contain linear conjugated systems of double and triple bonds whereas thiophenes are biosynthetically derived from polyacetylenes by cyclization of two conjugated triple bonds. These chemicals are found throughout the plant but most notably in the root, flower, and leaf tissues (Bohlmann et al, 1973).

The two substances used in the present study were alpha-terthienyl and phenylheptatriyne. Alpha-terthienyl (α -T) is a thiophene compound that consists of a series of three thiophene units bonded at the alpha position (Figure 1). Tagetes erecta L. is one of the better natural sources of α -T as the concentration can approach 0.1 percent of the root dry weight (Towers and Wat, 1978). Biosynthetically, α -T is known to be derived from pentayne-ene through a cyclization and sulphur addition by methionine (Jente et al, 1982). Phenylheptatriyne (PHT) is a polyacetylene compound with a phenyl group attached to a series of three conjugated triple bonds; it is found in the leaves of Bidens pilosa L.,

Figure 1: The structure of alpha-terthienyl and
phenylheptatriyne.

ALPHA TERTHIENYL

PHENYLHEPTATRIYNE

a pan-tropical weed that has become a serious problem in agriculture in the humid tropics (Gleissman, 1980). It has also been identified in root tissue of Cernua spp. and Coreopsis spp. (Table 1). Taquetes erecta and Bidens pilosa are known to occur in pure stands which is suggestive of possible allelopathic dominance (Gleissman, 1980).

Previous reports indicate several polyacetylenes have been known to exhibit very diverse toxic activity towards mammals, fungi, microorganisms, and insects (Robinson, 1980). Discovery of the potent nematicidal properties of α -T led to the original interest in this compound (Uilenbroek and Bijloo, 1958). It was subsequently shown that the nematicidal ability of α -T was enhanced in the presence of daylight and specifically the UV-A region of the spectrum (320nm to 400nm) (Gommers et al, 1973). Chan et al (1975) reported that α -T was phototoxic or bactericidal in UV-A, but caused no lethal effect in the dark. The photosensitizing effect was demonstrated for various thiophenes and polyacetylenic compounds against a wide range of organisms in addition to nematodes and include bacteria (Arnason et al, 1980), fungi (Dicosmo et al, 1982), algae (Arnason et al, 1981), and insects (Wat et al, 1981).

The photosensitizing effects of alpha-terthienyl, phenylheptatriyne, and related compounds has stimulated interest in the in vivo mechanism of action. Work on the mechanism of action has revealed that the action spectrum resembles

TABLE 1

NATURAL SOURCES OF POLYACETYLENES

higher plant families	algae	fungi
Asteraceae	<u>Cryptomonas</u> spp.	<u>Helminthosporium</u> spp.
Araliaceae	<u>Laurentia</u> spp.	<u>Streptomyces</u> spp.
Pilosporaceae	<u>Hemiselmis</u> spp.	<u>Rhododoturala</u> spp.
Umbelliferae	<u>Asterias</u> spp.	
Campanulaceae		
Euphorbiaceae		
Olacaceae		
Loranthaceae		
Lauraceae		
Santalaceae		
Cupressaceae		
Solanaceae		

TABLE 2

NATURAL SOURCES OF ALPHA-TERTHIENYL AND PHENYLHEPTATRIYNE
FROM THE ASTERACEAE

ALPHA-TERTHIENYL	PHENYLHEPTATRIYNE
<u>Tagetes</u> spp.	<u>Bidens</u> spp.
<u>Berkeya</u> spp.	<u>Cernua</u> spp.
<u>Flaveria</u> spp.	<u>Dahlia</u> spp.
<u>Eclipta</u> spp.	<u>Coreopsis</u> spp.
<u>Gaillardia</u> spp.	

the absorption spectrum with the photoreceptor molecule being the thiophene or polyacetylene (Arnason et al, 1980). Recent research has attempted to relate the activity of these compounds to known photosensitizers. There are two known groups of photosensitizers, the largest being the photodynamic compounds which require oxygen for the toxic process (Spikes, 1977). The second group does not require oxygen to exert damage and includes the furanocoumarins whose toxicity is caused by a cross-linking of DNA induced by near UV radiation (Spikes, 1977). Alpha-terthienyl has been shown to be photodynamic in vivo as anaerobic conditions inhibit the toxicity and quenchers of activated species of oxygen such as superoxide dismutase (SOD) and sodium azide (NaN_3) lower activity (Arnason et al, 1981). The in vitro studies of Bakker et al, 1979) have confirmed the photodynamic properties of α -T.

After absorption of near UV by alpha-terthienyl, the molecule in its triplet state interacts with oxygen in its ground state, and in an energy transfer reaction oxygen is converted to a high energy singlet state ($^1\text{O}_2$). Singlet oxygen is a very potent oxidizing agent which can readily diffuse relatively large distances within the cell to cause cellular damage related to lipid peroxidation (Rawls and van Santen, 1970), DNA degradation (Schutze and Lochmann, 1981), and amino acid oxidation (DeGroeij et al, 1976, and Yamamoto et al, 1979). Membranes appear to be the central target

site for photosensitization which stems from investigations on erythrocyte electrolyte leakage (Wat et al, 1980), damaged erythrocyte electron micrographs (Yamamoto et al, 1979), and selective activity studies against membrane enclosed lipid viruses (Downum et al, 1982).

PHT photosensitization is non-photodynamic in vivo (Arnason et al, 1980) but there is no cross-linking of DNA as with the furanocoumarins (Towers et al, 1977). It is thus believed that PHT represents a new class of photosensitization and it has been postulated that a free radical intermediate formed within the membrane is the toxic moiety (Arnason et al, 1980).

There are only two reports in the literature describing the effects of polyacetylenes on higher plants (Ichiyama et al, 1976, and Kobayashi et al, 1980). These workers demonstrated that C-10 polyacetylenes of the Asteraceae, especially cis-dehydromatricariaester and cis-lachnophylumester were inhibitory to plant growth and were potentially allelopathic substances of Solidago altissima L. The role of UV-A was not investigated. Many species within the Asteraceae have been evaluated for potential allelopathy and include Cirsium arvense (Wilson and Anderson, 1981), Ambrosia artemisiifolia (Kobayashi et al, 1975), Helianthus annuus (Leather, 1983), Artemisia tridentata (Groves and Anderson, 1981), and Centaurea spp. (Vezina, 1983). The active compounds involved in these studies were not identified and the possibility exists that polyacetylenes may be involved.

Allelopathy has been extensively reviewed by Rice (1973, 1981). From the wealth of papers published, there are many established examples of allelopathy. Questions that must be answered include chemical identification of the inhibitor and how does it get from the producer to the receiver plant to influence growth. Conclusive studies must show that allelopathy actually operates under field conditions. In experiments on allelopathy, extracts or exudates are often tested by applying them to young seedlings on filter paper in Petri dishes (Rice, 1973). The toxins most often reported are simple phenolics such as 4-hydroxybenzoic acid, ferulic, caffeic, and vanillic acid (Rice, 1981).

Allelopathy might be considered the counterpart in natural ecosystems, of weed control by herbicides in agroecosystems. In allelopathy the host plant influences the growth of another by means of a toxic chemical. Allelopathy is a possible means by which weeds could influence crops, crops influence weeds, or one crop influencing another which follows it. An applied aspect of allelopathy is that it might suggest new chemicals that could form the basis for herbicides or other agrochemicals.

Many synthetic biocides are coming under increasing scrutiny with regard to their possible long term effects in both natural and agroecosystems and as a consequence new and innovative methods of pest control are being developed. Natural products of plant origin provide a large source of

bioactive substances which have been sparingly exploited as synthetic models or as natural pesticides. The rapid development of powerful analytical tools such as HPLC, GC-MS, and related techniques have facilitated identification of natural products at an increasing pace. Among the best known natural products and one of great economic importance are the pyrethroid insecticidal components of Chrysanthemum spp. Pyrethrin I was an excellent prototype for the synthetic pyrethroids, which have improved photostable characteristics (Ohsumi et al, 1981). Several other active plant principles have been used as insecticides but with limited success. These include the rotenoids (Jacobsen, 1973) and the alkaloid nicotine (Robinson, 1980). A study of the natural products of tropical hardwoods has yielded very potent insecticidal benzoquinone compounds that may have economic importance. Synthetic analogues of the benzoquinones have been subsequently found to be superior (Jurd and Manners, 1980). Several insect antifeedants of plant origin hold promise and these include warburganal (Meinwald et al, 1978), and a triterpenoid, azadarachtin (Zanno et al, 1975).

If a plant contains a phytotoxic substance, it may be useful as a herbicide. A prime requirement is that it should be toxic to weed species and not to others (selectivity). Its efficacy should be comparable to presently established herbicides and possess low environmental persistence.

The lack of information on the phototoxic effects of thiophenes and polyacetylenes on higher plants catalysed the present investigation. It was proposed that alpha-terthienyl and phenylheptatriyne would be evaluated for their toxic and growth inhibitory activity against two selected forage plants (Phleum pratense and Trifolium pratense) and two problem weeds (Asclepias syriaca and Chenopodium album) in germinated seedling Petri plate hydroponic tests. It was also proposed that the role of near UV light in the phototoxic process would be investigated. In order to ascertain the allelopathic potential of α -T, soil samples from pure stands of Tagetes erecta were to be analyzed for their α -T content for comparison with the inhibitory activity observed in the hydroponic tests.

If the selectivity and activity observed under the seedling trials were found to be sufficiently high, it was proposed that the study be extended to evaluate these substances as herbicides in controlled environmental chamber trials using a standard Zea mays -broadleaf weed system (Chenopodium album and Amaranthus retroflexus) because of its agricultural relevance. The plants would be evaluated by a post-emergent spray application to the two, four, and six leaf stages of growth.

It was suggested that in order to accurately assess the efficacy of the substance, growth chamber trials would be extended to include outdoor field trials. Of major interest

would be the environmental persistence in the soil and on the exposed plant surface of the substance.

Chapter II

MATERIALS AND METHODS

Plant sources

Dehiscing pods of Asclepias syriaca were collected in Ottawa, Ontario, Canada (October, 1979). The seeds were stratified in distilled water at 3°C for three weeks to induce a high level of germination. Phleum pratense L., Trifolium pratense L., and Tagetes erecta L. were obtained from a local seed supplier. Tagetes erecta was grown in a greenhouse from seed, while Bidens pilosa was harvested from wild populations growing in vacant lots in Miami, Florida. Atrazine sensitive Chenopodium album L. and Amaranthus retroflexus L. were supplied by Dr. Suzanne Warwick (Agriculture Canada) after collection in southern Ontario.

Extraction and Purification of Alpha-Terthienyl and Phenylhelipatriyne from Plant Sources

Alpha-terthienyl from Tagetes erecta root tissue was extracted using the technique of Chan et al (1975). This method involved an ethanol extraction of fresh plant material. The tissue was ground in a Waring blender and the mixture filtered in a Buchner funnel using Whatman No. 1 filter paper. The filtrate was diluted 1:1 with distilled water and extracted three times with equal volumes of petroleum

ether (30-60°C) in a separatory funnel. The combined petroleum ether fractions were dried with anhydrous sodium sulphate, filtered and concentrated using a rotary evaporator. All procedures were conducted under dim light to avoid photodegradation of the extracted compounds. Purification of alpha-terthienyl was accomplished using column and thin layer chromatography. Columns of unactivated aluminum oxide (Baker Chemicals Chromatography Grade) were developed in an acetone:petroleum ether (30-60°C) solvent system (1:99). Development of the thiophene compounds on the column were monitored by their characteristic blue fluorescence under a hand held near ultraviolet light source. Fractions were collected, concentrated on a rotary evaporator and dissolved in analytical grade ethanol for UV spectroscopic analysis using a Pye Unicam (SP8-100) visible-UV spectrophotometer. The quantity of α -T in a sample was determined spectrophotometrically using the Beer-Lambert Law for the peak at 350nm (extinction coefficient $22,500 \text{ M}^{-1} \text{ cm}^{-1}$ in ethanol).

Thin layer chromatography was used for qualitative analysis and small scale isolation of compounds. Silica Gel G-100 plates (Baker Chemicals) were spotted with extracts and α -T standards and developed in one dimension in rectangular chambers using one of two solvent systems: petroleum ether (30-60°C):acetone (99:1) or hexane:chloroform (1:1).

Phenylheptatriyne was isolated and quantified from Bidens pilosa leaf tissue in an identical fashion to α -T extraction (Towers and Wat, 1978). This compound however does not fluoresce under long wave UV light and its progress cannot be monitored on a column of silica gel developed in petroleum ether. However, elution from a column can be detected spectrophotometrically. PHT was quantified utilising a spectral peak at 310nm (extinction coefficient $17,320 \text{ M}^{-1} \text{ cm}^{-1}$).

The Chemical Synthesis of Alpha-Terthienyl

The Ullmann reaction was the method used to produce α -T. This reaction involved the coupling of 2-iodothiophene (Aldrich Chemicals) in the presence of a copper powder catalyst under extreme reaction conditions (200-210°C) for thirty minutes in a three necked flask fitted with a condensing tube, thermometer, and a glass stopper. A typical reaction was as follows. Twenty grams of 2-iodothiophene were placed in a reaction vessel containing twenty grams of commercial grade copper powder (Aldrich Chemicals). The vessel was slowly heated to reaction temperature in an oil bath. Mechanical stirring was provided by a magnetic stir bar during the course of the reaction.

Various reaction conditions have been known to influence the yield of the Ullmann reaction and these included the activation state of the copper powder, and solvent influences (Yamazaki, 1976)). An activation process for copper powder

from Vogel (1957) was used in these experiments. One hundred grams of copper powder was treated with one litre of a two percent solution of iodine in acetone for five minutes. The product was filtered in a Buchner funnel and washed by stirring with a solution of hydrochloric acid in acetone (1:1). The residue was filtered and washed with acetone and dried in a vacuum desiccator. The copper was used immediately after this process. Aprotic solvents such as dimethylformamide (DMF) can improve yields (Vogel, 1958) for the Ullmann synthesis. As a consequence 200 ml of DMF were used in the reaction mixture. Since 2,2'-bithienyl was known to be an intermediate in the formation of α -T, 2,2'-bithienyl was added to the reaction mixture in an attempt to improve the yield (Yamazaki, 1976).

After completion of the reaction, the flask was cooled to room temperature. The thiophenes were extracted as follows. Two hundred ml of distilled water were added to the mixture followed by 200 ml of petroleum ether (30-60°C). The ether layer containing the thiophenes was isolated in a separatory funnel and concentrated in a rotary evaporator. This material was subsequently chromatographed on aluminum oxide columns to isolate crude α -T. This crude mixture was rechromatographed to further purify α -T. The final step in purification was a recrystallization from hot methanol. Verification of the identity and purity of the synthetic material was accomplished using mass spectroscopy, gas chroma-

tography, nuclear magnetic resonance spectroscopy, high performance liquid chromatography, and melting point determination. An α -T standard was supplied by Prof. G.H.N. Towers, Botany Department, University of British Columbia.

Seedling Mortality and Inhibition Curves

To determine the toxic effects of α -T and PHT on seedlings of Asclepias syriaca L., Chenopodium album L., Phleum pratense L., and Trifolium pratense L., germinated seedlings 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 light, one trial consisted of polyacetylenes or thiophenes, and near UV exposure. The near UV exposure was omitted in one trial to observe the non photosensitizing effects of the pure compounds. The toxic effects of near UV exposure were monitored by running trials without α -T or PHT treatment. Controls were run without UV exposure and without chemical treatment. The same volume of ethanol as used to dissolve PHT and α -T was added to the controls.

Solutions of the polyacetylenes were made from a one percent stock solution in ethanol and diluted in a standard Hoaglands nutrient solution to achieve the following concentrations: 10, 5, 1, 0.1, 0.8, 0.5, and 0.01 parts per million. Five milliliters were applied to each plate. There were 100 seedlings per trial.

The near UV exposure was made using four Westinghouse blacklight blue tubes (Westinghouse F20T12/BLB) with an em-

ission in the 300-400 nm range and a maximum at 350 nm. Using a YSI 65-A radiometer and a Kodak Wratten filter No.2A, incident UV radiation of less than 400 nm was estimated to be 3 W/m^2 . After a 16-hour exposure of the uncovered seedlings, all plates were then placed in a growth chamber with a 16-hour photoperiod (fluorescent and incandescent) at 30°C . Complete chlorosis became the criterion for seedling mortality after five days of incubation. The experiments were carried out with five replicates. From the data collected probit plots were obtained from which LC_{50} and LC_{90} values were determined for all species tested (Guthrie and Perry, 1980).

To test the effect of varying near UV exposure on the toxicity of α -T, Asclepias syriaca was chosen since it was the most sensitive of the seedlings tested. One hundred germinated seedlings were exposed to 0.3 ppm of α -T as described above. Plates were exposed to near UV light as before for periods varying from 2 to 24 hours. Seedling death was assayed after five days. The toxicity levels of α -T were considered to have reached saturation when additional near-UV exposure produced little increase in toxicity. Each experiment was carried out in triplicate.

In order to monitor the growth inhibiting effects of α -T and PHT, seedlings of the four species were placed in Petri plates containing Hoaglands solution with α -T and PHT concentrations of 100 ppm, 30 ppm, 10 ppm, 3 ppm, 1 ppm and 0.3

ppm. Treatments and controls were identical to the seedling mortality experiments. The near UV exposure was of 6 hours each day for 14 days. The fresh weight was determined on 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 Alpha-Terthienyl from Soil taken from Tagetes Stands

One hundred grams of soil were removed from the roots of Tagetes and run through a 2mm sieve to remove root particles. The resulting soil was suspended in 200 ml of ethanol and mixed in a shaker for 24 hours. 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 using the standard disc method of Chan et al (1975). The α -T was separated by thin layer chromatography (silica gel G-25 UV254) from 100 μ l 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 plates. A second solvent system (1:1 hexane and chloroform) was used to confirm the presence of α -T in the extract. Plates were observed under near UV light to detect α -T. To quantitate the relative amounts of α -T in the soil, the TLC plate fluorescent spots were removed, eluted with ethanol and determined spectrophotometrically. The complete procedure was repeated three times.

The Herbicidal Potential of α -T (Growth Chamber Trials)

The weed species chosen for testing were Amaranthus retroflexus and Chenopodium album L., two problem weeds of corn. The first tests were designed to illustrate and evaluate the efficacy of α -T as a post-emergent spray herbicide. Seeds of each species were placed into flats of a greenhouse soil mixture (vermiculite-peat moss) and germinated in an environmental growth chamber. The conditions in the chamber did not vary throughout the course of the experiment. The temperature regime was 20-30°C with a constant photoperiod of 16 hours and the humidity was constant at 40 percent. Upon germination, selected stages of development were chosen for herbicidal spraying of α -T. Flats (30cm by 30cm) containing 100 plants at the two, four, and six leaf stages were selected for herbicidal sprayings of α -T.

The growth chambers were fitted with a series of Vita-Lite lamps (Durotest Electric, No. 48T12). These lamps are solar simulating especially with respect to near UV content which is known to be the spectral band responsible for the photosensitizing properties of α -T. The intensity of the Vita-Lite emission at the test surface was measured at 456 W/m² using a YSI 65-A radiometer. The near UV intensity was determined to be 2 W/m² using the radiometer and the Kodak Wratter filter No. 2A.

The formulations used for the sprayings were prepared as follows. An accurately weighed quantity of α -T was deposit-

ed in a 200 ml applicator spray bottle to which 100 ml of distilled water were added and agitated manually. A commonly used herbicide wetting agent surfactant, Citowett Plus (B.A.S.F Corporation), was used in the formulations. The formulations were then spray-dispensed evenly over the flats and the resulting application rate was calculated as kilograms per hectare (kg/ha). Due to the cost and time involved in the synthesis of α -T, only a limited number of trials could be undertaken. The rates were varied from 1 to 30 kilograms per hectare for each of the three test species. After application, the plants were left for a two week period after which they were removed from the flat, oven dried, and weighed. Controls consisted of exposing the plants to the same formulation minus the test compound and/or the wetting agent. In this way it was possible to separate the growth effects of α -T from the wetting agent in the formulation. The two week post-application growth period was required to give quantitative information on the herbicidal damage of α -T. The efficacy of α -T was recorded as the growth of the test species versus the controls, expressed as a percentage.

In order to assess the potential of α -T as a herbicide, a standard was required for comparison of efficacy. Atrazine (technical grade Aatrex), an s-triazine herbicide, was selected because of its value in combatting Chenopodium album and Amaranthus retroflexus in corn. An identical protocol

was used in testing the post-emergent activity of atrazine. The chemical was obtained from W. Saidak (Agriculture Canada). The application rates differed from the a-T trials due to differences in herbicidal efficacy.

Since previous work on other organisms has shown that a-T has significantly higher activity in UV light, it was desirable to see if this affected its phytotoxic activity. One hundred weeds of Amaranthus retroflexus at the four leaf stage were exposed to a spray application of 15 kilograms per hectare of a-T. They were subsequently placed in regimes of total darkness (enclosed) or exposed under Vita-Lites. A sublethal assay of the number of necrotic lesions in each group were recorded after one hour intervals. This experiment was completed in triplicate.

The Herbicidal Potential of a-T (Field Trials)

The solar trials for a-T were performed on June 27, 1982 to optimize the near UV component (Thorington, 1976) involved in the phytotoxic process. The near UV content of the solar spectrum is maximized for Ottawa in late June. Ambient conditions were ideal on this date for spray application as there were no clouds, no wind, low humidity (21 percent), and a moderate temperature (22°C at 1 p.m.). The UV light irradiance was measured as previously described (global=928W/m², UV=11W/m²) at the ground surface. The test site was located in cultivated fields at Carleton University, Ottawa. Square plots (30 cm by 30 cm) were cultivated

and seeded with the three test species prior to the application date (June 6, 1982). In each plot, 100 plants at the four leaf stage were collected for testing. The controls were identical to the growth cabinet trials. Plants were collected after a two week growth period, oven dried and weighed. For each application rate there were three repetitions. Atrazine was again used as a reference standard herbicide.

Persistence studies

The persistence of α -T was measured in the soil and on the plant surface. The soil samples were supplied by S. Khan (Agriculture Canada). Two soil types classified according to their organic matter content (64 and 23 percent) as determined by flame crucible combustion of weighed samples, were selected for these experiments. The soils were brought to field capacity by trickling water through soil under gravitational filtration with Whatman No. 1 filter paper. Thirty grams of each were subsequently placed in 250 ml Ehrlenmeyer flasks. Ten milligrams of α -T were dispersed through the mixtures by vigorous stirring for five minutes. The samples were incubated at 23°C and at daily intervals three 1 g samples were withdrawn and accurately weighed. Each was suspended in 50 ml of ethanol (reagent grade), thoroughly mixed and filtered in a Buchner funnel fitted with Whatman No. 1 filter paper. The filtrate was diluted 1:1 with water and extracted with 100 ml of petroleum ether

three times. The ether layer was concentrated to 5 ml on a rotary evaporator. This solution was then banded on silica gel G-25 UV254 and developed in petroleum ether and acetone (99:1). The α -T band was removed, eluted with spectral grade ethanol and quantified spectrophotometrically.

The persistence of α -T on the plant surface under field conditions was tested as follows. The compound was applied to Amaranthus retroflexus (4 leaf stage) at 10 kg per hectare. Twenty five seedlings were then removed, weighed, and suspended in 100 ml of reagent grade ethanol. After maceration in a Waring blender the mixture was extracted as previously described into petroleum ether (30-60°C). The ether fraction was concentrated in vacuo and run on silica gel G-25 UV254 using an acetone:petroleum ether solvent system (1:99). The α -T band was removed, eluted, filtered, and quantified spectrophotometrically. In this manner the α -T residues were determined as micrograms per plant fresh weight. The procedure was repeated over a 14 day period to monitor the change in α -T residue content.

Standard errors were calculated from three trials, with each trial being the mean biomass of approximately 100 plants.

Chapter III

RESULTS

Preparation of α -T

During the extraction from plant material, it was found that alpha-terthienyl was very difficult to separate from a similar blue fluorescing compound (5-buten,3-1-ynyl-2,2'bithienyl) of Tagetes as indicated from the similarity in Rf values in TLC (Table 3). Structurally, the two compounds differ as terthienyl contains three thiophene units while the bithienyl possesses two thiophene units bonded to a conjugated triple bond-double bond system. The difference in UV maxima between the two compounds is only 2nm. Large scale columns (10 inches by 2 inches) could not resolve compounds using silica gel or activated aluminum oxide. For this reason, chemical synthesis became the method of choice for large scale preparation of α -T.

The high temperature Ullmann reaction yielded a series of polythienyl compounds which included monothienyl, bithienyl, terthienyl, quatrathienyl, pentathienyl, and hexathienyl. All of these compounds were characterized by a unique near UV fluorescence and chromatographic behavior on silica gel TLC. The data are shown in Table 4.

TABLE 3

CHEMICAL DATA OF EXTRACTED THIOPHENES FROM Tagetes erecta

compound	TLC Rf values	
	acetone:pet ether(99:1)	chloroform:hexane(1:1)
terthienyl	0.69	0.80
bithienyl	0.73	0.89

TABLE 4
THE CHEMICAL CHARACTERIZATION OF THE ULLMANN SYNTHESIS
PRODUCTS

compound	fluorescence under near UV	TLC Rf, values acetone:pet ether(1:99)
monothienyl	colourless	0.84
bithienyl	colourless	0.78
terthienyl	blue	0.69
quaterthienyl	green	0.54
pentathienyl	yellow	0.50
hexathienyl	orange	0.38
reaction components	percent yield of terthienyl	
100 g 2-iodothiophene 100 g copper powder	1.4	
100 g 2-iodothiophene 100 g copper powder(activated)	2.8	
50 g 2-iodothiophene 50 g bithienyl 100 g copper powder(activated)	4.9	
50 g 2-iodothiophene 50 g bithienyl 100 g copper powder(activated) 200 ml dimethylformamide	6.7	

Varying the reaction conditions and the reaction ingredients significantly altered the yield of α -T as revealed in Table 4. The 2-iodothiophene and commercial copper powder gave very low and inconsistent yields in the Ullmann reaction. Activation of the copper powder significantly increased the yield from 1.4 to 2.8 percent. Maximum yields were achieved by mixing 2,2'-bithienyl (Aldrich Chemicals) and 2-iodothiophene in dimethylformamide (DMF). Utilizing this synthesis, α -T was produced for the phytotoxic evaluations.

The identity and purity of the synthesized α -T was assessed and confirmed using mass spectroscopy, high performance liquid chromatography, gas chromatography, nuclear magnetic resonance spectroscopy, and melting point determination. The spectra of the synthesized α -T was identical to the standard and those observed in the literature. Thin layer chromatography (TLC) using multiple solvent systems yielded one band with no trace of impurity. From this assorted evidence, it was concluded that the synthesized α -T was pure enough to commence with the allelopathic and herbicidal tests. Chemical data are as follows; crystallized m.p. 93.5-94.5 lit 95°C (Zechmeister and Sease 1947); UV (ethanol) max 254 and 350 nm lit 254 and 350 nm (Chan et al, 1975); M/s 248 lit 248 (Gatsoni et al, 1982); NMR 7.10 lit 7.10 (Chan et al, 1975). The UV absorption spectra for α -T and PHT are given in Figures 2 and 3. The mass spectrum of α -T is shown in Figure 4.

Figure 2: The UV spectrum of alpha-terthienyl.

Figure 3: The UV spectrum of phenylheptatriyne.

Figure 4: The mass spectrum of synthesized alpha-terthienyl.

Seedling Mortality Curves

The probit plots (Figure 5) represent data points that lie between 10 and 90 percent seedling mortality for plants treated with α -T or PHT in the presence of near UV radiation. At least three points of intermediate toxicity were found for α -T and PHT for each of the four species. The symptoms of α -T and PHT damage were remarkably similar in all four test species. Asclepias syriaca revealed the most acute signs of damage, characterized by a severe necrosis of the roots and a chlorosis of the leaf tissue. The leaf response of Trifolium pratense L., Chenopodium album L., and Phleum pratense was also symptomized by severe chlorosis whereas the root necrosis was not as pronounced as that observed for Asclepias syriaca. The criterion used for seedling death was a complete chlorosis of leaf and stem tissue. Chlorotic and necrotic mottling was also observed on the leaf tissue of the seedlings that were not exposed to near UV light but the damage was not as severe. This was a clear indication of a dark effect. Probit analysis was not attempted for the near UV controls as complete chlorosis did not occur. The near UV treatments (minus α -T or PHT treatment) did not reveal signs of phototoxic damage and confirm that the observed damage to the seedlings was caused by PHT or α -T.

The lethal concentrations at fifty and ninety percent mortality (LC₅₀ and LC₉₀) as determined from the probit

Figure 5: Probit plots of seedling mortality of seedlings exposed to a-T and PHT. The log of seedling mortality over seedling survival for four plant species exposed to varying concentrations of a-T (triangles) and PHT (circles). Near UV exposure was 16 hours at $3W/m^2$. Data points represent intermediate toxicity between 100 percent seedling survival and 100 percent mortality. Standard error is included from three replicates.

plots (Figure 5) indicate that Asclepias syriaca was the most sensitive of the species tested followed by Chenopodium album, Trifolium pratense and the monocot Phleum pratense for both α -T and PHT (Table 5). Significant selectivity could be observed for α -T and PHT in this hydroponic system which is indicated in Table 5 by the difference in LC_{50} values for Asclepias syriaca and Phleum pratense.

Figure 6 represents the effects of varying the exposure time to near UV light of Asclepias syriaca treated with 0.3 ppm α -T. Minimal increases in seedling mortality were observed after 16 hours of near UV light exposure (3 W/m^2). Saturation was assumed to occur at this point, and all experiments had this minimum level of near UV exposure.

To test for the sublethal effects of α -T and PHT on the growth of four test species in Petri plate hydroponics, a daily eight hour near UV exposure period was used over a two week period. In this experiment on sub-saturation, the near-UV irradiance used approximated the natural period of intense near UV irradiation from the sun (Thorington, 1976). The fresh weight after the two week growth period was recorded for tests and controls. The results are expressed as the percent growth of the treated seedlings versus the control plotted against the concentration of PHT or α -T in ppm (Figures 7 and 8). The non-UV treated groups not exposed to the eight hour near UV treatment are shown on the same figure and reveal the growth inhibition of α -T and PHT without

Figure 6: The effect of near UV exposure on seedling survival of Asclepias syriaca treated with 0.3 ppm α -T. Near UV was 3 W/m². Standard error of three replicates is included.

Figure 7: The effects of α -T on seedling growth in Petri plate hydroponics. 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 eight hours per day for 14 days. Plants given no near UV treatment are represented by closed circles. Growth was monitored after 14 days.

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Figure 8: The effects of PHT on seedling growth in Petri plate hydroponics. The growth of four seedling species (fresh weight) as a percent of control growth, is plotted against the concentration of PHT in the growth medium. Near UV exposure (open symbols) was for eight hours per day for 14 days. Plants given no near UV treatment are represented by closed symbols. Growth was monitored after 14 days.

Seedling growth (percent of control)

TABLE 5

LETHAL CONCENTRATION LEVELS FOR SEEDLINGS TREATED WITH ALPHA-TERTHIENYL
AND PHENYLHEPTATRIYNE EXPOSED TO NEAR UV

test species	alpha-terthienyl	PHT	lethal conc.
<u>Asclepias</u>	0.15	0.66	LC ₅₀
<u>syriaca</u>	0.79	1.51	LC ₉₀
<u>Chenopodium</u>	0.27	0.83	LC ₅₀
<u>album</u>	0.83	1.57	LC ₉₀
<u>Phleum</u>	1.82	2.88	LC ₅₀
<u>pratense</u>	3.63	5.08	LC ₉₀
<u>Trifolium</u>	0.79 [±]	1.43	LC ₅₀
<u>pratense</u>	1.99	3.63	LC ₉₀

near UV exposure. The α -T and PHT growth inhibition was more dramatic under near UV light.

Asclepias syriaca was the most sensitive seedling while Phleum pratense was the most resistant which reflects the selectivity observed for the seedling mortality trials. Alpha-terthienyl's activity exceeded that of PHT.

Extraction of Active Compounds from the Soil

In order to determine the allelopathic potential of α -T, extracts of soil taken from pure stands of Tagetes erecta were analyzed for phototoxic activity and α -T concentration. The extracts were found to be phototoxic in assays using yeast-streaked plates as evidenced by the formation of clear halos upon plate incubation after initial exposure to near UV light (Chan et al, 1975). The non-UV treated plates did not reveal this zone of inhibition. Thin layer chromatography of the crude extract with authentic α -T revealed the presence of a fluorescing compound which was tentatively identified as α -T on the basis of its UV spectrum. The concentration of α -T in the soil was estimated to be 4.8 ± 0.9 (SE) ppm. This concentration could be high enough to inhibit newly emergent seedlings as observed for the hydroponic trials.

Post Emergent Trials in Environmental Control Chambers

The activity and selectivity of α -T under the hydroponic trials was high enough to warrant its full investigation as

a potential natural product herbicide. The α -T formulations were spray-dispensed to the weed species Chenopodium album, Amaranthus retroflexus and crop species corn (Zea mays) at the two, four, and six leaf states of growth. The damage caused by high application rates of α -T became immediately apparent as chlorotic and necrotic mottling of leaf and stem tissue were observed 15 minutes after application. The damage was identical for all three test species. At lower application rates, less severe foliar and stem damage was observed as necrotic lesion formation. These were characterized as brown spots of damaged tissue profused over the plant surface directly exposed to the formulation. The smallest application rate (1kg/ha) revealed the presence of necrotic lesions. The near UV intensity measured in the growth chamber from the Vita-Lites was 2 W/m². In order to see what role light played in the phytotoxic response, a sublethal assay of necrotic lesions formed under light or dark incubation after a six hour period was performed (Figure 9). The number of necrotic lesions per plant was plotted against the hours after spray application of α -T. From the curves it can be seen that light exposed Amaranthus retroflexus at the four leaf stage developed a greater number of necrotic lesions per plant than the dark incubated plants. A significant dark effect could be observed in the phytotoxicity.

Figure 9: The light and dark activated components of α -T sublethal effects on Amaranthus retroflexus. The number of necrotic lesions on the plant was recorded against time after application of α -T following placement in light (circles) and dark (triangles) regimes in Vita-Lite fitted growth cabinets. Ten plants were assayed for necrotic lesions per data point. The wetting agent in the formulation was 0.1 percent (v/v). Standard error of three replicates is indicated.

From Figure 10 the surfactant wetting agent Citowett Plus had inhibited growth at the two leaf stage. With α -T in the formulation at a spray application rate of 20 kg/ha, Citowett Plus increased the herbicidal effect of α -T until the concentration reached 0.1 percent when the activity levelled out. Any further increase in wetting agent content does not effect the activity of α -T. The formulation for subsequent trials consisted of 0.1 percent Citowett Plus for optimization of α -T's activity.

The herbicidal efficacy of α -T at the various stages of growth for Amaranthus retroflexus is shown in Figure 11. The efficacy is represented graphically as lines of best fit for growth inhibition versus the application rate since the standard growth response of a weed versus the herbicidal application rate is typically linear when plotted on logarithmic axes (Hanf and Bernhardt, 1978). From the linear regression lines, the ED₅₀ values were calculated by graphical extrapolation. The six leaf stage was the least sensitive, followed by the four and the two leaf stages. The four leaf stage was taken as being the most appropriate for herbicidal evaluation due to the effects of the wetting agent at the two leaf stage.

Figure 12 represents the herbicidal activity of α -T against corn and Chenopodium album at the four leaf stage. The activity for both of these species was very close to that observed for Amaranthus retroflexus which indicates a lack of selectivity.

Figure 10: The inhibitory effects of Citowett Plus (wetting agent in the formulation) on the growth of Amaranthus retroflexus in Vita-Lite fitted growth chambers. Triangles represent the growth effects (percent of control dry weight) of wetting agent without α -T in the formulation, to plants at the four leaf stage. Circles represent the growth effects at the two leaf stage, without α -T. Squares represent the effects of wetting agent concentration on the growth effects of α -T in the formulation at 20 kg/ha. The UV intensity was 2.5 W/m² in the growth cabinets. Data points represent the mean of three replicates. Standard error is included.

Figure 11: The herbicidal efficacy of α -T at the 2, 4, and 6 leaf stages of growth to Amaranthus retroflexus in Vita-Lite growth chamber trials. The effect of varying the application rate of α -T on the percent growth (dry weight) of Amaranthus retroflexus at the six (closed circles), four (triangles), and two (open circles) leaf stages of growth. The dry weight of the plants were recorded after a two week growth incubation in a Vita-Lite fitted growth chamber. The recorded UV intensity was 2 W/m². Lines of best fit were drawn after plotting data points on double logarithm axes. Standard error of three replicates is included.

Figure 12: Growth chamber trials of α -T against corn and Chenopodium album. Refer to Figure 11 for experimental details. Corn is represented by closed circles, and Chenopodium album by open circles, for the four leaf stage of growth.

The atrazine standard exhibited a much higher order of activity as seen in Figure 13. Corn was relatively unaffected by this treatment. The two broad leaf species upon atrazine exposure revealed a complete chlorosis of leaf and stem tissue which differs from the damage noted for α -T.

Post Emergent Field Trials

The field trials were conducted with the hope of increasing the near UV component involved in the phytotoxic process. The near UV intensity under field conditions was 11W/m^2 in contrast to the 2W/m^2 measured for the Vita-Lite fitted growth cabinets. The field trials revealed the same order of activity to the growth cabinet trials as seen in Figure 14. Despite increased intensity the efficacy was not altered under solar irradiation. The activity of atrazine in the field was also comparable to the activity in the growth chambers (Figure 15). The effective dose to cause a 50 percent reduction in dry weight (ED_{50}) was calculated directly from the linear plots and is listed in Table 6. The lack of selectivity of α -T is evident from this table and is in contrast to the earlier work in Petri dish trials.

Persistence in Soil and Plant Material

The persistence of α -T in high organic soil was much less than that observed for the low organic soil as seen in Figure 16. The extractable α -T residues in the soil were plotted against the days after the incorporation of α -T. Disap-

Figure 13: Herbicidal efficacy of atrazine against weeds at the 4 leaf stage against Chenopodium album and Amaranthus retroflexus in Vita-Lite chamber trials. The herbicidal effects of atrazine against corn (closed circles), Chenopodium album (triangles), and Amaranthus retroflexus (open circles) were determined under the same conditions as in figure 11.

Figure 14: The herbicidal efficacy of α -T against weeds in field trials. Alpha-terthienyl was applied to corn (open circles), Chenopodium album (closed circles), and Amaranthus retroflexus (triangles) at the four leaf stage of growth. The percent relative growth (dry weight) was recorded after a two week growth period. The recorded near UV intensity was 11 W/m² at the test site. Data points represent the mean of three replicates.

Figure 15: Field trial herbicidal efficacy of atrazine against weeds at the four leaf stage of growth. Chenopodium album (triangles), Amaranthus retroflexus (open circles), and corn (closed circles) were exposed to atrazine under similar conditions as in figure 14.

Figure 16: The persistence of *a*-T in the soil. The soil persistence of *a*-T in the soil was determined for a low organic (dashed lines) and a high organic soil (solid lines) after incorporation of *a*-T (calculated as being 333 ppm). The soil concentration was plotted against the days after incorporation. Soil samples were incubated in a growth chamber. One gram samples were removed for *a*-T extraction and quantification. Data points represent the mean of three replicates. Standard error is included.

TABLE 6

ED₅₀ VALUES FOR ATRAZINE AND ALPHA-TERTHIENYL IN GROWTH CHAMBER
AND FIELD TRIALS

test species	growth cabinet trials(ED ₅₀ kg/ha)		field trials (ED ₅₀ kg/ha)	
	alpha-T	atrazine	alpha-T	atrazine
<u>Amaranthus</u> <u>retroflexus</u>	15.6	0.12	18.3	0.61
<u>Chenopodium</u> <u>album</u>	15.8	0.33	29.6	0.84
<u>Zea mays</u> (corn)	21.2	--	29.9	--

pearance of α -T is probably a first order process, but this could not be discerned definitively because of the variability in the data. In the high organic soil a trace amount of α -T was detectable 13 days after incorporation, while in the low organic soil over 50 percent had disappeared after 13 days.

The persistence of α -T on the plant surface after spray application to Amaranthus retroflexus is shown in Figure 17. The residues were not detectable after 6 days which indicates a rapid breakdown under field conditions.

Figure 17: The persistence of α -T residues on the plant surface of Amaranthus retroflexus. Persistence was recorded after spray application, as micrograms of α -T per gram fresh weight of Amaranthus retroflexus. This was plotted against time after application. Alpha-Terthienyl was extracted from 25 weeds per trial. Data points represent the mean of three replicates. Standard error is included.

Figure 18

Chapter IV

. DISCUSSION

To demonstrate that a naturally occurring plant compound is allelopathic it must first be shown that it is inhibitory towards competing plants. The hydroponic tests clearly indicate that both α -T and PHT are toxic towards the seedlings of Asclepias syriaca, Chenopodium album, Phleum pratense and Trifolium pratense. This toxic activity was enhanced under near UV irradiation but significant effects were observed in non-UV treated groups with α -T and PHT exposure. The non-UV treated groups for both α -T and PHT revealed similar symptoms of damage to the near UV-test seedlings which included severe chlorotic and necrotic mottling.

The second stage in identifying an allelopathic compound is to demonstrate that the chemical is present in the soil in sufficient concentration to inhibit growth after being released from the plant body. There are four known ways by which plants can release these compounds into the soil (Turkey, 1969). They are 1) the decomposition of leaf litter through mechanical or biological means; 2) highly volatile compounds could vaporize and exert their effects on the foliage of other plants; 3) the roots could release the compounds by direct exudation or by mechanical or biological

decomposition and; 4) by leaching of the leaves by rain to the soil. For pure stands of Tagetes erecta, α -T was isolated in soil concentrations as high as 10 ppm. As seen from the hydroponic trials the concentrations found in the soil would be high enough to exert toxic effects on competing plant species even under dark conditions. The possible role of near UV light in the potential allelopathic action of α -T and PHT requires clarification. It is possible that these compounds might be absorbed from the soil by newly emergent competing seedlings to a degree where the sun's near UV rays could invoke photosensitized damage. Leachates from leaves of Bidens spp. may contain PHT which could fall on the developing understorey of competing plants to exert phototoxic inhibition upon exposure to the sun's rays. Penetration of near UV into the soil is negligible and phototoxic effects on the roots would not be possible. If α -T is to be thought of as a potential allelopathic substance, its dark effects would appear to have greater importance in the soil.

The last step in establishing that a compound is allelopathic is to show that it is active in inhibiting competing plants under field conditions. This step is the most difficult to show and has yet to be investigated for α -T or PHT. Another question that remains to be addressed, is how the host plant protects itself from the autotoxic effects of α -T and PHT. For polyacetylenes and thiophenes, it is believed that the compounds are stored in the resin ducts and the

waxy cuticle (Bohlmann et al, 1973), where the host plant could be immune to possible toxic and phototoxic effects. Near UV-screening compounds such as the lignins and flavanoids could also play a role in preventing photosensitization (Towers and Wat, 1977) caused by phototoxins found in exposed plant structures.

Membranes appear to be the target site of phototoxic activity for polyacetylenes and thiophene derivatives from studies of membrane disruption of erythrocytes (Wat et al, 1980) involving electrolyte leakage and gross morphology alterations upon exposure to near UV irradiation. Similar studies have been conducted on yeast and Escherichia coli (Arnason et al, 1980) and confirm the hypothesis that the membrane is the target site. The lipophilicity of α -T as evidenced from its highly non-polar chromatographic behaviour would tend to indicate that membranes would be a logical site for bioaccumulation of α -T in plants.

Sinclair and Arnason (1982) investigated the photosensitizing properties of α -T on Chlorella and isolated spinach chloroplasts. These workers found that photosynthesis as measured by oxygen evolution in Chlorella was more rapidly inhibited upon exposure to near UV light and 10 ppm α -T than respiration. The Hill reaction in uncoupled spinach chloroplasts using ferricyanide as the electron receptor is sensitive to photosensitization with α -T but electron transport through photosystem I showed no decrease in activity. This

has been interpreted as an indication of a site of action close to photosystem II.

Alpha-terthienyl behaved as a contact herbicide as only portions of the plants that were directly exposed to α -T revealed signs of damage. Translocation of the compound was not observed in the plants as evidenced by the contact nature of the phytotoxic damage. Spot application of compound to plants revealed necrotic patches that were restricted to the exposed plant surface. Photosensitizing agents such as rose bengal (Dodge, 1983) and anthracene (Zweig and Nathtig, 1975) have been investigated for their phytotoxic activity and revealed the same contact toxic properties. The bipyridylum herbicides are known to produce activated species of oxygen (superoxide and singlet oxygen) in the foliar tissue upon exposure to light (Summers, 1980). The herbicidal damage of paraquat and other members of this class (bipyridylum) is identical to that observed with the contact toxic symptoms noted for α -T (chlorotic mottling of exposed tissues).

Other photosensitizing compounds of plant origin have not been investigated for their phytotoxic properties and these include the linear furanocoumarins and extended quinones such as hypericin. Hypericin is a red quinone compound found in Hypericum perforatum in glandular structures on the underside of the leaves away from the potential damaging rays of the sun. Cercosporin, a structurally similar com-

pound to hypericin, is a photosensitizing pigment from a plant pathogenic fungi (Cercospora spp.). whose damage is similar to that observed for α -T (Daub, 1982). Cercosporin was found to be the agent responsible for the pathological condition as cercosporin-treated plants revealed identical symptomatic damage to the Cercospora -infected plants, and in the absence of light the damage was negligible (Daub, 1982).

The initial toxicity revealed by α -T and PHT under hydroponic conditions indicated that these compounds might have herbicidal value. Not only did the probit analysis indicate very potent efficacy under UV light for the four species tested but it also indicated a degree of species specificity. Asclepias syriaca was the most sensitive in this assay and the monocot forage species Phleum pratense was the least sensitive. Phytotoxic selectivity is an important factor in considering a compound's herbicidal potential and at this stage the results were encouraging. Alpha-terthienyl was the most active of the two compounds in the preliminary seedling tests and it was decided that this would be the compound for herbicidal evaluation.

A large quantity of alpha-terthienyl was required for herbicidal evaluation and chemical synthesis was the most efficient method for obtaining such quantities. The Ullmann reaction yielded very low quantities of α -T production in contrast to the results of Yamazaki et al (1976) where a 30

percent yield was reported from the identical reaction. The maximum yield received in this study was .7.6 percent. The yield of the reaction was variable and dependent upon the activation state of the copper powder, reaction temperature and solvent influences. Evidently, further chemical research is required.

A conventional weed-crop system was used to determine the efficacy of α -T. The wetting agent in the formulation (Citowett Plus) was found to exert inhibitory activity against the two leaf stage of Amaranthus retroflexus that was not observed at the four leaf stage. Growth effects caused by surfactant wetting agents are not uncommon in herbicide studies but their causes are largely unknown (Parr, 1980). Citowett Plus increases the activity of α -T by thirty percent for Amaranthus retroflexus at 20 kg per hectare of α -T. The heightened efficacy of α -T using a wetting agent could be due to the increased penetration of the compound into the foliage or alternatively more α -T could adhere to the leaf surface and exert toxic activity.

The post-emergent trials conducted in controlled environment chambers fitted with Vita-Lites indicated that α -T was not as effective against more advanced stages of plant growth. The stage of plant development affected the efficacy of α -T in the growth cabinet trials as the two leaf stage was the most sensitive followed by the four and the six leaf stages. This is a feature common to most herbicides where

resistance is increased as the weed becomes more advanced. Most post-emergent herbicides are applied at the two leaf stage for maximum control (Hanf and Bernhardt, 1978). The two leaf stage was deemed to be unreliable for herbicidal evaluation for α -T due to the inhibitory effects of the wetting agent while the low activity observed at the six leaf stage prevented its use.

The application rates for fifty percent control (ED_{50}) at the four leaf stage were extremely high for all three test species in comparison with atrazine whose activity is over twenty fold greater than for α -T. No selectivity could be observed for the three test species as corn was severely damaged by identical application rates to Amaranthus retroflexus. The lack of selectivity could be due to the high application rates used. This result was disappointing since selectivity is a very important attribute for a useful herbicide for post-emergence control, and atrazine is a prime example. Its activity was restricted to the broadleaf weeds while leaving the corn virtually unaffected. Non-selective commercial herbicides such as glyphosate and paraquat are seeing decreased use as more effective selective herbicides are developed (Hanf and Bernhardt, 1978).

A sublethal assay of the number of necrotic lesions formed after α -T application under Vita-Lite or dark incubation indicated that there was a light and dark activated component to the herbicidal effect of α -T. The dark effect was

less apparent in the toxic activity of α -T against mosquito larvae (Arnason et al, 1981), algae (Arnason et al, 1981), nematodes (Gommers, 1973), and various microbes (Arnason et al, 1980). This dark component may be a function of the quantity and range of concentrations being used for herbicidal evaluation in comparison to the limited range of conditions to other organisms. Substantial dark effects were also observed for the hydroponic tests.

The field trials were attempted to determine the efficacy of α -T under more realistic environmental conditions. The Near UV intensity was markedly higher than that observed in the Vita-Lite growth chamber trials and it was anticipated that this would improve the performance of α -T against Amaranthus retroflexus and Chenopodium album. The improved efficacy was not observed as the ED₅₀ values corresponded to those calculated for the growth cabinet trials. Apparently the increased activation of α -T was counterbalanced by a concomittant increase in the environmental degradation of α -T. For many herbicides undergoing evaluation, the efficacy under field conditions is substantially less than that observed for preliminary investigations under controlled environmental conditions (Allen, 1978). For α -T the field activity was a direct reflection of the growth chamber trials.

The herbicidal rating system used for the evaluation of α -T and atrazine involved dry weight growth. This rating method has been suggested as the most accurate of the rating

systems in weed science (Franz and Tolbert, 1977). The visual rating system is based on observation of percent coverage or extent of the damage symptoms of treated versus non treated plots. This system is not favoured because of its subjective and qualitative nature (Franz and Tolbert, 1977). Fresh weight determination is used extensively for efficacy evaluation because of the ease and rapidity of obtaining results (Allen, 1978). This method however is not a true representation of plant growth as many factors could influence the water content and hence the fresh weight of the test species.

Alpha-terthienyl was found to have very low persistence on solar exposed plant surfaces in the field as no traces could be observed after six days. This rapid degradation could be due to biological activity, evaporation losses (volatility), photo-oxidation, or catalytic polymerization reactions on the plant surface. This low persistence contrasts favourably with other herbicides such as atrazine and 2,4-D whose residues are still substantial on the plant tissue several months after application.

The apparent persistence in the soil was also found to be low as seen in figure 16. For the high organic soil, residues could not be detected 13 days after incorporation, while in the low organic soil over 50 percent disappeared over the same time span. This apparent disappearance of α -T observed for the high organic soil could be due to the forma-

tion of bound residues trapped in the humic material of the soil. This would decrease the extractability of unaltered α -T in the high organic soil which contains less humic material. Bound residue formation has been found to be important for the s-triazine herbicides of which atrazine is a member (Khan et al, 1980). The disappearance of α -T could also be due to microbial degradation or catalytic breakdown in the soil. The use of radioactive tracers and the identification of α -T metabolites in the soil could facilitate the identification of the major source for degradation.

Low persistence in the soil suggests that there must be a rapid turnover of α -T in pure Tagetes erecta stands and to maintain the concentrations observed, root exudation must be substantial. The exudation of large amounts of α -T from the roots has been reported by Tang and Towers, 1983). This could be taken as an indication of a potential ecological role for α -T in combatting competitors and pathogens in the soil.

While the low persistence of α -T would be a desirable attribute for a potential herbicide, this property could also be a significant factor contributing to its low efficacy in the field trials. Another factor restricting the efficacy of α -T is its lipophilic character which reduces the translocation of the compound within the plant. The chemical may be trapped in the lipid soluble materials of the foliage (cuticular waxes, suberins, and resins) which would prevent

passage to the water soluble free space for transport to other parts of the plant. Few herbicides with lipophilic properties have been developed due to this lack of translocability (Price, 1978). Trifluralin, the most lipophilic herbicide used commercially, has an octanol-water partition of 4.5 (Dearden and Townend, 1978) while α -T has a value which far exceeds this (McLachlan, 1983). Optimum lipophilicity is an important concept in herbicide development (Dearden and Townend, 1978).

A hydrophilic analogue of α -T is now being synthetically produced for herbicidal evaluation. This compound could potentially improve the efficacy by increasing the mobility within the plant and reduce the contact-toxic nature of α -T while retaining its photosensitizing and nonpersistent properties.

Alpha-terthienyl offers a unique mechanism of action for which it would be difficult for weeds to develop resistance. Plants require light and can not be cryptic so as to avoid the photosensitizing effects of α -T. Another photosensitizing compound, Erythrosin B, has been registered as an insecticide for chicken coop fly eradication (Pimprikar et al, 1980), and indicates that photosensitizing compounds offer applied potential.

The low persistence and the potential for minimal environmental impact of natural products makes them very attractive as pesticidal agents. However toxicology of natural

products must be evaluated on an individual basis. Alpha-terthienyl is nonmutagenic in the Ames test and sister chromatid exchange (MacCrae et al, 1980). It has an oral LD₅₀ of not less than 300 mg per kilogram body weight to mice which is a low order of toxicity (Laferiere et al, 1982).

However, there are problems associated with the use of α -T as a herbicide. The major problems would be the low efficacy encountered in the field trials and the inefficiency of the syntheses available for α -T production. The lack of selectivity for weed species would also restrict its usefulness. In addition, such photosensitizing compounds are not specific to effects on plants as evidenced from the large spectrum of phototoxic activity of α -T. Alpha-terthienyl is also known to photosensitize human skin at very high concentrations (Towers et al, 1979) and is symptomized by erythema and blister formation (Chan et al, 1977).

The problems associated with the use of α -T as a herbicide may be eliminated by the production and evaluation of analogue prototypes by retaining its photosensitizing capacity and persistence properties while increasing the performance. Structural alterations of novel chemicals undergoing herbicidal evaluation are not uncommon.

The ecological role of α -T appears to be a generalized defense against competitors and pathogens (McLachlan et al, 1982). It has antifungal, allelopathic, and insect antifeedant properties. Apparently, the Asteraceae have developed a

unique chemical protective system which has evolved over many years and perhaps we can utilize some of these natural products in our battle against agricultural pests. Only recently has the biorational approach been emphasized in the search to eliminate major pest problems. Exploitation of natural products pose a rational alternative within integrated pest management and offers a great potential which has yet to be fully explored.

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