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ABSTRACT

Three naturally occurring thiophenes (a monothiophene, a bithiophene, and α-terthienyl) and four polyacetylenes (phenylheptatriyne, phenylheptadiynene, phenylheptadiynene acetate, and matricaria lactone) were examined for phototoxicity and light-independent toxicity to two polyphagous lepidopterans, the darksided cutworm, Euxoa messoria and the European corn borer, Ostrinia nubilalis. Phenylheptatriyne and α-terthienyl were also tested against an oligophagous lepidopteran, the tobacco hornworm, Manduca sexta. All of the compounds except the monothiophene and matricaria lactone inhibited the growth of Euxoa messoria larvae without exposure to near-UV irradiation. Light-independent toxicity involved feeding deterrence against neonate and, to a lesser extent, older larvae, and long-term metabolic toxicity reflected in impaired nutrient utilization. Phenylheptatriyne, phenyl- phenylheptadiynene, and α-terthienyl (at 100 µg/g) were phototoxic with 2 w/m² near-UV, and α-terthienyl was lethal in conjunction with 4 w/m² near-UV irradiation. All of the compounds reduced the growth of Ostrinia nubilalis larvae without near-UV exposure the thiophenes were more toxic than the polyacetylenes and toxicity increased with the increasing number of thiophene rings.

Feeding deterrence was only a minor component of this light-independent activity. Behavioral adaptations, including spinning silk and boring into diet, allowed Ostrinia nubilalis larvae to avoid photosensitization. α-terthienyl at 100 µg/g strongly deterred feeding by neonate Manduca sexta, resulting in complete mortality. Phenylheptatriyne at 100 µg/g and α-terthienyl at 10 µg/g inhibited growth without near-UV exposure, and were also phototoxic. Phenylheptatriyne at 10 µg/g stimulated both feeding and growth.

Thiophene toxicity was expressed more rapidly than polyacetylene activity; the toxicity (in terms of growth reduction) of both classes of compounds was correlated with their lipophilicity but the relationship was different for polyacetylenes and thiophenes. Accumulated body burden data suggested that polyacetylenes are more rapidly metabolized than thiophenes. Feeding deterrence was correlated with the host plant range of the three test insects.

Résumé

Les polyacétylènes et leurs dérivés thiophènes sont des métabolites secondaires caractéristiques des Asteracés et on pense qu'ils ont un rôle à jouer dans la protection biochimique de ces plantes. On a étudié trois thiophènes (un monothiophène, un bithiophène, et α-terthienyl) et quatre polyacétylènes (phénylheptatriyne, phénylheptadiynène, phénylheptadiynene acétate et matricaria lactone) en fonction de leur phototoxicité et de leur toxicité sans lumière envers deux lépidopteres polyphages, le ver gris moissonneur Euxoa messoria, et la pyrale du maïs, Ostrinia nubilalis. On a aussi évalué le phénylheptatriyne et l'α-terthienyl envers un lépidoptere oligophage, le ver du tabac Manduca sexta. Tous les composés sauf le monothiophène et la matricaria lactone ont inhibé la croissance des larves d' Euxoa messoria sans exposition a l'irradiation UV proche. La toxicité indepeндante de la lumière implique l'anti-appétence envers les larves néonates, et a un degré moindre, les larves plus agées et la toxicité métabolique prolongée suggère une utilisation incomplète des substances nutritives. Le phenylheptatriyne, le phenylheptadiynene, et l'α-terthienyl (a 100 µg/g) étaient phototoxiques sous une irradiation de 2 w/m² UV-proche et l'α-terthienyl était mortel a une irradiation de 4 w/m² UV-proche. Tous les composés ont ralenti la croissance des larves d' Ostrinia nubilalis en l'absence d'UV. Les thiophènes étaient

plus toxiques que les polyacetylènes et la toxicité augmentait en fonction du nombre d'anneaux thiophènes. Des adaptations du comportement tel que le tissage de soie et l'enfouissement dans la diète ont permis a Ostrinia nubilalis d'éviter la photosensibilisation l'éventail de plantes dont se nourrit cet herbivore inclut un nombre d'Astéracés contenant des polyacétylènes. L'α-terthienyl a 100 µg/g a neonates de Manduca sexta, conduisant ainsi a la mort des larves. Le phenylheptatriyne a 100 µg/g et l'α-terthienyl a 10 µg/g ont inhibé la croissance en l'absence d'UV-proche et étaient également phototoxiques. Le phénylheptatriyne a 10 µg/g a stimulé la croissance et l'alimentation.

La toxicité au thiophènes s'est manifestée plus rapidment que l'activité du polyacetylène; la toxicité (en termes de réduction de croissance) des deux classes de composés montrait une corrélation avec leur lipophilie mais la relation était differente pour les polyacétylenes et les thiophènes. L'accumulation des produits toxiques suggère que les polyacétylènes sont métabolisés plus rapidement que les thiophènes. L'interférence dans le processus d'alimentation est discuté en relation avec l'éventail des plantes hôtes pour les trois insectes étudiés.

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Drs. J. Thor Arnason and Bernard J.R. Philogène, not only for their help and support throughout this project, but especially for introducing me to a field I find both fascinating and challenging. I am also grateful to my advisors, Drs. J. Picman and C. Nozzollilo, for their guidance during this work.

Dr. Jorgen Lam (Chemistry Dept., University of Aarhus, Denmark) provided several of the compounds used in this study. My own efforts at isolating and identifying similar compounds have taught me the greatest respect for those who have mastered this craft. Glen Campbell, Dr. J.T. Arnason, and Drs. P. Morand and L. Leach of the Chemistry Dept., University of Ottawa, provided synthetic α-T. Dr. H.H. Cheng, Agriculture Canada, Delhi, Ontario, cheerfully provided Euxoa messoria eggs whenever they were requested. Cathy McDougall, France Duval, and Natalie Donskov were generous in allowing me to periodically raid the Ostrinia nubilalis and Manduca sexta cultures. Natalie's help with the feeding deterrency tests was also greatly appreciated. Georges Bourque provided much useful advice on the care and feeding of word processors.

All of the students and staff of the third floor group contributed much towards creating a friendly and invigorating atmosphere. I would particularly like to acknowledge and thank David McLachlan, who is both a friend and an inspiration to me. Cathy McDougall, Seshadri Iyengar, and Scott Wilson, among many others, contributed to the coffee-room forum, where we regularly tackled not only those problems relating to our research, but the ills of the world at large.

Financial support for this work was provided by NSERC and Agriculture Canada, as operating and research grants to Dr. Arnason. This support is gratefully acknowledged.

Finally, I would like to thank Christy, my wife, who has been my partner in all things, and whose contributions to this work defy words. It is to her that this thesis is dedicated.

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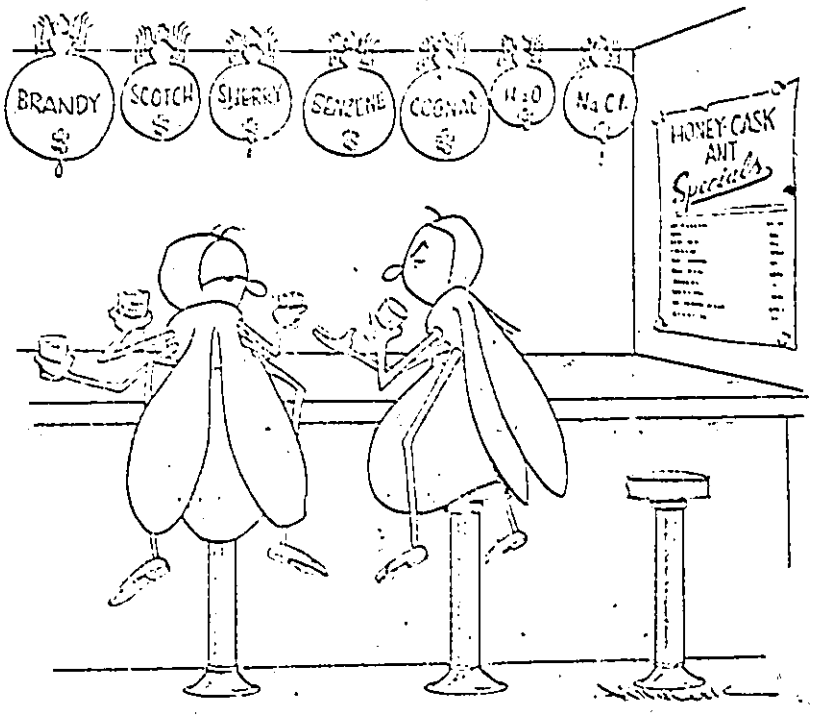


"Everything I drink has the same effect."

Chapter I

INTRODUCTION

Plants use a variety of defensive strategies to counteract the activity of herbivores. Morphological features including spines, trichomes, hairs, and tough leaves have long been recognized as an evolutionary response to the strong selective pressure to escape herbivory (eg. Darwin, 1859). That plants may also utilize chemistry to deter the activity of herbivores was first suggested by Stahl (1888) but did not gain widespread acceptance until Fraenkel's landmark paper of 1959. The chemicals involved are the "secondary metabolites" of plants, a highly diverse assemblage of over 10,000 known compounds belonging to about three major and sixteen minor structural classes based on biosynthetic origin (Swain, 1977) (Table 1).

The term "secondary metabolite" was applied when a role in primary metabolism could not be found for these compounds; they were originally considered to be storage products or metabolic wastes (Swain, 1977). More useful terms, which describe the more recently discovered ecological functions of these chemicals, are available. Allelopathic compounds are released as leaf or root exudates, as volatiles, and from decaying vegetation and inhibit the germination and

Table 1. Classes of plant secondary metabolites (from Swain, 1977)

Chemical class	Approx. no. of known structures	Examples	Active against
Polyacetylenes	750	Wyerone	Fungi
Alkaloids	4500	Lupanine	Mammals
Non-protein amino acids	250	Canavanine	Insects
Carotenoids	300	β-Carotene	Photoprotection
Coumarins	150	Scopoletin	Fungi
Cyanogenic glycosides	50	Linamarin	Molluscs
Flavonoids	1200	Morin	Insects
Glucosinolates	80	Sinigrin	Insects
Lignans	50	Excelsin	Insects
Lipids	100	Waxes	Fungi
Phenolic acids	100	Vanillic acid	Plants
Polyketids	500	Hircinol	Fungi
Quinones	200	Juglone	Plants
Terpenes	1100	Glaucolide-A	Insects
Steroids	600	Ecdysones	Insects
Miscellaneous	500	Tuliposide	Fungi
Proteins	?	Lectins	Insects
Polysaccharides	?	Acylated polysaccharides	Fungi
Other polymers	?	Cutin	Fungi

growth of neighbouring plants (Rice, 1974, 1982; Whittaker, 1970). Phytoalexins are low molecular weight compounds that are synthesized in response to fungal infection (Ingham, 1973).

Several terms apply to secondary metabolites that function in plant-herbivore interactions. Attractants and repellents are volatile plant products that result in oriented locomotion towards or away from the stimulus and aid in the location of suitable, and avoidance of unsuitable host plants (Dethier et al., 1960). Feeding deterrents affect the palatability of potential food plants and inhibit insect feeding activity (Schoonhoven, 1982). Antifeedants are a special case in which the inhibition is absolute at low concentrations and is not overcome by starvation. Phagostimulants, on the other hand, evoke a feeding response and are important "sign stimuli" for specialist insects (Fraenkel, 1959,1969; Dethier, 1970; Schoonhoven, 1982). Toxic allelochemicals, when ingested, intoxicate the insect or reduce the nutritive value of ingested food (Reese, 1979). A secondary metabolite which behaves as an allelochemical towards one species may be nontoxic, or even an essential nutrient to another species adapted to a host plant containing that compound (Brattsten, 1979). Feeding deterrents and toxic allelochemicals are discussed in more detail in sections 1-2 and 1-3.

It is not unusual for a secondary metabolite to be active against more than one group of target organisms. For example, some isoflavonoid phytoalexins are also insect feeding deterrents (Sutherland et al., 1980). Many feeding deterrents, as well, behave as toxins when ingested. Table 1 lists only the best established target organisms for the secondary metabolite classes listed, whereas in fact members of each class may prove toxic to a variety of taxa.

As a general rule the biological activity of only a few of the known compounds from each class have been examined. This is the case with the polyacetylenes and their biosynthetic derivatives, the thiophenes. This class of secondary metabolites is the subject of the present study.

1.1 Polyacetylenes and Thiophenes

1.1.1 Nature and Occurrence

Polyacetylenes are highly unsaturated compounds derived (in vascular plants) from oleic acid via a series of dehydrogenations to form triple bonds, and β-oxidations leading to chain shortening (Bohlmann et al., 1973). These compounds occur in about twenty vascular plant families but are found regularly only in the Araliaceae, Campanulaceae, Santalaceae, Apiaceae, and particularly in the Asteraceae (Composi-

tae), from which the majority of the more than 750 known polyacetylenes have been identified. The compounds are C_{10}, C_{13}, C_{17}, and C_{18} structures and may be aliphatic or, in the case of the Asteraceae, ring-stabilized and contain phenyl, furan, and spiroketalenol ether groups. Thiophenes are cyclized polyacetylenes which incorporate sulphur to form one to three thiophene rings. A number of terminal groups are possible including aldehyde, ester, acetate, alcohol, and methyl structures; as well, a few polyacetylenes are halogenated. Examples of thiophenes and phenyl acetylenes which were used in this study are given in Figure 4.

Fungi, particularly the Basidiomycetes, produce C_{10} polyacetylenes that tend to be all trans and highly polar (Bohlmann et al., 1973). Plant constituents which contain a monoacetylene group are widely distributed. Among the algae the Chrysophyceae, Bacillariophyceae, and Xanthophyceae contain acetylenic carotenoids (Bohlmann et al., 1973). The monoacetylenic constituents of higher plants have been reviewed (Ross, 1972); such compounds are generally derived from different biosynthetic pathways than the polyacetylenes.

Although all parts of a plant may contain polyacetylenes, particular compounds are usually confined to certain tissues, so that flowers, leaves, stem and roots may each have a unique polyacetylene profile (ex. Wrang and Lam, 1975). The acetylenes are usually most diverse and concentrated in

the roots (Bohlmann et al., 1973) but flowers may also be rich in these compounds. Leaves typically contain fewer acetylenes but these may be present in high concentrations. Bidens pilosa leaves contain up to 700 µg/g fresh weight phenylhepta-1,3,5-triyne (PHT) and traces of phenylhepta-1,3-diyne-5-ene (PHD) whereas roots contain mostly PHD (Wat et al., 1979 Bohlmann et al., 1973). Polyacetylenes are stored in the cuticle and resin canals but the site of their synthesis has not been identified (Towers and Wat, 1978). One enzyme involved in the biosynthesis of acetylenic compounds has been identified, an acetate esterase that converts unstable 5-(4-acetoxy-1-butinyl)-2,2'-bithiophene to the stable 5-(4-hydroxy-1-butinyl)-2,2'-bithiophene (Sutfield and Towers, 1982).

1.1.2 Biological Activity of Polyacetylenes and Thiophenes

The neurotoxic effects of some polyacetylenes have long been known: compounds with this activity include the fish poisons ichthyotherial and its acetate from leaves of Clibadium spp. and Ichthyothere spp. and roots of Dahlia coccinea (Bohlmann et al., 1973) and the long-chain acetylenic alcohols cicutitoxin from Cicuta virosa and oenanthotoxin from Oenanthe crocata (Robinson, 1980). The latter two compounds have been responsible for poisonings of livestock and humans

(Kingsbury, 1964). It has been suggested that these toxins uncouple oxidative phosphorylation (Clark, 1964; Puritch, 1975). Other polyacetylenes and thiophenes were found to be toxic to nematodes, including α-terthienyl and 5-(3-buten-1-ynyl)-2,2-bithienyl from Tagetes erecta (Uhlenbroek and Bijloo, 1959,1960), 1-tridecanene-3,5,7,9,11-pentayne from Helenium (Gommers, 1972), and 3-cis and 3-trans, 11-trans, trideca-1,3,11-triene-5,7,9-triyne from Carthamus tinictorius (Munakata, 1983). Carthamus has also yielded the phytoalexins safynol and dehydrosafynol (Allen and Thomas, 1971). Other phytoalexins include wyerone from the broad bean Vicia faba (Hargraves et al., 1976) and falcarinol, falcarindiol, and cis-tetradeca-6-ene-1,3-diyne-5,8-diol from tomato (Lycopersicon esculentum) leaves (DeWit and Koddle, 1981). Kawazu et al. (1977) identified cis-dehydromatricaria ester as an allelopathic compound from Solidago altissima. This activity was extended to other C acetylenes by Kobayashi et al. (1980).

The biological activities so far discussed occurred in the absence of light. That the toxic activity of thiophenes and polyacetylenes is enhanced in the presence of near-ultraviolet (near-UV or UV-A) light (300-400 nm) was discovered independently by Gommers (1972; Gommers and Geerligs, 1973) and by Chan et al. (1975). The physical-chemical basis of this photoactivation will be discussed in section

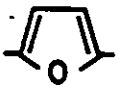
1.3.2. Light enhances the toxicity of these natural products to nematodes (Gommers, 1972; Gommers and Geerligs, 1973; Bakker et al., 1979 Wat et al., 1981), fungi (Wat et al., 1979 Towers, 1980; Bourque, 1984) and vascular plants (Campbell et al., 1982), indicating that earlier studies should be repeated with allowance for the role of light in the activity of these compounds. Phototoxicity to a variety of bacteria, yeasts, and fungi (Chan et al., 1975 Towers and Wat, 1978; Wat et al., 1979 Towers, 1980), trematodes (Graham et al., 1980), and marine and fresh-water algae (Arnason et al., 1981b) has been demonstrated. Alpha-terthienyl inhibits photosynthesis at a site near photosystem II (Sinclair and Arnason, 1982). Human skin (Towers et al., 1979) and fibroblast cells (Wat et al., 1979) can also be photosensitized.

The haemolysis of erythrocytes (Wat et al., 1980b Yamamoto et al., 1979) and the photoinactivation of membrane-bound but not membraneless viruses (Warren et al., 1980 Hudson et al., 1982) indicates that polyacetylenes act primarily on membranes. The site of action is not DNA (as is the case with the furanocoumarins) as these compounds do not photobind to DNA in vitro (Wat et al., 1979), do not induce cytogenetic damage (MacRae et al., 1980), and are equally toxic to wild-type and DNA-repair deficient Escherichia coli (Downum et al., 1982).

Figure 1. Structures of some biologically active polyacetylenes discussed in the text. Neurotoxins include cicutitoxin (a), oenanthotoxin (b), and ichthyotherial (h). Phytoalexins include wyerone (c), dehydrosafynol (d), and safynol (e). Pentaynene (f) and dehydromatricaria ester (g) are ovicidal.

a) $HOCH_2CH_2CH_2C{\equiv}C-C{\equiv}C-(CH{=}CH)_3CH(OH)CH_2CH_2CH_3$

b) $HOCH_2CH{=}CH-C{\equiv}C-C{\equiv}C-(CH{=}CH)_2CH_2CH_2CH(OH)CH_2CH_2CH_3$

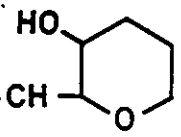
c) $CH_3-CH_2-CH{=}CH-C{\equiv}C-C(=O)$  $CH{=}CH-COOH$

d) $CH_3-(C{\equiv}C)_4-CH{=}CH-CH(OH)-CH_2(OH)$

e) $CH_3-CH{=}CH-(C{\equiv}C)_3-CH{=}CH-CH(OH)-CH_2(OH)$

f) $CH_3-(C{\equiv}C)_5-CH{=}CH_2$

g) $CH_3-(C{\equiv}C)_3-CH{=}CH-C(=O)-O-CH_3$

h) $CH_3-(C{\equiv}C)_3-CH{=}CH$ 

Polyacetylenes also have a variety of effects on insects. Of a total of twenty-seven acetylenes and thiophenes examined by Arnason et al. (1981) and Wat et al. (1981c), eight were highly phototoxic to larvae of the mosquito Aedes aegypti. Dehydromatricaria ester from Solidago altissima and pentaynene from Xanthium canadense had ovicidal activity against the housefly (Musca domestica) and fruitfly (Drosophila melanogaster) without photosensitization (Kawazu et al., 1977 Nakajima et al., 1977). Subsequently a number of polyacetylenes and thiophenes, and synthetic diphenylbutadienes, have been found to be phototoxic to insect eggs (Kagan et al., 1983 Kagan and Chan, 1983). Matricaria ester, a widespread aliphatic polyacetylene (Bohlmann et al., 1973). was toxic, without photosensitization, to three species of herbivorous lepidopterans (Binder et al., 1979). This compound and phenylheptatriyne (McLachlan et al., 1982) have significant feeding deterrent activity, as does a polyacetylene of insect origin, Z,Z-dihydromatricaria acid (Eisner et al., 1981).

1.2 Test Insects

The activity of a possible allelochemical should be tested against a variety of phytophagous insects as tolerance for an allelochemical may vary from one species to the next. In

general, polyphagous insects posses more labile MFO systems than do oligo- or monophagous species (Kreiger et al., 1971 Brattsten et al., 1977). I chose to work with two highly polyphagous insects, Euxoa messoria (whose diet includes no phototoxic Asteraceae) and Ostrinia nubilalis (which feeds on several phototoxic Asteraceae). One oligophagous species, Manduca sexta, which does not include any Asteraceae in its host plant range, was also used.

1.2.1 Euxoa messoria (Harris) (Lepidoptera:Noctuidae)

The darksided cutworm, Euxoa messoria, occurs in North America, from the southern North West Territories to southern California and from the eastern to the western coast (Hardwick, 1970; Bierne, 1971; Cheng, 1973). The species is univoltine (one generation per year) throughout its range. Oviposition occurs in the ground, particularly in sandy soil, in late summer and early fall (Harris and Svec, 1968; Cheng, 1973). Larvae overwinter in the egg (Hinks and Byers, 1976) and hatch in late January to early April. Newly emerged larvae feed on almost any available plant (Crumb, 1929; Harris and Svec, 1968); feeding larvae (first to seventh instars) occur in the field, in Ontario, from early April to early August (Cheng, 1973). Aestivating prepupae are found in July and August, adults emerge in mid-August to mid-October and egg laying commences.

Euxoa messoria is highly polyphagous: a list of known host plants includes representatives of at least twelve vascular plant families (Appendix 1A). The only Asteraceae noted is Lactuca sativa, a species almost devoid of polyacetylenes (Bohlmann et al., 1973). The darksided cutworm is an economically important pest on tobacco (Cheng, 1973). Its diet exposes it to a variety of known allelochemicals, including alkaloids and phenolics; in addition it has been resistant to cyclodiene insecticides since 1961 (Bierne, 1971).

1.2.2 Ostrinia nubilalis (Hbn.) (Lepidoptera: Pyralidae)

The European corn borer, Ostrinia nubilalis was introduced into North America around 1909 and spread rapidly, now occuring in all corn producing areas of the continent (Davidson and Lyon, 1979). The insect has two or three generations a year. Overwintering occurs as mature larvae, pupation takes place in the spring, and adult moths appear in May or June. Eggs are laid in masses of 15 to 35 on the underside of leaves; a female may produce over 500 eggs in all. First instar larvae feed on leaves of the host plants, but in the second instar the larvae bore into the stem or stalk, or in a few cases the fruit (see Appendix 1B).

European corn borers are the most significant pest of corn, but also damage pepper, beet, bean, potato, oat, soy-

bean, and wheat crops (Davidson and Lyon, 1979). An extensive list of 190 host plants of this very highly polyphagous insect was compiled by Caffrey and Worthley, (1927) (Appendix 1B). The list includes a number of Asteraceae, among them phototoxic species such as Bidens cernua, Dahlia spp., and Artemisia spp. Plants known to contain polyacetylenes and thiophenes are indicated in Appendix 1B.

1.2.3 Manduca sexta (L.) (Lepidoptera: Sphingidae)

The tobacco hornworm, Manduca sexta, occurs in South and Central America, Mexico, the West Indies, the United States, and southern Ontario and Quebec (Madden and Chamberlain, 1945). There are one to four generations per year, depending on the latitude (Davidson and Lyon, 1979). Manduca sexta overwinters in the soil as a pupa; adults emerge in the spring and begin ovipositing on the underside of leaves, one egg per leaf (Davidson and Lyon, 1979). Eggs hatch in three to six days and the larvae develop rapidly through five instars. Larval development requires about 30 days, during which time larvae will consume about 35 g artificial diet, most of it in the fourth and fifth instars (Bell and Joachim, 1976). Mature larvae weigh between 5 and 10 g. The aestivatory prepupal period is short: larvae construct a pupation chamber and pupate within 12 to 24 hr (Nijhout and Williams, 1974). In non-diapausing insects the life cycle

requires about 60 days, whereas diapausing larvae require about 130 days to complete development (Bell et al., 1975).

Manduca sexta is an oligophagous specialist on solanaceous plants, particularly tobacco (Nicotiana tabacum), but also including jimson weed (Datura spp.), ground cherry, tomato (Lycopersicon esculentum), Jerusalem cherry, potato (Solanum tuberosum), and pepper (Brinkley et al., 1975). Tobacco hornworms tolerate high concentrations of some alkaloids: growth is not affected by up to 1.5% nicotine in the diet (Parr and Thurston, 1972). Assimilated nicotine is prevented from reaching sensitive tissues by a blood-brain barrier (Self et al., 1964a,b). The host plants are not known to contain polyacetylenes or thiophenes, with one exception. Lycopersicon esculentum (as noted in Section 1.1.2) produces three polyacetylenes in response to fungal attack, but this plant is only rarely utilized.

1.3 Plan of Study

The effect of polyacetylenes and thiophenes, as a class, on insect herbivores, is still unclear. In particular, the importance of photosensitization in protecting plants containing these natural products has not been defined. The purpose of the present study is to answer some basic questions regarding the possible role of polyacetylenes and thiophenes as a part of the biochemical defence of plants:

Do these compounds affect the growth or survivorship of phytophagous insects? If so, is photosensitization an important part of this toxicity? What other mechanisms of toxicity are apparent?

These questions were initially approached by examining the effect of α-terthienyl (α-T), at two concentrations and two light levels, on the growth, development, and survivorship of the polyphagous noctuid Euxoa messoria. The study was subsequently expanded to include the effect of four representative polyacetylenes: phenylhepta-1,3,5-triyne (PHT, compound VI), phenylhepta-1,3-diyne-5-ene (PHD, compound IV), phenylhepta- 1,3-diyne-5-ene-7-acetate (PHD-acetate, compound V), and -(hex-4'-en-2'-ynylidenyl)- -but-2-enyrolactone (matricaria lactone, compound VII), and three thiophenes: 2-(1,3-pentadiynyl)-5- (methyl-3-chloro-1-butynoate) thiophene (monothiophene, compound I), 5-(3-buten-1-ynyl)-bithiophene (bithiophene, compound II), and α-terthienyl (α-T, compound III), at 100 µg/g, on Euxoa messoria and Ostrinia nubilalis, another polyphagous lepidopteran. A specialist insect, Manduca sexta, was reared on diet containing α-T and PHT. Rearing experiments were followed by an examination of the effect of the test compounds on feeding behavior of the three insect species, and on nutrient utilization in Euxoa messoria. The relationship between the toxicity of the compounds and their lipophilici-

ty, and the extent to which they accumulated in Euxoa messoria larvae, was determined.

1.4 Literature Review

1.4.1 Feeding Deterrents

Most insect herbivores utilize only a small fraction of the plant species available to them. Suitable host plants are selected on the basis of a spectrum of physical and chemical characteristics; the latter appears to be the more significant and chemical perception is the major sensory modality involved in host plant selection (Jermy, 1971; Chapman, 1974; Schoonhoven and Jermy, 1977; Schoonhoven, 1972,1982; Dethier, 1980,1982).

Plant chemicals are detected with contact or gustatory chemoreceptors located on the maxillae, labium, and tarsi (Schoonhoven 1968; Chapman and Blaney, 1979). Lepidoptera may in addition have receptors in the epipharynx and hypopharynx (DeBoer et al., 1977). Olfactory chemoreceptors, generally located on the antennae, detect volatile compounds which act as attractants or repellants. These chemicals aid in the location of suitable (or avoidance of unsuitable) host plants but are usually not involved in the initiation or maintainance of feeding (Schoonhoven, 1982; Dethier, 1980).

Verschaffelt (1910) first demonstrated that monophagous or oligophagous insects could be induced to feed on non-host plants if an extract, or a chemical characteristic of the host plant, was applied to the profferred leaves. This and subsequent work led to the belief that secondary metabolites behaved as feeding stimuli (phagostimulants): feeding would commence if an insect detected the appropriate chemical signal (Fraenkel, 1959,1969). Other workers (Dethier, 1953; Thorsteinson, 1960) suggested that monophagous insects respond positively to phagostimulants while polyphagous herbivores respond negatively to the presence of feeding deterrents in non-host plants.

Electrophysiological studies have supported the latter view, as chemoreceptors which respond specifically to putative phagostimulants have been identified only in monophagous and a few oligophagous species. For example, Chrysolina brunsvicensis, a monophagous beetle which feeds on Hypericum hirsutum, has a receptor cell that responds only to hypericin, an extended quinone characteristic of Hypericum spp. (Rees, 1969). This beetle is unusual in that it lacks a sucrose receptor, present in all other insects yet examined. Several oligophagous species occurring on cruciferous plants, including Pieris brassicae, have a glucosinolate receptor (Nielsen et al., 1979 Schoonhoven, 1967). On the other hand, Colorado potato beetles (Leptinotarsa decemlineata) restrict their feeding to potato leaves, apparently

despite the absence of any specific phagostimulant (Ritter, 1967; Hsiao, 1976).

Feeding deterrents are more significant determinants of behavior in polyphagous and oligophagous insects (Schoonhoven, 1982). Chapman et al., 1978) concluded that polyphagous acridoidea selected host plants primarily on the absence of feeding deterrents. Deterrency causes an insect to cease feeding (Ma, 1972; Blom, 1978) and often initiates locomotion or a search for a more appropriate food source (Jermy, 1971).

No "generalized deterrent receptor" capable of responding equally to all possible deterrents has been found (Dethier, 1980); rather each receptor is sensitive to a different range of secondary metabolites (Schoonhoven and Jermy, 1977; Schoonhoven, 1982; Dethier, 1980,1982). Pieris brassicae and other crucifer-feeding insects have one or more deterrent receptors (Ma, 1969; Schoonhoven, 1973, 1981a,b) that respond to low concentrations of a diverse array of allelochemicals including alkaloids, flavonoids, and terpenes (Ma, 1969; Schoonhoven, 1973, 1981a,b). The "bitter receptor" of Bombyx mori is equally catholic in sensitivity (Ishikawa, 1966). Manduca sexta's receptors respond to some alkaloids but not others, nor do they respond to flavonoids or the terpenoid azadirachtin (Schoonhoven 1982).

Many secondary metabolites with feeding deterrent activity operate by blocking the sugar and salt receptors, important in detecting phagostimulatory nutrients; included in this group are some tannins, terpenes, and a number of alkaloids (Dethier, 1982). Warburganal (Fig. 2), a powerful antifeedant (Kubo et al., 1976) reversibly inhibits the sugar receptor of the armyworm Spodoptera exempta and may bind to thiol groups, affecting energy transduction in the chemoreceptor (Ma, 1977). Brattsten (1983) suggested that mono- and diterpenes may interfere with a cytochrome P-450 like enzyme complex in the receptor cells. In such cases an insect may simply fail to perceive the plant as a potential food source.

It must be remembered that insects generally do not choose host plants as an all-or-none response to a single feeding deterrent (Schoonhoven, 1982; Dethier, 1982). A plant will usually stimulate a number of different receptors simultaneously, providing the insect with a wealth of chemosensory data. Cross-fibre patterning and central nervous system integration are involved in the choice process (Dethier, 1982). In general the ratio of phagostimulants (sugars, amino acids. etc.) to feeding deterrents determines the outcome of the choice. Moss and Dethier (1982) found that the ratio of sucrose to quinine hydrochloride determined the acceptance threshold of the mixture to Phormia regina and larvae of Danaus plexippus. The physiological

state of the insect, especially deprivated versus satiated, is also of importance: deprivated animals will accept a marginally deterrent plant that would be rejected by a satiated insect (Dethier, 1982).

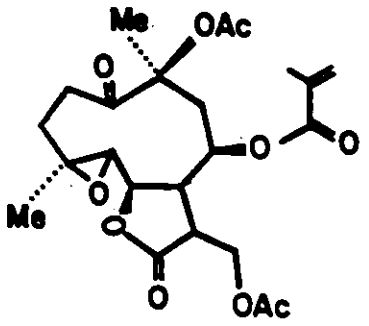
Table 2 summarizes selected insect antifeedants (structures for some are shown in Figure 2). Some of these compounds, particularly the triterpene, azadirachtin (Schmutterer et al., 1981) and the sesquiterpene, warburganal (Kubo et al., 1976) are effective at low concentrations to several insect species, and have attracted interest as antifeedants of potential agricultural significance (Jermy, 1983). The list includes a number of other terpenes, including the very large group of sesquiterpene lactones from the Asteraceae which are effective against insects (Burnett et al., 1974) but which may be more deterrent to mammalian herbivores (Picman et al., 1982 Mabry and Gill, 1979). The flavonoid antifeedants described by Sutherland et al. (1980) are noteworthy as all are also phytoalexins.

Few reports exist of polyacetylenes as feeding deterrents. The only quantitative study is that of McLachlan et al. (1982) who found that phenylheptatriyne (PHT) from Bidens pilosa inhibited feeding and weight gain of the cutworm, Euxoa messoria, at concentrations below those occurring in the plant. A polyacetylene fraction from the rabbitbush, Chrysothamnus nauseosus, inhibited feeding by Colorado potato beetle (Leptinotarsa decemlineata) larvae

and adults at a concentration of 35 μg/cm² (Jermy et al., 1981) the polyacetylene fraction consisted of four compounds (Rose et al., 1980). Binder et al. (1979) noted anecdotally that matricaria ester, a widely distributed acetylene in the Asteraceae, strongly reduced feeding by three polyphagous lepidopterans, Heliothis zea, Heliothis virescens, and Pectinophora gossypiella. Finally, a polyacetylene from Chanliognathus pennsylvanicus, an Asteraceae-feeding beetle, was found to be highly deterrent to predatory jumping spiders (Meinwald et al., 1968 Eisner et al., 1981). The marked activity of the isolated acetylene, Z,Z-dihyromatricaria acid (Fig. 1) led Eisner and his coworkers (1981) to suggest that acetylenes in plants might similarly deter herbivores.

Figure 2. Structures of some representative antifeedant and toxic allelochemicals. Compounds include the sesquiterpene lactones glaucolide A (a) and alantolactone (b), the isoflavanoid (+)-pisatin (c), the terpenoids warburganol (d) and limonin (e), and the alkaloid nicotene (e). Polyacetylenes include Z,Z-dihydromatricaria acid (g) and four compounds from Chrysothamnus nauseosus: methyl 2(Z),8(Z)-10-acetoxymatricariate (h), methyl 2(Z),8(Z)-10-hydroxy-matricariate (i), methyl 2(Z)-10-acetoxy-8,9-epoxydecen-4,6-diynoate (j), and methyl 2(z)-10-hydroxy-8,9-epoxydecen-4,6-diynoate (k).

a)



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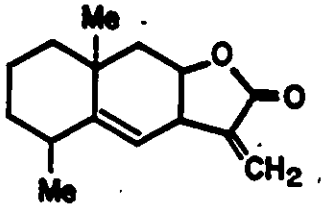

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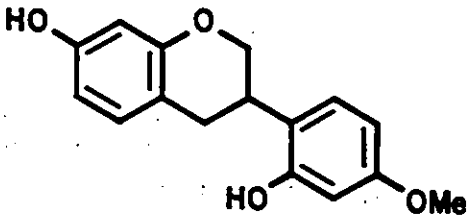

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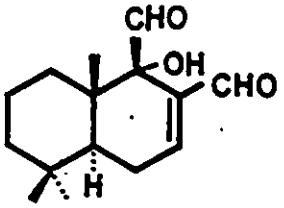

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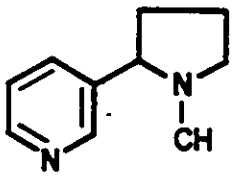

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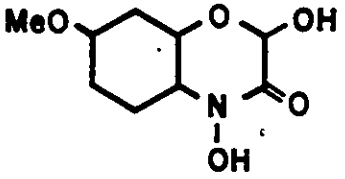


g) $CH_3CH{=}CHC{\equiv}C{-}C{\equiv}C{-}CH_2CH_2COOH$

h,i) $CH_3OOC{-}CH{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH_2OR$

h) R = Ac
i) R = H

j,k) $CH_3COOC{-}CH{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}CH{-}CH{-}CH_2OR$ (with epoxide O)

j) R = Ac
k) R = H

Table 2. Selected insect antifeedants.

Compound	Class	Test Insect	Effective Conc.	Reference
Azadirachtin	Triterpenoid	Schistocerca gregaria	0.04 ppm	Butterworth and Morgan, 1971
Azadirachtin	Triterpenoid	Spodoptera frugiperda	0.35 ppm	Warthen, 1979
Warburganal	Sesquiterpenoid	Spodoptera exempta	0.10 ppm	Kubo et al., 1976
Strychnine	Alkaloid	Pieris brassicae	5.00 ppm	Ma, 1972
Isopimpinelline	Furanocoumarin	Spodoptera litura	5.00 ppm	Yajima et al., 1977
Hydroxygrinellic acid	Diterpenoid	Schizaphis graminum	20.0 ppm	Rose et al., 1981
Piperone	Neolignan	Spodoptera litura	50.0 ppm	Matsui et al., 1976
Nomalin	Limonoid	Heliothis zea	6.6 ug/cm²	Klocke and Kubo, 1982
Obacunone	Limonoid	Heliothis zea	6.5 ug/cm²	Klocke and Kubo, 1982
Limonin	Limonoid	Heliothis zea	60.8 ug/cm²	Klocke and Kubo, 1982
3R-(-)-Vesitol	Isoflavonoid	Costelytra zealandica	8.35 ppm	Russel et al., 1978
(-)-Phaseollin	Isoflavonoid	Costelytra zealandica	1.0 ppm	Sutherland et al., 1980
(-)-Phaseollin	Isoflavonoid	Heteronychus arator	10.0 ppm	Sutherland et al., 1980
(+)-Pisatin	Isoflavonoid	Costelytra zealandica	100.0 ppm	Sutherland et al., 1980
Alantolactone	Sesquiterpene lactone	Tribolium confusum	1000.0 ppm	Picman et al., 1978
Glaucolide-A	Sesquiterpene lactone	Spodoptera eridania	500.0 ppm	Burnett et al., 1974

1.4.2 Allelochemicals: Toxicity and Phototoxicity

The chemical warning provided by feeding deterrents and repellents is not without substance: plants produce a diversity of compounds that are toxic to insects. Typically such allelochemicals reduce the survivorship, growth, or fecundity of herbivores (Rhoades and Cates, 1976; Swain, 1977). Often the same compound may function both as a feeding deterrent and as a toxin.

1.4.2.1 "Ground-State" Allelochemicals

Most known allelochemicals are active in the ground state (ie. without interaction with light). Reactions with biological substrates involve such processes as hydrogen or hydrophillic bonding, the formation of coordination complexes with cytochromes or hemes, or alkylation of target molecules. Two broad (and somewhat overlapping) groups of ground-state allelochemicals have been described, "digestability reducers" and "metabolic toxins".

Digestability reducers limit the nutritional value of ingested plant tissue by complexing with plant constituents or digestive enzymes, restricting the insect's ability to digest or assimilate nutrients. For example, saponins complex with steroids including cholesterol, (Applebaum and Birk, 1979); as insects cannot synthesize the steroidal nucleus these are essential nutrients (Bloch et al., 1956

Clark and Bloch, 1959). Protease and amylase inhibitors restrict an insect's ability to utilize polypeptides or starch (Ryan, 1979). Tannins (Feeny, 1970; Swain, 1979) and more recently phenolic resins (Rhoades and Cates, 1976) have been considered protein precipitators; however at the pH occurring in insect guts such compounds may operate by binding hydrophobically to polypeptides and cell wall constituents and sterically hindering digestive enzymes (Zucker, 1983). Such digestability-reducing compounds have a generalized mechanism of action, which led Feeny (1976) and Rhoades and Cates (1976) to suggest that they could not be countered by adapted insect herbivores. However, Bernays (1978) found that both condensed and hydrolysable tannins did not affect the efficiency with which several polyphagous acridid grasshoppers could digest diet. Gypsy moth (Lymantra dispar) larvae feed on leaves of several species rich in tannins (Matheson, 1984) and appear to be well adapted to the presence of these compounds.

Other secondary metabolites attack specific metabolic processes or target sites and so interfere with insect metabolism (Table 3). Non-protein amino acids are incorporated into proteins, where they disrupt structure and function (Rosenthal, 1982). Glucosinolates (Van Etten and Tookey, 1979; Ericson and Feeny, 1974) and cyanogenic glycosides (Conn, 1979) are enzymatically degraded to release hydrogen cyanide or thiocyanates which disrupt res-

piration by combining with the terminal cytochrome oxidase. Sesquiterpene lactones bind to thiol groups of proteins (Picman et al., 1981). Phytoecdysones and juvenile hormone analogues mimic endogenous insect hormones, altering moulting behaviour and development (Slama, 1979; Bowers, 1983). Many anti-acetylcholinesterases are known, including pyrethrins and isobutylamides (Jacobson and Crosby, 1971). Alkaloids are well known for their pharmacological effects on humans (Robinson, 1981) but few studies have addressed the role of these compounds in plant-insect interactions. Some alkaloids reduce insect growth and survivorship (Devitt et al., 1980), and toxic effects on insect neuroendocrinology have been demonstrated (Morris, 1984). A very different mechanism of action has been described for the alkaloid berberine, a photooxidative photogenotoxin (Philogene et al., 1984a) (Section 1.3.2).

These secondary metabolites may be characterized as metabolic poisons (Feeny, 1976; Rhoades and Cates, 1976) and are usually active at concentrations well below those at which digestibility reducers are toxic. Generally, toxins are active at concentrations below 1% whereas digestability reducers must be present at several percent to be effective.

Recently it has been found that some secondary plant metabolites can absorb light and be photoactivated to an excited state. Under appropriate conditions these secondary metabolites are capable of photosensitizing insect herbivores and other organisms.

Table 3. Selected toxic secondary metabolites.
ED_{50} concentrations are in % of diet fresh weight unless otherwise indicated. Superscripts denote: a) growth reduction to 50% of control; b) doubling of development time to pupa; and c) 50% mortality

Compound	Biosynthetic Class	Test Insect	ED_{50}	Reference
Gossypol	sesquiterpene	Heliothis zea	0.25^{a}	Shaver & Parrott, 1970
Gossypol	sesquiterpene	Heliothis virescens	0.35^{a}	Shaver & Parrott, 1970
Gossypol	sesquiterpene	Pectinophora gossypiella	0.35^{a}	Shaver & Parrott, 1970
Sinigrin	glucosinolate	Papillio polyxenes	0.01^{a}	Erikson & Feeny, 1974
DIMBOA	hydroxamic acid	Ostrinia nubilalis	$<0.032^{a}$	Klun et al., 1967
DIMBOA	hydroxamic acid	Schizaphis graminum	1.0 mM/l^{a}	Argandona et al., 1983
L-Canavanine	non-protein amino acid	Callosobruchus maculatus	1.00^{bc}	Janzen et al., 1977
L-Canavanine	non-protein amino acid	Manduca sexta	<2.5 mM^{bc}	Dahlman, 1977
Quercitrin	flavonoid	Heliothis zea	0.6^{a}	Shaver & Lukefahr, 1969
Quercitrin	flavonoid	Heliothis virescens	0.2^{a}	Shaver & Lukefahr, 1969
Quercitrin	flavonoid	Pectinophora gossypiella	0.1^{a}	Shaver & Lukefahr, 1969
Chlorogenic acid	flavonoid	Heliothis zea	2.5^{b}	Elliger et al., 1981
Chlorogenic acid	flavonoid	Heliothis zea	6.0 mM/kg^{a}	Isman & Duffey, 1982
Rutin	flavonoid	Heliothis zea	2.4^{a}	Elliger et al., 1981
Rutin	flavonoid	Heliothis zea	4.8 mM/kg^{ab}	Isman & Duffey, 1982
Quercetagetin DME	methylated flavonoid	Heliothis zea	4.8 mM/kg^{a}	Isman & Rodrigues, 1983
Quercetagetin DME	methylated flavonoid	Spodoptera exigua	2.4 mM/kg^{a}	Isman & Rodrigues, 1983
α-Tomatine	alkaloid	Heliothis zea	0.40^{d}	Elliger et al., 1981
α-Tomatine	alkaloid	Heliothis zea	0.90 mM/kg^{a}	Isman & Duffey, 1982
Berberine	alkaloid	Euxoa messoria	0.3^{ab}	Devitt et al., 1980
Nicotine	alkaloid	Euxoa messoria	0.3^{ab}	Devitt et al., 1980
Veratrine	alkaloid	Euxoa messoria	$<0.01^{c}$	Devitt et al., 1980
Coronopilin	sesquiterpene lactone	Heliothis zea	3.7 mM/kg^{a}	Isman & Rodrigues, 1983
Confertin	sesquiterpene lactone	Heliothis zea	2.8 mM/kg^{a}	Isman & Rodrigues, 1983
Tetraneurin-A	sesquiterpene lactone	Heliothis zea	1.5 mM/kg^{a}	Isman & Rodrigues, 1983

1.4.2.2 Photosensitizing Secondary Metabolites

A "photosensitizer" is a compound which interacts with another component of a system (ie. biological substrate) and causes it to react to the presence of light (Turro and Lamola, 1977). Photoactivation allows a secondary plant metabolite access to "excited state chemistry", which leads to more energetic and potentially damaging reactions, than occur in the thermally-driven reactions of ground state molecules (Arnason et al., 1983b McLachlan, 1984). Such photoreactions may involve covalent bond breaking and formation, and the generation of free radicals, superoxide radicals, and singlet oxygen (Spikes, 1977; Ito, 1983). Insects may be photosensitized by a variety of natural and synthetic compounds with very different mechanisms and sites of action (Arnason et al., 1983b). Photosensitizers may be classed by their mechanism of action although some overlap between mechanisms does occur.

Photosensitizers which require oxygen for toxicity are termed photodynamic and account for the majority of the 400 known phototoxins (Spikes, 1977). Photodynamic action is established by the absence of toxicity under anaerobic conditions (Turro and Lamola, 1977). Two general photooxidative mechanisms occur, termed Type I and Type II photodynamic action (Spikes, 1977). Type I is a relatively rare process involving an electron transfer from the excited sensitizer to molecular oxygen to yield superoxide radical, O_2^-.

The O_2^- reacts with and fully oxidizes susceptible substrates (biological molecules). Among natural products this process is restricted to the flavines. The more common Type II photosensitization involves an energy transfer from the sensitizer to molecular oxygen to yield singlet oxygen, $^1O_2$, the most reactive species of oxygen. The ground electronic state of oxygen is unusual in that it is a triplet and facilitates the production of $^1O_2$ by an interaction of triplet O_2 and the triplet sensitizer. Only 96 Kj/mole are required to reach the long-lived (2 μs in H_2O) first singlet state, $^{\Delta}g$. The second excited state $^{\Sigma}g$ requires 155 Kj/mole but is unstable as electron spins are not paired; internal conversion rapidly leads to a return to the $^{\Delta}g$ state. Such transition energies are readily available from most excited state sensitizers as even red light has an energy of over 166 Kj/einstein.

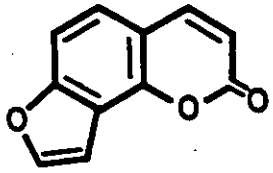
Type II photooxidations are characteristic of the acridine, thiazine, and xanthene dyes (ex. acridine orange, methylene blue, and rose bengal respectively), the first photosensitizers to be described (Raab, 1900). Naturally occurring type II photooxidants include the condensed anthrones hypericin and pseudohypericin from Hypericum spp. (Arnason et al., 1983b) and the isoquinoline alkaloid berberine (Philogene et al., 1984a). The thiophene α-terthienyl is strictly photodynamic (Bakker et al., 1979 Arnason et al., 1981a Downum et al., 1982 Gommers et al., 1982

McLachlan et al., 1984 McLachlan 1984) and is a particularly potent generator of $^1O_2$ (Arnason et al., 1981a McLachlan et al., 1984 McLachlan, 1984). This mechanism of action has recently been extended to other thiophene compounds including bithiophene (McLachlan, 1984; McLachlan et al., 1984 Gommers et al., 1982). Partially ring-stabilized polyacetylenes, such a as PHT and its derivatives, also generate $^1O_2$ but have a more complex mechanism of action; discussed below.

Some secondary plant metabolites, upon photoexcitation, form free radicals which react directly with susceptible substrates. Such a mode of action is less common (as far as is known) than type II photooxidations. Bakker et al. (1983) recently ascribed a free radical mode of action to several phytoalexin isoflavonoids. Rapid photopolymerization of aliphatic polyacetylenes, and the absence of protection under anaerobic conditions, led McLachlan et al. (1984) to suggest a free radical or other non-oxidative mechanism of action for these phototoxins. Partially ring-stabilized polyacetylenes, intermediate in structure between the thiophenes and the aliphatic polyacetylenes, apparently have access to both non-oxidative and photooxidative modes of action. Free radical additions can propagate polymerization reactions among suitably arranged unsaturated molecules; such a mechanism has been described in the case of synthetic diacetylenes (Lopez et al., 1982).

Figure 3. Structures of some phototoxic plant secondary metabolites (a-f) and synthetic dyes (g-i). Compounds include angelicin (a), xanthotoxin (8-methoxypsoralin) (b), berberine (c), harmaline (d), dictamnine (e), visnagin (f), methylene blue (g), rose bengal (h), and eosin Y (i).

a)



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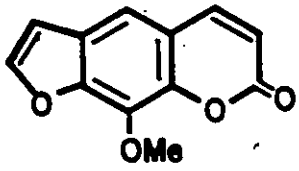

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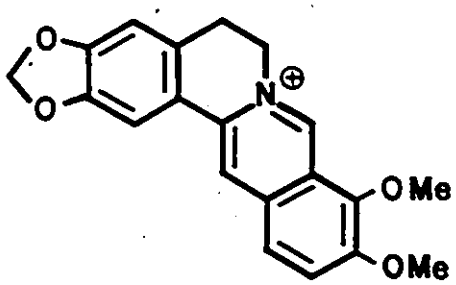

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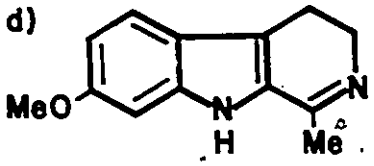

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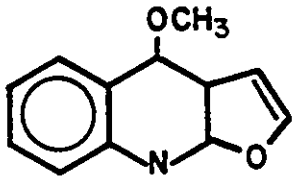

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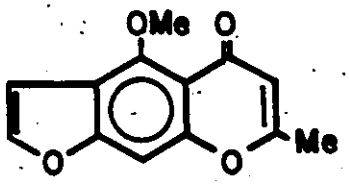

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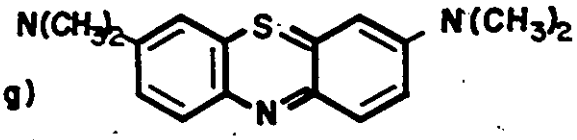

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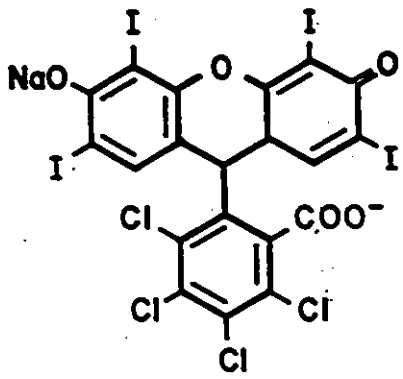
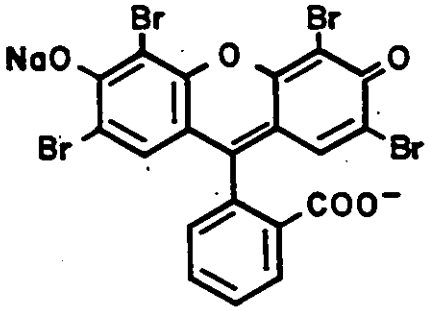


i)



A major component of the phototoxicity of any compound is the nature of susceptible or target substrates. Thiophenes and polyacetylenes both disrupt membranes, as indicated by the haemolysis of erythrocytes (Wat et al., 1980b). These lipophillic compounds intercalate into the hydrophobic region of membranes and attack membrane components by photooxidation, free radical addition, and possibly free radical polymerization of lipids (McLachlan, 1984). These reactions possibly produce locally rigid areas in an otherwise fluid membrane; shear stresses at the interface tear the membrane and result in loss of cell contents and the disruption of across-membrane gradients.

Other phototoxic secondary plant metabolites intercalate into and photosensitize DNA and may be termed photogenotoxic. Most photogenotoxins undergo cycloaddition to pyrimidine bases of DNA; the best known of these are the furanocoumarins. Linear furanocoumarins, such as 8-methoxypsoralin (xanthotoxin, Fig. 3) form difunctional crosslinks between thymidine bases, disrupting transcription and DNA synthesis (Musajo et al., 1974). Unadapted insects, such as the armyworm Spodoptera eridana are highly susceptible to photosensitization by these compounds (Berenbaum, 1978). Sterically hindered furanocoumarins and the angular furanocoumarins (ex. angelicin, Fig. 3) form monofunctional adducts to DNA (Coppey et al., 1979). These do not crosslink DNA but still interfere with transcription and DNA

replication. They are more easily repaired than are crosslinks but induce a higher frequency of mutation. Recently described photogenotoxins which also form monofunctional adducts include harmaline, a β-carboline alkaloid (McKenna and Towers, 1981), the furanoquinoline alkaloid dictamnine and the furochrome visnagen (Towers et al., 1981 Pfyffer et al., 1982 Pfyffer and Towers, 1982).

Berberine represents a different mode of photogenotoxicity in that it intercalates into DNA and oxidizes susceptible regions, rather than photobinding to bases (Philogene et al., 1984a).

1.4.3 Insect Response: Detoxification and Coevolution

Adapted insects possess a variety of enzyme systems which metabolize and facilitate the excretion of plant allelochemicals. The most active system is the microsomal mixed-function oxidase (MFO) system associated with the smooth endoplasmic reticulum in the midgut, fat body, Malphigian tubules, and other tissues (Brattsten, 1979). This system is able to accept a wide variety of lipophillic substrates; compounds are oxidized via cyclodiene epoxidation, aromatic and aliphatic epoxidation, oxidative desulfuration, thio ether oxidation, or tertiary amine N-oxidation (Nakatsugawa and Morelli, 1976). The basis of this remarkably versatile system is a family of cytochrome P-450's with overlapping substrate affinities (Wilkinson, 1983).

The MFO system can be rapidly induced by exposure to a number of allelochemicals (Brattsten et al., 1977). Limits to the response are imposed by the feeding habits of the species. Polyphagous insects, exposed to a diversity of natural products, elaborate high titres of diverse MFO's, whereas mono- or oligophagous insects are more limited in the range of compounds to which they can respond (Kreiger et al., 1971 Brattsten et al., 1977). Other enzymes also function in the metabolism of allelochemicals, including reductases, hydrolases, and a variety of group transferases (Brattsten, 1979).

Success in overcoming a plant's defences leaves that plant open to attack; selection then favors the elaboration of new allelochemicals, which in turn select further for resistant insects. The result is a coevolutionary "arms race" (Metcalf, 1979; Berenbaum, 1978; Gilbert and Raven, 1975). Such an "arms race" has apparently occurred between various Apiaceae and insects. Phototoxic linear furancoumarins are found in eight plant families, particularly the Apiaceae and Rutaceae; they are much more toxic to insects than is their biosynthetic precurser, umbelliferone, which occurs in over thirty families (Berenbaum, 1978). A limited fauna can metabolize the linear furanocoumarins (Ivie et al., 1983) and exploit plants containing them. However, these insects cannot utilize plants containing angular furancoumarins, found only in two advanced tribes of the

Apiaceae (Berenbaum and Feeny, 1981). Thus the evolutionary sequence from umbelliferone to linear furanocoumarins to angular furanocoumarins appears to represent successive escalations in the coevolution between these plants and their insect herbivores. This is supported by the observation that the insect fauna on umbelliferous plants is strongly correlated with the chemistry of those plants (Berenbaum, 1981,1983).

Chapter II

METHODS

2.1 Test Compounds

Structures for all thiophenes (compounds I-III) and polyacetylenes (compounds IV-VII) used in these experiments are given in Fig. 4, and Table 4 lists their molecular weights, λ_{max}, $\epsilon\lambda_{max}$, and log water/octanol partition coefficients (from McLachlan, 1984).

1-Phenylhepta-1,3,5-triyne (PHT) (compound VI) was extracted from the aerial parts of Bidens pilosa collected by Dr. J. Romeo at Miami, Florida. Plants were stored in 95% ethanol (EtOH), and subsequently ground in 95% EtOH in a Waring blender. The homogenate was filtered to remove particulate matter, combined with an equal volume of water, and extracted three times with an equal volume of petroleum ether (P.E.). The P.E. phase was dried with sodium sulfite and reduced to a small volume on a rotary evaporator (30°C). The concentrated extract was chromatographed on 100-200 mesh silica gel columns using P.E. as the elutant. Collected fractions were monitored for PHT with a Pye-Unicam SP8-100 spectrophotometer; PHT eluted just behind the solvent front

and ahead of the first carotenoid band. Fractions containing PHT were reduced in volume on a rotary evaporator. Final purification involved two recrystalizations, the first from P.E. and subsequently from 95% EtOH. Pure PHT was stored in 95% EtOH, in the dark, at about -5°C.

A similar procedure was followed to obtain α-terthienyl (α-T) (compound III) from Tagetes erecta roots. The roots (from plants grown at the Central Experimental Farm) were not ground as they had been stored in 95% EtOH for over a year. The ethanolic filtrate, rich in thiophenes, was extracted with P.E., and the concentrated P.E. extract was chromatographed on unactivated alumina with 1% acetone in P.E. as the elutant (Chan et al., 1977). Thiophene bands, which could be visualized by their strong blue-violet flourescence under short-wave UV, were collected and further purified by three recrystalizations, the final two from 95% ETOH.

Subsequently synthetic α-T was obtained from G. Campbell. This material was prepared from 2,2'-bithiophene and 2-bromothiophene by a modification of the Ullmann synthesis of Yamazaki et al, (1976) (Campbell, 1983 synthesis "a" in Philogene et al, 1984b)(6% yield). α-T was also obtained from Drs. L.C. Leach and P. Morand (Dept. of Chemistry, University of Ottawa), who developed a large-scale version of a Gringard synthesis (synthesis "b" in Philogene et al, 1984b) which produces α-T in good yield (85%).

Figure 4. Structures of thiophenes (I-III) and polyacetylenes (IV-VII) used in this study.

I. 2-(1,3-pentadiynyl)-5-(methyl-3-chloro-1-butynoate)

II. 5-(3-buten-1-ynyl)-bithiophene

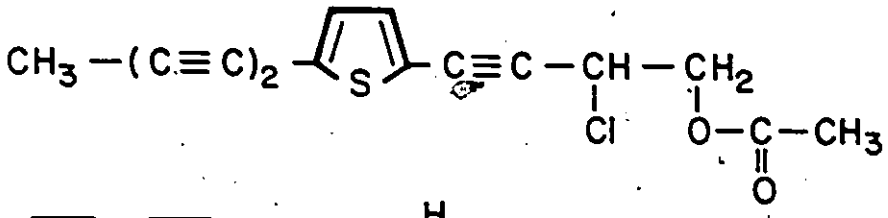
III. α-terthienyl

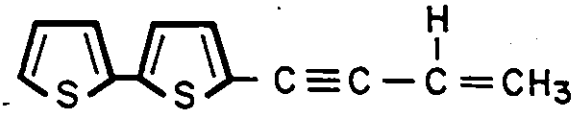
IV. phenylhepta-1,3-diyne-5-ene

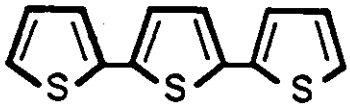
V. phenylhepta-1,3-diyne-5-ene-7-acetate

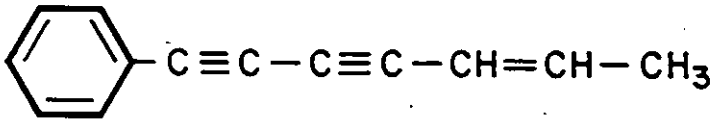
VI. phenylhepta-1,3,5-triyne

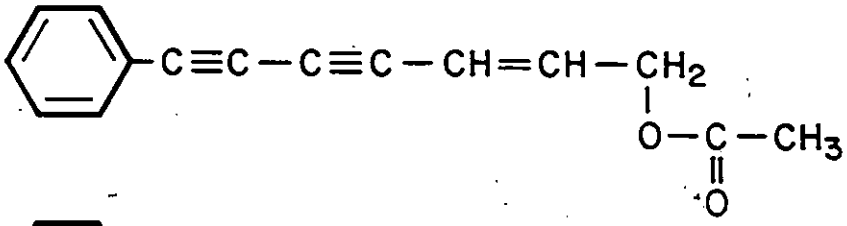
VII. γ-(hex-4'-en-2'-ynylidenyl)-γ-but-2-enyrolactone

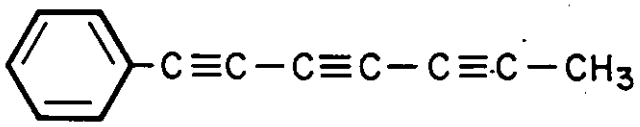
I 

II 

III 

IV 

V 

VI 

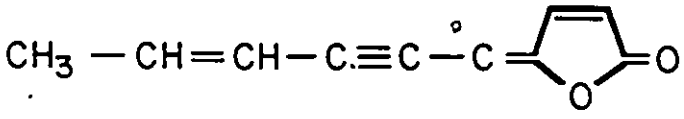
VII 

Table 4. Molecular weights, λ max, and $\epsilon\lambda$max for polyacetylenes and thiophenes used in this study.

Compound	Molecular Wt.	max	max
I	290.90	320	30,900
II	216.33	343	21,600
III	248.4	350	22,500
IV	166.25	299	22,650
V	208.27	299	32,400
VI	164.21	330	17,320
VII	160.17	347	21,200

All other compounds were obtained from Dr. J. Lam (Dept. of Chemistry University of Aarhus, Aauhus, Denmark). Compound II was extracted from Echinops sphaerocephalus and Echinops bannaticus, and compound IV from Dahlia pinnata. Sources of other compounds are given in McLachlan (1984).

Compound purity was assayed by HPLC and by conformity of UV spectra with published values. Concentrations were determined spectroscopically on a Pye-Unicam SP8 spectrophotometer.

2.2 Insect Cultures

2.2.1 Euxoa messoria

Euxoa messoria eggs were obtained from Dr. H.H. Cheng, Agriculture Canada, Delhi, Ontario except for one group obtained from Mr. J.P. Whistlecraft, Agriculture Canada, London, Ontario. Neonate larvae were removed from the sand and rye leaves in which they had been shipped and transferred to vials containing artificial diet. Initially stock larvae were maintained individually in polystyrene vials capped with perforated, opaque lids and lined with filter paper; subsequently stock insects were reared in glass dishes sealed with polyethylene film (Stretch n' Seal), with 25-40 larvae per dish. All larvae were transferred to individual

vials after the third instar. Hinks and Byers (1976) reported that some species of Euxoa were cannibalistic, but this did not occur with first to third instar Euxoa messoria larvae unless insufficient diet was provided. Larvae were maintained in a Conviron growth chamber at 27°C, 18 hour photoperiod and a relative humidity (R.H.) of 75-80%. Stock larvae were exposed to the same UV light levels as were utilized in the experiments. Larvae were judged to have reached the prepupal stage when they ceased feeding, lost weight on three consecutive weighings, and developed a translucent cuticle (especially ventrally) through which white fat body lobes could be observed (Devitt, 1978). Prepupae were transferred to clean vials filled with moist peat moss. Pupae turned dark shortly before adult emergence; such pupae were placed on moist peat moss in a 60 x 60 x 49 cm wire mesh cage for adult emergence. The artificial diet used was that developed by Devitt (1978; Devitt et al., 1980). The diet composition is given in Appendix 2A.

2.2.2 Ostrinia nubilalis

Ostrinia nubilalis eggs and larvae were obtained from a stock culture maintained in this laboratory. Larvae were reared in mass culture according to the method of Guthrie (1971) in a Conviron growth cabinet, under an 18 hour photoperiod and a R.H. of 80%. The temperature was 27°C in the

day and 16°C at night. The artificial diet used was that of Guthrie (1971) without corn cob grits (Appendix 2B).

2.2.3 Manduca sexta

Manduca sexta eggs and larvae were also obtained from a stock culture maintained in this labotatory. The larvae were reared as in Stewart (1981), that is, individually in 10 x 3.5 cm polystyrene vials capped with a foam plug. An 18 hour photoperiod and temperature of 26°C were used. The chamber used for the Manduca sexta stock culture had no provision for humidity control; however, humidity was high (ca 70%) and diets were changed frequently (initially every second day, every day in later instars) to prevent dehydration. The diet used was that of Stewart (1981) (Appendix 2C). Prepupal Manduca sexta ceased to feed, coated the inside of the vial with a mixture of diet, frass, and fluid to form a pupation chamber, and exhibited an obvious dorsal blood vessel through a transparent cuticle. Such larvae were placed in glass bottles filled with vermiculite and allowed to pupate in the dark. Pupae were placed in 60 x 60 x 48 cm cages for adult emergence; a tobacco plant was provided as an oviposition site.

Adults of all three species were fed a solution of 10% sucrose in water.

2.3 Test Diets

Appropriate amounts of the test compounds in 95% EtOH were placed in 50 or 100 ml beakers, to which was added warm liquid diet. The temperature of the diet was not more than 35° C at this time, in order to prevent degradation of the acetylenes. The diet and acetylenes in EtOH (or EtOH alone in the case of controls) were mixed vigorously by hand for about two minutes; the diet was then poured into 1 x1 x 0.6 cm molds to cool. The amount of 95% EtOH carrier in the diets did not exceed 2.5% (0.5 ml 95% EtOH in 25g diet).

Diets treated with α-T were examined to ensure even distribution of the compounds and to ensure against degradation of the phototoxin during diet preparation or during exposure under expriemental conditions. Diet cubes (Euxoa messoria diet) were placed on two Saboraud dextrose broth/agar plates that had been streaked with Sacchromyces cerevisiae, and were exposed to 4 hours near-UV or darkness (McLachlan, 1984). A prominent clearing zone around the diet cubes treated with α-T and near-UV was not present around control diets or those treated with α-T and not irradiated. This indicated the presence of the phototoxin in the diet in unaltered form. The clearing zone declined with time, presumably due to such processes as photodegradation or microbial action, and disappeared after 5 days. Consequently diets were changed frequently (every 2-3 days). Fresh diet for all insect species was prepared weekly and refrigerated at about 5°C until used.

2.4 Light sources and near-UV filters

Combinations of two light sources were used. Solar-simulating 48 inch Vita-light flourescent lamps (Duro-Test No. 48T12) were used in all tests to provide visible light and some near-UV irradiation. The light output of these lamps simulate solar radiation in spectral distribution but not intensity. In several experiments these lights were augmented with 24 inch, 20 watt Westinghouse blacklight-blue fluorescent lamps (FT20T12/BLB). Blacklight-blue lamps emit in the 300-400 nm range with the peak emission at 350 nm.

Treatment groups not exposed to near-UV were shielded by Kodak Wratten 2B filters (400 nm cut off) placed over the vials, or vertically between the vials and the near-UV light source. Radiant heating of vials under the filters was not detectable (< 0.25 C).

All materials used in the rearing of experimental insects, including the polystyrene vials, glass scintillation vials, petri dishes, and polyethylene film (Stretch-n'--Seal) were checked for UV adsorption and were found to be transparent to wavelights above 300 nm.

2.5 Growth Studies

All growth studies included four treatment groups: 1) those exposed to the test diet and UV light, 2) those fed test diet but not irradiated, 3) those fed control diet and exposed to UV light, and finally 4) those exposed to neither the phototoxin nor UV light. Insects were reared under the same conditions as described for the stock cultures except for variations in the amount of near-UV irradiation to which they were exposed. Treatments were compared according to Duncan's multiple range test unless otherwise indicated.

2.5.1 α-T Toxicity to Euxoa messoria: "High" UV

In the first experiment, Euxoa messoria larvae were exposed to combinations of 4 w/m^2 near-UV and diet containing 100 ug/g α-T. Fifteen larvae were used in each treatment group. Neonate larvae, 24 to 72 hours old, were individually transferred to clear polystyrene 2.5 x 4 cm vials containing a piece of diet cube. A strip of filter paper 1 cm tall encircled the inside bottom of the vial, which was capped with UV-transparent polyethylene film (Berenbaum, 1978). The cap was perforated with a no. .001 insect pin. This, and the filter paper, helped prevent condensation in which neonate larvae tended to drown. The perforations also helped reduce radiant heating inside the vials. Diet was changed, and larvae weighed, every two days until all of the

larvae exposed to both α-T and 4 w/m² near-UV died. The presence of shed exuvia was noted when diets were changed.

2.5.2 α-T Toxicity to Euxoa messoria: "Low" UV

Groups of thirty larvae were treated with combinations of 0.5 w/m² near-UV and diet containing 10 or 100 ug/g α-T. Larvae were reared individually in polystyrene vials as described previously. Fresh diet was provided, larvae weighed, and exuvia checked for every two days until all of the individuals in each treatment group had entered the prepupal stage. Prepupae were transferred to vials containing moist peat moss and checked every two to three days until pupation occurred, at which time pupae were weighed. Darkened pupae were transferred to 62 x 60 x 30 cm oviposition cages for adult emergence.

Light was provided by a bank of twelve Vita-lights 50 cm above the insects. The near-UV intensity to which the larvae were exposed was measured at 0.5 w/m².

2.5.3 Toxicity of Compounds I-VII to Euxoa messoria

Rearing larvae individually is practical only when relatively large amounts of the test compounds are available, as substantial amounts of unconsumed diet must be discarded every two to three days. It was noted that Euxoa messoria

larvae were not cannibalistic during the first three instars. As communal rearing makes for more efficient use of diet, growth studies for compounds I-VII were done using the following technique, adapted from Berenbaum (1978). Growth of control larvae under these conditions was very similar to that of individually reared larvae.

Diet cubes (two to four, depending on the age and size of the insects), containing 100 ug/g test compound, were cut in two and placed in a petri dish; the perimeter of the dish was lined with Watman # 1 glass fibre filter paper. Twenty-five Euxoa messoria larvae were placed in each dish, which was sealed with polyethylene film. One dish was prepared for each combination of test compound and light treatment (2 w/m^2 near-UV), due to limitiations in the amount of test compounds. The experiment was subsequently repeated but followed for two, instead of three weeks. Diets were changed, and dead larvae counted and removed every second day, and larvae were weighed every fourth day. The larvae were reared individually from day 18 to day 21, at which time the experiment was terminated (see Section 2.7).

The light system used for these experiments included a bank of eight Vita-lights augmented by four BLB lamps 54 cm above the insects. The near-UV contributed by the Vita-lights was negligible as these lamps were located above a UV-opaque plexiglass partition. The near-UV was contributed by the BLB lamps and was measured at 2.0 w/m^2.

2.5.4 α-T and PHT Toxicity to Manduca sexta

Manduca sexta larvae were also communally reared in petri dishes until the third instar. Larvae were presented with diet containing 10 and 100 μg/g PHT and α-T The experiments were begun with newly hatched neonates rather than with 24-72 hr old larvae.

Thirty or forty mature eggs were placed in each petri dish with an appropriate diet cube; the dishes were examined 24 hours later and unhatched eggs, and larvae in excess of 25 were removed. Irradiation was begun on day 5 of the test, to allow larvae to accumulate the test compounds and to facilitate distinction of light-independent effects from phototoxicity. Larvae were weighed on days 5, 7, 10, 14, and 15, at which time the experiment was terminated. Diets were changed every 2-3 days. The experiments were repeated twice, and each experiment included two replicates of the α-T and PHT treatments and three of the control.

The same light system as was described in Section 2.5.3 was used in these experiments (2 w/m² near-UV).

2.5.5 Toxicity of Compounds I - VI to Ostrinia nubilalis

Ostrinia nubilalis neonates were sufficiently small to escape the petri dishes, and displayed a penchant for eating polyethylene film. To screen compounds I to VI (at 100 μg/g) against Ostrinia nubilalis, egg masses at the black-

head (hatching) stage, containing 10 to 30 eggs each, were pinned to diet cubes in glass scintillation vials and stoppered with a cotton plug. Hatching neonates moved onto the diet cubes and began feeding. The following morning (15 hours later) larvae were distributed to six vials for each treatment group, five larvae to a vial. Thus all of the thirty larvae in each treatment group were of the same age, within 15 hours. Vials were laid on their side and exposed to the appropriate light treatment (2 w/m² near-UV). The diet used for Ostrinia nubilalis was relatively rich in antibiotics and remained free of bacterial contamination for extended periods. Diets were changed and larvae weighed every four to five days over a twenty day period.

2.6 Feeding Deterrency Tests

To test the effect of thiophenes and polyacetylenes on neonate feeding behaviour, test compounds were applied in acetone to both surfaces of a leaf disc. Control discs were treated with acetone alone. Lettuce (Lactuca sativa) leaves were used for Euxoa messoria, corn leaves (Zea mays cultivar Silver Queen) for Ostrinia nubilalis, and tobacco (Nicotiana tabacum cultivar Delhi 77) leaves for Manduca sexta. Leaf discs were weighed and placed in glass scintillation vials; five Euxoa messoria and Ostrinia nubilalis or two Manduca sexta neonates less than 24 hours old were placed on each

leaf disc and the vials were capped. Experiments were conducted in a Conviron ET growth cabinet at 26°C, 80% R.H. and 18 hour photoperiod. After 48 hours the surviving larvae in each vial were counted, and the leaf discs were dried for 24 to 48 hours at 80°C in a drying oven and weighed. Consumption was calculated as the difference in leaf dry weight; initial dry weights were calculated from regression equations based on not less than ten dry weight/fresh weight determinations for each plant species. The equations were:

d.w. = .0035541(f.w.) + .0036 r=0.975 (lettuce),

d.w. = .258(f.w.) - .481 r=0.95 (corn)

d.w. = .035(f.w.) + 6.88 r=0. (tobacco)

All compounds were tested at 100 µg/g (and in some cases 10 µg/g) concentrations. At least five replicates of each treatment were obtained. The design of these experiments was "no-choice".

More mature Euxoa messoria and Manduca sexta larvae were tested in no-choice and choice experiments. For no-choice tests preweighed 4th or 5th instar unstarved larvae were presented with an appropriately treated weighed cube of artificial diet. Consumption and frass production was measured after 24 and 48 hours, and larvae were reweighed. Diet weight loss due t to evaporation was corrected for on the basis of diet cubes not exposed t to insect feeding; evaporation accounted for a daily weight decline of about 16%. At least ten replicates of each concentration were obtained.

For Euxoa messoria fed 100 µg/g α-T trials with and without near-UV (4 w/m²) were conducted. For choice tests eight diet cubes were pinned around the perimeter of a 14 cm diameter desiccator. Each experiment included three control and five dosed diet cubes; the dosed cubes included two at each of two concentrations plus one at a third concentration. Diet cubes were arranged so that adjacent cubes were of differing treatments. Four Euxoa messoria larvae were released in the center of the desiccator. Experiments were conducted with both starved (18 hour) and unstarved larvae, but no difference in the behaviour of the two groups was apparent. Both α-T and PHT were tested at 0.1, 1.0, 10.0, and 100 µg/g. Each concentration was tested at least seven times. Feeding larvae were exposed to an 18 hour photoperiod of visible light without UV.

2.7 Nutritional Indices

Euxoa messoria larvae, from the test in which three thiophenes and four polyacetylenes were examined (Section 2.5.3) were weighed on day 18 of that test, then transferred to individual 3.5 x 6.0 cm clear polystyrene vials with a fresh, weighed diet cube dosed with the same compound (100 µg/g) to which the insects had been exposed. Larval weights, frass, and unconsumed diet were determined daily for the next three days. The same procedure was followed

with fourth instar larvae from the stock culture: such larvae were "naive" in that they had not previously been exposed to the test compounds. Remaining diet was dried at 80 C in a drying oven. Frass was dried for 48 hours under vacuum in a dessicator. The initial dry weight of the diet was calculated from the dry weight/fresh weight ratio (=0.2624), as were both the initial and final dry weights of the larvae (for unstarved larvae, dry weight = 0.1415 fresh weight). Nutritional indices determined included the consumption index (C.I.), the approximate digestibility (AD), the efficiency of conversion index (ECI), and the efficiency of conversion of digested food (ECD). Indices are defined in Results section 3.5 and follow Reese (1979) and Waldbauer (1968).

2.8 Body Burden and Excretion

After 21 days of exposure to diets containing compounds I to VII, Euxoa messoria larvae were analysed for polyacetylene or thiophene content. Larvae were starved for 36 hrs to clear the gut of diet containing unassimilated test compounds. The larvae were then reweighed, frozen in liquid nitrogen, and homogenized in 5 ml HPLC grade methanol (Fisher) with a teflon pestle. After extracting overnight in methanol, the homogenates were extracted 3X with 5 ml HPLC grade hexane (Fisher). Extracts were pooled and evaporated

to dryness under vacuum, in the dark and at room temperature. Extracts were taken up in 0.5 ml HPLC grade acetonitrile (Fisher), and 25 µl aliquots were analyzed for polyacetylene or thiophene content by reverse-phase HPLC. The HPLC system included Beckman 110A pumps, a 420 Beckman controller, and a Beckman 165 Variable Wavelength Detector. The column was a Beckman Ultrasphere 5µ ODS 4.6 mm x 25 cm ($RP\text{-}C_{18}$), and samples were eluted with 15% HPLC grade water in acetonitrile. Samples generally gave few peaks, and peaks attributable to the test compounds, if present, were distinct and did not overlap with other insect constituents. Concentrations of test compounds were determined according to standard curves; identity of the detected peaks with polyacetylenes or thiophenes was confirmed by determining UV spectra during elution, with the Beckman 165 detector, and comparing them with those of the original compounds.

Frass samples were dried in a dessicator, weighed, then extracted for two weeks in 5 ml HPLC grade acetonitrile. Extracts were concentrated to 1 ml, and 25 µl aliquots were analysed by HPLC as described for insect samples.

Chapter III

RESULTS

3.1 Effects of α-T and Near-UV on Euxoa messoria

When Euxoa messoria larvae were fed diet containing 100 µg/g α-T, the degree of toxicity expressed was dependent on the intensity of near-UV exposure; both phototoxic and non-phototoxic effects were observed.

3.1.1 α-T Toxicity to Euxoa messoria: "High" UV Test

Larvae exposed to α-T without near-UV showed markedly decreased growth compared to the control; at 14 days the mean weight of the α-T fed insects was only 20% of the control (Fig. 5). Relative weights were higher after 21 and 28 days as control larvae were entering plateau phase and α-T treated larvae were still gaining weight.

Similar results, although not as marked, were found for larvae exposed to near-UV (4 w/m²) without α-T; in this case larval weights closely approached those of the controls by day 21.

Cutworm larvae exposed to both α-T and near-UV (4 w/m²) did not differ significantly from larvae treated with α-T alone at days 7 and 14, although the mean weight of the irradiated larvae was consistantly lower. By day 21, however, larval weights were significantly depressed. (Duncan's Multiple Range Test, =.05, d.f.=45). Although the larvae were observed to be feeding, the growth rate was lower than in the other treatment groups. Mortality was higher in this group as well: on day 21 only 4 of the original 15 larvae were alive, compared to 11 to 14 survivors in the other treatment groups. Mortality reached 100% by day 28.

3.1.2 α-T Toxicity to Euxoa messoria: "Low" UV Tests

The sublethal effects of α-T on Euxoa messoria were further investigated when larvae were reared on diet containing 100 and 10 µg/g α-T and exposed to a low intensity (0.5 w/m²) of near-UV.

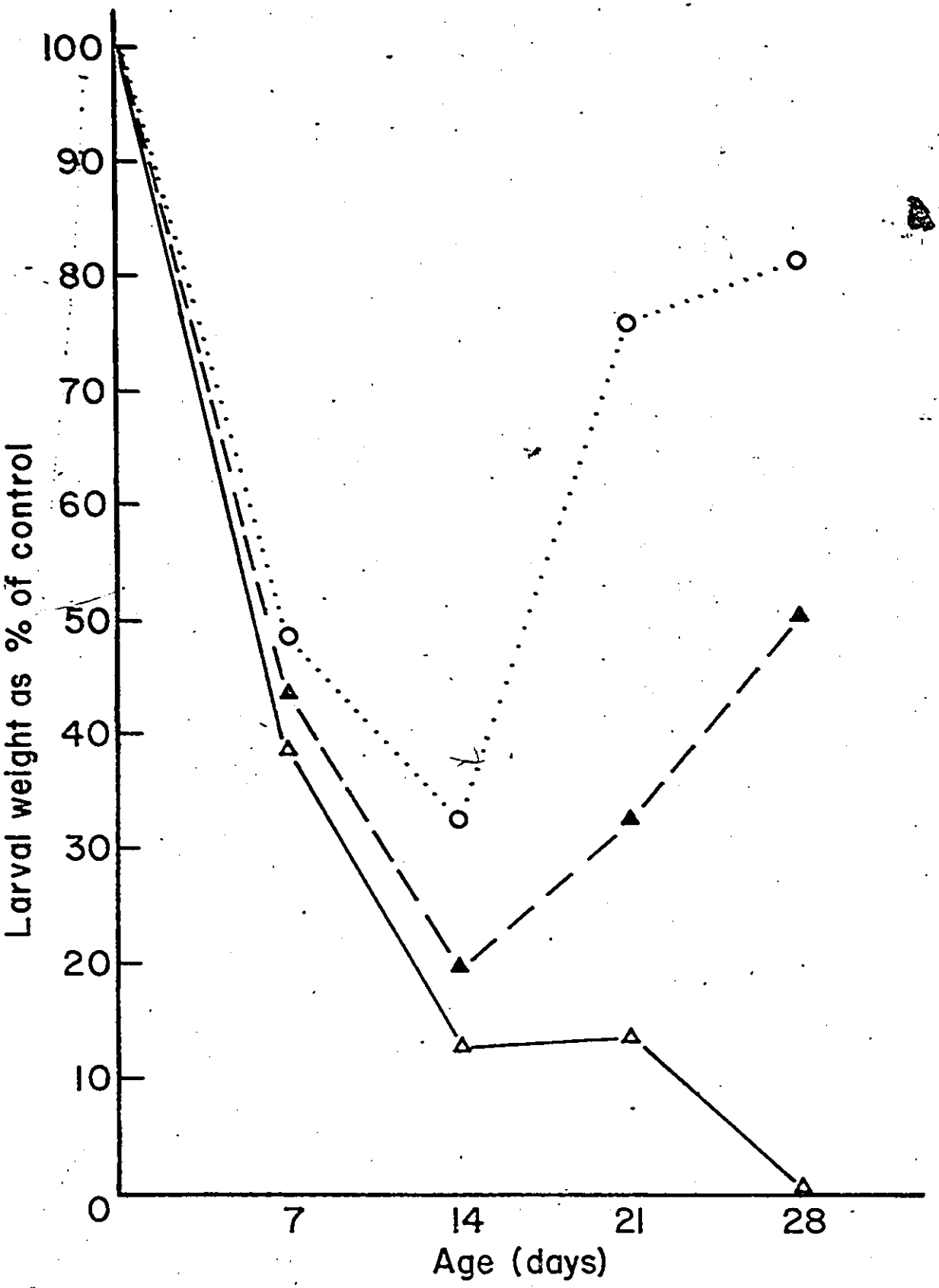
Larvae fed 100 ug/g α-T showed significantly depressed growth relative to the light and dark controls (Fig. 6) and development time to the prepupal stage (indicated by the leveling off of growth curves in Figures 6 and 7) was increased from 34 to 50 days (one-tailed t-test, t=3.96, d.f.=42, p <.01).

Figure 5. Euxoa messoria growth as percent of control with exposure to combinations of 100 ug/g α-T and 4 w/m near-UV.

O 4 w/m near-UV only.

▲ 100 ug/g α-T only.

△ 100 ug/g α-T plus 4 w/m near-UV.


100
90
80
70
60
50
40
30
20
10
0
Larval weight as % of control
7
14
21
28
Age (days)

Cutworm larvae exposed to both α-T and near-UV (4 w/m²) did not differ significantly from larvae treated with α-T alone at days 7 and 14, although the mean weight of the irradiated larvae was consistantly lower. By day 21, however, larval weights were significantly depressed. (Duncan's Multiple Range Test, =.05, d.f.=45). Although the larvae were observed to be feeding, the growth rate was lower than in the other treatment groups. Mortality was higher in this group as well: on day 21 only 4 of the original 15 larvae were alive, compared to 11 to 14 survivors in the other treatment groups. Mortality reached 100% by day 28.

3.1.2 α-T Toxicity to Euxoa messoria: "Low" UV Tests

The sublethal effects of α-T on Euxoa messoria were further investigated when larvae were reared on diet containing 100 and 10 µg/g α-T and exposed to a low intensity (0.5 w/m²) of near-UV.

Larvae fed 100 ug/g α-T showed significantly depressed growth relative to the light and dark controls (Fig. 6) and development time to the prepupal stage (indicated by the leveling off of growth curves in Figures 6 and 7) was increased from 34 to 50 days (one-tailed t-test, t=3.96, d.f.=42, p <.01).

Figure 6. Euxoa messoria growth with exposure to combinations of 100 ug/g α-T and 0.5 w/m² near-UV.

Error bars indicate one standard deviation.

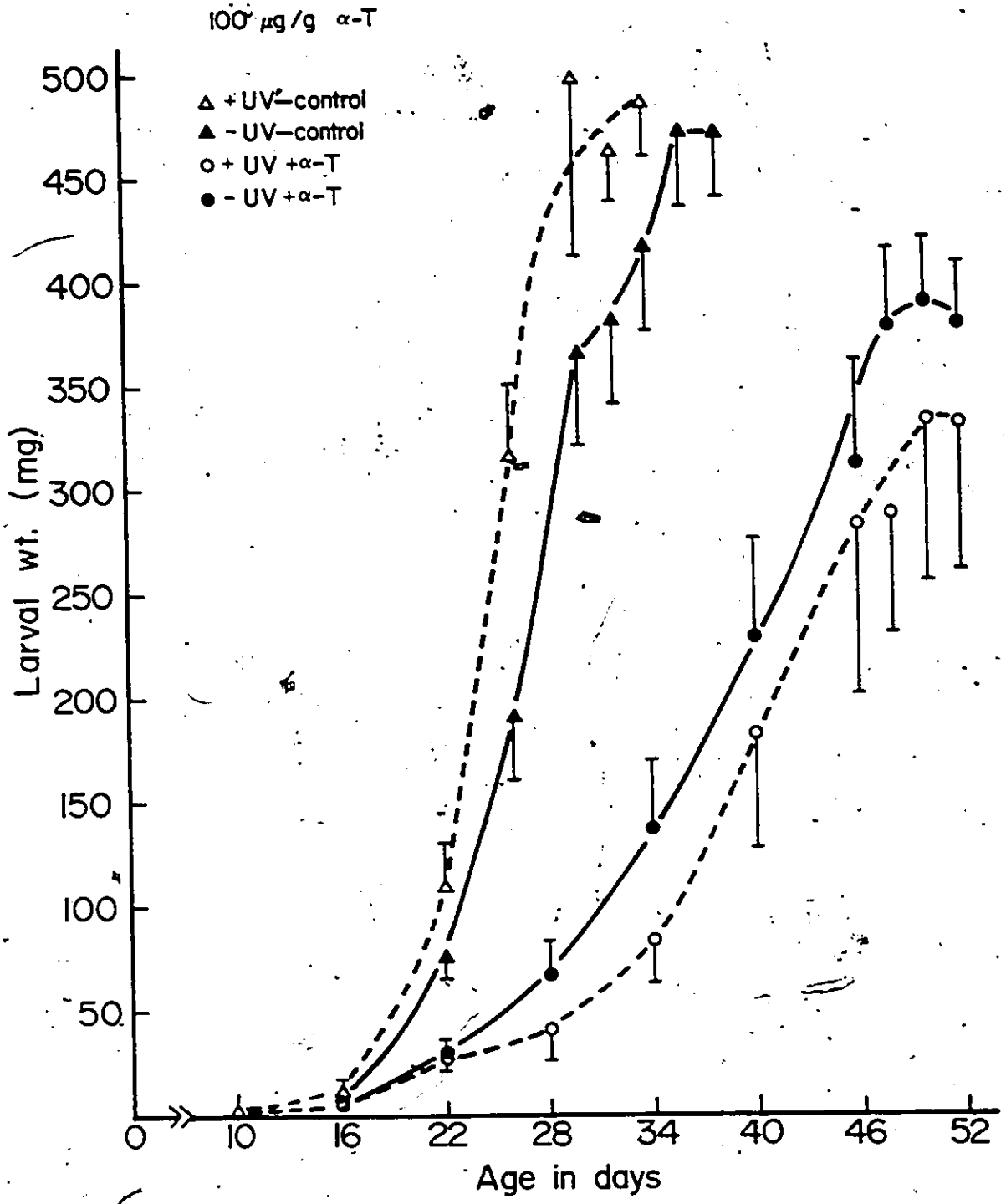
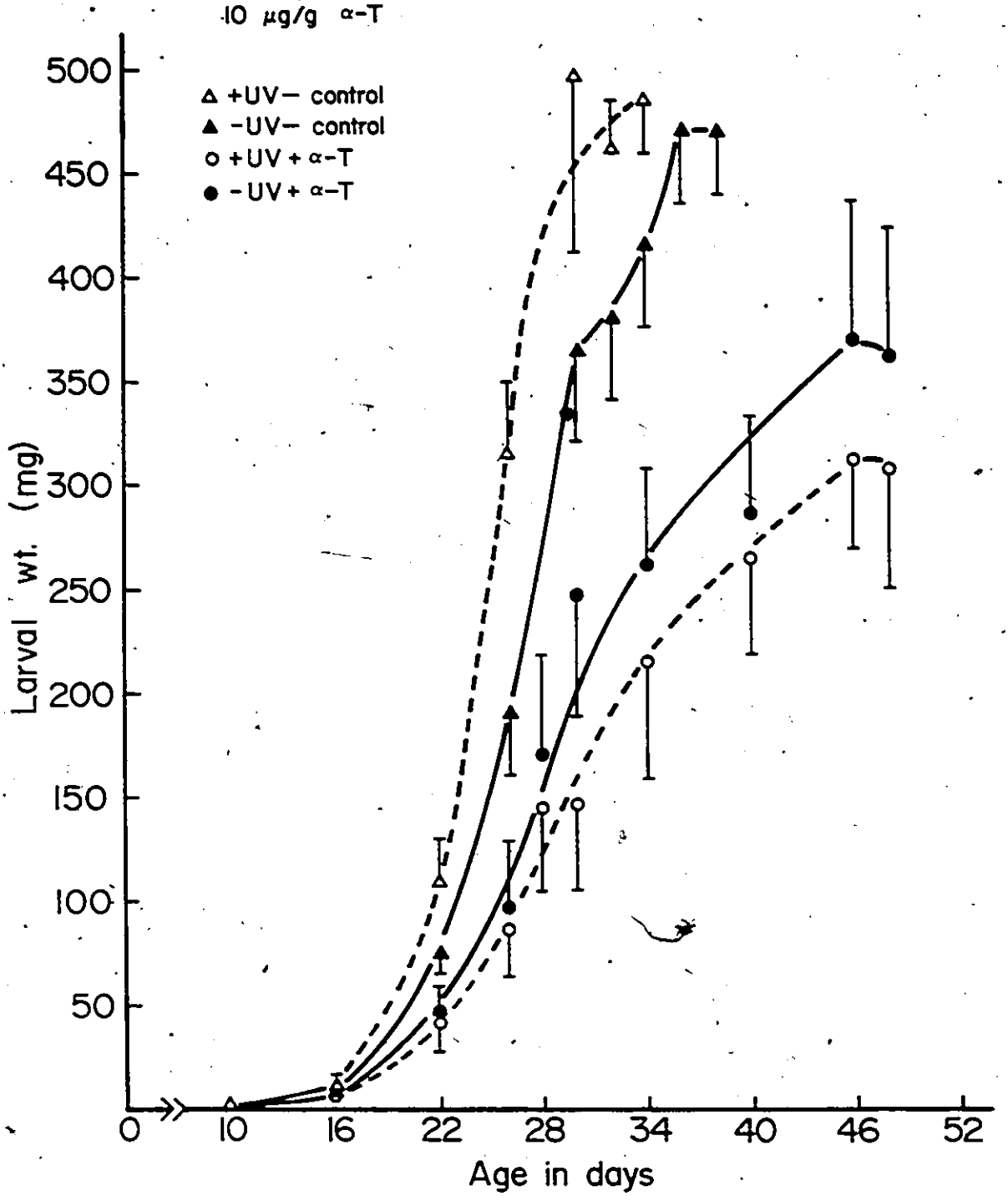

100 µg/g α-T
△ + UV–control
▲ - UV–control
○ + UV +α-T
● - UV +α-T
Larval wt. (mg)
500
450
400
350
300
250
200
150
100
50
0
10
16
22
28
34
40
46
52
Age in days

Figure 7. Euxoa messoria growth with exposure to combinations of 10 ug/g α-T and 0.5 w/m² near-UV.

Error bars indicate one standard deviation.


10 µg/g α-T
△ +UV− control
▲ −UV− control
○ +UV + α-T
● −UV + α-T
Larval wt. (mg)
500
450
400
350
300
250
200
150
100
50
0
10
16
22
28
34
40
46
52
Age in days

Maximum larval weight decreased from 478 mg to 385 mg (one-tailed t-test, t=3.02, d.f.=42, p<.05) (without irradiation) or 338 mg (irradiated) (one-tailed t-test, t=3.91, d.f.=40, p <.01). No increase in the number of individuals developing supernumary instars was noted. Pupal weights were similarly reduced. The mean weight of larvae treated with α-T and near-UV was consistently lower than that of larvae exposed to α-T alone, and a X^2 test indicated that the observed weights were lower than would be expected if α-T and near-UV light did not interact, from day 28 on.

Similar results were found when larvae were fed diet containing 10 μg/g α-T (Fig. 7). The growth of these larvae was depressed to a rate intermediate between the control groups and the larvae fed 100 μg/g α-T. These larvae reached the prepupal stage 4 days earlier than larvae treated with α-T at 100 μg/g but significantly later than control larvae (one-tailed t-test, +U.V. t=3.67, d.f.=45, p <.01; -U.V.: t=3.58, d.f.=44, p <.05). Maximum larval weights were similar to those observed for larvae treated at the higher α-T concentration. Pupal weights were significantly lower than the controls (one-tailed t-test, =U.V.: t=3.92, d.f.=45, p<.01; -U.V.:t=2.97, d.f.=44, p <.05). There were no significant differences in mortality rates between any of the treatment groups.

3.2 Toxicity of Thiophenes and Polyacetylenes to Euxoa messoria

Three thiophenes (compounds I-III) and four polyacetylenes (compounds IV-VII) were screened for allelochemical effects against Euxoa messoria (Fig. 8). Of the seven compounds tested, five (II, III, IV, V, VI,) caused long-term depression of growth; compounds III (-T), IV (PHD), and VI (PHT) were clearly phototoxic.

Compound I, a monothiophene, initially decreased growth in the absence of near-UV. Relative weights recovered to control levels by day 12 but subsequently declined as control larvae entered the most rapid growth phase. Growth of irradiated larvae closely matched the controls until day 18, and was essentially identical to non-irradiated larvae from day 12 until the experiment was terminated.

The weight of larvae fed compound II, bithiophene, declined to 55% of the controls over the course of the experiment. Photosensitization was not evident.

Compound III, α-T, rapidly reduced the growth of treated larvae. By day 9 irradiated larvae weighed 50%, and non-irradiated larvae 62%, of the controls. Photosensitization was evident as non-irradiated larvae gradually gained weight relative to the controls, whereas irradiated larvae consistently weighed about 20% less and did not recover.

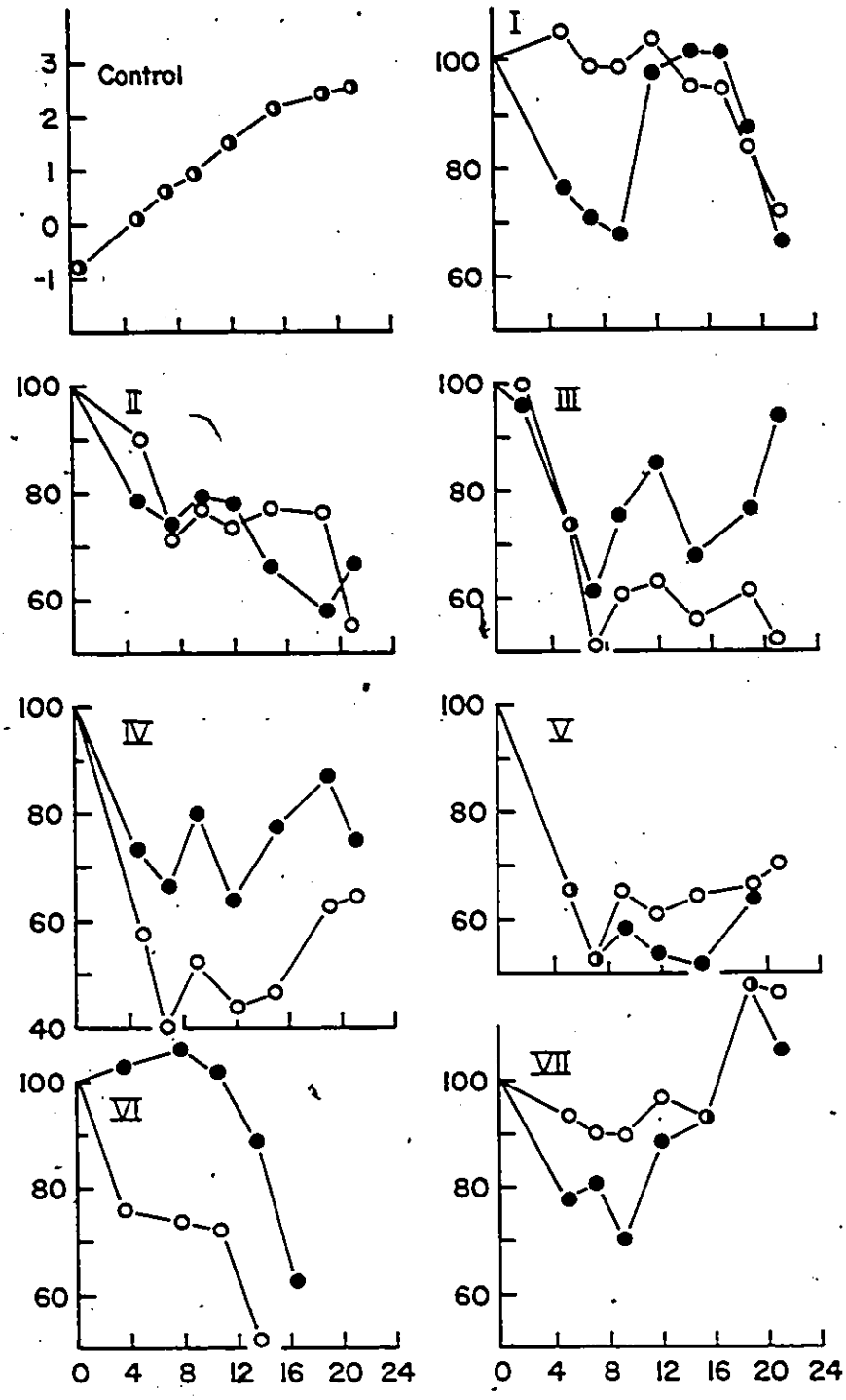
Compound IV, PHD, was also markedly phototoxic. PHD alone reduced larval weights by 25%, but PHD plus near-UV reduced weights to 45% of the controls.

Compound V, PHD-acetate, depressed growth rates as treated larvae weighed only 55% of the controls. However, irradiated larvae generally weighed about 20% more than larvae treated with compound V alone.

Figure 8. Euxoa messoria growth as percent of control with exposure to 2 w/m near-UV and diet containing 100 ug/g compounds I-VII. The growth of control larvae, as log wt (mg), is given in the top left figure.

O irradiated.

● unirradiated.


Control
I
II
III
IV
V
VI
VII
Days

Compound VI, phenylheptatriyne (PHT), was another phototoxic polyacetylene. Non-irradiated larvae showed significant relative weight decline only at day 17 of the test, compared to irradiated larvae which weighed 25% less than controls after four days. The relative weight of irradiated larvae declined to 50% of the controls by day 14 of the test.

Non-irradiated larvae fed compound VII, matricaria lactone, initially showed a small growth depression, but their mean weight exceeded the control on days 19 and 21 of the test. Irradiated larvae did not differ from the controls until days 19 and 21, when their weight exceeded the controls and was similar to the non-irradiated larvae.

All the compounds produced, in the absence of near-UV, a rapid initial growth inhibition which suggested feeding deterrence to neonate larvae (Section 3.6).

3.3 Toxicity of Thiophenes and Polyacetylenes to *Ostrinia nubilalis*.

The toxicity of three polycetylenes (IV, V, and VI) and three thiophenes (I, II, and III) to *Ostrinia nubilalis* at 100 µg/g was examined (Fig. 9).

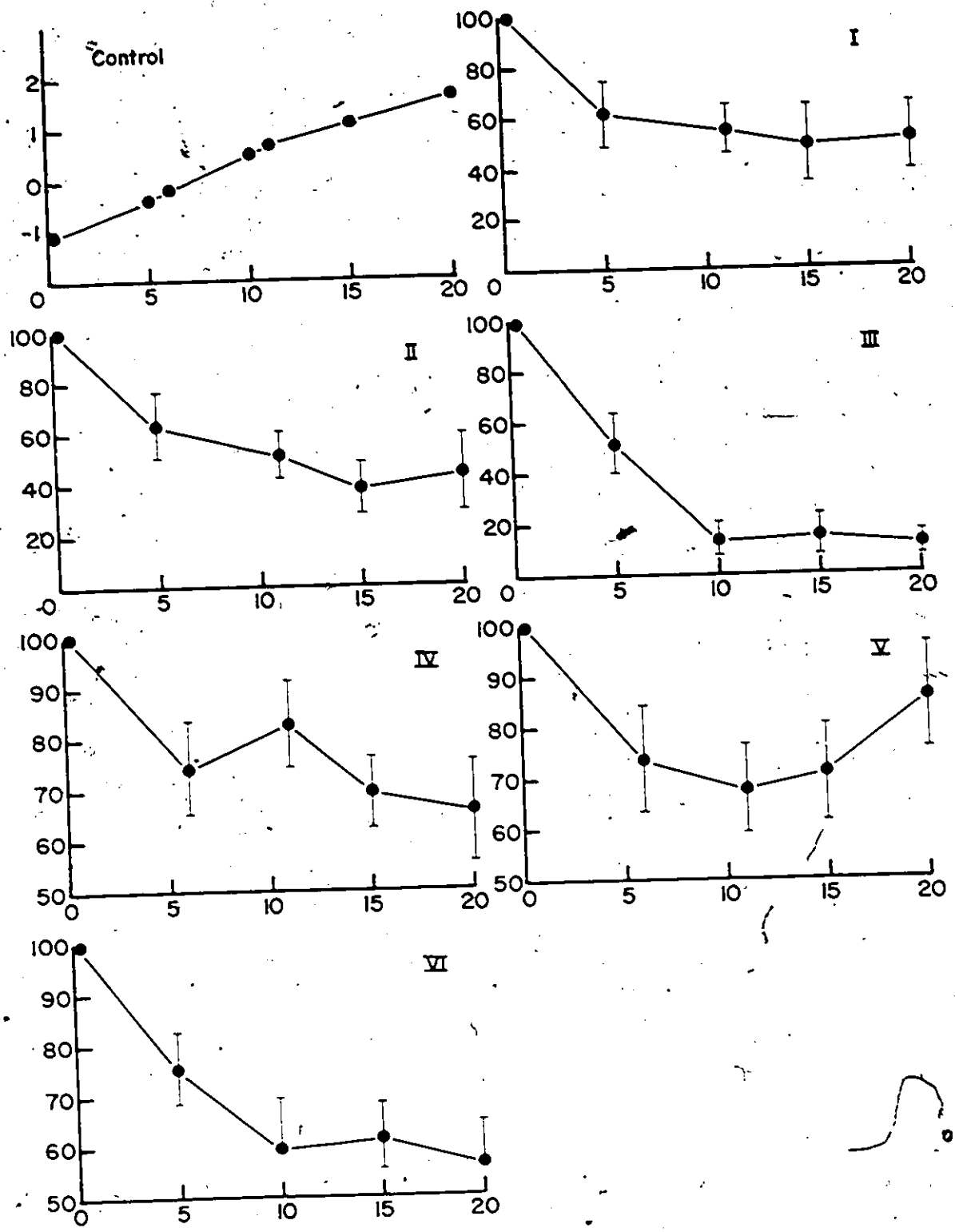
Ostrinia nubilalis control larvae grew more slowly than did *Euxoa messoria*. Growth was exponential during the 20 day study period. All larvae lined the rearing vials with

silk which collected frass and formed an opaque barrier, and many larvae also bored into the diet cubes. Consequently larvae could not be exposed effectively to near-UV irradiation. Weights determined for irradiated and non-irradiated treatments were always similar and were combined in Figure 9. This data represents only the "dark" toxicity of these compounds.

All of the compounds inhibited larval development to some extent. The thiophenes as a group were more toxic than the polyacetylenes: toxicity increased with increasing number of thiophene rings. At day 15 compound I had inhibited growth by 52%, compound II by 61%, and compound III (α-T) by 86%. Increased mortality was seen with compounds II (67%) and III (90%) (compared to 20% for the control) but not with compound I or any of the polyacetylenes.

Polyacetylene toxicity was less marked; over 15 days compound IV inhibited growth by 30%, compound V by 28%, and compound VI (PHT) by 40%. Larvae fed compound V showed improved growth by day 20; in all other treatment groups including the thiophenes relative weight declined througout the experiment.

Figure 9. Ostrinia nubilalis growth as percent of control with exposure to diet containing 100 ug/g compounds I-VI. The growth of control larvae, as log wt (mg), is given in the top left figure.


Control
I
II
III
IV
V
VI
Age (days)

3.4 Toxicity of α-T and PHT to Manduca sexta

α-T and PHT showed both phototoxicity and light-independent toxicity to Manuca sexta larvae.

Neonate larvae fed 100 μg/g α-T exhibited high mortality rates even without photosensitizing irradiation; after five days of exposure to α-T diet only 15% of the larvae survived (Fig. 10) and their weight was only 11% of the controls (Fig. 11). Exposure to near-UV began on day 5; all irradiated larvae died within two days, and non-irradiated larvae did not survive to day 10. Larvae in these treatment groups produced few fecal pellets, suggesting that death was due to starvation and not direct toxicity. A similar mortality rate was observed when Manduca sexta neonates were maintained without access to diet. Differences in survivorship between irradiated and non-irradiated treatments were not significant.

Mortality was slightly increased in the case of larvae fed 10 μg/g α-T (Fig. 10) but was not influenced by exposure to near-UV; note in Figure 10 that a difference in the "irradiated" and "non-irradiated" treatment groups existed by day 5, before the onset of irradiation. Rather, the major symptom was a markedly reduced growth rate (Fig. 11). By day 5 treated larvae weighed only 46% of the controls (one-tailed t-test, $t=2.85$, $d.f.=91$, $p<.05$) ; this declined to 30% in the case of non-irradiated larvae and 20% with UV-treated larvae over the next 10 days (one-tailed t-test,

+U.V.: t=4.09, d.f.=38, $p<.01$; -U.V.: t=3.11, d.f.=41, $p<.05$). Differences due to near-UV were small but significant ($p<.05$). About 20% of the larvae fed 10 µg/g α-T, and irradiated with near-UV, developed black lesions on their dorsal surface, concentrated in the cuticle and dermis along the mid-dorsal vein. These lesions were more evident in larvae fed 100 µg/g PHT.

α-T was less toxic when larvae were exposed in the fourth and fifth instar. Weight gain was depressed to 49% (one-tailed t-test, t=2.93, d.f.=28, $p<.05$) of control in the first 24 hrs by 100 µg/g α-T, but mortality was not increased. At 10 µg/g treated larvae did not differ from the controls. At the higher concentration some 5th instar larvae ceased feeding and entered the prepupal stage 1-3 days earlier than control larvae.

Manduca sexta neonate larvae fed 10 µg/g PHT initially grew more rapidly than control larvae (Fig. 11). By day 15, however, treated and control larvae were of similar weight. In contrast, PHT at 100 µg/g was toxic. Larval weights were reduced to 48% of controls before the initiation of irradiation on day 5 (one-tailed t-test, t=3.41, d.f.=41, $p<.05$) (Fig. 11). Non-irradiated larvae remained at about 50% of control larvae weight for the subsequent 10 days; the relative weight of irradiated larvae declined to 9.5% of the control over the same period. Irradiated and unirradiated larvae differed significantly from day 10 on. (one-tailed

t-test, t=3.23, d.f.=28, $p < .05$). The majority of larvae in this group developed a number of necrotic lesions on the dorsal surface, particularly along the mid-doral blood vessel. Control larvae, and larvae fed PHT but not exposed to near-UV, never developed these lesions.

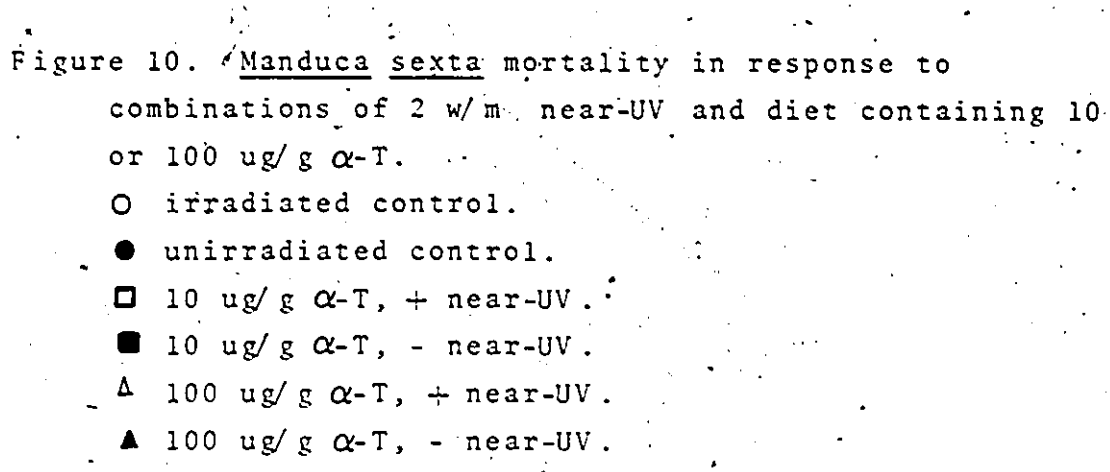


Figure 10. *Manduca sexta* mortality in response to combinations of 2 w/m near-UV and diet containing 10 or 100 ug/g α-T.

- ○ irradiated control.
- ● unirradiated control.
- □ 10 ug/g α-T, + near-UV.
- ■ 10 ug/g α-T, - near-UV.
- △ 100 ug/g α-T, + near-UV.
- ▲ 100 ug/g α-T, - near-UV.

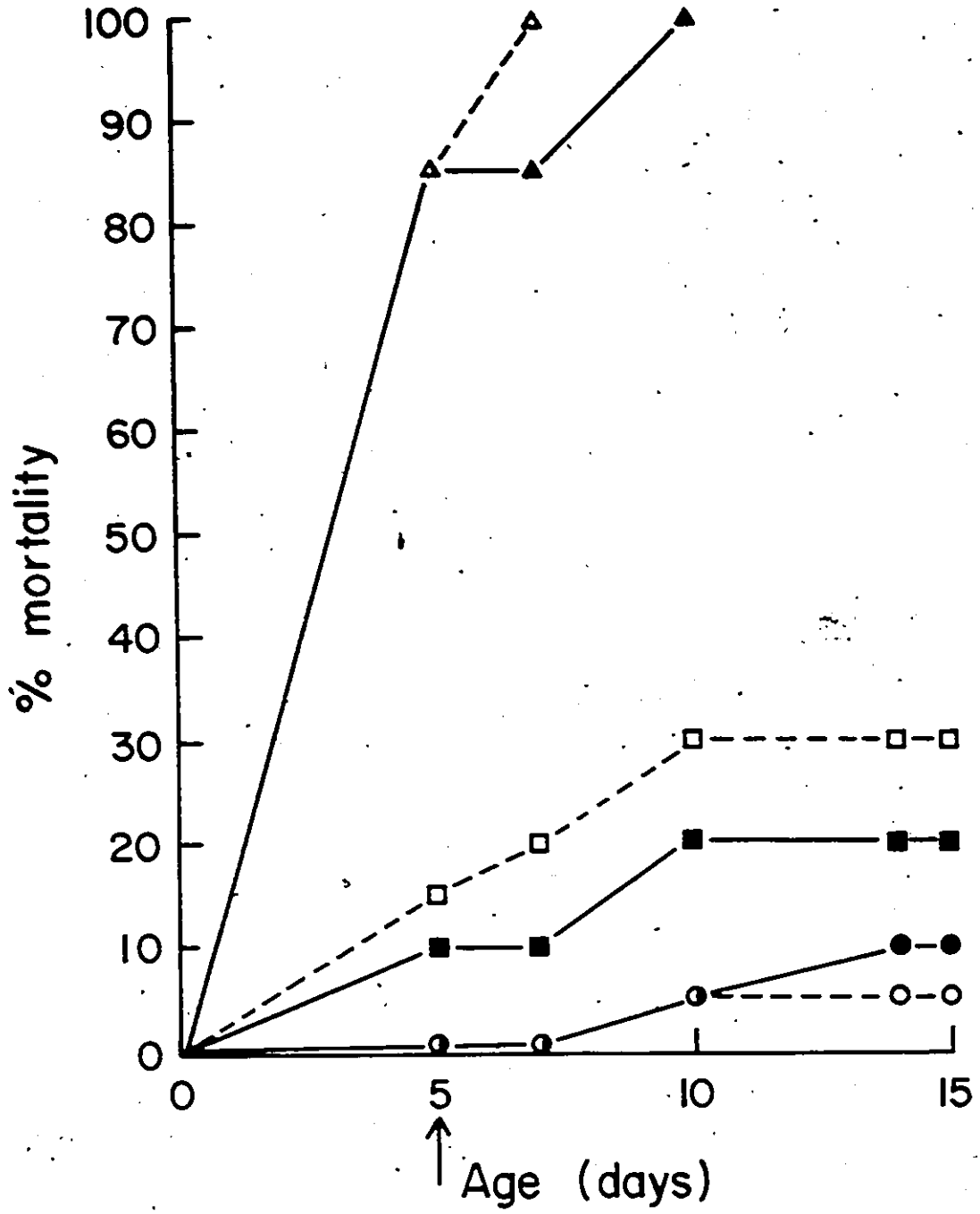
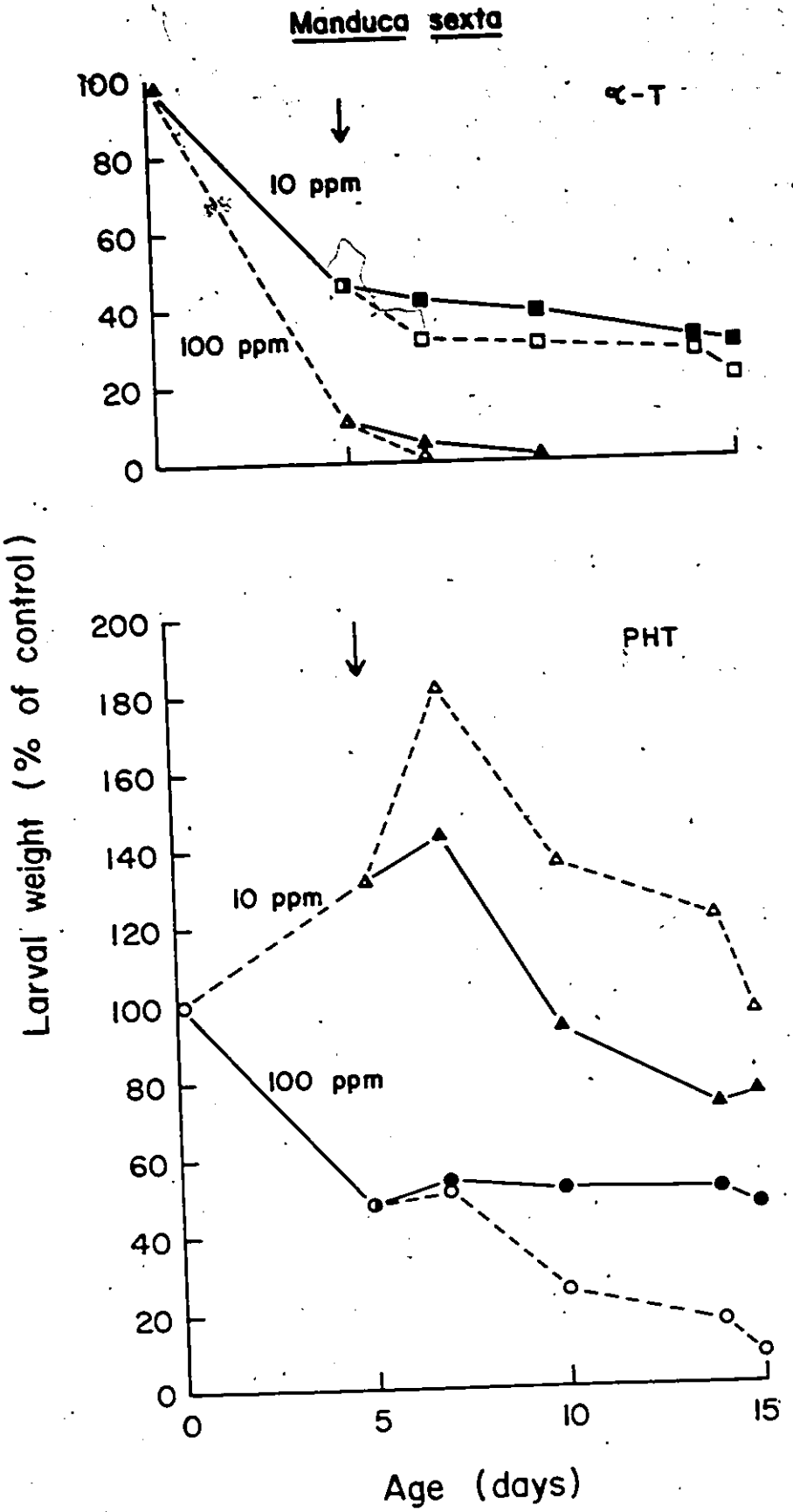

100
90
80
70
60
50
40
30
20
10
0
% mortality
0
5
10
15
Age (days)

Figure 11. Manduca sexta growth as percent of control with exposure to combinations of 2 w/m near-UV and: A) 10 and 100 ug/g α-T, and B) 10 and 100 ug/g PHT. The arrows indicate the initiation of irradiation on day five of the tests.

A) □ 10 ug/g α-T, + near-UV.
■ 10 ug/g α-T, − near-UV.
△ 100 ug/g α-T, + near-UV.
▲ 100 ug/g α-T, − near-UV.

B) △ 10 ug/g PHT, + near-UV.
▲ 10 ug/g PHT, − near-UV.
○ 100 ug/g PHT, + near-UV.
● 100 ug/g PHT, − near-UV.


Manduca sexta
α-T
10 ppm
100 ppm
100
80
60
40
20
0
PHT
10 ppm
100 ppm
200
180
160
140
120
100
80
60
40
20
0
0
5
10
15
Larval weight (% of control)
Age (days)

Larvae from one replicate of the experiment were exposed to direct sunlight for 50 minutes, and the number and size of the lesions were observed to increase substantially during this period. Control larvae exposed to direct sunlight, and PHT treated larvae exposed to sunlight without the near-UV component, failed to develop the lesions. Small lesions caused larvae no obvious distress, as they weighed as much as similarly treated larvae without lesions. However, larvae were unable to shed the cuticle past larger lesions at moulting. The partially moulted cuticle constricted the larvae, preventing the passage of gut contents and restricting the circulation of haemolymph. Such larvae stopped feeding and eventually died. This resulted in a 35% mortality over 15 days, compared to 10% mortality with larvae not exposed to PHT.

The concentration of the lesions along the dorsal blood vessel suggests that PHT is circulated with the haemolymph and binds to appropriate sites adjacent to the haemocoel. The dorsal surface is exposed directly to near-UV and is less pigmented than Euxoa messoria which did not develop lesions when exposed to the same near-UV and PHT treatment.

Manduca sexta larvae fed 10 μg/g α-T and PHT also developed small lesions in a few individuals, but such lesions were not lethal.

3.5 Nutritional Indicies

Both thiophenes and polyacetylenes were found to decrease the efficiency with which Euxoa messoria larvae converted ingested diet into insect biomass.

The simplest measure of the ability of an animal to utilize dietary items is the "Efficiency of Conversion Index" or ECI, which is defined as:

increase in larval biomass / diet consumed

on a dry weight basis (Waldbauer, 1968; Reese, 1979). Compounds II, III, IV, and V all reduced the ECI to 32-50% of the control in "habituated" larvae that had been exposed to the compounds for 15 days (Table 7). Reduction in the ECI was generally proportional to the decrease in growth rate. Thiophenes (II and III) and polyacetylenes were about equally efficient at reducing the ECI. Amongst the phototoxic compounds (III and IV) the irradiated larvae had a lower ECI than non-irradiated larvae. The ECI has two components, the "Approximate Digestibility" (AD), defined as:

(diet consumed - frass) / diet consumed

and the "Efficiency of conversion of digested food" or ECD, defined as:

increase in insect biomass / (diet consumed - frass)

Table 5. Nutritional indices for habituated Euxoa messoria exposed to diet containing 100 ug/g compounds II to V with and without near-UV irradiation. All larvae were exposed to the test compounds for the 18 day period preceeding the initiation of the three day test. Values are given as percent of control; values in a column followed by the same letter are not significantly different (Duncan's multiple range test).

Compound	Near-UV	ECI	AD	ECD	CI
Control	+	100 a	100 a	100 a	100 a
	-	98.2 a	103.3 a	95.8 a	108.4 a
II	+	49.8 b	89.4 a	51.7 b	92.4 a
	-	39.0 b	86.6 a	37.2 b	87.7 a
III	+	33.7 b	84.4 a	37.2 b	88.9 a
	-	40.3 b	93.5 a	39.0 b	91.5 a
IV	+	23.7 b	103.4 a	21.4 b	102.3 a
	-	38.8 b	96.4 a	37.3 b	98.7 a
V	+	38.8 b	96.4 a	29.9 b	110.3 a
	-	31.8 b	95.9 a	29.9 b	94.1 a

(Reese 1979). In all cases the AD was close to control values, demonstrating that the acetylenes and thiophenes did not interfere with the digestibility of the diet. However the ECD was lowered to 20-60% of control.

The values in Table 5 were obtained from habituated larvae exposed to thiophenes and acetylenes throughout their entire growth (15 days). A different pattern was seen when compounds were presented to "naive" 4th instar larvae (Table 6). Within 24 hours α-T (100 µg/g) reduced the ECI to 52% of the control value; this reduction was independent of UV exposure. In contrast, acetylenes IV and V did not affect the ECI during the first 24 hours of exposure. Acetylenes appear to lower the ECI more gradually than do thiophenes.

3.6 Antifeedant activity

The rapid initial decline in relative weight of neonate Euxoa messoria, Ostrinia nubilalis, and Manduca sexta seen with most of the acetylenes and thiophenes suggested that these compounds reduced the feeding activity of these insects.

Experiments in which leaf discs of appropriate plants were treated with 100 µg/g of compounds I to VII confirmed that all except VII deterred feeding by neonate Euxoa messoria (Table 7) in no-choice tests. Neonate Manduca sexta were strongly deterred by 100 µg/g α-T and PHT; 10 µg/g α-T was slightly deterrent whereas 10 µg/g PHT acted as a phagostimulant (Table 8).

Table 6. Mean ECI values for naive and habituated Euxoa messoria larvae after 24 hr feeding on diet containing 100 ug/g compounds III, IV, or V. Naive larvae were not exposed to the test compounds prior to the experiment, whereas habituated larvae had 20 days exposure to treated diets. Values in a column, followed by the same letter, were not significantly different (Duncan's multiple range test).

Compound	Near-UV	Naive larvae	Habituated larvae
Control	+	50.8 a	48.2 a
	-	43.6 a	47.4 a
III	+	23.6 b	16.2 b
	-	23.1 b	19.1 b
IV	+	--	11.4 b
	-	46.6 a	16.6 b
V	+	--	18.7 b
	-	42.2 a	15.1 b

Table 7. Effect of polyacetylenes and thiophenes on feeding behavior of neonate Euxoa messoria and Ostrinia nubilalis. Feeding rate is given as percent of control; all compounds were tested at 100 ug/g and without near-UV irradiation. Values in a column followed by the same letter are not significantly different (Duncan's multiple range test).

Compound	Euxoa messoria	Ostrinia nubilalis
Control	100 a	100 a
I	80.8 a	91.2 a
II	38.0 b	72.8 b
III	38.0 b	64.8 b
IV	32.8 b	103.2 a
V	25.1 b	108.0 a
VI	16.3 b	85.6 b
VII	80.1 a	94.4 a

Table 8. Effect of 10 and 100 ug/g α-T and PHT on feeding by neonate and 4th & 5th instar Manduca sexta. All values are as percent of control; numbers in a column followed by the same letter are not significantly different (Duncan's multiple range test).

Treatment	Neonate larvae	4th & 5th instar larvae
Control	100 a	100 a
10 ug/g -T	84.3 b	97.3 a
100 ug/g -T	0 c	50.0 b
10 ug/g PHT	120.2 d	---
100 ug/g PHT	5.0 c	---

Ostrinia nubilalis neonates were not affected by compounds IV, V, and VII, and were only slightly deterred by compounds I (9% inhibition), II (27%), III (35%) and VI (14%) (Table 7).

Polyacetylenes and thiophenes also reduced feeding by mature larvae, although not to the same extent. In a choice test, α-T reduced feeding by 5th instar Euxoa messoria larvae (Fig. 12). A regression line fitted to this data:

$$y=-17.96(\log \alpha\text{-T})+64.23 \qquad R=.99$$

where y (the consumption rate as % of control) indicates that feeding is reduced by 50% at 6.2 μg/g α-T. PHT is less efficient as an antifeedant: the regression

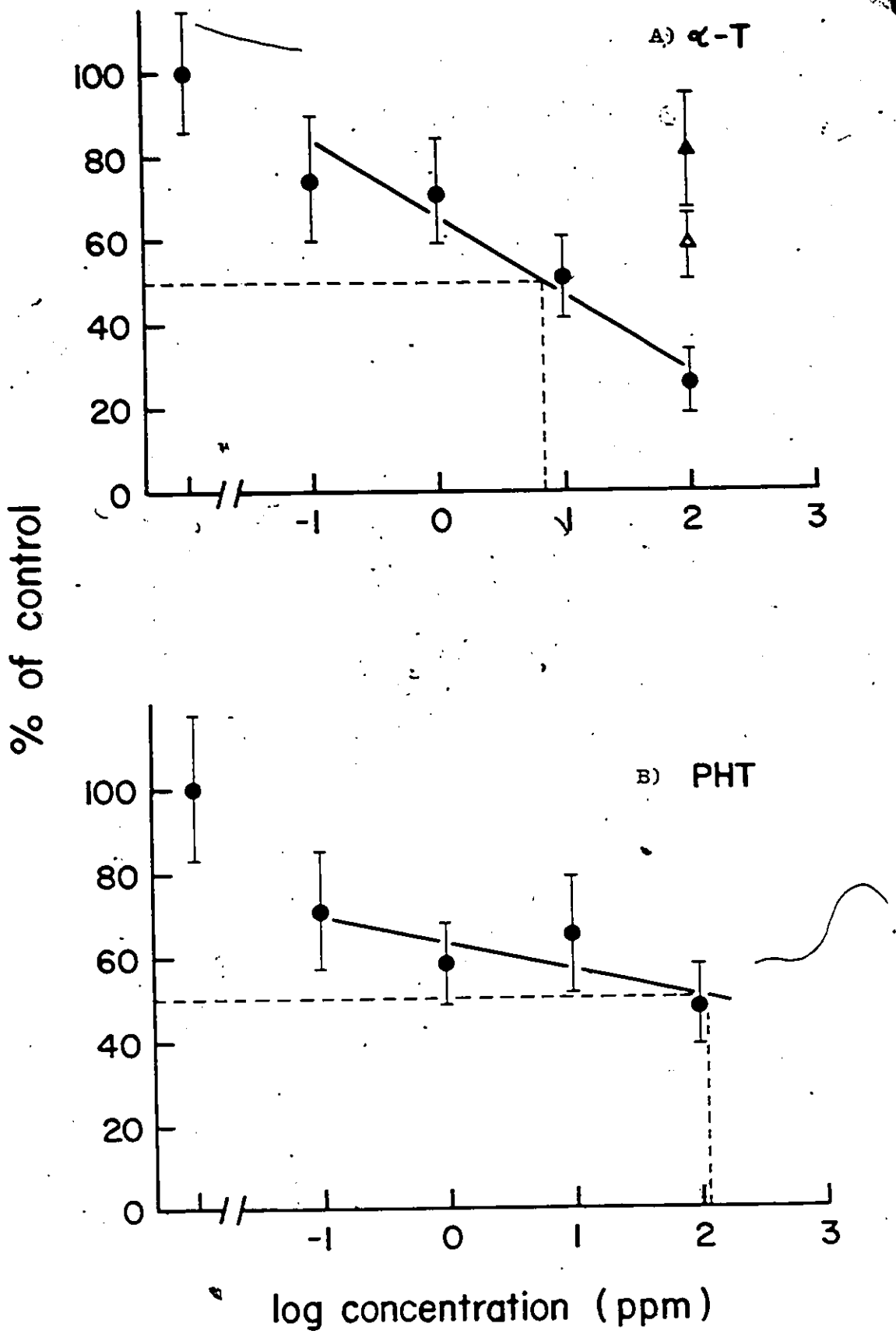
$$y=-6.5(\log \text{PHT})+63.5 \qquad R=.81$$

indicates that 50% feeding reduction occurrs at 119.4 μg/g, still well below levels occurring in plants.

In a no-choice test α-T was less deterrent to Euxoa messoria (Fig. 12): 100 μg/g reduced feeding by 20% over 24 hours when photosensitizing light was absent, but this activity was doubled by the presence of near-UV. That the antifeedant activity of α-T was enhanced by near-UV may indicate that toxic symptoms due to α-T photosensitization occur rapidly enough to affect feeding in a 24 hour trial. The presence of near-UV is a more realistic approximation of conditions likely to occur in the field.

Figure 12. Euxoa messoria feeding rate as percent of control in response to α-T and PHT. A) ● indicates feeding activity with 0-100 ug/g α-T in a choice test. ▲ indicates feeding on diet containing 100 ug/g α-T in a no-choice test without near-UV irradiation, and Δ indicates feeding, in a no-choice test, to a combination of 100 ug/g α-T and 4 w/m near-UV. B) Feeding activity in a choice test in response to diet containing 0-100 ug/g PHT.

In both cases the dashed line indicates the concentration required to produce a 50% decrease in feeding.


A) α-T
B) PHT
% of control
100
80
60
40
20
0
-1
0
1
2
3
log concentration (ppm)

Similarly, 100 µg/g α-T reduced feeding by fifth instar Manduca sexta larvae by 50% ($p<.05$), whereas this concentration abolished feeding by neonates. α-T at 10 µg/g had no effect on the feeding behavior of fifth instar Manduca sexta larvae, compared to a 15% decrease in feeding by neonate larvae.

3.7 Toxicity/Partition Coefficient Relationship

The toxicity of polyacetylenes and thiophenes, measured as the % growth reduction on day 15, was found to be related to their lipophilicity, measured as the log of the water/ octanol partition coefficient (log P_{cal}).

Only light-independent toxicity could be determined for Ostrinia nubilalis. Thiophene toxicity was highly correlated with lipophilicity; the relationship may be described as:

$$t = 56.8(\log P_{cal}) - 230.9 \qquad r = .999$$

where t is the toxicity. Polyacetylene light-independent toxicity was also correlated with lipophilicity, although less strongly so:

$$t = 7.1(\log P_{cal}) - 0.2 \qquad r = .750$$

Thiophene toxicity to Euxoa messoria was related to the water/ octanol partition coefficient in the presence of photosensitizing light:

$$t = 33.5(\log P_{cal}) - 149.2 \qquad r = .860$$

and in the dark:

$$t = 37.8(\log P_{cal}) - 169.9 \qquad r = .996$$

Polyacetylene toxicity was highly correlated to the partition coefficient in the light:

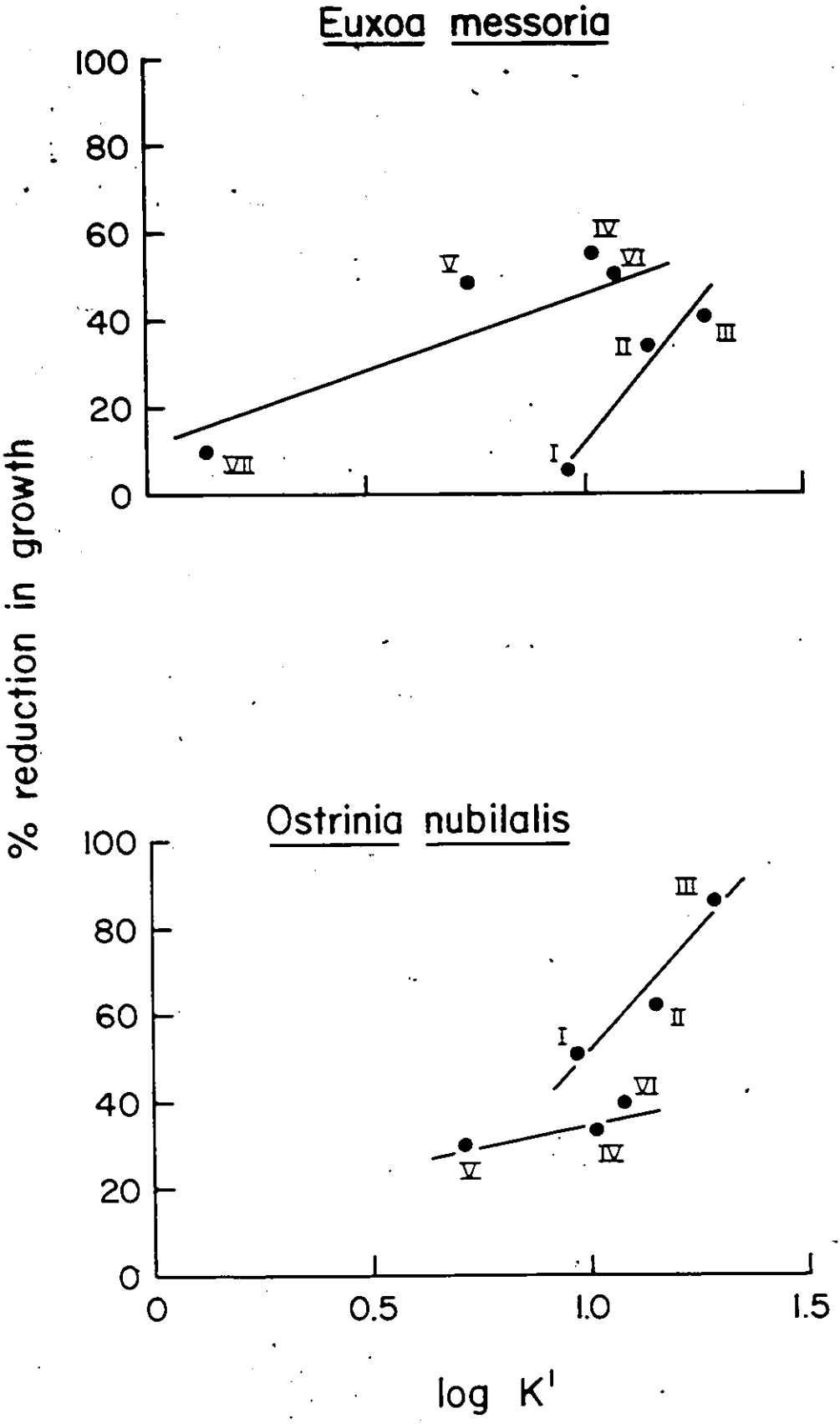
$$t = 16.7(\log P_{cal}) - 29.8 \qquad r = .996$$

but much less so in the dark:

$$t = 6.3(\log P_{cal}) + 1.4 \qquad r = .447$$

In these relationships it is noteworthy that the relationship between lipophilicity and toxicity is different for polyacetylenes and thiophenes, and the relationship appears to be independent of the test insect. This correlation is primarily described by the slopes of the regression equations, which are large for thiophenes (33.5 to 56.8) and small for polyacetylenes (6.3 to 16.7). The weak correlation for polyacetylene toxicity to Euxoa messoria in the dark was due to the toxicity of PHD-acetate (compound V), which was greater than expected on the basis of a regression equation derived from compounds IV, VI, and VII.

Figure 13. Polyacetylene and thiophene toxicity to A) Euxoa messoria, and B) Ostrinia nubilalis, in relation to their water/octanol partition coefficient.


Euxoa messoria
100
80
60
40
20
0
IV
V
VI
II
III
I
VII
Ostrinia nubilalis
III
II
I
VI
IV
V
0
0.5
1.0
1.5
% reduction in growth
log K'

3.8 Accumulated body burden and excretion

The extent to which thiophenes and polyacetylenes accumulate in the insect body was studied in Euxoa messoria (Table 9).

The highest body burdens were observed with α-T: concentrations as high as 660 ug/g insect fresh weight were found with irradiated larvae; the mean for this group was 490 µg/g. Larvae fed α-T and not irradiated accumulated 120 µg/g α-T on average. Considerable variability between individuals was noted, and both groups included individuals in which α-T was not detectable.

Compound IV was also detected, but at lower concentrations. Among larvae exposed to near-UV the mean body burden was 36 ug/g, but one larva had twice this level. Unirradiated larvae contained a mean of 17 ug/g compound IV. No individuals were free of polyacetylene.

Compound V was the only one to give clear evidence of metabolism, as larvae fed diet containing this polyacetylene accumulated large amounts of another polyacetylene, identified by HPLC retention time and UV spectrum as compound IV. Irradiated larvae accumulated 88 ug/g compound IV, and one larva had 140 ug/g. Without near-UV irradiation, an average body burden of 78 ug/g was observed, and one individual had accumulated 143 ug/g. No individuals were found without compound IV. Only one larva contained detectable levels of compound V, but in that case it was present at a high concentration, 93 ug/g.

PHT was found at levels comparable to compound IV. Irradiated larvae contained 28 ug/g PHT, and unirradiated larvae 15 ug/g.

Compounds I, II, and VII were not detected in any insects. There was no correlation between the water/octanol partition coefficients and observed body burdens.

Frass was examined for polyacetylenes and thiophenes to determine if Euxoa messoria larvae were excreting these compounds unmetabolized. Polyacetylenes and thiophenes were not detected in any of the frass samples examined.

Table 9. Polyacetylene and thiophene body burdens in Euxoa messoria after 21 days of exposure to diet containing 100 ug/g compounds I to VII. All values are in ug/g insect fresh weight; the numbers in brackets are one standard deviation. N.D. signifies "not detected". Only one of the unirradiated larvae yielded detectable amounts of compound III. The log of the water/octanol partition coefficient (from McLachlan, 1984) is indicated for comparison.

Compound	Near-UV	Body Burden	Log P_{cal}
I	+	N.D.	4.60
I	-	N.D.	
II	+	N.D.	5.15
II	-	N.D.	
III	+	490 (120)	5.57
III	-	120 (-)	
IV	+	36 (24)	4.76
IV	-	17 (9)	
V	+	88 (74)	3.88
V	-	78 (51)	
VI	+	28 (19)	4.91
VI	-	15 (12)	
VII	+	N.D.	2.09
VII	-	N.D.	

Chapter IV

DISCUSSION

This study has demonstrated that polyacetylenes and thiophenes are toxic to phytophagous insects in the absence of near-UV, and this toxicity is enhanced, in some cases, by the presence of near-UV. Toxicity is expressed as mortality or as a decreased growth rate, resulting in an extended development period and decreased pupal weight. Three components of this toxicity were elucidated: light-independent growth reduction involving both adverse effects on nutrient utilization and feeding deterrence, and photosensitization. Thiophenes and polyacetylenes differed in their toxicity, effect on nutritional indices, and relationship between toxicity and lipophilicity.

4.1 Light-Independent Mortality and Growth Reduction

At 100 µg/g, all of the thiophenes and polyacetylenes examined except compound VII reduced the growth of Euxoa messoria and Ostrinia nubilalis larvae in the absence of photosensitizing light. Manduca sexta growth was similarly inhibited by 10 µg/g α-T and 100 µg/g PHT; α-T at 100 µg/g

was lethal and growth was stimulated by 10 µg/g PHT. In the absence of photosensitization thiophenes were about ten times as toxic (in terms of both mortality and growth reduction) as polyacetylenes to Manduca sexta, about twice as effective at reducing the growth of Ostrinia nubilalis, and the two reduced the growth of Euxoa messoria to a similar extent. Thiophene toxicity increased with increasing number of thiophene rings, a relationship which was also found by McLachlan (1984).

Allelochemicals may interfere with the growth and survivorship of an insect by disrupting metabolic processes, by interferring with nutrient assimilation, or by deterring feeding. Two components of the light-independant activity of polyacetylenes and thiophenes were discerned in the present study: feeding deterrence and inhibition of nutrient utilization.

4.1.1 Feeding Deterrence

Feeding deterrence is an important component of the toxicity of many allelochemicals and may, in some cases, largely account for their activity (Isman and Rodrigues, 1983). This activity may explain much of the initial growth response of the three test insects to polyacetylenes and thiophenes.

Neonate Manduca sexta exhibited 100% mortality when provided with diet containing 100 µg/g α-T (Fig. 10). This may be accounted for by the antifeedant activity of this thiophene, as α-T at this concentration abolished feeding by Manduca sexta neonates (Table 11) and neonates given no diet at all had a similar rate of mortality. However, the deterrent activity of 10 µg/g α-T was insufficient to account for the inhibition of growth observed at that concentration, implying another component to the light-independent toxicity of this compound. PHT at 10 µg/g stimulated feeding, a phenomenon which is occasionally seen with very low doses of insecticides (Moriarty, 1975). The increase in feeding resulted in an accelerated initial growth rate (Fig. 11). Conversely, 100 µg/g PHT produced a marked initial retardation of growth, due initially to a 95% reduction in feeding. Normal feeding rates were gradually assumed after the initial period of starvation, but growth rates remained below controls, again suggesting another toxic mechanism.

Euxoa messoria neonate larvae were strongly deterred from feeding on diet containing 100 µg/g of compounds II to VI, (Table 7), and these compounds all produced an initial decrease in relative weight gain (Fig. 8). The two least deterrent compounds, I and VII, produced only a small and transient decline in relative weight, and that only in the absence of near-UV light. In these cases, and possibly with II and V as well, photodegradation of these light-unstable

compounds (McLachlan, 1984) may have led to a small decrease in the effective concentration of the allelochemical at the surface of the diet cube. This would primarily affect neonate larvae feeding on the surface of the diet, as more mature larvae typically ate into the cube to a greater depth than light could be expected to penetrate. In all cases feeding inhibition was abolished by 24 to 72 hours of starvation of neonates. Growth inhibition continued, however, and subsequently became evident in larvae fed compound I as well. This was again consistent with another light-independent toxic activity for polyacetylenes and thiophenes.

The growth of Ostrinia nubilalis was inhibited by 100 µg/g of compounds I to VI. However, only II, III, and VI inhibited feeding to any extent (Table 7), and this activity was insufficient to account for the observed growth reduction.

Euxoa messoria and Manduca sexta neonates were more strongly deterred than were more mature larvae by a given concentration of PHT or α-T. In particular, Manduca sexta neonates starved on leaf disks containing 100 µg/g α-T, but fifth instar larvae exhibited only a 40% decrease in feeding rate. That the neonate stage is the most sensitive to allelochemicals is well established (Reese, 1983), and many allelochemicals may operate primarily against this life stage (Isman and Rodrigues, 1983; Isman and Duffey, 1982).

α-T was also more deterrent if insects were given a choice between treated and undosed diet (choice test). Choice tests more accurately reflect the situation facing a mobile, polyphagous insect, such as Euxoa messoria. If an insect is immobile, monophagous, or occurs in an agricultural monocrop situation, no-choice tests are more likely to indicate it's behavior in the field (Schoonhoven, 1982).

Near-UV enhanced the feeding deterrent effect of α-T in a no-choice test with sixth and seventh instar Euxoa messoria. This may indicate photosensitization of insect chemoreceptors or other sensillae, or alternatively (or perhaps in addition) light-dependent toxicosis may be occurring quickly enough to affect feeding in a 24 hr trial. As an insect feeding in daylight will certainly be exposed to some near-UV, this result suggests that α-T (and perhaps other phototoxins) will be more effective as an antifeedant against diurnally active insect herbivores.

The feeding deterrent activity of polyacetylenes and thiophenes occurrs at concentrations well below those found in many Asteraceae. Arnason et al. (1983a) found 60 µg/g α-T in the leaves of Flaveria trinervis, and Downum and Towers (1983) reported that Tagetes erecta roots, flowers, and leaves contained over 1200 µg/g fresh weight (5 mM/g) α-T and very high levels of other thiophenes. Bidens pilosa leaves may have up to 700 µg/g PHT (Towers and Wat, 1978; Wat et al., 1979). These concentrations are likely to deter feeding by all but the highly adapted insects.

A correlation can be made between the response of the neonate larvae to polyacetylenes and thiophenes, and their host plant range. Manduca sexta larvae were the most strongly deterred, as the feeding inhibition due to α-T at 100 µg/g could not be overcome by starvation. As solanaceous host plants of this insect do not usually contain these compounds, the ability to detect and avoid them is probably an adaptation to allow Manduca sexta larvae to avoid ingesting potentially toxic non-host plants. At low concentrations, (10 µg/g), α-T and PHT had little deterrent effect or were phagostimulatory, even though α-T reduces growth at that level. Euxoa messoria larvae were also deterred by all of the test compounds except VII, the only one that did not reduce growth. The host plant range of this polyphagous insect is extensive but apparently includes only those Asteraceae that lack polyacetylenes and thiophenes. Again, avoidance of these compounds may lead the insect to avoid toxic non-host plants. Ostrinia nubilalis larvae were only slightly deterred by the thiophenes and PHT, and did not respond to the other acetylenes. This insect utilizes a number of phototoxic Asteraceae (Caffrey and Worthley, 1927) (Appendix 1B). First instar larvae feed on leaves but may be able to avoid resin canals, glands, and other sites with high levels of phototoxins. Such a behavior allows neonate Heliothis zea to avoid ingesting gossypol from cotton leaves (Reese et al., 1981). Later instar Ostrinia avoid photosen-

sitization by spinning silk and by boring into stems or, less frequently, fruit. As well, the concentration of polyacetylenes and thiophenes is frequently lower in the stems than in other plant tissues (Bohlmann et al., 1973 Towers and Wat, 1978). This may be one reason why Ostrinia nubilalis is able to exploit plants not accessible to Manduca sexta or Euxoa messoria or, presumably, many other insects.

The activity of polyacetylenes and thiophenes may be compared to other known feeding deterrents (Table 2). These compounds are not as active as azadirachtin and warburganal, which are antifeedants of potential commercial significance (Jermy, 1983; Schoonhoven, 1982). However, polyacetylenes and thiophenes are as efficient as several other limonoid and isoflavonoid antifeedants, and are considerably more effective than tannins, gossypol, or tomatine. In particular, several of the compounds tested in this study are effective antifeedants at much lower concentrations than are the sesquiterpene lactones, widely considered to constitute a major part of the biochemical defenses of many plants, especially in the Asteraceae (Burnett et al., 1974 Mabry and Gill, 1979).

4.1.2 Nutrient Utilization

Decreased growth persisted even after larvae assumed normal feeding rates. This led to an examination of the effect of thiophenes and polyacetylenes on some standard measures of the efficiency with which insects (Euxoa messoria in this case) assimilate and utilize their diet.

All of the compounds tested reduced the gross efficiency with which diet was converted to insect biomass (ECI) (Table 5). This effect did not involve changes in the assimilation (AD) of diet, indicating that the availability of diet components was not affected by the test compounds. These compounds do not, then, behave as digestibility reducers. Rather, the conversion of assimilated diet to biomass was strongly reduced, over 15 days, by all of the compounds tested. This is evidence of damage to metabolic processes: nutrients are diverted to the repair of such damage and do not contribute to new growth (Waldbauer, 1968).

Differences in the rate at which thiophenes and polyacetylenes affect the ECD were noted. α-T reduced this index by 48% within 24 hours whereas polyacetylenes required days to accomplish the same effect (Table 6). One possible explanation is that thiophenes may attack sites in the midgut and/or hindgut leading to rapid interference in nutrient utilization, and acetylenes may lower the ECI as a response to progressive damage during long-term exposure at the same

or another site. An observation consistent with this hypothesis is that fourth and fifth instar Manduca sexta fed diet containing α-T frequently produced liquid frass, an indication that the hindgut is failing to readsorb water. This effect was not noted when larvae were fed diet containing PHT. Sublethal doses of some synthetic insecticides (ex. fenitrothion and aminocarb) increase liquid loss from the digestive tract of insects by disrupting the epithelial membrane of the midgut and by interfering in the function of the rectal glands (Tauton and Kahn, 1978).

The effect of thiophenes and polyacetylenes (over the longer term) on the ECI and ECD was similar to that found by Beck and Reese (1976) for a variety of different allelochemicals. For example, thiophene effects were similar to those of highly toxic alkaloids such as L-DOPA. The gradual reduction of nutrient ulilization by polyacetylenes is similar to the effect of the non-protein amino acid canavanine on Manduca sexta (Dahlman, 1977). The toxicity of non-protein amino acids is expressed gradually, as insects accumulate defective proteins.

At concentrations higher than necessary for photosensitization, polyacetylenes and other photosensitizers, including xanthotoxin (Berenbaum, 1978), also display light-independent toxicity. Of 23 polyacetylenes and thiophenes tested for mosquito larvicide activity, three, including α-T, were active without near-UV (Arnason et al., 1981c Wat

et al., 1981). Both α-T and PHT have dark activity against trematodes (Graham et al., 1980) and vascular plants (Campbell et al., 1982). Alpha-T is antibiotic towards nematodes (Gommers, 1972) and PHT inhibits the growth of Fusarium mycelia in the absence of light (Bourque, 1984). A number of polyacetylenes are antibiotic against the yeast Candida albicans (Towers et al., 1979), and several species of algae (Arnason et al., 1981b). The neurotoxic acetylenes cicutotoxin, falcarinol, and ichthyothereal and its derivatives, are all active in the absence of light. Light-independent toxicity of rose bengal to the fire ant Solenopsis richteri, the boll weevil Anthonomus grandis, and the house fly Musca domestica has been reported (Broome et al, 1975 Callahan et al, 1977 Sakurai and Heitz, 1982). Methylene blue is toxic to Tenebrio molitor in the absence of light (Graham et al., 1972).

The physiological basis of the light-independent toxicity of photosensitizers has been studied only with reference to the dyes. Boll weevils, Anthonomus grandis, fed rose bengal showed growth inhibition, and had markedly depressed lipid and total protein levels (Broome et al., 1976 Callaham et al., 1976,1977a). Changes in the amino acid pool composition occurred, but essential amino acids were not perturbed more than others, indicating that assimilation of nutrients across the gut was not affected. Levels of the two enzymes monitered, lactate dehydrogenase and acetylcho-

linesterase, declined over time but not to a greater extent than the rest of the protein content (Callaham et al., 1977a,b). These results were interpreted as suggesting an "energy stress" on the insect. Many of the symptoms noted are also produced by exposure to sublethal doses of insecticides, including the botanical pesticide pyrethrin (Brown, 1963; Saxena and Sirvastava, 1968,1969). In particular, damage to the fat body reduces the lipid content of the insect. As the fat body is a primary site of metabolism of allelochemicals (Brattsten, 1979) damage at this site is likely to be reflected in increased susceptibility should subsequent exposure to allelochemicals occur.

Along with direct toxic effects on an insect's physiology, polyacetylenes and thiophenes may be capable of another mechanism of action in the field. Many insects depend on an extensive microflora of endosymbiotic bacteria to metabolize or digest allelochemicals and nutrients (Jones et al., 1981). In view of the pronounced antibacterial activity of many polyacetylenes and thiophenes, even in the dark, (Towers, 1980), an adverse effect on gut microflora and consequently on the insects nutritional ecology may be expected. This activity has been described in connection with other secondary metabolites. Jones et al., (1981) found that 2-furaldehyde, released as a volatile from baldcypress (Taxadium distichum), inhibited growth of silkworm (Bombyx mori) larvae. The 2-furaldehyde was toxic to the enteric microor-

ganisms of Bombyx and so reduced the insect's ability to utilize its diet, mulberry (Morus alba) leaves. Gossypol, caryophyllene, gallic acid, and tannic acid from cotton suppressed the growth of gut bacteria in Heliothus zea (Hedin et al., 1978). Isman and Duffey (1982) suggested that phenolic compounds, including rutin and chlorogenic acid, may inhibit the growth of Heliothis zea by restricting the gut microflora. This mechanism of action may be more widespread among plant allelochemicals than is presently realized. If so, plant-insect coevolution may, in part, involve selection for resistant genotypes among endosymbionts. The deterrent properties of non-host plants may be linked to the avoidance of "antiendosymbiont" allelochemicals as well as "anti-insect" compounds (Jones et al., 1981).

The ability of thiophenes and polyacetylenes to reduce the growth of phytophagous insect larvae, even in the absence of photosensitizing light, is comparable to well-established allelochemicals (Table 3). Thiophenes and polyacetylenes effectively impair growth at concentrations below the levels required to reduce growth by 50% with sesquiterpene lactones, methylated flavonoids, some triterpenoids, and various phenolic compounds. Effects produced by exposure to polyacetylenes and thiophenes resemble those produced by some alkaloids and nonprotein amino acids at concentrations an order of magnitude greater. These compounds would seem to constitute a potentially powerful com-

ponent of the plant's defense even without access to photochemical reactions.

4.2 Photosensitization of Insect Herbivores

Light-dependent increases in mortality were clearly observed in two instances in this study. PHT at 100 µg/g (0.3 µm/g) was markedly phototoxic to Manduca sexta larvae, and Euxoa messoria larvae fed 100 µg/g α-T (0.4 µm/g) did not survive past the fourth instar when exposed to 4 w/m² near-UV. Growth reduction resulted when Euxoa were fed 100 µg/g α-T and was enhanced by irradiation with 2 and 0.5 w/m² near-UV. Near-UV also increased the reduction in growth of this insect due to PHT and IV. As the near-UV component of sunlight at Ottawa may exceed 50 w/m² on a clear day, unadapted insects ingesting plant tissues containing α-T at 100 µg/g or more are likely to be lethaly photosensitized. The low levels of UV used in some tests are more representative of the light levels occurring under a leaf canopy, as leaves effectively filter UV light (Berenbaum, 1978). This indicates that photosensitized growth reduction may occur even in shaded microhabitats.

Behavioral adaptations allowed Ostrinia nubilalis to avoid photosensitization. Larvae spun silk around the diet cube and vial; the silk is fairly opaque and also accumulated frass to form a barrier to light. In many cases larvae

also bored into the diet and again escaped exposure to light. The possible significance of this strategy will be discussed in section 4.4.

The phototoxicity of α-T to Manduca sexta has also been reported by Downum et al. (1984) who examined the effect of α-T on the survivorship and growth of fifth instar Manduca sexta. At 25 and 50 μg/g α-T, larvae took longer to reach the prepupal stage, and maximum larval weights were 85 and 75 % of the control. Mortality occurred when hornworms were unable to develop a completely sclerotized cuticle at the pupal moult.

In some cases the basis of photosensitized mortality was evident. Manduca sexta larvae fed 100 μg/g PHT, and to a lesser extent 10 μg/g PHT and α-T, and exposed to near-UV (black lights or sunlight), developed necrotic lesions in the dorsal cuticle, particularly along the dorsal blood vessel. Large lesions prevented shedding of the cuticle after ecdysis; the old cuticle constricted the larvae anterior to the lesion, preventing the passage of gut contents and restricting circulation of the haemolymph. Eventually the anterior part of the larva became turgid, feeding ceased, and the larva died. Similar effects were noted by Downum et al (1984) when α-T was fed (25 and 50 μg/g) or topically applied to fifth instar Manduca sexta larvae. The rapid development of lesions on exposure to UV light, and the distribution of the injury along the dorsal blood vessel, indi-

cate that α-T and PHT may be transported through the haemolymph, bind to tissues adjacent to the haemocoel, and photosensitize those tissues if sufficient UV light is available.

Increased mortality and photosensitized growth reduction in Euxoa was not accompanied by exterior evidence of tissue damage. This was also the case with xanthotoxin toxicity to Spodoptera eriandia (Berenbaum, 1978) and with various photosensitizing dyes (Lavielle, 1983; Clement et al., 1980). Dye-sensitized photooxidation reactions have been demonstrated in the boll weevil, Anthonomous grandis, the imported fire ant, Solenopsis richteri (Broome, et al., 1975), and the house fly, Musca domestica (Yoho et al., 1971,1973), but the target tissues involved are not well defined. Photosensitization may be associated with the inhibition of enzymatic processes, as Callaham et al. (1975, 1977) found that dye sensitizers inactivated acetylcholinesterase and lactic dehydrogenase in vitro. However, In vivo effects were more ambiguous, as light did not appear to have an effect on this activity. The phototoxic effect of compounds in insects may be modified by several factors, including the opacity of the cuticle and the amount of phototoxin which can reach susceptible target sites. For example, the cuticle of Euxoa messoria is more heavily pigmented than that of Manduca sexta, and only transmits about 2% (A=1.8) of the incident light at the max of α-T, 350 nm. The body burden of α-T in Euxoa messoria was 160 to 420 μg/g indicating that this com-

pound is rapidly metabolised and excreted. Tissue concentrations of α-T in Manduca were not quantified but α-T is detectable, by HPLC, in the haemolymph. Perhaps due to its oligophagous feeding habits this insect lacks the ability to rapidly metabolize unfamiliar allelochemicals. That polyphagous insects are more adept than specialists at responding to unfamiliar secondary compounds is well established (Brattsten, 1979).

The phototoxicity of α-T and PHT is comparable to other naturally occurring photosensitizers. The linear furanocoumarin xanthotoxin was highly phototoxic to Spodoptera eridania larvae at 0.1 and 1.0% concentrations (Berenbaum, 1978). These concentrations are an order of magnitude greater than were necessary for photosensitization with α-T and PHT. Berenbaum did not specify the light intensity to which the Spodoptera eridania were exposed, but her light system (General Electric Cool White bulb) was comparable to the vita-lights used in the present study. Subsequently, Berenbaum and Feeny (1981) demonstrated that the angular furanocoumarin angelicin was more toxic than xanthotoxin to the umbelliferous specialist Papilio polyxenes at a concentration of 0.05 % dry weight. Larvae showed delayed female development, reduced female pupal weight, and reduced fecundity, but mortality was not significantly increased. Although angelicin is phototoxic to microorganisms, this experiment did not include non-irradiated treatments, so the

light and dark components of angelicin toxicity were not resolved.

The phototoxicity of α-T and PHT is comparable, as well, to the photodynamic dyes. The activity of a number of dyes against herbivorous and non-herbivorous insects is given in Table 10. This list includes one registered pesticide: erythrosine B is used for fly control in chicken coops (Pimprikar et al., 1980).

Quantitative comparison of thiophene and polyacetylene phototoxicity to that of synthetic dyes is complicated by differences in experimental designs and test organisms. Except for one study (Lavielle, 1983) dye concentrations exceeded those used in the present study by at least an order of magnitude. In all cases the intensity of irradiation exceeded those of the present study manyfold. Insects were exposed to light briefly (1-12 hours) after ingesting dye, whereas insects in this study were exposed to long periods of low levels of near-UV. Dye-induced mortality occurred rapidly, whereas mortality due to α-T or PHT occurred only after several days or weeks of exposure. In the two studies in which dyes were injected, the concentrations used exceeded the observed body burden of α-T in Euxoa messoria. Despite these experimental differences it seems clear that α-T and PHT, at least, are comparable to the photodynamic dyes, including one registered pesticide, in their ability to photosensitize insects.

Table 10. Insecticidal activity of photodynamic dyes.
All dyes were administered to insects in diet except the last two listed, which were injected at the concentrations given.

Dye	Test Insect	Dose	Light	Mortality	Reference
Methylene blue	Pieris brassicae	50 ug/g	9.0 w/m²	66 %	Lavialle, 1983
Methylene blue	Pieris brassicae	150 ug/g	9.0 w/m²	90 %	Lavialle, 1983
Methylene blue	Tenebrio molitor	1000 ug/g	4.2 w/m²	90 %	Graham et al., 1972
Chlorophyllin	Myzus persicae	10 ug/g	9.0 w/m²	16 %	Lavialle, 1983
Chlorophyllin	Myzus persicae	50 ug/g	9.0 w/m²	90 %	Lavialle, 1983
Rose bengal	Agrotis ipsilon	5 mM	9.2 w/m²	95 %	Clement et al., 1980
Rose bengal	Solenopsis richteri	.49-4.9 mM	3.8 w/m²	44-100 %	Broome et al., 1975
Erythrosine B	Agrotis ipsilon	5 mM	9.2 w/m²	15 %	Clement et al., 1980
Phloxin B	Agrotis ipsilon	5 mM	9.2 w/m²	59 %	Clement et al., 1980
Methylene blue	Tenebrio molitor	135 ug/g	4.2 w/m²	100 %	Graham et al., 1972
Rose bengal	Musca domestica	10 nM	3.8 w/m²	100 %	Sakurai and Heitz, 19

As many thiophenes, polyacetylenes, other natural photosensitizers and dyes are lethal photosensitizers of mosquito and blackfly larvae at very low concentrations, these insects provide a convenient bioassay for phototoxic activity. Of fourteen polyacetylenes and thiophenes tested, nine were phototoxic to Aedes aegypti at 0.5 ppm (Wat et al, 1981). In a subsequent screening of twenty-four compounds, Arnason et al (1981c) identified several that were sufficiently toxic to be of interest as potential mosquito larvicides, including α-T and PHT. Alpha-T has been further investigated as a mosquito and blackfly larvicide (Philogene et al., 1984b) and has been patented for this application (Towers et al., 1983). Synthetic 1,3-butadiynes, similar to natural thiophenes, were also phototoxic to Aedes aegypti larvae (Kagan et al., 1983).

Three of the compounds utilized in the present study have been tested for mosquito larvicidal activity (Arnason et al., 1981c Wat et al., 1981). Matricaria lactone, compound VII, was inactive in both studies, whereas α-T and PHT were highly toxic to both mosquito larvae and herbivorous insects. However, the herbivores were able to tolerate phototoxin concentrations far higher than were lethal to mosquito larvae. A similar situation occurs with the phototoxic alkaloid berberine. This compound is toxic to Aedes atropalpus larvae at 8.8 ppm in the light and 250 ppm in the dark (Philogene et al., 1984a) but is tolerated at

dietary concentrations of 0.1% (1000 ppm) by Euxoa messoria larvae (Devitt et al., 1980). As the latter study utilized Cool White flourescent lamps which provide low levels of near-UV and berberine has a minor absorbtion peak in the visible range, photosensitization was possible; however there were no unirradiated controls. The difference in toxicity is likely due to the difference in routes of entry into the insect: in the mosquito tests phototoxins in aqueous solution bind to and pentrate the cuticle to reach target tissues which are accessable to UV light, whereas ingested phototoxins may be excreted or metabolized and must translocate through the insect to reach susceptible sites.

4.3 Evolutionary Considerations

Chemotaxonomic and biosynthetic evidence indicates strongly that the series fatty acid to polyacetylene to thiophene represents an evolutionary elaboration (Bohlmann et al., 1973). The toxicity of the substances appears to be correlated with their position in this evolutionary sequence.

Fatty acids, particularly oleic acid, are ubiquitous among vascular plants (Robinson, 1980). Some of these compounds are toxic to herbivorous and non-herbivorous insects; this led to their early use in insecticidal soaps (Puritch,

1975). The C10-C12 fatty acids and oleic ($C_{18:1}$) acid are the most toxic. Binder et al. (1979) and Binder and Chan (1982) examined the toxicity of a series of C_{10} to C_{12} fatty acids to three phytophagous lepidopterans, Pectinophora gossypiella, Heliothis zea, and Heliothis virescens. Concentrations required to reduce larval growth by 50% (ED50) ranged from 0.1 to 0.6%, with some variation between species. Toxicity increased with increasing unsaturation.

Polyacetylenes are derived from the fatty acids (oleic acid) via β-oxidations, and occur in a more limited range of plant families, particularly the Asteraceae and the Apiaceae (Bohlmann et al, 1973). This study has determined that several of these compounds markedly reduce the growth of lepidopteran herbivores at a concentration of 0.01%. In addition, matricaria ester, a widely distributed polyacetylene in the Asteraceae, was the most toxic of the compounds examined by Binder et al. (1979), with an EC50 of 0.0046 to 0.017%. Polyacetylenes appear to be an order of magnitude more toxic to insects than their fatty acid precursors.

Thiophenes represent a further evolutionary step: they are derived from polyacetylenes by cyclization with sulphur; these compounds are restricted to advanced tribes of the Asteraceae including the Veronieae, Eupatorieae, Inuleae, Heliantheae, Helenieae, Anthemideae, Senecioneae, Cynareae, and particularly the Tageteae (Bohlmann et al., 1973). The more toxic bithiophene and terthiophene compound are

restricted to the last three tribes listed. In this study these compounds were an order of magnitude more toxic than the polyacetylenes to Manduca sexta, about twice as toxic to Ostrinia nubilalis, and equally toxic to Euxoa messoria.

The increasing toxicity of this evolutionary sequence of secondary metabolites indicates that they have been selected for on the basis of their activity against herbivores and other plant enemies. This supports the hypothesis that the function of polyacetylenes and thiophenes is in the defense against herbivores and pathogens.

4.4 Ecological Considerations

Ostrinia nubilalis was able to avoid photosensitization by polyacetylenes and thiophenes toxicity by a series of behaviors including spinning silk and boring into the diet. Berenbaum (1978, 1982) has suggested that silk spinning and leaf rolling by umbelliferous specialists, including Agonopterix spp. and Depressaria spp. is an adaptation to avoid photosensitization by furanocoumarins. To this may be added the stem boring habit of Ostrinia and many other insects. Avoidance of photo sensitization may be of importance in allowing Ostrinia to exploit host plants rich in phototoxins, many of which are included in a list of its host plants (Appendix 1B). The nocturnal habits of some phytophagous

insects, including Euxoa messoria and other Noctuidae, may constitute another adaptation to avoid photosensitization. This behavior has been considered a mechanism for avoiding avian predators, but it may also constitute an adaptation allowing such insects to exploit plants which contain phototoxins.

The toxicity of allelochemicals is often evaluated in terms of induced mortality. However, impairment of growth and a prolonged development period, may have a number of different consequences for insect herbivores. Exposure to abiotic and biotic causes of mortality may be increased. The increased duration of the sensitive larval stages, and prolonged feeding bouts, increase exposure to predators and parasitoids (Feeney, 1975; Rausher and Feeney, 1982; Price et al, 1980). Stress can decrease an insect's resistance to pathogens. For example, Stubblebine and Langenheim (1977) found Spodoptera exempta fed artificial diet containing Hymenaea courbaril leaf resin (1.6%) were more susceptible to viral infection. If development is sufficiently prolonged, insects may fail to reach the appropriate life stage to avoid the consequences of adverse climatic conditions . For example, Euxoa messoria must reproduce before winter as this species has an egg diapause (Hinks and Byers, 1976). Adult moths are normally active from mid-August to mid-October (Cheng, 1973); an increase in development time could result in increased mortality of pupae and adults due to

early frost. This strategy provides a part of the rational behind the development of juvenile hormone analogues for pest control (Bowers, 1983). Growth reduction may be a particularly effective adaptation for plants which appear or reproduce late in the season; this includes many local Asteraceae. Manduca sexta and Ostrinia nubilalis diapause as pupae or late instar larvae and consequently escape this risk.

Growth reducion may also have adverse effects on insect fecundity. Pupal weights are highly correlated with fecundity and fertility (Campbell, 1962). Insect populations may develop asynchronously, reducing the number of adults present in the field at any one time, and therefore the probability of mating. However, Devitt (1978) noted that although Euxoa messoria fed various alkaloids exhibited growth reduction and prolonged larval development, the prepupal period was decreased so that adult emergence was not significantly delayed. A similar phenomena was noted in the case of Manduca sexta fed sublethal doses of the synthetic insecticide fenitrothion (Stewart, 1981). Increased generation time can dramatically reduce a population's intrinsic rate of increase (Cole, 1954); this rate may be represented as:

$$di/dx = (n \times R \times Fe \times Fr)$$

where n is the initial population size, R is the proportion of the population that is female, Fe is the average fecundi-

ty (number of eggs), and Fr is the average fertility (egg viability), and V is the voltinism, the number of generations per year. Any change in generation time primarily affects v, and so may decrease the exponential growth rate to a greater extent than does a change in n , Fe, or Fr (which of course may also be affected). In the field insect populations rarely increase exponentially; rather they stabilize at an equilibrium between the rate of production of new individuals and the mortality rate. As growth reducers decrease the rate of population growth and increase exposure to mortality from biotic and abiotic, they may cause populations of phytophagous insects to stabilize at a lower level. Decreased abundance of insect herbivores would increase the likelyhood of a given plant escaping detection or significant damage.

4.5 Toxicity-Partition Coefficient Relationships

That polyacetylenes and thiophenes differ in their site or mechanism of light-independent toxicity is also indicated by differences in the relationship between their activity as growth inhibitors and their lipophilicity (water/octanol partition coefficient). This relationship is best described by the slopes of regression equations derived by plotting activity against lipophilicity (Section 3.7). The slopes for thiophenes were large (33.5 to 56.8) compared to the

values obtained for polyacetylenes (6.3 to 16.7). That this holds for two different insect species, one of them somewhat adapted to the presence of dietary polyacetylenes, suggests that the difference in slopes may be indicative of a property of the compounds, and is not simply a reflection of the idiosyncracies of one insect's metabolism.

The lipophilicity of a compound is related to the ability of the compound to penetrate and accumulate in membranes and lipid-rich sites, and is often correlated with toxicity (Moriarty, 1975). The relatively low toxicity of compounds I and VII may be due to their low lipophilicity, which would likely lead to reduced penetration of the peritrophic and epithelial membranes of the midgut, and to relatively rapid excretion (Brattsten, 1979). However, these compounds are also unstable (McLachlan, 1984), and may not be persistent enough to accumulate to toxic levels (see also Section 4.6).

4.6 Body Burdens and Metabolism

Euxoa messoria larvae were examined for the presence of polyacetylenes and thiophenes in body tissues, after 21 days of exposure to diet containing 100 μg/g of compounds I to VII. The highest concentrations were found in the case of α-T, which was the only compound to accumulate to greater concentrations than were present in the diet. Polyacetylenes, in contrast, were present at much lower levels. This

may indicate that polyacetylenes, with their accessable unsaturated regions, are more acceptable substrates for detoxification enzymes than the more cyclized compounds. However, the MFO system also oxidizes fully cyclized ring structures (Nakatsugawa and Morelli, 1976) and α-T in susceptable, in synthetic chemistry, to additions at the alpha positions of the outer rings (Morand and Arnason, 1984).

Compounds IV and V, PHT, and α-T were present at higher concentrations in the tissues of larvae exposed to near-UV. One possible explanation is that these compounds may bind to and/or photosensitize the cytochrome P-450 of the MFO enzymes; some monoacetylene compounds are able to block MFO functioning and are used as pesticide synergists (Casida, 1970). However, most of the MFO activity in insects is associated with the midgut and fat body (Brattsten, 1979), and it seems unlikely that significant amounts of near-UV could penetrate to these tissues. Nutrient and energy stresses are also known to affect the MFO titre in insects (Brattsten, 1979). The phototoxic activity of polyacetylenes and thiophenes may, therefore, possibly be able to decrease the MFO titre without directly attacking the MFO enzymes.

Polyacetylenes and thiophenes may also undergo hydroxylations and other reactions without the intervention of enzyme systems at the pH occurring in insect digestive tracts. Lepidopteran midguts are typically alkaline, with a

pH of 8.4 to 9.8 (House, 1964). Addition of hydroxyl radicals to the unsaturated bonds of polyacetylenes, and to a lesser extent to the thiophene rings of α-T, might possibly occur at this pH. At least one bithiophene compound is known to be unstable under acidic and basic conditions (Sutfield and Towers, 1982) whereas α-T is relatively stable under these conditions (Arnason et al., 1983). This may account for the absence of detectable levels of polyacetylenes and thiophenes in Euxoa messoria frass, and for the absence of compounds I, II, and VII, which are relatively unstable (McLachlan, 1984), in body tissues.

Compound V was apparently metabolised to IV, most likely by a hydrolase (Brattsten, 1979), which would cleave the terminal acetate group. Compound IV was more toxic than V to Euxoa messoria occasionally insect detoxification systems will produce metabolites that are more toxic than the parent compound, a phenomenon termed hypertoxification (Brattsten et al., 1977). In this case the increased toxicity may be related to the increased lipophilicity of the metabolite.

The observed body burdens of polyacetylenes and thiophenes may, in part, account for the toxicity/lipophilicity relationships discussed in Section 4.6. As α-T appears to be metabolized more slowly than the polyacetylenes, lipophilicity may be a primary factor governing the degree to which α-T accumulates in insect tissues. The toxicity of

polyacetylenes, on the other hand, may largely be governed by the rate at which these compounds are metabolized and excreted; increased lipophilicity would result in a greater amount of compound being assimilated but detoxification systems may be selectively removing much of the assimilate polyacetylene.

4.7 Future Work

Two major questions need to be addressed with regard to the activity of polyacetylenes and thiophenes against phytophagous insects: what is the physiological basis of both the light-dependent and light- independent activity of these compounds, and how significant are these compounds in plant defense in nature? Any approach to the first question should include fluorescence microscopy and histological studies to determine the tissues and sites most susceptible to damage by these compounds. Analysis of lipid membrane components and the activity of membrane-bound enzyme systems, particularly those involved in the synthesis of cuticle components and the detoxification of allelochemicals, would be of value. The metabolism of these compounds by insects would be an interesting area of research; radiolabelled compounds could be used to identify the metabolic products. It may also be possible to follow the pharmacokinetics of these secondary substances *in vivo* using C_{13} NMR

techniques, should these highly constrained molecules have distinctive C_{13} NMR spectra. The feeding deterrent activity of thiophenes and polyacetylenes should be persued in electrophysiological studies.

The second question is in some ways more difficult to approach. The insecticidal and feeding deterrent activity of polyacetylenes and thiophenes under natural conditions, particularly sunlight, should be examined further. An exhaustive study of the insect fauna associated with a community of endemic Asteraceae may reveal correlations between the fauna and the phototoxic secondary metabolite chemistry of the plants. If so, those insects using phototoxic plants as hosts should be examined for the biochemical or behavioral adaptations which allow them to exploit phototoxic plants.

Chapter V

REFERENCES

Allen, E.H. and C.A. Thomas. 1971. Trans trans-3,11 tridecadiene- 5,7,9-triyne-1,2-diol, an antifungal polyacetylene from diseased safflower (Carthamus tinctorius). Phytochem. 10: 1579-1582.

Applebaum, S.W. and Y. Birk. 1979. Saponins. In "Herbivores: Their Interaction with secondary plant metabolites". pp 539-566. G.A. Rosenthal and D.H. Janzen, Eds. Academic Press, N.Y., N.Y.

Argandona, V.H., L.J. Corcuera, H.M. Neimeyer and B.C. Campbell. 1983. Toxicity and feeding deterrency of hydroxamic acids from Graminae in synthetic diets against the greenbug, Schizaphis graminum. Ent. Exp. Appl. 34: 134-138.

Arnason, T., C.-K. Wat, K. Downum, E. Yamamoto, E. Graham and G.H.N. Towers. 1980. Photosensitization of Escherichia coli and Saccromyces cerevisia by phenylheptatriyne from Bidens pilosa. Can. J. Microbiol. 26: 698-705.

Arnason, T., G.F.Q. Chan, C.-K. Wat, K. Downum and G.H.N. Towers. 1981a. Oxygen requirement for near-UV mediated cytotoxicity of α-terthienyl to Escherichia coli and Saccharomyces cerevisia. Photochem. Photobiol. 33: 821-824.

Arnason, T., J.R. Stein, E. Graham, C.-K. Wat, G.H.N. Towers and J. Lam. 1981b. Phototoxicity to selected marine and fresh-water algae of polyacetylenes from species in the Asteraceae. Can. J. Bot. 59:54-58.

Arnason, T., T. Swain, C.-K. Wat, E. Graham, S. Partington, G.H.N. Towers and J. Lam. 1981c. Mosquito larvicidal activity of polyacetylenes from species in the Asteraceae. Biochem. System. Ecol. 9: 63-68.

Arnason, T., P. Morand, J. Salvador, I. Reyes, J. Lambert and G.H.N. Towers. 1983a. Phototoxic substances from Flaveria trinervis and Simira salvadorensis. Phytochem. 22:594-595.

Arnason, T., G.H.N. Towers, B.J.R. Philogene and J. Lambert. 1983b. The role of natural photosensitizers in plant resistant to insects. In "Plant Resistance to Insects" ACS Symposium series 208. pp 139-151. Am. Chem. Soc., Washington, D.C.

Bakker, J., F.J. Gommers, I. Nieuwenhuis and H. Wynberg. 1979. Photoactivation of the nematocidal compound

α-terthienyl from roots of marigolds (Tagetes species). J. Biol. Chem. 254: 1841- 1844.

Bakker, J., F.J. Gommers, L. Smits, A. Fuchs and F.W. DeVries. 1983. Photoactivation of isoflavonoid phytoalexins: Involvement of free radicals. Photochem. Photobiol. 38: 323-329.

Beck, S.D., and J.C. Reese. 1976. Insect-plant interactions: Nutrition and metabolism. Rec. Adv. Phytochem. 10: 41-92.

Bell, R.A., C.G. Rasul and F.G. Joachim. 1975. Photoperiodic induction of pupal diapause in the tobacco hornworm, Manduca sexta. J. Insect Physiol. 21: 1471-1480.

Bell, R.A. and F.G. Joachim. 1976. Techniques for rearing laboratory populations of tobacco hornworms. Ann. Entomol. Soc. Am. 19: 365- 373.

Berenbaum, M. 1978. Toxicity of a furanocoumarin to armyworms: A case of biosynthetic escape from insect herbivores. Science 201: 532-534.

Berenbaum, M. 1981. Patterns of furanocoumarin distribution and insect herbivory in the Umbelliferae: Plant chemistry and community structure. Ecology 62: 1254-1266.

Berenbaum, M. 1983. Coumarins and caterpillars: A case for coevolution Evolution 37: 163

Berenbaum, M. and P. Feeny. 1981. Toxicity of angular furanocoumarins to swallowtail butterflies: Escalation in an coevolutionary arms race? Science 212: 927-929.

Bernays. E.A. 1978. Tannins: an alternative viewpoint. Ent. Exp. Appl. 24: 44-53.

Bernays, E.A., R.F. Chapman, J. Horsey and E.M. Leather. 1974. The inhibitory effect of seedling grasses on feeding and survival of Acridids (Orthoptera). Bull. Ent. Res. 64: 413-420.

Bierne, B.P. 1971. Pest insects of annual crop plants in Canada: I Lepidotera, II Diptera, III Coleoptera. Mem. Ent Soc. Can. #78 123 pp.

Binder, R.G. and B.G. Chan. 1982. Effects of cyclopropanoid and cyclopropenoid fatty acids on growth of pink bollworm, bollworm, and tobacco budworm. Ent. Exp. Appl. 31: 291-295.

Binder, R.G., B.G. Chan and C.A. Elliger. 1979. Antibiotic effects of C_{10}-C_{12} fatty acid esters on pink bollworm, bollworm and tobacco budworm. Agric. Biol. Chem. 43: 2467-2471.

Blau, P.A., P. Feeny and L. Contardo. 1978. Allylglucosinolate and herbivorous caterpillars: A contrast in toxicity and tolerance. Science 200: 1296-1298.

Bloch, K., R.G. Langdon, A.J. Clark and G. Fraenkel. 1956. Impaired steroid biogenesis in insect larvae. Biochem. Biophys. Acta 21:176

Blom, F. 1978. Sensory activity and food intake: A study of input- output relationships in two phytophagous insects. Neth J. Zool. 28: 277-340.

Bohlmann, F., T. Burkhardt and C. Zdero. 1973. Naturally occurring acetylenes. Academic Press, N.Y., N.Y. 547 pp.

Bourque, G. 1984. Photosensitization of Fusarium culmorum by phenylheptatriyne from the Asteraceae. M.Sc. Thesis, University of Ottawa. 112 pp.

Bowers, W.S. 1983. The search for fourth-generation pesticides. In "Current Themes in Tropical Science Vol. 2: Natural Products for Innovative Pest Management". D.L. Whitehead and W.S. Bowers, Eds. Pergamon Press.

Brattsten, L.B. 1979. Biochemical defense mechanisms in herbivores against plant allelochemicals. In "Herbivores: Their interaction with secondary plant metabolites" G. Rosenthal and D. Janzen, Eds., pp.200-270.

Brattsten, L.B. 1983. Cytochrome P-450 involvement in the interactions between plant terpenes and insect herbivores. In "Plant Resistance to Insects." ACS Symposium Series # 208. Washington, D.C.

Brattsten, L.B., C.F. Wilkinson and T. Eisner. 1977. Herbivore-plant interactions: Mixed-function oxidases and secondary plant substances. Science 196: 1349-1352.

Brinkley, S.W., W.A. Dickerson and W.R. West. 1975. The tobacco hornworm. Carolina Tips 38: 45-48.

Broome, J.R., M.F. Callaham, W.E. Poe and J.R. Heitz. 1976. Biochemical changes in the boll weevil induced by rose bengal in the absence of light. Chem. Biol. Interactions 14: 203-206.

Brown, A.W.A. 1963. Chemical injuries. In "Insect Pathology." E.A. Steinhaus, Ed. pp 65-131. Dekker, London.

Burnett, W.C.Jr., S.B. Jones Jr., T.J. Mabry and W.G. Padolina. 1974. Sesquiterpene lactones: insect feeding deterrents in Veronia. Biochem. System. Ecol. 2: 25-29.

Butterworth, J.H. and E.D. Morgan. 1971. Investigations of the locust feeding inhibition of the seeds of the neem

tree, Azadirachta indica. J. Insect Physiol. 17: 969-977.

Caffrey, D.J. and L.H. Worthley. 1927. A progress report on the investigations of the European corn borer. USDA Dept. Bull. 1476. 155 pp.

Callaham, M.F., L.A. Lewis, M.E. Holloman, J.R. Broome and J.R. Heitz. 1975. Inhibition of the acetylcholinesterase from the imported fire ant, Solenopsis richteri (Forel), by dye-sensitized photo- oxidation. Comp. Biochem. Physiol. 51C: 123-128.

Callaham, M.F., J.R. Broome, W.E. Poe and J.R. Heitz. 1977a. Time dependence of light-independent biochemical changes in the boll weevil, Anthonomus grandis, caused by dietary rose bengal. Environ. Ent. 6: 669-673.

Callaham, M.F., C.O. Palmertree, J.R. Broome and J.R. Heitz. 1977b. Dye-sensitized photoinactivation of the lactic dehydrogenase and acetylcholinesterase from the boll weevil, Anthonomus grandis. Pest. Biochem. Physiol. 7: 21-27.

Campbell, G., J.D.H. Lambert, T. Arnason and G.H.N. Towers. 1982. Allelopathic properties of α-terthienyl and phenylheptatriyne, naturally occurring compounds from the Asteraceae. J. Chem. Ecol. 8:961-972.

Campbell, I.M. 1962. Reproductive capacity in the genus Choristoneura Led. (Lepidoptera:Tortricidae) I. Quantitative inheritance and genes as controllers of rates. Can. J. Genet. Cytol. 4: 272-288.

Casida, J.E. 1970. Mixed-function oxidase involvement in the biochemistry of insecticide synergists. J. Agric. Food Chem. 18: 753-772.

Chan, G.F.Q., G.H.N. Towers and J.C. Mitchell. 1975. Ultraviolet- mediated antibiotic activity of thiophene compounds of Tagetes. Phytochem. 14:2295-2296.

Chapman, R.F. 1974. The chemical inhibition of feeding by phytophagous insects: A review. Bull. Ent. Res. 64:339-363

Chapman, R.F. and W.M. Blaney. 1979. How animals percieve secondary compounds. In "Herbivores: Their interaction with plant secondary metabolites." G. Rosenthal and D. Janzen, Eds. Academic Press, N.Y., N.Y. 718 pp.

Cheng, H.H. 1973. Observations on the bionomics of the darksided cutworm, Euxoa messoria (Lepidoptera: Noctuidae), in Ontario. Can. Ent. 105: 311-322.

Clark, A.J. 1969. Biochem. Pharmacol. 13: 73.

Clark, A.J. and K. Bloch. 1959. The absence of sterol synthesis in insects. J. Biol. Chem. 234: 2578-2582.

Clement, S.L., R.S. Schmidt; G. Szatmari-Goodman and E. Levine. 1980. Activity of xanthene dyes against black cutworm larvae. J. Econom. Entomol. 73: 390-392.

Conn, E.E. 1979. Cyanide and cyanogenic glycosides. In "Herbivores: Their interaction with secondary plant metabolites." pp 387-412. G.A. Rosenthal and D.H. Janzen, Eds. Academic Press, N.Y., N.Y.

Coppey, J., D. Averbeck and G. Moreno. 1979. Herpes virus production in monkey kidney cells and human skin cells treated with angelicin or 8-methoxypsoralen plus 365 nm light. Photochem. Photobiol. 29: 797-801.

Crumb, S.E. 1929. Tobacco cutworms. Tech. Bull. USDA 88.

Dahlman, D.L. 1977. Effect of L-Canavanine on the consumption and utilization of artificial diet by the tobacco hornworm, Manduca sexta. Ent. Exp. Appl. 22: 123-131.

Darwin, C. 1859. The Origin of Species. Harvard Facsimile 1st Edn. 1964

Davidson, R.H. and W.F. Lyon. 1979. Insect pests of farm, garden, and orchard. 7th Edition. John Wiley & Sons, Toronto.

DeBoer, G., V.G. Dethier and L.M. Schoonhoven. 1977. Chemoreceptors in the preoral cavity of the tobacco hornworm, Manduca sexta, and their possible function in feeding behavior. Ent. Exp. Appl. 21: 287-298.

Dethier, V.G. 1953. Host plant perception in phytophagous insects. Trans. 9th Int. Congr. Entomol. 2: 81-88.

Dethier, V.G. 1970. Chemical interactions between plants and insects. In "Chemical Ecology." E. Sondheimer and J.B. Simeone, Eds. pp 83-102. Academic Press, N.Y., N.Y.

Dethier, V.G. 1982. Mechanism of host-plant recognition. Ent. Exp. Appl. 31: 49-56.

Dethier, V.G. 1980. Evolution of receptor sensitivity to secondary plant substances with special referance to deterrents. Am. Nat. 115: 45-66.

Dethier, V.G., L. Barton-Brown and C.N. Smith. 1960. The designation of chemicals in terms of the responses they elicit from insects. J. Econom. Ent. 53: 134-136.

Devitt, B.D. 1978. Effects of some alkaloids on the dark-sided cutworm Euxoa messoria (Lepidoptera: Noctuidae). M.Sc. Thesis, University of Ottawa 155pp.

Devitt, B.D., B.J.R. Philogene and C.F. Hinks. 1980. Effects of veratrine, berberine, nicotine, and atropine on developmental characteristics and survival of the dark-sided cutworm Euxoa messoria (Lepidoptera: Noctuidae). Phytoprotection 61:88-102.

DeWitt, P.J.G.M. and E. Koddle. 1981. Induction of polyacetylenic phtoalexins in Lycopersicon esculentum after inoculation with Cladosporium fulvum (syn. Fulvia fulva). Physiol. Plant Path. 18: 143-148.

Dicosmo, F.,R. Norton and G.H.N. Towers. 1982. Fungal culture filtrate elicits aromatic polyacetylenes in plant tissue culture. Naturwiss. 69: 50-551.

Downum, K.R., R.E.W. Hanncock and G.H.N. Towers. 1982. Mode of action of α-terthienyl on Eschrichia coli: Evidence for a photodynamic effect on membranes. Photochem. Photobiol. 36: 517-532.

Downum, K.R., G.A. Rosenthal and G.H.N. Towers. 1984. Phototoxicity of the plant metabolite, α-terthienyl, to larvae of Manduca sexta (Sphingidae).. Pest. Biochem. Phsiol. 22: 104-109.

Downum, K.R., and G.H.N. Towers. 1983. Analysis of thiophenes in the Tagetae (Asteraceae) by HPLC. J. Nat. Prod. 46: 98-103.

Eisner, T., D. Hill, M. Goetz, S. Jain; D. Alsop, S. Camazine and J. Meinwald. 1981. Antifeedant action of Z-dihydromatricaria acid from soldier beetles (Chauliognathus spp.). J. Chem. Ecol. 7: 1149-1157.

Elliger, C.A., Y. Wong, B.G.Chan and A.C. Waiss, Jr. 1981. Growth inhibitors in tomato (Lycopersicon) to tomato fruitworm (Heliothis zea). J. Chem. Ecol. 7: 753-758.

Ericson, J.M. and P. Feeny. 1974. Sinigrin: A chemical barrier to the black swallowtail butterfly, Papilio polyxenes. Ecology 55: 103-111.

Feeny, P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51: 565-581.

Feeny, P. 1975. Biochemical coevolution between plants and their insect herbivores. In "Coevolution of animals and plants." L.E. Gilbert and P.H. Raven, Eds. University of Austin Press, Austin. 246 pp.

Feeny, P. 1976. Plant apparency and chemical defense. Rec. Adv. Phytochem. 10: 1-40.

Fondren, J.E. and J.R. Heitz. 1978. Xanthene dye induced toxicity in the adult face fly, Musca autumnalis. Environ. Entomol. 7: 843-846.

Fondren, J.E., B.R. Norment and J.R. Heitz. 1978. Dye-sensitized photooxidation in the house fly, Musca domestica. Environ. Entomol. 7: 205-208.

Fox, L.R. 1981. Defense and dynamics in plant-herbivore systems. Am. Zool. 21: 853-864.

Fraenkel, G. 1959. The raison d'etre of secondary plant substances. Science 129: 146-170.

Fraenkel, G. 1969. Evaluation of our thoughts on secondary plant metabolites. Ent. Exp. Appl. 12: 473-486.

Gilbert, L.E. and P.H. Raven. 1975. Coevolution of animals and plants. University of Texas Press, Austin. 246 pp.

Gommers, F.J. 1972. Increase of the nematicidal activity of α-terthienyl and related compounds by light. Nematologica 18: 458-462.

Gommers, F.J., J. Bakker and H. Wynberg. 1982. Dithiophenes as singlet oxygen sensitizers. Photochem. Photobiol. 35: 615-619.

Gommers, F.J. and J.W.G. Geerligs. 1973. Lethal effect of near- ultraviolet light on Pratylenchus penetrans from roots of Tagetes. Nematologica 19: 389-393.

Gorz, H.J., F.A. Haskins and G.R. Manglitz. 1972. Effect of coumarin and related compounds on blister beetle feeding in sweetclover. J. Econ. Ent. 65: 1632-1635.

Graham, K., E. Wrangler and L.H. Aasen. 1972. Susceptability of the mealworm, (Tenebrio molitor (L.)) to photodynamic injury by methylene blue. Can. J. Zool. 50: 1625-1629.

Graham, K., E.A. Graham and G.H.N. Towers. 1980. Cercaricidal activity of phenylheptatriyne and α-terthienyl, naturally occurring compounds in species of the Asteraceae (Compositae). Can. J. Zool. 58: 1955-1958.

Guthrie, W.D. 1971. Resistance of maize to second-brood European corn borers. Rept. 26th Ann. Corn Sorghum Res. Conf., Amer. Seed Assoc. public. 26. pp 165-179.

Hardwick, D.F. 1970. The genus Euxoa (Lepidoptera: Noctuidae) in North America. L. Subgenera Orasagrotis, Longivesica, Choizagrotis, Pleonectopoda and Crassivesica. Mem. Ent. Soc. Can. 67.

Hargraves, J.A., J.W. Mansfield, D.T. Coxon and K.R. Price. 1976. Wyerone epoxide as a phytoalexin in Vicia faba and its metabolism by Botrytis cinerea and B. Fabae in vitro. Phytochem. 15: 1119- 1121.

Harris, G.R. and H.J. Svec. 1968. Toxicological studies on cutworms. 1. Laboratory studies on the toxicology of insecticides to the dark-sided cutworm. J. Econ. Ent. 61: 788-792.

Hedin, P.A., O.H. Lindig, P.P. Sikorowski and M. Wyatt. 1978. Suppressants of gut bacteria in the boll weevil from the cotton plant. J. Econ. Ent. 71: 394-396.

Hinks, C.F. and J.R. Byers. 1976. Biosystematics of the genus Euxoa (Lepidoptera: Noctuidae) V. Rearing procedures, and life cycles of 36 species. Can. Ent. 108: 1345-1370.

Hosozawa, S., N. Kato and K. Munakata. 1974. Antifeeding active substances for insect in Caryopteris divaricata Maxim. Agric. Biol. Chem. 38: 823-826.

House, H.L. 1965. Digestion. In "The Physiology of Insecta." Vol.3 M. Rockstein, Ed. pp 815-862. Academic Press, N.Y., N.Y.

Hsiao, T.H. 1976. Chemical and behavioral factors influencing food selection of Leptinotarsa beetle. Symp. Biol. Hung. 16: 95-99.

Hudson, J.B., E.A. Graham and G.H.N. Towers. 1982. Nature of the interaction between the photoactive compound phenylheptatriyne and animal viruses. Photochem. Photobiol. 36: 181-185.

Ichihara, K.-I., T. Kawai, M. Kaji and M. Noda. 1976. A new polyacetylene from Solidago altissima L. Agric. Biol. Chem. 40: 353-358.

Ingham, J.L. 1973. Disease resistance in higher plants: Concept of preinfectional and postinfectional resistance. Phytopath. Z. 78: 314-335.

Ishikawa, S. 1966. Electrical response and function of a bitter substance receptor associated with the maxillary sensilla of the silkworm, Bombyx mori L. J. Cell Physiol. 67: 1-11.

Isman, M.B. and S.S. Duffey. 1982. Toxicity of tomato phenolic compounds to the fruitworm, Heliothis zea. Ent. Exp. Appl. 31: 370-376.

Isman, M.B. and E. Rodriguez. 1983. Larval growth inhibitors from species of Parthenium (Asteraceae). Phytochem. 22: 2709-2713.

Ito, T. 1983. Photodynamic agents as tools for cell biology. In "Photochemical and photobiological reviews". Vol 7. pp 141-186. K.C. Smith, Ed. Plenum Press, N.Y., N.Y.

Ivie, G.W., D.L. Bull, R.C. Pryor and E.H. Oertii. 1983. Metabolic detoxification: Mechanism of insect resistance to plant psoralens. Science 221: 374-376.

Janzen, D.H., H.B. Juster and E.A. Bell. 1977. Toxicity of secondary compounds to the seed-eating larvae of the bruchid beetle Callosobruchus maculatus. Phytochem. 16: 223-227.

Jermy, T. 1971. Biological background and outlook of the antifeedant approach to insect control. Acta Phytopath. Acad. Sci. Hung. 6: 253-260.

Jermy, T. 1983. Multiplicity of insect antifeedants in plants. In "Current Themes in Tropical Ecology Vol. 2. Natural Products for Innovative Pest Management. D.L. Whitehead and W.S. Bowers, Eds. pp 223-236. Pergamon Press.

Jermy, T., B.A. Butts and L. McDonough. 1981. Antifeedants for the Colorado potato beetle I.: Antifeeding constituents of some plants from the sagebrush community. Insect Sci. Appl. 1: 237-242.

Jones, C.G., J.R. Aldrich and M.S. Blum. 1981. Baldcypress allelochemics and the inhibition of silkworm microorganisms: Some ecological considerations. J. Chem. Ecol. 7: 103-114.

Kagan, J. and G. Chan. 1983. The photoovicidal activity of plant components towards Drosophila melanogaster. Experientia 39: 402-403.

Kagan, J., G. Chan, S.N. Dhawan, S.K. Arora and I. Prakash. 1983. The effect of ultraviolet light on the toxicity of natural products toward the eggs of Drosophila melanogaster. J. Nat. Prod. 46: 646-650.

Kawazu, K., M. Ariwa and Y. Kii. 1977. An ovicidal substance, cis-dehydromatricaria ester from Solidago altissima. Agric. Biol. Chem. 41: 223-224.

Kingsbury, J.M. 1964. Poisonous plants of the United States and Canada. Prentice-Hall, Englewood Cliffs, New Jersey.

Klocke, J.A. and I. Kubo. 1982. An insect growth regulator from Trichilia roka (Meliaceae). Experientia 38: 639-640.

Klun, J.A., C.L. Tipton and T.A. Brindley. 1967. 2,4-Dihydroxy 7-methoxy-1,4-benzoxazin-3-one (DIMBOA), an active agent in the resistance of maize to European corn borers. J. Econom. Ent. 60: 1529-1533.

Kobayashi, A., S. Morimoto, Y. Yamashitka and M. Numata. 1980. C10 polyacetylenes as allelopathic substances. J. Chem. Ecol. 6: 119-136.

Kreiger, R.I., P.P. Feeny and C.F. Wilkinson. 1971. Detoxification enzymes in the guts of caterpillars: An evolutionary answer to plant defenses? Science 172: 579-582.

Kubo, I., Y.W. Lee, M. Petteri, F. Pilkiewicz and K. Nakanishi. 1976. Potent armyworm antifeedants from the east African Warburgia plants. J. Chem. Soc. Chem. Comm. 1013-1014.

Lavialle, M. 1983. Photodynamic action in two insects, the European cabbage butterfly, Pieris brassicae (L.) and the green aphid, Myzus persicae (Sulzer). Sonderdruck aus Bd. 95: 133-140.

Lopez, E., D.F. O'Brian and T.H. Whitesides. 1982. Effects of membrane composition and lipid structure on the photopolymerization of lipid diacetylenes in bilayer membranes. Biochem. Biophys. Acta 693: 327-335

Ma, W.C. 1969. Some properties of gustation in larvae of Pieris brassicae. Ent. Exp. Appl. 12: 584-590.

Ma, W.C. 1972. Dynamics of feeding responses in Pieris brassicae Linn. as a function of chemosensory input: A behavioral, ultrastructural, and electrophysiological study. Meded. Landbouw. Wagen. 72/11 162 pp.

Ma, W.C. 1977. Alterations of chemoreceptor function in armyworm larvae (Spodoptera exempta) by a plant-derived sesquiterpenoid and by sulfhydryl reagents. Physiol. Ent. 2: 199-207.

Ma, W.C. and L.M. Schoonhoven. 1973. Tarsal contact chemosensory hairs of the large white butterfly, Pieris brassicae, and their possible role in oviposition behavior. Ent. Exp. Appl. 16: 343-357.

Mabry, T.J. and J.E. Gill. 1979. Sesquiterpene lactones and other terpenoids. In "Herbivores: Their interaction with secondary plant metabolites". pp. 502-538. G.A. Rosenthal and D.H. Janzen, Eds. Academic Press, N.Y., N.Y.

MacRae, W.D., G.F.Q. Chan, C.-K. Wat, G.H.N. Towers and J. Lam. 1980. Examination of naturally occurring polyacetylenes and α-terthienyl for their ability to induce cytogenetic damage. Experientia 36: 1096-1097.

Madden, A.H. and F.S. Chamberlain. 1945. Biology of the tobacco hornworm in the southern cigar tobacco district. USDA Tech. Bull. 896. 51 pp.

Matsui, K. and K. Munakata. 1976. Insect antifeeding substances in Parabenzoin preacor and Piper futokadzura. Agric. Biol. Chem. 40: 1045-1046.

McKenna, D.J. and G.H.N. Towers. 1981. Ultra-violet mediated cytotoxic activity of β-carboline alkaloids. Phytochem. 20: 1001-1004.

McLachlan, D.G. 1984. Mechanism of action of polyacetylene and thiophen photosensitization. M.Sc. Thesis, University of Ottawa. 144 pg.

McLachlan, D., T. Arnason and J. Lam. 1984. The role of oxygen in photosensitization with polyacetylenes and

thiophene derivitives. Photochem. Photobiol. 39: 177-182.

McLachlan, D., J.T. Arnason, B.J.R. Philogene and D. Champagne. 1982. Anti-feedant activity of the polyacetylene phenylheptatriyne (PHT), from the Asteraceae to Euxoa messoria (Lepidoptera: Noctuidae). Experientia 38: 1061-1062.

Meinwald, J., Y.C. Meinwald, A.M. Chalmers and T. Eisner. 1968. Dihydromatricaria acid: Acetylenic acid secreted by soldier beetles. Science 160: 890-892.

Meisner, J., K.R.S. Ascher and M. Zur. 1977. Phagodeterrency induced by pure gossypol and leaf extracts of a cotton strain with high gossypol content in the larva of Spodoptera littoralis. J. Econ. Ent. 70: 149-150.

Metcalf, R.L. 1979. Plants, chemicals, and insects: Some aspects of coevolution. ESA Bull. 25: 30-34.

Montgomery, M.E. 1984. Is the effect of tannins on gypsy moth caterpillars digestion inhibition? PSNA abstracts, PSNA Newsletter 24(2).

Moss, B.E. and V.G. Dethier. 1982. Unpublished data, cited in Dethier, 1982.

Morand, P. and J.T. Arnason. 1984. Unpublished data.

Moriarty, F. 1975. Exposure and residues. In "Organochlorine Insecticides: Persistant Organic Pollutants." F. Moriarty, Ed. Academic Press, N.Y., N.Y. 302 pp.

Morris, C.E. 1984. Electrophysiological effects of cholinergic agents on the CNS of a nicotine-resistant insect, the tobacco hornworm (Manduca sexta). J. Exp. Zool. 229: 361-374.

Munukata, K. 1977. ACS Symposiun Series 62: 185-196.

Munakata, K. 1983. Nematicidal natural products. In "Current Themes in Tropical Science Vol. 2: Natural Products for Innovative Pest Management. pp 299-310. D.L. Whitehead and W.S. Bowers, Eds. Pergamon Press.

Musajo, L., G. Rodighiero, G. Caporale, F. Dall'Acqua, S. Marciani, F. Bordin, F. Baccichette and R. Bevilacqua. 1974. Photoreactions between skin-photosensitizing furanocoumarins and nucleic acids.- In "Sunlight and Man". pp 389-409. T.B. Fitzpatrick, Ed. University of Tokyo Press, Tokyo.

Nakajima, S. and K. Kawazu. 1977. Tridec-1-ene-3,5,7,9,11 pentyne, an ovicidal substance from Xanthium canadense. Agric. Biol. Chem. 41: 1801

Nakatsugawa, T. and M.A. Morelli. 1976. In "Insecticide Biochemistry and Physiology." C.F. Wilkinson, Ed. Plenum Press, N.Y.

Neilsen, J.K., L. Dalgaard, L.M. Larsen and H. Sorensen. 1979. Host plant selection of horse-radish flea beetle Phyllotetra armora (Coleoptera: Chrysomelidae): Feeding response to glucosinolates from several crucifers. Ent. Exp. Appl. 25: 227-239.

Nijhout, H.R. and C.M. Williams. 1975. Control of moulting and metamorphosis in the tobacco hornworm, Manduca sexta: Growth of the last instar larvae and the decision to pupate. J. Exp. Biol. 61: 481-492.

Parr, J.C. and R. Thurston. 1972. Toxicity of nicotine in synthetic diets to larvae of the tobacco hornworm. Ann. Ent. Soc. Amer. 65: 481-492.

Picman, A.K., R.H. Elliot and G.H.N. Towers. 1978. Insect feeding deterrent activity of alantolactone. Biochem. System. Ecol. 6: 333-335.

Picman, A.K., R.H. Elliot and G.H.N. Towers. 1981. Cardiac-inhibiting properties of the sesquiterpene lactone, parthenin, in the migratory grasshopper, Melanoplus sanguinipes. Can. J. Zool. 59: 285-292.

Picman, J., A.K. Picman and G.H.N. Towers. 1982. Effects of the sesquiterpene lactone, helenin, on feeding rates of the tundra redback vole Clethrionomys rutilus. Biochem. System. Ecol. 10: 269-273.

Pfyffer, G.E., B.U. Pfyffer and G.H.N. Towers. 1982. Monoaddition of dictamnine to synthetic double-stranded polydeoxyribonucleotides in UVA and the effect of photomodified DNA on template activity. Photochem. Photobiol. 35: 793-797.

Pfyffer, G.E. and G.H.N. Towers. 1982. Photochemical interaction of dictamnine, a furoquinoline alkaloid, with fungal DNA in vitro and in vivo. Can. J. Microbiol. 28: 468-473.

Philogene, B.J.R., J.T. Arnason, G.H.N. Towers, Z. Adambrowski, F. Campos, D. Champagne and D. McLachlan. 1984a. Berberine: A naturally occurring phototoxic alkaloid. J. Chem. Ecol. 10: 115-123.

Philogene, B.J.R., J.T. Arnason, C.W. Berg, F. Duval, D. Champagne, R.G. Taylor, L. Leitch and P. Morand. 1984b. Evaluation of the naturally occurring phototoxin, alpha-terthienyl as a control agent for larvae of Aedes intrudens and Aedes atropalpus (Diptera: Culicidae) and of Simulium verecundum (Diptera: Simuliidae). Accepted in J. Econom. Ent.

Pimprikar, G.D., B.R. Norment and J.R. Heitz. 1979. Toxicity of rose bengal to various instars of Culex pipiens quinquefasciatus and Aedes trisreiatus. Environ. Ent. 8: 856-859.

Pimprikar, G.D., J.E. Fondren and J.R. Heitz. 1980. Small- and large-scale field tests of erythrosine B for house fly control in caged layer chicken houses. Environ. Ent. 9: 53-58.

Price, P.W., C.E. Bouton, P. Gross, B.A. McPheron, J.N. Thompson and A.E. Weis. 1980. Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. Ann. Rev. Ecol. Syst. 11: 41-65.

Proksch, P., M. Proksch, G.H.N. Towers and E. Rodriguez. 1983. Phototoxic and insecticidal activities of chromenes and benzofurans. J. Nat. Prod. 46: 331-334.

Puritch, G.S. 1975. The toxic effects of fatty acids and their salts on the balsam wooly aphid, Adelges piceae (Ratz.). Can. J. For. Res. 5: 515-522.

Raab, O. 1900. Ueber die wirkung fluorescirender stoffe auf infusorien. Z. fur Biol. 39: 524

Rausher, M.D. and P. Feeny. 1980 Herbivory, plant chemistry, and plant reproductive success: the effect of Battus philenor on Aristolochina reticulata. Ecology 61: 905-917.

Rees, C.J.C. 1969. Chemoreceptor specificity associated with choice of feeding site by the beetle Chrysoli-

na brunsvicensis on it's foodplant, Hypericum hirsutum. Ent. Exp. Appl. 12: 565-583.

Reese, J.C. 1979. Interactions of allelochemicals with nutrients in herbivore food. In "Herbivores: Their interaction with secondary plant metabolites. pp 309-330. G.A. Rosenthal and D.H. Janzen, Eds. Academic Press, N.Y., N.Y.

Reese, J.C. 1983. Nutrient-allelochemical interactions in host plant resistance. In "Plant Resistance to Insects." P.A. Hedin, Ed. ACS Symposium Vol. # 208. pp 321-244. ACS, Washington.

Reese, J.C., B.G. Chan, N.R. Malm and A. Waiss. 1981. Feeding sites of bollworm larvae on cotton. Environ. Entomol. 10: 81-84.

Rhoades, D.F. 1979. Evolution of plant chemical defense against herbivores. pp. 4-54 in "Herbivores: Their interaction with secondary plant metabolites." G. Rosenthal and D. Janzen, Eds.

Rhoades, D.F. and R.G. Cates. 1976. A general theory of plant anti- herbivore chemistry. Rec. Adv. Phytochem. 10: 168-213.

Rice, E.L. 1974. Allelopathy. Academic Press, N.Y., N.Y.

Rice, E.L. 1982. Allelopathy. Bot. Rev. 48: 690-703.

Ritter, F.J. 1967. Feeding stinulants for the Colorado beetle. Meded. Rijksfac. Landbouwwet. Gent. 32: 291-305.

Robinson, T. 1980. The Organic Constituents of Higher Plants. 4th Edn. Cordus Press, Amherst, Mass.

Robinson, T. 1981. Molecular Biology, Biochemistry, and Biophysics Vol. 3: The Biochemistry of Alkaloids. 2nd Edn. Springer- Verlag, N.Y., N.Y.

Rose. A.F., B.A. Butt and T. Jermy. 1980. Polyacetylenes from the rabbitbush, Chrysothamnus nauseosus. Phytochem. 19: 563-566.

Rose, A.F., K.C. Jones, W.F. Haddon and D.C. Dreyer. 1981. Frindelane diterpenoid acids from Grindelia humilis: feeding deterrency of diterpene acids towards aphids. Phytochem. 20:3349-2255.

Rosenthal, G.A. 1982. Plant Nonprotein and Imino Acids. Academic Press, N.Y., N.Y. 273 pp.

Rosenthal, G.A. and D.H. Janzen. 1979. Herbivores: Their interaction with secondary plant metabolites. Academic Press, N.Y., N.Y. 718 pp.

Ross, R.A.M. 1972. Natural acetylenes of special biosynthetic interest. Phytochem. 11: 221-227.

Ryan, C.A. 1979. Proteinase inhibitors. In "Herbivores: Their interaction with secondary plant metabolites. pp 599-618. G.A. Rosenthal and D.H. Janzen, Eds. Academic Press, N.Y., N.Y.

Russell, G.B., O.R.W. Sutherland, R.F.N. Hutchins and P.E. Christmas. 1978. Vesitol: A phytoalexin with insect feeding deterrent activity. J. Chem. Ecol. 4: 571-579.

Sakurai, H. and J.R. Heitz. 1982. Growth inhibition and photooxidative toxicity in the house fly, Musca domestica L., caused by xanthene dye in larval growth medium and after injection. Environ. Ent. 11: 467-470.

Saxena, S.C. and J.P. Sirvastava. 1968. On the histology and histo- chemistry of insecticide treated insects. Pyrethrum Post 9: 9-13.

Saxena, S.C. and J.P. Sirvastava. 1969. The histopathology and histochemistry of insecticide treated insects. III. The phospho- lipids in the midgut of Periplaneta americana (L.) treated with pyrethrum. Pyrethrum Post 10: 5-7.

Schmutterer, H., K.R.S. Ascher and H. Rembold, Eds. 1981. Natural pesticides from the Neem tree (Azadirachta indica A. Juss.) Proc. First Int. Neem Conf. Publ.

by German Agency for Technical Cooperation (GTZ). 297 pp.

Schoonhoven, L.M. 1967. Chemoreception of mustard oil glucosides in larvae of Pieris brassicae. Proc. K. Ned. Akad. Wet. 70: 556-568.

Schoonhoven, L.M. 1968. Chemosensory basis of host plant selection. Ann. Rev. Entomol. 13: 115-136.

Schoonhoven, L.M. 1972. Secondary plant substances and insects. Rec. Adv. Phytochem. 5: 197-224.

Schoonhoven, L.M. 1981a. Chemical mediators between plants and phytophagous insects. In "Semiochemicals: Their Role in Pest Control." D.A. Norlund, R.L. Jones and W.J. Lewis, Eds. pp 31-50. Wiley, London.

Schoonhoven, L.M. 1981b. Perception of azadirachtin by some lepidopterous larvae. In "Natural Pesticides From the Neem Tree (Azadirachta indica A. Juss.)" H. Schmutterer, K.R.S. Ascher and H. Rembold, Eds. pp 105-108. Proc. First Int. Neem Conf. Publ. by German Agency for Technical Cooperation (GTZ).

Schoonhoven, L.M. and T. Jermy. 1977. A behavioral and electrophysio- logical analysis of insect feeding deterrents. In "Crop Protection agents- Their biological evaluation." N.R. McFarlane, Ed. Academic Press, N.Y., N.Y.

Schoonhoven, L.M. 1982. Biological aspects of antifeedants. Ent. Exp. Appl. 31: 57-69.

Self, L.S., F.E. Guthrie and E. Hudson. 1964a. Adaptations of tobacco hornworms to the ingestion of nicotine. J. Insect Physiol. 10: 907-914.

Self, L.S., F.E. Guthrie, and E. Hudson. 1964b. Metabolism of nicotine by tobacco feeding insects. Nature 204: 300-301.

Shaver, T.N. and M.J. Lukefahr. 1969. Effect of flavonoid pigmants and gossypol on growth and development of the bollworm, tobacco budworm, and pink bollworm. J. Econom. Ent. 62: 643-646.

Shaver, T.N. and W.L. Parrott. 1970. Relationship of larval age to toxicity of gossypol to budworms, tobacco budworms, and pink bollworms. J. Econom. Ent. 63: 1802-1804.

Sinclair, J. and J.T. Arnason. 1982. The effect of alpha-terthienyl on photosynthesis. Can. J. Bot. 60: 2565-2569.

Slama, K. 1979. Insect hormones and antihormones in plants. In "Herbivores: Their interaction with secondary plant metabolites." pp 683-706. G.A. Rosenthal and D.H. Janzen, Eds. Academic Press, N.Y., N.Y.

Spikes, J.D. 1977. Photosensitization. In "The science of photobiology pp 87-112. K.C. Smith, Ed. Plenum Press, N.Y., N.Y.

Stahl, E. 1888. Pflanzen und Schnecken: ein biologische Studie uber die Schutzmittel der Pflanzen gegen Schneckenfrass. Jena. Z. Med. Naturwiss. 22: 557-684.

Stewart, J.G. 1981. The development of the tobacco hornworm as influenced by diet and sublethal doses of fenitrothion. M.Sc. Thesis, University of Ottawa. 144 pg.

Stubblebine, W.H. and J.H. Langenheim. 1977. Effects of Hymenaea courbaril leaf resin on the generalist herbivore Spodoptera exigua. J. Chem. Ecol. 3: 633-647.

Sutfield, R. and G.H.N. Towers. 1982. 5-(4-acetoxy-1-butinyl)-2,2'- bithiophene: acetate esterase from Tagetes patula. Phytochem. 21: 479-501.

Sutherland, O.J., G.B. Russell, D.R. Biggs and G.A. Lane. 1980. Insect feeding deterrent activity of phytoalexin isoflavonoids. Biochem. System. Ecol. 8: 73-75.

Swain, T. 1977. Secondary compounds as protective agents. Ann. Rev. Plant Physiol. 28: 479-501.

Swain, T. 1979. Tannins and lignins. In "Herbivores: Their interaction with secondary plant metabolites". pp 657-682. G.A. Rosenthal and D.H. Janzen, Eds. Academic Press, N.Y., N.Y.

Tauton, M.T. and S.M. Khan. 1978. Effects of fenitrothion and aminocarb, at doses giving low mortality, on surviving eggs and larvae of the Eucalypt-defoliating chrysomelid beetle Paropsis atomaria Ol. III Histological changes in treated larvae. Aust. J. Zool. 26: 139-146.

Thorsteinson, A.J. 1960. Host selection in phytophagous insects. Ann. Rev. Ent. 5: 193-218.

Tietz, H.M. 1972. An index to the described life histories, early stages, and hosts of the Macrolepidoptera of the United States and Canada. Allyn Museum of Entomology, Sarasoto, Fla. 1041 pp.

Towers, G.H.N. 1980. Photosensitizers from plants and their photodynamic action. Progress in Phytochemistry 6: 183-202.

Towers, G.H.N., J.T. Arnason, C.K. Wat, E.A. Graham, J. Lam and J.C. Mitchell. 1979. Phototoxic polyacetylenes and their thiophene derivatives (effects on human skin). Contact Dermatitis 5: 140-144

Towers, G.H.N., E.A. Graham, I.D. Spenser and Z. Abramowski. 1981. Phototoxic furanoquinolines of the Rutaceae. Planta Medica 41: 136-142.

Towers, G.H.N. and C.K. Wat. 1978. Biological activity of poly- acetylenes. Rev. Latinoam. Quim. 9: 162-170.

Towers, G.H.N., C.K. Wat, E.A. Graham, R.J. Bandoni, G.F.Q. Chan, J.C. Mitchel and J. Lam. 1977. UV mediated antibiotic activity of species of Compositae caused by polyacetylenic compounds. Lloydia 40: 487-498.

Towers. G.H.N., J.T. Arnason, C.K. Wat and J.D.H. Lambert. 1984. Pesticidally active composition and methods for controlling pests. Canadian patents, accepted Jan. 1984. U.S. patent pending.

Turro, N.J. and A.A. Lamola. 1977. Photochemistry. In "The science of photobiology". pp 63-86. K.C. Smith, Ed. Plenum Press, N.Y., N.Y.

Uhlenbroek, J.H. and D.J. Bijloo. 1959. Investigations on nematicides II: Structure of a second nematicidal principle isolated from Tagetes roots. Recueil 78: 382-390.

Uhlenbroek, J.H. and D.J. Bijloo. 1960. Investigations on nematicides III: Polythienyls and related compounds. Receuil. 79: 1181-1196.

Van Etten, C.H. and H.L. Tookey. 1979. In "Herbivores: Their interactio with secondary plant metabolites". pp 471-501. G.A. Rosenthal and D.H. Janzen, Eds. Academic Press, N.Y., N.Y.

Verschaffelt, E. 1910. The cause determining the selection of food in some herbivorous insects. Proc. Kon. Ned. Acad. Wtsch. 13: 536-542

Wada, K., K. Matsui, Y. Enomoto, O. Ogiso and K. Munakata. 1970. Insect feeding inhibitors in plants. Part 1: Isolation of three new sesquiterpenoids from Parabenzoin trilobum Nakai. Agric. Biol. Chem. 34: 941-945.

Waldbauer, G.P. 1968. The consumption and utilization of food by insects. Adv. Insect Physiol. 5: 229-288.

Warren, R.A.J., J.B. Hudson, K. Downum, E.A.Graham, R. Norton and G.H.N. Towers. 1980. Bacteriophages as indicators of the mechanism of action of photosensitizing agents. Photobiochem. Photobiophys. 1: 385-389.

Warthen, J.D. 1979. Azadirachta indica: A source of insect feeding inhibitors and growth regulators. USDA Agric. Rev. and Man. ARMNE-4, 21 pp.

Wat, C.K., R.K. Biswas, E.A. Graham, L. Bohm, G.H.N. Towers and E.R. Waygood. 1979. Ultraviolet-mediated cytotoxic activity of phenylheptatriyne from Bidens pilosa L. J. Nat. Prod. 42: 103-111.

Wat, C.K., T. Johns and G.H.N. Towers. 1980a. Phototoxic and antibiotic activities of plants of the Asteraceae used in folk medicine. J. Ethnopharm. 2: 279-290.

Wat, C.K., W.D. McRae, E. Yamamoto, G.H.N. Towers and J. Lam. 1980b. Phototoxic effects of naturally occurring polyacetylenes and α-terthienyl on human erythrocytes. Photochem. Photobiol. 32: 167-172.

Wat, C.K., S.K. Prasad, E.A. Graham, S. Partington, T. Arnason, G.H.N. Towers and J. Lam. 1981. Photosensitization of invertebrates by natural polyacetylenes. Biochem. System. Ecol. 9: 59-62.

Whittaker, R.H. 1970. The biochemical ecology of higher plants. In "Chemical Ecology." E. Sondheimer and J.B. Simeone, Eds. pp 43-70. Academic Press, N.Y.

Wilkinson, C.F. 1983. Role of mixed-function oxidases in insecticide resistance. In "Pest Resistance to Insecticides: Challenges and Prospects." G.P. Georghiou and M. Saho, Eds. Plenum Press, N.Y.

Wrang, P.A. and J. Lam. 1975. Polyacetylenes of Chrysanthemum leucanthemum. Phytochem. 14: 1027-1035.

Yajima, T., N. Kato and K. Munakata. 1977. Isolation of insect anti- feeding principles in Orixa japonica Thunb. Agric. Biol. Chem. 41: 1263-1268.

Yamamoto, E., C.K. Wat, W.D. MacRae, G.H.N. Towers and C.F.Q. Chan. 1979. Photoinactivation of human erythrocyte enzymes by α-terthienyl and phenylheptatriyne, naturally occurring compounds in the Asteraceae. FEBS Letters 107: 134-136.

Yoho, T.P., L. Butler and J.E. Weaver. 1971. Photodynamic effect of light on dye-fed house flies: preliminary observations of mortality. J. Econom. Ent. 64: 972-973.

Yoho, T.P., J.E. Weaver and L. Butler. 1973. Photodynamic action in insects. I. Levels of mortality in dye-fed light-exposed house flies. Environ. Ent. 2: 1092-1096.

Zucker, W.V. 1983. Tannins: Does structure determine function? An ecological perspective. Am. Nat. 121: 335-365.

Appendix 1A. Food plants of larval Euxoa messoria (from Tietz, 1972).

Acer saccharinum L.
Allium cepa L.
Beta vulgaris L.
Brassica oleracea L.
Brassica rapa L.
Fagopyrum sagittatum Gilib
Fragaria chiloensis (L.)
Ipomoea batatas (L.)
Lactuca sativa L.
Lilium sp.
Malus pumila Mill.
Nicotiana tabacum L.
Phaseolus vulgaris L.
Pisium sativum L.
Portulaca oleracea L.
Prunus persica (L.)
Raphanus sativus L.
Ribes sativum Syme
Solanum lycopersicum L.
Solanum tuberosum L.
Spinaca oleracia Mill.
Trifolium spp.
Vitis vinifera L.
Zea mays L.

Appendix 1B. Food plants of larval Ostrinia nubilalis (from Caffrey and Worthley, 1927). Members of the Asteraceae are indicated with an "A", and those known to contain polyacetylenes (according to Bohlmann et al., 1973) are indicated with an "*".

I. Plants severly attacked.

Echinochola crus-galli (L.)(Beauv.)
Bidens spp. (including B. cernua L.) A,*
Holcus sorghum L.
Xanthium spp. A,*
Zea mays L.
Dahlia spp. A,*
Rumex spp.
Iva xanthifolia Nutt. A,*
Cannabis sativa L.
Humulus japonicus Sieb. & Zucc.
Amaranthus retroflexus L.
Polygonum orientale L.
Ambrosia spp. A,*
Rheum rhaponticum L.
Polygonum spp.
Chenopodium ambrosioides L.

II. Plants frequently attacked.

Hordeum vulgare L.
Phaseolus spp.
Beta vulgaris crassa Alef.
Panicum miliaceum L.
Arctium spp. A
Celeri graveolens (L.)(Britton)
Callistemma chinense A
Chrysanthemum spp. A,*
Gossypium hirsutum L.

Appendix 1B. (con't)

Vigna sinensis (L.) Endl.
Erigeron canadensis L. A,*
Polygonum sieboldii DeVriese
Datura spp.
Capsicum annum L.
Solanum tuberosum L.
Holcus sorghum L. var. Sorgo
Helianthus annuus L. A
Beta vulgaris cicla Moq.
Tanacetum vulgare L. A,*
Euchlaena mexicana Schrad.
Artemisia spp. A,*
Zinnia elegans Jacq. A

III. Plants occaisionally attacked.

Aster spp. A,*
Boltonia asteroides (L.) L'Her. A,*
Panicum spp.
Fagopyrum vulgare Hill
Calendula officinalis L.
Canna spp.
Celosia cristata L.
Celosia argentea L.
Coleus spp.
Cosmos bipinnatus Cav. A
Syntherisma sanguinalis (L.) Dulsac.
Solanum melongena L.
Chrysanthemum parthenium (L.) Bernh. A,*
Erechites hieracifolia (L.) Raf. A
Galinsoga spp. A,*
Pelargonium hortorum
Gladiolus spp.

Appendix 1B. (con't)

Rudbeckia laciniata L. var Golden Glow A
Solidago spp. A,*
Althaca rosea Cav.
Humulus lupulus L.
Helianthus tuberosus L. A
Chenopodium album L.
Malva spp.
Leonurus cardiaca L.
Brassica spp.
Urtica lyallii Wats
Avena sativa L.
Atriplex sp.
Lactuca sp. A
Portulaca oleracea L.
Salvia splendens Ker-Gawl.
Sonchus spp. A
Helichrysum bracteatum Andr.
Cirsium spp. A,*
Lycopersicon esculentum Mill.
Abutilon theophrasti Medic.
Achillea millefolium L. A,*

Appendix 2A. Euxoa messoria artificial diet composition (from Devitt et al., 1980)

Ingredients	Weight (g)
Agar	1.4
Distilled water	82.3
Wheat germ oil	0.7
Wheat germ	2.8
Casein	3.2
Soybean protein	3.2
Sucrose	3.2
Salt mixture W	0.9
Cholesterol	0.06
Sorbic acid	0.06
Methylparaben	0.1
Vandezant Vitamen mixture	1.0
Ascorbic acid	0.13
10% KOH	1.0 ml
Formalin	0.2 ml

Appendix 2B. Ostrinia nubilalis artificial diet composition (from Guthrie, 1971.

Ingredients	Weight (g)
Water	130
Agar	2.8
Wheat germ	5.2
Dextrose	4.0
Casein	4.4
Cholesterol	0.32
Salt Mixture 2	1.44
Vitamen Supplement	0.92
Ascorbic acid	1.2
Aureomycin	9 teaspoon
Fumiail B	0.069
Methyl p hydroxybenzoate	0.75 ml
Propionic acid	0.86 ml
Formaldehyde	0.07 ml
Sorbic acid	0.80 ml

Appendix 2C. *Manduca sexta* artificial diet composition (from Stewart, 1981).

Ingredients	Weight (g)
Premix	
Wheat germ	50.00
Casein	22.50
Sucrose	20.00
Brewers yeast	15.00
Wesson's salt mixture	7.50
Ascorbic acid	2.00
Sorbic acid	0.75
Methyl-p-hydroxybenzoate	0.50
Cholesterol	0.25
Vitamens	
Nicotinic acid	5.00 mg
Calcium pantothenate	5.00 mg
Riboflavin	2.50 mg
Thiamine HCl	1.65 mg
Pyridoxine HCl	1.65 mg
Folic acid	0.10 mg
Biotin	0.10 mg
Others	
Agar	10.00
Formalin	10.00 ml
Raw linseed oil	2.00 ml
Distilled water	750.00 ml