INSECT GROWTH INHIBITORS FROM ASTERACEOUS PLANT EXTRACTS

by

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ABSTRACT

Petrol and ethanolic extracts of six asteraceous weeds were added to artificial diet and screened for inhibition of larval growth on variegated cutworm, Peridroma saucia (Hbn.). Petrol and ethanolic extracts of Artemisia tridentata and Chamomilla suaveolens and ethanolic extracts of Chrysothamnus nauseosus and Centaurea diffusa were highly inhibitory at five times the naturally occurring concentrations. The two C. suaveolens extracts and the ethanol extract of A. tridentata were active at the natural concentration (100%) and were further examined at 20, 40, 60, and 80% of this level. Inhibition of larval growth was directly related to concentration for each of the three extracts tested. EC_{50}'s (effective concentration to inhibit growth by 50% relative to controls) for the three extracts were 36-42% of the naturally occurring level in the plants.

Nutritional indices were calculated for second instar P. saucia feeding on the active ethanolic A. tridentata extract and the petrol extract from C. suaveolens . The relative growth rate (RGR) of P. saucia larvae fed the ethanolic extract of A. tridentata in artificial diet was significantly lower than that in larvae fed diet with the petrol extract of C. suaveolens and larvae on control diet. Dietary utilization was significantly lower for larvae fed the A. tridentata extract.

Results of a field trial indicated that a single treatment of A. tridentata extract at the equivalent of 0.2 g/ml could protect cabbage significantly better than the carrier solvent (30% aq ethanol) or distilled water as measured by a visual damage estimate. An insecticide standard, deltamethrin (17.9 µg/l with 0.4% Superspred^{TM}), suppressed pest damage significantly better than the A. tridentata-extract treatment.

A residual oviposition deterrency to Pieris rapae was found in the field results. Caged experiments in the laboratory confirmed the contact oviposition deterrency of the A. tridentata extract at 0.2 g/ml.

Offspring of field-collected P. saucia larvae grew 2.5-fold heavier than larvae from the laboratory colony. However, diet with the A. tridentata extract inhibited both field-collected and laboratory reared P. saucia larvae equally when compared to their respective controls fed untreated diet.

In summary, these results indicate the potential benefit of using specific unrefined plant extracts for growth inhibitors and oviposition deterrents against insect pests. The contribution of individual phytochemicals in the A. tridentata ethanolic extract to growth inhibition or oviposition deterrency is currently speculative.

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I. Introduction

Present day crop protection strategies often rely heavily on the liberal use of broad-spectrum insecticides. Novel pest management tactics are beginning to reduce the use of these non-selective biocides (Huffaker 1980). Improvements to crop protection methods are neccesitated by health, environmental, and economic concerns associated with the use of synthetic insecticides (Luck et al. 1977, Basol 1980, Metcalf 1980).

Public concern regarding insecticides is often the result of chemophobia or the fear that widespread use of chemicals will damage the environment and human health. The fear is justifiable, for example, where toxic insecticides have been misused causing environmental damage, including groundwater contamination (Zitter 1984). Agricultural agencies could better inform legitimate interests (i.e., the public) of the risks and benefits of insecticide use (Czerwinski and Isman 1986) and be able to provide options and alternatives to the use of biocides.

The cost of insecticide usage is increasing for several reasons. Where insecticides are used intensively, insects often develop resistance to insecticides (Luck et al. 1977). Almost invariably, broad-spectrum insecticides increase the development of resistant pest genotypes. When insecticides are sprayed, major or minor pest species may become a serious problem if their natural enemies are eliminated. When pest populations resurge or develop resistance to an insecticide, farmers and other applicators, may use higher doses, increase the frequency of spraying, or change control chemicals. New insecticides are thus needed to replace those no longer effective. Insects that have aquired resistance to existing insecticides are often predisposed to develop cross-resistance to new insecticides (Devonshire and Moore 1982). Cross-resistance has

decreased the average lifespan of a new insecticide to about two years (Metcalf 1980). Additional economic problems, such as cost (about $25 million) and development time (8-10 years) for new products (Kinoshita 1985), suggest that innovative approaches are needed to control insect pests and manage extant insecticides (Croft 1982).

Natural products from plants are potential sources of useful crop protection agents and novel pest control strategies. Plant products, such as vegetable oils, are known to have been used for crop protection in early Greek and Roman agriculture (Smith and Secoy 1975). More recently, plant extracts have been investigated for control of both viral (Verma and Abid Ali Khan 1984) and fungal (Kuc and Shain 1977, El-Shazly et al. 1981) crop diseases. Numerous plant extracts have also been examined for their acute insecticidal properties (McIndoo and Sievers 1924, Jacobson 1958). However, extensive screenings for acutely toxic phytochemicals have produced few commercially exploited botanical insecticides (Jacobson and Crosby 1971).

A. Objectives of Thesis

The primary objective of this investigation was to screen crude extracts from asteraceous weeds as potential insect control agents. Plants in the Asteraceae were selected because of their richness in secondary metabolites (Herout 1970). The insect used in the extract screening was the variegated cutworm, Peridroma saucia (Hbn.), because it is a serious insect pest of many crops in Canada (Beirne 1971), and is reared consistently and available in large quantities in Dr. M. B. Isman's laboratory. The plants chosen (Table I) were all weedy species with no current economic value. The dried plant powders were extracted with both polar and non-polar solvents to assess biological activity of fractions

containing different phytochemical mixtures. Dose-response of the most growth inhibiting extracts showed the relative effectiveness of the extracts as larval growth inhibitors and the nature of the growth inhibitory response. To distingish between gross behavioral and physiological effects, growth inhibition was further studied by determination of nutritional indices for larvae feeding on artificial diet admixed with extracts.

The next objective was to prepare a formulation of the most active extract to assess the extract's field efficacy against cabbage insect pests. A short term phytotoxic test on cabbage prior to the field test insured the survival of the cabbage plants for the field trial. Two laboratory experiments were performed subsequent to the field trial. One was to confirm the field observation of lowered Pieris rapae egg-counts in the cabbage treated with Artemisia tridentata extract using caged butterflies in the laboratory. The other compared the effects of the ethanolic A. tridentata extract on field-collected VC larvae relative to larvae from the laboratory colony.

The final section of this thesis involved an examination of the phytochemistry in the most growth-inhibiting extract. Chromatographic separation in conjunction with an insect bioassay investigated whether growth inhibition was attributable to one or several groups of compounds. Chromatographic comparisons of pure compounds isolated from closely related plants with active fractions allowed tentative identification of the chemical class of compounds responsible for the growth inhibition.

II. Literature Review

The literature on the interactions between plants and their insect herbivores is large. This includes numerous reviews and books on the breeding of plants resistant or tolerant to insect attack (Painter 1951, Maxwell and Jennings 1980, Hedin 1983), identification of plant characteristics conferring insect resistance (Thorsteinson 1960, Chapman 1974, Rosenthal and Janzen 1979, Dethier 1980, Hedin 1983), and the physiological factors that allow insects to perceive and metabolize phytochemicals (Fraenkel 1959, Brattsten 1979, Bell and Carde 1984). In addition, many classes of secondary compounds that influence insect behavior and physiology have been examined (Chapman 1974, Rosenthal and Janzen 1979, Hedin 1983, Whitehead and Bower 1983).

Several studies have considered sublethal ways of manipulating insect populations with phytochemicals, including inhibition of feeding (Jermy et al. 1981), growth (McMillian et al. 1969) and oviposition (Mitchell and Heath 1985). These chemicals are often termed 'allelochemics', defined by Whittaker (1970) as chemicals that mediate non-nutritional interspecific interactions.

Many phytochemicals have been shown to reduce insect growth, and prolong the life cycle (Chapman 1974, Reese and Beck 1976). Extending an insect's life cycle has been shown to increase exposure to predators (Wesloh et al. 1983) and other environmental hazards (Courtney 1986). Phytophagous insects that develop more slowly than normal are more likely to die of disease (Courtney 1981) and the early onset of winter (Chew 1975). Natural mortality of first instar Ostrinia nubilalis L. is about

90% (Beck 1960). Introduction of new, and enhancement of existing mortality factors at this vulnerable stage may play an important role in its population biology and thus its effect in agricultural systems.

Oviposition interference is another sublethal mode through which allelochemicals aid plants in escaping insect herbivory. Both host and non-host plant extracts have been shown to deter oviposition of several insect species (Tingle and Mitchell 1984, Renwick and Radke 1985). Many authors have recognized the importance of using phytochemical disruptions of insect behavior to protect crops from herbivory (Munakata 1970, Chapman 1974, Jermy 1983). Plant allelochemicals that have sublethal effects on insect pests are potentially useful for agriculture as valuable breeding criteria or as applied protectants, provided that they are non-toxic to humans, environmentally sound, economical to produce and convienient to apply.

A. Chemical Basis for Plant Resistance to Insects

Some insects feed on several different plant families whereas others rely on a restricted number of plants or even a single host species (Thorsteinson 1960). In addition, some plant species are rarely attacked by insects. Several factors influence the pattern of insect attack on the available plant species. Plant architecture, including size, shape, and color, as well as plant habitat and distribution, are important considerations in host selection by phytophagous insects. However, the chemical content of the plant is often considered the most important factor determining host-plant specificity (Haniotakis and Voyadjoglou 1978, Hardman and Ellis 1978, Rosenthal and Janzen 1979).

Many classes of phytochemicals have been shown to play an important role in insect-plant interactions. Sugars, amino acids and proteins are

important for insect growth and development and can play a role as feeding stimulants (Bernays and Simpson 1982, Dethier 1973). These ubiquitous constituents of plants are commonly known as primary metabolites. Their broad distributions in the plant kingdom make them unlikely candidates for insect host specificity or plant defensive chemistry (Bernays and Chapman 1978).

The other major group of phytochemicals are called "secondary" metabolites, either because they are poorly understood or because they are not involved in primary metabolism, or both. Numerous secondary metabolites are known to mediate insect-plant interactions (Chapman 1974, Hedin 1983). These important phytochemicals are usually classified according to their structure or biosynthetic pathways.

The effects of a single allelochemical on insects may differ depending on a variety of biotic and abiotic factors. The insect-allelochemical interaction may be influenced by the insect species examined (Eisner 1964, Dethier 1973, Chew 1980), insect growth stage (Reese 1979, Chew 1980, Isman and Duffey 1982), previous exposure to allelochemicals (Jermy et al. 1982), as well as concentration, route of entry and the simultaneous occurrence of other phytochemicals (Bernays and Chapman 1978). Isman and Duffey (1982) have shown that a phytochemical may elicit a growth inhibitory reponse at one concentration and a toxic reaction at a higher dose. Nepetalactone, a monoterpenoid, is known to repel some insects but have little or no effect on other insects in the same order (Eisner 1964).

The distribution of secondary chemicals is frequently limited to one plant taxon, while occurring sporadically in systematically unrelated groups. Glucosinolates or mustard oil glycosides provide a good example, occurring often in the Brassicaceae and other Capparales families, but occurring occasionally in the unrelated plant family Caricaceae (Bjorkman

1976). Sesquiterpene lactones are another example, occurring widely in the Asteraceae and less frequently in the Apiaceae and Magnoliaceae (Heywood et al. 1977).

Specific allelochemicals (e.g., oviposition cues) can have a beneficial effect on oligophagous insects (i.e., insects having a restricted range of food plants of related plant orders or even of a single genus), while at the same time contributing to the plant's chemical defence against more general herbivores. Allylglucosinolate, present in many Brassicaceae plants, is innocuous to the growth of the crucifer specialist Pieris rapae, but inhibits the growth of the polyphagous Spodoptera eridania , and is acutely toxic when fed to the Apiaceae specialist Papilio polyxenes (Blau et al. 1978). Even compounds present in their host plants may cause antibiosis (sensu Painter 1951) or reduce the fitness and vigor of oligophagous insects. The triterpenoid cucurbitacins of the Cucurbitaceae deter feeding by the cucurbit-specialist Epilachna tredecimnotata, but act as attractants to the striped cucumber beetle, Acalymma vittata (Carroll and Hoffman 1980).

Very few insects rely entirely on specific chemical cues for feeding or oviposition. Most oligophagous insects do not appear to rely on the presence or absence of a single compound for host acceptability, but on their chemosensory response to the total phytochemical mixture (Dethier 1973). Studies of food consumption by cruciferous flea beetles, Phyllotreta spp., show that the amount consumed is usually dependent on the balance of stimulant and deterrent chemicals (Nielsen 1978).

Although the effects of phytochemicals have often been investigated individually, an insect's sensory perception of their natural habitat must include the complexity of chemical mixtures in non-host plants. Many

plants have compounds that, in combination with other phytochemicals, increase insect antibiosis more so than a single compound alone (Adams and Bernays 1978, Kubo et al. 1984, Berenbaum and Neal 1985).

B. Insect Bioassays of Phytochemicals

Several bioassays have been used to detect phytophagous pest control agents in plant extracts. Bioassays may measure a variety of factors, including mortality (Freedman et al. 1979), feeding and oviposition punctures, larval and egg counts (Jacobson et al. 1978), growth, dietary utilization (Isman and Proksch 1985), reproductive potential (Robert and Blaisinger 1978) and consumption (Bentley et al. 1982).

Laboratory evaluation of behaviorally active allelochemicals may occur in the form of choice or 'no-choice' experiments. Choice experiments offer the test insect a selection of two or more substrates on which to feed or oviposit. In the so called no-choice experiments the insect has a single feeding or ovipositional substrate. Bioassays may be designed so that the insects are evaluated by either an 'all-or-none' or a graded type of response. Larval growth, for example, is usually a graded measure and mortality is an 'all or none' response.

Larval feeding and growth inhibition may be examined by incorporating plant powders or extracts in artificial diets and feeding the diets to test insects (Hsiao and Fraenkel 1968). After feeding, the surviving larvae are counted and weighed. Bernays (1983) and Smith (1978) discuss criteria for choosing between different bioassay methods.

The process of extracting allelochemicals from plant tissue and incorporating them into artifical diets could alter allelochemicals, potentially increasing or decreasing their effectiveness (Bernays and Chapman 1978). An artificial diet may mask the presence of feeding

deterrents (Bernays and Chapman 1977). The masking may be a result of increased feeding stimulation, absence of other fitness-reducing compounds or a better nutrient source than host plants. However, insects fed artificial diets provide a good relative measure of larval growth inhibition using extracted phytochemicals. The use of artificial diets alleviates problems inherent in the use of live plant materials and allows direct comparisons with other chemically defined food sources. Experimental error may increase with the use of live plant material due to the potential for large allelochemical differences (Risch 1985), and physical differences like leaf toughness (Reese 1983).

Although many compounds in different chemical classes have been shown to inhibit insect growth and increase development time, bioassays do not often distingish between grossly different modes of action. Fecal pellet counts, for example, may be excellent for detecting feeding inhibitors but they do not distingish between behavioral and physiological differences between treatments. Behavioral reasons for reduced fecal pellet counts in a treatment include reduced phagostimulation and feeding deterrency. Possible physiological causes for reduced fecal pellet counts relate to toxicity, including reduced food utilization and inhibition of metabolism. Cockroaches are known to increase consumption to compensate for food diluted with a non-nutritive, non-toxic cellulose filler (Bignell 1978). In addition, Risch (1985) has shown that feeding preferences can change, depending upon whether leaf disks or whole leaves are used in feeding bioassays.

Unless behavioral and physiological effects can be separated, the mode of action at even a superficial level remains speculative. Dietary efficiency studies are used to distingish between behavioral and physiological components of insect growth inhibition (Reese 1979, Isman and

Duffey 1982, Isman and Rodriguez 1984) as well as determining the adequacy of an insect's food source (Soo Hoo and Fraenkel 1966, Waldbauer 1968, Kogan and Cope 1974). Indices of dietary utilization are calculated from measurements of food consumption, weight gain, and excreta production. The growth rate may then be separated into its constituents, consumption rate and dietary utilization. Some of the technical difficulties with this bioassay method have recently been examined by Schmidt and Reese (1986).

C. Behavioral Response to Plant Defence Chemicals

Feeding and oviposition in phytophagous insects are regulated by several factors, such as chemostimulants (Hsiao 1969) and deterrents (Shurr and Holdaway 1970, Renwick and Radke 1985) in host and non-host plants (Jermy 1966, Bernays 1983, Thibout and Auger 1983). The term 'non-preference' has often been used to describe a behavioral non-event but is less than ideal due to its anthropocentric bias. 'Deterrent' is used to specify a substance that when contacted prevents or interrupts behavioral activity, including feeding or oviposition (sensu Schoonhoven 1982). A 'stimulant' for the purpose of this discussion is the antonym of deterrent and is a substance that, when physically contacted, incites a positive behavioral response such as feeding or oviposition.

Two different neural events may produce the same behavioral response. A deterrent may act directly on a chemoreceptor (Schoonhoven 1982) or by masking the effect of a chemostimulant (Mitchell and Sutcliffe 1984). Chemoreceptors in silkworm, Bombyx mori, larvae contain specialist cells that respond directly to both stimulants and deterrents (Ishikawa 1966).

Sparteine, a phytoalkaloid feeding inhibitor, is responsible for inhibiting the response of the sugar-sensitive cell. A lack of response from the receptor detecting behavioral stimulants will produce the same

response as a deterrent (Mitchell and Sutcliffe 1984). Both the central and the peripheral nervous system have important roles in the feeding behavior of herbivorous insects (Dethier 1980).

Many insects examined do not have specific receptors for individual chemicals. Dethier (1973, 1980) hypothesizes that neuro-reception of feeding deterrents involves the processing of information centrally from several receptors. Herbivore generalists and specialists may have a similiar capacity to detect chemicals but the information may be processed differently (Dethier 1980). Phytochemicals may thus serve as feeding stimulants to some phytophagous insects while inhibiting feeding and growth in non-adapted species.

Plant resistance to insects may often be traced to phytochemical defenses. Host-plant chemicals may aid plant breeders by focusing their attention on factors contributing to arthropod resistance. Allelochemicals that may prevent arthropod attack have been identified in several crops including tomatoes and cucumbers (Patterson et al. 1975, de Ponti 1977). Gramine, an alkaloid from barley, is responsible for resistance to the aphid Schizaphis graminum (Zuniga et al. 1985). Colorado potato beetles, Leptinotarsa decemlineata, are deterred from feeding by glycoalkaloids present in Solanum chacoense (Sinden et al. 1986).

Oviposition deterrents, present in many plants (Gupta and Thorsteinson 1960, Hsiao and Fraenkel 1968a), can occur as cuticular components or as chemicals that are released upon feeding. Field tests with the melonworm, Diaphania hyalinata, and the pickleworm, Diaphania nitidalas, showed that the principle mechanism of resistance in two varieties of butternut squash, Curcurbita moschata, was oviposition deterrency (Elsey 1985). European corn borer, O. nubilalis, females avoid ovipositing in fields where damage to corn releases host volatiles (Shurr and Holdaway 1970).

Oviposition and feeding deterrents are often present in non-host plants. Spraying plant extracts as insect control agents has inhibited mating and oviposition of oligophagous (Robert and Blaisinger 1978, Dover 1985) and polyphagous insects (Burnett and Jones 1978). The non-host sesquiterpene lactone, glaucolide A, present in Vernonia spp. (Asteraceae), deters feeding by larvae of several species of polyphagous lepidopterans (Burnett et al. 1974).

Phytochemicals may show behavioral deterrency only as naturally occurring mixtures. Woodhead and Bernays (1977) have shown that several non-toxic phenolic compounds at natural concentrations produce feeding deterrency only when combined. Even where dominant compounds have been isolated, they seldom account for host discrimination even in oligophagous insects (Berenbaum 1985). Although glucosinulates are known to stimulate some crucifer feeding caterpillars, the total response can be attributed to more than one group of phytochemicals (Gupta and Thorsteinson 1960). Furthermore, Nielsen (1978a) states that the acceptability of their crucifer host plants to flea beetles, Phyllotreta spp., could not be accounted for solely on the basis of glucosinolate content, but was likely due to a composite of allelochemicals.

Plants commonly contain feeding deterrents (Woodhead and Bernays 1977, Isman and Duffey 1982). Insects, however, do not always avoid plants because of toxic phytochemicals. Insects may be deterred from feeding on harmless plants and conversely may be intoxicated by consuming poisonous plants. Certain tomato cultivars that contain the toxic glycoalkaloid, tomatine, are consumed with impunity by Heliothis zea larvae because of the antidotal effect of foliar sterols (Campbell and Duffey 1981). With cauterized chemoreceptors, tobacco hornworm larvae, Manduca sexta, readily consume non-toxic plants previously avoided (de Boer et al. 1977).

Phytochemicals, such as feeding deterrents, that protect a plant and allow survival of susceptible insect genotypes may protect plants longer than potent chemicals that quickly select for resistant insect genotypes. Gould (1986), in his simulation model to predict the durability of wheat germplasm resistant to the Hessian fly, Mayetiola destructor, indicated that the most durable resistance would occur if totally susceptible cultivars are planted with a resistant cultivar. This suggests that plant resistance will be more durable when insects are not under severe selection pressure, such as would occur when a monoculture of a resistant cultivar is planted. Plant extracts used in the field may mimic the phytochemical profile of a mixed cropping system.

D. Phytochemicals and Physiological Stress

Plant defense chemicals cause many adverse physiological effects on insects, for example reduced digestion, suppression of microsomal enzymes, disruption of endosymbiotic organisms, interference with hormonal processes, reduction of reproductive capacity and death.

Immature insects are confronted with chemical and physical plant defenses when obtaining vital nutrients. Dietary nitrogen and water, are often the most important factors limiting larval growth (Mattson 1980, Scriber and Slansky 1981).

Many adult female insects oviposit on or near potential food plants. The gravid female must be genetically 'wired' to discriminate among potential larval food plants and the larvae must be able to detect and avoid ingestion of toxins. In fact, neonate lepidopteran larvae are highly susceptible to allelochemicals (Reese 1979) possibly because they have less active or fewer detoxificative enzymes, a thin peritrophic gut membrane or lack endosymbiotic organisms for xenobiotic detoxification.

The ubiquitous mixed-function oxidase (MFO) system is the major detoxification system in insects (Ahmad 1986, Dauterman and Hodgson 1978). Adaptation to an allelochemical may result from an increased efficiency of the nonspecific MFO system.

Polyphagous insects, such as some lepidopteran larvae, are potentially exposed to a broad range of sublethal plant toxins and may be better able to detoxify ubiquitous fitness-reducing allelochemicals than oligophagous insects, as the former possess higher gut MFO levels (Krieger et al. 1971). Specific allelochemicals, however, may be better dealt with by specialist insects feeding on their host plants (Blau et al. 1978).

How does the metabolism of xenobiotics affect insect resistance to insecticides? Insect resistance often involves increased enzymatic activity and when this occurs, cross-resistance to chemically unrelated compounds is quite common (Agosin and Perry 1974, Devonshire and Moore 1982). Spider mites bred for tolerance to an insect resistant cucumber variety were, interestingly, cross-resistant to several insecticides and a variety of unrelated plants, but the mechanism of resistance was not investigated (Gould et al. 1982).

Specialists and generalists may sequester a wide variety of toxic phytochemicals. Sequestration may effectively prevent the xenobiotic from causing damage to the insect. The monarch butterfly, Danaus plexippus, which sequesters cardiac glycosides from its milkweed host, is an excellent example (Roeske et al. 1976).

Nicotine is highly toxic to many insects but some insect pests of tobacco avoid toxicity by efficient metabolism and excretion (Self et al. 1964, Brattsten 1979). L-Canavanine is an abundant non-protein amino acid found in seeds of the Central American legume, Dioclea megacarpa, and is highly toxic to most insects. However the bruchid, Caryedes brasiliensis,

not only feeds on D. megacarpa seeds but uses the arginine analog, L-canavanine, in protein production (Rosenthal et al. 1982).

One must be cautious when drawing analogies between the arthropod response to insecticides, and their response to phytochemical mixtures. Insects resistant to a single pyrethroid can develope strong cross-resistance to other pyrethroids (Priester and Georghiou 1980) and several other classes of insecticides (Funaki and Motoyama 1986). In contrast, detoxification of phytochemical mixtures in plants has received scant attention. However, Gould et al. (1982) have shown that organophosphorous resistant mites are as sensitive to a toxic host plant as are susceptible strains. These results suggest that the detoxification mechanism of allelochemical mixtures is different from organophosphorous insecticides and that other mechanisms may be involved.

E. Plant Extracts in Crop Protection

Plant products have been used to control insects since man first began cultivating plants. An extract from the flowers of Chrysanthemum cinerariaefolium, called pyrethrum, is perhaps the most widely used insecticidal plant product. Pyrethrum was first sold in North America in 1916 (Mallis 1982). Rotenone, from Derris spp. and Lonchocarpus spp., and nicotine, from Nicotiana rustica, are other commercially available insecticidal plant constituents. Other botanical insecticides are used mainly where they are indigenous, including Ryania speciosa, tung seed (Aleurites spp.) and sabadilla from Schoenocaulon spp.(Jacobson and Crosby 1971).

Recently, crude extracts and isolated phytochemicals from the neem tree, Azadirachta indica, have been investigated as insect control agents. Azadirachtin, a limonoid isolated from neem, completely inhibited feeding

of the migratory locust, Schistocerca gregaria, at levels as low as 1 ng/cm^2 on leaf disks (Kubo and Nakanishi 1977). The Environmental Protection Agency of the United States has registered a patented formulation (Larson 1985) of neem oil for use on non-food crops, and registration on food crops is pending (Jacobson 1986).

Both pyrethrum and neem oil contain more than one insecticidal compound. The pyrethrum activity is derived from six pyrethrin esters (Elliot and Janes 1973) and the insecticidal effects of neem oil are a result of several tetranortriterpenoids (Jacobson 1986).

Botanicals may provide entomologists with novel crop protection agents. Mixtures of defensive chemicals have evolved in plants and some evidence suggests plants mitigate damage by having combinations of phytochemicals (Berenbaum 1985). Adams and Bernays (1978) showed that fourteen phytochemicals in naturally occurring concentrations did not produce a measurable effect on Locusta migratoria feeding when presented alone but deterred feeding when presented as a mixture. If plants, having evolved over millions of years, use mixtures of chemicals to defend against herbivory perhaps we can also use this 'novel' strategy.

Naturally occurring insect growth inhibitors (e.g., feeding deterrents) may provide effective tools for crop management by protecting crops from herbivory while avoiding destruction of beneficial insects (Bernays 1983). Recent studies suggest that synthetic insecticides confer part of their benefit due to sublethal effects. Aldicarb at sublethal doses reduces the ability to fly and probe, as well as the fecundity, of potato aphids, Macrosiphum euphorbiae (Boiteau et al. 1985). Reduced flying and probing also decreased the ability of this aphid to transmit viral diseases. The carbamate, methomyl, inhibits the growth and development of fall armyworm, Spodoptera frugiperda, larvae at sublethal

concentrations (Javid and All 1984). Some of the pyrethroids show promise at sublethal doses because they deter insect feeding and inhibit development (Dobrin and Hammond 1985, Kumar and Chapman 1984).

Physiological or behavioral stress impeding optimal larval growth reduces insects' resistance to disease (Boucias et al. 1984) and increases their susceptibility to natural enemies. For example, feeding deterrents used in conjunction with insect pathogens may increase larval infection rates. Trichoplusia ni larvae in the early instars are more susceptible to infection by the entomopathogenic fungus Spicaria rileyi (Ignoffo et al. 1975). If a deterrent can maintain an insect in an early instar through growth inhibition, then other mortality factors can play a greater role in population regulation. Laboratory studies provide evidence that chronic sublethal effects may have an important but deferred impact on insect populations (Reese and Beck 1976).

Field use of feeding deterrents has been limited to a few compounds. The best example of a feeding deterrent tested on a large scale is the synthetic compound, 4'-dimethyltriazeno-acetanilide (cited in Bernays 1983). This product was an effective feeding deterrent in field tests against several herbivorous insects including, the cabbage looper, T. ni, the cotton leafworm, Alabama argillacea, and the boll weevil, Anthonomus grandis. However, no control was observed for several other pest insects, such as the pink bollworm, Pectinophora gossypiella, and the codling moth, Cydia pomonella. These results emphasize that feeding and oviposition deterrents are often species specific.

The use of feeding and oviposition deterrents integrate well with contemporary integrated pest management (IPM). IPM requires the monitoring of pests to determine when biocidal agents are needed. The application of deterrents could be prophylactic or applied when pest populations or crop

damage reach an economic threshold. Advantages of applying phytochemical deterrents include selective pest control and minimal environmental disturbances.

Many host and non-host phytochemicals are known to inhibit growth and oviposition. Laboratory reports on the isolation of insect fitness-reducing phytochemicals are numerous (e.g., Trial and Dimond 1979, Delle Monache et al. 1984). Most of the studies are not directly concerned with the application of the chemicals in pest control situations, but deal with ecological or physiological considerations. Most phytochemicals examined in the laboratory have not been studied in field tests and rarely with the ultimate aim of developing a useful agricultural product. The lack of experimental field data on the use of sublethal phytochemicals undermines the many laboratory studies on the subject.

III. MATERIAL AND METHODS

A. Plant extracts

The plants were all collected from southern British Columbia, air-dried and finely ground in a Wiley™ mill. Plant species, parts extracted, location of harvest, and harvest dates are listed in Table I. Powdered plant material (200 g) was thoroughly mixed with 1 liter of either petrol (petroleum ether, boiling range 30-60°C) or 95% aq ethanol (EtOH) and soaked for 24 h at room temp (21°C). The slurry was filtered and rinsed, then the extracts were reduced under vacuum to 10-60 ml depending on their respective viscosities.

B. Biological Screenings

1. Initial Screening

Extracts 5-fold of those naturally occurring, calculated as the dry weight of plant powder extracted to the dry weight of artificial diet (dwt/dwt), were admixed with the dry portion of the artificial diet (Bioserv Inc., Frenchtown, NJ, no. 9682) and the carrier solvent was removed in a fume hood. Controls consisted of artificial diet similarly treated with the carrier solvents alone (petrol and EtOH). Upon hatching, 2 neonate *P. saucia* larvae from a laboratory colony were placed on about 2 g (wwt) aliquots of diet in 30 ml plastic cups at room temperature. The rearing cups were placed in plastic boxes with moistened paper towels to prevent desiccation of larvae and diet. Using live larval weights (n=30), larval growth was measured as a percentage of the controls after 14 days. The larval gravimetric data was $\log_{10}$ transformed prior to statistical analysis in each experiment.

Table I. Plant species, components extracted, harvest locations in British Columbia and dates of material bioassayed in initial screening

Plant Species	Components Extracted	Location	Harvest date
Artemisia tridentata	stms, lvs, fls[1]	Summerland	10-83
Centaurea diffusa	stms, lvs, fls	Kamloops	10-83
Chrysothamnus nauseosus	stms, lvs	Keromeos	05-84
Chamomilla suaveolens	whole plant	Vancouver	05-84
Senecio jacobaea	stms, lvs	Abbottsford	05-84
Tragopon dubius	stms, lvs, fls	Hedley	05-84

[1] stms=stems, lvs=leaves, fls=flowers

2. Bioassay of Unextracted and Extracted Plant Material

The plant residue remaining from the initial extraction, hereafter referred to as the 'marc', and their respective unextracted plant powders were assayed for biological activity against P. saucia neonates to determine the efficiency of the extraction process. The dry ingredients of the artificial diet consisted of Bioserv no. 9682 with an equivalent portion of marc or plant powder (1:1 w/w). Control diet was prepared using one part powdered cellulose (alphacel) to one part artificial diet. An additional treatment consisted of the control diet without cellulose. This treatment was used to determine the effect of diluting the control diet with cellulose on larval growth. The experimental design was the same as in the previous experiment.

3. Second Screening

The most inhibitory extracts to P. saucia larval growth were selected for a further bioassay. Artificial diets were freshly prepared using natural concentrations (dwt/dwt) of the plant extracts. Control diets were treated with the carrier solvent. P. saucia neonates were individually placed on ca. 1 g of diet (n=25) and allowed to feed for 11 days and then weighed. All surviving larvae were placed on control diet on day 11 and allowed to continue feeding to determine the persistence of growth inhibitory effects through pupation and emergence.

C. Dose-Response Bioassays

1. Dose-Response Bioassay on P. saucia Neonates

Four concentrations (20, 40, 60, and 80% of natural conc. dwt/dwt) of the ethanolic extract of A. tridentata and both extracts of C. suaveolens were assayed as above with ethanol and petroleum ether solvent controls. After 15 days P. saucia larvae were counted, weighed, and then allowed to

feed on the control diet until pupation. Rates of pupation and emergence were recorded for each treatment. EC_{50}s (effective concentrations inhibiting larval growth by 50% relative to controls) were calculated using probit analysis (Finney 1971).

2. Dose-Response Bioassay using the Alfalfa Looper, Autographa californica Speyer

Another dose-response bioassay was performed to determine if the biological effect of extracts on P. saucia was also evident for other noctuid species. Field collected A. californica larvae were reared to maturity on artificial diet (Bioserv no. 9682). The resulting F_1 neonates were used for this experiment (n=25); the bioassay and data analysis were the same as described in the neonate P. saucia dose-response experiment.

3. Sensitivity of Older P. saucia Larvae

To determine how the biological activity of the plant extracts was influenced by larval age, another dose-response experiment was initiated with older caterpillars. Neonate P. saucia larvae were fed for six days on the standard control diet. The resulting second instar larvae (ca. 7 mg)were then transferred to the treatment diets (n=25). The bioassay and data analysis were as previously described in the neonate P. saucia dose-response experiment.

D. Final Determination of Extract for Field Trial

1. Detailed Growth Analysis of P. saucia Larvae Feeding on A. tridentata and C. suaveolens Extracts

To distinguish between behavioral and physiological contibutions to larval growth inhibition, a detailed growth analysis was initiated on second instar P. saucia. Larvae (10.9 ± 1.5 mg, n=15) were fed diets at their natural concentrations (100% dwt/dwt). An EtOH extract of A.

tridentata and a petrol extract of C. suaveolens were compared with the standard diet treated with petrol. The duration of the experiment was 48 h, although larvae were weighed at 24 h as well as 48 h to determine relative feeding and growth rates over the two 24 h periods. Except where otherwise indicated, all measurements are based on dry weights. Growth indices were calculated as described by Scriber and Slansky (1981).

2. Formulation of Active Extracts for Foliar Application and Stability of Crude Extracts

Biological activity of two aqueous extracts and a 20% aq EtOH extract were compared to the original EtOH extract (8 months old) and a freshly prepared EtOH extract from the original plant material for both A. tridentata and C. suaveolens. The two aqueous extracts were prepared by adding 10 g of ground plant powder to 40 ml of distilled water. One of these slurries was brought to a rolling boil and then both were kept at room temp for 24 hrs before being filtered. The 20% aq EtOH extracts were prepared following the same procedure as the room temp water extracts. The aqueous extracts were then lyophilized to reduce their volume. Control larvae fed on artificial diets treated with water or EtOH. Neonate P. saucia larvae (n=25) were used for this bioassay as described above.

E. Field Trial of the A. tridentata Extract on Cabbage

An experiment was designed to test the field efficacy of the A. tridentata extract relative to its carrier (30% aq EtOH), water and the pyrethroid insecticide, deltamethrin. Cabbage (cv. Early Marvel) was seeded on May 27, 1985. Plots of cabbage were assigned to four complete blocks and treatments were randomized within each block. Plots consisted of a row of seven cabbage plants (11.7 m^2) and were separated by an equivalent row of unsprayed cabbage. In addition, guard plants were

situated at both ends of each plot. Spray applications were made with a hand-held trigger sprayer and the nozzle was calibrated to deliver an equivalent volume (14 ml) of solution to each plant. The nozzle was calibrated by measuring 10 pulls of the trigger into a graduated cylinder as a fine mist. Four trigger pulls were executed from directly over the plant and three pulls for coverage to the sides and three pulls for the lower side of the leaves. The A. tridentata extract was formulated in 30% aq EtOH. This carrier gives an even foliar coverage without the addition of a spreader or sticker. The resulting solution was the equivalent of 0.2 g/ml. The 3 other treatments consisted of the carrier solvent, 30% aq EtOH, distilled water, and the standard pest control agent, deltamethrin (Decis™ 2.5 EC, Hoechst) at 17.9 µg ai./l plus 1 ml/l of the spreader-sticker, Superspred™ (Decis™ and Superspred™ were provided courtesy of Dr. Robert S. Vernon, Agriculture Canada, Vancouver, B.C). Spray applications were made to all plots on July 24, 1985 at 6:30 am. with the cabbage at post-heading (59 days from seed). The above ground parts of all experimental plants were surveyed 2 days before spraying to establish the baseline insect populations in each of the experimental plots. Once the trial was initiated, all experimental plants were monitored 1, 6, 9, and 25 days after spraying to assess the effects on the major insect pests of cabbage. The pre-count survey and the first three post-treatment counts were non-destructive visual observations of both sides of all non-head leaves. The final insect count at 25 days post-treatment was a destructive sampling of the above ground cabbage. The pests monitored were the cabbage looper (CL), T. ni, imported cabbageworm (ICW), P. rapae, and diamondback moth larvae (DBM), Plutella xylostella L. Cabbage pests were analyzed separately where numbers warranted and together as cabbage looper

equivalents (CLE) : 1 CLE = 1 CL = 1.5 ICW = 20 DBM (Shelton et al. 1982). Data from the field trial was analyzed in a completely randomized block design with repeated observations over time. The 'treatment' degrees of freedom were partitioned with individual treatment contrast comparisons. The 'day' effect was partitioned with polynomial expansion coefficients. Then the sum of squares from the 'treatment X day' interaction was partitioned for the linear, quadratic and residual variation. Significant 'treatment X day' interactions established differences in variation among pest population levels with the four spray treatments during the five survey dates.

A quality estimate was obtained by rating each of the cabbage heads on the last day of the experiment (25 days post-spray). The rating was based on the amount of visible damage present in and on each head after the wrapper leaves were removed (4=marketable cabbage, no exterior damage; 3=sauerkraut grade, exterior damage only, no holes; 2=garden grade, one hole into head; 1=unmarketable, more than one hole; 0=no head remaining). An analysis of variance was performed on the visual damage estimates and means were compared by partitioning the treatment sum of squares.

F. Laboratory Evaluation of the Oviposition Deterrence of A. tridentata Extract on Caged P. rapae

A field collected colony of P. rapae larvae was reared to pupation on cabbage (cv. Early Marvel). Eight adults of each sex were placed in a 50 X 50 X 50 cm screened cage and fed a solution of 10% sucrose. The cage was on a laboratory bench and late summer light from the south and west was supplemented with a 100 watt lamp for 16 h each day. On the following day six cabbage leaves in 50 ml flasks filled with water were offered to the butterflies in a choice oviposition experiment. Two leaves from each of

the following treatments were included: A. tridentata extract at the equivalent of 0.2 g/ml, 30% aq EtOH, and distilled water. The solutions were hand-painted onto the leaves. The leaves and the flasks were arranged in a circle in a random order. Eggs were counted after 48 h and the experiment was repeated. Results from the 2 replicates were pooled.

G. Growth Comparison of Laboratory Reared and Wild Colonies of P. saucia Larvae on Standard Diet and Diets with Addition of an Ethanolic A. tridentata Crude Extract

A chronic feeding bioassay was used to establish the response of a natural P. saucia population in comparison with the P. saucia laboratory population on standard artificial diet and diets with the addition of the EtOH-A. tridentata extract. Neonate F_1 larvae from field collected P. saucia and laboratory reared P. saucia (>20 generations) were divided into two groups (n=30). One group from each colony was allowed to feed on either the standard control diet or diet admixed with a ethanolic A. tridentata extract (50% nat. conc. dwt/dwt). As in previous experiments the neonates were each placed in individual feeding containers with ca. 500 mg of diet. After 8 days the larvae were counted and weighed. The larval weights were $\log_{10}$ transformed and analyzed by a two way analysis of variance.

H. Phytochemical Investigation

1. Chromatographic Separation of the Ethanolic A. tridentata Extract

Centrifugal thin layer chromatography was used to separate the active component(s) of the crude ethanolic extract of A. tridentata. An aliquot of extract equivalent to eight grams of plant powder was eluted on a 1.5 x 10 cm silica gel column (60-200 mesh) with EtOH and reduced to 3 ml. The reduced extract was loaded onto a Chromatatron™ plate (2 mm) and developed

with successively more polar solvents (hexane, hexane:$CHCl_3$ (1:1), $CHCl_3$:acetone (6:1), $CHCl_3$, EtOH, and MeOH) to give ten 10-ml fractions, 77 5-ml fractions and 2 50-ml fractions. The first 5 fractions were hexane eluates followed by 44 largely acetone and $CHCl_3$ eluates and 40 EtOH-MeOH fractions. The eighty-nine fractions were spotted on silica gel thin layer chromatography (TLC) plates containing a fluorescent indicator (Baker-flex™, IB2-F). The plates were developed using a standard method for sesquiterpene lactones (Picman et al. 1980) with $CHCl_3$-acetone (6:1). TLC plates were observed under short- and long- wave ultraviolet light and then sprayed with a vanillin spray reagent. Fractions containing similar compounds based on TLC observations were pooled into 5 major fractions according to their phytochemical constituents and then reduced in volume under vacuum. Pooled fractions consisted of: major fraction no. 1 containing hexane eluates (fractions 1-5), major fraction nos. 2,3 and 4 were mainly $CHCl_3$ eluates (fractions 6-13,14-26,27-48) and major fraction no. 5 contained EtOH and MeOH fractions 49-89. Major fraction no. 3 was further separated on the Chromatatron™ as it contained TLC spots common to both major fractions nos. 2 and 4. Major fraction no. 3 was eluted with hexane:$CHCl_3$ (2:1,1:1,1:2,1:6) and $CHCl_3$ giving 60 fractions that were spotted on TLC plates, developed and analyzed as above. The 60 fractions of major fraction no. 3 were divided between major fraction nos. 2 (1-35) and 4 (36-60). The resulting 4 major fractions were bioassayed with neonated P. saucia larvae (n=20) using an 80% conc (dwt/dwt) of the extract major fraction. A positive control consisting of the original extract was included as well as a solvent ($CHCl_3$) control.

2. Chromatography of Sesquiterpene Lactones Previously Isolated from A. tridentata

To determine the chemical nature of the highly active A. tridentata extract, pure sesquiterpene lactone standards were chromatographed with the most active major fractions from the previous experiment. Ten sesquiterpene lactones were obtained from Dr. Richard G. Kelsey, Dept. of Chemistry, University of Montana that had previously been isolated from A. tridentata and its closely related subspecies (Kelsey and Shafizadeh 1979). Dehydroleucodin, dihydrosantamarin, arbusculin A, arbusculin B, arbusculin C, (tatridin A - purity questionable), matricarin, deacetoxymatricarin, deacetylmatricarin, and dehydroreynosin were chromatographed with the major fractions obtained in the previous experiment and the original ethanolic A. tridentata extract. The two solvent systems used consisted of $CHCl_3$:acetone (6:1) (Picman et al. 1980) and petroleum ether:$CHCl_3$:Et_2OAc (2:2:1)(Greissman and Griffin 1971). Plates were developed in either saturated or non-saturated TLC tanks and examined under long- and short-wave ultraviolet light, and treated with the vanillin colour reagent as described above.

IV. RESULTS

A. Laboratory Screenings

Table II shows the growth inhibitory effects on P. saucia larvae of crude plant extracts added at 5 times the natural concentration (dwt/dwt) to artificial diet. Six of the 12 extracts exhibited sufficient antibiosis (sensu Painter 1951) to proceed to the next screening. Since the extract concentrations were high, only the six treatments that completely or severely inhibited growth advanced to the second screening. No larvae survived on the diets with ethanolic and petrol extracts of A. tridentata and C. suaveolens; the ethanolic extract from C. nauseosus and the ethanolic extract from C. diffusa were similarly active. The only other extract significantly different from the controls was the S. jacobaea ethanol extract, but at the high concentration used in the bioassay it was not considered to be sufficiently active for further screening.

The results of the unextracted plant powder and marc bioassay are shown in Table III. The efficiency of the extraction process in removing growth inhibitory phytochemicals is determined by testing the extracted plant residue for larval growth inhibition. In cases where diet with the unextracted plant powder severely inhibited P. saucia larval growth, at least one of the respective marcs was shown to have had the growth inhibitory agents removed.

Table II. Effects of weed extracts[1] incorporated into artificial diet on growth and survival of neonate *P. saucia* in an initial screening bioassay

PLANT SPECIES	GROWTH (% OF CONTROL)		SURVIVORSHIP (%)[2]	
	Petrol	Ethanol	Petrol	Ethanol
A. tridentata	0c[3]	0c	0	0
C. diffusa	106a	8c	63	3
C. nauseosus	80ab	0c	67	0
C. suaveolens	0c	0c	0	0
S. jacobaea	105a	18bc	90	37
T. dubius	107a	72ab	80	80
control	100a	100a	77	73

[1]Extract concentrations were five times the natural conc.(dwt/dwt).

[2]N=30

[3]Treatments followed by the same letter are not significantly different (Tukey's studentized range (HSD) test, p=0.05).

Table III. Growth and survival of P. saucia neonate larvae fed on artificial diets with unextracted plant powder or on the extracted marcs[1].

PLANT	POWDER		ETHANOL MARC		PETROL MARC	
SPECIES	GROWTH[2]	SURVIVAL[3]	GROWTH	SURVIVAL	GROWTH	SURVIVAL
A. tridentata	0g[4]	0	121abc	50	19fg	53
C. diffusa	47cdef	87	34defg	77	31efg	70
C. nauseosa	149ab	40	163ab	90	34bcde	50
C. suaveolens	18g	30	114abcd	77	190ab	80
S. jacobaea	15g	30	98abcd	73	145ab	77
T. dubius	96abcd	67	147ab	43	277a	73
Standard diet			272a	70		
Control (with cellulose)			100abcd	80		

[1]Plant material incorporated with artificial diet (1:1 dwt/dwt).

[2]Taken as the percentage of larval growth of the control treatment with cellulose filler simulating the plant material.

[3]Percentage of total larval survivors (N=30).

[4]Treatments followed by the same letter are not significantly different (Tukey's studentized range (HSD) test;p=0.05).

Specifically, the diet containing the unextracted C. suaveolens and S. jacobaea powders produced significantly smaller larvae and higher mortality than both of their marcs. Larvae fed the marc diets from these plants grew as well, or better than control larvae. Antibiosis was similiarly high among the P. saucia larvae fed the diets with unextracted A. tridentata powder, in which no survivors were observed. Furthermore the weights of larvae fed the A. tridentata ethanol marc diet did not differ significantly from the controls. The extracts from A. tridentata and C. suaveolens that produced these marcs were also shown to be the most active P. saucia larval growth inhibitors (Table IV). These results demonstrate that in nearly every case where P. saucia growth inhibitors were present in the unextracted plant powders they were removed by one or both of the extracting solvents.

P. saucia larvae fed diet without cellulose were, on the average, over two and a half times heavier than those fed diet containing cellulose (50% dwt). The dietary addition of plant powders or marcs, however, did not always reduce larval growth. Larvae fed seven of the treatment diets resulted in heavier larvae on average than the control larvae fed the standard diet with cellulose.

The results of the second screening experiment at natural concentrations are comparable to those of the plant powder and marc experiment. Even at the reduced concentration (100% dwt/dwt) the diet containing the A. tridentata ethanolic extract resulted in 100% P. saucia larval mortality (Table IV).

Table IV. Effects of selected weed extracts incorporated into artifical diet at natural concentrations on the weight and survival of neonate *P. saucia* fed for 11 days

PLANT SPECIES	EXTRACT	LARVAL WEIGHT (% OF CONTROL)	SURVIVORSHIP (% OF TOTAL)[1]
A. tridentata	PETROL	63ab[2]	51
A. tridentata	ETHANOL	0d	0
C. diffusa	ETHANOL	44ab	92
C. nauseosus	ETHANOL	31bc	96
C. suaveolens	PETROL	9d	36
C. suaveolens	ETHANOL	12cd	8
control		100a	96

[1]N=25 neonate *P. saucia* larvae

[2]Treatments followed by the same letter are not significantly different (p=0.05) using Tukey's studentized range (HSD) test.

In addition, larvae fed diets with both C. suaveolens extracts grew significantly less and had reduced survivorship compared to larvae fed on control diet. These results agree with the plant powder and marc experiment showing that diets incorporating plant powders of A. tridentata and C. suaveolens are the most biologically active towards P. saucia larvae.

Larvae surviving the second screening were transferred to control diet to examine latent effects of neonatal growth inhibition on later larval growth and development. Between 50 and 100% of the surviving P. saucia larvae emerged as adults from all treatments. These treatments, therefore, do not appear to cause any obvious physiological damage which persists through the pupal to the adult stage.

Fig. 1 shows the results of the dose-response experiment using ethanol and petrol extracts of C. suaveolens and the A. tridentata ethanolic extract at four concentrations. P. saucia larval weight was inversely related to the plant extract concentration for all 3 crude extracts. The resulting EC_{50}'s were 36, 39, and 42% for the C. suaveolens EtOH- and petrol extract, and A. tridentata EtOH-extract, respectively. Similar results were obtained when this experiment was replicated. EC_{50}'s for the petrol extract of C. suaveolens and the EtOH extract of A. tridentata were 37 and 35%, respectively.

Survival of P. saucia larvae was mainly concentration-dependent for each extract. Survivorship showed a linear response for the A. tridentata ethanolic and C. suaveolens petrol extracts (with r^2 values of 0.84 and 0.87, rspectively),but survivorship on the C. suaveolens ethanolic extract did not correlate well with the linear equation (r^2= 0.52) (Fig. 1). Pooled results from two dose-response experiments suggest that low dietary

concentrations of the plant extracts (0-40% natural conc.), had little effect on survival. The highest dietary concentration (80%) usually resulted in greatly reduced survivorship. Fig. 1 shows mortality is largely uneffected until the concentration increased between 40% and 60% for the three extracts.

Antibiosis increased with concentration as shown by the results of both survival and growth inhibition (Fig. 1). Dose-dependant antibiosis was evident initially as larval growth inhibition and at higher doses as both increased growth inhibition and mortality. The mode of action of the bioactive plant extracts on P. saucia larvae was examined further in a later experiment measuring consumption and growth rates along with dietary utilization. As in the previous experiment, surviving larvae placed on control diet, allowed to pupate and emerge, showed no obvious persistent physiological effects.

Age-dependent effects were also examined in a dose-response experiment. Six-day old, second instar larvae appeared more tolerant to the plant extracts than neonates when tested at the same concentrations. For example, even at an extract concentration of 80% the mortality was consistently 15% or less relative to the controls (Fig.2). Furthermore, growth was 33, 52 and 72% of controls for the A. tridentata EtOH and the C. suaveolens EtOH- and petrol extracts, respectively, at this concentration.

The biological activity of these extracts is not restricted to P. saucia larvae. Larvae of another polyphagous noctuid, the alfalfa looper, A. californica, were also tested in a chronic feeding dose-response experiment (Fig. 3). The EC_{50}'s of A. californica neonates were 10-20%

Figure 1. Percent growth (o) relative to control growth and percent total mortality (•) of P. saucia neonates fed ethanolic extracts from A) A. tridentata , and B) C. suaveolens, and a petrol extract from C) C. suaveolens admixed to artificial diets (n=25 larvae per concentration with each extract). Error bars on the growth points are the standard deviation.

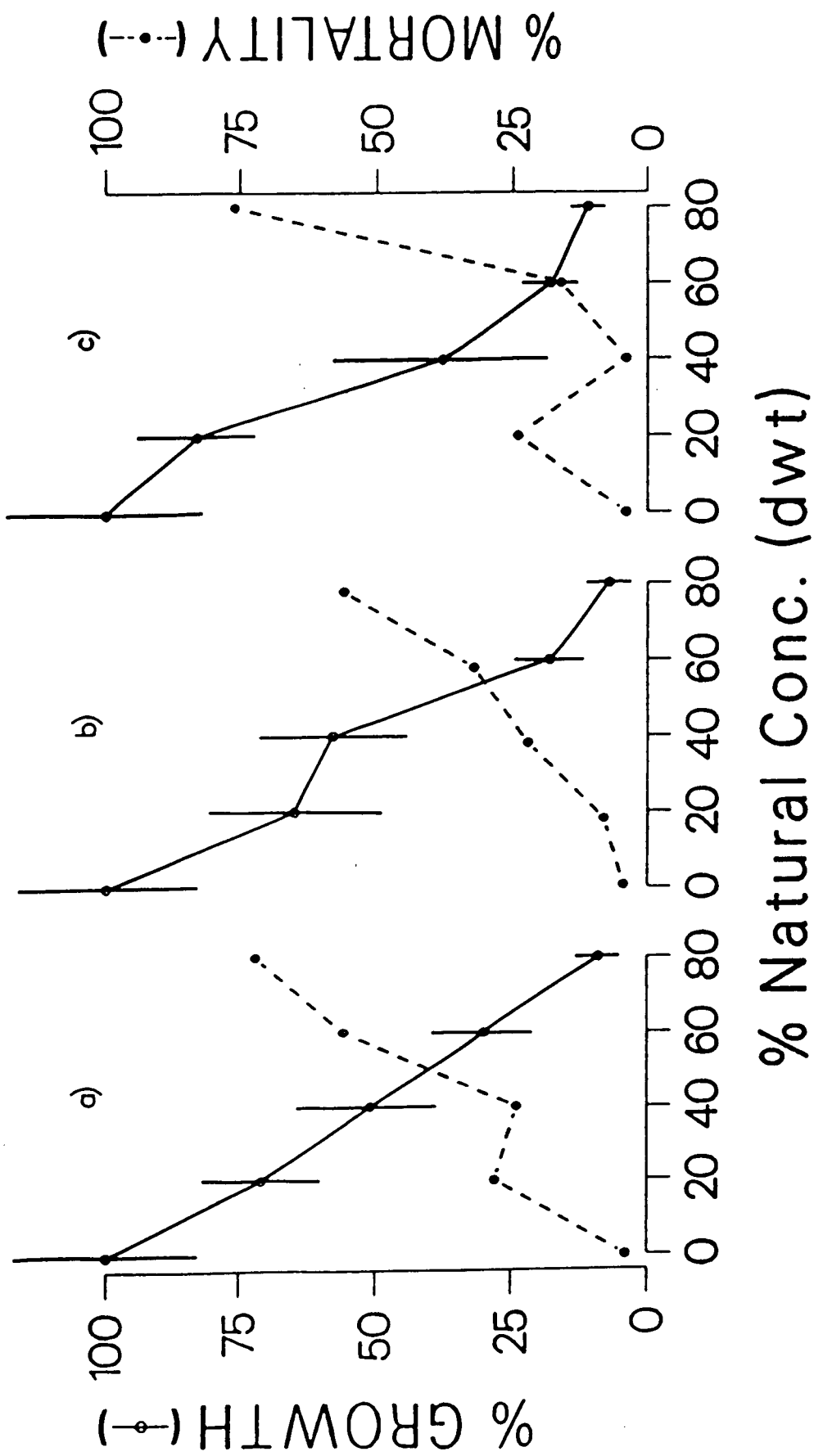

% MORTALITY (---•---)
100
75
50
25
0
c)
b)
a)
0 20 40 60 80
% GROWTH (—o—)
% Natural Conc. (dwt)

Figure 2. Percent growth (o) relative to control growth and percent total mortality (●) of six day-old *P. saucia* larvae fed ethanolic extracts from A) *A. tridentata* , and B) *C. suaveolens*, and a petrol extract from C) *C. suaveolens* admixed to artificial diets (n=25 larvae per concentration with each extract). Error bars on the growth points are the standard deviation.

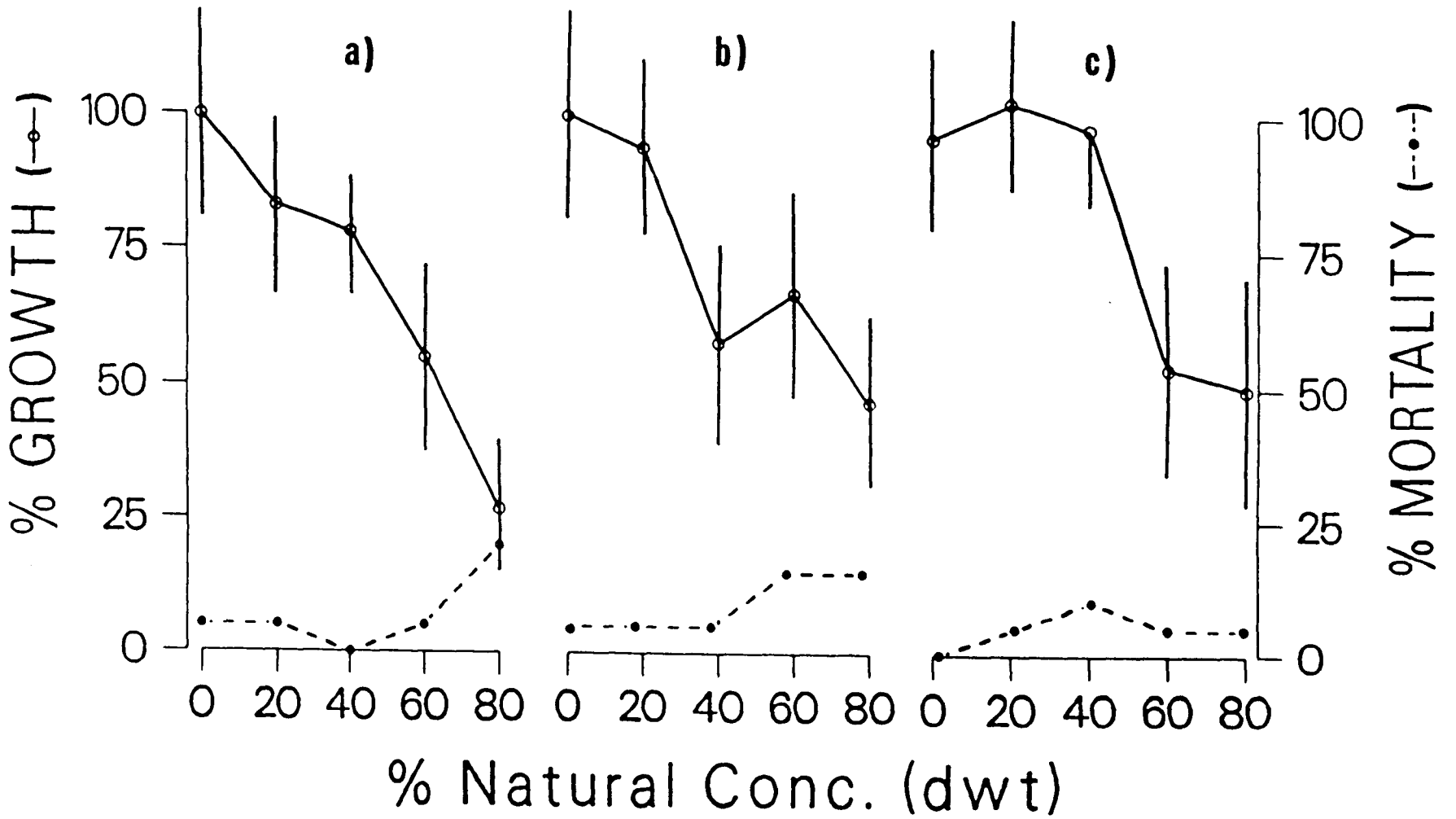
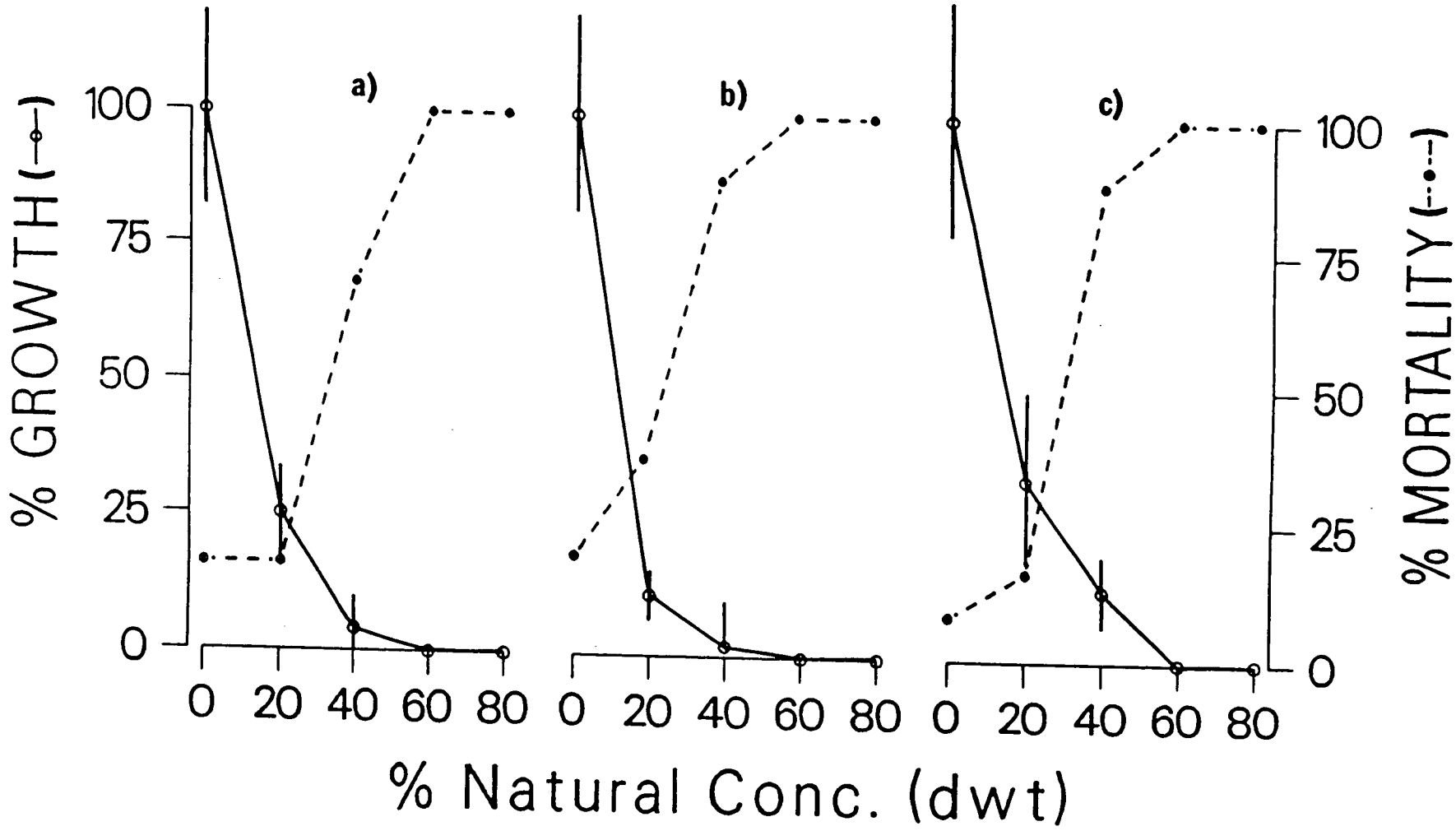

a)
b)
c)
% GROWTH (—o—)
% MORTALITY (--•--)
100
75
50
25
0
0 20 40 60 80
% Natural Conc. (dwt)

Figure 3. Percent growth (o) relative to control growth and percent total mortality (•) of neonate A. californica fed ethanolic extracts from A) A. tridentata , and B) C. suaveolens, and a petrol extract from C) C. suaveolens admixed to artificial diets (n=25 larvae per concentration with each extract). Error bars on the growth points are the standard deviation.


% GROWTH (—o—)
100
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a)
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0 20 40 60 80
0 20 40 60 80
0 20 40 60 80
100
75
50
25
0
% MORTALITY (---•---)
% Natural Conc. (dwt)

(natural conc.) for all three extracts. These EC_{50}'s are about half the observed EC_{50}'s for P. saucia larvae and no A. californica larvae survived the 60 or 80% concentrations for any of the extracts.

B. Detailed Growth Analysis of P. saucia Larvae on the C. suaveolens and A. tridentata Extracts.

The results of detailed growth analysis of second instar P. saucia, fed diets containing the A. tridentata EtOH extract and the C. suaveolens petrol extract, are shown in Table V. The approximate digestibility (AD) for the three larval cohorts did not differ significantly. The relative growth rate (RGR) is a product of relative consumption rate (RCR) and dietary utilization. The RGR for larvae fed diets with the C. suaveolens petrol extract was 70% of the control-diet fed larvae. The majority of this growth inhibition appears to be associated with behavioral factors as indicated by the significantly lower RCR, whereas dietary utilizations do not differ significantly from the controls. The P. saucia larvae fed diets with the A. tridentata EtOH extracts produced even lower growth rates than larvae fed the C. suaveolens extract. This severe growth inhibition, however, appears largely due to physiological effects. This is indicated by the extremely low net (ECI) and gross (ECD) dietary utilization even while the consumption rate remained about 60% of the control.

Separate consideration of the RGRs over the 48 h experiment reveal an interesting phenomenon. The RGR for larvae fed the C. suaveolens petrol extracts during the first 24 h period was severely retarded relative to the controls, but in the second 24 h period the growth rate accelerated to equal the RGR of the control.

Table V. Effects of dietary A. tridentata extract (EtOH) and C. suaveolens extract (petrol) on second instar P. saucia digestibility of food (AD), relative growth rate (RGR), relative consumption rate (RCR), and gross (ECI)[1] and net (ECD)[1] dietary utilizations.

Nutritional Index	Dietary Supplement		
	C. suaveolens	A. tridentata	Control
n=	14	13	15
AD ±SD[2] (mg dwt/mg dwt-day x 100)	59.6 ± 15.2	58.2 ± 33.8	51.8 ± 14.6nsd[3]
RGR ±SD (mg dwt/mg dwt-day)	0.44 ± 0.09 b[4]	0.03 ± 0.15 c	0.58 ± 0.10 a
RCR ±SD (mg dwt/mg dwt-day)	2.2 ± 0.6ab	1.6 ± 1.2 b	2.7 ± 0.4 a
ECI ±SD (mg dwt/mg dwt-day x 100)	20.4 ± 6.3 a	-3.6 ± 24.6 b	22.0 ± 5.1 a
ECD ±SD (mg dwt/mg dwt-day x 100)	44.9 ± 48.2 ab	0.8 ± 59.5 b	52.9 ± 44.6 a

[1]ECI=efficiency of conversion of ingested food
ECD=efficiency of conversion of digested food

[2]SD = standard deviation

[3]nsd = not significantly different

[4]Means in a row followed by the same letter are not significantly different (Tukey's studentized range (HSD) test, p=0.05).

The stability of crude extracts is important for reliable reproduction of laboratory experiments and for insect controls in the field. The growth inhibitory response of P. saucia to extracts kept at 4°C for eight months was numerically though not significantly different ($p<0.05$) from freshly prepared extracts (Table VI). However, in both treatments of the fresh as compared to the stored extracts, larval mortality was significantly reduced (orthogonal comparisons; $p<0.05$). Freshly prepared extracts were subsequently used in further experiments.

The results of the formulation trials of crude A. tridentata and C. suaveolens extracts are shown in Table VI. There was an almost complete loss in activity of the C. suaveolens extracts when diluted with water. The consistent growth inhibitory activities of the A. tridentata extracts formulated in 20% aq EtOH and the added physiological component of growth inhibition determined its selection for the field trial. The undissolved tar and suspended particulates in the water formulated extracts did not present problems in the laboratory bioassay because the extract was admixed with the artificial diet. Field spraying, however, required a more soluble medium, thus a 30% aq EtOH solution was used to dissolve most of the extract residues.

Table VI. Mean growth and percent survival of neonate P. saucia larvae fed artificial diets incorporating fresh and eight month old ethanolic extracts, hot water, room temp water and 20% aq ethanol formulations of A. tridentata and C. suaveolens for 16 days

Treatment	% Survival (n=25)	Mean weight ± SD[1]
A. tridentata		
95% ethanol		
fresh	24	2.6 ± 2.5fg[2]
aged[3]	68	13.8 ± 6.6def
water	96	34.9 ± 25.5cd
Hot water	100	41.9 ± 23.3bc
20% aq EtOH	40	8.7 ± 5.3ef
C. suaveolens		
95% ethanol		
fresh	12	1.1 ± 0.6g
aged[3]	52	5.8 ± 5.3fg
water	100	98.2 ± 45.7a
Hot water	100	121.5 ± 62.4a
20% aq EtOH	100	79.4 ± 26.6ab
Control	96	101.7 ± 35.6a

[1]SD=standard deviation

[2]Means followed by the same letter are not significantly different (Tukey's studentize range (HSD) test, p=0.05).

[3]Extracts were kept at 4°C for eight months.

C. Field Trial of the A. tridentata Extract on Cabbage

The results of the field spraying are illustrated in Figures 4, 5, and 6. Significant differences between treatments were observed in the aggregate number of cabbage pests computed as CLEs (Fig. 4, Appendix I). When treatments were compared for the entire duration of the experiment, the cabbage treated with A. tridentata extract had significantly ($p<0.05$) fewer CLEs compared with the carrier solvent (30% aq EtOH) treatment and the water sprayed cabbage. There was no significant difference in CLEs between the 30% aq EtOH and the water sprayed cabbage. In addition, the insecticide treatment of deltamethrin was shown to give excellent control of the lepidopteran cabbage pests. The significant linear effect indicates that there was a general increase in the number of pest larvae on the cabbage over the duration of the experiment. The significant residual in the polynomial analysis shows that the first-order polynomial did not account for all the variation in the experiment. The 'treatment X day' interaction was significantly different ($p<0.001$) and the separation of the sum of squares showed that the linear interaction of the deltamethrin, in contrast with the rest of the treatments, accounts for most (62%) of the variation. The significant quadratic component measures the additional improvement due to fitting the second-order polynomial. This shows that the variation in the CLE of the deltamethrin treatment contrasted against the rest of the treatments does not closely follow the linear relationship.

Separate considerations of the key cabbage pests reveal highly significant differences ($p<0.001$) in the larval counts of ICW between the A. tridentata treatment and the 30% aq EtOH and water treated cabbage plots (Fig. 5, Appendix II). About 35% of the variation in ICW larval counts is due to this significant difference. The plots treated with deltamethrin

had significantly ($p<0.001$) fewer ICW larvae than all the other treatment plots. The deltamethrin contrast, however, accounted for 62% of the total variation. ICW larval population counts were not significantly different between the water treated plants and the 30% EtOH treated plots. Separation of 'treatment X day' interaction sum of squares for the deltamethrin versus the other treatments in Appendix II again shows significant linear and quadratic variation.

One of the most encouraging results comes from the analysis of the ICW egg counts. Low numbers of ICW eggs on the A. tridentata sprayed cabbage suggest an oviposition deterring effect (Fig. 6). The A. tridentata sprayed cabbage had significantly ($p<0.001$) fewer ICW eggs than the 30% aq EtOH and water sprayed plants (Appendix III). Interestingly, plants exposed to the deltamethrin-spreader treatment had significantly ($p<0.001$) higher ICW egg counts than all other treatments. Reduced numbers of ICW eggs in the A. tridentata treated plots relative to plots treated with its carrier suggest one reason for the continued suppression of ICW larvae in the A. tridentata treated plots (Fig. 5).

Figure 4. Percentage change in cabbage looper equivalents (CLE) after field spraying cabbage with a) 30% aq ethanolic solution of A. tridentata (0.2 g/ml), b) 30% aq ethanol, c) deltamethrin 2.5 EC (17 µg/l a.i.) with 0.1% Superspred or d) distilled water, July 24, 1985.

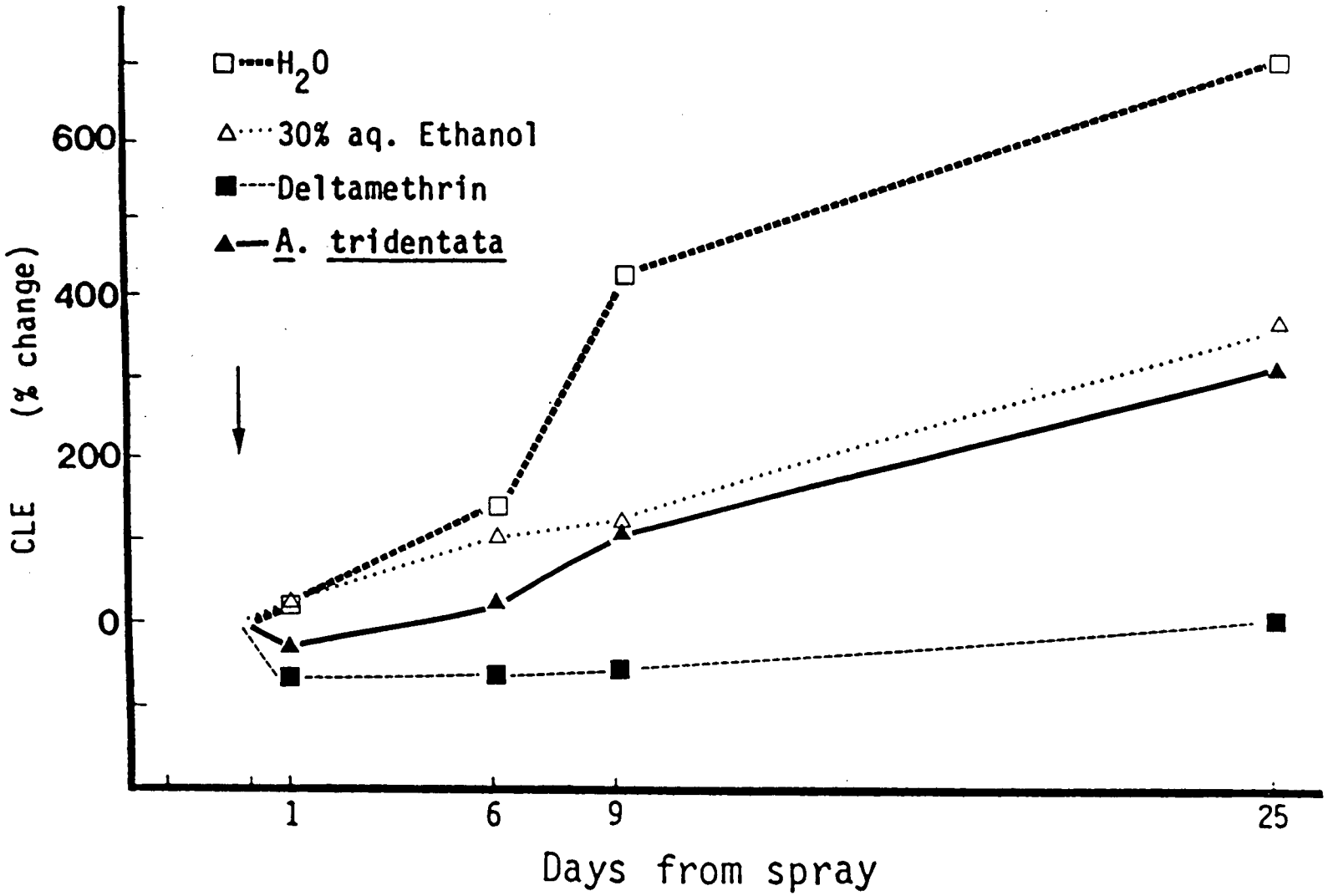

□----H_2O
△····30% aq. Ethanol
■----Deltamethrin
▲—A. tridentata
600
400
200
0
CLE (% change)
1
6
9
25
Days from spray

Figure 5. Percent change in imported cabbageworm, P. rapae larval populations before and after field spraying cabbage with a) 30% aq ethanolic solution of A. tridentata (0.2 g/ml), b) 30% aq ethanol, c) deltamethrin 2.5 EC (17 µg/l a.i.) with 0.1% Superspred or d) distilled water, July 24, 1985.

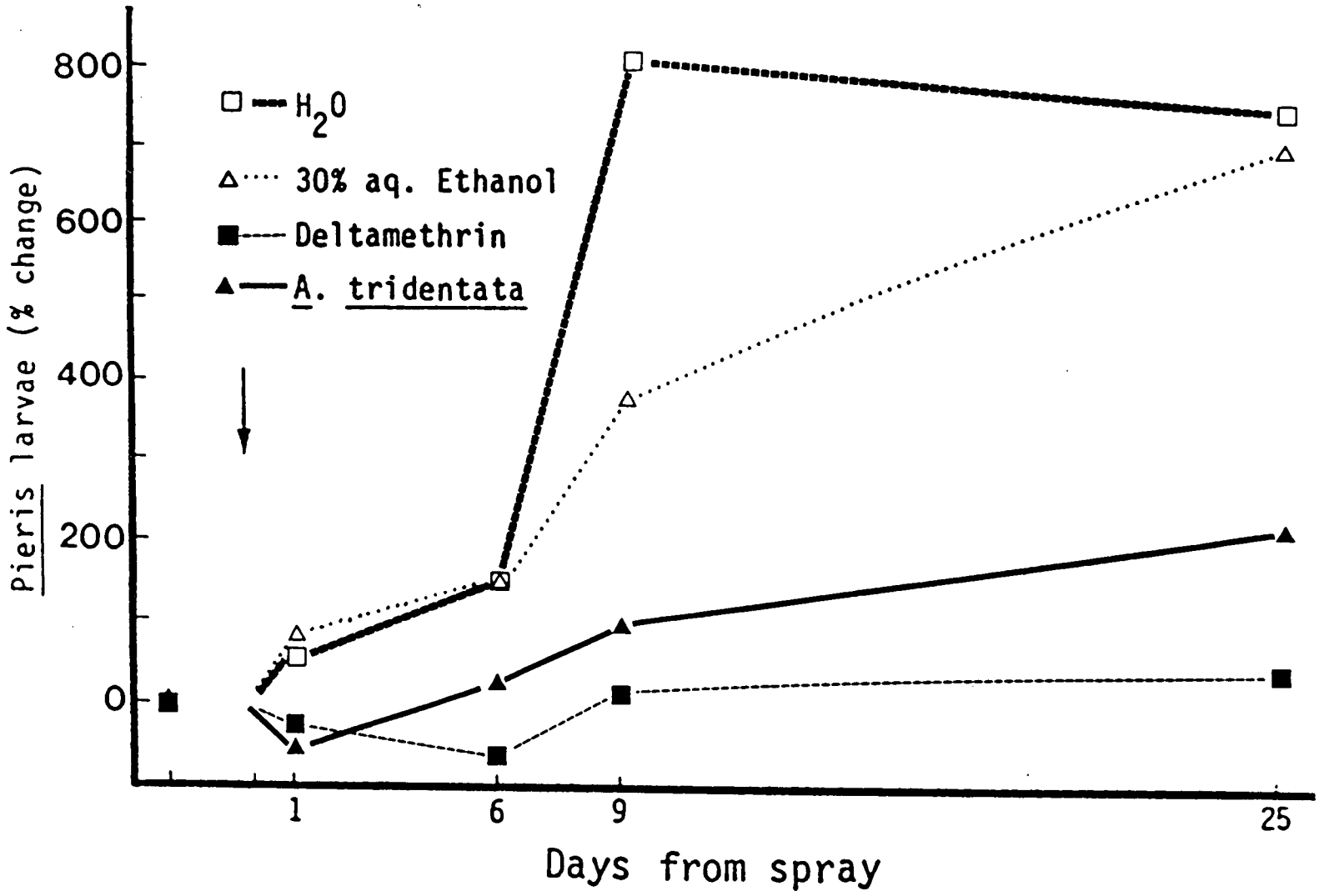
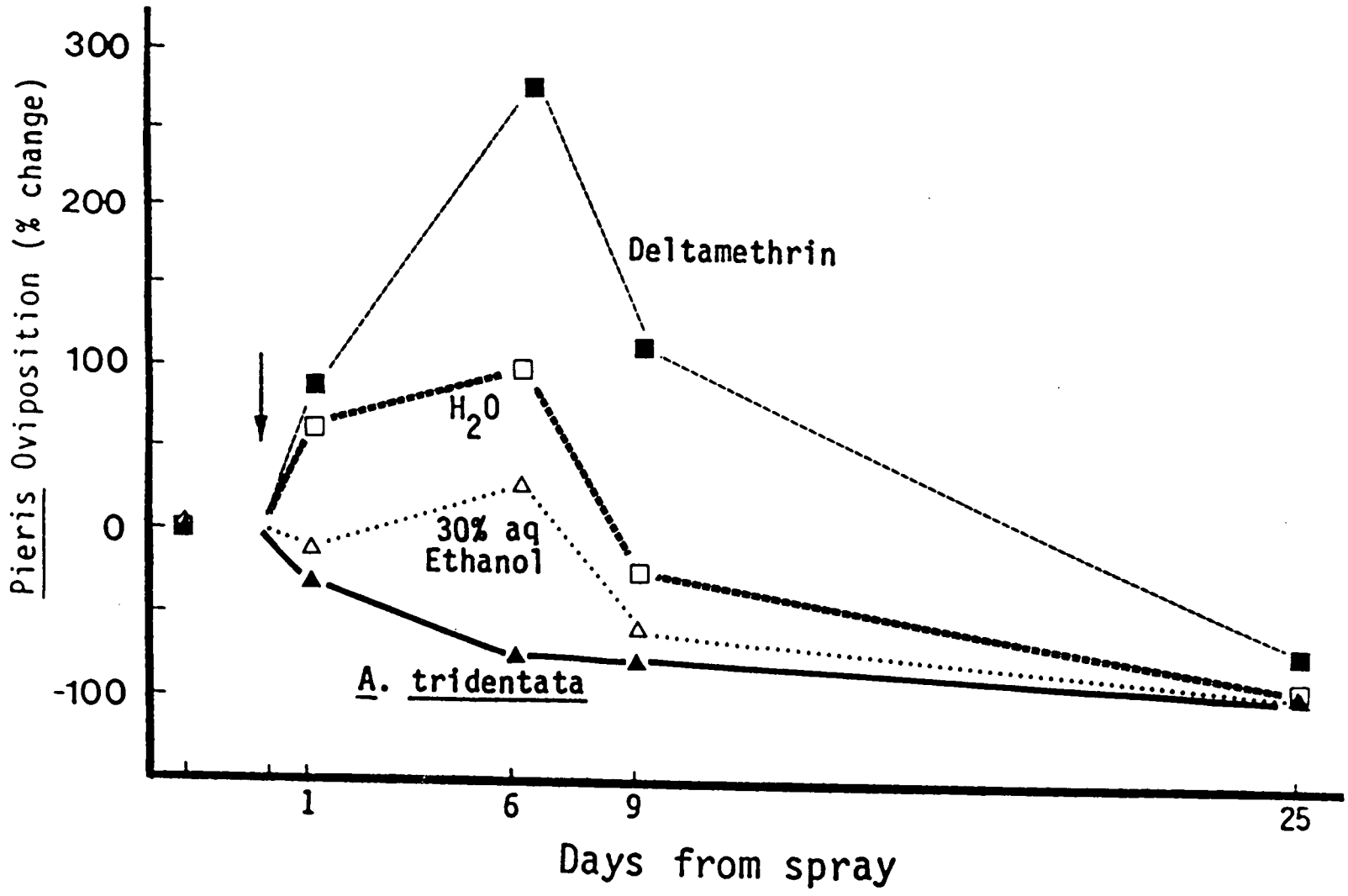

800
600
400
200
0
Pieris larvae (% change)
H2O
30% aq. Ethanol
Deltamethrin
A. tridentata
1
6
9
25
Days from spray

Figure 6. Percent change in imported cabbageworm, P. rapae, oviposition surveyed before and after field spraying cabbage with a) 30% aq ethanolic solution of A. tridentata (0.2 g/ml), b) 30% aq ethanol, c) deltamethrin 2.5 EC (17 µg/l a.i.) with 0.1% Superspred™ or d) distilled water, July 24, 1985.


Pieris Oviposition (% change)
300
200
100
0
-100
Deltamethrin
H_2O
30% aq
Ethanol
A. tridentata
1
6
9
25
Days from spray

D. Oviposition of P. rapae: Laboratory Experiment

The oviposition deterring effect of the A. tridentata extract in the field was confirmed in a controlled laboratory experiment. While a total of over 105 eggs were laid in the two experimental trials only two eggs were laid on the cabbage leaves painted with the A. tridentata extract. Leaves with ethanol and water solutions received almost all of the eggs, 58 and 45 respectively. Female P. rapae were observed alighting on the extract sprayed cabbage leaves but without ovipositing either in the laboratory or in the field experiments.

E. Quality of Cabbage Heads from the Field Trial

The visual quality estimate of the field sprayed cabbage (Table VII) showed that the single spraying of the A. tridentata extract produced cabbage of significantly ($p<0.05$) higher quality than the 30% EtOH and water sprayed plants. The cabbage sprayed with deltamethrin, however, received the highest visual quality estimate, which was significantly higher ($p<0.001$) than all the other treatments. No significant difference ($p=0.7$) was detected between the EtOH and water sprayed treatments.

F. Comparison of Wild Versus Laboratory Reared P. saucia Larvae

A comparison of the growth response of two separate populations of P. saucia fed diets with and without a growth inhibiting extract are shown in Table VIII. The F_1 P. saucia larvae from the field-collected population grew significantly faster than larvae from the laboratory colony. After feeding on the standard diet for 8 days the P. saucia from the field population had grown an average of 137 mg versus 57 mg for the lab-reared larvae. The P. saucia larvae fed diet with A. tridentata extract grew, as

Table VII. Mean visual quality estimates of cabbage treated with an A. tridentata ethanolic extract, deltamethrin, 30% aq ethanol, and water, recorded 25 days post-treatment.

Treatment	Concentration	Mean estimate ±SD[1]
A. tridentata (30% aq EtOH)	0.2 g-eq	1.6 ± 0.7b[2]
Deltamethrin	17.9 µg/l	2.5 ± 0.7a
Ethanol	30% aq	1.3 ± 0.8c
distilled water		1.3 ± 0.6c

[1]Visual quality estimates ± standard deviation; based on a scale from 0-4. The scale is an estimate of market quality ie. 0=no head remaining, 1=unmarketable more than one hole in the head, 2=garden grade, one hole into head, 3=sauerdraut grade, exterior damage only, no holes, 4=marketable cabbage, no exterior or interior damage.

[2]Means followed by the same letter are not significantly different ($p>0.05$, means separated by orthogonal contrasts).

Table VIII. Mean P. saucia larval weight of a F_1 field collect population compared to the laboratory colony fed the standard artificial diet and diet containing a 50% A. tridentata ethanolic extract (dwt/dwt) for 8 days.

Diet Treatment Source of larvae	% Survival (n=30)	Mean weight ±SD[1] (mg)	%RC[2]
Standard diet			
Lab colony	90	56.9 ± 27.9	
Field colony	100	136.6 ± 43.6	
A. tridentata diet[3]			
Lab colony	90	13.5 ± 8.7	23.7
Field colony	80	34.8 ± 14.7	25.5

[1] standard deviation

[2] % of respective control

[3] 50% (dwt/dwt) concentration

Two-way analysis of variance

Source of Variation	DF	SS	F	Probability
Model	3	16.9	25.6	0.0001
between populations	1	8.3[a]	37.8	0.0001
between diets	1	7.5	34.0	0.0001
popul. * diets	1	0.2	0.8	0.3833
Error	104	22.9		

[a] Sum of squares of larval growth are adjusted for mortality.

expected, significantly less than the larvae fed the standard diet. However, growth of the field P. saucia larvae was 4-fold more than the lab-reared P. saucia on extract-treated diet.

Interestingly, there was no significant interaction between larval origin and response to dietary A. tridentata extract. In other words, the proportional growth inhibition (75%) was not significantly different between the two larval populations, thus indicating that percentage of control larval growth is a valid expression for laboratory experiments.

G. Preliminary Phytochemical Investigation

The chromatographically separated ethanolic extract from A. tridentata was pooled into four major groups with generally distinct chemical profiles: a non-polar hexane fraction, two groups from $CHCl_3$ eluates, and a final group of EtOH and MeOH eluates. Table IX shows the growth and mortality of P. saucia fed diets incorporating these elutions. The two $CHCl_3$ fractions accounted for most of the growth inhibition and mortality in P. saucia larvae. There was no significant difference among the other fractions or the control.

The direct chromatography of sesquiterpene lactones with the two biologically active fractions is shown in Figs. 7,8,9, and 10. The high biological activity of the two chromatographically separated major fractions of A. tridentata could not be consistently correlated with any of the ten pure sesquiterpene lactones available. Although some of the R_f values and colour reactions corresponded in one solvent system, there were no unambiguous matches in both solvent systems or tank arrangements.

Table IX. Mean larval weight of neonate P. saucia fed artificial diet admixed with chromatographically separated fractions of an A. tridentata ethanolic extract compared to the original extract at ecological concentrations and the standard diet[1].

TREATMENT	% SURVIVAL (n=20)	% LARVAL WEIGHT (of control)
A. tridentata		
ELUANT		
#1-Hexane	95	63.5a[2]
#2-$CHCl_3$	40	2.8b
#4-$CHCl_3$	30	1.3b
#5-MeOH,EtOH	95	74.2a
Original extract	0	0.0c
standard diet	95	100.0a

[1]The standard diet was treated with petrol

[2]Larval growth followed by the same letter are not significantly different, Tukey's studentized range (HSD) test (p=0.05).

Major fraction no. 2 is a highly complex phytochemical mixture containing seven major constituents, of which five give a positive colour reaction with the vanillin reagent. Ten other spots were visible in short- and long-wave ultraviolet light and using colour reactions with the vanillin reagent.

Major fraction no. 4 appears as a chemically simpler mixture of five major TLC spots. Two of the spots fluoresced blue with long-wave ultraviolet light and the other 3 spots gave positive colour reactions with vanillin reagent. Six other minor constituents were also detected in major fraction no. 4.

Figure 7. Thin-layer chromatograph of fractions 2 (fr#2) and 4 (fr#4) from the separation of a crude ethanolic A. tridentata extract chromatographed with phytochemicals from Artemisia spp. The pure sesquiterpene lactones compared to the A. tridentata fractions were: Dehydroleucodin (dhl), dihydrosantamarin (dhs), arbusculin A (abA), arbusculin C (abC), matricarin (mat), deacetoxymatricarin (dom), deacetylmatricarin (dam), and dehydroreynosin (dhr). Non-fluorescent colours occurred after developing the plate with a vanillin reagent and the arrows indicate a colour shift after 24 h. TLC developed with petroleum ether:$CHCl_3$:Et_2OAc (2:2:1) in a non-saturated tank.

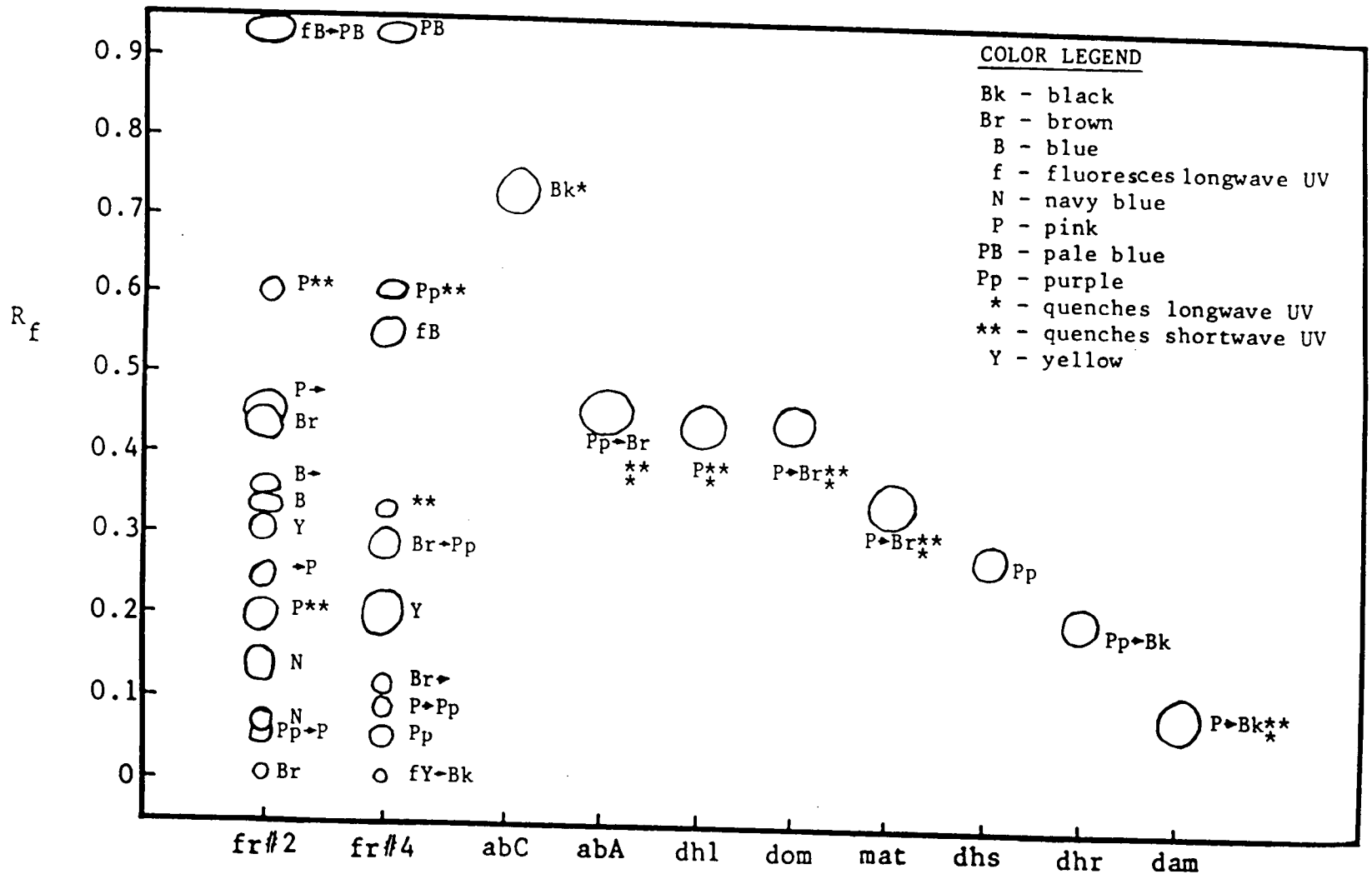

R_f
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
fB←PB
PB
Bk*
P**
Pp**
fB
P←
Br
B←
B
Y
←P
P**
N
N
Pp←P
Br
**
Br←Pp
Y
Br←
P←Pp
Pp
fY←Bk
Pp←Br
**
*
P**
*
P←Br**
*
P←Br**
*
Pp
Pp←Bk
P←Bk**
*
COLOR LEGEND
Bk - black
Br - brown
B - blue
f - fluoresces longwave UV
N - navy blue
P - pink
PB - pale blue
Pp - purple
* - quenches longwave UV
** - quenches shortwave UV
Y - yellow
fr#2
fr#4
abC
abA
dhl
dom
mat
dhs
dhr
dam

Figure 8. Thin-layer chromatograph of fractions 2 (fr#2) and 4 (fr#4) from the separation of a crude ethanolic A. tridentata extract chromatographed with phytochemicals from Artemisia spp. The pure sesquiterpene lactones compared to the A. tridentata fractions were: dehydroleucodin (dhl), dihydrosantamarin (dhs), arbusculin A (abA), tatridin A (ttA), matricarin (mat), deacetoxymatricarin (dom), and deacetylmatricarin (dam). Non-fluoresent colours occurred after developing the plate with a vanillin reagent and the arrows indicate a colour shift after 24 h. TLC developed with CHCl3:acetone (6:1) in a saturated tank.

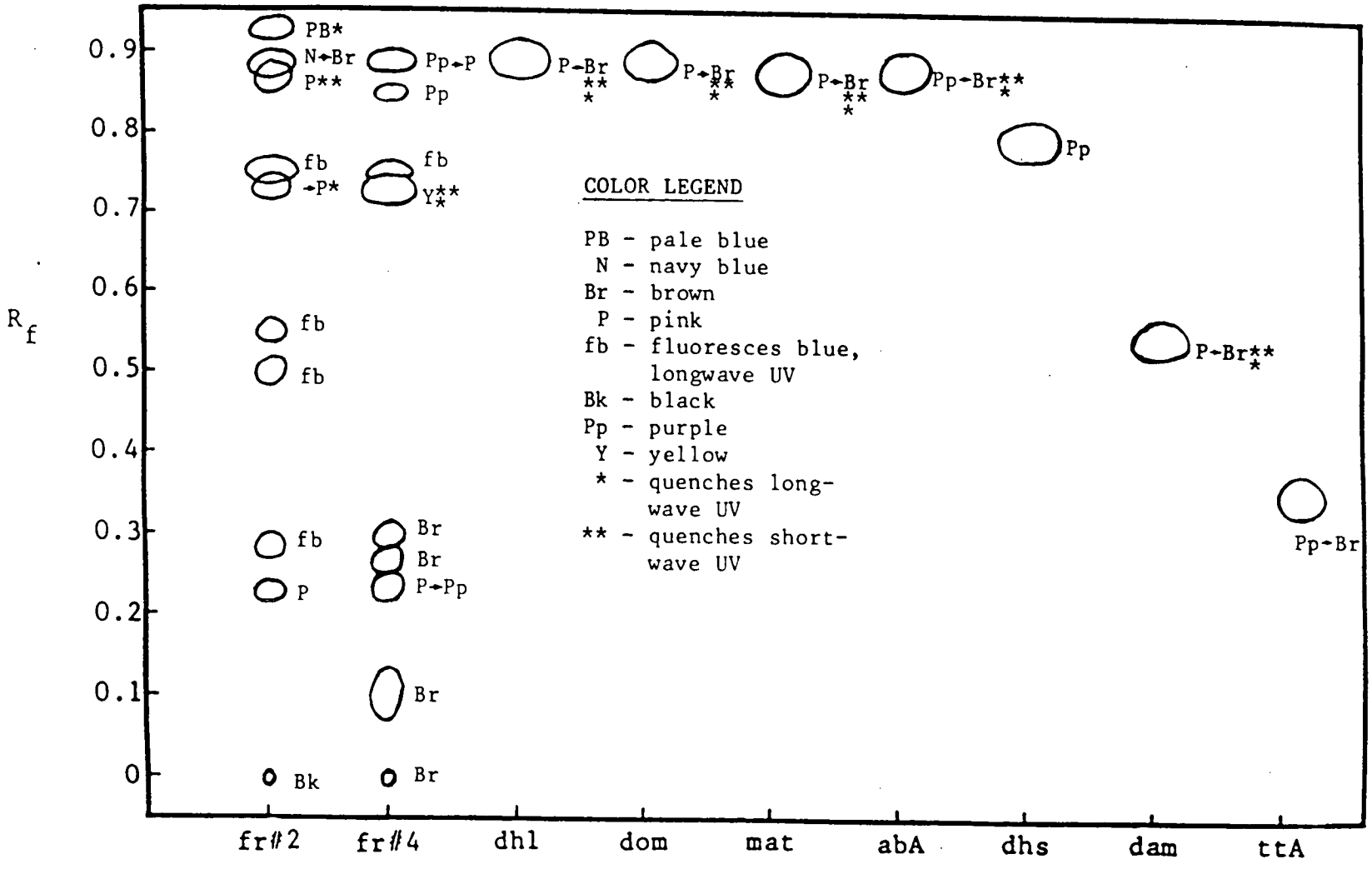

R_f
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
PB*
N→Br
P**
Pp→P
Pp
P→Br **
*
P→Br **
*
P→Br **
*
Pp→Br**
*
Pp
fb
→P*
fb
Y**
*
fb
fb
fb
P
Br
Br
P→Pp
Br
Bk
Br
P→Br**
*
Pp→Br
COLOR LEGEND
PB - pale blue
N - navy blue
Br - brown
P - pink
fb - fluoresces blue, longwave UV
Bk - black
Pp - purple
Y - yellow
* - quenches long-wave UV
** - quenches short-wave UV
fr#2
fr#4
dhl
dom
mat
abA
dhs
dam
ttA

Figure 9. Thin-layer chromatograph of fractions 2 (fr#2) and 4 (fr#4) from the separation of a crude ethanolic A. tridentata extract chromatographed with phytochemicals from Artemisia spp. The pure sesquiterpene lactones compared to the A. tridentata fractions were: Dehydroleucodin (dhl), arbusculin A (abA), arbusculin B (abB), arbusculin C (abC), tatridin A (ttA), matricarin (mat), deacetoxymatricarin (dom), deacetylmatricarin (dam), and dehydroreynosin (dhr). Non-fluorescent colours occurred after developing the plate with a vanillin reagent and the arrows indicate a colour shift after 24 h. TLC developed with $CHCl_3$:acetone (6:1) in a saturated tank.

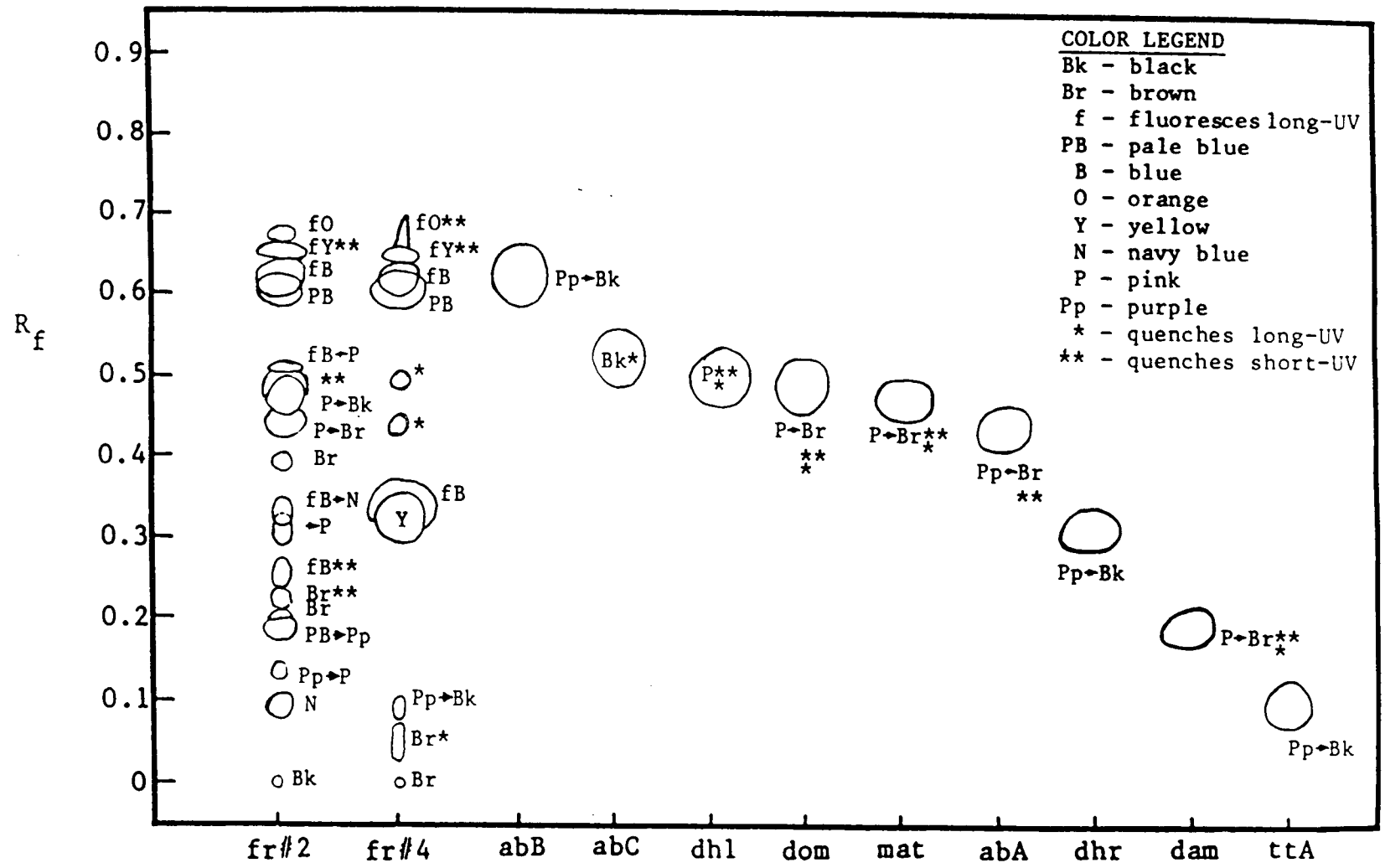
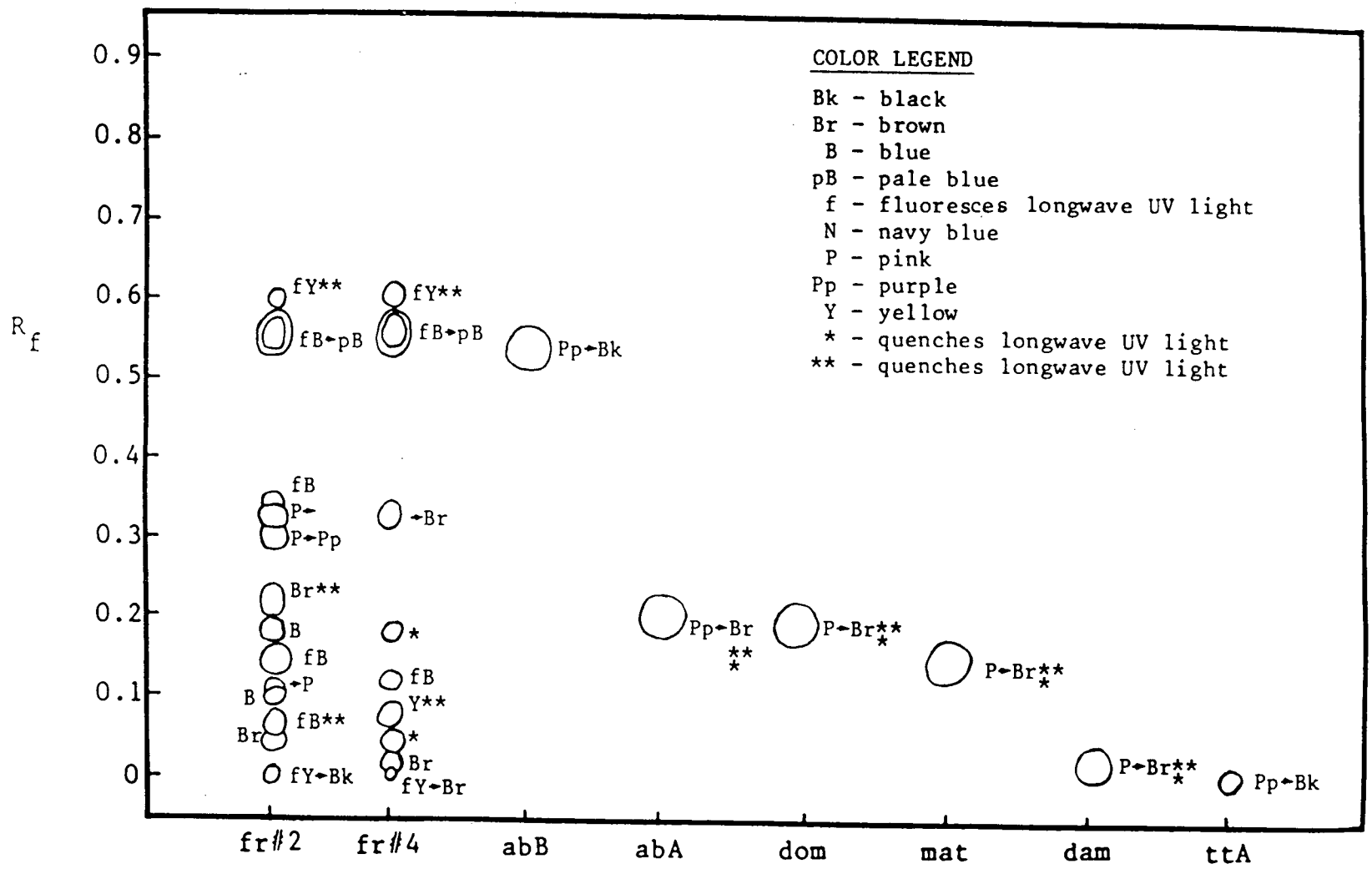

COLOR LEGEND
Bk - black
Br - brown
f - fluoresces long-UV
PB - pale blue
B - blue
O - orange
Y - yellow
N - navy blue
P - pink
Pp - purple
* - quenches long-UV
** - quenches short-UV
R_f
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
fO
fY**
fB
PB
fO**
fY**
fB
PB
Pp→Bk
fB→P
**
P→Bk
P→Br
Br
*
*
Bk*
P**
*
P→Br
**
*
P→Br**
*
Pp→Br
**
fB→N
→P
Y
fB
fB**
Br**
Br
PB→Pp
Pp→P
N
Pp→Bk
Br*
Bk
Br
Pp→Bk
P→Br**
*
Pp→Bk
fr#2
fr#4
abB
abC
dhl
dom
mat
abA
dhr
dam
ttA

Figure 10. Thin-layer chromatograph of fractions 2 (fr#2) and 4 (fr#4) from the separation of a crude ethanolic A. tridentata extract chromatographed with phytochemicals from Artemisia spp. The pure sesquiterpene lactones compared to the A. tridentata fractions were: arbusculin A (abA), arbusculin B (abB), tatridin A (ttA), matricarin (mat), deacetoxymatricarin (dom), and deacetylmatricarin (dam). Non-fluorescent colours occurred after developing the plate with a vanillin reagent and the arrows indicate a colour shift after 24 h. TLC developed with petroleum ether:$CHCl_3$:Et_2OAc (2:2:1) in a non-saturated tank.


COLOR LEGEND
Bk - black
Br - brown
B - blue
pB - pale blue
f - fluoresces longwave UV light
N - navy blue
P - pink
Pp - purple
Y - yellow
* - quenches longwave UV light
** - quenches longwave UV light
R_f
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
fr#2
fr#4
abB
abA
dom
mat
dam
ttA
fY**
fB→pB
fY**
fB→pB
Pp→Bk
fB
P→
P→Pp
→Br
Br**
B
fB
B
→P
Br
fB**
fY→Bk
*
fB
Y**
*
Br
fY→Br
Pp→Br
**
*
P→Br**
*
P→Br**
*
P→Br**
*
Pp→Bk

V. DISCUSSION

A. Screening Asteraceous Extracts for Insect Growth Inhibitors

Plant extracts from many families have been screened as insect control agents. Many large screenings for botanical insecticides occurred prior to the advent of synthetic insecticides (Jacobson and Crosby 1971). Recent public interest in alternatives to conventional insecticides has supported the scientific effort to find environmentally sound insect controls (Abivardi & Benz 1984, Bernays 1983). Screening for botanical insect controls has often focused on acute toxicity but the past ten years have seen a renewed interest in materials with more subtle actions, such as growth regulators, larval growth inhibitors, and oviposition deterrents.

Extracts from certain asteraceous plants are reported to inflict a variety of deleterious effects on insects; some extracts from the Asteraceae have been shown to act as insect repellents (Hwang et al. 1985), feeding inhibitors (Isman and Rodriguez 1984, Nawrot et al. 1982) oviposition deterrents (Lundgren 1975, Burnett and Jones 1978) and contact insecticides (Jacobson and Crosby 1971). The insect growth inhibitory activity and chronic toxicity of ethanolic and petrol extracts of six weeds in the Asteraceae (Table I) have also been assessed.

Many factors determine an insect's response to plant allelochemicals, such as insect species, stage of development, concentration of the allelochemical, and the chemical context in which the allelochemical is presented to the insect. Lepidopteran larval sensitivity to growth inhibitors has been shown previously to be inversely correlated with larval age (Reese 1983, Isman and Duffey 1982). These results follow this pattern in that younger P. saucia larvae were more sensitive to effects of the phytochemical growth inhibitors than older larvae (Fig 1 & 2). Neonate P.

saucia larvae grew less, and suffered greater mortality than, second instar larvae fed artificial diet containing the same levels of asteraceous extracts (Fig 1 and 2). Younger lepidopteran larvae may possess fewer endosymbiotic microorganisms needed for detoxification (Jones et al. 1981), or lower levels of constituative detoxifying enzymes (Ahmad 1986).

Insect growth inhibition could result from behavioral factors (e.g., feeding deterrency), physiological factors (e.g., microsomal enzyme suppression) or both. Schroeder (1976) has shown that decreases in larval food utilization can be induced by food deprivation. Starvation or behavioral food aversion resulting in a lower RCR may also decrease nutrient utilization. Table V shows that when P. saucia larvae were fed the A. tridentata extract, even though the RCR was 60% of the controls, the ECI of those larvae was only 5% of the controls (Table V). Thus it is likely that the severely reduced food utilization of the A. tridentata fed larvae was due to physiological factors rather than a lower consumption rate.

Larval growth on C. suaveolens-petrol diet is initially inhibited as shown by a RGR of 51% of that of the controls for the first 24 hrs of the 48 hr nutritional experiment. In the second half of the experiment these larvae recovered from the prior inhibition and attained an RGR equal to the control fed larvae. In contrast, the RGR for P. saucia larvae fed an A. tridentata-ethanolic diet was significantly lower (Tukey's studentized range (HSD) test; $p=0.05$) than the controls for both 24 hr periods and remained essentially the same at 27 and 29% of control fed larval RGR, respectively. This implies that growth inhibitors that function mainly as behavioral feeding deterrents can be readily overcome by insects, whereas growth inhibitors that decrease nutrient utilization may protect plants better because of their more persistent activity.

The ability of the extraction process to remove insect growth inhibitors is an important step in an efficient screening process. Table III shows that solvent extraction removed insect growth inhibitors in nearly every case where inhibitors were present in the unextracted plant powders. Increases in larval growth on the marc-diets indicate that the extraction process was efficient in removing insect growth inhibitors from the plant material.

The dilution of insect diet with a non-nutritive, non-deterrent substance has been shown to increase food consumption in a number of insects (Dadd 1970). Cockroaches have been shown to increase feeding in response to dietary dilutions of cellulose (Bignell 1978). In contrast, growth is reduced in P. saucia larvae by dietary additions of cellulose (Table III). This may result from a decrease in available nutrition, phagostimulation, or both. Increased consumption does not necessarily imply increased fitness but may lead to decreased dietary utilization, ultimately resulting in growth reduction. Results in Table III show that P. saucia larvae fed many of the diets (e.g., the ethanolic marc-diet from C. nauseosa and the petrol marc-diet from C. suaveolens and T. dubius) grew more than did larvae fed the cellulose containing control diet. Artificial diets including plant material may possess additional nutrients or phagostimulants lacking in the cellulose containing control diet.

Insect growth inhibition and feeding deterrent activity has previously been reported in asteraceous plants. The growth inhibition and feeding deterrency, however, appears to be species specific rather than broad spectrum. Nawrot et al. (1982) screened 23 extracts from asteraceous plants against three coleopteran pests of stored products and found that 7 extracts possessed strong feeding deterrency. Interestingly, there was no consistency between the extracts' activity amongst the insect species

tested. These authors later confirmed that sesquiterpene lactones were in part responsible for the feeding deterrency of the asteraceous extracts (Nawrot et al. 1984, Harmatha and Nawrot 1984). Table IV shows that of the extracts examined, C. suaveolens and A. tridentata had the strongest inhibitory activity on P. saucia larval growth. An ethanolic extract of A. tridentata was chosen for field evaluation because of superior performance against A. californica larvae (Fig. 3) and because it significantly lowered the RGR and ECI of the P. saucia larvae (Table V).

Several investigators have chosen extracts of Artemisia species as potent insect growth and feeding inhibitors. Villani and Gould (1985) investigated crude extracts from twelve Asteraceae and found that two, Artemisia dracunculus and Santolina virens, deterred feeding by corn wireworm, Melanotus communis. Suomi and associates (1986) examined eleven Asteraceae (and 14 other plants) for feeding deterrency to larval codling moth, Cydia pomonella. They found the strongest deterrency in the Asteraceae extracts from Artemisia absinthium, Chrysothamnus nauseosus and Tanacetum vulgare. Yang (1983) has reported that two phenlylalkynes from the buds of A. capillaris were feeding deterrents for the imported cabbageworm, Pieris rapae. In choice experiments Jermy et al. (1981) reported that Colorado potato beetle (Leptinotarsa decemlineata) larvae were inhibited from feeding on leaf disks coated with an ethanolic extract from A. tridentata. In my study I have shown that an ethanolic extract of A. tridentata strongly inhibits larval growth of two lepidopteran larvae, A. californica and P. saucia, in feeding bioassays. The above results indicate that extracts of Artemisia species have broad spectrum activity on phytophagous insect pests.

B. Phytochemicals and Insect Growth Inhibition

Research on insect growth inhibitors and feeding deterrents has often focused on the isolation of specific phytochemicals. Many investigators have emphasized individual compounds and single classes of phytochemicals as the key to insect-plant interactions. In nature however, phytophagous insects are always exposed to complex mixtures of phytochemicals. Considering the within plant diversity of chemicals, interactions among phytochemicals may be a common determining factor in insect/plant relations (Berenbaum 1985).

Plant defense strategies using chemical mixtures probably occur more frequently than defensive strategies using single allelochemicals. Most plants contain more than one defensive phytochemical (Berenbaum 1985, Harborne 1982), but, few studies have examined the growth inhibitory activities among co-occurring phytochemicals. In the limited number of cases where co-occurring chemicals have been examined, the results underscore the importance of phytochemical interactions. Adams and Bernays (1978) examined the effects of fourteen simple phenolic chemicals from Sorghum bicolor fed to Locusta migratoria at naturally occurring concentrations. These phytochemicals produced a measurable feeding deterrency only when combined. When feeding deterrents from unrelated chemical groups were combined in binary combinations (e.g., sinigrin and tomatine) deterrent effects were often additive (Adams and Bernays 1978).

Phytochemicals presented as a mixture may have a greater than additive effect on insects. Berenbaum and Neal (1985) report the synergistic effects of the methylene dioxyphenyl compound, myristicin and the co-occurring furanocoumarin, xanthotoxin, at naturally occurring concentrations. Insect growth may be reduced more effectively by a

chemical mixture causing different behavioral and physiological activity than a single deterrent chemical.

Polyphagous pests are generally more resistant to growth inhibitors than insects with a narrow host range (Bernays 1983) and thus may provide evidence of a broader spectrum of growth inhibitory activity. In the present thesis, the growth of the highly polyphagous P. saucia larvae was significantly reduced relative to the controls by ethanolic extracts from five of the six plants investigated but only two of six petrol extracts tested at five times the natural concentration. This indicates that the growth inhibitors in the plants chosen (Table I) contain mostly polar compounds. The greater proportion of the ethanol extracts exhibiting potent activity support the results of Freedman et al. (1979).

A. tridentata is known to contain many phytochemicals (Table X) and some are reported to have insect growth and feeding inhibitory activity. Kelsey and Shafizadeh (1979) have isolated several sesquiterpene lactones from A. tridentata and its subspecies. Jermy et al. (1981) bioassayed one of these, deacetylmatricarin. They reported good feeding deterrent activity against larval Colorado potato beetle but noted that significant feeding deterrent activity remained in the extract even after removal of deacetylmatricarin. Wisdom et al. (1983) tested five sesquiterpene lactones against H. zea and found that only a guaianolide from A. tridentata, dehydroleucodin, significantly reduced growth.

Sesquiterpene lactones from other plants have been shown to affect insect growth and feeding. Isman and Rodriguez (1983) found that several sesquiterpene lactones extracted from Parthenium species (Asteraceae) inhibited larval growth of H. zea. Burnett and co-workers (1974) reported that of six lepidopteran larval species examined, four were deterred from feeding on Vernonia spp. (Asteraceae) containing sesquiterpene lactones.

TABLE IX: Phytochemical constituents previously isolated from *Artemisia tridentata*

Monoterpenes[1]	Sesquiterpene lactones[2]	Coumarins[3]
camphor	matricarin	esculin
1,8-cineole	tatridin A, B, C	umbelliferone
delta-3-carene	deacetoxymatricarin	cichoriin
santolinyl ester	deacetylmatricarin	isoscopoletin
alpha-pinene	ridentin	scopoletin
camphene	detatin A, B	scoparon
	dehydroleucodin	esculetin
	arbusculin A, B, C	artelin

Flavonoids[4]

quercetagetin 3,6-dimethyl ether
quercetagetin 3,6,7-trimethyl ether
kaempferol 3,6,7-trimethyl ether
luteolin
luteolin-7-O-glucoside
6-methoxy luteolin
axillarin

[1]Buttkus et al. (1977) The listed monoterpenes comprise 80% of the essential oils

[2]Seaman (1982)

[3]Brown et al. (1975)., Murray et al. (1982) These compounds are 80% of the phenolic fraction of an *A. t.* spp. *vaseyana* extract.

[4]Rodriguez et al. (1972)

However, Jones et al. (1979) reported that cabbage looper, T. ni, and yellow woollybear, Spilosoma virginica, were not inhibited from feeding on diet containing the sesquiterpene lactones, glaucolide-A. The above results indicate that several sesquiterpene lactones have insect growth inhibitory and feeding deterrent properties. However, not all sesquiterpene lactones are effective and those that are do not show activity against all insect species tested.

In the present study four fractions of a chromatographically separated ethanolic extract of A. tridentata were assayed, and two fractions accounted for nearly all of the growth inhibitory activity of the initial extract (Table IX). Thin layer chromatographic separations (Figs. 8-11) showed that several major spots reacted to a vanillin reagent, suggesting that these were sesquiterpene lactones (Picman et al. 1980).

Camphor and 1,8-cineole, major monoterpenes in the essential oil of A. tridentata have previously been shown to be highly active against insects. Camphor is reported to be a mosquito repellent (Hwang et al. 1985), and 1,8-cineole has been shown to repel the American cockroach, Periplaneta americana (Scriven and Meloan 1984). Thin layer chromatographic (TLC) study of the two major fractions in this thesis revealed several spots that quenched ultraviolet light that may be monoterpenes (Croteau and Ronald 1983) in the most growth inhibitory fractions (Figs. 7-10).

Jermy et al. (1981) examined the feeding deterrency of several coumarins reported from A. tridentata and found that none of these reduced feeding of Colorado potato beetle larvae. Coumarin has, however, been shown to inhibit larval growth and development as well as adult fertility in the cotton leafworm, Spodoptera littoralis (Mansour 1982). In Figures 7-10 several TLC spots showed a weak blue fluorescence in the active fractions of A. tridentata that could be coumarins. Isman and Rodriguez

(1983) reported that quercetagetin 3,7-dimethy ether, a flavonoid from guayule (Parthenium argentatum) was a larval growth inhibitor of H. zea and S. exigua whereas a closely related 6-hydroxykaempferol 3,6,7-trimethyl ether was stimulatory to both insect species. The other phenolic compounds in A. tridentata have not been investigated for insect activity.

The aerial parts of A. tridentata are known to possess a wide range of biologically active compounds. Indigenous peoples of British Columbia used A. tridentata foliage as a disinfectant, insect repellent and as a deodorant when handling corpses (Turner 1979). Volatile (Nagy and Tengerdy 1967) and non-volatile (Ramirez 1969) components of the leaves are known to possess antibacterial activity. In addition, allelopathic activity has been reported from volatile and non-volatile leaf fractions (Groves and Anderson 1981).

Seasonal (Kelsey et al. 1982) and intraspecific (Shafizadeh et al. 1971) variations in the terpenoid content of A. tridentata have been reported; until the active ingredients are known and bioassayed with co-occurring compounds, one must be cautious in interpreting insect growth inhibitory and oviposition deterrence activity.

Kelsey and co-workers (1983) suggest that the biological activity of A. tridentata may be a result of synergism between the volatile essential oils and other secondary compounds like sesquiterpene lactones and phenolics. Although this hypothesis is intriguing it is nonetheless speculative.

C. Field Trials of Plant Extracts

The proper selection of botanicals as field-active control agents requires the evaluation of extracts in the target area. Since laboratory and greenhouse studies have not always predicted the effects in the field

(Obrycki and Tauber 1984, Haverty and Robertson 1982), field studies are an essential part of a complete screening procedure. While laboratory screenings of crude plant extracts for growth inhibitors and feeding deterrents are not uncommon, reports of field trials on extracts is scarce, save for work on neem extracts. Figures 4-6 show the results of a field trial on cabbage using an ethanolic extract of A. tridentata selected in the laboratory.

The suitable bioassay for both the pest and intended application should be carefully chosen when screening plant extracts against pest insects. For example, the acridid, Locusta migratoria, was substantially more sensitive to a wider range of compounds than four lepidopteran pest species tested (Simmonds et al. 1985). The anthranoid, harunganine, was a phagodeterrent to the polyphagous cotton leafworm Spodoptera littoralis when presented on cabbage, whereas the same compound was ineffective when the host plant was wheat, despite cabbage being a preferred plant (Simmonds et al. 1985).

The difference in response to a treatment can depend more on the test method than on the product tested. For example, larvae fed supra-optimal diets treated with moderate amounts of allelochemicals may exaggerate the larval growth response of treated versus control larvae. Another important factor to consider when using bioassays to screen pesticidal or inhibitory products is the number of choices given an insect. A dual choice feeding bioassay resulted in 100% deterrency of Pieris brassicae compared with only 19% deterrence in a single choice bioassay when fed an equivalent concentration of an Artemisia absinthium extract applied to leaf discs (Abivardi and Benz 1984). Results from laboratory screenings should be

substantiated in the target area (e.g., greenhouse or field). Field testing candidates selected in the laboratory may determine the relevance of the bioassay for screening suitable control agents.

The relevance of using results from laboratory reared insects to calculate concentration levels for field trials should be addressed when screening insect control products. Laboratory reared insects may respond differently than field populations of the same species (Brattsten et al. 1986), just as products that function well in the laboratory may not be stable under field conditions. I have shown (Table VIII) that P. saucia larvae from an F_1 generation of a field collected population were 2.5 times heavier than larvae from a two-year-old laboratory reared colony fed the same artificial diet with 50% (dwt/dwt) of an ethanolic A. tridentata extract. Therefore, a realistic estimation of the extract concentration needed should be performed on field collected insects or their offspring. Another part of this experiment showed the accuracy of the laboratory bioassay for reporting relative growth inhibition. There was no significant growth differences ($p=0.05$) between the laboratory reared or field collected populations of P. saucia larvae (Table VIII) fed diet containing A. tridentata extract when compared to their respective controls.

Natural plant defenses may be of use in agriculture for the management of pest populations. Several researchers have noted that the spraying of crude plant extracts should not present insurmountable problems (Jacobson 1983, Jermy et al. 1981), particularly for underdeveloped countries where variable efficacy is acceptable. The results reported herein show the feasibility of field spraying A. tridentata extracts formulated in 30% ethanol.

My field trial with an A. tridentata extract against cabbage insect pests using a single application resulted in a higher quality cabbage yield and lower larval pest counts than the solvent treated controls (Table VII). The standard insecticide spray of deltamethrin and Superspred™ gave the highest quality cabbage. The benefits of the A. tridentata extract were evident for the first week after spraying, but integrating other control options such as spraying more frequently or combining control strategies may make the A. tridentata extract more effective.

Cabbage looper equivalents (CLEs) have been used for measuring pest damage on cabbage from the three major lepidopteran cabbage pests (Shelton et al. 1982). In the present thesis, A. tridentata sprayed cabbage was significantly less damaged by major insect pests, estimated as CLEs, than the solvent treated controls (Fig. 4). Deltamethrin was shown to be a good choice as an insecticide standard as it resulted in significantly lower CLEs than any of the other treatments. Deltamethrin sprays, however, are reported to decrease the populations of several predatory arthropods such as carabids, staphilinids, spiders and phytoseiid mites (Matcham and Hawkes 1985, Basedow et al. 1985, Samsoe-Petersen 1985). Bernays (1983) suggests that one of the potential advantages of spraying plants with growth inhibitory compounds is that they may avoid damage to non-target organisms.

An important result of the field experiment was observed in the significantly lower larval ICW counts on the A. tridentata extract sprayed cabbage (Fig. 6). The lower counts of larval P. rapae may be due to an indirect mode of action of the A. tridentata extract. Reduced P. rapae egg counts could explain most, if not all, of the reduced larval populations infesting the cabbage foliage.

In plants, phytochemicals that deter oviposition are the first line of chemical defense against herbivorous insects. When females oviposit on plants, they determine larval food choice and survival of the succeeding generation. To optimize larval survivorship, adult oviposition preference and larval food suitability should be synchronized. Non-host plant extracts applied to crop plants may deter oviposition, and thus protect the crop from herbivory, if larvae have limited mobility as in the case of P. rapae. Lundgren (1975) tested several plant extracts, including Artemisia absinthium and A. abrotanum, for their ability to deter oviposition of three Pieris species in two-choice tests, and found significantly fewer eggs laid on extract treated cabbage leaves. In this thesis an A. tridentata extract applied to field grown cabbage resulted in significantly fewer ($p<0.05$) P. rapae eggs compared to cabbage treated with the carrier solvent alone and the deltamethrin-surfactant treatment (Fig. 7). The deltamethrin-surfactant treated cabbage received the most P. rapae eggs. (The surfactant, Triton-X-100^{TM}, has been shown to increase Plutella xylostella oviposition on Brussel sprouts [Perrin and Phillips 1978], but the surfactant effect was not isolated in this experiment.) Laboratory experiments confirmed the oviposition deterrency of the A. tridentata extract. Observations indicated the mode of oviposition deterrency of the A. tridentata treated cabbage was due to a contact chemoreception rather than repellency, because cabbage butterflies were not inhibited from alighting on the treated plants.

However, whether the P. rapae females were deterred from ovipositing, or if the plants were unrecognizable as host plants, is not clear. For example, cuticular components in tobacco have been shown to stimulate oviposition of H. virescens (Cutler et al. 1986). Tarsal contact with cabbage foliage was found to have important influences on the oviposition

behavior of P. rapae, whereas host-plant odor, ovipositor tip contact and previously laid eggs showed no influence (Traynier 1979). In the field and laboratory experiments reported in this thesis it remains to be determined whether spraying the cabbage masked oviposition stimulating cuticular chemicals, blocked P. rapae chemoreceptors, or activated deterrent receptors.

Non-host plant extracts and specific phytochemicals have been examined as oviposition deterrents to insect pests of cabbage. Non-host cruciferous and non-cruciferous extracts have been reported as oviposition deterrents for P. rapae (Renwick and Radke 1985). Tabashnik (1985) showed that coumarin sprayed cabbage deterred oviposition by P. xylostella. In the field experiment reported herein (Fig. 4), the large population of P. xylostella larvae on A. tridentata sprayed plants indicated that if coumarins were present in the extract, they were not factors in the field at the concentration sprayed.

Field trials of oviposition deterrents without the aid of field cages are rare. Most investigators that have ventured into the field have resorted to the use of cages and laboratory reared insects (e.g., Williams et al. 1986). The effects of the controlled environments and laboratory reared insects may have little bearing on the actual field situation. Nonetheless, Burnett and Jones (1978) using cage experiments with Vernonia plants, with and without sesquiterpene lactones, showed that oviposition preference depended on the species of moth. Yellow woollybear, Spilosoma virginica, showed no oviposition preference, the cabbage looper, T. ni, showed a preference for the two plants containing sesquiterpene lactones and the other three lepidopterans, (fall, southern and yellowstriped armyworms, Spodoptera frugiperda, S. eridania, and S. ornithogalli) showed a preference for the sesquiterpene lactone lacking Vernonia species.

Burnett and Jones (1978) also showed that the fall armyworm was significantly inhibited from ovipositing on the Vernonia species lacking sesquiterpene lactones when 1% glaucolide-A was applied to the foliage. The sesquiterpene lactones in the A. tridentata extract could therefore be responsible for the oviposition deterrency reported in this thesis on P. rapae.

Combining control strategies (ie. insecticide and plant extract) could have advantages over the use of either agent alone. Combinations of insecticides may prolong the use of existing and novel control techniques by slowing the rate of insect resistance (Georghiou 1983). Reduced synthetic insecticide use would lower the insecticide load on the crop and in the environment and may permit the return of beneficial organisms.

Growth inhibitors and oviposition deterrents may enhance the action of natural enemies if they are not adversely affected by these compounds. The use of growth inhibitors could, for example, be used in conjunction with the release of insect parasites and predators. Weseloh et al. (1983) have shown that release of the parasitic wasp, Apanteles melanoscelus, and field sprays of the lepidopteran pathogen, Bacillus thuringiensis, acted synergistically to control gypsy moth because the bacteria maintained the larvae longer in the second instar, which is the host stage preferred by the parasite. Velvet bean caterpillar, Anticarsia gemmatalis, and soybean looper, Pseudoplusia includens, are more susceptible to the entomophagous pathogen, Nomuraea rileyi, in the early instars (Boucias et al. 1984). If growth inhibitors maintain insects in stages vulnerable to predators and parasites, they may provide another tool for protecting crops in intensively managed agricultural systems.

Mammalian toxicity is an important factor when considering the merits of a pesticide. While there are no studies on mammalian toxicity of ethanolic A. tridentata extracts, big sagebrush is used as a major winter forage by pronghorn antelope (Cronin et al. 1978), mule deer (Hansen and Reid 1975), and pygmy rabbits (White et al. 1982). In addition, sesquiterpene lactones, one of the principle classes of secondary compounds in A. tridentata, have been investigated as antitumor agents (Lee et al. 1977).

Plant-derived chemicals are most often biodegradable and thus they might prove to be preferred alternatives to synthetic chemicals that persist in the environment. A. tridentata is listed as one of four weeds in the United States with the most potential for crop development and commercialization for sources of insect attractants, repellents or toxicants (Jacobson 1983). This species is drought tolerant (Rickard and Warren 1981) and can support a winter shoot removal of about 50% (Fetcher 1981). The resilience of A. tridentata shrubs adds to their potential as a new crop for exploiting semi-arid marginal lands (Jacobson 1983).

Insects that are rapidly developing resistance to synthetic insecticides create problems for pest control, while plants, with their multichemical defenses, may contain solutions to some of these problems. Reliance on mono-chemical pest control is inadequate and thus it is an opportune time to study pest control methods that have evolved in plants.

VI. CONCLUSIONS

The objectives of this thesis were to select a potent growth inhibitory extract from an asteraceous weed, to assess the extract for deleterious effect on insects, to evaluate the field efficacy of the most potent growth inhibitor and to explore the chemistry of insect growth inhibitory activity.

The results show that:

1. Of six asteraceous weeds extracted in EtOH and petrol, six of the 12 extracts inhibited P. saucia larval growth by more than 90% compared to the growth of control larvae (at five times naturally occurring concentrations).

2. Naturally occurring concentrations of an ethanolic extract from the leaves and flowers of A. tridentata and two of its chromatographic fractions significantly inhibited early larval growth of P. saucia.

3. Feeding bioassays showed a significant dose-response by P. saucia and A. californica larvae to A. tridentata and C. suaveolens extracts, but, no significant differences were found between these extracts. Extracts from both plants inhibited growth more in A. californica larvae than in P. saucia larvae and the A. tridentata ethanolic extract inhibited growth more in A. californica larvae than the C. suaveolens extracts.

4. Second instar P. saucia larvae were less sensitive to the growth inhibitory effects of the extracts than neonatal P. saucia.

5. Results obtained from the field trial suggest that the 30% aq A. tridentata ethanolic extract at 0.2 g/ml protected cabbage from insect pest damage significantly better than the water or EtOH controls. Insect pest damage to cabbage, however, was significantly less with the deltamethrin spray at 17.9 µg/l than in all other treatments.

6. Residual oviposition deterrency to P. rapae was suggested from results obtain in the field trial. Laboratory experiments with caged P. rapae appear to confirm a contact oviposition deterrency due to the A. tridentata ethanolic extract at 0.2 g/ml on cabbage.

7. An F_1 generation of field-collected P. saucia grew significantly better than the larvae from the laboratory colony. However, the growth inhibition of P. saucia larvae by the A. tridentata extract was not significantly different between the two populations relative to their respective controls.

My findings revealed that using insect growth inhibitory extracts, selected in laboratory bioassays, should constitute only the first stage in the development of novel botanical pest controls. The next stage should invariably be testing the products on target plants and insects in the field. Further investigations using more intensive phytochemical techniques may help elucidate the specific chemicals or chemical mixtures responsible for both the growth and oviposition inhibitory activity.

The extraction and screening of asteraceous weeds has advanced the potential use of these natural products in insect pest control programmes. Effective insect growth inhibitors and oviposition deterrents may be used in combination with other pest control options provided they are non-toxic to humans, economical to produce, and harmless in the environment.

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VIII. APPENDICES

APPENDIX I

Analysis of Variance Table including the separation of individual degrees of freedom using orthogonal contrasts for Cabbage Looper Equivalents, for the four treatments, four blocks and five survey days from the field trial on cabbage, July 25, 1985

SOURCE	DF	SUM SQ	F-VALUE	PROBABILITY
BLOCKS	3	0.928	0.897	0.4487
TREATMENTS	3	27.646	26.715	<0.0001
Deltamethrin vs. OTHERS[1]	1	25.596	74.203	<0.0001
A. tridentata vs. CONS[2]	1	1.744	5.055	0.0284
30% aq EtOH vs. H2O	1	0.306	0.886	0.3506
DAYS	4	45.650	33.085	<0.0001
TREATMENT * DAYS	12	14.464	3.494	<0.0007
Deltamethrin vs. OTHERS * LIN	1	8.524	24.712	<0.0001
Deltamethrin vs. OTHERS * QUA	1	2.819	8.173	0.0059
Deltamethrin vs. OTHERS * DEV	1	0.141	0.409	0.5251
A. tridentata vs. CONS * LIN	1	0.324	0.937	0.3367
A. tridentata vs. CONS * QUA	1	0.213	0.619	0.4348
A. tridentata vs. CONS * DEV	1	0.031	0.091	0.7646
30% aq EtOH vs. H2O * LIN	1	0.541	1.569	0.2155
30% aq EtOH vs. H2O * QUA	1	0.353	1.024	0.3159
30% aq EtOH vs. H2O * DEV	1	0.754	2.187	0.1447
ERROR	57	19.662		
TOTAL	79	108.35		

[1]OTHERS = the three other treatments, namely, A. tridentata extract in 30% aq EtOH, 30% aq EtOH, and H_2O

[2]CONS = the two controls, the carrier solvent 30% aq EtOH, and H_2O

APPENDIX II

Analysis of Variance Table including the separation of individual degrees of freedom using orthogonal contrasts for imported cabbageworm, P. rapae, larval counts for four treatments, four blocks and five survey days from the field trial on cabbage, July 25, 1985

SOURCE	DF	SUM SQ	F-VALUE	PROBABILITY
BLOCKS	3	2.475	2.986	0.0386
TREATMENTS	3	19.790	23.880	<0.0001
Deltamethrin vs. OTHERS[1]	1	12.173	44.067	<0.0001
A. tridentata vs. CONS[2]	1	6.896	24.964	<0.0001
30% aq EtOH vs. H2O	1	0.721	2.609	0.1118
DAYS	4	34.009	30.779	<0.0001
TREATMENT * DAYS	12	10.984	3.314	<0.0007
Deltamethrin vs. OTHERS * LIN	1	4.283	15.505	0.0002
Deltamethrin vs. OTHERS * QUA	1	2.001	7.245	0.0093
Deltamethrin vs. OTHERS * DEV	1	0.031	0.111	0.7403
A. tridentata vs. CONS * LIN	1	1.319	4.775	0.0330
A. tridentata vs. CONS * QUA	1	0.784	2.840	0.0974
A. tridentata vs. CONS * DEV	1	0.012	0.045	0.8327
30% aq EtOH vs. H2O * LIN	1	0.129	0.468	0.4967
30% aq EtOH vs. H2O * QUA	1	0.546	1.964	0.1653
30% aq EtOH vs. H2O * DEV	1	0.593	2.148	0.1482
ERROR	57	15.746		
TOTAL	79	83.003		

[1]OTHERS = the three other treatments, namely, A. tridentata extract in 30% aq EtOH, 30% aq EtOH, and H_2O

[2]CONS = the two controls, the carrier solvent 30% aq EtOH, and H_2O

APPENDIX III

Analysis of Variance Table including the separation of individual degrees of freedom using orthogonal contrasts for imported cabbageworm, P. rapae, egg counts, for the four treatments, four blocks and five survey days from the field trial on cabbage, July 25, 1985

SOURCE	DF	SUM SQ	F-VALUE	PROBABILITY
BLOCKS	3	9.363	4.270	0.0094
TREATMENTS	3	31.476	14.353	<0.0001
Deltamethrin vs. OTHERS[1]	1	17.901	24.488	<0.0001
A. tridentata vs. CONS[2]	1	12.287	16.809	0.0002
30% aq EtOH vs. H2O	1	1.288	1.763	0.1906
DAYS	4	77.694	26.572	<0.0001
TREATMENT * DAYS	12	23.006	2.6227	0.0089
Deltamethrin vs. OTHERS * LIN	1	0.008	0.010	0.9194
Deltamethrin vs. OTHERS * QUA	1	8.195	11.211	0.0016
Deltamethrin vs. OTHERS * DEV	1	0.599	0.819	0.3700
A. tridentata vs. CONS * LIN	1	0.563	0.771	0.3843
A. tridentata vs. CONS * QUA	1	5.515	7.544	0.0085
A. tridentata vs. CONS * DEV	1	3.808	5.209	0.0269
30% aq EtOH vs. H2O * LIN	1	0.675	0.924	0.3413
30% aq EtOH vs. H2O * QUA	1	0.149	0.204	0.6536
30% aq EtOH vs. H2O * DEV	1	0.037	0.051	0.8230
ERROR	57	35.087		
TOTAL	79	191.43		

[1]OTHERS = the three other treatments, namely, A. tridentata extract in 30% aq EtOH, 30% aq EtOH, and H_2O

[2]CONS = the two controls, the carrier solvent 30% aq EtOH, and H_2O