This is to certify that the

dissertation entitled

MANIPULATING OVIPOSITION OF THE ONION FLY, *DELIA ANTIQUA* (MEIGEN): A STIMULO-DETERRENT DIVERSIONARY APPROACH

presented by

RICHARD STEVEN COWLES

has been accepted towards fulfillment of the requirements for

Doctoral **degree in** Entomology

James R. Miller
Major professor

Date May 21, 1990

MANIPULATING OVIPOSITION OF THE ONION FLY, *DELIA ANTIQUA* (MEIGEN): A STIMULO-DETERRENT DIVERSIONARY APPROACH

By

Richard Steven Cowles

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Entomology

1990

ABSTRACT

MANIPULATING OVIPOSITION OF THE ONION FLY, *DELIA ANTIQUA* (MEIGEN): A STIMULO-DETERRENT DIVERSIONARY APPROACH

By

Richard Steven Cowles

Onions and onion models were used to bioassay onion fly host acceptance behavior, with the goal of developing strategies for controlling onion fly (*Delia antiqua* (Meigen)) oviposition. Stimulo-deterrent diversion (SDD) was developed, where the valued crop is treated with chemical deterrents, and simultaneously, a highly stimulatory ovipositional resource (onion culls) is deployed to concentrate eggs away from the crop.

A wide range of non-onion chemicals deterred onion fly oviposition. In laboratory choice experiments, pungent spices deterred oviposition by 88 to 100%, but were ineffective in no-choice conditions. Compounds with appreciable deterrency are: C8 to C13, intermediate in polarity, and possess either oxygen-containing or nitrile functional groups. When formulated in polyethylene pellets, (*E*)-cinnamaldehyde had a BR_{90} (concentration eliciting 90% deterrency) of 1.0% and (*E*)-4-methoxycinnamaldehyde had a BR_{90} of 0.38%. The air concentration of (*E*)-cinnamaldehyde at its BR_{90} was 1.7 ng/ml. Deterrents alone may not be sufficient for control; increased oviposition due to deprivation would require high deterrent concentrations.

The interaction of visual (red) and chemical (cinnamaldehyde) deterrent stimuli fit a purely multiplicative model, consistent with separate processing of host stimuli from different modalities during distinct host examining behaviors. Video

recordings of examining behavior revealed that red foliage decreased overall activity on a resource, while cinnamaldehyde diminished transitions to ovipositor probing. A greenhouse test of SDD also revealed a multiplicative response when deterrents plus culls protected seedling onions. Total eggs laid on seedlings were: seedlings-only (3185), seedlings + culls (1531), seedlings + deterrent (127), and seedlings + deterrent + culls (69). The probability of an onion fly accepting seedlings was reduced independently by the presence of deterrent and culls.

SDD can reduce pest density in a valued crop. It is suggested that increased pest densities in a diversionary crop will enhance biological control. A population genetics model using two-allele loci for avoidance and physiological resistance traits suggested that SDD combined with conventional insecticide could prevent or reverse pesticide resistance development. Requirements are: 1) higher suitability of the diversionary crop, 2) high finding of the diversionary crop, and 3) deterrents to which a pest is preadapted to respond.

To Elizabeth, for her love and her faith in my abilities,

to my parents, for nurturing my interest in plants and insects,

and to Jim, for being the quintessential mentor.

ACKNOWLEDGEMENTS

I would like to thank my committee: Drs. George Ayers, Guy L. Bush, William Mattson, Edward Grafius, and especially James R. Miller for having patiently helped revise manuscripts generated through this work. I would especially like to thank Dr. George Ayers for having stimulated thought on the importance of the diversionary crop concept.

I would like to thank co-workers, who have shared in the toil and less in the spoils. They have been co-authors on some manuscripts: James R. Miller, James E. Keller, and Robert M. Hollingworth, from Michigan State University, and George Matolcsy, Ferenc Szurdoki, Taha Abdel-aal, and K. Bauer from the Plant Protection Institute, Budapest, Hungary. I would also like to thank David F. Grant for bringing my attention to Fred Gould's population genetics models so early in my work with Jim Miller. Special thanks go for the technical assistance from James E. Keller, Brian K. Mitchell, David Arnosky, and Michael G. Rashid. They helped make my work easier. JEK and MGR have gone the extra mile and have also made substantive suggestions for improving my manuscripts.

TABLE OF CONTENTS

LIST OF TABLES x

LIST OF FIGURES xii

General Introduction 1
- Introduction and Overview 1
- Perspective on the Problem 2
- Host Finding and Acceptance 5
- The Nature of Deterrents 8
- The Onion/Onion Fly System 9
- *Delia antiqua* Colonization of Onions 12
- Stimulo-deterrent diversion 14

Chapter 1. The effects of depth of planting, physical damage, and larval infestation on acceptability of cull onions to *Delia antiqua* 15

- Introduction 16
- Methods and Materials 17
  - Experiment 1 - Depth of Planting 17
  - Experiment 2 - Damage and Infestation 18
- Results and Discussion 20
  - Experiment 1 - Depth of Planting 20
  - Experiment 2 - Damage and Infestation 24
- General Discussion 26

Chapter 2. Evaluating choice-test methods for onion fly ovipositional deterrents 28

- Introduction 29
- Methods and Materials 30
  - Onion flies 30
  - Deterrent 30
  - Bioassays 31
  - Experiment 1 - Treatment:Contol Ratio 31
  - Experiment 2 - Two - vs. Multiple-Choice 32
  - Data analysis 32
- Results and Discussion 32
  - Experiment 1 - Treatment:Contol Ratio 32
  - Experiment 2 - Two - vs. Multiple-Choice 34
- General Discussion 36

Chapter 3. Deterrency of pungent spices and capsaicin-containing products to *Delia antiqua* 38

Introduction 39

Methods and Materials 40
Delia antiqua culture 40
Laboratory ovipositional bioassays 40
Botanicals 40
Dose-response series 41
No-choice test 41
Field trials 42

Results and Discussion 43
Botanicals 43
Dose-response series 44
No-choice test 47
Field trials 49

General Discussion 50

Chapter 4. Cinnamyl derivatives and monoterpenoids as non-specific ovipositional deterrents of the onion fly 53

Introduction 54

Methods and Materials 55
Delia antiqua culture 55
Chemicals 55
Bioassays 55
Vapor concentration 59

Results and Discussion 59
Dose-response series 59
Discriminate-dosage series 62
Vapor concentration 62

Discussion 64
Advantages of discriminate bioassay 64
Structure-activity relationships 64
Biological activity and concentration 69
Behavioral effects and volatility 70
Suggested mode of action 70
Consideration of stimulus interaction 71
Ecological interpretations 72
Practical considerations 74

Chapter 5. Interaction of visual and chemical onion fly ovipositional deterrents 75

Introduction 76

Methods and Materials 78
General methods 78

Experiment 1 - Chemical synergism 79
Experiment 2 - Visual-chemical synergism 79
Experiment 3 - Video analysis 80

Results and Discussion 81
Experiment 1 - Chemical synergism 81
Experiment 2 - Visual-chemical synergism 83
Experiment 1 - Video analysis 84

General Discussion 88

Chapter 6. Stimulo-deterrent diversion for manipulating onion fly oviposition: a greenhouse test 90

Introduction 91

Methods and Materials 92

Results and Discussion 94

Chapter 7. Integrating stimulo-deterrent diversion into pesticide resistance management: Directing pest evolution towards sustainable control 102

Introduction 103

Pesticide management approaches 104
Mixtures and rotation 104
Pesticide plus synergist 105
High dose and selective timing 105
Pesticide strategy assumptions 106
High vs. low selection strategies 108

Stimulo-Deterrent Diversion 109
Modeling the impact of SDDS on Resistance 110

Simulation results and discussion 117
Interpretation of figures 117
Genetic factors 120
Operational factors 121

Practical considerations for SDDS 124

Summary 125

Thesis Summary 127

Diverting oviposition with cull onions 128
Significance of ovipositional deterrents 128
Interactions and host-plant acceptance 129
SDD and natural systems 130
General conclusions 131
Important unanswered questions 132

References 133
Appendix 1 BASIC Population genetics model 146
Appendix 2 Voucher specimen depository forms 149

LIST OF TABLES

Chapter 1.

Table 1. Means for the ln(x+1) transformed and back-transformed (B. T.) means for the numbers of eggs laid upon cull onions planted at varying depths. The standard error for all ln(x+1) transformed values is 0.22 21

Table 2. Average stand counts and egg count totals for 2.5 meter long double row plots of cull onions planted at varying depths 22

Table 3. Mean ± S.E. *Delia antiqua eggs* laid per 24 hours on variously treated cull onions in the laboratory 25

Chapter 2.

Table 1. The effect of treated:control ratio on the measured ovipositional deterrency of 1% cinnamaldehyde in PEG. Deterrency is the difference between ln(x+1) transformed treatment and control egg counts..... 33

Chapter 4

Table 1. Estimated mean and 95% confidence limits of the BR_{90} ovipositional deterrency of cinnamaldehyde derivatives..... 60

Table 2. Estimated mean and 95% confidence limits of the BR_{90} ovipositional deterrency of monoterpenoids 63

Table 3. Estimated percent ovipositional deterrency (stimulation) for compounds tested at 0.1% in PEG 65

Chapter 5.

Table 1. Treatments, means for egg counts (ln(x+1) transformed), and back-transformed deterrency values for testing binary mixtures of three compounds for synergism..... 82

Table 2. Totals (and expected totals from X^2 analysis) for behavioral events in two-choice assays analyzing the effects of visual and chemical deterrents on behavioral transitions. 86

Chapter 6.

Table 1. Analysis of variance for ln(x+1) transformed numbers of onion fly eggs deposited on seedling onions (valued crop) protected by deterrent (cinnamaldehyde) and diversionary (cull onion) treatments. Rows represent sequential presentation of all four treatment combinations while columns coded the order of treatment presentation. 95

Chapter 7.

Table 1. Parameters used in a two-locus, two-allele model investigating the effects of physiological resistance and behavioral avoidance on the evolution of pesticide resistance. 113

LIST OF FIGURES

Chapter 2.

Figure 1. Dose response relationships for methyl salicylate generated when all deterrent concentrations are presented simultaneously in a bioassay chamber (multiple choice) or when single concentrations are compared to control cups (two-choice). Mean deterrency and standard errors are from back-transformed logarithmic values 35

Chapter 3.

Figure 1. Number of eggs laid with 5-7 mg of the following spices placed within 1.5 cm of surrogate onion in choice tests: CON, control; GIN, ginger; DIL, dill; PPK, paprika; RPP, red pepper; CHL, chili powder; BPP, black pepper. Means followed by the same letter are not significantly different (SNK Test, 2 df)..... 45

Figure 2. Dose response relationship for oviposition when ground cayenne pepper was placed in choice tests within 1 cm of surrogate onion foliage. Straight line fit by eye. Confidence interval based on 7 df..... 45

Figure 3. Dose response curve for oviposition when substrate was sprayed in choice tests with 0.175 ml of Agrigard Insect Repellent solutions. Standard error based on 6 df. 46

Figure 4. Dose response relationship for oviposition with synthetic capsaicin placed within the top cm of sand..... 46

Figure 5. Cumulative oviposition for 2x2 factorial experiment investigating pre-reproductive and reproductive exposure to GCP. --◊--, no exposure; --□--, exposure only pre-reproductively; --Δ--, exposure only reproductively; ——o——, continual exposure. 48

Figure 6. Percent damage to seedling onions by onion fly. W, water control; VG, Vaporguard control; AGR, Agrigard; SBR, Sevana Bird Repellent Powder; CHE, conventional chemical control (0.05 g chlorpyriphos m^{-1}). Amounts given are for 3 m of row. Means followed by the same letter are not statistically different (SNK Test, 5 df)..... 48

Chapter 4

Figure 1. Method for determining the BR_{90} from the dose-relationship, as demonstrated with cinnamonitrile (see text for further explanation). 58

Chapter 5.

Figure 1. Sample data collection sheet for transcribing behavioral transitions from videotape recordings of onion fly preovipositional examining and egg deposition behaviors. 85

Chapter 6.

Figure 1. Theoretical forms of interactions between deterrent and diversionary treatments, with non-transformed and log-transformed hypothetical data. (A) - no interactions when non-transformed; slightly negative interactions when transformed. (B) - Positive interaction. The log-transformed data show no interaction, hence, the factors are independent. (C) - Negative interaction. 96

Figure 2. Experimental data for interactions between cinnamaldehyde deterrent and cull onions, as both non-transformed (A) and (B) log-scale graphs. These data are consistent with Figure 1 B, a synergistic effect with an additive model but with parallel lines indicating treatment independence with log-transformation 99

Chapter 7.

Figure 1. Schematic for the life cycle of a pest and its biology relevant to the pesticide resistance simulation model. - - - - Immature stages (egg through pupae) are exposed to selection; —— Adult stage: mates randomly yet chooses habitat to lay eggs based on A allele 112

Figure 2. Relationship between log(concentration) of a pesticide and percent mortality for rr, Rr, and RR genotypes. Arrows labeled A, B, and C correspond to pesticide concentrations simulated in Figures 3 A-C 114

Figure 3. Genetic factors influencing development of avoidance (A allele) or physiological resistance (R allele). (A) R allele is recessive; (B) R allele is dominant; (C) A allele is dominant; (D) A allele is recessive; (E) no fitness costs; (F) fitness cost exists for R allele; (G) fitness cost exists for a allele; (H) fitness cost exists for R and a alleles. 118

Figure 4. Operational factors influencing development of avoidance (A allele) or physiological resistance (R allele). (A-C) pesticide is applied at low, moderate, and high concentrations, respectively (see arrows in Fig. 1); (D-F) diversionary crop suitability is greater than, equal to and less than the suitability of the valued crop; (G) high availability of diversionary crop; (H) Low availability of diversionary crop and high dicrimination by Aa and AA genotypes; (I) an SDDS situation, including low availability but high discrimination of Aa and AA genotypes for the diversionary crops and low survivorship in both diversionary and valued crops. 119

"... I shall be telling this with a sigh
Somewhere ages and ages hence:
Two roads diverged in a wood, and I-
I took the one less traveled by,
And that has made all the difference."

- Robert Frost, The Road Not Taken

Introduction and Overview

Today's farmers increasingly face a predicament. The pesticides on which they depended for the last fifty years are becoming ineffective, unavailable, and unacceptable in the market-place (Dover and Croft, 1984; Schneider, 1989). A major reason for loss of insecticides is physiological resistance. Under strong selection, many pests have proven capable of adapting to new pesticides faster than products with new modes of action can be registered (Dover and Croft, 1984). The process of product registration has become increasingly arduous and expensive, requiring eight to ten years of research and development at a cost of 25 to 40 million dollars (Patton, et al., 1982; Storck, 1984). Partly to limit overall registration costs, manufacturers limit new pesticide registration to those materials that are marketable on the most profitable major crops. Growers of minor use crops are thus often forced to use older and less efficacious compounds (Patton, et al., 1982).

Though Alar^{R} is a plant regulator rather than a pesticide, its recent withdrawal from sale has foreshadowed an era in which scare tactics and consumer demand for residue-free produce set more stringent pesticide limits than those set

by the Environmental Protection Agency. Following the Alar^{R} scare, several national grocery chains declared that, by the year 1995, they will not sell produce sprayed with 64 "hazardous" pesticides. Such action would close large markets to those growers following conventional and entirely legal practices (Ames and Gold, 1989; Schneider, 1989).

Having this century started down the road of using biocides, is there any way growers can switch to a different and "less traveled road" that promotes pesticide alternatives? This thesis investigates how behavioral manipulation of the onion fly could assist the transition to more rational pest control. I report in this thesis studies of ovipositional deterrents and cull onions, which could be used for manipulating onion fly distribution. This, in turn, has important implications for: i) reducing maggot damage to seedling onions, ii) maintaining biological control agents, and iii) managing pesticide resistance. Together, these developments suggest that manipulating pest behavior is crucial to sustainable pest management.

Perspective on the Problem

Pesticide advantages - From an ecological point of view, pesticides are simply human-applied antibiotics (Gould, 1984; Brattsten, 1988). By design, the most successful insecticides, like DDT or chlorpyrifos, have broad-spectrum activity. The manufacturer benefits from broad-spectrum toxicity, both because of the larger sales volumes and the concomitant decrease in unit costs of production. The relatively low cost of pesticides extended to growers partly explains their prevalence today. In apple production, for example, multiple pests can be controlled with a single reduced-rate application of synthetic pyrethroid insecticide at a chemical cost of *ca.* $2.00 per acre (Cowles, unpublished data).

Applying pesticides as defensive compounds differs significantly from natural systems where insects may coevolve with plant defences and slowly be selected for

detoxification adaptations. When insecticides with varying modes of action are available, a grower can alternate or combine these chemicals. This leads to a situation where pests may not be able to adapt genetically to changes in synthetic protective chemistry (Curtis, 1985).

Effective pesticides give agriculturists a distinct marketing advantage. Farms freed from normal ecological constraints can change so that maximal yields of the most valued cultivar can be grown in monoculture with the same sort of efficiency of scale that benefits pesticide producers. Ultimately, consumers realize short-term economic benefit from pesticides, because market forces decrease the cost of produce.

Pesticide disadvantages - Large-scale, high-yield agriculture is conducive to pest outbreaks. Monoculture, high soil fertility, and susceptible crops stray from natural conditions, in which populations often are suppressed by spatial heterogeneity, low host-plant availability and nutritional quality, toxins and genetic diversity in host-plants, as well as by predators, parasitoids, and pathogens (Edens et al., 1985; Tahvanainen and Root, 1972; Atsatt and O'Dowd, 1976; Kogan, 1988; Bernays and Graham, 1988).

Although pests adapted to monoculture crops live on an ecological "easy street," their biological control agents can be severely disadvantaged. Monoculture and use of broad-spectrum pesticides together can accelerate the spiralling loss of biological control agents. A hypothetical and simplistic relationship might be that ecological suppression of pest populations is proportional to species diversity (Atsatt and O'Dowd, 1976; Price et al., 1980) and inversely proportional to the extent of pesticide suppression (Levins, 1986).

Insecticides are not the only biocides destroying biological control. Herbicides may reduce competition between weeds and crop plants, but they also decrease within-field diversity, and in some cases may be directly toxic to beneficial

insects (Carruthers et al., 1985). Eliminating weeds may improve host-plant finding by some insects (Tahvanainen and Root, 1972; Thiery and Visser, 1987). In addition, parasitoids lose sources of pollen and nectar (Atsatt and O'Dowd, 1976), and ground-cover, favoring predatory insects, also disappears (Ryan et al., 1980; House and Alzugaray, 1989). Fungicides controlling plant diseases have broad-spectrum activity, so they often disrupt insect pathogens. For example, onion flies often are decimated by epizootics of *Entomophthora muscae*, until fungicides are applied to control *Botrytis* leaf blight (Carruthers and Haynes, 1986).

Entomophagous insects are directly and indirectly affected more adversely by most insecticides than their phytophagous hosts (Croft and Brown, 1975). Gordon (1961) suggested that since predators and parasitoids are one trophic level above the plant origin of defensive compounds, they are less likely to have detoxification enzyme preadaptations that could allow survival in pesticide-laden environments. Furthermore, since these beneficials depend on pest populations as food, they may starve when pests are suppressed to low levels (Croft and Brown, 1975).

Production "improvements" made possible by applying pesticides, such as monoculture, high fertility, and large fields, are unlikely to be abandoned by growers who have made large capital investments in equipment specific to these large scale practices. When pest control fails, the usual response is to find a new and more effective pesticide. The "pesticide treadmill" phenomenon (Van den Bosch, 1978) results, where the negative impact of this strategy on biological control agents exacerbates dependence on pesticides.

Alternatives - On first appearances, growers have "burned their bridges." Current practices guarantee that if pesticides fail, biological control alternatives will not respond quickly enough to prevent severe crop losses. Today's agriculture

critically needs approaches that will allow conventional agronomic practices to use biological control and other biotic means for pest suppression.

One possible approach toward enhancing biocontrol would be to subdivide the pest population into two habitats, consisting of a valued crop (initially protected by insecticides), and a diversionary crop (where pesticides are not used). Concentrating the pest in a pesticide-free diversionary crop should have three major impacts: 1) direct reduction of pest pressure on the valued crop, 2) facilitation of host finding and use by biological control agents, and 3) providing refugia for insecticide-susceptible pest genotypes. Reduced insecticide applications on the valued crop favors survival of biocontrol agents immigrating from the diversionary crop; this would further reduce the need for insecticides. This positive feedback could reduce or eliminate the need to apply insecticides to the valued crop.

The key to implement this concept is efficient concentration of the pest in a diversionary crop. This requires designing the maximum preference differential between the two habitats, perhaps by simultaneously deploying deterrents on the valued crop while planting a maximally stimulatory diversionary crop.

Host Finding and Acceptance

Principles of host finding and acceptance have been reviewed by Dethier, 1982; Miller and Strickler, 1984; Courtney et al., 1989; and Courtney and Kibota, 1989. Host colonization is now divided into: finding, examining, and consuming (Miller and Strickler, 1984). Host finding is the process of arriving at or near host resources. Behaviors that enable host stimuli to be sensed and processed constitute examining. Consuming consists of end result behaviors, such as feeding or oviposition, which indicate that the host has been accepted.

Host finding, the process of arriving at or near host resources, can be governed by two major strategies. Sensory stimuli that operate at a distance, such as

vision and olfaction could direct movement to a host (Prokopy and Owens, 1983). Otherwise, insects may follow movement patterns that are independent of host-associated stimuli, and simply be arrested when host stimuli are encountered. Tracking insects in natural or semi-natural environments is difficult. Nevertheless, the actual path taken by an insect must be analyzed to distinguish between host-finding mechanisms.

Examining involves active sampling of host stimuli, usually at close quarters, and leads to host acceptance or rejection. Host acceptance is a complex process involving integration of physiological state with external stimuli (Dethier, 1982). The sensory capabilities of each insect shapes how a potential host is perceived. Thus we should expect each species to respond to a complex of plant stimuli in some unique manner, and possibly with some degree of genetic variation (Singer, 1982). Physiological state or experience adds another dimension, individuals may not always perceive stimuli the same way (Visser, 1983).

Although the physical process involved in the integration of sensory inputs leading to host acceptance or rejection is not well understood, Miller and Strickler's (1984) rolling fulcrum model provides a physical representation of the behavioral process without assuming a particular underlying mechanism. In this model, all sensory information (visual, olfactory, tactile, etc.,) is processed together as a *Gestalt*. The relative "weight" of external excitatory inputs are balanced against the "weight" of the external inhibitory inputs (deterrents). The likelihood that the insect will respond to these stimuli, such as by laying eggs, is also affected by the internal physiological milieu. Ovipositional activity is most likely when a female is mated, reproductively mature and presented with all pertinent host stimuli (Spencer, unpublished data). Lack of food required to mature eggs can cause females to be unresponsive to host stimuli (Klowden, 1989). Conversely, females deprived of a

host can be induced to display ovipositional behaviors even when some host stimuli are lacking (Harris and Miller, 1991).

Other models of host acceptance behaviors have recently been developed. The rolling-fulcrum model has been reworked from the perspective of end-result predictions of individual host selection, and renamed the heirarchy-threshold model (Courtney et al., 1989). In this model, the rank order of potential hosts is genetically determined. A highly ranked host will always be accepted by a gravid female, while lower-ranked hosts will only be accepted following deprivation. If a low-ranked host is accepted, then any higher-ranked host will be acceptable, while the opposite is not true.

Experience (learning) can have subtle effects if included with the heirarchy-threshold concept. Jaenike (1983) has observed cross-induction in acceptance of low-ranked hosts, e.g., an insect is more likely to accept a low-ranked host following oviposition on a different low-ranked host. The heirarchy-threshold model predicts cross-induction if acceptance of a low-ranked host is explained as an accompanying change in specificity (Courtney and Kibota, 1989). Host ranking, the core of the heirarchy-threshold model, can be dramatically altered through early adult experience. The change in ranking of apple over hawthorne (Papaj and Prokopy, 1988) by *Rhagoletis pomonella* is only compatible with the heirarchy-threshold model if experience re-programs how an individual insect perceives potential hosts.

A radically different approach for modeling host choice has been pioneered by Mangel (1989a; 1989b; Mangel and Roitberg 1989). He modifies standard optimal foraging models (Stephens and Krebs, 1986) by replacing expected energy values with the expected increase in lifetime fitness. A dynamic model is generated by using a state-variable approach to maximize lifetime fitness (Mangel, 1989b). This model is important because it predicts changes in ovipositional behavior in individuals based on the assumption that evolutionary processes have selected for

behaviors that maximize reproductive success. Mangel's dynamic state-variable approach quantitatively predicts how fast the "fulcrum rolls" (Miller and Strickler, 1984) relative to the rate of egg maturation and the life expectancy of an individual female.

The Nature of Deterrents

The complexity of host examining behaviors and use of a *Gestalt* for host recognition suggest there may be multiple opportunities for manipulating stimuli to effect rejection of a potential hostplant. Manipulation could involve host color, if cultivars with poorly accepted reflectance spectra are developed (Prokopy et al., 1983). When less-accepted host cultivars are not available, the entomologist may achieve desired preference differentials by applying deterrents. Theoretically, almost any sensory stimulus could become deterrent under selective pressure (Landis and Gould, in press).

Most research in the area of ovipositional deterrents has been shaped by the assessment of Dethier (1980) and earlier workers that secondary ("defensive") plant compounds are well suited, through insect-plant evolution, for use as deterrents. Examples of work in this area are investigations on how the composition of secondary compounds in crucifers determines host specificity of Pierid butterflies (Renwick and Radke, 1987); and various works (Tabashnik, 1987; Tingle and Mitchell, 1984; 1986; Lundgren, 1975), on the possibility of using non-host secondary compounds to deter oviposition in *Pieris* species and *Heliothis virescens* (F.).

The mode(s) of action of chemical deterrents and repellents is not well understood. Early discussion of "deterrent receptors," (Jermy and Szentesi, 1978) suggested a similarity to "token stimuli" and the "labeled line hypothesis" then invoked for explaining host acceptance (Fraenkel, 1959). Under this hypothesis, single stimuli will produce a stereotyped (reflexive) response independent of other

factors. This idea had to be modified when neurophysiological work (Dethier, 1980) proved that receptors once thought to be specific are indeed more broadly sensitive. Broad tuning implies that single compounds elicit complex responses from several receptor populations whose output must then be interpreted by the central nervous system.

Davis (1985) proposed several modes of action for repellents. Interference with perception of host-attractant signals can be brought about by: 1) exciting a receptor for competing behavior, 2) switching a sensory message from attraction to repulsion, 3) activating several different receptor systems so that the repellent, in effect, "jams" significant sensory information, and/or 4) exciting a repellent (i.e., a noxious substance) receptor.

A concept common in deterrency literature is that certain compounds may "mask," host odors (Jermy and Szentesi, 1978; Stadler, 1983; Visser, 1983) "Masking," is not yet a defined physiological process; so, like "repellents," this operational description cannot serve indefinitely in the place of understanding the underlying sensory-behavioral mechanisms (Davis, 1985).

The Onion / Onion Fly System

Onion, *Allium cepa* (L.), is a member of the Family Liliaceae. Approximately 2900 ha are planted to onions in Michigan each year (Clement, 1987), almost exclusively on muck soil. Onions here are mostly grown from seed planted from mid-April to the first week in May. Germinating seedlings first pass through the "loop" and "flag" or "seven" stages, descriptive of the appearance of the cotyledon. For the remaining interval of active growth, the onion develops an additional new leaf approximately every week. These tubular leaves emerge from the center of the plant, displacing the next oldest leaf in an alternating pattern. In late August through September, these leaves die back as metabolic resources are

stored as swollen leaf bases, or bulb scales, which form concentric layers in the familiar onion bulb (Raven and Curtis, 1970). This process is accelerated by uprooting the onion and allowing the plant to dry in the field in preparation for harvest (a common practice in New York, but not in Michigan).

The major onion insect pest, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae), usually completes three generations each year in Michigan. Onion fly adults start emerging from overwintering pupae in mid-April, and feed for one to two weeks before ovipositing (Loosjes, 1976). Eggs are laid in the soil at the base of onion plants. When larvae hatch, they first may feed on or around smaller roots, then usually enter the base of the onion at the meristematic disk. This tissue is so critical for plant survival that the plant rapidly shrivels and dies. During the first maggot generation, the number of plants required to complete development depends on the quantity of tissue available in each plant. When onions are in the loop stage, as many 28 seedlings may be consumed by a single larva (Workman, 1958). Typically though, several eggs are laid in a batch, and 10 to 20 adjacent seedlings are destroyed by each group of larvae (Loosjes, 1976). The first generation larvae requires about one month to develop, so they are present during the cotyledon to six- or seven-leaf stage of onions.

Second generation flies begin emergence in late June through July. Onions may have 6-10 leaves at this time, so second-generation larvae often do not kill the plant (Kendall, 1932; Workman, 1958). Damage from second generation larvae is largely restricted to plants earlier damaged but not killed by first generation larvae (Kendall, 1932), or by farm equipment (Finch, et al, 1986b). Third generation flies are active through harvest and up until a killing freeze (late October or November). These flies predominantly lay eggs on bulbs damaged by earlier generation larval feeding or farm machinery, and on sprouting onions left in the field after harvest (Finch and Eckenrode, 1985; Finch, et al., 1986b). Pupae from this generation, and

a small number of diapausing pupae left from the first and second generations, overwinter to the next growing season (Perron and LaFrance, 1961; Finch and Eckenrode, 1985).

Current control practices - Control of *D. antiqua* is achieved by using in-furrow insecticides targeting first generation larvae. These soil pesticides contact larvae traveling from egg-to-plant and plant-to-plant (Loosjes, 1976). Currently available in-furrow insecticides, chlorpyrifos and fonofos, are losing (or have lost) efficacy at labeled rates (Grafius, et al., 1987).

Adulticide sprays used to be directed at first, second, and third generation adults, often on intensive 3 to 5 day schedules. Spraying contact insecticides has largely been discontinued; they were ineffective because of insecticide resistance and because the diurnal movement of flies out of fields coincided with the usual timing of spray applications (Finch et al., 1986a).

Howitt (1958) and Guyer and Wells (1959) gave the first reports of onion fly insecticide resistance. These cases reported cross resistance to a large number of chlorinated hydrocarbons insecticides in the Pacific Northwest and Michigan, respectively. Harris et al. (1982) reviewed onion fly insecticide resistance in Michigan and Ontario, and concluded from comparisons of laboratory selected vs. field collected flies that field populations could rapidly be selected for high levels of resistance to organophosphate and carbamate insecticides.

Other areas that have been investigated for control of onion flies include control with natural enemies. Much of this work has been done at MSU under the purview of the IPM approach of the 1970's and early 1980's. Several beneficial insects are of importance, including *Aleochara bilineata* (Gryll.) (Coleoptera: Staphylinidae), *Aphaereta pallipes* (Say) (Hymenoptera: Braconidae), and *Bembidion quadrimaculatum* (Coleoptera: Carabidae) (Groden, 1982; Grafius and Warner, 1990). Conditions affecting *Entomophthora muscae*, an entomopathogen

attacking calypterate muscoid flies, have been studied by Carruthers (1981; Carruthers and Haynes, 1986). Intensive work in the Netherlands focused control efforts on sterile male onion fly release (Loosjes, 1976), a method that is theoretically feasible but expensive and technologically demanding. A more complete discussion of current control methods for *Delia* flies is given in Finch (1989).

Delia antiqua colonization of onions

Onion fly habitat finding has been studied by Martinson et al. (1988). Flies emerging from overwintering puparia tend to emigrate from fields more than second generation adults. This difference could be caused by diapause vs. non-diapause conditioned flies, or alternatively to differences in the quality of host habitat. First generation onion flies were captured up to 1.5 km from likely overwintering sources, supporting Loosjes's (1976) calculation of a fivefold increase in movement rates in non-host habitat.

Onion fly habitat finding probably fits the model, discussed earlier, of non-directed movement followed by arrestment by host odors. This assessment is supported by: 1) good fit of an exponential function, relating damage to distance from overwintering source of flies (Martinson et al., 1988), 2) fly movement out of fields when onions are producing small quantities of volatiles (Martinson et al., 1988), and rapid movement between onion fields (Loosjes, 1976; Martinson et al., 1988).

In contrast to Martinson et al. (1988), Judd and Borden (1989) suggest that long-distance host finding is mediated by anemotactic response to onion volatiles. Using 14-day old virgin females, these workers demonstrated slightly cross-wind biased flight for flies exposed to low n-propyl disulfide concentrations and upwind flight at higher concentrations (Judd and Borden, 1989). These observations may be subject to another interpretation, however. Considering that some mating

encounters occur on the host-plant (Cowles, personal observation) and that the age of onion fly mating in lab conditions normally occurs at 5 or 6 days (Joseph Spencer, Michigan State University), we can speculate that the onion fly females used by Judd and Borden were highly mating-deprived. The observed response to onion volatiles may have been more closely related to mate-finding rather than host-finding behavior.

Odor and vision may both play a role in short-distance host finding. Onion flies made short, upwind, non-zigzag flights to a source of volatile compounds, most likely a combination of onion odors combined with microbial products (Dindonis and Miller, 1980; Hausmann and Miller, 1989b). Harris and Miller (1988) conclude, based on the use of surrogate onion foliage, that color, shape and size all influence alighting behavior in the onion fly.

Onion fly host examining is characterized by stereotyped preovipositional behaviors, such as foliar runs, substrate runs and substrate probing. During these behaviors, there is concomitant repetitious sampling by mouthparts and ovipositor, and very likely visual and tarsal stimulation as well (Harris and Miller, 1988). Harris and Miller (1988, et ante) have established that optimal release of ovipositional behavior requires simultaneous presentation of a combination of chemical, visual and tactile stimuli. For example, host models with sub-optimal chemical and visual stimuli will not receive as many eggs in choice tests as the optimal 4 mm diameter green or yellow cylinders, emitting dipropyl disulfide from both foliage and substrate.

Harris and Miller (1982) noted that there was a significant interaction ("synergism") between visual and chemical cues. Stimulus interaction suggests that onion flies either integrate these cues via central nervous system cross-fiber patterning or by temporal processing of information.

The consumatory stage, oviposition, for *Delia antiqua* has been examined in great detail through analysis of video records (Mowry et al., 1989b). Unlike preovipositional behaviors, which have complex feedback to previous activities, egg depositional behaviors appear to be deterministic. Egg depositional behavior begins with subsurface probing with the ovipositor. Once an egg begins moving to the bursa copulatrix, all ancillary behaviors (such as grooming) cease, and the fly becomes stationary. The increasingly deterministic nature of behaviors, as a fly approaches egg deposition, suggests that behaviors occuring late in preovipositional sequences may be more difficult to avert (with deterrents) than earlier preovipositional examining activity.

Stimulo-deterrent diversion

The objective of the work embodied in thesis was to assay onion fly host acceptance behavior, with the goal of developing strategies for preventing oviposition on young seedling onions. Stimulo-deterrent diversion (SDD) was developed, where the valued crop is treated with chemical deterrents, and simultaneously, a highly stimulatory ovipositional resource (sprouting cull onions) is deployed to concentrate eggs away from the crop. Basic and applied questions are addressed in this work. Use of deterrents to protect crops is not a well developed field, and with the exception of work by Rice (1986; Pyke et al., 1987), I am not aware of other workers considering the importance of bipolar manipulation of pests' consumatory behaviors.

The thesis is divided into chapters corresponding to units involved with SDD: Chapter 1 involves stimulatory ovipositional resources, Chapters 2 through 5 investigate deterrent stimuli and their interactions, Chapter 6 describes a greenhouse test of SDD, and Chapter 7 discusses how SDD may prevent or reverse pesticide resistance.

Chapter 1

Acceptability of cull onions to *Delia antiqua* (Meigen) oviposition: the effects of planting depth, physical damage, and previous larval infestation

Introduction

Onion maggot (*Delia antiqua* (Meigen)) is the principle insect pest of onions in temperate regions (Loosjes, 1976; Eckenrode, 1988). In Michigan, onion maggots are typically trivoltine, with spring, mid-summer, and autumn larval development (Whitfield, 1981). Young seedlings are especially susceptible to damage caused by the spring generation of maggots; a single larva typically consumes four to ten onions (Kendall, 1932; Workman, 1958). Manipulating onion fly ovipositional activity of *D. antiqua* on waste onion bulbs (cull onions), may protect seedlings by: 1) reducing the overwintering population and 2) diverting oviposition away from seedlings.

Third generation densities ranging from 10,000 to 600,000 overwintering pupae per hectare may result from colonization of onions left in the field after harvest (Drummond, 1982; Finch and Eckenrode, 1985). Preventing oviposition on culls left after harvest, and making these bulbs unsuited for larval development should be effective cultural control practices (Finch and Eckenrode, 1985; Eckenrode and Nyrop, 1986).

Sprouting cull onions are 60 to 200 times more acceptable to onion flies than small seedlings (Lovett, 1923; Hammond, 1924; Mowry, unpublished; Chapter 6). This ovipositional preference may be exploited by planting cull onions in the same field as small seedlings, either as a trap crop (Lovett, 1923; Hammond, 1924), or a diversionary crop (Miller and Cowles, 1990). Maximizing preference of onion flies for the sprouted onion bulb should enhance these strategies for protecting seedlings.

This chapter investigates physical and biological effects on ovipositional preference of onion flies for sprouted onion bulbs. Both physical and maggot

feeding damage to onion bulbs were predicted to enhance oviposition through increased production of chemicals stimulatory to onion flies (Dindonis and Miller, 1981; Hausmann and Miller, 1989). Investigating the effect depth of planting has for stimulating *D. antiqua* oviposition on cull onions simultaneously addresses: 1) how we should manage bulbous onion residues after harvest to minimize the overwintering population, and 2) how we may best use these onions to manipulate ovipositional behavior of spring adults.

Methods and Materials

Experiment 1. Planting cull onions at varying depths - Large sprouting red onion bulbs (*ca.* 6 cm diam., unknown cultivar) were obtained from Riley Farms, Plainwell MI. To ascertain which planting depth stimulated the most onion fly oviposition, these bulbs were planted so the neck heights were at 5 cm above (+5 cm), even with (0 cm), 5 cm below (-5 cm), and 12 cm (-12 cm) below the soil surface

Plots were laid out in a commercial onion field (Bath, MI, Clinton Co.), within the previously planted rows. Three rows, 50 meters apart, were used to lay out experimental plots. Four replicates were placed in each row, for a total of 12 replicates in a randomized complete block design (RCBD). Blocks were spaced 20 meters apart and were located at least 20 meters from the edge of the onion field. To enhance the element of fly "choice," it was desireable to maintain close proximity and nearly equal distances between the four depth treatments. This was accomplished by arranging treatments in a rectangle. Two treatments were located end-to-end within one row, and the other two treatments were laterally located two rows (1 meter) away.

Seeds and fonofos granular insecticide previously planted by the grower were removed along with soil by digging trenches 25 cm wide, 2.5 meters long and 5, 10, or 17 cm deep. Bulbs were planted touching each other in two rows 10 cm apart for

each depth. For the +5 cm treatment, pesticide-free soil was transported from the edge of the field to fill the 5 cm deep trench before the bulbs were pushed into the loose soil. For the other treatments, bulbs were placed at the bottoms of the trenches, then covered with pesticide-free soil. Blocks were planted between April 21 through 23.

Eggs deposited near cull onions were sampled on May 20-21, June 3-4, and June 18. A plastic spoon was used to remove soil and eggs from the proximity of onion plants. Two subsamples of eggs laid near three onions were taken from within the middle third of the plots (to avoid edge effects). Eggs and muck soil were stored in one liter wax paper cups for up to five days at 4° C until the eggs were counted. Stand counts of bulbs with green foliage were taken May 20 and June 3.

Eggs were separated from muck soil by flotation in water. Residual floating muck and eggs were passed through a 1 mm mesh sieve to remove the larger muck fragments; the resulting eggs and fine muck were deposited on a nylon filter. This residue was washed into a black enamel pan. The contrast of the white eggs against the black background enabled the eggs to be easily counted as they were removed with a vacuum aspirator.

Egg counts required $\ln(x+1)$ transformation to establish homogeneity of variance (Bartlett's test, $P > 0.1$). Egg counts from each sample date were subjected to analysis of variance and the means separated by the Student-Newman-Keuls' test (Steel and Torrie, 1980). The average number of eggs per plant was multiplied by the stand count to estimate the total eggs per treatment plot. These total eggs per plot were then averaged to give average eggs per plot for each planting depth.

Experiment 2. Effects of bulb damage and larval infestation - The main effects and interaction of bulb damage and larval infestation on onion fly oviposition were studied via a two x two factorial design. Larval and adult onion flies were obtained from a laboratory culture initiated in September, 1986, from pupae

collected from onions left in harvested fields in Grant, MI (Newaygo Co.). Flies were housed in 80 x 65 x 60 cm screened cages provisioned with food (Schneider et al., 1983), water, and ovipositional resources (Harris et al., 1987a). The rearing room was held at 16:8 L:D photoperiod, 21 ± 1° C, and 70 ± 5% RH. Larvae were reared on bisected onions. Flies used in this experiment were reared in the lab for *ca.* eight generations.

Neonate larvae were obtained by collecting eggs from the culture cage by flotation from sand, and holding the eggs on damp filter paper at 4° C. Under these conditions, egg hatch is prolonged, so larvae were available for four to five days.

Commercially obtained yellow onion bulbs (unknown cultivar) were refrigerated at 4° C from July 27 until September 14, 1987 to break bulb dormancy. Bulbs were then planted in a 50 x 35 x 10 cm flat filled with steam sterilized muck soil. Bulbs were positioned with their necks at the soil surface, and allowed to grow within a 1.5 x 1.5 x 3.5 m screened cage in a greenhouse. Plants were kept within a cage to prevent infestation with onion maggots prior to the start of the experiment. On September 30, sprouted onions were then blocked by bulb size and foliar development into groups of four. Two bulbs from each of the 11 blocks were subjected to damage by sagittally slicing a 1 cm thick piece from the side of the bulb. Single bulbs were planted so the necks were 5 cm beneath the surface of steam sterilized muck soil held in 20 cm x 20 cm diameter pots.

Half of the bulbs from the damaged and undamaged treatments were then subjected to larval infestation. Twenty neonate larvae were transferred with a fine camel hair brush to the foliar-soil interface of one damaged and one undamaged bulb treatment from a replicate, two weeks before an ovipositional bioassay.

Bioassays were conducted in a one meter x one meter diameter cylindrical cage held in a growth chamber under the same environmental conditions as for maintaining cultures. The cage had a plywood base and a suspended floor, into

which pots could be inserted. The soil level in pots placed in the cage was even with the suspended cage floor. Sides of the cage were made of Mylar™ plastic and was ventilated at the top with aluminum window screen.

Fifty females and 50 male onion flies of mixed ages were transferred from the onion fly culture to the bioassay cage provisioned with food and water. The four treatment-combinations in one replicate of the factorial design were placed in randomized order within the cage. After allowing the flies to oviposit for *ca.* 20 hr, the pots were removed, dead flies were replaced, and the next block of pots was introduced to the cage.

Eggs were sampled from the soil in the same manner as for Experiment 1, except that all the muck to a depth of 4 cm was removed, and onion plants were dissected to wash eggs from leaf axils. Egg counts did not require transformation before analysis of variance.

Results and Discussion

Experiment 1. Effects of the depth of planting of cull onions - Depth of cull planting greatly affected onion fly oviposition (Table 1). F-tests for treatment effects were highly significant both for the late May and early June egg sample dates ($F_{(3,33)}$ = 14.55 and 20.6, respectively; $P < 0.0001$). For the May 21 - 22 egg counts, the rank order of preference (descending order) was -5, 0, -12, and 5 cm treatments. The June 3 egg counts had a rank order of -12, -5, 0, and 5 cm treatments. On the June 18 sample date, very few eggs were found and there were no significant differences between treatments ($F_{(3,33)} = 2.75$, $P > 0.05$).

The preference of onion flies for these treatments probably was determined by the number and quality of plant parts projecting through the soil surface. Cull onions pressed into the soil surface (+5 cm) were the least accepted treatment. These sprouting bulbs made a broad circumference of soil contact and were not

Table 1. Means for the ln(x+1) transformed and back-transformed (B. T.) means for egg numbers laid upon cull onions planted at varying depths. The standard error for all ln(x+1) transformed values is 0.22.

Neck Height Relative to Soil Surface	Mean Eggs					
	May 21-22		June 3-4		June 18	
	B. T. eggs	Mean Mean	B. T. eggs	Mean Mean	B. T. eggs	Mean
5	1.212	2.4 c	1.170	2.2 d	1.482	3.4 a
0	3.242	24.5 a	1.936	5.9 c	0.861	1.4 a
-5	3.755	41.7 a	3.405	29.1 b	0.966	1.6 a
-12	2.211	8.1 b	4.022	54.8 a	1.070	1.9 a

*Means within columns followed by the same letter are not significantly different (SNK test, 9 d.f., $P < 0.05$).

Table 2. Average stand counts and egg count totals for 2.5 meter long double row plots of cull onions planted at varying depths.

Neck height relative to soil surface	Plants per plot	Eggs per plot		
	Mean (± S.E)	5/21 Sample	6/3 Sample	Total
5	26.8 (3.1)	111	173	284
0	24.6 (2.7)	681	851	1532
-5	18.1 (1.9)	859	1470	2329
-12	7.8 (1.2)	159	825	984

green, two characteristics that make poorly accepted host models (Miller and Harris, 1985). Variation in acceptability among the remaining treatments probably was due to the varying numbers of leaves projecting through the soil at different sampling dates. The deepest planted bulbs were the most stimulatory, once their leaves reached the soil surface. For the June 3 - 4 sample dates, this treatment had an unsurpassed mean of 55 eggs per plant (Table 1). However, fewer bulbs in this treatment had leaves reaching the soil surface; thus, fewer eggs were laid per plot than for -5 cm (Table 2). Egg counts per plant were lower on -12 cm for the May sample date (Table 1), probably because so few leaves per plant reached the soil surface.

The decreased relative acceptance of bulbs planted at -5 cm compared to -12 may also have been due to the changing qualities of leaves at the soil surface. When cull onion foliage first breaks through the soil, a large number of foliar resources may be present upon which the onion fly may lay eggs. As the onion grows, however, the floral stalk emerges and the leaves, which earlier were at the soil surface, are well above ground. An onion thus changes from having a large number of leaves that are a nearly optimal diameter for eliciting onion fly oviposition, to having one or two large diameter (suboptimal) floral stalks. Based on these observations, -12 cm on June 3 probably closely resembled the -5 cm treatment on May 21.

In addition to foliar cues, chemical stimuli influencing acceptability of culls probably were changing. Colonization of bulbs by onion maggots and microbial decomposition each initially increase the production of onion fly ovipositional stimulants (Hausmann and Miller, 1989a; 1989b). Maximal production of these volatiles probably was reached within two weeks of foliage having reached the soil surface. After this time, bulb resources may have become depleted and microsuccessional changes in bacteria would have diminished ovipositional

acceptability. By June 3, the acceptability of the -5 cm treatment probably were diminished compared to the May 21 sample date.

Experiment 2. Effects of bulb damage and larval infestation - The mean numbers of eggs laid upon each of the four treatment combinations were not significantly different from each other (Table 3, $F_{(3,30)} = 0.08$, $P > 0.1$). Partitioning variability into one degree of freedom main effects and interaction suggested that neither damage to the bulbs, larval infestation, nor their interaction caused significant differences in oviposition on bulbous onions ($F_{(1,30)} = 0.08$, 0.09, and 0.34, respectively; $P > 0.1$).

This experiment suggests that sprouting bulbous onions planted at an optimal depth will remain highly stimulatory under highly variable conditions. This greenhouse experiment also confirmed some personal field observations on the range of ovipositional resources accepted by onion flies. Most strikingly, treatments with foliage that was becoming flaccid from maggot feeding remained highly attractive to gravid onion flies as long as the leaves were green and moist. In two replicates, foliage from the damaged + infested bulbs had become detached from the bulb and had dried out; these plants did not receive eggs. However, when five other pots (both damaged and undamaged bulbs) had been fed upon sufficiently to cause leaves to detach and begin to dry, ovipositional activity was not diminished. It is possible that increased chemical cues generated by feeding larvae offset lower quality foliar cues, so that differences between treatments were not detected. This experiment only offered a single time point for assaying ovipositional acceptance. By allowing larvae to develop for two weeks, both damaged and maggot infested bulbs may have passed their peak chemical acceptability to ovipositing onion flies (Hausmann and Miller, 1989a; 1989b). Earlier bioassays could then have generated treatment differences caused by damage and/or infestation.

Table 3. Mean ± S.E. *Delia antiqua* eggs laid per 24 hours on variously treated cull onions[1] in the laboratory.

INFESTATION EFFECTS	DAMAGE EFFECTS	
	No Damage	Damage
0 larvae	62 ± 16 a[2]	54 ± 19 a
20 larvae	59 ± 17 a	66 ± 16 a

[1] 2 x 2 factorial design with 11 replicates.

[2] Means within the table followed by the same letter are not significantly different, SNK test, $P < 0.05$.

General Discussion

Important features of bulb onions that elicit oviposition from *D. antiqua* are: 1) their sprouted leaves projecting through the soil surface, 2) large quantities of characteristic volatiles from decomposing onion bulbs, which are known to attract adults (Dindonis and Miller, 1981; Hausmann and Miller, 1989) and 3) crevices in the soil that facilitate ovipositor probing (Mowry, et al., 1989a), generated by the expansion of below-ground onion foliage. The upright, ca. 4 mm diameter yellow to green colored leaves projecting through the soil surface closely resemble optimally stimulatory surrogate onion foliage (Harris and Miller, 1991; *et ante*), and probably had the greatest influence in eliciting oviposition.

Eckenrode and Nyrop (1986) found damage to bulbs to be of great importance in colonization by the overwintering population of onion maggot in New York State. Experiment 2 would suggest that damage to bulbs may increase overwintering populations more through enhanced bulb suitability to onion flies rather than through increased acceptability as an ovipositional resource. However, the onions in the New York study were lifted from the soil, exposing damaged onion tissues more than in Experiment 2.

To minimize the overwintering population of onion maggots, I recommend from this study and other work (Finch and Eckenrode, 1985; Eckenrode and Nyrop, 1986), that bulbous onion crop residue should be damaged as little as possible, to restrict colonization of the onion tissues, and should either be: 1) plowed deeply under the soil, so that sprouting foliage will not reach the soil surface or 2) be removed from the field and placed in deep cull piles (Finch and Eckenrode, 1985). Leaving bulbs on the surface of the field is not recommended, because some bulbs will sprout and will be colonized. However, this last option is probably better than shallowly discing the residues. Damage to the bulbs during discing causes maximal sprouting of bulbs and allows entry by maggots.

The preference of onion flies for deeply planted sprouted bulbs over small seedling onions has been documented since the 1920's (Lovett, 1923; Hammond, 1924). Deeply planted sprouting onion bulbs elicit 60 to 200 times as much onion fly oviposition as small onion seedlings (Hammond, 1924; Chapter 6; Mowry, unpublished data). To maximize the number of eggs laid around cull onions used as either a trap or a diversionary crop, bulbs should be planted so that the largest number of leaves is emerging through the soil surface when seedlings need to be protected. For the field work in this experiment, planting cull onions with their necks 5 cm below the soil surface gave optimal conditions for deploying them as a trap crop.

Chapter 2

Evaluating choice test methods for onion fly ovipositional deterrents

Introduction

Investigators wishing to evaluate deterrents of insect oviposition by counting numbers of eggs deposited on treated resources face further methodological choices: the single treatment ("no-choice") test offers the greatest assurance of freedom from treatment interactions, and over time can address important shifts in acceptance resulting from ovipositional deprival. Weaknesses are high demands on time and insect stocks, and difficulties in comparing activities of various compounds. By contrast, multiple-treatment ("choice") tests offer the converse strengths and weaknesses.

Experience in searching for deterrents of onion fly (*Delia antiqua* (Meigen)) oviposition (Cowles, et al., 1990) has led us to favor the "choice" test in primary screening for the most deterrent compounds. Vast time savings can be realized when testing is limited to discriminating dosages. Follow-up "no-choice" tests with the most promising deterrents can address the influence of ovipositional deprival.

Prokopy, *et al.* (1988), reviewed efficiency of choice bioassays for tephritid flies, and concluded that multiple-choice assays with continual behavioral recording were efficient and more sensitive (could detect ovipositional discrimination better) than two-choice assays. Throughout that study, however, non-observed flies biased deterrency measurements due to deposition of oviposition-deterring pheromone during assays. Girolami, *et al.* (1981) ran ovipositional deterrent trials with *Dacus oleae* using only two-choice arenas. They explained that in preliminary multiple-choice trials, there were too few eggs deposited on deterrent treatments to conduct analyses, because of competition from non-deterrent treatments. These two studies illustrate complexities inherent with designing deterrent choice-tests;

communicational cues deposited during ovipositional activities, and the physical number of treatment and control resources can limit and bias measures of deterrency. The present study tested the quality of onion fly ovipositional choice-test data. Specific questions of interest were: 1) Are measures of percent deterrency in a "choice test" influenced by the number of negative controls included?, and 2) Do two-choice and multiple-choice dose-response assays give similar deterrency estimates and precision?

Methods and Materials

Onion flies - An onion fly culture was established in September 1987 from field collected pupae and maintained as described earlier (p. 18 - 19). Flies used in experiments were reared in the lab for 5-10 generations.

Deterrent - Methyl salicylate (98%, Sigma Chemical) was diluted in polyethylene glycol (PEG)(Carbowax PEG 8000, Fisher Scientific) in five decade steps to a 0.001% concentration (w/w). (*E*)-Cinnamaldehyde (99+% purity) was purchased from Aldrich Chemical and formulated in PEG at a 1% concentration. PEG made an appropriate release matrix, as upon moderate heating it readily dissolved these compounds and after cooling retarded their evaporation. Pre-weighed PEG was melted at *ca.* 56°C, deterrent compound was added, and the mixture was vortexed. The mixture was dispensed from a Pasteur pipette onto aluminum foil to form *ca.* 10 mg pellets easily weighed for subsequent dilution steps. These pellets were coarsely crushed, then stored at -18°C until bioassayed. Experiments used 20 mg formulated mixture per ovipositional cup.

Bioassays - Ovipositional bioassays were conducted in a walk-in environmental growth chamber having conditions identical to the rearing chamber except the bioassay room was free of onion volatiles. Experiments were conducted in 30 cm diameter x 30 cm cylindrical top-ventilated plastic cages provisioned with

diet and water and stocked with *ca.* 10 male and 20 female flies. These cages rotated on their axis once every 13 min; thus, environmental gradients were distributed evenly over all treatments (Weston and Miller, 1985).

Ovipositional resource - Each ovipositional dish consisted of 50 g white silica sand (Unimin Granusil, Grade 40, Oregon, IL) moistened with 3 ml distilled water, then tamped into a 4 cm diameter x 4 cm plastic cup. Standing upright in the center of each dish was a surrogate onion leaf consisting of a 0.4 cm diameter x 12 cm glass tube painted onion-green with oil pigments (Winsor and Newton Cadmium Yellow, Flake Everwhite No. 2, Winsor Green, and Lamp Black in the weight ratio 132:7:5:2) and coated with paraffin wax containing 0.05% n-propyl disulfide (Harris et al., 1987). The surrogate onion was swiveled in the sand to provide a 1 mm space around its base for ovipositor probing (Mowry et al., 1989a). This surrogate onion foliage is competitive with similarly sized onion foliage (Harris and Miller, 1991), and offers a highly controlled ovipositional resource. Deterrents were evenly dispensed on the sand within 1 cm of the juncture of foliage and soil, where the most critical host examining behaviors and oviposition occur (Harris et al., 1987). PEG granules applied on moist sand partly dissolved and became incorporated in the top 1 mm layer.

Experiment 1. Treatment:control ratio - Four cups were placed in each of the five rotating cages. Combinations were: 1) four control (blank PEG) cups, 2) three control and one cinnamaldehyde-treated cup, 3) two control and two treated cups, 4) one control and three treated cups, and 5) four treated cups. "Combinations" 1 and 5 were included to contrast no-choice conditions with the choice assays. Combinations 1 through 5 were randomly assigned each day to five cages. There were ten replicates in this randomized complete block design (RCBD).

Experiment 2. Two-choice vs. multiple choice presentation - The dose-response relationship for methyl salicylate was compared from two-choice (two types of treatment per cage) versus multiple choice protocols. For the multiple-choice test, the five decade-step concentrations of methyl salicylate and a control (blank PEG) were randomly ordered in a single cage, constituting one replicate in a five-replicate RCBD. To maintain the same fly densities in the cages, yet avoid fly crowding on oviposition cups, the number of resources in the two-choice protocol was kept the same as in the multiple-choice experiment; e.g., three treated cups were compared with three control cups. Treated cups (one concentration per cage) and control cups were alternated in a hexagonal pattern in the cages. Egg counts within treatment and cage were pooled, constituting one replicate. There were three replicates of each concentration tested, for the same five concentrations as in the multiple-choice assay. The five replicates of the multiple choice series and the three replicates of the five concentrations of the two-choice tests were randomly assigned to the five rotating cages over four days.

Data analysis - Log-transformed ($\ln(x+1)$) egg counts fulfilled homogeneity of variance assumptions for analysis of variance (Bartlett's test, $P > 0.1$). Therefore, deterrency was measured as the difference between $\ln(x+1)$ transformed treatment and control egg counts. This difference is readily converted to percent deterrency (percent deterrency $\approx \{1-e^{[(trt+1)-\ln(con+1)]}\} \times 100\%$).

Results and Discussion

Experiment 1. Treatment:control ratio - The ratio of treatment:control cups significantly affected the logarithmic deterrency measurement ($F_{(3,27)}=8.58$, $P < 0.0001$). Converting from logarithms yielded 87.6, 92.5, and 94.6% deterrency for one, two, and three treated cups per cage, respectively (Table 1). The linear

Table 1. The effect of treated:control ratio on the measured ovipositional deterrency of 1% cinnamaldehyde in PEG. Deterrency is the difference between ln(x+1) transformed treatment and control egg counts.

Treated:Control Ratio	Total Eggs			Deterrency (S.E)	Mean % Deterrency*
	Treated	Control	Combined		
0:4	-	1300	1300	-	-
1:3	91	1692	1783	-2.085 (0.314)b	88 %
2:2	181	1856	2037	-2.591 (0.299)ab	93
3:1	369	1972	2341	-2.925 (0.249)a	95
4:0	995	-	995	-0.246 (0.314)c	22

*Means followed by the same letter are not significantly different (SNK test, 9 d.f., $P < 0.05$).

orthogonal contrast (Steel and Torrie, 1980) demonstrated systematic bias in the deterrency measurements ($F_{(1,27)}=6.19$, $P < 0.025$).

This increase in measured deterrency with increasing treatment:control ratio would not be expected to hold if the total number of cups increased so the one control cup became less findable. This condition would probably mimic the no-choice situation. Cinnamaldehyde at 1% concentration in PEG was only 22% deterrent in a no-choice comparison (compared to the 90+% deterrency from the choice test).

A possible explanation for this increase in deterrency with increasing treatment:control ratio is that ovipositing onion flies became more concentrated on a single resource and that there is a "group effect" where ovipositional behaviors of flies or perhaps pheromonal substances associated with freshly laid eggs stimulate further oviposition. This hypothesis is consistent with observations of intense oviposition by aggregations of onion flies in culture cages. Two way analysis of variance and linear, quadratic, and cubic orthogonal contrasts were conducted on control egg counts to investigate this possible source of deterrency bias. If pheromonal cues biased deterrency, a significant inverse linear relationship should exist between the number of control cups and the number of eggs laid on the controls. This linear orthogonal contrast was suggestive but not statistically significant ($F_{(1,27)} = 1.30$, $P > 0.25$), so the source of the bias in deterrency remains unexplained.

Experiment 2. Two-choice vs. multiple choice presentation - The standard errors in the two-choice test were slightly smaller (Figure 1) than for the multiple-choice test, even though there were two more replicates for the latter. The higher precision in the two-choice test largely can be attributed to the pooled triplicate subsamples.

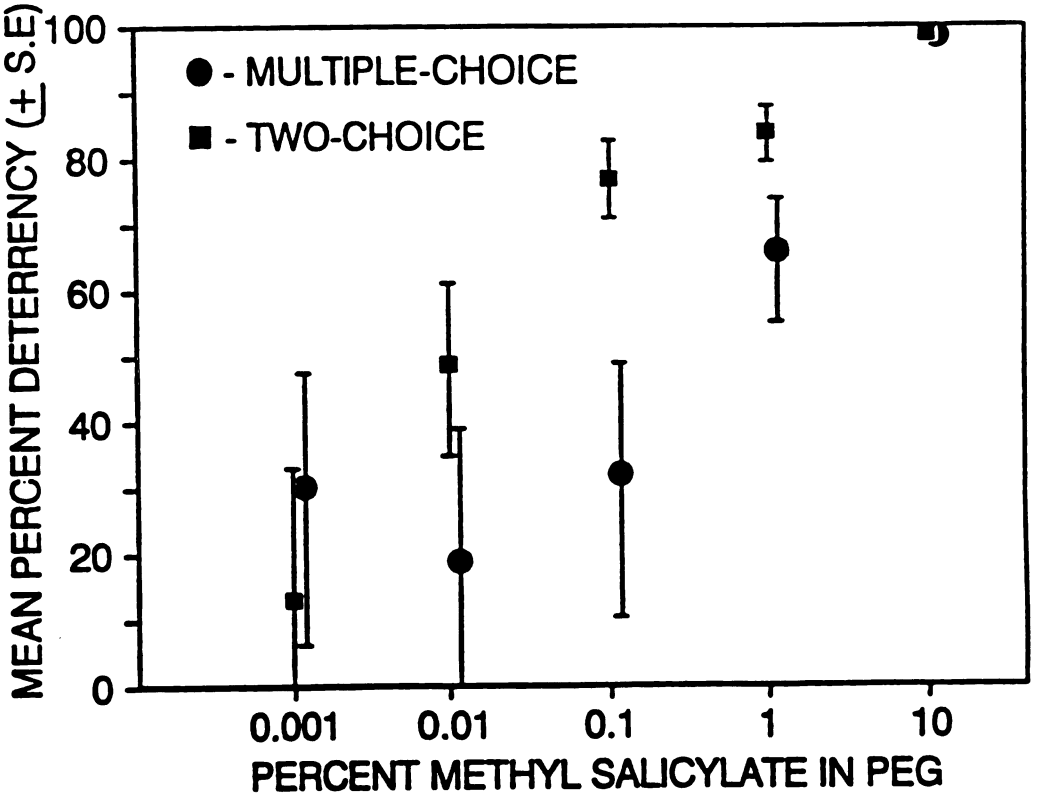


Figure 1. Dose-response relationships for methyl salicylate generated when all deterrent concentrations are presented simultaneously in a bioassay chamber (multiple-choice) or when single concentrations are compared to control cups (two-choice). Mean deterrency and standard errors are from back-transformed logarithmic values.

To compare dose response curves, the deterrency for each chemical concentration was calculated separately for the two protocols, using the difference between ln(x+1) transformed treated and control egg counts, then the two sets of deterrency values were subjected to a paired t-test. There was not a significant over-all bias in deterrency ($t_{(4\ df)} = 0.901$, $P > 0.4$). However, there was a trend toward higher deterrency for intermediate concentrations in the two-choice assay (Figure 1). This effect is biologically consistent with: 1) adaptation or habituation to low concentrations of deterrent when presented with high concentrations, and 2) greater ability of the flies to discriminate between treatments when they are physically close, so that intervals are short between examining bouts on the different "choices" (Mowry et al., 1989a).

Having multiple deterrent treatments in dose-response choice tests of deterrents should cause increased acceptance of low concentration treatments. Flies examining low-deterrency treatments after having visited high-deterrency treatments should be more likely to lay eggs than had she previously just visited an untreated control. This influence could readily be tested by designing an experiment in which each deterrent concentration in a multiple-choice assay is alternated with an untreated control. If the cause of deviation from a two-choice assay is deprivationally-caused, then there will no longer be differences in deterrency compared to the two-choice assay. If differences in deterrency are caused by habituation due to high ambient concentrations of deterrents, then the differences between multiple-choice and two-choice tests will persist.

General Discussion

The results of choice are context-dependent, because measures of deterrency are a function of the number and quality of all the host resources present. The quantitative differences in deterrency shown in Experiment 1 were small, however,

and probably detectable only because of the high experimental precision. Experiment 2 suggested that two-choice assays may eliminate underlying interactions caused by either deprivation or habituation. Furthermore, two-choice tests can give a standard response unaffected by the ratio of treatment:contol ovipositional resources.

Multiple-choice assays have the advantages of allowing direct comparisons between all treatments (rather than comparisons through a control), as is necessary for conducting factorial designs. Also, multiple choice assays allow removal of experimental variation due to differences in overall oviposition activity, and are less labor intensive. The dose-response assays generated by multiple-choice and two-choice tests converge at low and high concentrations of deterrents; this suggests estimates of threshold responses and agronomically "practical" levels of deterrency may be estimated at nearly the same deterrent concentrations with either bioassay method. The advantages of multiple-choice assays are probably most readily realized in discriminating-dosage bioassays (Cowles, et al., 1990). In a descriminating-dosage assay, several treatments are compared via a multiple-choice design. However, unlike the dose-response assay in this paper, concentrations are kept low enough to prevent deprivation or habituation.

For the chapters to follow, multiple choice is justified as deterrency measures were similar enough between two-choice and multiple choice assays to satisfy the requirements of large chemical screening tests.

CHAPTER 3

Deterrency of Pungent Spices and Capsaicin-containing Products to *Delia antiqua* (Meigen)

Introduction

In accepting hosts, insects integrate via multiple sensory modalities a diversity of external excitatory and inhibitory inputs with internal excitatory and inhibitory inputs, that establish internal "physiological status" (Dethier, 1982; Miller and Strickler, 1984). As for many insect herbivores, external excitatory inputs affecting onion fly oviposition have been investigated much more extensively (Harris *et al.*, 1987; *et ante*), than external inhibitory inputs. Distinctive allelochemicals (Levin, 1971) from non-hostplants could potentially interfere with normal processing of host cues if brought into the region where intensive host examining behaviors occur.

After ascertaining that various spices deter *D. antiqua* oviposition in choice tests, ground cayenne pepper was chosen for detailed study involving both choice and no-choice conditions because: (1) a synthetic analogue of the principal flavor ingredient was readily available, (2) commercial products, including an insect repellent mixture available for field use, contain capsicum oleoresin as a principal ingredient, and (3) at least in vertebrates, capsaicinoids cause stimulation of chemoreceptors, heat and pain receptors (Virus and Gebhart, 1979), making animal behavioral responses likely.

Materials and Methods

D. antiqua culture - An onion fly culture was established in September 1986 from field collected pupae and maintained as described earlier (p. 18 - 19). Flies used in experiments were reared in the lab for 3-5 generations.

Laboratory ovipositional bioassays - Ovipositional assays were conducted in a second walk-in environmental growth chamber having conditions identical to the rearing chamber except this room was free of onion volatiles. All choice experiments were conducted in 57 cm diameter x 57 cm tall cylindrical cages stocked with *ca.* 50 male and 50 female flies provisioned with diet and water. These cages rotated once every 13 min, thus experimental error was minimized by distributing environmental gradients evenly over all treatments (Weston and Miller, 1985).

Ovipositional dishes and onion foliar surrogates were as previously described (p. 31).

With the exception of the no-choice experiment, all tests were randomized complete block designs, with treatments placed 5 cm from the perimeter of the cage in randomized order and blocked over time intervals, usually by day. Replicates with combined treatment counts of less than 50 eggs were pooled to minimize sampling error. Egg counts were not distributed normally, however log-transformed ($\ln(x+1)$) counts fulfilled assumptions for analysis of variance. A two-way analysis of variance general linear models procedure (SAS Institute, Cary, NC) was used, and means were separated with the Student-Newman-Keuls' Test for multiple comparisons.

Botanicals - Pungent spices were presented as choices in one cage, along with a foliar surrogate control. Treatments consisted of 5-7 mg of each of the following spices scattered within 1.5 cm of the surrogate onion foliage: crushed red pepper, consisting of 3 x 5 mm flakes with seeds; chili powder, containing chili

pepper, onion, cumin, garlic, oregano, cayenne pepper, black pepper, caraway and silicon dioxide; dill weed, flakes 1 cm x 1 mm; ground ginger; and coarsely ground black pepper (R. T. French Co., Rochester, NY 14692). This experiment was replicated three times, flies were allowed to oviposit 5, 6, and 48 h, respectively for each replicate.

Dose response series - Dose response choice tests were conducted in three cages, each cage with various concentrations of one test material. Ground cayenne pepper (GCP) (McCormick & Co., Baltimore, MD) or Sevana Bird Repellent Powder (SBR) (Sevana Co., Fresno, CA) were applied in quantities of 0 (control), 1, 2, 5, 10, 22 and 46 mg, placed within 1 cm of the surrogate onion. Agrigard Insect Repellent (AGR) (Sevana Co., Fresno, CA) diluted to 1, 3.2, 10, 32, 100 and 320 ppt in deionized distilled water was sprayed (*ca.* 0.175 ml) on the sand surface with a TLC atomizer.

Ovipositional deterrency of synthetic capsaicin was assayed in a dose response choice test with 50 males and 50 females per cage. Synthetic capsaicin (97% *n*-vanillyl-*n*-nonamide, Pfaltz & Bauer, Waterbury, CT) was dissolved in 95% ethanol and diluted to 6.32, 20.0, 63.2, 200, 632, 2000 and 6320 ppm. Twenty ml of each solution was added to 200 g white silica sand, mixed thoroughly and allowed to air dry. Final concentrations were 0.316, 1.00, 3.16, 10.0, 31.6, 100 and 316 μg/g sand. Each sand treatment was moistened with 10 ml deionized distilled water and added in a 1 cm layer on top of clean sand in oviposition cups.

No-choice test - GCP was assayed in a no-choice context that employed a two x two factorial completely randomized design that quantified effects of exposures during both pre-reproductive and reproductive periods. Pre-reproductive exposure was effected by placing 120 flies (not sexed, less than 24 hr post-eclosion) for 5 days in a cage with food, water, and a foliar surrogate treated with 10 mg GCP. A second

group received similar treatment but no GCP. Females from these two groups were transferred individually to 9 cm diameter screen sided cages and provided with 2 males, food, water and an ovipositional dish. Half of the females experiencing GCP pre-reproductively were provided standard foliar surrogate; half were provided foliar surrogate plus 10 mg GCP. Similar reproductive exposure treatments were provided to females that had previously not experienced GCP.

Egg counts were taken daily from day 6 to day 15 post eclosion, to measure days until first oviposition as well as daily oviposition. Onion flies in this experiment tended to lay most eggs on alternating days, which hindered data analysis because of zero counts. This problem was eliminated by pooling counts every two days. Egg counts for flies that died during the experiment (18% of total flies) were not used in the analysis, however their ovipositional record was included when comparing days until first oviposition. Days to first oviposition data could not be analyzed by parametric methods due to non-homogeneity of variance. These data were therefore analyzed with the Kruskal-Wallis Test (Steel and Torrie, 1980); treatment comparisons were then conducted with the Wilcoxon-Mann-Whitney Test (Steel and Torrie, 1980).

We hypothesized that if habituation or adaptation to GCP had occurred in the no-choice experiment, that flies with the greatest exposure would show diminished GCP deterrence in a follow-up choice test, *e.g.*, as when compared to flies not exposed or exposed only pre-reproductively. Therefore, groups of 3 females were pooled from each treatment and placed in 9 cm diameter screen cages with food, water and two oviposition cups, one with 10 mg GCP, one without. The percent ovipositional deterrency was then compared for the 4 treatment groups.

Field trials with capsaicin-based products - Field trials of AGR and SBR ovipositional deterrency to *D. antiqua* were carried out in 1986 in a commercial

onion field (Eaton Rapids, MI) with historically moderate onion fly population pressure. Plots consisted of 3 m of treated row, separated by 3 m of buffer row, in a linearly arranged randomized complete block design replicated 6 times. Blocks were separated by *ca.* 35 m. SBR treatments were 0.05, 0.5 or 5 g per plot, applied to the soil at the base of onion plants with salt shakers. AGR treatments were 1.5 mg, 15 mg, 150 mg, 1.5 g or 15 g plus 15 mg Vaporguard (Miller Chemical Co., Hanover, PA), in 70 ml distilled water per plot, using Chapin compressed air sprayers (Model No. 110, R. E. Chapin Manufacturing Works, Inc., Batavia, NY 14020), with Teejet 730077 flat fan nozzle at 20 psi (Spray Systems Co., North Ave, Wheaton, IL 60188). Negative controls for these treatments were 70 ml water and 15 mg Vaporguard in 70 ml water applied in 3 m plots in each block. There were 9 applications made of SBR and AGR, on a 7 to 10 day schedule starting May 27. Damage estimates in conventionally pesticide treated rows (liquid chlorpyriphos, *ca.* 1.1 kg A.I./ha) were made in 3 m of row 1 m laterally from untreated plots. Stand counts were made May 27 and at 2 week intervals. Analyses were conducted on percent damage [(stand count on August 6 / stand count May 27) x 100%]; these data did not require transformation.

Results and Discussion

Botanicals - In choice experiments, pungent spices all significantly deterred onion fly oviposition (Figure 1). Mean percent ovipositional deterrencies of these commercial spices were: paprika (88.6%), red pepper (95.9%), ginger (99.0%), dill (99.3%), chili powder (99.8%), and black pepper (100%).

Black pepper, red pepper and ginger were pungent spices that showed great promise in choice tests. Unfortunately, the pungent flavor components of black pepper, predominantly piperine, are suspected carcinogens (Buchanen, 1978), while gingerol, the pungent principle ingredient of ginger (Merck Index, 1976), is

relatively expensive. Red pepper was chosen for further studies based on its relatively well known physiological effects on taste perception in mammals and the availability of synthetic capsaicin. Capsaicinoids, the pungent principal flavor ingredients in red pepper, cause bursts of activity from contacted chemoreceptors and nociceptors (Virus and Gebhart, 1979). Capsaicinoids have some similarities to warburganal, a potent *Spodoptera exempta* anti-feedant from the bark of an African tree (Nakanishi, 1980). Both capsaicin and warburganal have a pungent flavor and are used as spices (Todd, et al, 1977; Nakanishi, 1980); in both cases these chemicals cause rapid firing from chemoreceptors that then become unresponsive to stimulation (Virus and Gebhart, 1979; Ma, 1977). It may be that such non-specific activity at the level of gustatory chemoreceptors enables these spicy substances to interfere with normal host acceptance behavior.

Dose-response series - In dose-response choice tests, oviposition of onion flies was reduced 78 to 99% by the presence of 1 to 46 mg GCP (Figure 2) (F = 26.1; df=6,24; $P<0.001$). Increasing quantities of GCP clearly caused greater reductions in the number of eggs laid next to treated surrogate foliage. Agrigard deterred oviposition at the highest rates tested (F = 10.0; df = 6,36; $P<0.001$), with 98% deterrence at 100 ppt and 100% deterrence at 320 ppt (Figure 3); recommended field rates correspond approximately to 32 ppt, which was not significantly different from the control. Sevana Bird Repellent showed no deterrence, even at the highest rate tested (F = 0.58; df = 6,36). Synthetic capsaicin incorporated into the top 1 cm of ovipositional substrate significantly deterred oviposition when present at concentrations greater than 600 ppm (F = 35.4; df = 7,77) (Figure 4).

As summarized by Dethier (Dethier, 1947), many chemicals are deterrent or repellent at high concentrations, including host-plant chemicals that at lower

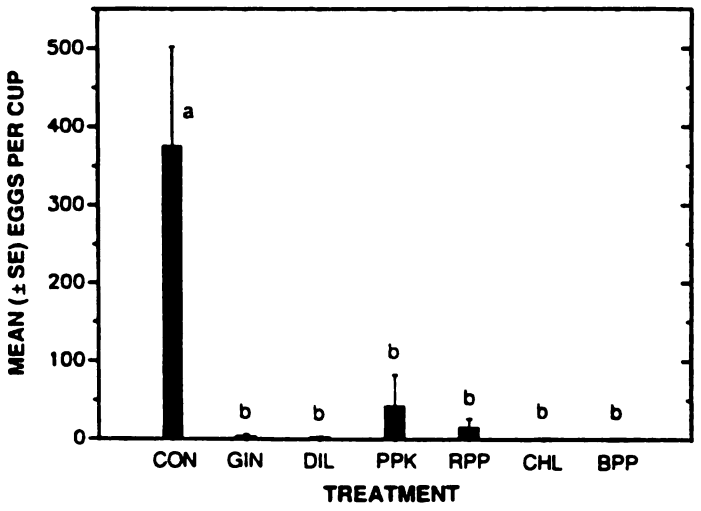


FIG. 1. Number of eggs laid with 5-7 mg of the following spices placed within 1.5 cm of surrogate onion in choice tests: CON, control; GIN, ginger; DIL, dill; PPK, paprika; RPP, red pepper; CHL, chili powder; BPP, black pepper. Means followed by the same letter are not significantly different (SNK test, 2 *df*).

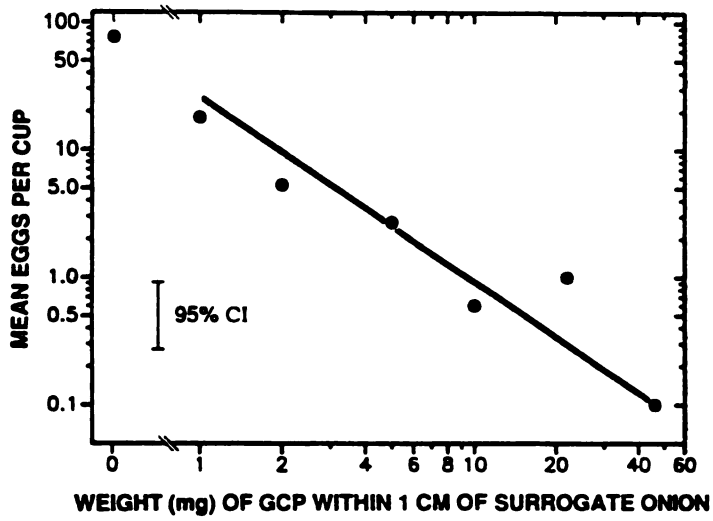


FIG. 2. Dose-response relationship for oviposition when ground cayenne pepper was placed in choice tests within 1 cm of surrogate onion foliage. Straight line fit by eye. Confidence interval based on 7 *df*.

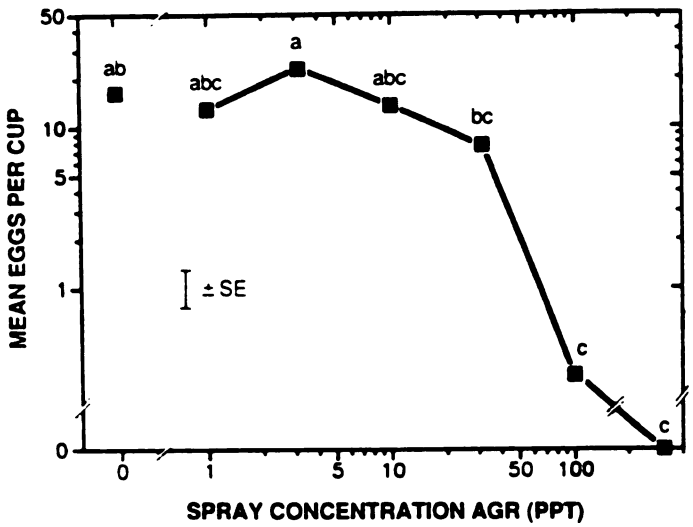


FIG. 3. Dose-response curve for oviposition when substrate was sprayed in choice tests with 0.175 ml of Agrigard Insect Repellent solutions. Standard error based on 6 *df*.

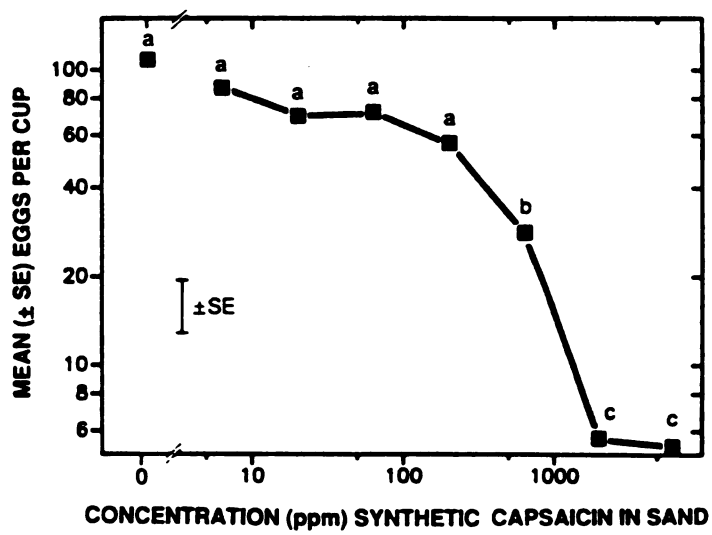


FIG. 4. Dose-response relationship for oviposition with synthetic capsaicin placed within the top centimeter of sand.

concentrations are involved with host acceptance. These dose response choice tests were conducted to be certain that low rates of a promising material did not stimulate oviposition. Any material showing stimulatory activity at low concentrations would cause logistical problems when applied in the field, because chemical decomposition would eventually decrease the concentration to levels that could stimulate insect damage. Dose response experiments with capsaicin-based products elicited no increased oviposition through the range of rates tested, unlike dipropyldisulfide, which shows deterrent activity at high concentrations (Matsumoto and Thorsteinson, 1968) and stimulatory activity with an optimum concentration in surface wax of about 0.05% (Harris, et al, 1987). Direct visual observations of fly behavior suggested that even at the highest rates tested, onion flies did not orient away from GCP; so, the end result appears to be mediated by deterrency upon contact rather than repellency.

No-choice test - In the no-choice experiment, there was no evidence that GCP deterred oviposition (Figure 5). Pre-reproductive exposure showed no significant effect on two-day egg counts (F = 0.19; df = 1,39); exposure during peak reproductive activity also was not significant (F = 0.39; df = 1,39). There were significant differences between treatments in days to first oviposition (Kruskal-Wallis Test, $^{H}/_{D}$ = 11.25, $P<0.025$) (Steel and Torrie, 1980). Multiple comparison of treatments showed only one significant difference, flies not exposed to GCP laid eggs sooner (mean of 6.9 days) than flies of the reproductive-exposure-only treatment (mean of 7.8 days) (Wilcoxon-Mann-Whitney Test, T'= 89.5, n_1 = 10, n_2 = 14, $P<0.05$) (Steel and Torrie, 1980).

No-choice tests of deterrents are a rigorous test of deterrency, because insects under these conditions face increasing internal excitatory inputs that can override the presence of external inhibitory inputs. No-choice situations may

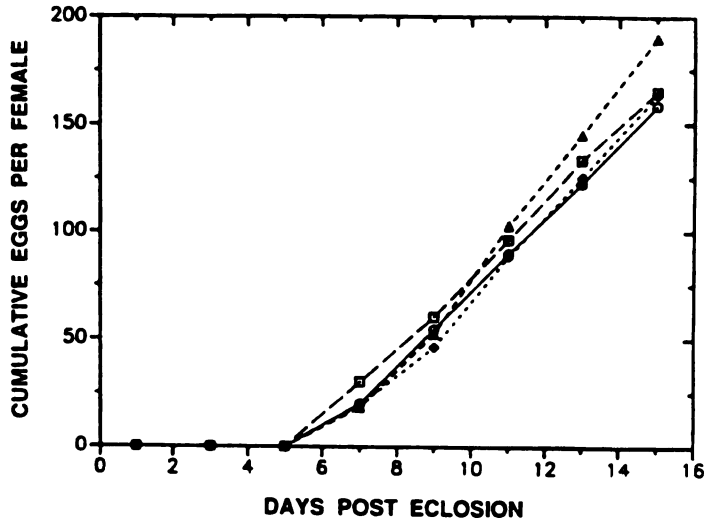


FIG. 5. Cumulative oviposition for 2 × 2 factorial experiment investigating prereproductive and reproductive exposure to GCP. --◊--, no exposure; --□--, exposure only prereproductively; --△--, exposure only reproductively; —○—, continual exposure.

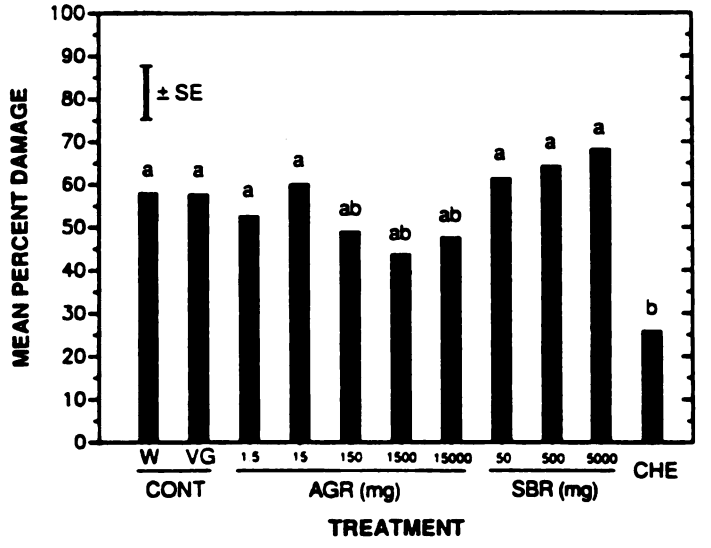


FIG. 6. Percent damage to seedling onions by onion fly. W, water control; VG, Vaporguard control; AGR, Agrigard; SBR, Sevana Bird Repellent powder; CHE, conventional chemical control (0.05 g chlorpyriphos/m). Amounts given are for 3 m of row. Means followed by the same letter are not statistically different (SNK test, 5 *df*).

simulate some field situations (where alternative acceptable ovipositional substrates may not be available), and also assist in determining whether choice test deterrency is caused predominantly by sensory vs. sublethal physiological effects (Landis and Gould, in press). Our no-choice tests effectively ruled out the possibility that effects of GCP seen in choice tests were caused by sublethal toxicity, since all no-choice groups laid similar numbers of eggs. This is in contrast to preliminary screening with pyrethroids, in which "deterrent" concentrations were accompanied by convulsions or tremors in onion flies (unpublished data).

In the choice test bioassay of flies previously used in the no-choice experiment, all groups laid similar percentages of eggs on the control (84 ± 2.7%, mean ± SE) (F = 0.49, df = 3,6), implying that these groups retained the same ability to sense and respond to the presence of GCP. Whether habituation or adaptation were responsible for the lack of differences in the no-choice experiment is still unclear; either dishabituation occurred rapidly or else another mechanism was responsible for acceptance of GCP treated ovipositional substrates. Perhaps flies simply tolerated deterrents because they were becoming deprived.

Field trials - Field tests of AGR and SBR in 1986 for the most part agreed well with laboratory studies, however the trends in field data were not statistically significant (Figure 6). There was a trend toward higher mean damage for all three rates of SBR in the field than in the control plots. These field results suggested that SBR may have stimulated oviposition, in contrast to the lack of response (stimulatory or deterrent) to SBR in the laboratory. Under the wet field conditions experienced in 1986, there exists the possibility that SBR, which consists of 10% ground red peppers and 4% ground garlic, could have decomposed to form microbial products stimulatory to onion fly oviposition (Coley-Smith and King, 1969; Dindonis and Miller, 1980).

The most highly concentrated sprays of AGR suppressed damage to a level intermediate to the untreated controls and the conventionally pesticide treated areas. These sprays were irritating to the applicators, and at elevated rates caused onions to have stunted, yellow foliage. These field results with AGR agree with the laboratory studies that indicated that ovipositional deterrency only occurred at concentrations exceeding the field recommended rates.

General Discussion

Ovipositional and feeding deterrents should be tested with hosts or host models that accurately reflect normal sensory input (Städler, 1983). Using natural stimuli allow normal sequences of behaviors to proceed. Allowing the full repertoire of behaviors involved with host acceptance to be expressed implies that each behavior has an opportunity to be influenced by the presence of deterrents. When using highly artificial substrates, internal excitatory inputs may override inputs associated with normally non-stimulatory substrates to provide abnormal behavioral responses. An example demonstrating how ovipositional substrates can influence results while studying ovipositional deterrents is the recent work of Tingle and Mitchell (1986). Their data show that in choice tests, elderberry extracts were more deterrent to *Heliothis virescens* when applied to tobacco (a preferred host) compared to a standard paper towel substrate.

Ovipositional deterrents have not previously been tested for the onion fly with host models. Wiens, et al, (1978) were able to reduce oviposition by ca 78% over controls in choice tests using hydrated bean (*Phaseolus vulgaris*) extracts. Alfaro, et al (1981), using cedar (*Thuja plicata*) leaf oil found an 84% ovipositional deterrency. Both of these experiments used inverted beaker ovipositional bioassays (Vernon et al, 1977). Deterrency assays with pine oil (Javer et al, 1987) similarly used halved onion bulbs rather than onion foliage or foliar models.

External excitatory inputs for the onion fly, *Delia antiqua* (Meigen), involve synergism between visual and chemical stimuli (Harris and Miller, 1982). Yellow or green vertical cylinders ca 4 mm diameter that emit *n*-dipropyl disulfide from surface waxes elicit pre-ovipositional behaviors, including foliar runs, ovipositor examining of foliage and soil, and ovipositor probing (Harris, *et al.*, 1987). These surrogate onions provided a highly standardized ovipositional resource that accurately simulated host stimuli. We suggest that they are highly suitable and convenient for studying ovipositional deterrency.

Loss of GCP deterrency in a no-choice situation is an excellent demonstration of the dynamic influence physiological state has on the acceptance of otherwise deterrent hosts (Dethier, 1982; Miller and Strickler, 1984). Other examples of how deprivation affects oviposition are available from fruit fly literature. Fitt (1986) deprived three species of Dacine fruit flies (Family Tephritidae) for 4 to 7 days and observed the host range that flies oviposited into. In *Dacus tryoni* Frogg, a rapid increase in egg load was accompanied by a propensity to accept *Solanum mauritianum* fruits which normally would not be acceptable. The other two species tested, *D. jarvisi* and *D. cacuminatus* apparently have a physiological feedback mechanism that allows resorption of eggs to take place; these species did not show altered host acceptance patterns when deprived. Effects of deprivation of suitable hosts can affect ovipositional preferences over a much shorter time scale. Roitberg and Prokopy (1983) found that *Rhagoletis pomonella* accepted hosts marked with ovipositional deterring pheromone following only a 5-10 min deprivation of fruits.

Loss of GCP deterrency in no-choice situations also indicates that there may be great difficulties in obtaining control of onion flies in the field. It may be necessary to make highly accepted alternative hosts available, in the form of trap or diversionary crops, so that flies do not enter an ovipositionally deprived state. The

low efficacy of capsaicin-containing products in 1986 field tests may have been due partially to extremely wet weather or to the lack of a such an alternative ovipositional resource.

While pungent spices appear to be highly deterrent materials for onion fly oviposition in laboratory choice tests, and could perhaps be of some use in the small home garden, I do not feel that they hold much promise for commercial field use.

Chapter 4

Cinnamyl derivatives and monoterpenoids as non-specific ovipositional deterrents of the onion fly

Introduction

Previous work with ovipositional deterrents for *Delia* flies has largely focused on mixtures of compounds from essential oils or other plant products. Havukkala (1982) had little success in field trials using turpentine-soaked stakes to control *D. radicum*, while Den Ouden and Theunissen (1980) achieved control of *D. brassicae* approaching standard chemical treatment in trials using slow-release formulations containing 2 - 8 g naphthalene per plant. Wiens et al., (1978) reported that hydrated bean seed extracts reduced onion fly oviposition by *ca.* 78% over controls in choice tests. The active constituent was a non-volatile gustatory deterrent and was not further characterized. Alfaro et al., (1981), found that high levels (300 μg per ovipositional site) of red cedar leaf oil deterred *D. antiqua* oviposition by 84%. This activity was attributed to thujones, the major monooxygenated monoterpenoid components of this oil. Pine oil, a mixture of terpenes and oxygenated terpenoids, significantly reduced onion fly oviposition in a no-choice situation when 5 mg was applied per onion bulb (Javer et al., 1987).

Cowles et al., (1989) showed that black pepper, ginger, dill, and various materials containing red pepper deterred onion fly oviposition. Synthetic capsaicin, an analog of the principal flavor ingredient of red peppers, deterred oviposition by 95% when present at 320 ppm in the top cm of sand. Red pepper's efficacy disappeared in no-choice conditions, however, and was not effective in field tests.

In this study, I quantified the ovipositional deterrency of a range of cinnamyl, cinnamoyl, monoterpenoid, and phenethyl alcohol derivatives presented around surrogate onions exposed to *D. antiqua* in the laboratory. The goal was to explore

structure-activity relationships and to target those compounds most promising for field assays.

Materials and Methods

D. antiqua culture - Onion flies were maintained as described earlier (pp.18-19) in a culture started in 1987. Flies used in all experiments were reared in the lab for 3-10 generations.

Chemicals - Most cinnamyl, cinnamoyl, and phenethyl derivatives were purchased from Aldrich Chemical Co., (Milwaukee, WI, 53233) or synthesized (Szurdoki et al., in prep.) in the Plant Protection Institute (Budapest, Hungary), according to published methods.

Compounds were diluted in polyethylene glycol as described earlier (p. 30), in five decade steps to a 0.001% concentration (w/w). Experiments used 20 mg formulated mixture per treatment.

Bioassays - Ovipositional assays were conducted in a walk-in environmental growth chamber having conditions identical to the rearing chamber except the bioassay room was free of onion volatiles. Experiments were conducted in 30 cm diameter x 30 cm cylindrical cages provisioned with diet and water and stocked with *ca.* 10 male and 20 female flies. These cages rotated on their axis once every 13 min; thus, experimental error was minimized because environmental gradients were distributed evenly over all treatments (Weston and Miller, 1985).

Ovipositional dishes and surrogate onion foliage used in bioassays are described on p. 31.

For initial screening, the deterrency (percent reduction in the number of eggs laid on a treatment *vs.* the control) of *ca.* 1 mg of neat compound was compared with an untreated control. Four treatments were tested simultaneously per cage. If

there was at least 80% deterrency in the first replicate, the compound progressed to dose-response analysis. If there was less than 80% deterrency recorded after three replicates, the BR_{90} (concentration required to elicit 90% behavioral response) was considered unattainable and the compound was listed as inactive.

Dose-response choice tests were conducted in a randomized complete block design. A replicate consisted of egg counts from six ovipositional resources (5 decade-step dilutions of a compound in PEG, plus a PEG control) placed 5 cm from the perimeter of the cage in randomized order and blocked by day. Five compounds were tested each day (one chemical in each of five cages) and flies were used once per compound; this ensured reasonable replicate independence and minimal fly learning. Replicates with combined treatment counts of less than 50 eggs were discarded to minimize sampling error. Every concentration series was replicated ten times. Phenethyl alcohol was tested separately from the other compounds listed in Table 1. It was tested two days apart; all five cages were simultaneously used to obtain the ten replicates.

Egg counts were not distributed normally; however, log-transformed ($\ln(x+1)$) counts fulfilled homogeneity of variance assumptions for analysis of variance (Bartlett's test, $P > 0.1$; excluding treatments with all zero egg counts and therefore zero variances). A two-way analysis of variance (general linear models procedure, SAS Institute, 1985) generated the treatment variance (mean square error). Ninety-five percent confidence limits were calculated for percent eggs relative to the control for each concentration in the dosage response relationship for a compound. A procedure analogous to calculating the least significant ratio (LSR) (Steel and Torrie, 1980) was used, except that the confidence limit values (mean $\bar{x} / + e^{(t_{\alpha/2} \sqrt{(MSE/r)})}$) were calculated. By connecting the upper limits, and similarly connecting the lower limits, a 95% confidence belt was constructed for each dose-response relationship. The intersection of the confidence belt with the

90% deterrency response graphically estimated the concentration of chemical eliciting 90% ovipositional deterrency (BR_{90}) for a compound (Figure 1). This graphical method was used instead of regression analysis because: 1) dose response functions were non-linear, 2) there were few deterrent concentration levels; therefore the regression degrees of freedom were low and confidence limits inflated, and 3) the graphical method makes better use of the knowledge that at high concentrations behavioral response converged towards absolute deterrency, generating zero variance. Differences between apparently stimulatory responses of compounds at low concentrations and the control treatment were compared via the Student-Newman-Keuls' test.

Upon establishing that only the most active compounds at 0.1% were detectably different from control PEG, this concentration was chosen for conducting discriminate-dosage bioassays. In one large experiment, the activity of 35 additional compounds was ranked. Four randomly chosen materials and two blank PEG controls were placed in each of five rotating cages; cumulative presentation of all compounds constituted one replicate. Thirty female and 15 male flies were used for each of the four replicates of this modified randomized complete block design. Data were analyzed under the assumption that the number of eggs laid on each treatment relative to the number laid on the control is independent of the presence of other treatments (see pp. 36-37). This allowed direct comparison of each compound to the control via a paired t-test. When these data were log(x+1) transformed, the value used to test the null hypothesis, [TRT-(CON1+CON2)/2] (SAS Institute, 1985), was a convenient transformation of percent acceptance, (TRT/CON)x100%, since subtraction with logarithms is equivalent to forming this ratio. One slight difference is that averaging the two blank PEG controls in the former equation is equivalent to taking the geometric mean of the non-transformed counts. Using the difference between log-transformed egg counts satisfactorily

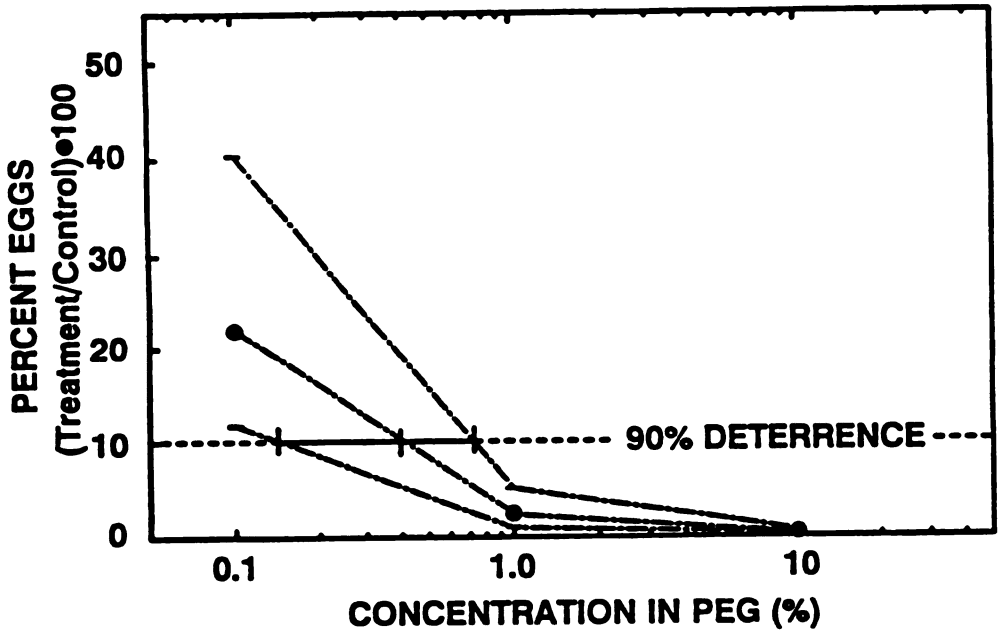


Figure 1. Method for determining the BR_{90} from the dose- response relationship, as demonstrated with cinnamonitrile (see text for further explanation).

transforms, and is symmetrical, for both deterrent (negative values) and stimulatory treatments (positive values). These data were then transformed to percent deterrency (or stimulation), for presentation in Table 3.

Vapor concentration required for deterrency - Ovipositional cups were prepared with 20 mg of 1% (w/w) (*E*)-cinnamaldehyde or (*E*)-cinnamonitrile in PEG placed on moistened sand, but without an onion foliar surrogate. One cup was placed in each of four cylindrical cages (the same as used for bioassays), and allowed to equilibrate in the 21° C conditions for one hour. A one ml air sample was withdrawn over a 45 sec interval with a Hamilton gas-tight syringe needle positioned within 5 mm of the PEG granules. The syringe needle was immediately inserted into a gas chromatograph injector port. The syringe was heated for 30 sec with a hand-held hair dryer, to assure that the vapors did not condense on the syringe wall, then the plunger was immediately depressed to inject the sample. The Varian 3700 gas chromatograph was equipped with a 30 m x 0.32 mm (ID) DB-5 capillary column. Running conditions were 120° C isothermal, 1 ml/min He carrier gas flow rate, with a flame ionization detector and HP3390A integrator. Standard curves were generated by injecting 1, 2, or 3 ng of each of the two test compounds in 1 to 2 μl of CS_2 (3 replicates).

Results

Dose response series - Among cinnamyl related compounds (Table 1), allyl benzene (**1**), (*E*)-β-methyl styrene (**2**), (*E*)-cinnamic acids (**3**, **4**, **5** and **6**), and (*E*)-cinnamamide (**7**) were less than 80% deterrent in the initial screening, so the BR_{90} was not attained. The mean BR_{90}'s for active compounds ranged from 0.38% in PEG for (*E*)-4-methoxycinnamaldehyde (**20**) to 3% for (*E*)-cinnamaldehyde dimethyl acetal (**8**).

Table 1. Estimated mean and 95% confidence limits of the BR_{90} ovipositional deterrency of cinnamaldehyde derivatives.

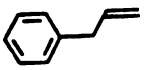
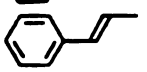
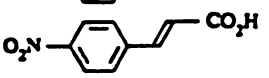
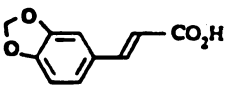
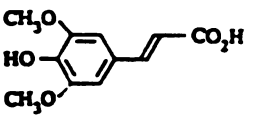
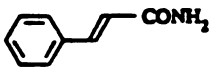
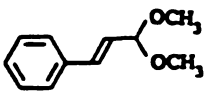
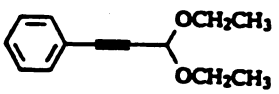
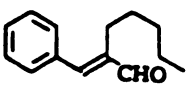
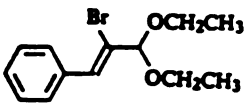
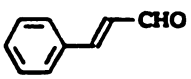
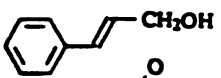
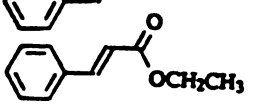
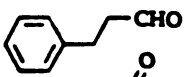
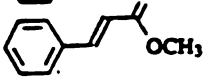
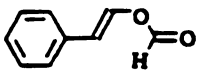
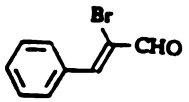
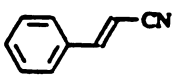
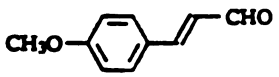
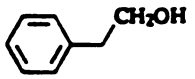
	Compound	b.p./pres. [°C]/[mm] (m.p.) [°C]	Source, Purity	Structure	% of Compound in PEG: Mean BR_{90}	95% C. I.
1	allyl benzene	156	A, 98%		Not active	
2	(*E*)-β-methyl styrene	175	A, 99		Not active	
3	(*E*)-cinnamic acid	300	A, 99+		Not active	
4	(*E*)-4-nitrocinnamic acid	(285-286) dec.	H, 93		Not active	
5	(*E*)-3,4-methylenedioxy-cinnamic acid	(242-243) dec.	H, 97		Not active	
6	sinapinic acid 3,5-dimethoxy-4-hydroxycinnamic acid [predominantly (*E*)]	(203-205) dec.	A, 98		Not active	
7	cinnamamide [predominantly (*E*)]	(147)	A, 97		Not active	
8	(*E*)-cinnamaldehyde dimethyl acetal	257*	I, 90		3.0%	1.3 - 5.0%
9	phenylpropargyl aldehyde diethyl acetal	110-115/15	H, 95		2.5	0.92 - 5.6
10	α-pentyl cinnamaldehyde	287	A, 97		2.6	0.99 - 4.8
11	(*Z*)-α-bromocinnam-aldehyde diethyl acetal	145-150/5	H, 95		2.1	0.91 - 4.2
12	(*E*)-cinnamaldehyde	248	A, 99		1.0	0.60 - 2.8
13	(*E*)-cinnamyl alcohol	250	A, 98		0.88	0.60 - 1.8
14	ethyl cinnamate [predominantly (*E*)]	271	A, 99		0.80	0.50 - 1.4
15	hydrocinnamaldehyde	224	A, 95		0.84	0.50 - 1.2
16	(*E*)-methyl cinnamate	260	A, 99		0.70	0.35 - 0.96

Table 1 (cont'd.).

17 2-phenylvinyl formate		N, 41		0.50	0.13 - 0.70
18 (*Z*)-α-bromo cinnamaldehyde	(67-68)	H, 98		0.44	0.22 - 0.64
19 cinnamonitrile [predominantly (*E*)]	255	A, 97		0.43	0.15 - 0.74
20 (*E*)-4-methoxy cinnamaldehyde	(59-61)	H, 95		0.38	0.16 - 0.60
21 2-phenethanol	220	A, 99		0.32	0.12 - 0.54

* Boiling point corrected to 760 mm.

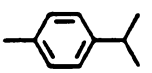
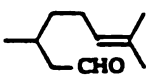
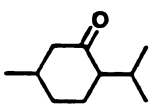
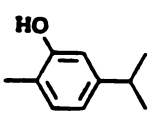
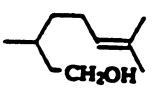
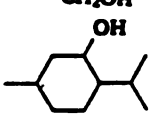
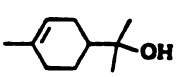
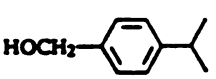
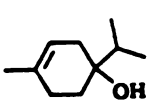
Chemical sources: A, Aldrich Chemical, Milwaukee, WI, 53233; H, Plant Protection Institute, Budapest, Hungary; I, Internat. Flavors and Fragrances, Inc., NY, NY; N, Dr. M. G. Nair, Horticulture Dept., Michigan State Univ.

Ovipositional deterrent activity among terpenoids paralleled that of cinnamyl derivatives with similar functional groups (Table 2). The non-oxygenated monoterpene *p*-cymene (**22**), having the same carbon skeleton as terpenoids **24**, **25**, and **27** - **30** in Table 2, was inactive in preliminary screening. The remaining oxygenated compounds had overlapping 95% confidence limits for their BR_{90}'s, so their *D. antiqua* ovipositional deterrency did not differ statistically from each other.

Discriminate-dosage tests - Compounds tested at 0.1% concentration are ranked by increasing deterrency in Table 3. Because compounds were relatively dilute and variability was high, only the most deterrent compounds were significantly different from the control. Mean percent deterrency given in parentheses (compounds **31** - **37**) reveals instances when more eggs were laid on the chemical treatment than the control. Though none of these compounds was statistically different from the blank PEG, the 67% mean percent stimulation with benzalpinacolone (**31**) suggests that this compound is a likely ovipositional stimulant. Similarly, the close agreement of *ca.* 34% increase in numbers of eggs with 4-nitrophenethyl alcohol and (*E*)-4-nitrocinnamyl alcohol suggests that they are possible stimulants at 0.1%. Compounds **34** through **50**, with standard errors approaching or overlapping 0% deterrency, are probably neutral at the tested concentration. The remaining chemicals in the table were variably deterrent. Among those that were significantly different from the control, (*E*)-cinnamaldehyde dimethyl acetal (**54**) was 58% deterrent (lowest) while hydrocinnamonitrile (**64**) was 88% deterrent (highest).

Vapor concentration required for deterrency - The concentration of active compounds in air was quantified by gas chromatography. (*E*)-Cinnamaldehyde at its BR_{90} (1% formulation in PEG) was present at 1.7± 0.3 ng (mean ± S. E.) per ml of air. (*E*)-Cinnamonitrile is more active than cinnamaldehyde, so the 1% formulation

Table 2. Estimated mean and approximate 95% confidence limits of the BR_{90} ovipositional deterrency of monoterpenoids.

	Compound	b.p./pres. [°C]/[mm]	Source, Purity	Structure	% of Compound in PEG: Mean BR_{90}	95% C. I.
22	*p*-cymene 4-isopropyltoluene	176-178	A, 96%		Not active	
23	citronellal	207	S, 95		3.7%	1.4 - 5.8%
24	menthone [23% isomenthone]	210	A, 75		1.0	0.80 - 2.8
25	carvacrol 5-isopropyl-2-methylphenol	236-237	A, 98		0.90	0.62 - 1.7
26	citronellol	222	G, 92		0.88	0.72 - 1.2
27	menthol	216	A, 99		0.86	0.64 - 1.0
28	α-terpineol	217	A, 98		0.85	0.52 - 2.4
29	cumic alcohol 4-isopropylbenzyl alcohol	135-136/26	A, 97		0.66	0.40 - 0.85
30	terpinene-4-ol	210	A, 97		0.46	0.12 - 0.90

Chemical sources: A, Aldrich Chemical, Milwaukee, WI, 53233; G, Givaudan Corp., Clifton, NJ, 07014; S, SCM Organic Chemicals, Jacksonville, FL, 32201

would give greater than 90% deterrency; its vapor concentration was measured at 2.3± 0.3 ng/ml.

Discussion

Advantages of discriminate-dosage bioassay - Besides being able to select compounds with high activity, the discriminate-dosage bioassay could be completed approximately eight time faster than a full dose-response series. Furthermore, being able to test a large number of compounds in one experiment permitted a larger number of compounds to be compared directly. The 90% deterrency estimate for (*E*)-4-methoxycinnamaldehyde (**20**), which was included as a positive control in half of the discriminate-dosage replicates, agreed well with the estimated BR_{90} concentration of 0.4% from the dose-response assays.

A large amount of structure-activity information was obtained by combining data from dose-response and discriminate-dosage bioassays. Instead of using the BR_{90} values from Tables 1 and 2, deterrency observed at the 0.1% PEG concentration in dose response assays was directly compared to the discriminate-dosage tests.

Structure-activity relationships - Key features investigated in this study were: 1) unsaturation and length of alkyl side chains, 2) functional groups at the end of an alkyl side chain, 3) ring substitution, and 4) presence of an aromatic ring. Unsaturation and the accompanying conjugation between the aromatic ring and carbonyl in cinnamaldehyde were not necessary for activity. (*E*)-Cinnamaldehyde (**12**) and hydrocinnamaldehyde (**15**) had similar BR_{90}'s of about 0.9% (Table 1), and had similar dose-response profiles. Hydrocinnamonitrile (**64**) and cinnamonitrile (**19**) had similar deterrency (*ca.* 85%) at the 0.1% concentrations. However, the alkyne bond in phenylpropargylaldehyde (**59**) and its dimethyl

Table 3. Estimated percent ovipositional deterrency (stimulation) for compounds tested at 0.1% concentration in PEG.

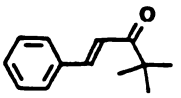
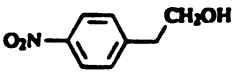
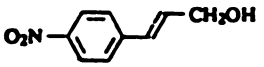
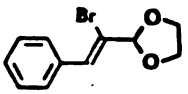
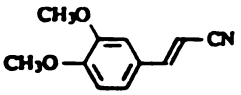
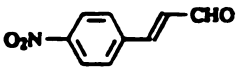
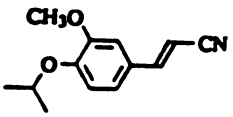
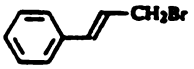
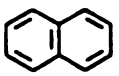
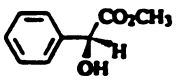
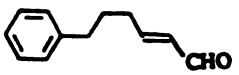
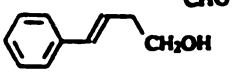
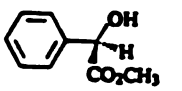
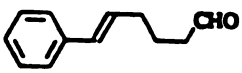
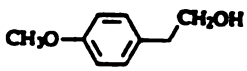
	Compound	b.p./pres. [°C]/[mm] (m.p.)[°C]	Source, Purity	Structure	% Deterrency (Stim.) Mean	S.E.
31	benzalpinacolone 2,2-dimethyl-5-phenyl-4-penten-3-one	(41-43)	H, 95%		(67)%	(87)-(16)%
32	4-nitrophenethyl alcohol	177/16	A, 99		(37)	(50)-(21)
33	(*E*)-4-nitrocinnamyl alcohol	(122-125)	H, 90		(31)	(47)-(10)
34	(*Z*)-α-bromocinnamaldehyde ethylene acetal		H, 95		(26)	(42)-(5)
35	(*E*/*Z*)-3,4-dimethoxy cinnamonitrile		A, 97		(26)	(34)-(17)
36	(*E*)-4-nitrocinnamaldehyde	(139-141)	H, 95		(14)	(47)- 28
37	(*E*/*Z*)-4-isopropoxy-3-methoxy-cinnamonitrile (*E*/*Z* - 1:1)		H, 95		(5)	(28)- 20
38	(*E*)-cinnamyl bromide	100-105/20	H, 90		4	(32)- 38
39	naphthalene		P, 99		6	(36)- 44
40	(S)-(+)-methyl mandelate	171†	A, 99+		8	(53)- 60
41	(*E*)-6-phenyl-2-hexenal		H, 96		11	(14)- 32
42	(*E*)-4-phenyl-3-buten-1-ol		H, 95		21	(14)- 46
43	(R)-(-)-methyl mandelate	171†	A, 99+		21	(15)- 48
44	(*E*/*Z*)-6-phenyl-5-hexenal (*E*/*Z* - 8:2)		H, 90		22	(20)- 51
45	4-methoxyphenethyl alcohol	334	A, 99		23	(10)- 47

Table 3 (cont'd.).

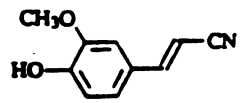
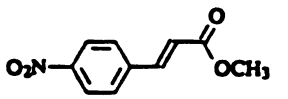
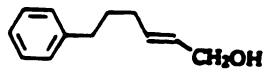
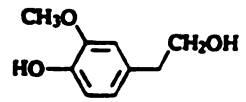
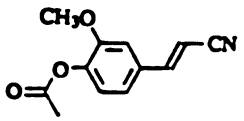
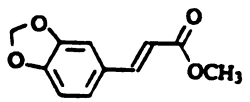
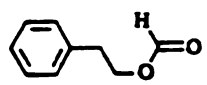
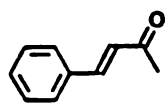
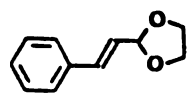
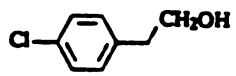
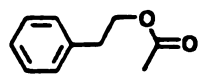
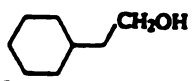
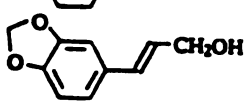
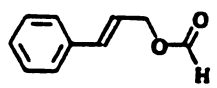
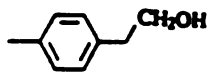
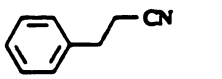
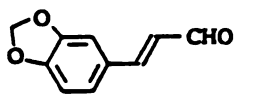
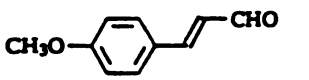
46	ferulonitrile (*E*/*Z*)-4-hydroxy-3-methoxy-cinnamonitrile (*E*/*Z* - 1:1)	160/1	H, 95		24	(22)- 56
47	(*E*)-methyl 4-nitrocinnamate	(158-161)	H, 90		30	(12)- 44
48	(*E*)-6-phenyl-2-hexen-1-ol		H, 91		30	(10)- 56
49	homovanillyl alcohol		A, 99		31	1 - 52
50	(*E*/*Z*)-4-acetoxy-3-methoxy-cinnamonitrile (*E*/*Z* - 1.4:1)		H, 95		32	4 - 51
51	(*E*)-methyl 3,4-methylenedioxycinnamate	(131-133)	H, 93		48	9 - 70
52	β-phenethyl formate		P, 95		50	18 - 70
53	benzalacetone (*E*)-4-phenyl-3-buten-2-one	(38-41)	H, 97		52	38 - 65
54	(*E*)-cinnamaldehyde ethylene acetal		H, 95		58*	43 - 69
55	4-chlorophenethyl alcohol	310†	A, 99		59	21 - 79
56	phenethyl acetate	232-235	H, 97		62*	45 - 73
57	2-cyclohexylethanol	207	A, 99		63	13 - 84
58	3,4-methylenedioxy cinnamyl alcohol	(71-74)	H, 97		64*	64 - 71
59	phenylpropargylaldehyde	118/13	A, 96		69**	68 - 70
60	(*E*)-5-phenyl-4-pentenal		H, 92		71*	60 - 79
61	cinnamyl formate		P, 97		76*	74 - 77
62	4-methylphenethyl alcohol	224	A, 99		78	50 - 90

Table 3 (cont'd.).

63	1-nonanol	215	A, 99		81*	68 - 88
64	hydrocinnamonitrile	250†	A, 99		88*	78 - 93
65	(*E*)-3,4-methylenedioxy-cinnamaldehyde	(84-86)	H, 95		88	70 - 95
20	(*E*)-4-methoxy-cinnamaldehyde	(59-61)	H, 95		90**	86 - 92

*$P < 0.05$, **$P < 0.001$; for t-tests of ln(x+1) treatment eggs versus controls, n=4 for all compounds except **20**, n=20 for **20**.

† Boiling point corrected to 760 mm.

Cinnamaldehyde: 3-phenyl-2-propenal; cinnamic acid: 3-phenyl-2-propenoic acid; cinnamyl: 3-phenyl-2-propen-1-yl

Chemical sources: A, Aldrich Chemical, Milwaukee, WI, 53233; H, Plant Protection Institute, Budapest, Hungary; P, Pfaltz and Bauer, Inc., Waterbury, CT, 06708.

acetal (**9**) appeared to enhance activity compared to the saturated or alkene compounds (**15, 12, 8**).

Increasing the length of the alkyl side chain of aromatic compounds appeared to decrease activity. (*E*)-5-phenyl-4-pentenal (**60**) was deterrent while (*E*)-6-phenyl-5-hexenal (**44**) was not. An alkyl side-chain also decreased activity of **10** *vs.* **12**. Phenethyl alcohol (**21**), included as a comparison to cinnamyl alcohol (**13**), had the highest activity (BR_{90} of 0.32%), suggesting that a two-carbon side chain is no less active than a three-carbon side chain. Part of the loss in activity with alkyl group size is probably due to the attendant decrease in volatility. The vapor pressure of these compounds is inversely related to the boiling points given in the tables.

Moderately polar functional groups appear to be required for ovipositional deterrency. Hydrocarbons (**1, 2, 22**) were inactive, at the extreme for non-polar compounds. Carboxylic acids (**3 - 6**) and cinnamamide (**7**) are relatively polar compounds, and also were inactive. Functional groups associated with deterrency were ketones, aldehydes, alcohols, nitriles, and esters. The contrast between aldehydes and alcohols (**12** *vs.* **13, 23** *vs.* **26**), seems to indicate that alcohols had greater activity.

Modifications of aldehydes included formation of their acetals and α-bromination. Acetals (**8, 11, 34,** and **54**) were investigated because they might allow slow chemical release (Pickett et al., 1984) of the parent aldehydes (**12, 18**). Acetalation reduced activity; this could be attributed to reduced fit to a receptor and/or decreased volatility. α-Bromo derivatives (**18, 11**) were more active than the non-halogenated compounds (**12, 8**).

Ring substitution at the *para* position of phenethyl alcohol (**21, 32, 45, 55, 62**), decreased deterrency in the following order: unsubstituted, methyl, chloro, methoxy, nitro. Nitro-substitution converted deterrent phenethyl alcohol (**21**),

cinnamaldehyde (**12**), and cinnamyl alcohol (**13**), into possibly oviposition-stimulating chemicals (**32**, **36**, and **33**, respectively). Except for the 3,4-methylenedioxy-substituted cinnamaldehyde (**65**), multiple ring substitution seemed to reduce deterrency compared to unsubstituted or monosubstituted rings. For example, none of these compounds (**37**, **46**, **49**, **50**, and **51**) were significantly deterrent in the discriminate assay. One half as much terpinene-4-ol (**30**) was required as carvacrol (**25**) to achieve 90% deterrency, suggesting that the OH ring position may affect deterrency.

The closely similar activity of carvacrol (**25**) and citronellol (**26**) suggests that the aromatic ring is not a key deterrent feature. This is confirmed by the deterrency of 1-nonanol (**63**).

Biological activity as influenced by chemical concentration - As summarized by Dethier (1947), many chemicals are deterrent or repellent at high concentrations, including host-plant chemicals that at lower concentrations mediate host acceptance. For example, n-propyl disulfide is deterrent at high concentrations (Matsumoto and Thorsteinson, 1968; Harris et al., 1987) and stimulatory at an optimum concentration in onion surface wax of about 0.05%. Demonstrating that these compounds were not simply deterrent because of elevated dosages was particularly important because phenethyl alcohol, the most active deterrent in these assays, had been described by other workers as an attractant for both onion and seed-corn flies (Ishikawa et al., 1983). The dose-response choice tests were well suited to ascertain deterrent potency at a range of concentrations; the discriminate-dosage bioassays also address stimulation at low concentrations. Any material that is stimulatory at low concentrations would be problematic when applied in the field, because the concentration eventually would decrease to levels stimulating undesirable insect behaviors. None of the cinnamaldehyde or terpenoid derivatives

tested in dose-response assays elicited increased oviposition through the range of rates tested, though, as mentioned above, some nitro-containing compounds assayed via discriminate-dosage may be somewhat stimulatory.

The concentration of compounds from both groups of compounds required to deter ovipositional activity, and the threshold for detecting EAG response to these compounds (Cowles, unpublished data), indicate that onion flies have approximately a 10-100 fold greater sensitivity to the stimulatory compound, n-propyl disulfide, than to these deterrents. These generalizations agree well with Dethier's (1980) proposition that receptors sensitive to deterrents should be relatively less specialized, and therefore have higher sensitivity thresholds, than receptors that are adapted for detecting host-specific stimuli.

Behavioral effects of these deterrents and volatility - At the highest dosages of deterrent compounds, flies were observed to move away from the source after approaching to within *ca.* 1 cm. Thus, they act as repellents. Part of the reason why some compounds in this study are more active than capsaicin-containing products (Cowles et al., 1989) is that deterrency could be mediated not only by short range gustation (as with capsaicin) but also through olfaction.

Deterrency is not a simple function of volatility, however. This is demonstrated with hydrocarbons, which are highly volatile but inactive, and by the high deterrency of less volatile compounds, such as 4-methoxycinnamaldehyde (**20**) and α-bromocinnamaldehyde (**18**).

Suggested mode of action for *D. antiqua* ovipositional deterrents - Deterrents can be hypothesized to have various modes of action (Davis, 1985). They could: 1) block perception of host stimuli by binding to and inhibiting their receptors (Davis, 1985), 2) cause more permanent damage to chemoreception by cross-liking or otherwise disturbing receptor proteins (Gothilf and Bar-Zeev, 1972), 3) act upon

specialized deterrent receptors responsive to non-host odors or eidectic pheromones (Prokopy, 1981) or, 4) be detected by generalized receptors and interpreted as deterrents by the CNS. The broad array of deterrent compounds, including monoterpenoid ketones and alcohols, substituted phenethyl alcohols, 1-nonanol, and cinnamyl-related aldehydes, their acetals, alcohols and nitriles, is unsupportive of the idea that *D. antiqua* deterrency requires functional group specificity, and thus is unsupportive of hypotheses 1, 2, and 3 (above). Indeed, the similar deterrency ranking of positional (**41** and **44**) or optical (**40** and **43**) isomers argues against highly specific receptor geometry. The large variation in deterrent structure, both in size and functional moieties, requires that *D. antiqua* possess either a very broadly tuned population of one chemoreceptor type, or more likely, a variety of receptor types that detect these compounds. For onion fly ovipositional deterrency, I favor hypothesis 4 (above), and recognize that dealing with multiple deterrent receptors complicates optimization of compound "fit" to receptors via structure-activity relationship analyses, particularly if stimulus inputs interact in the CNS.

Consideration of stimulus interaction - Host odors can act synergistically during host finding or acceptance (Finch, 1978; Visser, 1983; Davis and Sokolove, 1976), probably via central integration of signals from various receptors (Visser, 1983). At the ecological level, integrating signals from various receptors provides far greater information than relying on signals from one receptor type. Combining inputs, both within and across sensory modalities (Miller and Strickler, 1984) thus reduces uncertainty in the host acceptance decision-making process. Such a scheme also holds for interpreting compounds as deterrents, hence presentation of single deterrent compounds may not be the best way to maximize deterrence. Instead, synergistic combinations of compounds that act upon several receptor populations might be most deterrent.

Complex involvement of multiple receptor populations could help explain inconsistencies in the rank order for deterrency based on specific chemical modifications. For example, *para*-methoxy-substitution (**20**, **45**) increased activity for (*E*)-cinnamaldehyde (**12**), but not for phenethyl alcohol (**21**). Another example is the reduced activity of 3,4-methylenedioxycinnamyl alcohol compared to the aldehyde (**58** *vs.* **65**), which reverses the trend seen with other alcohol/aldehyde comparisons.

Ecological interpretations - Why are onion flies deterred by such a broad array of chemicals? The phenylpropenoids, phenolics, and terpenoids tested in this study are well known allelochemicals. In plants, they prevent competition by inhibiting root growth or seed germination (Rice, 1984; Nair et al., 1988), inhibit microbes (Levin, 1971; Mitscher, 1975; Mandava et al., 1980), and are toxic to or block detoxification enzymes in insects (Brattsten, 1983; Singh et al., 1989). These same compounds can be sequestered by or synthesized by insects for defense (Eisner, 1970) and can act as cues for the presence of interspecific competitors (Jones et al., 1988). More complex multitrophic interactions (Howard et al., 1988) can be governed by plant defensive compounds. Indeed, the ability of herbivores or predators to sense these chemicals allows them to function as interspecific warning signals (Lovett et al., 1989). Such signals serve the evolutionary interests of both the sender, who benefits by deterring enemies, and the receiver, who benefits by avoiding a source of potentially toxic substances. For the insect not adapted to detoxify phenolics or terpenoids in their host plant or environment, these chemicals could, over evolutionary time, become grouped to indicate poor suitability.

Sensitivity to phenolics and terpenoids appears to be a general phenomenon in insects, and suggests that chemoreceptors for these compounds might be ancient. Mosquito feeding repellents (Bunker and Hirschfelder, 1925) and ovipositional

deterrents (Klocke et al., 1989), have structure-activity relationships very similar to those of the present study; activity is largely governed by oxygenation of C_7 to C_{13} compounds. Thus, the 'oxygenated' derivatives of inactive hydrocarbons (aldehydes, esters, ketones and alcohols) exhibited strong repellent activity. If terpenoids and phenylpropenoids are detected by the same receptors, then I would expect the magnitude of insect response (attraction or avoidance) to be correlated with fit of compounds to those receptors. In some cases, species are attracted to the same compounds that are deterrent to onion flies. For example, in trapping experiments using bait pails for codling moths, citronellal and methyl cinnamate have been shown to be attractants (Dethier, 1947). Specific structure-activity relationships have been elucidated for attraction of *Diabrotica* species to cinnamyl derivatives. Among these derivatives, *Diabrotica* species responded most strongly to 4-methoxycinnamaldehyde, 4-methoxycinnamonitrile, cinnamyl alcohol, and cinnamaldehyde (Metcalf and Lampman, 1989), the same compounds with greatest onion fly deterrency.

Though general sensitivity of insects to these compounds may be exploitable, specific ecological relationships are certain to fine tune the behavioral response and re-order the ranking of activity. For example, even though codling moths are sensitive to the same classes of compounds as are onion flies, their rank order for sensitivity to acids, esters, and alcohols (Dethier, 1947) is the reverse of what I observed for onion flies, probably because they are adapted to fruit feeding. Recently, Jones et al., (1988) discovered that 3,5-dimethoxy-4-hydroxycinnamic acid, produced in the frass of a lepidopteran pest of cabbage, deters oviposition by *D. radicum*, suggesting that this *Delia* species may have greater sensitivity to carboxylic acids than does the onion fly.

Practical considerations - There are practical advantages and disadvantages to the finding that deterrents or repellents act upon fairly generalized chemoreceptors. On one hand, generality could allow a single product to be broadly useful in agriculture because of the wide range of pests that could be deterred. On the other hand, higher concentrations are required than for detecting host cues. Field studies with thymol, eugenol, cinnamaldehyde and its dimethyl acetal, phenethyl alcohol, and cinnamyl formate (Cowles, unpublished data) suggest that practical use of ovipositional deterrents will rely on placing these compounds in close enough proximity to a host plant, and at sufficiently high concentrations. For the volatile compounds (*E*)-cinnamaldehyde and (*E*)-cinnamonitrile, the concentration of active material required for at least 90% deterrency appears to be 1 to 2 ng/ml of air, which is 9 orders of magnitude more concentrated than for obtaining the BR_{50} for some lepidopteran pheromones (Miller, unpublished data). Because of the high concentrations of these compounds needed for control, care will be required that formulations be designed to avoid phytotoxicity.

I believe that these ovipositional deterrents should be explored further as possible agents of pest control. It is not yet clear whether deployment would involve controlled release formulations of synthetics, companion plants chosen for release of these deterrents, or using plant breeding or genetic engineering to incorporate these agents into the crop plants. Whatever the deployment method, it will be important that onion flies are given highly attractive alternative sites in which to oviposit (i.e., the stimulo-deterrent diversionary strategy, Miller and Cowles, 1990), so that pest deprival does not lead to loss of deterrency.

Chapter 5

Interaction of visual and chemical onion fly ovipositional deterrents

Introduction

Acceptance of host-plants by many phytophagous insects is triggered by particular combinations of chemical, visual, and physical stimuli sensed during examining behaviors (Miller and Strickler, 1984; Papaj, 1986; Prokopy, 1986). The onion fly is an excellent example: oviposition is stimulated by a combination of dipropyl disulfide released at optimal concentrations from foliage and substrate, and by yellow or green cylindrical foliage 4 mm in diameter standing upright in soil (Harris and Miller, 1991).

The nature of stimulus interaction during host examining is not well understood. Miller and Harris (1985) likened the neurophysiological decision making process to an electronic lock, in which pushing buttons (receiving modality-specific stimuli) opens the lock (signifying host-plant acceptance). There are at least four distinct models that can be described with this analogy; all have the same possible end-result of integrating sensory information across-modalities. 1) In "classical behavioral chaining", sequential behaviors leading to host-plant acceptance are released by "sign stimuli" (Miller and Harris, 1985; Kennedy, et al., 1961). Modality-specific sensory information is serially processed: particular behaviors are responsible for collecting specific types of information. The electronic lock "opens" when separate buttons are pushed in an unvarying order. 2) Across-modality stimulus summation requires convergence of information from different sensory modalities at an interneuron (Miller and Harris, 1985; Camhi, 1984; Meredith and Stein, 1983). Stimulation of this cell then triggers host-plant acceptance. An insect then may be able to perceive the host *Gestalt* instantaneously

because of parallel processing of sensory information. The electronic lock, in this case, opens when a particular combination of buttons are simultaneously pushed. 3) The "classical" behavioral chain concept does not apply when specific examining behaviors may be followed by a number of other behaviors. A Markov-chain process may be more appropriate, in which a behavioral web replaces the linear "classical behavioral chain." Markov-chains have probabilities associated with transitions between any two behaviors (Hoppensteadt, 1982). When modality-specific sensory input is associated with each behavioral state, the result may be temporal summation. The electronic lock opens when required buttons are pushed in any order. 4) Last, a mixed model may apply, in which Markov-chain transitions between examining behaviors are involved with processing sensory information across sensory-modalities. The electronic lock opens when specific combinations of buttons are pushed at various times.

These models can be evaluated with respect to the expected behavioral patterns they would generate. Models 1 and 2 would be characterized by highly deterministic behaviors, perhaps appearing to the viewer like a fixed action pattern (Dawkins, 1983). Models 3 and 4 involve highly probabilistic examining behaviors.

Manipulative experiments involving combinations of stimuli affecting different sensory modalities should be able to distinguish between models involving parallel sensory processing (Models 2 and 4) vs. serial processing (Models 1 and 2). Complex interactions may take place at a recognition interneuron in Models 2 and 4, so any number of outcomes can be programmed for a combination of sensory inputs (Moore and Christensen, 1985). Under these conditions, marginal acceptance probabilities for factors affecting different modalities should be dependent. With temporal summation (Models 1 and 3), the probability of acceptance is the product of probabilities that each behavior has occurred. This

model predicts independent marginal acceptance probabilities for factors affecting different sensory modalities.

A detailed investigation of onion fly examining behaviors has suggested that temporal summation (Miller and Harris, 1985) of sensory information occurs through behavioral webs, but did not distinguish between Models 3 or 4. This paper extends work on onion fly host acceptance by investigating interactions between combinations of deterrent visual and chemical stimuli. Chemical deterrents with different polar functional groups were tested either singly or in mixtures constituting a test for synergistic interactions within gustatory and olfactory modalities. A factorial design investigated the between-modality interaction of visual and chemical deterrents. Behavioral observations from videotape recordings were then used to study whether specific *D. antiqua* examining behaviors were primarily responsible for assessing chemical vs. visual sensory stimuli.

Methods and Materials

General methods - Flies were from the same population as reported in pp. 18-19 and were reared in the lab for 10-15 generations.

Phenethyl alcohol, hydrocinnamonitrile, and (*E*)-cinnamaldehyde were purchased at 99% purity from Aldrich Chemical. (*E*)-Cinnamyl formate was purchased from Pfaltz and Bauer (Waterbury, CT, 06708) at 97% purity. These compounds were diluted on a weight/weight basis in polyethylene glycol (PEG), according to the same methods as in Chapter 2 (p. 30). Experiments used 20 mg formulated mixture per treatment.

Ovipositional dishes were the same as described on p. 31. Deterrents were evenly dispensed on the sand within 1 cm of the juncture of foliage and soil, where most host examining behaviors and oviposition occur (Harris et al., 1987). PEG

granules applied on moist sand partly dissolved and became incorporated in the top 1 mm layer.

Experiment 1. Synergism of chemical deterrents - Ovipositional bioassays were conducted in a growth chamber with the same rotating cylindrical cages and standard foliar surrogates painted with oil pigments as described on p. 30.

Chemical treatments were the single compounds, phenethyl alcohol (0.4%), hydrocinnamonitrile (0.4%), or cinnamyl formate (1%) in PEG. Binary mixtures consisted of these compounds at halved concentrations, for all pairwise combinations of chemicals. Mixtures were formulated at half the single compound concentrations to test for synergism, following the logic that if a single compound were used as a control for testing synergism, the full dosage would have been reconstituted for the "combination" treatment. Synergism would be supported if a combination were more deterrent than a full dose of a single compound, while additivity would generate deterrency intermediate to the single compound deterrencies.

There were three assays (Table 1, p. 82). Each binary mixture was presented in a cage with the corresponding single compounds and one control (blank PEG). The four cups were placed on the cage floor in a square pattern 15 cm apart. Flies were allowed to lay eggs for 24 hours, then the eggs were removed from the sand by flotation and counted. Analysis of variance was conducted on $\ln(x+1)$ transformed egg counts, which established homogeneous variances. Each assay was replicated ten times in a randomized complete block design (RCBD).

Experiment 2. Synergism of visual and chemical deterrents - Bioassay location, cages, and foliar surrogates were the same as Experiment 1, except foliar surrogates were painted with chromium oxide green or napthol crimson acrylic

paints (Binney and Smith, Inc., Easton, PA, 18042). Cinnamaldehyde was formulated at 1% concentration in PEG.

The four treatment-combinations deployed in a two x two factorial design were: green onion + blank PEG, green onion + 1% cinnamaldehyde in PEG, red onion + PEG, and red onion + cinnamaldehyde. The four cups were arranged in random order in a 15 cm. square pattern, for ten replicates in a RCBD. Flies were allowed to lay eggs for 24 hours, then the eggs were removed from the sand by flotation and counted. Data were subjected to analysis of variance on ln(x+1) transformed egg counts, which established homogeneous variance. X^2 contingency analysis was also conducted, on treatment totals, to confirm independence of main effects.

Experiment 3. Video analysis of visual x chemical synergism - Green onion + 1% cinnamaldehyde in PEG, red onion + blank PEG, and red onion + cinnamaldehyde were treatments paired with the green onion + PEG control for observations of predepositional examining behaviors. A pair of treatments was centered 8 cm apart in a 15 x 15 x 25 cm glass-fronted plexiglass cage, having screened top and sides. The cage was provisioned with food and water, and had a 10 x 4 cm mirror obliquely positioned behind the oviposition dishes to observe flies eclipsed by the foliage. Four female onion flies were removed from the onion surrogate foliage in the main culture cage at 1:00 pm and added to the observational cage. The observations were conducted in a different room than Experiment 2, but with similar temperature and light conditions. A JVC Model BY-110 VHS video camera was positioned to view the surface of the sand in the two cups, the entire length of the foliar surrogates, and the image of the foliage in the mirror. Video recording was conducted for 6 hrs, flies were removed the next morning and the cage prepared for another pairwise comparison of ovipositional treatments.

The following behavioral states and transitions between them were recorded: arrive on substrate, rest or walk on sand, land on foliage, rest or walk on foliage, foliar run, ovipositor probing of substrate, oviposition, and leaving the ovipositional resource. Focal animal sampling, e.g., observing one animal for a specified duration (Martin and Bateson, 1986; Harris and Miller, 1991) was not necessary, because video tapes could be reviewed for all fly visits to ovipositional resources. Transitions between these behaviors were transcribed from video tape and subjected to X^2 analysis for differences in transitional probabilities.

Results and Discussion

Experiment 1. Synergism of chemical deterrents - In all three experiments, the deterrency was slightly, though not significantly, greater for the mixtures than the single compounds ($P > 0.05$, SNK test on $\log(x+1)$ egg counts, Table 1). The consistently greater deterrency for combinations of deterrents provides weak evidence for synergistic activity. Differences between treatments at high deterrency are not expressed well with percent deterrency. Expressing these data as number of deposited eggs or percent acceptance relative to the control highlights the possible importance for synergism at high percent deterrency. For Combination 2, the mixture received half as many eggs as either cinnamyl formate or phenethyl alcohol alone.

Synergistic interactions within the chemical senses of gustation and olfaction have been noted for plant allelochemicals involved with hostplant finding and acceptance by insects (Visser, 1983). Such interactions have basic significance because they indicate likely across-fiber pattern involvement in processing sensory information (Dethier, 1982). The presence of similar interactions between deterrent compounds would be significant, because mixtures of low concentrations of deterrents could be applied to protect plants from phytophagous insects rather

Table 1. Treatments, means for egg counts (ln(x+1) transformed), and back-transformed deterrency values for testing binary mixtures of three compounds for synergism.

Treatment	Concentration in PEG (%)	Mean ln(eggs+1) (±S.E)	% Deterrency
Combination 1.			
Control	-	5.028 (0.20) a	-
phenethyl alcohol (P)	0.4	1.845 (0.30) b	95.8
hydrocinnamonitrile (H)	0.4	1.514 (0.39) b	97.0
P + H	0.2 + 0.2	1.128 (0.50) b	97.9
Combination 2.			
Control	-	4.755 (0.20) a	-
cinnamyl formate (F)	1.0	2.195 (0.35) b	92.2
phenethyl alcohol (P)	0.4	2.081 (0.30) b	93.1
F + P	0.5 + 0.2	1.262 (0.28) b	96.9
Combination 3.			
Control	-	4.850 (0.43) a	-
cinnamyl formate (F)	1.0	2.565 (0.38) b	89.8
hydrocinnamonitrile (H)	0.4	1.435 (0.39) c	96.7
F + H	0.5 + 0.2	1.381 (0.41) c	96.9

*Means not followed by the same letter are not significantly different, SNK test, $P > 0.05$.

than high concentrations of single compounds. The implications have a parallel significance for natural plant-herbivore interactions; i.e., plants would be sufficiently protected by generating an array of defensive compounds at low concentrations rather than high concentrations of single compounds.

The lack of significant synergism in this experiment could be due to: no differences or extremely broad overlap in receptor response to these deterrent compounds, or if these chemicals were perceived as being different, non-interacting neural processing of chemical sensory cues could lead to an additive behavioral response. A critical follow-up experiment would be to test the physiological basis for lack of synergism. This could be accomplished by analyzing differential receptor saturation via electroantennography. If antennal receptors do not distinguish these compounds as being different, then receptors saturated with one odor will not generate further stimulation when exposed to a second odor (Payne and Dickens, 1976; Miller et al., 1977).

Experiment 2. Synergism of visual and chemical deterrents - Mean eggs deposited on the four treatment combinations (back-transformed from $\ln(x+1)$ counts) were: green foliage + PEG, 148; green foliage + 1% cinnamaldehyde in PEG, 17.3; red foliage + PEG, 79.1; and red foliage + cinnamaldehyde, 4.52.

The synergistic interaction was explored with analysis of variance on log-transformed egg counts $\ln(x+1)$ (LTANOVA). A significant interaction term in LTANOVA signifies lack of independence of main effects, similar to X^2 analysis, however it allows block-to-block variation to be partitioned. Analysis of variance suggested independence of main effects ($F_{(1,27)} = 1.01$, $P > 0.3$). Independence implies that the deterrent foliar color, red, decreased oviposition by approximately 45% compared to green foliage, irrespective of the chemical treatment. Similarly,

cinnamaldehyde decreased egg counts by 86% compared to blank PEG, irrespective of foliar color.

Contingency X^2 analysis did not confirm main effect independence. The calculated X^2 was 4.14, significant at $P < 0.05$ (Tabular $X^2_{(1\ df)}$ 0.05 = 3.84), indicating significant non-independence. Contingency analysis is designed for testing probabilities of independent events. One concern when applying contingency analysis to these egg-count totals is that a single acceptance event is the act of initiating an ovipositional bout, rather than deposition of a single egg. There are approximately 2.2 eggs deposited per bout (Mowry, et al., 1989b); dividing egg count totals by 2.2 eggs per bout transforms these data to the approximate number of egg deposition bouts. The revised X^2 agrees with the conclusion from LTANOVA that the vision and chemical deterrents act independently ($X^2_{(1\ df)}$ = 1.838, $P > 0.1$). This is good support for separate examining behaviors being responsible for detecting host cues of different sensory modalities (Model 3 from the introduction).

Experiment 3. Video analysis of visual-chemical deterrent synergism - Row totals from behavioral transition data tables (Figure 1) gave the number of times a behavioral event occurred. Because individual cells in the transition matrix had small counts, behaviors were grouped by physical location or by function. Visits (sand arrive + stem land), sand (sand rest/walk + sand run), foliar rest/walk, foliar run, and probe + oviposit were the resulting categories. When totals from each choice comparison (Table 2) were subjected to X^2 analysis, only the red foliage + 1% cinnamaldehyde in PEG yielded significant differences in transition probabilities ($X^2_{(4\ d.f.)}$ = 32.68, $P < 0.001$) from the green foliage control. Most behavioral frequencies were similar on red foliage + cinnamaldehyde and on green foliage, when these treatments were compared. For example, the numbers of visits

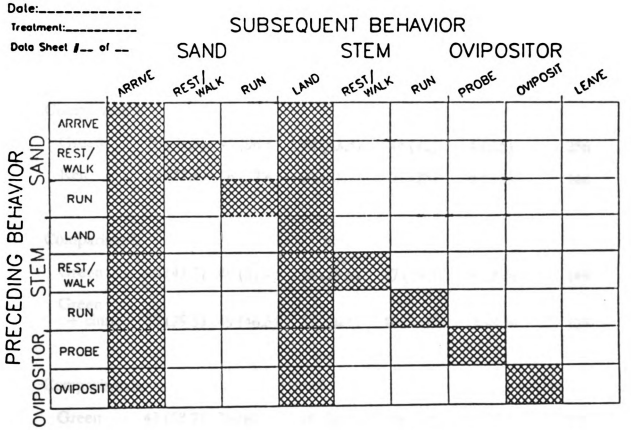


Figure 1. Sample data collection sheet for transcribing behavioral transitions from videotape recordings of onion fly preovipositional examining and egg deposition behaviors.

Table 2. Totals (and expected totals from X^2 analysis) for behavioral events in two-choice assays analyzing the effects of visual and chemical deterrents on behavioral transitions.

	Numbers of Behavioral Events					
Treatment	Visit	Sand	Stem rest/ walk	Stem run	Probe + Oviposit	Marginal Totals
Comparison 1.						
Green	70 (71.5)	98 (96.6)	38 (43.3)	46 (42)	4 (2.5)	256
Red	44 (42.5)	56 (57.4)	31 (25.7)	21 (25)	0 (1.5)	148
Comparison 2.						
Green	45 (43.5)	49 (51.4)	24 (28.9)	17 (16.3)	14 (8.9)	149
Green + cinn.	38 (39.5)	49 (46.6)	31 (26.1)	14 (14.7)	3 (8.1)	135
Comparison 3.						
Green	47 (58.7)	76 (82.2)	18 (21.7)	48 (40)	36 (22.3)	225
Red + cinn.	53 (41.2)	64 (57.8)	19 (15.2)	20 (28)	2 (15.7)	158

Green, green foliar onion surrogate + blank polyethylene glycol (PEG); Red, red foliar surrogate + PEG; + cinn., 20 mg. of 1% cinnamaldehyde in PEG.

to ovipositional cups were 53 and 47 for the two treatments, respectively. The difference between probing + oviposition between these two treatments suggested that these behaviors may have contributed to the significant differences between treatments. When this behavioral category was removed from analysis, there were still significant differences in behavioral frequencies ($X^2_{(3\ d.f.)} = 9.86$, $P < 0.05$). Further removing the effect of foliar runs generated an insignificant X^2 value of 1.31. Therefore, combined red foliage and cinnamaldehyde significantly affected the foliar run and ovipositor probing behaviors.

Separating the effects of visual versus chemical deterrents can be accomplished by examining the remaining choice comparisons. Though the combined $X^2_{(4\ d.f.)}$ value of 8.23 was not significant in the green vs. green + cinnamaldehyde comparison, the probe + oviposition category was by itself significant ($X^2_{(1\ d.f.)} = 6.474$, $P < 0.02$). All other activities on these two resources occurred at approximately the same frequency, therefore we can conclude that cinnamaldehyde mostly influenced behavioral transitions to ovipositor probing and any subsequent transitions to egg deposition. The lack of a significant X^2 value for behaviors in the red vs. green foliage may at first glance be misleading. If there were a general reduction in activity on cups that had red foliage, proportionally distributed across all behaviors, then the X^2 analysis would not detect differences between red and green foliage treatments. The total level of activity on cups is measured by the marginal totals used for X^2 analysis, and indeed suggests overall reductions in activity on treatment cups with red foliage. A paired t-test on general activity level was performed by pairing the number of events for each behavior in the red vs. green foliage treatments. Both the red + cinnamaldehyde vs. green foliage + cinnamaldehyde and the red vs. green foliage comparisons were used, yielding ten pairs of behavioral event totals. There was indeed a reduced level of activity on cups with red foliage (paired t-test = 3.079, $P < 0.02$). The stem rest +

stem walk behavioral frequencies were similar between treatments; the most consistent effect of the presence of red foliage was to decrease the transitions to foliar runs.

General discussion - Possible synergism or multiplicative effects from using combinations of deterrents are important, both from basic and applied interests. Multiplicative effects have been demonstrated with across-modality stimuli that release onion fly oviposition (Harris and Miller, 1988). It appears that the across-modality interactions are as important with deterrent stimuli as for excitatory cues.

Why do multiplicative models for between-modality interactions make sense? Neurophysiological models for integrating information from different sensory modalities and observations of behavioral transitions suggest that onion flies probably process visual and chemical stimuli during different behaviors. The probability of accepting a host model consequently is determined by the joint probability (a multiplicative function) that color and odor are acceptable. A temporal summation interpretation of across-modality stimulus synergism by onion flies is as follows: The presence of dipropyl disulfide increases the probability an onion fly will alight on vertical objects (Harris and Miller, in manuscript). Once having alighted on a leaf, sitting and grooming behaviors could be involved with assessment of foliar color (evidence from this work), which increases the probability of a transition to a foliar run. Changing foliar color from green to red would decrease the probability of a fly proceeding from landing to foliar runs. Decreasing dipropyl disulfide presence would "synergize" the deterrent effect of a red onion leaf because, by reducing the probability that an onion fly lands, it is less likely that fly will assess foliar color. The presence of deterrent would synergize decreased dipropyl disulfide concentration and red foliage, because the probability eggs would

be deposited in this combination would jointly be reduced by foliar landing, transition to foliar runs, and transition to ovipositior probing.

While the evidence from both excitatory and inhibitory cues is consistent with temporal summation of sensory stimuli in onion flies, there are additional complexities that need to be addressed. One concern with this simple model is that behavioral categories used in Experiment 3 were low resolution, allowing behavioral components like proboscis extension and touching the ovipositor tip to the foliar surface to be lumped under "foliar run." Would high resolution behavioral analysis correlate with the interpretations of across-fiber or with temporal summation? Manipulative experiments with very fine resolution behavioral observations, or neuroethological methods may have to be applied for us to know how the onion fly really perceives a host plant.

Chapter 6

Stimulo-deterrent diversion for manipulating onion fly oviposition: a greenhouse test

Introduction

Onion fly, *Delia antiqua* (Meigen), females deposit about 60 times more eggs on onion plants grown from deeply planted large bulbs than on small onion seedlings (Hammond, 1924; Finch and Eckenrode, 1985; Cowles and Miller, unpublished). However, onion seedlings are usually the only hosts available for onion flies to colonize. Damage to small seedlings from the first larval generation may be extensive, as small plants may be completely consumed and are highly prone to dehydration and microbial infection. Moreover, each larva may destroy *ca.* 10 adjacent seedlings while completing development (Workman, 1958; Loosjes, 1976). The result is economically injurious non-random thinning of onion stands.

Cull onions, consisting of unmarketably small or sprouting bulbs, are a waste product from the onion industry. It has been suggested that highly susceptible onion seedlings could be protected by planting cull onions as a trap crop, to which onion fly oviposition would be diverted (Hammond, 1924; Lovett, 1923).

Another idea for non-insecticidal control of onion maggot is treating seedlings or adjacent soil with deterrents. In laboratory studies, onion fly oviposition is suppressed by a wide variety of inexpensive and readily available compounds including phenolics and monoterpenoids; however, the dosages required for control are several orders of magnitude greater than that for insecticides (Alfaro, et al., 1981; Javer, et al., 1987; Cowles et al., 1990). As is true for consumatory behaviors for various insect/plant interactions, (Raffa and Frazier, 1988; Chapman, 1988; Jermy, 1971), onion fly ovipositional deterrents can lose their efficacy when females are given no ovipositional outlet (Cowles et al., 1989). Thus, it is doubtful that onion fly can be controlled by deterrents alone.

Miller and Cowles (1990) proposed stimulo-deterrent diversion (SDD) and reported preliminary data suggesting that behavioral forces of "push" and "pull" might be multiplicative rather than simply additive. We predicted for onion flies that presence of culls would improve the protection of deterrent-treated seedlings, by both diverting oviposition and preventing the gravid onion flies from becoming ovipositionally catholic due to deprivation. In the present greenhouse study, a factorial design was used to test the stimulo-deterrent diversion concept. *D. antiqua* oviposition on onion seedlings was assessed for the following treatments: 1) seedlings-only, 2) seedlings + culls, 3) seedlings + deterrent, and 4) seedlings + deterrent + culls.

Methods and Materials

The laboratory culture of onion flies was initiated in 1987 and maintained as described on pp. 18-19. Flies used in experiments were reared in the lab for 10-15 generations.

About 100 onion seeds, 'Sassy Brassy' cultivar (Ferry-Morse Seed Co., Modesto, CA 95354), were planted in muck soil per 13 cm diameter pot. To prevent undesired encounters with *D. antiqua*, onions were grown and kept in a large screened cage held in a greenhouse. Onion plants were watered on alternate days. Seedlings were transplanted when at the two-leaf stage, four per 13 cm diameter pot, in a 5 cm square pattern. Seedling onions used in experiments were at the 4-5 leaf stage.

Onion bulbs ('Spartan Banner') were collected from a grower's field in May and September 1989, and stored at 3°C until use. Culls collected in May had been held in the grower's storage through the winter and spread back into the field. Bulbs collected in September were gleaned after harvest. Two days before use, two sprouted cull onions were planted in non-sterile muck soil, in a 19 cm pot. The

onion neck was positioned 5 cm below the soil surface, the optimal depth for maximizing *D. antiqua* oviposition (unpublished).

The deterrent used was cinnamaldehyde (Aldrich Chemical, Milwaukee, WI 53233) 50% (w/w) in activated charcoal (aquarium charcoal). Granules (5 mm diameter) were formulated in May 1988, and stored in a Nalgene^{R} bottle at 20°C until needed. These large granules were coarsely crushed to form <2 mm diameter granules. About 50 mg of formulated material was placed within 2 cm of the base of each onion seedling.

To assay for a possible interaction between deterrents and culls when protecting seedlings, 100 mature females and 20 males were placed in a 1.5 x 1.5 x 3.5 m screened cage, held within a greenhouse. This large cage was provisioned with food, water, and one of the four onion treatments. To minimize possible treatment-treatment interactions, the four treatment combinations resulting from the 2 x 2 factorial design were sequentially presented at two day intervals according to a Latin square protocol. Four pots of seedlings were linearly positioned at 50 cm spacing. When culls were present their pot was positioned at the center of the row, with 50 cm to the seedling pots on each side. After allowing flies to oviposit for two days, the potted seedlings and culls were removed and the next onion treatment was placed in the cage.

The eggs from the top 4 cm layer of soil in each pot were collected and separated from muck soil by flotation in water. Residual floating muck and eggs were passed through a 1 mm mesh sieve to remove the larger muck fragments; the resulting eggs and fine muck were deposited on a nylon filter. This residue was washed into a black enamel pan. The contrast of the white eggs against the black background enabled the eggs to be easily counted as they were removed with a vacuum aspirator.

Egg counts were analyzed for significant main effects and their interaction with analysis of variance. The numbers of eggs laid on seedlings were not distributed normally, however, logarithmic (ln(x+1)) transformation established homogeneous variances (Bartlett's Test, $P > 0.1$, Steel and Torrie, 1980). Contingency X^2 analysis of treatment-combination totals was used to verify independence of treatment effects.

Results and Discussion

The total numbers of eggs laid on seedlings for the four treatments were 3185 (seedlings-only), 1531 (seedlings + culls), 127 (seedlings + deterrent), and 69 (seedlings + deterrent + culls). Both main effects were significant ($P < 0.01$, analysis of variance on log-transformed egg counts, LTANOVA, Table 1). Because calculating the differences between logarithms is equivalent to forming the ratio for non-transformed data, main effects generated from LTANOVA are a transformation of percent eggs laid on treated vs. control seedlings. The numbers of eggs laid on seedlings were reduced 96 ± 1 and 58 ± 10 percent (mean ± S.E.), by cinnamaldehyde and culls, respectively.

The total numbers of eggs laid on culls were 3867 and 4211 when deterrent was absent or present on seedlings, respectively. The slightly (but not significantly) greater number of eggs laid on culls when cinnamaldehyde was present argues against possible whole-cage ovipositional depression when deterrent was present.

Analysis of the interaction between the deterrent and culls was a major objective of this study. Possible relationships are non-interaction, positive interaction (synergism), and negative interaction (see Figure 1). The graphs in the right column of Figure 1 are logarithmically transformed data from the graphs in the left column. Non-interaction (Figure 1A) would be expected if each factor, culls or deterrent, prevented some absolute number of eggs from being laid on seedlings,

Table 1. Analysis of variance for ln(x+1) transformed numbers of onion fly eggs deposited on seedling onions (valued crop) protected by deterrent (cinnamaldehyde) and diversionary (cull onions) treatments. Rows represent sequential presentation of all four treatment combinations while columns coded the order of treatment presentation.

Source	df	Sums of Squares	Mean Square	F Value	Pr > F
Row	3	12.472	4.157	37.92	0.0003[1]
Column	3	1.471	0.490	4.47	0.057
Treatments	3	42.805			
Cinnamaldehyde	1	39.755	39.755	362.56	0.0001
Culls	1	2.982	2.982	27.19	0.002
Cinn. x Culls	1	0.068	0.068	0.62	0.460
Error	6	0.657	0.109		
Total	15	57.406			

[1] Significant row effect may indicate differences in relative stimulatory quality of culls collected in springtime (Rows 1 and 2) and autumn (Rows 3 and 4).

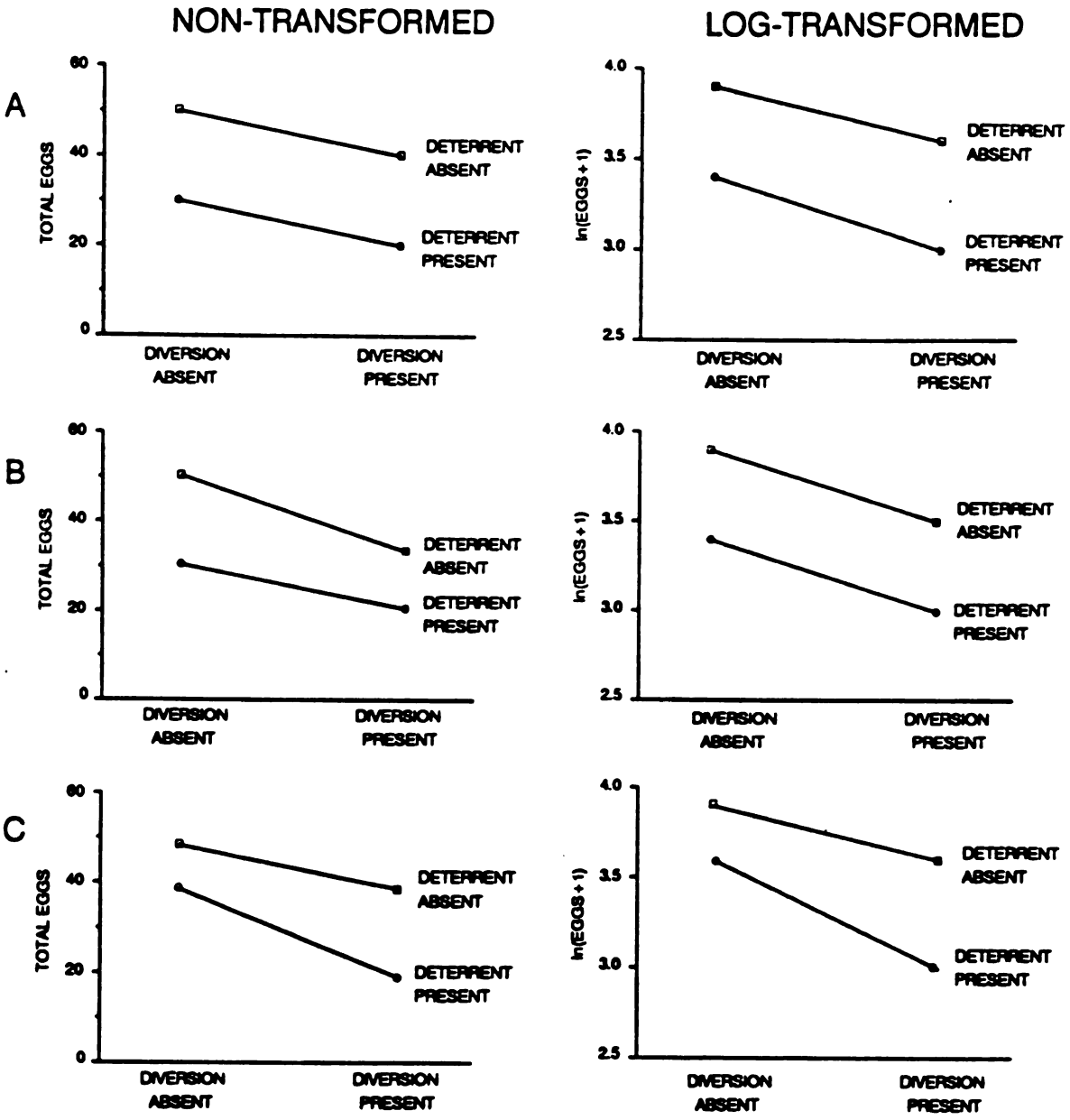


Figure 1. Theoretical forms of interactions between deterrent and diversionary treatments, with non-transformed and log-transformed hypothetical data. (A) - no interactions when non-transformed; slightly negative interaction, indicating dependence, when transformed. (B) - Positive interaction. The log-transformed data show no interaction, hence, the factors are independent. (C) - Negative interaction.

irrespective of the presence or absence of the other factor. This pattern generates parallel lines in the interaction diagram showing non-transformed egg counts.

Synergism can be concluded if there is a positive value for the culls x deterrent interaction in non-transformed egg counts (Fig 1B). The linear contrast (Snedecor and Cochran, 1967) calculates the mean for this interaction. For the present experiment this value is: (3185-127-1531+69)/8 = 199.5. The conclusion from this additive model is that approximately 200 fewer eggs were laid per 16 seedlings in the deterrent+culls treatment than expected from the main effects. Hence, there was a synergistic interaction between deterrent and culls. Synergism may result when independent factors (in the probabilistic sense) govern insect behavior. In this experiment, culls could reduce the probability of an onion fly finding a seedling, while deterrent applied to a seedling could, independently of host finding, reduce the probability of acceptance. The seedling + deterrent treatment was expected to cause some degree of ovipositional deprivation among gravid onion flies. This deprivation would increase the probability of an onion fly accepting seedlings in this treatment relative to the other three treatment combinations, and thus cause non-independence.

Contingency X^2 and LTANOVA detect non-independence between diversionary treatments and deterrents, because both analyses reveal non-parallel lines in log-transformed interaction diagrams (Figure 1A and C). LTANOVA has additional advantages: the calculated direction of the interaction should be negative when calculated from log-transformed values if there were significant deprivation; contingency analysis doesn't indicate the type of interaction. Also, LTANOVA partitions experimental variation from the Latin square design.

This predicted negative interaction (non-independence) for deterrent-treated seedlings presented in the no-choice situation was *not* detected by either LTANOVA (Table 1) or by X^2 analysis ($P > 0.25$). In fact, the slight interaction

illustrated in the log-transformed diagram of the experimental data (Figure 2), is positive, though not statistically significant. All analyses consistently support a multiplicative model for ovipositional response of onion flies to the combined presence of culls and deterrent.

Implications for field deployment of SDD - Unlike pesticidal control strategies, SDD involves several main components: valued crop, diversionary crop, deterrent, and pest. Relative to a pesticidal approach, the time frame for control is extended with SDD, because the adult pest is allowed to survive and reproduce. Multiple components and a long time-frame for control suggest that understanding component interactions is crucial for implementation of SDD.

The good fit of data in this greenhouse study to a multiplicative model for the interaction of deterrents and culls is encouraging for understanding how to apply the stimulo-deterrent diversionary strategy in the field. For example, if the present growth state of culls and seedling onions gave a 100:1 preference ratio, we would need at least 83% (5:1 ratio) deterrency to reach the overall goal of a 500:1 preference ratio.

The crop area that should be planted to diversionary culls, and the optimal distance and distribution of culls relative to the valued crop are unknown. Marsolan and Garrett's (1977) simulation model determined that the critical variables for optimizing a trap crop are maximum distance between trap crop resources, insect diffusivity, and ovipositional preference. The proximity of seedlings and culls in our experimental arena (maximum distance was 1 meter), may have maximized the "comparison" of treated seedlings and culls, and may have contributed to the effectiveness of the deterrent + cull combination in this small-scale test. However, the probability that a gravid onion fly would accept deterrent-treated seedlings did not change over two days, meaning that there may be a long time interval during

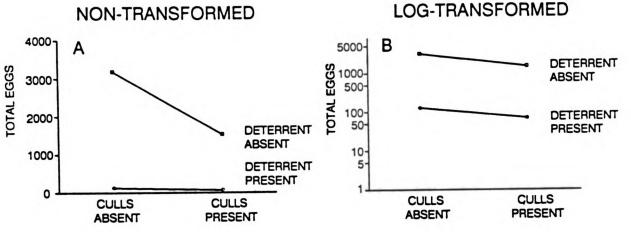


Figure 2. Experimental data for interactions between cinnamaldehyde deterrent and cull onions, as both non-transformed (A) and log-scale (B) graphs. These data are consistent with Figure 1 B, a synergistic effect with an additive model but with parallel lines indicating treatment independence with log-transformation.

which "choice" may be executed. Over a two-day interval, onion flies within crop habitat would be expected to move on the order of 100 to 300 meters, via multiple, short flights (Loosjes, 1976; Martinson et al., 1988). During movement, several encounters with highly acceptable culls could elicit oviposition, reset the physiological state, and consequently maintain sensitivity to deterrents. Less effective deterrents than in the present study would be expected to become more rapidly acceptable for oviposition; hence, the diversionary crop would have to be more closely spaced to increase the frequency of encounters.

So that the results could legitimately be subjected to X^2 analysis (Cochran, 1954), relatively large seedlings and moderate deterrent dosage were chosen to allow appreciable oviposition on the cull + deterrent protected seedlings. Four to five-leaf stage onions, as used in this experiment, stimulate more oviposition than the small seedlings to be protected in the field (Groden, 1982; Harris, et al., 1987). The 480:1 preference ratio of eggs laid on culls:seedlings (on a per-plant basis) measured in this experiment is close to the 500:1 theoretical value calculated for commercially acceptable crop protection (Miller and Cowles, 1990).

While the total picture for field deployment of SDD for controlling *D. antiqua* is complex, a major piece of the puzzle is in place. Specifically, a simple multiplicative model adequately described the interaction between deterrents and culls. Such a simple model suggests that the complexities of the multifactorial SDD approach may be decomposed into deterrency and diversion. These main components can be studied separately, and their combined effect predicted to be the product of the two separately determined probabilities of acceptance (where acceptance is defined as 1 minus the percent reduction in number of eggs laid on seedlings). Although this greenhouse test was only a microcosm of the field, the high mobility of onion flies in the field and lack of deprivation in this experiment over a two-day interval suggest that the simultaneous use of ovipositional deterrents

and culls has promise for field manipulation of onion fly oviposition, provided that more potent deterrents can be found and formulated to last for several weeks. An alternative to applying deterrents would be to generate onion cultivars antixenotic to onion flies. SDD may have greater potential for controlling other mobile and discriminating pests, for which formulated deterrents or antixenotic crop varieties are known, and where the plant/pest interaction is shorter than the *ca.* six weeks over which onion seedlings must be protected from first generation onion maggot larvae.

Chapter 7

Integrating stimulo-deterrent diversion into pesticide resistance management: directing pest evolution toward sustainable control

Introduction

Synthetic organic insecticides have demonstrated tremendous utility for over fifty years (Metcalf, 1980). The very nature of their success is the ability to kill large portions of treated pest populations. This ability, unfortunately, is a built-in flaw with respect to evolutionary processes. Successively removing susceptible individuals from a population may rapidly select for physiological resistance to an insecticide. As early as 1946, houseflies could no longer be controlled with DDT, and by 1980, more than 400 species of arthropods were resistant to insecticides (Georghiou and Mellon, 1983). Reports of pest resistance to the most recently developed classes of insecticides, such as pyrethroids, formamidines, insect growth regulators, and *Bacillus thuringiensis* endotoxin, make questionable the premise that resistance can be mitigated by substituting new modes of action (Dover and Croft, 1984; Georghiou and Mellon, 1983; Miller et al., 1983; Sparks and Hammock, 1983; Van Rie, et al., 1990).

In response to the possible threat of losing insecticides as a pest management tool, various strategies have been suggested for slowing or reversing pesticide resistance development. These approaches may be subdivided into *pesticide* and *ecological* management schemes (Dover and Croft, 1984). Pesticide management refers to tactics such as applying insecticide mixtures, rotating insecticides, applying insecticides with synergists, using high doses, applying low doses, or using short residual materials. Ecological management approaches endeavor to replace insecticide suppression of pests with cultural, physical, or biological control practices. These ecological approaches distribute selection pressure among several

mortality factors. The result is minimized use of insecticides, which in turn implies reduced selection pressure for insecticide resistance.

Tabashnik's recent (1989) review of resistance management concluded that pesticide combinations may not consistently prevent resistance development. In fact, pesticide combinations may be more disruptive than approaches that minimize pesticide use. This paper supports his critique of pesticide management strategies, and contrasts these strategies with an ecological approach we call the stimulo-deterrent diversion (SDD) (Miller and Cowles, 1990). SDD manipulates pest feeding or oviposition by simultaneously deploying a highly acceptable diversionary crop while protecting the valued crop with deterrents. This chapter addresses the theoretical consequences to pesticide resistance management of being able to manage pest distribution.

Pesticide management approaches

Mixtures and rotation - Successfully avoiding resistance by using insecticide mixtures assumes that different mechanisms are responsible for causing resistance to each insecticide (Ozaki, 1983; Curtis, 1985; MacDonald, et al., 1983; Van Rie, et al., 1990). If this assumption were true, and the genes coding for these resistance traits were present at low frequencies, the probability of finding individuals resistant to both pesticides should then be extremely small (Curtis, 1985; Tabashnik, 1989). Applying a mixture containing component insecticides at concentrations sufficient to kill homozygous susceptibles would be expected to leave rare double heterozygote and the extremely rare double homozygote resistant survivors. After selecting a population with an insecticide mixture, immigration of susceptible immigrants can decrease the gene frequency for resistance. The impact of gene flow on delaying resistance is contingent on immigrant arrival between the time that spraying occurred and mating of the resident population. If resistant individuals mate with

susceptible immigrants, then resistance alleles will mostly be maintained among heterozygotes (Curtis, 1985).

Using chemicals in rotation arose from the idea that chemicals may have negatively correlated cross resistance, meaning that selection with one compound would increase susceptibility to the other compound(s). While one chemical is being used to control a pest, susceptibility to another chemical would increase, so using the compounds in alternation could conceivably indefinitely prevent resistance (Georghiou, 1983). Straightforward genetic mechanisms could cause genetic trade-offs. One possibility is that different and independent resistance loci could be responsible for resistance to each pesticide, and each resistance allele has an associated fitness cost. Another possibility is that the resistance mechanism involves different, recessive alleles at the same locus. Selection for resistance to one compound would then preclude resistance to the second compound.

Pesticide plus synergist - A synergist is a compound that, when combined with an insecticide, increases the toxicity in more than an additive manner. By blocking detoxification mechanisms, synergists may restore insecticidal activity after resistance has occurred (Ranasinghe and Georghiou, 1979; Ozaki, 1983). Use of synergists has also been suggested for preempting resistance development by blocking in advance a likely detoxification pathway (Georghiou, 1983).

The use of synergists may readily extend the life of some insecticides. However, in an evolutionary view, depending on synergists presumes that 1) variants in detoxification enzyme insensitive to synergists do not exist (or are mutually incompatible with detoxifying the insecticide), and 2) that the blocked enzyme is the only important route available for adaptive change.

High dose and selective timing - Comins (1977) and Taylor and Georghiou (1979) developed a strategy for pesticide resistance management based on high pesticide doses. If resistance is controlled by one, two-allele locus with co-dominant

inheritance, then a concentration of insecticide could be applied that kills both homozygous susceptible and heterozygous genotypes. If the gene frequency for resistance is low, then there should be few if any homozygous resistant individuals present. These few individuals would be expected to survive a high dose insecticide application; their mating with susceptible immigrants would maintain most resistance genes among heterozygotes.

In closed pest populations, the probability of resistance development would be dependent on the initial resistance gene frequency and on mate finding, rather than on immigration. If the resistance allele is common, mating between resistant individuals is likely and the gene frequency for resistance would rapidly increase. Thus, high insecticide doses can be a prescription for immediate and high level resistance development. On the other hand, if the population is susceptible and potential resistance traits have low gene frequencies, then the high dose strategy could effectively suppress the pest population.

Pesticide strategy assumptions - Rotation, mixtures, and synergist strategies assume that pests have negatively correlated cross resistance or lack alternate adaptive routes. These assumptions are questionable, based on experience with: 1) the presence of cross resistance, even when selecting populations with insecticides of differing modes of action, and 2) the multiple forms of resistance possible when selecting with a single compound.

The first experiences with cross-resistance suggested that selection with one compound preselects the population for resistance to related compounds (Hoskins and Gordon, 1956). Cross resistance may explain why, in the case of organophosphate and carbamate insecticides applied to control rice leafhoppers, the effective field life for carbamates was only four years (Ozaki, 1983). In this situation, the assumption of independent modes of action was clearly violated by

synergistic interactions at the physiological level between these classes of compounds (Ozaki, 1983).

Cross-resistance can also be expected between unrelated compounds; ignoring this possibility can lead to poor predictions of evolutionary response. For example, in 1967 Williams predicted that insects would not develop resistance to juvenile hormone (JH) growth regulators used as insecticides (Sparks and Hammock, 1983). Williams' claim may have been based on an expectation that coordinated changes in JH target site and hormone structure would be an improbable mode of resistance development. However, within five years, strains of insects resistant to juvenile hormone applications were selected (Dyte, 1972; Cerf and Georghiou, 1972). The JH resistant strains had mechanisms of decreased penetration and increased metabolism; mechanisms pre-selected with conventional insecticides (Sparks and Hammock, 1983).

Houseflies have multiple resistance adaptations to toxins. DDT resistance in houseflies may be due to any combination of increased dehydrochlorinase or α-hydroxylation, decreased penetration, or target site insensitivity (Fukuto and Mallipudi, 1983; Matsumura, 1983). When the dehydrochlorination detoxification pathway was blocked with a synergist, selection with DDT lead to high resistance due to α-hydroxylation (Georghiou, 1983).

Correlated resistance traits is a weakness of all the high-kill strategies. Chemically unrelated compounds should be expected to cause cross resistance to each other because relatively non-specific resistance mechanisms exist, like decreased absorption or increased activity of generalized detoxification enzymes (Dyte, 1972; Brattsten et al., 1986; Tabashnik et al., 1987). Detoxification enzymes can be genetically correlated without being genetically linked, either through pleiotropy or coordinated gene expression (Plapp and Wang, 1983; Grant, 1986; Via, 1986).

Genetic correlation is expected for a deeper reason. Genes do not exist by themselves, rather they interact with all other genes that constitute the genetic endowment of an individual, the unit of selection. Changes in one enzyme, for example, should impact the milieu in which related enzymes function. Complex feedback through physiological machinery implies that positive and negative interactions exist. When a population is exposed to selection with pesticides, evolution should optimize population fitness by maximizing survivorship and minimizing reproductive disadvantages for the environmental conditions. If one enzyme system is unavailable to selection, perhaps because it is blocked by a synergist, then selection should act upon any remaining resistance traits. After a particular resistance allele is nearly fixed, continued selection should allow gene frequencies for alleles interacting through physiological processes to shift, so that the population will approach a new adaptive peak (Crow, 1957).

High vs. low selection strategies - Clearly, the pesticide management techniques of rotation, mixtures, synergists, high doses, and selective timing all are based on high mortality, and consequently on high selection potential. Tabashnik and Croft (1982) have pointed out several practical flaws with these high mortality strategies. Immigration of pests with susceptible genotypes are largely responsible for delaying resistance in these strategies, however, these individuals may not survive long enough in the treated habitat to mate and reduce the number of homozygous resistant individuals. In addition, I would add a concern that local mate choice from cohorts surviving pesticide sprays could change the expected genotype frequency. Inbreeding would greatly increase the number of homozygous or double heterozygote resistant progeny.

The most troublesome aspect of high-kill strategies is that they add to both environmental contamination and insecticide costs (Tabashnik and Croft, 1982; Tabashnik, 1989). Replacing insecticide sprays with photosynthetically derived crop

resistance traits is compatible with sustainable agriculture principles (e.g., preservation of natural enemies and environmental quality). However, whether a high level of antibiosis is caused by pesticides or plant traits (Gould, 1986), ecologically unsophisticated applications of high mortality strategies are likely to be short lived.

Pesticide management approaches using low doses, short residual materials, and fewer sprays are all consistent with reduced selection intensities, but may less effectively suppress pest populations (Georghiou, 1983). These approaches are more consistent with ecological aspects of integrated pest management, in which mutually compatible and varied techniques, such as cultural and biological control, replace some insecticide use.

Stimulo-Deterrent Diversion

The remainder of this paper examines the potential of an ecological management approach, stimulo-deterrent diversion (SDD) (Miller and Cowles, 1990), as a tool for pesticide resistance management. The generality of the pesticide resistance model used to evaluate SDD may contribute to increased interest in crop ecosystem management and behavioral manipulation for managing pesticide resistance.

Crop protectants have largely been limited to toxins. Insecticides are lethal agents applied to suppress insect pest populations, so they are ecologically analogous to naturally produced antibiotics found in plants (Gould, 1984; Brattsten, 1988). Other possible modes of action for protectants are antixenosis or tolerance (Painter, 1968).

Antixenosis, or deterrency, commonly manifests itself as differential acceptance of a crop or variety by a pest insect (Hokkanen et al., 1986; Riley, 1871, cited in Casagrande, 1987). Though a few examples are known, deterrents are rarely effective in no-choice situations (Painter, 1968). Miller and Cowles (1990)

have suggested deploying highly stimulatory alternative host resources to prevent deprivation-caused failure of deterrents protecting a valued crop. This bipolar behavioral approach has been named stimulo-deterrent diversion. SDD affects the distribution of individuals colonizing the two sub-habitats. If the diversionary crop is not treated with pesticides, it can serve as a refuge for susceptible genotypes in a pest population, divert pests from damaging the valued crop, and act as a nursery for biological control agents.

Modeling the Impact of SDD on Pesticide Resistance - The impact of refugia on the development of pesticide resistance has been modeled by Gould (1984) and Tabashnik and Croft (1982). Gould (1984) modeled the interactions between behavioral and physiological resistance to pesticides, a convenient starting point for modeling the effect of SDD on pesticide resistance development. In my model, the refuge is a diversionary crop designed as part of the pest management system; the pesticide-treated areas are the valued crop of the SDD system. The purpose of this model was to gauge the relative importance of genetic, management, and ecological parameters when designing SDD crop systems to prevent or reverse physiological resistance development.

The assumptions for my population genetics model were: the pest population is diploid and has two unlinked autosomal genes, each with two alleles that control: i) physiological resistance (R) to the pesticide residues, or ii) avoidance (A) to either pesticide residues, an applied deterrent, or an antixenotic valued crop. Adults emerging from pesticide-treated valued-crop and untreated diversionary crop habitats randomly mate and belong to an infinitely large population (i.e., there is no drift; thus, this a deterministic model). The behavioral effect of the A allele was implemented by assigning probabilities that avoiding and non-avoiding homozygote females lay eggs in the valued crop (variables X and Y in the model). Mortality occurs in the immature stage, considered to be incapable of moving between crops.

Differences in fitness were assumed to be solely determined by survivorship of the immature stage (Figure 1).

Instead of assigning survivorships for each genotype in the pesticide treated and untreated habitats (Gould, 1984), survivorship in this model was composed of: habitat suitability (THS and UHS for treated and untreated habitat suitability, respectively), fitness costs for carrying physiological and/or avoidance alleles (FCR and FCA), and an explicit dose-response model for pesticide mortality (PMORT, Figure 2). Pesticide concentration, mode of inheritance of R and A alleles, valued and diversionary crop suitability and availability, and fitness costs for R and *a* alleles were each varied to determine the impact of SDD systems on evolution of physiological resistance genotypes. Parameters used for all the simulations are summarized in Table 1.

The pesticide component of this model calculated mortality for rr, Rr, and RR genotypes, based on a linear log(concentration) vs. probit mortality relationship (Taylor and Georghiou, 1979). The slope and LD_{50} values for each genotype and the concentration of pesticide were program variables. Functional dominance or recessiveness was modeled with co-dominant R inheritance and low or high pesticide concentrations, respectively (Taylor and Georghiou, 1979). Dominant or recessive inheritance for the R allele was modeled by setting the slope and LD_{50} for the heterozygote genotype equal to the RR or rr genotypes, respectively. The program calculated the standard normal deviation from the LD_{50} concentration for the pesticide concentration, which then determined PMORT, the probability of

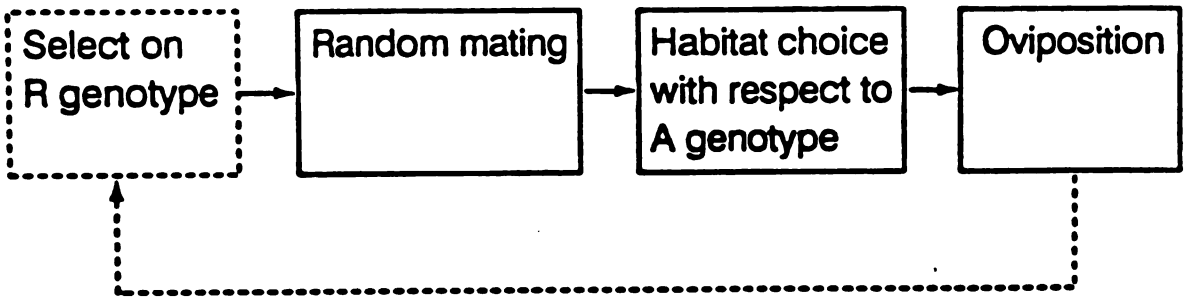


Figure 1. Schematic for the life cycle of a pest and its biology relevant to the pesticide resistance simulation model. - - - - Immature stages (egg through pupae) are exposed to selection; ———— Adult stage: mates randomly yet chooses habitat to lay eggs based on A allele.

Table 1. Parameters used in a two-locus, two-allele model investigating the effects of physiological resistance and behavioral avoidance on the evolution of pesticide resistance.

	PMORT			Proportion in valued crop*			Habitat Quality		Fitness Costs	
Figure	rr	Rr	RR	aa	Aa	AA	UHS	THS	FCR	FCA
Genetic Factors										
2A	0.92	0.92	0.10	0.9	0.65	0.4	0.5	0.5	0.0	0.0
B	0.92	0.10	0.10	0.9	0.65	0.4	0.5	0.5	0.0	0.0
C	0.92	0.54	0.10	0.9	0.4	0.4	0.5	0.5	0.0	0.0
D	0.92	0.54	0.10	0.9	0.9	0.4	0.5	0.5	0.0	0.0
E	0.92	0.54	0.10	0.9	0.65	0.4	0.5	0.5	0.0	0.0
F	0.92	0.54	0.10	0.9	0.65	0.4	0.5	0.5	0.2	0.0
G	0.92	0.54	0.10	0.9	0.65	0.4	0.5	0.5	0.0	0.2
H	0.92	0.54	0.10	0.9	0.65	0.4	0.5	0.5	0.2	0.2
Operational Factors										
3A	0.23	0.02	0.00	0.9	0.65	0.4	0.5	0.5	0.0	0.0
B	0.92	0.54	0.10	0.9	0.65	0.4	0.5	0.5	0.0	0.0
C	0.99	0.86	0.39	0.9	0.65	0.4	0.5	0.5	0.0	0.0
D	0.92	0.54	0.10	0.9	0.65	0.4	0.8	0.2	0.0	0.0
E	0.92	0.54	0.10	0.9	0.65	0.4	0.5	0.5	0.0	0.0
F	0.92	0.54	0.10	0.9	0.65	0.4	0.2	0.8	0.0	0.0
G	0.92	0.54	0.10	0.6	0.35	0.1	0.5	0.5	0.0	0.0
H	0.92	0.54	0.10	0.9	0.5	0.1	0.5	0.5	0.0	0.0
I	0.92	0.54	0.10	0.9	0.5	0.1	0.3	0.2	0.0	0.0

* Proportions for AA and aa are variables X and Y, respectively.

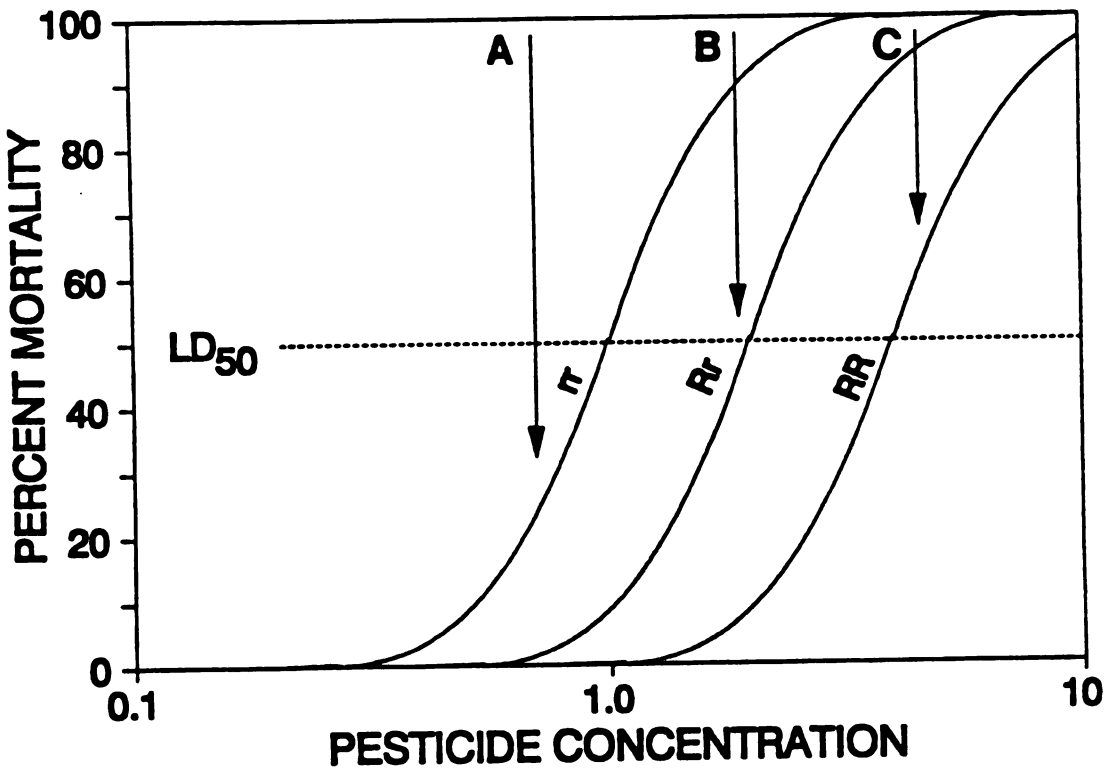


Figure 1. Relationship between log(concentration) of a pesticide and percent mortality for rr, Rr, and RR genotypes. Arrows labeled A, B, and C correspond to pesticide concentrations simulated in Figures 3A-C.

mortality for the RR, Rr, and the rr genotypes in the pesticide treated habitat (Figure 2 and Table 1).

Fitness costs for carrying R or *a* alleles reflect direct or indirect (pleiotropic) effects of these alleles on survivorship and reproduction. In this model, if the fitness of the RR genotype is 0.8 relative to the "wild-type" rr, the "fitness cost" for the R allele is defined to be 0.2. For this example, the relative survivorship of rr, Rr, and RR genotypes would be 1, 1-0.1, and 1-0.2, respectively.

Mean survivorship was calculated for the AARR genotype as follows:

$$S_{AARR} = [X*(1-PMORT_{RR})*THS + (1-X)*UHS]*FCR$$

The probability of finding an AARR genotype larva in the valued crop (treated habitat) was X; the remaining portion of this genotype (1-X) was attributed to the diversionary crop (untreated habitat). Survivorship in the treated environment was governed by both the presence of a pesticide ($1-PMORT_{RR}$), and also by the quality of the host resources (THS), while the survivorship in the untreated habitat was only governed by the quality of that resource (UHS). The fitness cost for carrying the R allele, FCR, was calculated to cause the same proportional reduction in survivorship within both habitats.

Equations calculating survivorship of the other eight genotypes were:

$S_{AaRR} = [(X+Y)/2*(1-PMORT_{RR})*THS + (1-(X+Y)/2)*UHS]*FCR*(1+FCA)/2$

$S_{aaRR} = [Y*(1-PMORT_{RR})*THS + (1-Y)*UHS]*FCR*FCA$

$S_{AARr} = [X*(1-PMORT_{Rr})*THS + (1-X)*UHS]*(1+FCR)/2$

$S_{AaRr} = [(X+Y)/2*(1-PMORT_{Rr})*THS + (1-(X+Y)/2)*UHS]*(1+FCR)*(1+FCA)/4$

$S_{aaRr} = [Y*(1-PMORT_{Rr})*THS + (1-Y)*UHS]*(1+FCR)/2*FCA$

$S_{AArr} = X*(1-PMORT_{rr})*THS + (1-X)*UHS$

$S_{Aarr} = [(X+Y)/2*(1-PMORT_{rr})*THS + (1-(X+Y)/2)*UHS]*(1+FCA)/2$

$S_{aarr} = [Y*(1-PMORT_{rr})*THS + (1-Y)*UHS]*FCA$

The genotype frequency for the first generation in each simulation was assumed to be at Hardy-Weinberg and linkage equilibrium. Subsequent generations had non-equilibrium genotype frequencies (due to linkage disequilibrium). To calculate the genotype frequencies following each generation of selection, the gamete frequencies were first calculated. For example:

$freq_{AR} = [S_{AARR} + .5*(S_{AARr} + S_{AaRR}) + .25*S_{AaRr}]/TOTAL$, where TOTAL is the sum of all genotype survivorships. After the frequencies of Ar, aR, and ar gametes were calculated, the genotype frequencies for the subsequent generation were calculated, based on random mating [i.e., $freq_{AaRr} = (freq_{ar} * freq_{AR}) + (freq_{Ar} * freq_{aR})$].

The multiplicative model for calculating survivorship, and the genotype times environment interaction present when dividing the population into valued and diversionary crops caused an over-all epistatic effect between the physiological resistance and behavioral avoidance loci. Selection under epistatic conditions generated linkage disequilibrium, hence there were multiple genotype frequencies possible for particular gene frequency combinations. Adaptive landscapes (Gould, 1984) therefore could not predict evolutionary outcomes (Lewin, 1988). Easily interpreted graphs (Figures 3 and 4) of evolutionary results were generated instead. Four regions may be present in these graphs, delineating the absorbing states aarr, aaRR, AArr, and AARR (the four corners of the graphs), to which populations evolve.

To generate such graphs, trajectories of gene frequencies from successive generations of a population were calculated, and initial gene frequencies for populations were investigated at 0.01 gene frequency intervals. Typically, within 10 to 20 generations, each trajectory asymptotically approached one of the four absorbing states. When gene frequencies approached a corner, the genotype to which the population evolved was determined. Two successive trajectories with

different final genotypes indicated that a transition between end results had occured, and the initial gene frequency from the last trajectory was stored as a transition point. When trajectories from all 10,000 initial gene frequency combinations had been calculated, the gene frequencies identified as transition points were written to a file, and later used for generating Figures 3 and 4.

Simulation results and discussion

Interpretation of figures - For all the graphs generated from the simulation model, there are either three or four corners to which all the initial gene frequencies will eventually evolve. For example, in Figure 4A four regions are demarcated. The lines separating the regions are boundaries (unstable equilibria); populations initialized at gene frequencies on either side of these lines will evolve to different genotypes (different corners).

The evolutionary state most desirable for pest and pesticide resistance management is selection to the AArr genotype (upper left corner, Figures 3 and 4). The AArr pest genotype would be most easily managed. Most of this genotype would develop in the diversionary crop, because of their avoidance trait; the few individuals developing in the valued crop would be physiologically susceptible to the applied insecticide. The pest management strategy would be evolutionarily stable when a pest population is selected to this genotype.

Selection to the AARR genotype (upper right corner) would diminish the value of applying an insecticide in the valued crop, because of physiological resistance. However, expression of avoidance would reduce pest density in the valued crop.

Selection to the aaRR genotype (lower right corner) yields a non-avoiding, physiologically resistant population, the worst evolutionary consequence for pest management. Finally, selection to aarr may occur in rare, low selection intensity

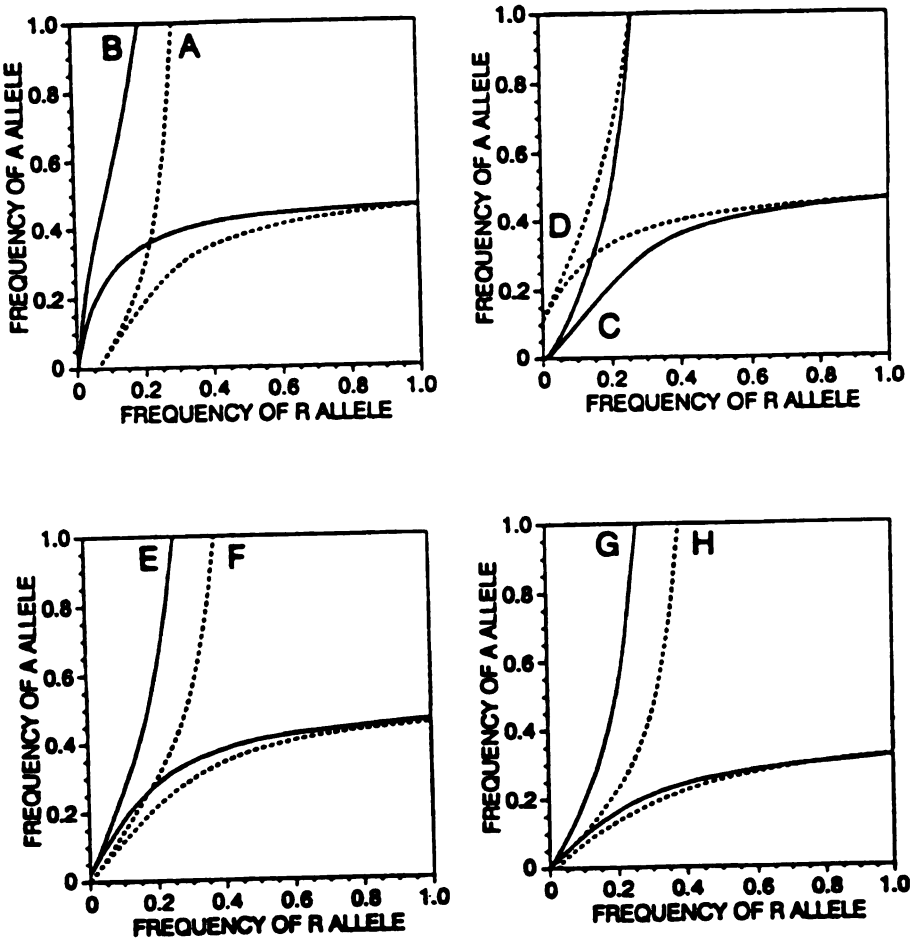


Figure 3. Genetic factors influencing development of avoidance (A allele) or physiological resistance (R allele).
(A) R allele is recessive; (B) R allele is dominant; (C) A allele is dominant; (D) A allele is recessive; (E) no fitness costs; (F) fitness cost exists for R allele; (G) fitness cost exists for a allele; (H) fitness costs exist for R and a alleles.

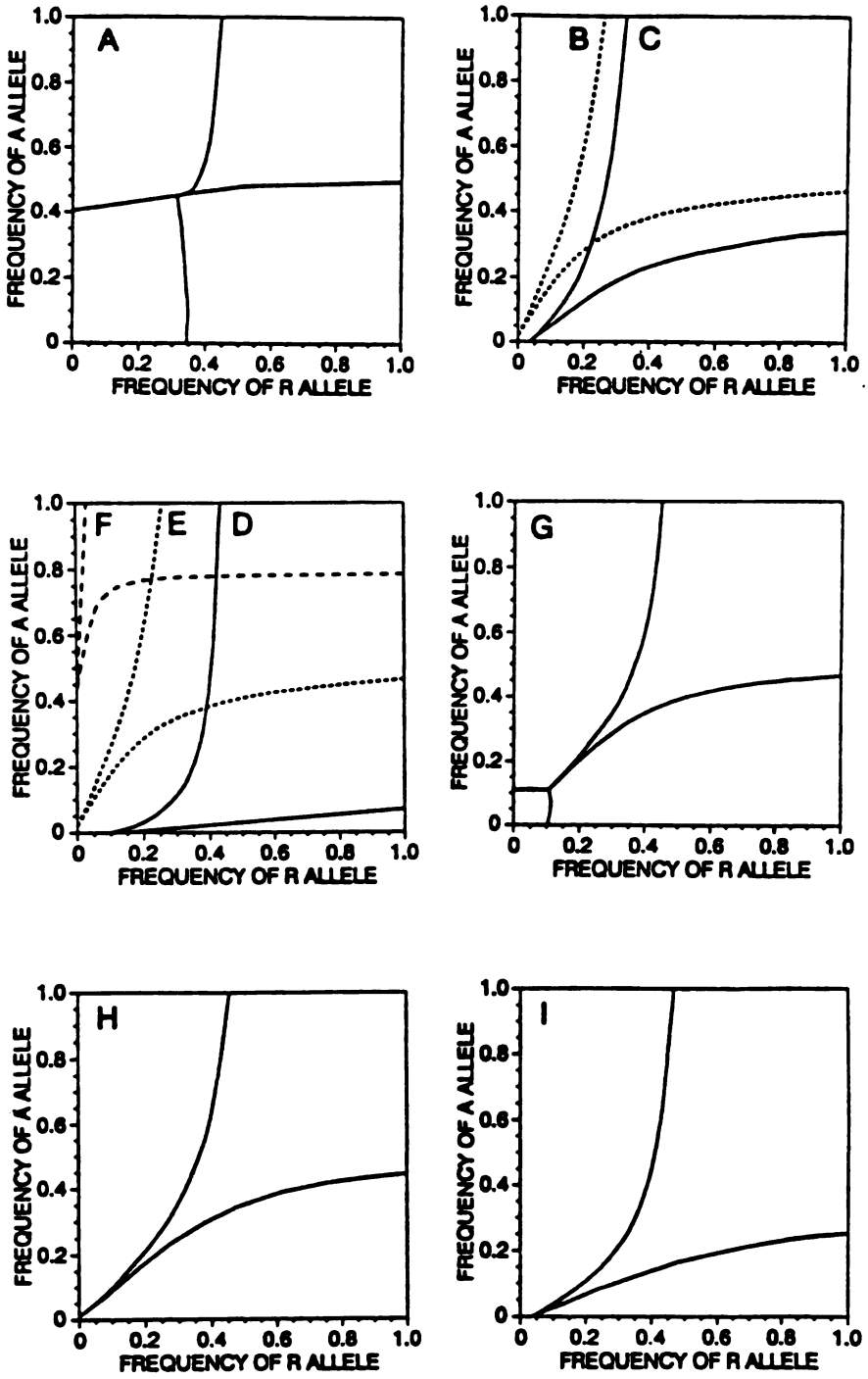


Figure 4. Operational factors influencing development of avoidance (A allele) or physiological resistance (R allele). (A-C) pesticide is applied at low, moderate, and high concentrations, respectively (see arrows in Fig. 1); (D-F) diversionary crop suitability is greater than, equal to and less than the suitability of the valued crop; (G) High availability of diversionary crop; (H) Low availability of diversionary crop and high discrimination by Aa and AA genotypes; (I) an SDDS situation, including low availability but high discrimination of Aa and AA genotypes for the diversionary crops and low survivorship in both diversionary and valued crops.

situations. This genotype would be easily controlled with insecticides, because it is physiologically susceptible, even though it does not avoid the valued crop.

The simulation results have been divided between genetic (mode of inheritance and fitness costs for R and A alleles, Figure 3) and operational factors (pesticide concentration, habitat quality, habitat availability, and an SDD example, Figure 4). The distinction is made because operational factors are management-determined variables (Tabashnik and Croft, 1982).

Genetic factors - Dominance of the R allele shifted the line separating the AArr- and AARR-destined regions further to the left (Figure 3 B vs. 3 A), meaning fewer gene frequency combinations were selected to the AArr genotype. Dominance of the A allele (Figures 3 C and 3 D) had less effect on selection than dominance of the R allele; the boundary shifted significantly only when A and R alleles were both at low frequencies. The co-dominant expression of R and A alleles (Figure 3 E) yielded results intermediate to recessive and dominant inheritance.

If fitness of individuals carrying resistance alleles were greater than that for carriers of susceptible alleles, gene frequencies for resistance would increase without selection with insecticide, making these resistance alleles common in unselected populations. Insecticides are not labeled for preadapted pests, so resistance alleles for pesticides are usually at very low initial frequencies. Hence, fitness of resistance alleles less than or equal to susceptible alleles should be a robust assumption. Fitness costs have been measured in several instances; they reduced intrinsic rates of growth in resistant strains by as much as 20 percent (Crow, 1957; Argentine, et al., 1989). Costs for carrying A or *a* alleles should also be possible. Pests may develop greater sensitivity to insecticides, and evolve avoidance responses (Gould, 1984; Lockwood, et al., 1984; Sparks, et al., 1989). A more efficient use of avoidance would be to take advantage of a pre-existing common

behavioral response. By the same reasoning used for insecticides above, applying a deterrent would not be practical unless the target pest is preadapted to respond; hence evolution of non-avoidance and fitness costs for the *a* allele should be considered.

Fitness costs subtly biased selection in these simulations. The greatest effect of a fitness cost for an *a* allele (Figures 3 G vs. 3 E and Figures 3 H vs. 3 F) was to shift the proportion of initial gene frequencies evolving to AARR rather than to aaRR. The effect of including a fitness cost for the R allele (Figure 3 F vs. 3 E and Figure 3 H vs. 3 G), was an increase in the proportion of gene frequencies evolving to the AArr genotype. Fitness costs for both the R and *a* alleles (Figure 3 H vs. 3 E) simply combined the effects mentioned above.

Operational factors - Pesticide concentration (Figure 2, arrows A, B, and C) greatly affected selection of R and A alleles (Figure 4 A-C). At low pesticide concentrations (Figure 2, arrow A) and low to moderate gene frequencies for physiological resistance, selection operates to diminish physiological resistance (Figure 4 A). Interestingly, if the A allele is also at low frequency, selection may drive the population to the aarr genotype. This simulation corresponds to a functionally dominance R allele, because at low pesticide concentrations, the Rr and RR genotypes suffer similar mortality (Taylor and Georghiou, 1979).

The greatest selection intensity for physiological resistance occurs when pesticide concentrations maximally discriminate between rr, Rr, and RR genotypes (Taylor and Georghiou, 1979). At a moderate pesticide concentration (Figure 2, arrow B; Figure 4 B) selection was greatest for the R allele and the AARR genotype. At a still higher pesticide concentration (Figure 2, arrow C and Figure 4 C), a greater number of initial gene frequency combinations evolved to the AArr genotype (note that the upper-left region in Figure 4 C is larger that the same region in Figure 4 B).

Habitat suitability is a measure of the quality of the environment for pest development. Suitability is itself a composite of abiotic and biotic factors contributing to survivorship within a habitat. To be realistic, suitability should include the effects of predators, parasitoids, pathogens, host-plant quality, competitors, sub-optimal abiotic conditions, and even density-dependent development restraints.

Maintaining a moderate pesticide dosage and changing the relative habitat quality of valued and diversionary crop demonstrated the profound influence of the habitat suitability on selection of the avoidance trait (Table 1; Figures 4 D-F). When the valued crop was more suitable for pest development than the diversionary crop (not considering the effect of pesticides), avoidance was selected against unless R alleles were absent (Figure 4 F). Lower survivorship in the diversionary crop may be common when the only "diversionary crop" in conventional agriculture is wild hosts.

As diversionary crop suitability improved relative to the valued crop, the region representing gene frequencies evolving to the AArr state greatly increased, and the number of gene frequency combinations evolving to aaRR was greatly reduced (Figure 4 D-F). If physiological resistance was below a critical value, the AArr genotype can evolve rather than physiological resistance genotypes.

Variables X and Y in the BASIC model were the probabilities AA and aa genotypes will lay eggs in the valued crop. Low values imply a complementarily high probability the eggs would be deposited in the diversionary crop. Manipulation of these values represents changes in the availability of or relative discrimination by the avoidance genotypes for the diversionary crop. Figure 4 B represents low availability of diversionary crop habitat (X=0.4, Y=0.9). Figure 4 G represents higher availability of diversionary crop (X=0.1, Y=0.6) relative to Figure 4 B.

Figure 4 H simulates low availability of diversionary crop, but very highly discriminating avoidance genotypes (X=0.1, Y=0.9).

There is an interaction between habitat availability and the avoidance trait. Having a readily available diversionary crop reduced selection for physiologically resistant genotypes, as expected from the reduced selection intensity on that trait (Figure 4 G vs. 4 B). Nearly the same selection diagram was generated in Figure 4 H as in 4 G, implying that greater discrimination by Aa and AA genotypes can be exchanged for greater availability of diversionary habitat. The greatest difference between these two graphs occurred when A and R alleles were at low frequencies. In Figure 4 G, this region evolved to the aarr genotype, while in Figure 4 H, the same initial gene frequencies evolve mostly to the aaRR or AArr genotypes.

SDD optimized both pest and pesticide resistance management when: 1) the diversionary crop habitat was of higher quality for pest development than the valued crop, 2) an insecticide was used to protect the valued crop, and 3) a deterrency trait was exploited. Under these conditions, a great number of initial gene frequencies will evolve to the most desired genetic state, an AArr population (Figures 4 D and 4 I vs. Figure 4 B).

An operational factor hidden in the graphs is choice of a deterrent to which the pest is or is not preadapted to respond. If a pest is preadapted to avoid a deterrent, then the population would have a large initial A allele gene frequency; non-preadaptation implies low initial avoidance. In all the simulation conditions, the line separating the areas evolving to the AArr *vs.* AARR genotypes slopes to the right. This is important - if a deterrent is chosen to which the pest is not adapted to respond, the A allele is at low gene frequency and there is a high probability that the population will be evolve to AARR. If a deterrent is chosen to which the pest is preadapted to respond, the population is likely to evolve to the AArr genotype.

Practical considerations for SDD

Deploying suitable diversionary crops for pests permits expression of normally precluded selective differences. Diversionary crops have been criticized because they may act as breeding grounds for the pest (Cromartie, 1981). This need not be a problem, if the number of pests emerging from the diversionary crop can be managed by destroying part of the infested crop. Manipulating pest survivorship differences in valued vs. diversionary crops should be viewed as necessary for both pest and pesticide resistance management. This is one feature distinguishing SDD strategy from previous pesticide resistance management models exploiting pest populations developing on wild hosts or abandoned orchards (Tabashnik and Croft, 1982).

Suppressing the pest population in a diversionary crop should be facilitated by first using behavioral modifying cues (chemical or physical) to confine the pest population. Under high pest densities, intraspecific competition may reduce survivorship. At somewhat lower populations, pest densities may exceed Reed-Frost epizootic thresholds (Hoppenstaedt, 1982), or facilitate host finding by predators or parasitoids.

One major advantage of SDD is that pesticides remain useful tools. Pesticides might be compatible with biological control and SDD because the majority of beneficial agents would be maintained in the non-treated habitat. Insecticides in an SDD system could actually help select for continued control, by driving selection for avoidance of the valued crop, and hence, improving partitioning of the population to the diversionary crop. If SDD partitioned the pest away from the valued-crop and reduced the pest density below economic thresholds, pesticides rates could be reduced or intermittently deleted.

Idealized use of resources (deterrents, pesticides, space used for diversionary crops) for accomplishing pest and pesticide resistance management may be very

challenging. Obviously it requires a thorough knowledge of a particular pest's behavioral and physiological traits, as well as the economics for growing the crop. Because selection requires several generations, temporal optimization is also possible; e.g., allocation of space for diversionary crops and pest emergence allowed from this habitat could initially be great enough to maximize evolutionary rate toward the AArr genotype, while simultaneously reestablishing viable populations of biological control organisms. Later, as the AArr genotype is being selected, space allocation to the diversionary crop, pesticide concentrations in the valued crop, and/or pest emergence from the diversionary crop could be reduced.

In a broader, sustainable view of pest management, plant breeders and genetic engineers should eventually replace insecticide applications with antibiotic and antixenotic crop traits. These traits have the same selective consequences for pest populations as conventional pesticides. Thus ecological management tools, such as SDD, should be evaluated to prevent squandering the limited genetic resource of pest susceptibility (Gould, 1984; 1986; Dover and Croft, 1984).

Summary

High-kill strategies for managing insecticide resistance may be short lived. Multiple resistance mechanisms make eventual failure of these approaches nearly certain, unless isolated populations lack genetic variance sufficient to respond to the high selection, or if a continual swamping of resistance genes occurs due to immigration of susceptible individuals. These pesticide management approaches are also incompatible with ecological closure aspects of sustainable agriculture, unless antibiosis crops are substituted for insecticide applications (Edens and Koenig, 1980). Even then, resistance development is likely unless ecological aspects of pesticide resistance are taken into consideration.

Ecological management of pesticide resistance through SDD is revolutionary. By taking advantage of behavior, pests could be manipulated to be

less damaging to the valued crop and concentrated in a diversionary crop, where biological control can be effective. If an SDD approach is implemented before gene frequencies for physiological resistance pass a critical value beyond which the population would be selected to the AARR genotype, and a deterrent is used on the valued crop, then avoidance behavior will be selected for and physiological resistance selected against. The consequence is an evolutionarily stable pest management strategy that removes conflict between the agriculturist and pest. Such an ecological approach redefines the time scale for successful pesticide resistance management, and re-castes the IPM practitioner as a selective breeder for beneficial pest traits. The critical role of host acceptance behavior for implementing SDD emphasizes the role insect behavior can play in generating new ecologically based IPM and sustainable agriculture methods.

Thesis Summary

Thesis Summary

Onions and onion models were used to bioassay onion fly host acceptance behavior, with the goal of developing strategies for controlling onion fly (*Delia antiqua* (Meigen)) oviposition. Stimulo-deterrent diversion (SDD) was developed, where the valued crop (seedling onions) are treated with chemical deterrents, and simultaneously, highly stimulatory ovipositional resources (sprouting cull onions) are deployed to concentrate eggs away from the crop.

Diverting oviposition with cull onions - Sprouting onion bulbs planted with the neck at *ca.* 5 cm below the soil surface stimulated more oviposition in a field experiment than bulbs planted either shallowly or at greater depth (Chapter 1). The stimulatory nature is probably attributable to the large number of leaves that project through the soil surface, each of which is an ovipositional resource. The visual and physical characteristics of these leaves closely match optimally stimulatory surrogate onion foliage (Harris, et al., 1987). In addition to the visual/physical stimuli, chemical cues generated through larval feeding and microbial decomposition of the bulb also enhance onion fly oviposition (Hausmann and Miller, 1989a; b). A factorial experiment investigating bulb damage and larval feeding on onion fly oviposition in the greenhouse suggested that bulbs remain highly stimulatory under great latitude of plant conditions. As long as onion bulbs are planted at *ca.* 5 cm depth, they can be expected to divert eggs from being deposited near seedlings.

Basic and applied significance of *Delia antiqua* ovipositional deterrents - Onion flies were sensitive to a wide array of deterrent compounds (Chapter 3 and 4), such as pungent spices, monoterpenoids and cinnamyl derivatives. Appreciably deterrent compounds were C8 to C13, intermediate in polarity, and possessed either

oxygen-containing or nitrile functional groups. In general these compounds were active at 10-100 fold higher concentrations than is dipropyl disulfide, a strong ovipositional stimulant (Harris and Miller, 1988). Broad but low sensitivity to phytochemicals does not mean such chemicals are unimportant in shaping insect-plant relationships. In an environment where there is a veritable pharmacopoeia of secondary compounds present in non-hosts, sensory apparatus for stenophagous herbivores like onion flies may detect "foreign" compounds when they are at their usual high, and consequently potentially toxic concentrations.

At a practical level, broad but low sensitivity has trade-offs. Various insect pests may respond (Dethier, 1947; Jermy, 1983) to compounds that deter onion flies, making these deterrents interesting because they could have "broad spectrum" activity. To be effective though, these deterrents may need to be applied at concentrations that currently make commercial development a challenge. A more practical approach might be to engineer deterrency into the crop plants. Traits such as antixenotic color (Prokopy, et al., 1983), or physical structure could enhance host-plant chemical deterrents. An alternative to deterrent host-plants could be the use of intercrops that are themselves repellent (Atsatt and O'Dowd, 1976).

Interactions and host-plant acceptance - Phytophagous insects integrate information from different sensory modalities to assess host-plant quality (Miller and Strickler, 1984; Miller and Harris, 1985). The neurophysiological basis for sensory integration is not well understood; paradigms for sensory integration and their expected behavioral characteristics are described in Chapter 5. Deterrents are expected to be most effective under the paradigm of "classical behavioral chaining" (Miller and Harris, 1985). Deterministic behavioral sequences could be disrupted at multiple points, each of which is necessary for the end result of host-plant acceptance. Deterrents are expected to be less effective when sensory information is temporally integrated via probabilistic behavioral transitions. Behavioral webs

allow some behavioral sequences to proceed to oviposition, in spite of the presence of suboptimal cues.

Interactions were studied within sensory modalities with chemical deterrents (Chapter 5), between modalities for chemical and visual deterrents (Chapter 5), and between positive and negative stimuli (Chapter 6). Behavioral observations supported temporal summation of across-modality sensory cues, which would generate independence of marginal acceptance probabilities and multiplicative effects. Analysis of variance on log-transformed egg counts was an innovative approach for studying end results of across-modality interactions and testing their multiplicative nature.

Chapters 2, 3, and 6 discussed interactions between external sensory and internal factors in governing host-plant acceptance. The loss of deterrency under conditions of ovipositional deprival suggests that deterrents may only be practical in field conditions if pests are offered highly acceptable ovipositional alternatives (SDD). A greenhouse test of SDD confirmed that the combination of deterrents applied to seedlings and adjacent sprouted cull onions more effectively protects seedlings from oviposition than either deterrents or culls alone (Chapter 6). The multiplicative effect for reduced numbers of eggs laid on seedlings can be explained through independently reduced acceptance probabilities when deterrent or culls are present.

SDD and natural systems - A population genetics model using two-allele loci for avoidance and physiological resistance traits (Chapter 7) suggested that SDD combined with conventional insecticides could prevent or reverse pesticide resistance development. Requirements were: 1) higher suitability of the diversionary crop, 2) high finding of the diversionary crop, and 3) deterrents to which a pest is preadapted to respond.

This model has important parallels in natural systems, if suitability of plants and adaptation to toxins are substituted for the pesticide/physiological resistance component. The model generated three or four "adaptive peaks" in which the homozygous populations of AArr, AARR, or aaRR genotypes had higher fitness than heterozygotes. These results suggest that evolution of specialization may be driven by the quality and quantity of alternative host-plant resources and gene frequencies for physiological and behavioral traits. The fitness disadvantage for heterozygotes implies that assortative mating may evolve; the avoidance allele could facilitate mate finding, thus causing assortative mating to take place. Hence, there is the possibility for host races to form on host plants with different suitabilities, and the pesticide resistance model converges with models for sympatric speciation (Bush and Diehl, 1982).

The population genetics model covered situations in which the quantities and qualities of alternative host-plant resource types were constant over time. In natural conditions, resource types may vary over time. Under these conditions, populations may respond genetically, through changes in gene frequencies for traits allowing adaptation to different host plant types. An alternative to genetic change is offered by behavioral flexibility. The observed effects of deprivation, and learning in other insects (Papaj, 1986), suggest that individual ovipositing insects can respond to paucity of suitable host resources by ovipositing on sub-optimal plants.

General conclusions - Cull onions, or deterrents applied to seedling onions by themselves probably would not provide economic control of onion flies unless the onion fly population is already very low, or the onion seedlings had strong antixenotic/antibiotic properties. The combined use of deterrents/antixenosis and stimulatory resources (SDD) could prevent economic damage from onion maggots. This SDD approach may have general appeal for other crops and pests, especially when: 1) deterrents or antixenotic cultivars are already available, 2) trap crop

species have already been identified, 3) reduced pesticide application is highly desirable, or 4) biological control agents require pest densities that cause economic damage under conventional agronomic practices.

The expected importance of SDD concepts for implementing biological control and pesticide resistance management suggests that behavioral manipulation can play a central role in developing a transition from conventional chemocentric management practices to sustainable agricultural systems.

Important unanswered questions - Does stimulus summation occur within or across sensory modalities, at the neurophysiological level?

Do onion flies vary the number of eggs laid per depositional bout in response to host quality? If they do, this argues for either 1) the fly having a *Gestalt* (across modality summation) perception of host plant quality or 2) concurrent examining and depositional behaviors.

Is there genetic variation for deterrency? How can this genetic variation best be measured, considering that acceptance is dynamically influenced by deprivation? Is there deterrency to pesticides in onion flies? If there is, is it an evolved response?

What are the implications of quantitative genetics for SDD? Could intermediate levels of avoidance or physiological resistance evolve, rather than fixation at physiological or behavioral extremes?

What would the population genetics model predict under conditions of overdominance in the behavioral trait? Would host races form if mating is associated with choice of ovipositional habitat? If host races are formed, would the the race specialized on the valued crop be *more* difficult to control than if the SDD approach had not been used?

LIST OF REFERENCES

LIST OF REFERENCES

Alfaro, R. I., Pierce, H. D., Jr., Borden, J. H., and Oehlschlager, A. C. 1981. Insect feeding and oviposition deterrents from western red cedar foliage. *J. Chem. Ecol.* 7:39-48.

Ames, B. N. and Gold, L. S. 1989. Pesticides, risk, and applesauce. *Science* 244:755-757.

Argentine, J. A., Clark, J. M., and Ferro, D. N. 1989. Relative fitness of insecticide-resistant Colorado potato beetle strains (Coleoptera: Chrysomelidae). *Environ. Entomol.* 18:705-710.

Atsatt, P. R. and O'Dowd, D. J. 1976. Plant defense guilds. *Science* 193:24-29.

Bernays, E. and Graham, M. 1988. On the evolution of host specificity in phytophagous arthropods. *Ecology* 69:886-892.

Brattsten, L. B. 1983. Cytochrome P-450 involvement in the interactions between plant terpenes and insect herbivores. pp. 173-195 *In*: Plant Resistance to Insects. P. A. Hedin (ed.).Amer. Chem. Soc. Symp. Series 208. ACS. Washington, DC.

Brattsten, L. B. 1988. Enzymic adaptations in leaf-feeding insects to host-plant allelochemicals. *J. Chem. Ecol.* 14:1919-1939.

Brattsten, L. B., Holyoke, C. W., Jr., Leeper, J. R., and Raffa, K. F. 1986. Insecticide resistance: challenge to pest management and basic research. *Science* 231:1255-1260.

Buchanen, R. L. 1978. Toxicity of spices containing methylene dioxybenzene derivatives: a review. *J. Food Safety* 1:275-293.

Bunker, C. W. O. and Hirschfelder, A. D. 1925. Mosquito repellents. *Am. J. Trop. Med.* 5:359-383.

Bush, G. L. and Diehl, S. R. 1985. Host shifts, genetic models of sympatric speciation and the origin of parasitic insect species. pp. 297-305 *In*: Proc. 5th Internat. Symp. Insect-Plant Relationships. J. H. Visser and A. K. Minks, (eds.). PUDOC. Wageningen, Netherlands.

Camhi, J. M. 1984. Neuroethology: Nerve Cells and the Natural Behavior of Animals. Sinauer. Sunderland, MA.

Carruthers, R. I. 1981. The biology and ecology of *Entomophthora muscae* (Cohn) in the onion ecosystem. Ph.D. Dissertation. Michigan State University. 171pp.

Carruthers, R. I. and Haynes, D. L. 1986. Temperature, moisture, and habitat effects on *Entomopthora muscae* (Entomopthorales: Entomopthoraceae). Conidial germination and survival in the onion ecosystem. *Environ. Entomol.* 15:1154-1160.

Carruthers, R. I., Whitfield, G. H., and Haynes, D. L. 1985. Pesticide-induced mortality of natural enemies of the onion maggot, Delia antiqua (Dip.:Anthomyiidae). *Entomophaga* 30:151-161.

Casagrande, R. A. 1987. The Colorado potato beetle: 125 years of mismanagement. *Bull. Entomol. Soc. Am.* 33:142-150.

Cerf, D. C. and Georghiou, G. P. 1972. Evidence of cross-resistance to a juvenile hormone analogue in some insecticide-resistant houseflies. *Nature* 239:401.

Chapman, R. F. 1988. Sensory aspects of host-plant recognitin by Acridoidea: questions associated with the multiplicity of receptors and variability of response. *J. Insect Physiol.* 34:167-174.

Clement, D. B. 1987. NOA summer regional meeting, optimistic attitudes prevailed. *Onion World* 3(5):6-10.

Cochran, W. G. 1954. Some methods for strengthening the common Chi squared tests. *Biom.* 10:417-451.

Coley-Smith, J. R. and King, J. E. 1969. The production by species of *Allium* of alkyl sulphides and their effect on germination of sclerotia of *Sclerotium cepivorum* Berk. *Ann. Appl. Biol.* 64:289-301.

Comins, H. N. 1977. The development of resistance in the presence of migration. *J. Theor. Biol.* 64:177.

Courtney, S. P., Chen, G. K. and Gardner, A. 1989. A general model for host selection. *Oikos* 55:55-65.

Courtney, S. P. and Kibota, T. T. 1989. Mother doesn't always know best: selection of hosts by ovipositing insects. *In*: Insect-Plant Interactions, Vol. 2. E. A. Bernays (ed.). CRC Press. Boca Raton, Florida.

Cowles, R. S., Keller, J. E. and Miller, J. R. 1989. Pungent spices, ground red pepper, and synthetic capsaicin as onion fly ovipositional deterrents. *J. Chem. Ecol.* 15:719-730.

Cowles, R. S., Miller, J. R., Hollingworth, R. M., Abdel-aal, M. T. Szurdoki, F., Bauer, K., and Matolcsy, G. 1990. Cinnamyl derivatives and monoterpenoids as non-specific ovipositional deterrents of the onion fly. *J. Chem. Ecol.* In press.

Croft, B. A. and Brown, A. W. A. 1975. Response of arthropod natural enemies to insecticides. *Ann. Rev. Entomol.* 20:285-355.

Cromartie, W. J., Jr. 1981. The environmental control of insects using crop diversity. pp. 223-251 *In*: CRC Handbook of Pest Management in Agriculture, Vol. 1. D. Pimentel (ed.). CRC Press. Boca Raton, FL.

Crow, J. F. 1957. Genetics of insect resistance to chemicals. *Ann. Rev. Entomol.* 2:227-246.

Curtis, C. F. 1985. Theoretical models of the use of insecticide mixtures for the management of resistance. *Bull. Entomol. Res.* 75:259-265.

Davis, E. E. 1985. Insect repellents: concepts of their mode of action relative to potential sonsory mechanisms in mosquitoes (Diptera: Culicidae). *J. Med. Entomol.* 22:273-243.

Davis, E. E. and Sokolove, P. G. 1976. Lactic acid-sensitive receptors on the antennae of the mosquito, *Aedes aegypti*. *J. Comp. Physiol.* 105:43-54.

Dawkins, M. S. 1983. The organization of motor patterns. pp. 75-99 *In*: Animal Behaviour, Vol. 1. T. R. Halliday and P. J. B. Slater. (eds.). W. H. Freeman. 228 pp.

Den Ouden, H. and Theunissen, J. 1980. Controlled release of naphthalene: a repellent against oviposition of the cabbage root fly, *Delia brassicae*. *Neth. J. Pl. Path.* 86:17-25.

Dethier, V. G. 1947. Chemical Insect Attractants and Repellents. Blakiston. Philadelphia, PA. 289 pp.

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

Dethier, V. G. 1982. Mechanism of host-plant recognition. *Entomol. exp. appl.* 31:49-56.

Dindonis, L. L. and Miller, J. R. 1980. Host finding responses of onion and seedcorn flies to healthy and decomposing onions and several synthetic constituents of onion. *Environ. Entomol.* 9:467-472.

Dindonis, L. L. and Miller, J. R. 1981. Onion fly and little house fly host finding selectively mediated by decomposing onion and microbial volatiles. *J. Chem. Ecol.* 7:419-426.

Dover, M. and Croft, B. 1984. Getting Tough: Public Policy and the Management of Pesticide Resistance. World Resources Institute. Washington, D. C. 80 pp.

Drummond, F. 1982. Post-harvest biology of the onion maggot, *Hylemya antiqua*. M.S. Thesis, Michigan State University.

Dyte, C. E. 1972. Resistance to synthetic juvenile hormone in a strain of flour beetle, *Tribolium castaneum*. *Nature* 238:48.

Eckenrode, C. J. 1988. *Delia antiqua* (Meigen): an increasing threat in North America. *In*: International Symposium on Diptera pests in vegetable crops. J. Narkiewicz-Jodko and B. Nawrocka, (eds.). pp. 27-30.

Eckenrode, C. J. and Nyrop, J. P. 1986. Impact of physical injury and commercial lifting on damage to onion bulbs by larvae of onion maggot (Diptera: Anthomyiidae). *J. Econ. Entomol.* 79:1606-1608.

Edens, T. C., Fridgen, C. and Battenfield, S. L. (eds.) 1985. Sustainable Agriculture and Integrated Farming Systems. Michigan State University Press.

Edens, T. C. and Koenig, H. E. 1980. Agroecosystem management in a resource-limited world. *Bioscience* 30:697-701.

Eisner, T. 1970. Chemical defense against predation in arthropods. pp. 157-217 *In*: Chemical Ecology. E. Sondheimer and J. B. Simeone (eds.). Academic Press.

Finch, S. 1978. Volatile plant chemicals and their effect on host plant finding by the cabbage root fly (*Delia brassicae*). *Entomol. exp. appl.* 24:150-159.

Finch, S. 1989. Ecological considerations in the management of *Delia* pest species in vegetable crops. *Ann. Rev. Entomol.* 34:117-138.

Finch, S., Cadoux, M. E., Eckenrode, C. J., and Spittler, T. D. 1986a. Appraisal of current strategies for controlling onion maggot (Diptera:Anthomyiidae) in New York State. *J. Econ. Entomol.* 79:736-740.

Finch, S. and Eckenrode, C. J. 1985. Influence of unharvested, cull-pile, and volunteer onions on populations of onion maggot (Diptera: Anthomyiidae). *J. Econ. Entomol.* 78:542-546.

Finch, S., Eckenrode, C. J., and Cadoux, M. E. 1986b. Behavior of onion maggot (Diptera:Anthomyiidae) in commercial onion fields treated regularly with parathion sprays. *J. Econ. Entomol.* 79:107-113.

Fitt, G. P. 1986. The influence of a shortage of hosts on the specificity of oviposition behaviour in species of *Dacus* (Diptera, Tephritidae). *Physiol. Entomol.* 11:133-143.

Fraenkel, G. 1959. The *raison d'etre* of secondary plant substances. *Science* 129:1466-1470.

Fukuto, T. R. and Mallipudi, N. M. 1983. Suppression of metabolic resistance through chemical structure modification. pp. 557-578 *In* Pest Resistance to Pesticides. G. P. Georghiou and T. Saito (eds.). Plenum. NY.

Georghiou, G. P. and Mellon, R. B. 1983. Pesticide resistance in time and space. pp. 1-46 *In* Pest Resistance to Pesticides. G. P. Georghiou and T. Saito (eds.). Plenum. NY.

Georghiou, G. P. 1983. Management of resistance in arthropods. pp. 769-792 *In* Pest Resistance to Pesticides. G. P. Georghiou and T. Saito (eds.). Plenum. NY.

Girolami, V., Vianello, A., Strapazzon, A., Ragazzi, E. and Veronese, G. 1981. Ovipositional deterrents in *Dacus oleae*. *Ent. exp. & appl.* 29:177-188.

Gordon, H. T. 1961. Nutritional factors in insect resistance to chemicals. *Ann. Rev. Entomol.* 6:27-54.

Gothilf, S. and Bar-Zeev, M. 1972. Effect of vapor repellents on the electrical response of insect antennae. *Experientia* 28:601-603.

Gould, F. 1984. Role of behavior in the evolution of insect adaptation to insecticides and resistant host plants. *Bull. Entomol. Soc. Am.* 30:34-51.

Gould, F. 1986. Simulation models for predicting durability of insect-resistant germ plasm: Hessian fly (Diptera: Cecidomyiidae)-resistant winter wheat. *Environ. Entomol.* 15:11-23.

Grafius, E., Hayden, J., and Jenkins, M. 1987. Management of insect pests of onions. 1987 Research Report to the Michigan Onion Research Committee.

Grafius, E. and Warner, F. W. 1990. Predation by *Bembidion quadrimaculatum* (Coleoptera: Carabidae) on the eggs of the onion maggot (*Delia antiqua*). *Environ. Entomol.* In Press.

Grant, D. 1986. Glutathione S-transferase in *Aedes aegypti*. PhD. Dissertation. Michigan State University.

Groden, E. 1982. The interactions of root maggots and two parasitoids *Aleochara bilineata* (Gryll.) and *Aphaereta pallipes* (Say). Master's Thesis, Michigan State University. 152 pp.

Guyer, G. and Wells, A. 1959. Evaluation of insecticides for control of the chlorinated hydrocarbon resistant onion maggot. *Quar. Bull. Mich. Agric. Expt. Sta.* 41(3):614-623.

Hammond, G. H. 1924. The onion maggot in the Ottawa District. *A. Rep. Entomol. Soc. Ontario* 1923 55:39-41.

Harris, C. R., Tolman, J. H. and Svec, H. J. 1982. Onion maggot (Diptera:Anthomyiidae) resistance to some insecticides following selection with parathion or carbofuran. *Can. Ent.* 114:681-685.

Harris, M. O., Keller, J. E., and Miller, J. R. 1987. Responses to n-dipropyl disulfide by ovipositing onion flies: Effects of concentration and site of release. *J. Chem. Ecol.* 13:1261-1277.

Harris, M. O. and Miller, J. R. 1982. Synergism of visual and chemical stimuli in the oviposition behavior of *Delia antiqua*. pp. 117-122 *In*: Proc. 5th Int. Symp. Insect Plant Relationships. J. H. Visser and A. K. Minks (eds.). Wageningen, The Netherlands. PUDOC, Wageningen.

Harris, M. O. and Miller, J. R. 1983. Color stimuli and ovipositional behavior of the onion fly, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae). *Ann. Entomol. Soc. Am.* 76:766-771.

Harris, M. O. and Miller, J. R. 1984. Foliar form influences ovipositional behavior of the onion fly. *Physiol. Entomol.* 9:145-155.

Harris, M. O. and Miller, J. R. 1988. Host acceptance behavior in an herbivorous fly, *Delia antiqua*. *J. Insect Physiol.* 34:179-180.

Harris, M. O. and Miller, J. R. 1991. Quantitative analysis of onion fly egglaying behavior: effects of a host-plant chemical. *J. Insect Behavior* In Press.

Harris, M. O., Miller, J. R., and de Ponti, O. M. B. 1987. Mechanisms of resistance to onion fly egg-laying. *Entomol. exp. appl.* 43:279-286.

Hausmann, S. M. and Miller, J. R. 1989a. Ovipositional preference and larval survival of the onion maggot (Diptera: Anthomyiidae) as influenced by previous maggot feeding. *J. Econ. Entomol.* 82:426-429.

Hausmann, S. M. and Miller, J. R. 1989b. Production of onion fly attractants and ovipositional stimulants by bacterial isolates cultured on onion. *J. Chem. Ecol.* 15:905-916.

Havukkala, I. 1982. Deterring oviposition of the cabbage root fly, *Delia radicum* (Diptera:Anthomyiidae), by non-chemical methods. *Acta Entomol. Fenn.* 40:9-15.

Hokkanen, H., Granlund, H., Husberg, G.-B., and Markkula, M. 1986. Trap crops used successfully to control *Meligethes aeneus* (Col., Nitidulidae), the rape blossom beetle. *Ann. Entomol. Fenn.* 52:115-120.

Hoppenstaedt, F. C. 1982. Mathematical methods of population biology. Cambridge Univ. Press. NY. 149 pp.

Hoskins, W. M. and Gordon, H. T. 1956. Arthropod resistance to chemicals. *Ann. Rev. Entomol.* 1:89-122.

House, G. J. and Alzugaray, M. R. 1989. Influence of cover cropping and no-tillage practices on community composition of soil arthropods in a North Carolina agroecosystem. *Environ. Entomol.* 18:302-307.

Howard, J. J., Cazin, J., Jr., and Wiemer, D. F. 1988. Toxicity of terpenoid deterrents to the leafcutting ant *Atta cephalotes* and its mutualistic fungus. *J. Chem. Ecol.* 14:59-69.

Howitt, A. J. 1958. Chemical control of *Hylemia antiqua* (Meig.) (Diptera: Anthomyiidae) in the Pacific Northwest. *J. Econ. Entomol.* 51:883-887.

Ishikawa, Y., Ikeshoji, T., Matsumoto, Y., Tsutsumi, A., and Mitsui, Y. 1983. 2-Phenethanol: an attractant for the onion and seed-corn flies, *Hylemya antiqua* and *H. platura* (Diptera: Anthomyiidae). *Appl. Ent. Zool.* 18:270-277.

Jaenike, J. 1983. Induction of host preference in *Drosophila melanogaster*. *Oecologia* 58:320-325.

Javer, A., Wynne, A. D., Borden, J. H., and Judd, G. J. R. 1987. Pine oil: an ovipositional deterrent for the onion maggot, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae). *Can. Ent.* 119:605-609.

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

Jermy, T. and Szentesi, A. 1978. The role of inhibitory stimuli in the choice of oviposition site by phytophagous insects. *Entomol. exp. appl.* 24:458-471.

Jermy, T. 1983. Multiplicity of insect antifeedants in plants. pp. 223-226 *In*: Natural Products for Innovative Pest Management. Vol. 2, Current Themes in Tropical Science. D. L. Whitehead and W. S. Bowers (eds.). Pergamon Press, NY, 586 pp.

Jones, T. H., Cole, R. A. and Finch, S. 1988. A cabbage root fly oviposition deterrent in the frass of garden pebble moth caterpillars. *Entomol. exp. appl.* 49:277-282.

Judd, G. J. R. and Borden, J. H. 1989. Distant olfactory response of the onion fly, *Delia antiqua*, to host-plant odour in the field. *Physiol. Entomol.* 14:429-441.

Kendall, E. W., Jr. 1932. Notes on the onion maggot (*Hylemya antiqua*, Meigen). A. Rep. Entomol. Soc. Ontario 1931 62:82-84.

Kennedy, J. S., Booth, C. O. and Kershaw, W. J. S. 1961. Host finding by aphids in the field. III. Visual attraction. *Ann. Appl. Biol.* 49:1-21.

Klocke, J. A., Balandrin, M. F., Barnby, M. A. and Yamasaki, R. B. 1989. Limonoids, phenolics, and furanocoumarins as insect antifeedants, repellents, and growth inhibitory compounds. *In*: Insecticides of Plant Origin, ACS Symposium Series 387. J. T. Arnason, B. J. R. Philogne, and P. Morand (eds.). Amer. Chem. Soc., Washington, D. C.

Klowden, M. J. 1989. Influence of the ovaries and fat body on the initiation and termination of pre-oviposition behavior in the mosquito, *Aedes aegypti*. *J. Insect Physiol.* 35:567-570.

Kogan, M. 1988. Integrated pest management theory and practice. *Entomol. exp. appl.* 49:59-70.

Landis, D. A. and Gould, F. Some considerations in the screening and use of feeding deterrents for crop protection. *Environ. Entomol.* In press.

Levin, D. A. 1971. Plant phenolics: an ecological perspective. *Amer. Nat.* 105:157-181.

Levins, R. 1986. Perspectives in integrated pest management: from an industrial to an ecological model of pest management. pp. 1-8 *In*: Ecological theory and integrated pest management practice. M. Kogan (ed.). Wiley. NY. 362 pp.

Lewin, R. 1988. The uncertain perils of an invisible landscape. *Science* 240: 1405-1406.

Lockwood, J. A., Sparks, T. C., and Story, R. N. 1984. Evolution of insecticide resistance to insecticides: a reevaluation of the roles of physiology and behavior. *Bull. Entomol. Soc. Amer.* 30(4):41-51.

Loosjes, M. 1976. Ecology and Genetic Control of the Onion Fly, *Delia antiqua* (Meigen). PUDOC, Wageningen.

Lovett, A. L. 1923. The onion maggot. *Oreg. Agric. Expt. Stn. Circ.* 37.

Lovett, J. V., Ryuntu, M. Y., and Liu, D. L. 1989. Allelopathy, chemical communication, and plant defense. *J. Chem. Ecol.* 15:1193-1202.

Lundgren, L. 1975. Natural plant chemicals acting as oviposition deterrents on cabbage butterflies (*Pieris brassicae* (L.), *P. rapae* (L.) and *P. napi* (L.)). *Zool. Scripta* 4:253-258.

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

MacDonald, R. S., Surgeoner, G. A., Solomon, K. R., and Harris, C. R. 1983. Effect of four spray regimes on the development of permethrin and dichlorvos resistance in the laboratory by the house fly (Diptera: Muscidae). *J. Econ. Entomol.* 76:417-422.

Mandava, N. B., Orellana, R. G., Warthen, J. D., Jr., Worley, J. F., Dutkey, S. R., Finegold, H. and Weathington, B. C. 1980. Phytotoxins in *Rhyzoctonia solani*: Isolation and biological activity of *m*-hydroxy and *m*-methoxyphenylacetic acids. *J. Agric. Food Chem.* 28:71-75.

Mangel, M. 1989a. An evolutionary interpretation of the "motivation to oviposit." *J. Evol. Biol.* 2:157-172.

Mangel, M. 1989b. Evolution of host selection in parasitoids: Does the state of the parasitoid matter? *Amer. Naturalist* 133:688-705.

Mangel, M. and Roitberg, B. D. 1989. Dynamic information and host acceptance by a tephritid fruit fly. *Ecol. Entomol.* 14:181-189.

Marsolan, N. F. and Garrett, D. L. 1977. Optimal sizing of trap crops. *Proc. Pittsburgh Conf. on Modeling and Simulation* 8:751-754.

Martin, P. and Bateson, P. 1986. Measuring Behaviour. Cambridge Univ. Press. 200 pp.

Martinson, T. E., Nyrop, J. P., and Eckenrode, C. J. 1988. Dispersal of the onion fly (Diptera:Anthomyiidae) and larval damage in rotated onion fields. *J. Econ. Entomol.* 81:508-514.

Matsumoto, Y. and Thorsteinson, A. J. 1968. Effects of organic sulfur compounds on oviposition in onion maggot *Hylemia antiqua* (Diptera: Anthomyiidae). *Appl. Ent. Zool.* 3:5-12.

Matsumura, F. 1983. Penetration, binding and target insensitivity as causes of resistance to chlorinated hydrocarbon insecticides. pp. 367-386. *In*: Pest Resistance to Pesticides. G. P. Georghiou and T. Saito (eds.). Plenum. NY.

Merck Index. 1976. M. Wildholz (ed.). 9th Edition. p. 570. Merck & Co., NJ, USA.

Meredith, M. A. and Stein, B. E. 1983. Interaction among converging sensory inputs in the superior colliculus. *Science* 221:389-391.

Metcalf, R. L. 1980. Changing role of insecticides in crop production. *Ann. Rev. Entomol.* 25:219-256.

Metcalf, R. L. and Lampman, R. L. 1989. Estragole analogues as attractants for corn rootworms (Coleoptera: Chrysomelidae) *J. Econ. Entomol.* 82:123-129.

Miller, J. R. and Cowles, R. S. 1990. Stimulo-deterrent diversion: A concept and its possible application to onion maggot control. *J. Chem. Ecol.* In Press.

Miller, J. R. and Harris, M. O. 1985. Viewing behavior-modifying chemicals in the context of behavior: lessons from the onion fly. pp. 3-31 *In*: Semiochemistry: Flavors and Pheromones. T. E. Acree and D. M. Soderlund (eds.). Walter de Gruyter & Co., Berlin.

Miller, J. R. and Strickler, K. L. 1984. Finding and accepting host plants. pp. 127-155 *In*: Chemical Ecology of Insects. W. J. Bell and R. T. Cardé, (eds.). Chapman and Hall, NY.

Miller, J. R., Mori, K., and Roelofs, W. L. 1977. Gypsy moth field trapping and electroantennogram studies with pheromone enantiomers. *J. Insect Physiol.* 23:1447-1453.

Miller, T. A., Salgado, V. L., and Irving, S. N. 1983. The *kdr* factor in pyrethroid resistance. pp. 353-366 *In*: Pest Resistance to Pesticides. G. P. Georghiou and T. Saito (eds.). Plenum. NY.

Mitscher, L. A. 1975. Antimicrobial agents from higher plants. *Recent Adv. Phytochem.* 9:243-283.

Moore, L. E. and Christensen, B. N. 1985. Synaptic integration in a single neuron. pp. 323-334 *In*: Contemporary Sensory Neurobiology. Correia, M. J. and Perachio, A. A. (eds.). Liss. NY.

Mowry, T. M., Keller, J. E., and Miller, J. R. 1989a. Oviposition of *Delia antiqua* (Diptera: Anthomyiidae) as influenced by substrate holes and particle size. *Ann. Entomol. Soc. Am.* 82:126-131.

Mowry, T. M., Spencer, J. L., Keller, J. E., and Miller, J. R. 1989b. Onion fly (*Delia antiqua*) egg depositional behaviour: pinpointing host acceptance by and insect herbivore. *Insect Physiol.* 35:331-339.

Nair, M. G., Epp, M. D. and Burke, B. A. 1988. Ferulate esters of higher fatty alcohols and allelopathy in *Kalanchoe diagremontiana*. *J. Chem. Ecol.* 14:589-603.

Nakanishi, K. 1980. Insect antifeedants from plants. pp. 603-611 *In*: Insect Biology in the Future "VBW 80". M. Locke and D. S. Smith (eds.). Academic Press. NY.

Ozaki, K. 1983. Suppression of resistance through synergistic combinations with emphasis on planthoppers and leafhoppers infesting rice in Japan. pp. 595-614 *In*: Pest Resistance to Pesticides. G. P. Georghiou and T. Saito (eds.). Plenum. NY.

Painter, R. H. 1968. Insect Resistance in Crop Plants. University Press of Kansas. 2nd edition. 520 pp.

Papaj, D. R. 1986. Conditioning of leaf-shape discrimination by chemical cues in the butterfly *Battus philenor*. *Anim. Behav.* 34:1281-1288.

Papaj, D. R. and Prokopy, R. J. 1988. The effect of prior adult experience on components of habitat preference in the apple maggot fly (*Rhagoletis pomonella*). *Oecologia* 76:538-543.

Patton, S., Craig, I. and Conway, G. R. 1982. The Pesticide industry. pp. 58-73 *In*: Pesticide Resistance and World Food Production. G. R. Conway (ed.) Imperial College Centre for Environmental Technology. London.

Payne, T. L. and Dickens, J. C. 1976. Adaptation to determine receptor specificity in insect olfactory communication. *J. Insect Physiol.* 22:1569-1572.

Perron, J. P. and LaFrance, J. 1961. Notes on the life history of the onion maggot, *Hylemya antiqua* (Meig.) (Diptera: Anthomyiidae), reared in field cages. *Can. Ent.* 93:101-106.

Pickett, J. A., Dawson, G. W., Griffiths, D. C., Xun, L., Macaulay, E. D. M., and Woodcock, C. M. 1984. Propheromones: an approach to the slow release of pheromones. *Pestic. Sci.* 15:261-264.

Plapp, F. W. and Wang, T. C. 1983. Genetic origins of insecticide resistance. pp. 47-70 *In*: Pest Resistance to Pesticides. G. P. Georghiou and T. Saito (eds.). Plenum. NY.

Price, P. W., Bouton, C. E., Gross, P., McPherson, B. A., Thompson, J. M., and Weis, A. E. 1980. Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Ann. Rev. Ecol. Syst.* 11:41-46.

Prokopy, R. J. 1981. Oviposition-deterring pheromone system of apple maggot flies. pp. 477-494 *In*: Management of Insect Pests with Semiochemicals. E. R. Mitchell (ed.). Plenum Press. NY.

Prokopy, R. J. 1986. Visual and olfactory stimulus interaction in resource finding by insects. pp. 81-89 *In*: Mechanisms in Insect Olfaction. T. L. Payne, M. C. Birch, and C. E. J. Kennedy (eds.). Clarendon Press. Oxford.

Prokopy, R. J., Collier, R. H. and Finch, S. 1983. Leaf color used by cabbage root flies to distinguish among host plants. *Science* 221:190-192.

Prokopy, R. J. and Owens, E. D. 1983. Visual detection of plants by herbivorous insects. *A. Rev. Ent.* 28:337-364.

Prokopy, R. J., Powers, P. J., Heath, R. R., Dueben, B. D., and Tumlinson, J. H. 1988. Comparative laboratory methods for assaying behavioral responses of *Rhagoletis pomonella* flies to host marking pheromone. *J. Appl. Entomol.* 106:437-443.

Pyke, B., Rice, M., Sabine, G., and Zalucki, M. 1987. The push-pull strategy - behavioral control of *Heliothis*. The Australian Cotton Grower, May-July.

Raffa, K. F. and Frazier, J. L. 1988. A generalized model for quantifying behavioral de-sensitization to antifeedants. *Entomol. exp. appl.* 46:93-100.

Ranasinghe, L. E. and Georghiou, G. P. 1979. Comparative modification of insecticide resistance spectrum of *Culex pipiens fatigans* Weid. by selection with temephos and temephos/synergist combinations. *Pestic. Sci.* 10:502.

Raven, P. H. and Curtis, H. 1970. Biology of Plants. Worth. N.Y.

Renwick, J. A. A. and Radke, C. D. 1987. Chemical stimulants and deterrents regulating acceptance or rejection of crucifers by cabbage butterflies. *J. Chem. Ecol.* 13:1771-1776.

Rice, E. L. 1984. Allelopathy. Academic Press. NY.

Rice, M. 1986. Semiochemicals and sensory manipulation strategies for behavioral management of *Heliothis* spp. Ochsenheimer (Lepidoptera: Noctuidae), pp. 27-45 *In*: *Heliothis* Ecology Workshop 1985 Proceedings. M. P. Zalucki and P. H. Twine (eds.). Queensland Department of Primary Industries, Brisbane, Australia.

Rodriguez, E. and Levin, D. A. 1976. Biochemical parallelism of repellents and attractants in higher plants and arthropods. *Recent Adv. Phytochem.* 10:214-270.

Roitberg, B. D. and Prokopy, R. J. 1983. Host deprivation influence on response of *Rhagoletis pomonella* to its oviposition deterring pheromone. *Physiological Entomology* 8:69-72.

Ryan, J., Ryan, M. F. and McNaedhe, F. 1980. The effect of interrow plant cover on populations of the cabbage root fly *Delia brassicae* (Weidemann). *J. Appl. Ecol.* 17:31-40.

SAS Institute Inc. 1985. SAS User's Guide: Statistics, Version 5 Edition. Cary, NC: Sas Institute Inc. 956 pp.

Schneider, K. 1989. Five supermarket chains open effort against pesticide use. *The New York Times* Vol. 138. Sept. 12, 1989. p. B9.

Schneider, W. D., Miller, J. R., Breznak, J. A. and Fobes, J. F. 1983. Onion maggot, *Delia antiqua*, survival and development on onions in the presence and absence of microorganisms. *Entomol. exp. appl.* 33:50-56.

Singer, M. C. 1982. Quantification of host preferences by manipulation of oviposition behavior in the butterfly, *Euphydryas editha*. *Oecologia* 52:224-229.

Singh, D., Siddiqui, M. S. and Sharma, S. 1989. Reproduction retardant and fumigant properties in essential oils against rice weevil (Coleoptera:Curculionidae) in stored wheat. *J. Econ. Entomol.* 82:727-733.

Snedecor, G. W. and Cochran, W. G. 1967. Statistical Methods. 6th Edition. Iowa State Univ. Press. 593 pp.

Sparks, T. C. and Hammock, B. D. 1983. Insect growth regulators: resistance and the future. pp. 615-668 *In*: Pest Resistance to Pesticides. G. P. Georghiou and T. Saito (eds.). Plenum. NY.

Sparks, T. C., Lockwood, A. J., Byford, R. L., Graves, J. B., and Leonard, B. R. 1989. The role of behavior in insecticide resistance. *Pestic. Sci.* 26:383-399.

Städler, E. 1983. Attractants, arrestants, feeding and oviposition stimulants in insect-plant relationships: application for pest control. pp. 243-258 *In:* Natural Products for Innovative Pest Management. D. L. Whitehead and W. S. Bowers (eds.). Vol. 2 of Current Themes of Tropical Science. Pergamon Press, NY.

Steel, R. G. D. and Torrie, J. H. 1980. Principles and Procedures of Statistics, 2nd Edition. McGraw-Hill. NY.

Stephens, D. W. and Krebs, J. R. 1986. Foraging theory. Princeton University Press. Princeton, N.J.

Storck, W. J. 1984. Pesticides head for recovery. *Chem. and Engin. News* 62:35-59. April 9, 1984.

Tabashnik, B. E. 1987. Plant secondary compounds as oviposition deterrents for cabbage butterfly, *Pieris rapae* (Lepidoptera: Pieridae). *J. Chem. Ecol.* 13:309-316.

Tabashnik, B. E. 1989. Managing resistance with multiple pesticide tactics: theory, evidence, and recommendations. *J. Econ. Entomol.* 82:1263-1269.

Tabashnik, B. E. and Croft, B. A. 1982. Managing pesticide resistance in crop-arthropod complexes: interactions between biological and operational factors. *Environ. Entomol.* 11:1137-1144.

Tabashnik, B. E., Cushing, N. E., and Johnson, M. W. 1987. Diamondback moth (Lepidoptera: Plutellidae) resistance to insecticides in Hawaii: intra-island variation and cross-resistance. *J. Econ. Entomol.* 80:1091-1099.

Tahvanainen, J. O. and Root, R. B. 1972. The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae), *Oecologia (Berlin)* 10:321.

Taylor, C. E. and Georghiou, G. P. 1979. Suppression of insecticide resistance by alteration of gene dominance and migration. *J. Econ. Entomol.* 72:105-109.

Thiery, D. and Visser, J. H. 1987. Misleading the Colorado potato beetle with an odor blend. *J. Chem. Ecol.* 13:1139-1146.

Tingle, F. C. and Mitchell, E. R. 1986. Behavior of *Heliothis virescens* (F.) in presence of oviposition deterrents from elderberry. *J. Chem. Ecol.* 12:1523-1531.

Tingle, G. C. and Mitchell, E. R. 1984. Aqueous extracts from indigenous plants as oviposition deterrents for *Heliothis virescens* (F.). *J. Chem. Ecol.* 10:101-113.

Todd, P. H., Jr., Bensinger, M. G. and Biftu, T. 1977. Determination of pungency due to capsicum by gas-liquid chromatography. *J. Food Sci.* 42:660-665.

Van den Bosch, R. 1978. The Pesticide Conspiracy. Doubleday. Garden City, NY. 226 pp.

Van Rie, J., McGaughey, W. H., Johnson, D. E., Barnett, B. D., and Van Mellaert, H. 1990. Mechanism of insect resistance to the microbial insecticide *Bacillus thuringiensis*. *Science* 247:72-74.

Vernon, R. S., Borden, J. H., Pierce, H. D., Jr. and Oehlschlager, A. C. 1977. Host selection by *Hylemia antiqua* laboratory bioassay and methods of obtaining host volatiles. *J. Chem. Ecol.* 3:359-368.

Via, S. 1986. Quantitative genetic models and the evolution of pesticide resistance. *In* Pesticide Resistance: strategies and tactics for management. National Academy of Science, Washington, D. C.

Virus, R. M. and Gebhart, G. F. 1979. Pharmacological actions of capsaicin: apparent involvement in substance P and serotonin. *Life Sci.* 25:1273-1284.

Visser, J. H. 1983. Differential sensory perceptions of plant compounds by insects. *In*: Plant Resistance to Insects. pp. 215-229. Amer. Chem. Soc. Symp. Ser. 208. P. A. Hedin (ed.). ACS. Washington, DC.

Weston, P. A., and Miller, J. R. 1985. Influence of cage design on precision of tube trap bioassay for attractants of the onion fly, *Delia antiqua*. *J. Chem. Ecol.* 11:435-440.

Whitfield, G. H. 1981. Spatial and temporal population analysis of the onion maggot, *Hylemya antiqua*, in Michigan. Ph.D. thesis. Michigan State University, East Lansing.

Wiens, M. N., Rahe, J. E., Vernon, R. S., and McLean, J. A. 1978. Ovipositional deterrents for *Hylemya antiqua* in hydrated seeds of *Phaseolus vulgaris*. *Environ. Entom.* 7:165-167.

Workman, R. B., Jr. 1958. The biology of the onion maggot, *Hylemya antiqua* (Meigen), under field and greenhouse conditions. Ph. D. Thesis, Oregon State College.

APPENDICES

APPENDIX 1

BASIC Population genetics model

APPENDIX 1

BASIC Population genetics model

```
10 CLS
20 DIM LC50(3) : DIM B(3) : DIM PMORT(3) : DIM STDCON(3)
30 A$="1" : DIM DIST(2) : DIM G(4) : DIM CORNER(2)
40 DIM P(3,3): DIM NS(3,3) : DIM S(3,3)
50 TOTAL=0 : CORNER(1)=0
60 INPUT "Path:filename.ext for datafile on disk is:";Z$
70 OPEN "O",#1,Z$
80 INPUT "Untreated habitat quality";UHS
90 INPUT "Treated habitat quality";THS
100 INPUT "Fitness cost for R allele";FCR
110 FCR=1-FCR
120 INPUT "Fitness cost for a allele";FCA
130 FCA=1-FCA
140 INPUT "Probability of AA being in treated area=";X
150 INPUT "Probability of aa being in treated area=";Y
160 INPUT "Do you want to use default toxicity values";J$
170 IF J$ = "n" THEN GOTO 200
180 LC50(1)=1 : LC50(2)=2 : LC50(3)=4
190 B(1)=2 : B(2)=2 : B(3)=2 : GOTO 280
200 PRINT "Enter the LC50 for the following genotypes:"
210 INPUT "rr";LC50(1)
220 INPUT "Rr";LC50(2)
230 INPUT "RR";LC50(3)
240 PRINT "Enter the slope for the ln(conc)-probit mortality curves"
250 INPUT "for the following genotypes: rr"; B(1)
260 INPUT "Rr";B(2)
270 INPUT "RR";B(3)
280 INPUT "Concentration of pesticide";CONC
290 FOR I = 1 TO 3
300 IF CONC<=.00005 THEN PMORT(I)=0 : GOTO 410
310  STDCON(I)=B(I)*(LOG(CONC)-LOG(LC50(I))) : FLG = 0
320  IF STDCON(I) >=0 THEN 340
330  FLG = 1 : STDCON(I) = ABS(STDCON(I))
340  IF STDCON(I) < 3.7 THEN 360
350    PMORT(I)=1 : GOTO 400
360  IF STDCON(I) < 2.64 THEN 380
370    PMORT(I)= .959658+.0216767*STDCON(I)-.00291*STDCON(I)^2 : GOTO 400
380  PMORT(I)= .49528+.47095*STDCON(I)-.14163*STDCON(I)^2+.01347*STDCON(I)^3
390  IF PMORT(I)>1 THEN PMORT(I)=1
400  IF FLG = 1 THEN PMORT(I) = 1 - PMORT(I)
410 NEXT I
420 S(1,1)=(X*(1-PMORT(3))*THS+(1-X)*UHS)*FCR
430 S(1,2)=((X+Y)/2*(1-PMORT(3))*THS+(1-(X+Y)/2)*UHS)*FCR*(1+FCA)/2
440 S(1,3)=(Y*(1-PMORT(3))*THS+(1-Y)*UHS)*FCR*FCA
450 S(2,1)=(X*(1-PMORT(2))*THS+(1-X)*UHS)*(1+FCR)/2
460 S(2,2)=((X+Y)/2*(1-PMORT(2))*THS+(1-(X+Y)/2)*UHS)*(1+FCR)*(1+FCA)/4
470 S(2,3)=(Y*(1-PMORT(2))*THS+(1-Y)*UHS)*(1+FCR)/2*FCA
```

```
480 S(3,1)=X*(1-PMORT(1))*THS+(1-X)*UHS
490 S(3,2)=((X+Y)/2*(1-PMORT(1))*THS+(1-(X+Y)/2)*UHS)*(1+FCA)/2
500 S(3,3)=(Y*(1-PMORT(1))*THS+(1-Y)*UHS)*FCA
510 FOR A= 0 TO 100
520 CLS
530 PRINT "Simulation is ";A;" percent done."
540 FOR R= 0 TO 100
550 GFR=R/100
560 GFA=A/100
570 FOR I=1 TO 3
580   FOR J=1 TO 3
590     IF I=1 THEN PR=GFR^2
600     IF I=2 THEN PR=2*GFR*(1-GFR)
610     IF I=3 THEN PR=(1-GFR)^2
620     IF J=1 THEN PA=GFA^2
630     IF J=2 THEN PA=2*GFA*(1-GFA)
640     IF J=3 THEN PA=(1-GFA)^2
650     P(I,J)=PR*PA
660   NEXT J
670 NEXT I
680 FOR I=1 TO 3
690  FOR J=1 TO 3
700   NS(I,J)=P(I,J)*S(I,J)
710   TOTAL=TOTAL+NS(I,J)
720  NEXT J
730 NEXT I
740 DIST(1)=GFR^2+GFA^2
750 GFR=(NS(1,1)+NS(1,2)+NS(1,3)+.5*(NS(2,1)+NS(2,2)+NS(2,3)))/TOTAL
760 GFA=(NS(1,1)+NS(2,1)+NS(3,1)+.5*(NS(1,2)+NS(2,2)+NS(3,2)))/TOTAL
770 DIST(2)=GFR^2+GFA^2
780 IF ABS(DIST(1)-DIST(2))<.00005 THEN GOTO 940
790 G(1)=(NS(3,3)+.5*(NS(3,2)+NS(2,3))+.25*NS(2,2))/TOTAL
800 G(2)=(NS(1,3)+.5*(NS(1,2)+NS(2,3))+.25*NS(2,2))/TOTAL
810 G(3)=(NS(3,1)+.5*(NS(2,1)+NS(3,2))+.25*NS(2,2))/TOTAL
820 G(4)=(NS(1,1)+.5*(NS(2,1)+NS(1,2))+.25*NS(2,2))/TOTAL
830 P(1,1)=G(4)^2
840 P(1,2)=G(2)*G(4)
850 P(1,3)=G(2)^2
860 P(2,1)=G(3)*G(4)
870 P(2,2)=G(1)*G(4)+G(2)*G(3)
880 P(2,3)=G(1)*G(2)
890 P(3,1)=G(3)^2
900 P(3,2)=G(1)*G(3)
910 P(3,3)=G(1)^2
920 TOTAL = 0
930 GOTO 680
940 CORNER(2) = INT(GFR+GFA*2+.5)
950 IF CORNER(1)<>CORNER(2) THEN GOTO 970
960 GOTO 1030
970 IF R= 0 THEN GOTO 990
980 PRINT #1, R/100;",";A/100
990 CORNER(1) = CORNER(2)
1000 IF CORNER(2)<>1 THEN GOTO 1030
1010 LET R=0
```

```
1020 GOTO 1040
1030 NEXT R
1040 NEXT A
1050 CLOSE
1060 INPUT "Would you like to run another simulation";I$
1070 IF I$ = "n" THEN END
1080 GOTO 50
```

APPENDIX 2

Voucher specimen depository forms

APPENDIX 2

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 1990-05

Title of thesis or dissertation (or other research projects):

Manipulating Oviposition of the Onion Fly, Delia antiqua (Meigen): A Stimulo-deterrent Diversionary Approach

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed)
Richard Steven Cowles

Date 5-20-90

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.
Copies: Included as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 2.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Delia antiqua (Meigen)	Lab culture, MICH: Newaygo Co. Grant, 1987 15 May 1990 R. S. Cowles					10	10		

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Richard Steven Cowles

Date 5-20-90

Voucher No. 1990-05

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Roland H. Fischer 16 April 1990

Curator Date