# Investigations of Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] pest management including : sampling strategies for insecticide resistance detection, development of a knowledge-based expert system and the physiology of cold tolerance.

Anne Marie Tisler

A dissertation submitted in partial fulfillment of the requirements of
Doctor of Philosophy in Entomology

Virginia Polytechnic Institute and State University
Blacksburg, Virginia

G.W. Zehnder, Chair

D.G. Cochran

C.D. Heatwole

D.E. Mullins

D.G. Pfeiffer

June 1991
Blacksburg, Virginia 24061

# **Investigations of Colorado Potato Beetle [*Leptinotarsa decemlineata* (Say)] Pest Management Including : Sampling Strategies for Insecticide Resistance Detection, Development of a Knowledge-Based Expert System and the Physiology of Cold Tolerance.**

Anne Marie Tisler

Committee Chair: Geoffrey W. Zehnder
Entomology

(ABSTRACT)

Within-field variation in mortality of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), in a filter-paper insecticide bioassay was determined in three Virginia potato fields in 1989 and five fields in 1990. Bioassays were performed for each of three different insecticides on Colorado potato beetle larvae collected from 12-16 different locations (equal sized blocks) per field. Comparisons of 95% mortality confidence intervals between all block combinations per field indicated that field size had no influence on variation in Colorado potato beetle mortality in the bioassay. No apparent relationship existed between the level of Colorado potato beetle insecticide susceptibility ($LC_{90}$ value) and percentage of mortality confidence interval overlap among field blocks. Bioassay data from the sample fields indicated that a single bioassay (of potato beetles from one location per field) will yield at least a 0.50 probability (> 0.90 in six of the eight fields) of estimating the mean mortality response (± 5%) of Colorado potato beetle in the entire field. The probability of sampling potato beetles from one location which differed in mean

mortality ($P \leq 0.05$) from individuals collected in another location within the same field decreased as the number of locations sampled increased (i.e., the probability is 0.12 if three locations are sampled).

Filter paper insecticide bioassays were performed on Colorado potato beetles collected from three commercial potato fields to determine the most cost-effective number of bioassay sample units (filter paper disks, 10 larvae per disk). Relative net precision values from three different insecticide bioassays were used as an indication of sample size efficiency, and were based on variation in larval mortality and sample cost. Greatest sample size efficiency in all insecticide bioassays was achieved from a sample of two to five filter paper disk sample units (20-50 total larvae). Additional insecticide bioassays were performed on Colorado potato beetle larvae collected at different times during the potato growing season (from a commercial potato field and from experiment station plots) to determine whether the larval generation sampled or previous insecticide application affected results of the bioassay. Although trends in mortality were not always consistent among first generation larvae sampled on different dates (from insecticide-treated and untreated plots), first generation larvae exhibited significantly ($P < 0.05$) greater mortality in the bioassay compared with second generation larvae. Based on these results, we recommend that bioassays to estimate the effectiveness of a particular insecticide against Colorado potato beetle be performed on the target generation immediately before the planned insecticide treatment.

PIES (potato insect expert system) is a knowledge-based system for Colorado potato beetle insecticide management in commercial potato fields on the Eastern Shore of Virginia. PIES was written using VP-Expert, a rule-based expert system shell, and uses Colorado potato beetle lifestage, potato growth stage, percent defoliation, and other factors to decide if an insecticide application is necessary to prevent tuber yield loss due to Colorado potato beetle pressure. Field tests at the Eastern Shore Agricultural Experiment Station compared PIES with conventional spray thresholds based on the number of Colorado potato beetles per stem. Tuber yields were not significantly different ($P \leq 0.05$) between the two methods, while PIES recommended, on average, 3.7 insecticide applications and conventional thresholds required six insecticide applications. An additional benefit of PIES is that scouting requirements are simpler and quicker than the conventional stem counts.

Supercooling points determined for Colorado potato beetle populations from Maine, Washington, and Prince Edward Island, Canada, suggest these populations are freeze-tolerant, that is, they can sustain ice formation within their body fluids. Colorado potato beetles collected from Virginia supercooled at a significantly ($P \leq 0.05$) lower temperature than the other Colorado potato beetle populations. The temperature at which Virginia Colorado potato beetle supercooled is indicative of freeze-sensitive species and may indicate divergence in the mechanisms Colorado potato beetle are using for cold tolerance.

Two polyhydric alcohols (polyols) were isolated in the hemolymph of Colorado potato beetle using high performance thin layer chromatography. The polyols were tentatively identified as inositol and xylitol.

## Acknowledgement

I would like to thank my major professor and committee chair, Dr. Geoffrey Zehnder for his financial support and editing efforts throughout my graduate studies; Dr. Donald Mullins for providing laboratory space and advising for the cold tolerance study; Dr. Conrad Heatwole and his Knowledged-Based Systems course for being the necessary catalyst for the PIES project; and Drs. Donald Cochran and Douglas Pfeiffer for serving on my committee and providing editorial comments for this manuscript.

I very much admire Dr. Joella Killian, Associate Professor of Biology at Mary Washington College. Her great enthusiasm for entomology, outstanding teaching abilities, and interest in sharing both, began me on my way to becoming an entomologist.

Without the help of Jack Speese, Cathy Stokes, Drew Savage and Scott Williams, all of the field work for this project would have never been completed in the allotted time. Their encouragement, antics and song-writing helped pass long, hot, humid days on the Eastern Shore . Additionally, I would like to thank all the faculty and staff at the Eastern Shore Agricultural Experiment Station for treating me as a professional entomologist, listening to my complaints and teaching me about production agriculture.

Nancy and Pete Davis provided a much needed home-away-from-home for me at Glebe Farm, and I appreciate them taking me under their wings. I couldn't have asked for kinder, more loving "country parents."

My Mom and Dad, as well as my brothers, Andrew (and his wife, Debbie), Tim, Mike and Matt (and Mr. Raisin), have always been just a phone call away when I needed encouragement, to complain about school, laugh or cry. It's great to be loved by such a wonderful bunch of people and I'm really lucky to have a fantastic family! I would also like to thank my friend Marie Blain for sharing Girl Scouting with me, and for believing in what I was doing, and my Grandma and Aunts Molly, Susan and Jean, for keeping my mailbox full despite the address changes. To these and the many, many other people who have shared my journey, I am grateful and hope I was able to give back some of what they gave to me.

Last, but certainly not least, I would like to thank my fiance, Bill Vencill, for showing me what a joy life can be. Words are not adequate to express what he means to me. I look forward to 20 July, 1991, when we will begin our life and family together.

This dissertation is dedicated to the memory of Vincent Tisler, who was my favorite uncle and became my friend.

# Table of Contents


Abstract ii

Acknowledgement v

Table of Contents vii

List of Tables ix

List of Figures xi

Introduction 1

- Historical Background 2
- Life History 4
- Diapause 4
- Overwintering and Cold Tolerance 6
- Colorado Potato Beetle Management 8
- Expert Systems 10
- Literature Cited 13

Within-Field Variation 22

- Materials and Methods 23
  - Sample Sites 23
  - Insecticide Bioassay 24
  - Data Analysis 26
- Results and Discussion 26
- Literature Cited 34

Sample Size and Temporal Variation 36

- Materials and Methods. 37
  - Insect Sample Size 37
  - Temporal Variation 40

Results and Discussion 41
Insect Sample Size 41
Temporal Variation 42
Literature Cited 52
PIES 55
Materials and Methods 57
Model Development 57
PIES Model 57
Model Evaluation 59
Results and Discussion 60
Model Implementation 60
Model Evaluation 61
References Cited 70
Appendix 1 74
Cold Tolerance Mechanisms 87
Materials and Methods 89
Samples 89
Supercooling Point 90
Polyol Analysis 91
Results and Discussion 93
Supercooling 93
Polyol Analysis 95
Literature Cited 101
Conclusions 105
Vita 110

# List of Tables

Chapter-Table page

2-1 Diagnostic dosages [$LC_{90}$ = (ml insecticide/50 ml acetone)] used in the Colorado potato beetle filter paper insecticide bioassay and percentage overlap among field blocks of 95% confidence intervals for mean mortality. 30

2-2 Confidence intervals (95%) for mean mortality (no. dead of 10 larvae per filter paper disk) of Colorado potato beetle larvae exposed to permethrin in a filter paper bioassay. 31

2-3 Confidence intervals (95%) for mean mortality (no. dead of 10 larvae per filter paper disk) of Colorado potato beetle larvae exposed to azinphosmethyl in a filter paper bioassay. 32

2-4 Confidence intervals (95%) for mean mortality (no. dead of 10 larvae per filter paper disk) of Colorado potato beetle larvae exposed to oxamyl in a filter paper bioassay. 33

3-1 Mean no. dead (y), standard error (SE), processing time (Cs), relative variation (Rv), and relative net precision values (RNP) for first instar Colorado potato beetle exposed to azinphosmethyl in a filter paper bioassay. 45

3-2 Mean no. dead (y), standard error (SE), processing time (Cs), relative variation (Rv), and relative net precision values (RNP) for first instar Colorado potato beetle exposed to oxamyl in a filter paper bioassay. 47

3-3 Mean no. dead (y), standard error (SE), processing time (Cs), relative variation (Rv), and relative net precision values (RNP) for first instar Colorado potato beetle exposed to permethrin in a filter paper bioassay. 48

3-4 Influence of Colorado potato beetle generation on larval mortality in filter paper insecticide bioassay. 50

3-5 Influence of previous insecticide spray and Colorado potato beetle generation on larval mortality in a filter paper bioassay, Eastern Shore Agricultural Experiment Station, Painter, Va. 51

4-1 Insecticide spray schedule for conventional and PIES plots in four fields at ESAES. 64

4-2 Mean number of Colorado potato beetle per potato stem in conventional and PIES plots at ESAES. 65

4-3 Comparison of mean tuber yield and number of insecticide applications for conventional and expert system plots at ESAES. 66

5-1 Mean whole body and hemolymph supercooling points (in $^{o}C$) for overwintered female and male Colorado potato beetles (Virginia). 97

5-2 Comparison of $R_f$-values of polyols and components of Colorado potato beetle hemolymph on silica gel HPTLC plates using three solvent systems and sulfuric acid charring for detection. 98

## List of Figures


4-1 Flow chart for the Colorado potato beetle insect expert system. 67

4-2 Mean per stem Colorado potato beetle counts (small larvae, large larvae, adults), threshold levels (small larvae, large larvae, adults), and insecticide applications for conventional treatments in fields at ESAES. 68

4-3 Mean percent defoliation per stem, insecticide applications, and threshold levels in PIES treatments in fields at ESAES. 69

5-1 Whole body and hemolymph supercooling points (in °C) for Colorado potato beetle collected from Painter, Va. 99

5-2 Whole body and hemolymph supercooling points (in °C) for four populations of Colorado potato beetle collected in February, 1991. 100

# Introduction

The Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] is the most important pest of potatoes (*Solanum tuberosum* L.) in the eastern United States. Current control measures for Colorado potato beetle rely almost exclusively on the application of insecticides. The development of insecticide resistant Colorado potato beetle populations, legislative restrictions and bans, and consumer concern about chemical inputs in the food supply, requires alternative strategies be evaluated for Colorado potato beetle control.

The status of alternative Colorado potato beetle control measures is unclear. Although some natural enemy species exist (Chittenden 1907, Tamaki 1981, Drummond et al. 1984, Ferro 1985) none present in Eastern Shore potato fields maintain Colorado potato beetle populations below damaging levels. The use of Colorado potato beetle active strains of *Bacillus thuringiensis* have been shown to be effective against small larvae (< 0.5 cm), however do not adequately control large larvae (> 0.5 cm) or adult stages (Ferro & Gelernter 1989, Zehnder & Gelernter 1989). Timing of insecticide applications (Zehnder 1986, Boiteau 1988), crop rotation (Lashomb & Ng 1984, Wright 1984), tillage practices (Zehnder & Linduska 1987) and planting date (Boiteau 1986) also have an influence Colorado potato beetle control. The incorporation of some or all of these tactics, and as of yet, unidentified tactics will comprise the Colorado potato beetle management programs of the future.

This research focuses on several aspects of Colorado potato beetle management, including insecticide resistance sampling strategies and the development of an expert system to computerize and standardize Colorado potato beetle insecticide recommendations. Additionally, I examined two aspects of Colorado potato beetle cold tolerance, supercooling point and polyhydric alcohol composition.

## Historical Background

The Colorado potato beetle has a colorful history and a reputation as one of the most notorious insect pests in the United States. As early naturalists explored the western regions of the U.S., they encountered the now infamous Colorado potato beetle. Thomas Nuttal was the first reported collector of Colorado potato beetles, when in 1811, he noticed them feeding on buffalo-bur, *Solanum rostratum* L. Colorado potato beetles were subsequently collected and described as *Doryphora decemlineata* by Thomas Say in 1824 (Jacques 1988).

Colorado potato beetles remained a virtually unknown and unimportant insect until 1859, when a grower near Omaha, Nebr. reported total defoliation of a potato field by the "ten-striped spearman" (Tower 1906, but see Jacques 1988). Colorado potato beetles became well adapted to potatoes and attained pest status rapidly, and spread eastward. Early outbreaks of Colorado potato beetles reported by Tower (1906) and Chittenden (1907), occurred along heavily travelled pioneer trails, near railroad right-of-ways, and in the direction of prevailing winds.

The common name, Colorado potato beetle, was not immediately equated with *Leptinotarsa decemlineata* Say. Early reports referenced Colorado potato beetles as: the "ten striped spearman", the "ten-lined potato beetle", the "potato bug" and the "new potato bug" (Pope & Madge 1984). The name Colorado was associated with this devastating insect, when B.D. Walsh (1865) noted several of his colleagues had spotted large numbers of potato beetles feeding on buffalo-bur in the new Colorado territory. Walsh assumed the potato beetle populations feeding on potatoes in Nebraska originated in the Colorado territory, and began to use the term, the "new Colorado potato bug". It was C.V. Riley however, who first used the term "Colorado potato beetle" in an 1863 article for the Prairie Farmer, and this common name has been recognized ever since.

Westward expansion of settlers and shipments of potatoes from west to east, spread Colorado potato beetles throughout the potato growing regions of the U.S. (Tower 1906). Colorado potato beetles are highly mobile insects. They are able to walk several hundred meters in search of a food source, and are capable of sustained, wind-assisted flight. Mass movements of Colorado potato beetles may be associated with the degradation or removal of host material, often a result of potato harvest (May & Ahmad 1983).

Colorado potato beetle was well established in the eastern U.S. by the 1870s, and was a major economic pest of potatoes on the Eastern Shore of Virginia by the 1890s (Gauthier et al. 1981). Eastward expansion of Colorado potato beetle populations was not limited to North America. Transatlantic shipments of infested seed potatoes spread Colorado potato beetle populations to the European continent where they reached pest status by the 1880s (Tower 1906). Although Colorado potato beetle populations originated on an non-agronomic species of Solanaceae, successful adaptation to cultivated potatoes produced an economic pest which spans the globe (Tower 1906, Jolivet 1991).

## Life History of Colorado Potato Beetle

As a member of the family Chrysomelidae, Colorado potato beetles are oligophagous foliage feeders (Chittenden 1907, Hsiao & Fraenkel 1968, Hsiao 1982). Larval and adult stages often feed and develop on the same food source. Colorado potato beetles are capable of feeding on many species of Solanaceous plants including: *Solanum tuberosum* L., *S. rostrum* Dunal., *S. dulcamara* L., *S. elaeagnifolium* Cav., *S. melongena, S. triquetrum, S. angustifolium, Physalis* sp., *Lycopersicon esculentum* Miller, and *Datura* sp. (Chittenden 1907, Jacques 1988).

Colorado potato beetles have two generations annually, although in warmer locations, a third partial generation may occur, and in more northern climates, a single generation (Chittenden 1907, Harcourt 1971). Adult beetles diapause 20 to 25 cm below the soil surface and remain underground until early spring, re-emerging about the time potato plants emerge from the ground. Oviposition begins three to four days after spring emergence and extends for about one month (Chittenden 1907, Harcourt 1971). Peak ovipositional period in Virginia occurs from mid-May to early June (personal observation.). Eggs hatch four to seven days after being laid; and larvae pass through four instars. Prior to pupation, fourth instars burrow into the ground and construct a pupal cell. Adult emergence occurs seven to twelve days after pupation (Chittenden 1907).

## Colorado Potato Beetle Diapause

Colorado potato beetles overwinter in a reproductive diapause, characterized by the arrest of oogenesis and adaptive behavioral changes (de Wilde et al. 1959). Photoperiod appears to be critical in the induction of diapause, and temperature, while important in life cycle development, appears to play a relatively minor role (de Wilde et al. 1959). de Kort (1990) suggests it is appropriate to classify diapause as an alternative developmental program in an insect's life cycle. Based on this definition, Colorado potato beetle adults are predisposed for non-diapause or diapause, depending on whether they develop under long (non-diapause) or short-day (diapause) conditions (de Kort 1990).

Colorado potato beetles which are predisposed to diapause differ physiologically from non-diapause adults in food utilization, flight muscle development and hemolymph protein composition (de Wilde & de Boer 1961, de Kort 1969, de Kort 1990). Food utilization is aimed at building up nutritional reserves in the fat body, instead of being used for reproduction, as in non-diapause Colorado potato beetle (de Kort 1990). Flight muscles in both non-diapause and diapause Colorado potato beetles are immature upon spring emergence, containing only small microfibrils and a few mitochondria. Continued muscle development occurs rapidly under long day conditions, and flight muscles degenerate rapidly after a few days of development under short day conditions, as they are not necessary for diapause (de Wilde & de Boer 1961, de Kort 1969). Colorado potato beetles developing under short-day photoperiods produce a complex of physiological mechanisms which may induce diapause, a gradual decrease of the titre of juvenile hormone, inactivation of the corpora allata, and changes in ecdysteroid concentrations (de Kort et al. 1987, de Kort 1990).

Colorado potato beetles are also capable of repeated and prolonged diapause. Repeated diapause occurs when post-diapause adults re-enter diapause in successive years. Prolonged diapause occurs when beetles are "dormant" for more than one year, and has been reported in Colorado potato beetle populations from New Mexico, Canada, Poland, Arkansas and Transcarpathia (Tower 1906, Biever & Chauvin 1990). Moisture, as well as temperature, appears to play a role in termination of diapause and may be the driving extrinsic factor in prolonged diapause (Biever & Chauvin 1990, de Kort 1990). Although the physiological mechanisms of prolonged dormancy have not been worked out, Biever & Chauvin (1990) suggest three possibilities exist: 1) beetles are in diapause before emergence and do not emerge, thus producing prolonged diapause; 2) beetles emerge after the winter, and not finding a suitable food source, re-enter diapause; and 3) beetles are in a post-diapause state and will emerge pending adequate moisture. Due to the origin of the species in Mexico (Tower 1906), where there is a yearly wet and dry cycle, and because moisture is a component in the termination of diapause, Biever and Chauvin (1990) hypothesize the last mechanism may account for the increased incidence of prolonged diapause they found in Colorado potato beetle populations collected near Yakima, Wash.

## Overwintering and Cold Tolerance

A number of insect orders (Coleoptera, Collembola, Dictyoptera, Diptera, Hymenoptera, and Lepidoptera) have mechanisms which allow exposure to subzero temperatures without extensive population mortality (Mansingh & Smallman 1972, Rains & Dimock 1978, Duman 1979, Block & Zettel 1980, Zachariassen 1980, Hamilton et al. 1985). Insects may be classified as freeze-sensitive or freeze-tolerant based on their supercooling strategy.

Freeze-sensitive insects are unable to tolerate freezing. These insects have developed a high capacity for supercooling and are able to remain unfrozen at temperatures of -20 to -30 $^{o}$C (Salt 1965, Danks 1978, Zachariassen 1985). Antifreeze agents are often present in the hemolymph of freeze-sensitive insects. Low-molecular-weight polyhydric alcohols (polyols) and sugars, high-molecular-weight peptides and proteins act as antifreeze agents (Duman 1982). Additionally, the selection of a thermally buffered overwintering site (in soil, logs, leaf litter, etc.) is important in the prevention of freezing.

Freeze-tolerant insects are able to sustain extracellular ice formation, and freeze around -5 $^{\circ}$C (Salt 1965, Danks 1978, Zacharissen 1985). These insects tend to have strong ice-nucleating agents present in the hemolymph during the period of the year when low temperatures are likely to be encountered (i.e. winter) and may lack ice-nucleating agents at other times of the year (i.e. summer) (Zachariassen 1985).

It is hypothesized that the physiological function of ice nucleating agents is to establish extracellular freezing before freezing is established by other nucleating agents in other insect organs (such as in cells and the gut). Extracellular ice-nucleating agents have high capacity for the initiation of ice formation at temperatures above that of other nucleating agents, especially those in the gut and cells (Zachariassen 1985).

Cold tolerance has been studied in several pest species of chrysomelid beetles, including: *Dibrotica virgifera virgifera* LeConte, *D. undecimpunctata howardi* Barber, *D. balteata* LeConte, and *Leptinotarsa decemlineata* (Say) (Mail & Salt 1933, Zachariassen 1980, Gustin & Wilde 1985, Elsey 1989). Knowledge of cold tolerance may be valuable for the identification of overwintering areas and the prediction of early season field infestations by these pests (Elsey 1989).

## Colorado Potato Beetle Management

An estimated $ 20-30 million are spent annually on pesticides used to control potato pests (arthropods, nematodes, pathogens and weeds) in the northeastern U.S. (Leach et al. 1986). Insecticides are the primary control used for Colorado potato beetles in this region and frequently, fields receive more than one insecticide application per season. Colorado potato beetle has become an increasing problem in potato production primarily because it has developed increasing levels of resistance to most classes of insecticides (Forgash 1981, Gauthier et al. 1981, Ferro 1985, Forgash 1985).

Insecticide resistance is widespread in Colorado potato beetle populations and was first reported in 1952 in Long Island, N.Y., when DDT control failure occurred (Post 1954). Subsequent reports of DDT control failures followed in Virginia, Minnesota and North Dakota (Forgash 1985) and resistance to dieldrin and other chlorinated hydrocarbons was first recognized in 1958 (Hoffmaster et al. 1967). By the mid-1970s, populations of Colorado potato beetles had developed resistance to all classes of registered insecticides (Ferro 1985). Reduced Colorado potato beetle sensitivity to insecticides has resulted in control failures and complete crop loss in some eastern U.S. potato-growing areas. In Virginia, the severity of Colorado potato beetle resistance to organophosphate, carbamate and pyrethroid insecticides varies greatly among local populations (Tisler & Zehnder 1990).

The ability of Colorado potato beetle to rapidly develop resistance to insecticides requires that steps be taken to prevent or delay selection and proliferation of resistant populations in potatoes. One approach to manage insecticide resistance involves the judicious selection of insecticides based on a knowledge of the resistance status of the target Colorado potato beetle population.

Resistance monitoring is essential for determination of resistance status in insecticide management programs (Roush & Miller 1986). Guidelines have been proposed for the development of sampling methods to detect insecticide resistance in insect populations, and include operational costs of sampling and bioassay, sampling objectives, and optimal dose required to produce statistically accurate results (Roush & Miller 1986, Sawicki & Denholm 1987, McCutchen et al. 1989, Halliday & Burnham 1990). Residue assay methods for detection of Colorado potato beetle resistance have been developed for both larval and adult life stages (Morrow & Grafius 1986, Heim et al. 1990, Roush et al. 1991).

Bioassays used as decision aids for insecticide selection or resistance monitoring must be used in conjunction with a statistically sound sampling strategy which is acceptable to end users (growers, pest management consultants, county extension agents, etc.) (French & Kennedy 1992). Factors which may influence bioassay results include within-field and temporal variation in insecticide susceptibility of a pest population, and insect-induced selection for resistance which may increase during the growing season.

## Expert Systems for Colorado Potato Beetle Management

In addition to statistically sound and economical sampling strategies for evaluation of Colorado potato beetle insecticide resistance, a number of other factors including: tillage practice (Zehnder & Linduska 1987), planting date (Boiteau 1986), crop rotation (Lashomb & Ng 1984, Wright 1984, Roush et al. 1990,) and insecticide timing and rotation (Zehnder 1986, Boiteau 1988) are components of an integrated management system for Colorado potato beetle. Incorporating sound pest management and agronomic practices into control schemes may delay or hinder development of Colorado potato beetle resistance to currently used insecticides. It is also likely future management strategies developed for Colorado potato beetle control will focus on biologically-based systems which will help to reduce dependence on insecticides.

The evolution of Colorado potato beetle management requires continual information exchange between researchers, growers and extension personnel. As state agricultural funding is reduced, money for travel and personnel disappear, and crop science technology continues to advance, the provision of up-to-date information for growers becomes increasingly difficult. The use of computer technology for data storage, retrieval and recommendations may provide information in a timely and cost effective manner.

Expert systems are computer programs designed to carry out a task usually performed by human experts (such as making insecticide recommendations based on current field conditions, weather predictions, etc.) (Parsaye & Chignell 1988). Knowledge-based, or expert systems, are more flexible alternatives to mathematical equations, which were used represent knowledge (Plant & Stone 1991), and have many potential uses in agricultural systems, including Colorado potato beetle management.

Expert system development began in the mid-1970s and the first "true" expert system, MYCIN, was a program designed to diagnose bacterial infections from human blood samples, and recommend a treatment based on available remedies and patient history (Shortliffe 1976). The success of MYCIN ushered in a new era of computer programming and information access.

A wide variety of expert systems have been developed for insect management in agricultural cropping systems (Roach et al. 1985, Lemmon 1986, Palmer 1986, Beck & Jones 1987, Roach et al. 1987, Batchelor et al. 1989, Heinemann et al. 1989). User input is an essential component of expert systems and most decisions are made based on user response to questions. Expert systems also contain an inference engine, the part of the system which performs reasoning functions, a knowledge base, and a user interface. Additionally, external programs, data bases and other computer support modules may be attached to the system and called up by subroutines within the expert system program (Parsaye & Chignell 1988).

The needs of the end user must be kept in mind when developing an expert system, and in many agricultural systems, the ultimate end user is the grower. It is imperative that the expert system be developed in a medium available to the user (e.g. a microcomputer), and that the user be able to easily respond to questions put forth by the system without assistance. Answers requiring typed responses, rather than selecting a choice from a list may be intimidating to persons not familiar with computer use.

Knowledge-based systems can play an important role in the future of agriculture. The incorporation of such systems into farm practices may provide the critical link between knowledge and decision making (Plant & Stone 1991).

## Literature Cited

Batchelor, W.D., R.W. McClendon, J.W. Jones & D.B. Adams. 1989. An expert simulation system for soybean insect pest management. Am. Soc. Agric. Eng. 32: 335-342.

Beck, H.B. & P. Jones. 1987. SOYBUG: an expert system for soybean insect pest management. Paper #89-4502. Am. Soc. Agric. Eng. St. Joseph, MI.

Biever, K.D. & R.L. Chauvin. 1990. Prolonged dormancy in a Pacific Northwest population of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera:Chrysomelidae). Can. Entomol. 122: 175-177.

Block, W. & J. Zettel. 1980. Cold hardiness of some alpine Collembola. Ecol. Entomol. 5: 1-9.

Boiteau, G. 1986. Effect of planting date and plant spacing on field colonization by Colorado potato beetle, *Leptinotarsa decemlineata* (Say), in New Brunswick. Environ. Entomol. 15: 311-315.

Boiteau, G. 1988. Timing of insecticide applications for control of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera:Chrysomelidae), on potatoes in New Brunswick. Can. Entomol. 587-591.

Chittenden, F.H. 1907. The Colorado potato beetle. U.S. Dept. Agric. Circ. 87: 1-15.

Danks, H.V. 1978. Modes of seasonal adaptation in the insects. Can. Entomol. 110: 1167-1205.

de Kort, C.A.D. 1969. Hormones and the structural and biochemical properties of the flight muscles in the Colorado potato beetle. Med. Landb. Hogesch. Wag. 69: 1-63.

de Kort, C.A.D., M. A. Kahn & A.B. Koopmanschap. 1987. Juvenile hormone and the control of adult diapause in the Colorado potato beetle *Leptinotarsa decemlineata*. Insect Biochem. 17: 985-988.

de Kort, C.A.D. 1990. Thirty-five years of diapause research with the Colorado potato beetle. Entomol. Exp. Appl. 56: 1-13.

de Wilde, J., C.S. Duintjer & L. Mook. 1959. Physiology of diapause in the adult Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] - photoperiod as a controlling factor. J. Insect Physiol. 3: 75-85.

de Wilde, J. & J.A. de Boer. 1961. Physiology of diapause in the adult Colorado potato beetle. II Diapause as a case of pseudo-allatectomy. J. Insect Physiol. 6: 152-161.

Drummond, F.A., R.L. James, R.A. Casagrande & H. Faubert. 1984. Development and survival of *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), a predator of the Colorado potato beetle (Coleoptera:Chrysomelidae). Environ. Entomol. 13: 1283-1286.

Duman, J.G. 1979. Thermal-hysteresis-factors in overwintering insects. J. Insect Physiol. 25: 805-810.

Duman, J.G. 1982. Insect antifreezes and ice-nucleating agents. Cryobiology 19: 613-627.

Elsey, K.D. 1989. Cold tolerance of adult spotted and banded cucumber beetles (Coleoptera:Chrysomelidae). Environ. Entomol. 18: 1112-1116.

Ferro, D.N. 1985. Pest status and control strategies of Colorado potato beetle. pp. 1-8. **In** D.N. Ferro and R.H. Voss (eds.) Proceedings of the Symposium on the Colorado Potato Beetle, XVII International Congress on Entomology. Mass. Agric. Exp. Stn. Res. Bull. #704.

Ferro, D.N. & W.D. Gelernter. 1989. Toxicity of a new strain of *Bacillus thuringiensis* to Colorado potato beetle (Coleoptera:Chrysomelidae). J. Econ. Entomol. 82: 750-755.

Forgash, A.J. 1981. Insecticide resistance of the Colorado potato beetle *Leptinotarsa decemlineata* (Say). pp. 34-46. **In** J.H. Lashomb & R. Casagrande (eds.) Advances in Potato Pest Management. Hutchinson Ross, Stroudsburg, Pa.

Forgash, A.J. 1985. Insecticide resistance in the Colorado potato beetle. pp. 33-53. **In** D.N. Ferro and R.H. Voss (eds.) Proceedings of the Symposium on the Colorado Potato Beetle, XVII International Congress on Entomology. Mass. Agric. Exp. Stn. Res. Bull. #704.

French, N.M. & G.G. Kennedy. 1992. Insecticide resistance monitoring techniques and novel decision aid for managing Colorado potato beetle (Coleoptera:Chrysomelidae) populations in North Carolina. J. Econ. Entomol. 85 (In Press).

Gauthier, N.L., R.N. Hoffmaster & M. Semel. 1981. History of Colorado potato beetle control. pp. 13-33. In J.H. Lashomb and R. Casagrande (eds.) Advances in Potato Pest Management. Hutchinson Ross, Strudsburg, Pa.

Gustin, R.D. & G.W. Wilde. 1985. Cold hardiness of western corn rootworm (Coleoptera:Chrysomelidae) eggs from northern and southern zones of the corn belt. J. Kans. Entomol. Soc. 57: 722-725.

Halliday, W.R. & K.P. Burnham. 1990. Choosing the optimal diagnostic dose for monitoring insecticide resistance. J. Econ. Entomol. 83: 1151-1159.

Hamilton, R.L., D.E. Mullins & D.M. Orcutt. 1985. Freezing-tolerance in the woodroach *Cryptocercus punctulatus* (Scudder). Experientia 41: 1535-1537.

Harcourt, D.G. 1971. Population dynamics of *Leptinotarsa decemlineata* (Say) in eastern Ontario. Can. Entomol. 103: 1049-1061.

Heim, D.C., G.G. Kennedy & J.W. VanDuyn. 1990. Survey of insecticide resistance among North Carolina Colorado potato beetle (Coleoptera:Chrysomelidae) populations. J. Econ. Entomol. 83: 1229-1235.

Heinemann, P.H. 1989. An expert system for apple orchard management. Paper #89-7038. Am. Soc. Agric. Eng. St. Joseph, MI.

Hoffmaster, R.N., R.L. Waterfield, & J.C. Boyd. 1967. Insecticides applied to the soil for control of eight species of insects on Irish potatoes in Virginia. J. Econ. Entomol. 60: 1311-1318.

Hsiao, T.H. & G. Fraenkel. 1968. Selection and specificity of the Colorado potato beetle for solanaceous and nonsolanaceous plants. Ann. Entomol. Soc. Am. 61: 493-503.

Hsiao, T.H. 1982. Geographic variation and host plant adaptation of the Colorado potato beetle. pp. 315-324.Proc. 5th Int. Symp. Insect-Plant Relationships, Pudoc, Wageningen.

Jacques, R.L. 1988. The Potato Beetles. E.J. Brill, NY.

Jolivet, P. 1991. Le doryphore menace l'Asie *Leptinotarsa decemlineata* (Say) 1824 (Col. Chrysomelidae). L'Entomologiste 47: 29-48.

Lashomb, J.H. & Y. Ng. 1984. Colonization by Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Coleoptera:Chrysomelidae) in rotated and nonrotated potato fields. Environ. Entomol. 1352-1356.

Leach, S.S., W.E. Fry, R.T. Jones, R. Loria, R.H. Storch, R.D. Sweet, J.P. Tette, G.B. White & R.J. White. 1986. Integrated systems for managing potatoes in the northeast. Maine Agric. Exp. Stn. Bull. 116.

Lemmon, H. 1986. Comax: an expert system for cotton crop management. Science 233: 29-33.

Mail, G.A. & R.W. Salt. 1933. Temperature as a possible limiting factor in the northern spread of the Colorado potato beetle. J. Econ. Entomol. 26: 1068-1075.

Mansingh, A. & B.N. Smallman. 1972. Variation in polyhydric alcohol in relation to diapause and cold-hardiness in the larvae of *Isia isabella*. J. Insect Physiol. 18: 1565-1571.

May, M.L. & S. Ahmad. 1983. Host location in the Colorado potato beetle: Searching mechanisms in relation to oligophagy. pp 173-199. **In** S. Ahmad (ed.) Herbivorous Insects. Academic Press, N.Y.

McCutchen, B.F., F.W. Plapp, Jr., S.J. Nemec, & C. Campanhola. 1989. Development of diagnostic monitoring techniques for larval pyrethroid resistance in *Heliothis* spp. (Lepidoptera: Noctuidae) in cotton. J. Econ. Entomol. 82: 1502-1507.

Morrow, M. & Grafius, E. 1986. CPB dip test measures insect resistance. Am. Veg. Grower. March: 36-38.

Palmer, R.G. 1986. How expert systems can improve crop production. Agric. Eng. 67: 28-29.

Parsaye, K. & M. Chignell. 1988. Expert Systems for Experts. Wiley & Sons, N.Y.

Plant, R.W. & N.D. Stone. 1991. Knowledge-Based Systems in Agriculture. McGraw-Hill, Inc., N.Y.

Pope, R.D. & R.B. Madge. 1984. The when and why of the Colorado potato beetle. Antennae, Bull. Entomol. Soc. London 8: 175-177.

Post, R.L. 1954. DDT-resistant Colorado potato beetle? Proc. North Central Branch, Entomol. Soc. Am. 9: 94-45.

Rains, T.D. & R.V. Dimock, Jr. 1978. Seasonal variation in cold hardiness of the beetle *Popilus disjunctus*. J. Insect Physiol. 24: 551-554.

Roach, J.W., Virkar, R.S., M.J. Weaver, & C.R. Drake. 1985. POMME: a computer-based consultation system for apple orchard management using Prolog. Expert Systems 2: 56-68.

Roach, J.W., Virkar, R.S., C.R. Drake & M.J. Weaver. 1987. An expert system for helping apple growers. Computers and Electronics in Agric. 2: 97-108.

Roush, R.T. & G.L. Miller. 1986. Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol. 79:293-298.

Roush, R.T., C.W. Hoy, D.N. Ferro, & W.M. Tingey. 1990. Insecticide resistance in the Colorado potato beetle (Coleoptera:Chrysomelidae): influence of crop rotation and insecticide use. J. Econ. Entomol. 83: 315-319.

Roush, R.T., P. Beckley, D.N. Ferro, C.W. Hoy, & W.M. Tingey. 1991. A simple and rapid bioassay method to assess pesticide resistance in the Colorado potato beetle. J. Econ. Entomol. 84 (in press).

Salt, R.W. 1965. Factors influencing nucleation in supercooled insects. Can. J. Zool. 44: 117-132.

Sawicki, R.M. & I. Denholm. 1987. Management of resistance to pesticides in cotton pests. Trop. Pest Manag. 33: 262-272.

Shortliffe, E.H. 1976. Computer-Based Medical Consultations: MYCIN. American Elsevier/North-Holland, N.Y.

Tamaki, G. 1981. Biological control of potato pests. pp 178-192. **In** J.H. Lashomb & R. Casagrande [eds.] Advances in Potato Pest Management. Hutchinson Ross, Stroudsburg, Pa.

Tisler, A.M. & G.W. Zehnder. 1990. Insecticide resistance in the Colorado potato beetle (Coleoptera:Chrysomelidae) on the Eastern Shore of Virginia. J. Econ. Entomol. 83: 666-671.

Tower, W.L. 1906. Evolution in chrysomelid beetles of the genus *Leptinotarsa*. Carnegie Institute, Washington, DC.

Walsh, B.D. 1865. The new potato-bug and its natural history. The Practical Entomol. 1: 1-4.

Wright, R.J. 1984. Evaluation of crop rotation for control of Colorado potato beetle (Coleoptera:Chrysomelidae) in commercial potato fields on Long Island. J. Econ. Entomol. 77: 1254-1259.

Zachariassen, K.E. 1980. The role of polyols and nucleating agents in cold-hardy beetles. J. Comp. Physiol. 140: 227-234.

Zachariassen, K.E. 1985. Physiology of cold tolerance in insects. Physiol. Rev. 65: 799-832.

Zehnder, G.W. 1986. Timing of insecticides for control of Colorado potato beetle (Coleoptera:Chrysomelidae) in eastern Virginia based on differential susceptibility of life stages. J. Econ. Entomol. 79: 851-856.

Zehnder, G.W. & J.J. Linduska. 1987. Influence of conservation tillage practices on populations of Colorado potato beetle (Coleoptera:Chrysomelidae) in rotated and non-rotated tomato fields. Environ. Entomol. 16: 135-139.

Zehnder, G.W. & W.D. Gelernter. 1989. Activity of the M-ONE formulation of a new strain of *Bacillus thuringiensis* against the Colorado potato beetle (Coleoptera:Chrysomelidae): relationship between susceptibility and insect life stage. J. Econ. Entomol. 82: 756-761.

## Within-Field Variation in Susceptibility of Colorado Potato Beetle (Coleoptera:Chrysomelidae) to Insecticides: Implications for Sampling Methods in an Insecticide Resistance Bioassay.

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the primary pest of potato, *Solanum tuberosum* L., in the eastern U.S. The application of frequent insecticide sprays during the growing season has resulted in the development of Colorado potato beetle resistance to most classes of insecticides (Gauthier et al. 1981, Ferro 1985, Forgash 1985). Increased tolerance of Colorado potato beetle to insecticides has resulted in control failures and complete crop loss in some eastern U.S. potato-growing areas. In Virginia, the level of Colorado potato beetle resistance to organophosphate, carbamate, and pyrethroid insecticides varies greatly among local populations (Tisler & Zehnder 1990).

The ability of the Colorado potato beetle to develop rapid resistance to insecticides requires that measures be taken to prevent or delay the selection of resistant populations in potatoes. An approach to manage insecticide resistance may involve the judicious selection of insecticides based on a knowledge of the level of susceptibility of the target potato beetle population. Resistance monitoring programs may be used to determine the resistance status of a pest population before appropriate management decisions are made (Roush & Miller 1986). Several factors must be considered in the development of sampling methods to detect insecticide resistance in insect populations. These include a knowledge of the operational costs involved with sampling and bioassay, and the optimal dose (diagnostic dose or concentration) required to produce statistically accurate results (Roush & Miller 1986, Sawicki & Denholm 1987, McCutchen et al. 1989, Halliday & Burnham 1990). A simple and effective filter paper bioassay for larvae has been developed for monitoring Colorado potato beetle resistance to different classes of insecticides (Heim et al. 1990). This bioassay has been implemented in North Carolina and, if successful, may gain commercial use in other states. However, the number of locations within a field that must be sampled to obtain bioassay results which represent the level of insecticide susceptibility of the entire Colorado potato beetle field population is not known.

Several factors, including insect migration patterns and insecticide use history, may result in sub-populations of insects within a field with varying levels of susceptibility to insecticides. Our study was done to determine whether Colorado potato beetle mortality data from the filter paper insecticide bioassay varied with field collection site, and whether within-field variation in insecticide susceptibility should be considered in the development of sampling methods for resistance monitoring programs.

## Materials and Methods

**Sample Sites.** Colorado potato beetle samples were collected in three potato fields in 1989 (AmesI, Belote and Kellam), and five potato fields in 1990 (AmesII, Custis, Drewer, Pungo and Titter). Fields were located in Accomack and Northampton Counties on the Eastern Shore of Virginia and ranged in size from 0.34 to 9.61 ha (Table 1) and were representative of potato fields on the Eastern

Shore. Field size has historical precedence in this region, with the maximum size usually representing the amount of land a farmer and mule could plow in one day. Therefore, the average potato field on the Eastern Shore may be smaller than in other potato-growing areas. A previous insecticide resistance survey demonstrated that potato beetle populations from the areas where the sample fields were located differed in susceptibility to carbamate, organophosphate, and pyrethroid insecticides (Tisler & Zehnder 1990).

Six of the fields (AmesII, Belote, Custis, Drewer, Pungo, Titter) were divided into 16 blocks of equal size, with each block representing 6.25% of the total field area. The AmesI and Kellam fields were divided into 12 blocks of equal size, with each block representing 8.33% of the total field area. Colorado potato beetle egg masses were collected from several locations within each field block from mid-May to mid-June, the period of peak oviposition by overwintered females. The eight fields received from zero to three insecticide applications before sampling. Records of specific insecticides used were not kept, however the eight fields encompassed a variety of potato production practices (and insecticide programs) typically used in Virginia.

**Insecticide Bioassay.** Field collected Colorado potato beetle egg masses (20 to 50 egg masses per field block) were taken to the laboratory where excess foliage was removed to prevent possible exposure of larvae to insecticide residue. Egg masses were placed in plastic boxes with screened lids (separate boxes for each field block) and stored in an insect rearing room at 27 °C, 65% RH and 16:8 (L:D) photoperiod until hatch. Neonates from several different egg masses per field block were transferred to fresh, untreated potato foliage and allowed to feed for 24 h before treatment.

A filter paper insecticide bioassay (Heim et al. 1990) was used to measure Colorado potato beetle susceptibility to various insecticides within each field block. Filter paper disks (#P5, Fisher Scientific, Pittsburgh, Pa.) were treated with 0.5 ml of a predetermined diagnostic concentration ($LC_{90}$) of azinphosmethyl (Guthion 2S, Mobay Corp., Kansas City, Mo.), oxamyl (Vydate L, Dupont Co., Wilmington, Del.) and permethrin (Ambush 2E, ICI Americas, Wilmington, Del.); insecticides commonly used by Virginia potato growers.

It was necessary to determine a diagnostic insecticide concentration for each field for use with the bioassay because of variation in mortality among Colorado potato beetle field populations. Preliminary experiments demonstrated that an $LC_{90}$ was the most effective diagnostic concentration because lower concentrations often resulted in insignificant mortality. The $LC_{90}$ was determined by probit analysis (SAS Institute 1985) of dosage mortality data from five concentrations of each insecticide (N=20 first instars per concentration, 10 larvae per filter paper disk). The lowest concentration produced no mortality and the highest dose > 90% mortality. Larvae used to determine diagnostic concentrations were collected from 12-16 locations throughout each field.

Laboratory methods were the same in the bioassay to determine the $LC_{90}$, and in the bioassay to determine within field variation in mortality response to the diagnostic concentration. Treated filter paper disks were placed in glass Petri dishes; ten first instars were transferred to each disk using a sable-hair brush. Dishes were covered with Parafilm (American Can Co., Neenah, WI) and placed in a dark environmental chamber at 27 °C and mortality was evaluated at 24 h. Larvae were considered dead if they did not respond to probes with the brush. Bioassays to determine within-field variation in mortality were replicated four times (40 total larvae) per block for each insecticide treatment.

**Data Analysis.** Mean mortality and standard error values and 95% confidence intervals were calculated for each field block and insecticide treatment (SAS Institute 1985). Mortality confidence interval overlap or non-overlap was determined for all possible block-pair comparisons within a field. Mortality of Colorado potato beetle larvae from different blocks within a field was not considered significantly different ($P \leq 0.05$) between blocks if overlap of mortality confidence intervals occurred.

## Results and Discussion

The data in Table 1 indicate that field size did not influence within-field variation in Colorado potato beetle mortality in the filter paper bioassay. If field size affected within-field variation in mortality, it may be expected that most variation would occur in larger fields (>4 ha) and least variation would occur in smaller fields. However, in our study, percentage overlap in mortality confidence intervals was often similar in both large and small fields (mortality confidence intervals for each insecticide and field block are given in Tables 2-4).

In tests with azinphosmethyl and permethrin, greater than 90% block overlap of confidence intervals occurred in six of the eight fields tested (Table 1). Therefore, the probability of estimating the mean mortality response of Colorado potato beetles in all field blocks by sampling from only one block (within 95%

confidence limits) was greater than 0.90. Results with oxamyl were not greatly different, in that five of the eight fields had greater than 90% block overlap in mortality confidence intervals.

We found no apparent relationship between the level of susceptibility of the Colorado potato beetle field population ($LC_{90}$ value) and the percentage overlap of mortality confidence intervals. This is shown in Table 1, where for each insecticide, fields with either low or high $LC_{90}$ values often yielded the same percentage overlap in mortality confidence intervals between blocks. This indicates that the level of insecticide susceptibility of a Colorado potato beetle field population does not affect within-field variability in bioassay results. Heim et al. (1990) reported that variation in insecticide susceptibility among subpopulations of Colorado potato beetles within a field was greatest when the level of insecticide-induced mortality was intermediate (e.g., genes for insecticide resistance are not fixed in the population). It is possible that some of our sample potato beetle populations possessed an intermediate level of resistance to one or more insecticides. The greater variation in bioassay percentage of overlapping 95% mortality confidence intervals among blocks within individual fields.

Mortality associated with intermediate resistance would result in larger confidence intervals, and a higher percentage overlap among block mortality confidence intervals. We do not believe that the high percentage confidence interval overlap in the present study was the result of intermediate levels of Colorado potato beetle insecticide resistance in our sample fields. One reason is that our sample potato beetle populations exhibited different levels of insecticide susceptibility, as indicated by the range in $LC_{90}$ values among fields for each insecticide (Table 1). In addition, the mortality confidence intervals were relatively narrow for bioassays representing some field blocks (Tables 2-4), indicating a homogeneous response.

This data indicate that in these sample fields, Colorado potato beetle mortality in the filter paper bioassay does not vary greatly among different locations. A single bioassay (of Colorado potato beetles from one location per field) yielded at least a 0.50 probability ( $>$ 0.90 in six of the eight fields) of estimating the mean mortality response (within 95% confidence limits) of Colorado potato beetles from all field locations. In the "worst case scenario", where approximately 50% of the field blocks yielded overlapping mortality confidence intervals, the probability of sampling Colorado potato beetles from one location which differs in mean mortality ($P \leq 0.05$) from Colorado potato beetles in another location within the same field was 0.50 (7.5/15, N=16 blocks; 15 possible block pair comparisons). The probability decreases as the number of locations sampled increases: two locations = 0.27, ([7.5/15][7/14]); three locations = 0.12, ([7.5/15][7/14][6.5/13]).

The sample size used in an insecticide resistance bioassay should be determined based on the purpose of the bioassay and the precision required (Halliday & Burnham 1990). A bioassay to detect rare resistant individuals in a pest population requires a greater number of samples (Roush & Miller 1986) than one in which a diagnostic concentration is used to prevent increased selection for resistance and future control failures. Based on the number of bioassay samples North Carolina potato growers and field scouts are willing to process, French & Kennedy (1992) recommend that the Colorado filter paper bioassay be implemented using one egg mass per treated filter paper disk as the sample unit (larval mortality is determined 24 h after hatch), with five sample units per field. A concurrent study done to determine the cost/precision ratios of various sample sizes in the potato beetle filter paper bioassay demonstrated that a two to five filter paper disk sample size (20-50 larvae) was most cost effective (see following chapter). The results of the present study are compatible with these recommendations, given that the mean larval mortality most often did not differ significantly ($P \leq 0.05$) between field blocks. However, because our data demonstrate that the probability of making an inaccurate estimate of the mortality response of the entire Colorado potato beetle field population decreases as the number of field locations sampled increases, we recommend that the egg mass or larval samples in the bioassay be collected from different sites within each field.

Table 1. Discriminating dosages [$LC_{90}$ = ml insecticide/50 ml acetone)] used in the Colorado potato beetle filter paper insecticide bioassay and percentage overlap among field blocks of 95% confidence intervals for mean mortality.

| | | Permethrin | | Azinphosmethyl | | Oxamyl | |
|---|---|---|---|---|---|---|---|
| Field | Size (ha) | $LC_{90}$ (95% CI) | % Overlap | $LC_{90}$ (95% CI) | % Overlap | $LC_{90}$ (95% CI) | % Overlap |
| Ames I | 3.6 | 0.62 (0.00-4.21) | 92.0 | 2.15 (0.17-4.13) | 100.0 | 1.35 (0.94-3.05) | 100.0 |
| Ames II | 4.9 | 1.44 (0.15-18.98) | 90.0 | 0.26 (0.13-0.78) | 68.3 | 0.64 (0.23-3.25) | 94.2 |
| Belote | 6.2 | 0.47 (0.32-1.05) | 94.0 | 1.93 (1.50-2.30) | 94.0 | 1.13 (0.86-1.87) | 94.0 |
| Custis | 7.1 | 1.34 (0.89-5.37) | 100.0 | 0.23 (0.19-0.30) | 50.8 | 1.50 | 53.3 |
| Drewer | 0.3 | 0.59 (0.20-1.96) | 83.1 | 1.91 (0.40-22.37) | 96.7 | 0.62 (0.48-0.94) | 60.8 |
| Kellam | 2.5 | 1.37 (0.00-3.69) | 94.0 | 1.07 (0.80-1.81) | 96.7 | 2.49 (1.47-22.03) | 94.0 |
| Pungo | 5.3 | 0.70 (0.56-1.28) | 100.0 | 0.26 (0.17-1.86) | 100.0 | 0.50 (0.07-1.04) | 90.0 |
| Titter | 9.6 | 1.11 (0.86-1.73) | 76.9 | 0.39 (0.12-2.01) | 98.3 | 0.52 (0.41-0.85) | 65.0 |

Table 2. Confidence intervals (95%) for mean mortality (no. dead of 10 larvae per filter paper disk) of Colorado potato beetle larvae exposed to permethrin in a filter paper bioassay.

| | Field Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Block | Ames I[a] | Ames II | Belote | Custis | Drewer | Kellam[a] | Pungo | Titter |
| 1 | 3.61-9.39 | 7.42-10.08 | 1.83-7.16 | 0.05-4.11 | 0.39-6.10 | 7.91-10.58 | 3.02-6.97 | 0.39-6.10 |
| 2 | 1.47-6.53 | 8.69-10.31 | 1.16-5.33 | -0.05-1.44 | 6.16-10.33 | 3.97-10.52 | 6.08-10.91 | 6.16-10.33 |
| 3 | 4.06-5.44 | 7.42-10.08 | 3.39-8.10 | 1.05-2.44 | 2.78-9.72 | 7.86-10.13 | 3.61-10.38 | 2.78-9.72 |
| 4 | 2.67-6.83 | 9.06-10.44 | 1.83-7.16 | -0.60-4.10 | 8.11-10.88 | 6.50-9.99 | 5.83-11.16 | 8.11-10.88 |
| 5 | 3.00-6.50 | 8.69-10.31 | 4.16-8.33 | -0.30-3.30 | 2.61-8.38 | 8.05-9.44 | 4.02-7.97 | 2.61-8.38 |
| 6 | 1.67-7.83 | 7.86-10.14 | 7.02-10.97 | -0.49-2.99 | 10.00-10.00 | 4.86-7.13 | 5.41-8.08 | -.-NT-.- |
| 7 | 1.69-3.31 | 7.02-10.97 | 5.11-7.88 | 0.16-4.32 | -0.10-9.60 | 6.69-10.30 | 4.86-7.13 | -0.10-9.60 |
| 8 | 1.47-6.53 | 7.86-10.10 | 2.94-11.55 | -0.60-4.10 | 10.00-10.00 | 8.00-8.00 | 0.89-10.60 | -.-NT-.- |
| 9 | 2.00-8.00 | 5.02-8.97 | 7.41-10.08 | -0.30-3.30 | 1.69-5.30 | 6.11-8.88 | 5.11-7.88 | 1.69-5.30 |
| 10 | 2.92-5.58 | 6.92-9.58 | 5.89-10.60 | -0.88-1.88 | 1.41-4.08 | -.-NT-.- | 1.33-10.66 | 1.41-4.08 |
| 11 | 2.25-10.25 | 9.05-10.44 | 1.91-10.08 | 0.69-2.30 | 5.41-8.08 | 8.05-9.44 | 0.11-7.38 | 5.41-8.08 |
| 12 | 4.86-7.14 | 10.00-10.00 | 2.75-10.74 | -0.30-3.30 | 5.89-10.60 | 5.02-8.97 | 4.47-9.52 | 5.89-10.60 |
| 13 | | 9.06-10.44 | 6.66-10.83 | 0.55-1.94 | 1.50-4.99 | | 2.66-6.83 | 1.50-4.99 |
| 14 | | 10.00-10.00 | 3.08-10.91 | -0.13-2.13 | 3.69-5.30 | | 6.38-9.61 | 3.69-5.30 |
| 15 | | 7.67-11.83 | 5.66-9.83 | 0.86-3.13 | -.-NT-.- | | 6.69-10.30 | -.-NA-.- |
| 16 | | 4.67-8.83 | 10.00-10.00 | 0.05-1.44 | -.-NT-.- | | 4.66-10.83 | -.-NA-.- |

[a]Ames 1 and Kellam fields were divided into 12 blocks.
NT = Not tested.

Table 3. Confidence intervals (95%) for mean mortality (no. dead of 10 larvae per filter paper disk) of Colorado potato beetle larvae exposed to azinphosmethyl in a filter paper bioassay.

| | Field Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Block | Ames I[a] | Ames II | Belote | Custis | Drewer | Kellam[a] | Pungo | Titter |
| 1 | 0.70-4.30 | 0.39-3.60 | 5.39-11.00 | 7.91-10.50 | 2.50-10.49 | 7.06-8.44 | -0.13-2.13 | 2.50-10.49 |
| 2 | -0.07-4.16 | 3.83-9.16 | 8.56-9.94 | 9.05-10.44 | 4.39-9.10 | -.-NA-.- | -0.61-5.10 | 4.39-9.10 |
| 3 | 0.86-3.13 | 0.50-3.99 | 8.11-10.89 | 8.11-10.89 | 2.80-11.69 | 6.02-9.97 | 4.66-8.83 | 2.80-11.69 |
| 4 | 0.69-2.30 | -0.11-6.24 | 0.67-4.83 | 9.06-10.44 | 7.17-11.38 | 7.38-10.61 | -0.38-5.38 | 6.11-11.38 |
| 5 | 0.08-4.91 | -0.71-3.49 | 7.92-10.58 | 10.00-10.00 | 4.39-10.10 | 5.91-8.58 | 2.16-6.33 | 4.39-10.10 |
| 6 | 1.11-3.89 | 6.08-10.91 | 3.61-10.38 | 9.06-10.44 | 6.07-9.91 | 2.25-10.24 | 3.78-10.72 | 4.61-9.88 |
| 7 | 0.17-4.33 | -0.16-2.97 | 2.33-11.66 | 8.70-10.31 | 3.38-6.61 | 4.86-7.14 | -1.19-5.19 | 3.38-6.61 |
| 8 | -0.04-2.08 | -0.16-2.97 | 3.16-10.38 | 8.70-10.31 | 4.08-11.94 | 5.41-8.08 | 3.55-4.94 | 2.05-11.94 |
| 9 | 0.92-3.58 | 4.89-10.60 | 6.50-9.99 | 0.29-3.97 | 6.69-10.30 | 5.38-8.68 | 1.19-7.30 | 6.69-10.30 |
| 10 | -0.04-2.83 | 7.91-10.58 | 7.86-10.14 | 0.91-3.58 | 4.39-10.10 | -.-NT-.- | 0.08-4.91 | 4.39-10.10 |
| 11 | 1.86-4.14 | -0.68-4.83 | 6.69-10.30 | 0.89-5.60 | 5.83-11.16 | 4.39-9.01 | -0.22-6.72 | 0.89-11.60 |
| 12 | -0.35-4.11 | -0.93-5.16 | 4.11-11.38 | 2.05-3.44 | 9.05-10.44 | 5.39-10.11 | 2.50-5.99 | 0.50-13.99 |
| 13 | | 2.53-11.96 | 7.86-10.14 | 0.001-3.49 | 6.69-8.30 | | 0.38-9.19 | 6.69-8.30 |
| 14 | | 5.69-9.30 | 7.00-10.49 | 1.69-3.31 | 5.66-9.83 | | 2.61-8.38 | 5.66-9.83 |
| 15 | | -0.04-2.08 | 7.91-10.58 | -1.32-3.13 | 4.47-9.52 | | 4.11-6.88 | 5.51-9.52 |
| 16 | | 1.00-1.00 | 5.19-11.80 | 2.25-12.24 | 9.05-10.44 | | 2.80-11.69 | 9.05-10.44 |

[a]Ames 1 and Kellam fields were divided into 12 blocks.
NT=Not tested.

Table 4. Confidence intervals (95%) for mean mortality (no. dead of 10 larvae per filter paper disk) of Colorado potato beetle larvae exposed to oxamyl in a filter paper bioassay.

| | Field Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Block | Ames I[a] | Ames II | Belote | Custis | Drewer | Kellam[a] | Pungo | Titter |
| 1 | 1.00-3.80 | -0.58-7.08 | 4.11-6.88 | 7.91-10.58 | -0.13-2.13 | -0.08-8.08 | 6.66-10.83 | -0.13-2.13 |
| 2 | 2.16-6.33 | 7.86-10.13 | 2.50-10.49 | 9.05-10.44 | 0.05-1.44 | 3.69-7.88 | 2.08-9.91 | 0.05-1.44 |
| 3 | -1.08-9.08 | -0.97-2.97 | 2.28-9.22 | 8.11-10.88 | 1.41-4.08 | 5.05-6.44 | 4.39-10.10 | 1.41-4.08 |
| 4 | 2.86-5.13 | -.-NT-.- | -0.13-2.13 | 9.05-10.44 | 2.28-9.22 | 4.16-10.33 | 2.69-6.30 | 2.28-9.22 |
| 5 | 1.86-4.13 | 0.89-5.60 | 6.08-10.91 | 9.05-10.44 | -0.58-2.08 | 0.75-8.74 | 2.91-5.58 | -0.58-2.08 |
| 6 | 0.91-3.58 | 1.41-8.58 | 3.00-8.98 | 10.00-10.00 | 2.66-8.83 | 4.56-5.94 | 2.91-11.08 | -0.33-8.83 |
| 7 | -0.10-4.60 | -1.88-9.88 | 6.16-10.32 | 8.69-10.30 | 1.00-1.00 | 2.66-6.83 | 2.66-8.83 | 0.05-1.44 |
| 8 | 1.69-3.30 | -0.08-2.58 | 2.97-9.52 | 10.00-10.00 | 0.89-5.60 | 2.23-6.99 | 6.05-7.44 | 0.89-5.60 |
| 9 | 2.05-3.44 | 3.41-6.08 | 4.11-11.38 | 0.02-3.97 | 10.00-10.00 | -0.83-5.33 | 0.19-6.80 | 10.00-10.00 |
| 10 | 1.00-6.99 | 2.02-5.97 | 3.66-9.83 | 0.91-3.58 | 0.55-1.94 | 1.80-6.38 | 2.69-4.30 | -0.80-5.80 |
| 11 | 2.39-7.10 | -0.83-3.33 | 2.39-7.11 | 0.89-5.60 | 0.55-1.94 | 2.89-8.60 | -0.10-4.69 | 0.55-1.94 |
| 12 | 3.11-8.38 | -4.44-9.44 | 2.61-8.39 | 2.05-3.44 | 10.00-10.00 | 1.32-6.52 | 1.66-5.83 | 10.00-10.00 |
| 13 | | 2.25-10.24 | 0.00-10.55 | 0.001-3.49 | 0.55-1.94 | | 5.11-7.88 | 0.55-1.94 |
| 14 | | 0.41-7.58 | 1.00-11.50 | 1.69-3.30 | -0.97-2.97 | | 6.75-6.75 | -0.97-2.97 |
| 15 | | -0.23-4.99 | 0.00-12.66 | 0.00-3.13 | 0.11-2.88 | | 4.39-10.10 | 0.11-2.88 |
| 16 | | -0.13-2.13 | 5.20-11.80 | 2.25-12.24 | 6.11-11.38 | | -1.88-8.88 | 6.11-11.38 |

[a]Ames 1 and Kellam fields were divided into 12 blocks.
NT=Not tested.

## Literature Cited

Ferro, D.N. 1985. Pest status and control strategies of Colorado potato beetle. pp. 1-8. **In** D.N. Ferro and R.H. Voss (eds.). Proceedings of the Symposium on the Colorado Potato Beetle, XVII International Congress of Entomology. Mass. Agric. Exp. Stn. Res. Bull. #704.

Forgash, A.J. 1985. Insecticide resistance in the Colorado potato beetle. pp. 33-53. **In** D.N. Ferro and R.H. Voss (eds.). Proceedings of the Symposium on the Colorado Potato Beetle, XVII International Congress of Entomology. Mass. Agric. Exp. Stn. Res. Bull. #704.

French, N.M. & G.G. Kennedy. 1992. Insecticide resistance monitoring techniques and novel decision aid for managing Colorado potato beetle (Coleoptera:Chrysomelidae) populations in North Carolina. J. Econ. Entomol. 85 (In Press).

Gauthier, N.L., R.N. Hoffmaster, & M. Semel. 1981. History of Colorado potato beetle control. pp. 13-33. **In** J.H. Lashomb and R. Casagrande (eds.) Advances in Potato Pest Management. Hutchinson Ross, PA.

Halliday, W.R. & K.P. Burnham. 1990. Choosing the optimal diagnostic dose for monitoring insecticide resistance. J. Econ. Entomol. 83: 1151-1159.

Heim, D.C., G.G. Kennedy, & J.W. VanDuyn. 1990. Survey of insecticide resistance among North Carolina Colorado potato beetle (Coleoptera:Chrysomelidae) populations. J. Econ. Entomol. 83: 1229-1235.

McCutchen, B.F., F.W. Plapp, Jr., S.J. Nemec, & C. Campanhola. 1989. Development of diagnostic monitoring techniques for larval pyrethroid resistance in *Heliothis* spp. (Lepidoptera: Noctuidae) in cotton. J. Econ. Entomol 82: 1502-1507.

Roush, R.T. & G.L. Miller. 1986. Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol. 79: 293-298.

SAS Institute. 1985. SAS User's Guide: Statistics. SAS Institute, Carey, N.C.

Sawicki, R.M. & I. Denholm. 1987. Management of resistance to pesticides in cotton pests. Trop. Pest Manag. 33: 262-272.

Tisler, A.M. & G.W. Zehnder. 1990. Insecticide resistance in the Colorado potato beetle (Coleoptera:Chrysomelidae) on the Eastern Shore of Virginia. J. Econ. Entomol. 83: 666-671.

# Sample Size and Temporal Variation Effects on Colorado Potato Beetle (Coleoptera: Chrysomelidae) Mortality in a Filter Paper Insecticide Bioassay

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), has become an increasing problem in potato production primarily because of increasing levels of resistance to most classes of insecticides (Gauthier et al. 1981, Ferro 1985, Forgash 1985). Although many studies have been done to develop alternative control strategies (Leach et al. 1986, Wright et al. 1987, Roush et al. 1990, Zehnder & Hough-Goldstein 1990, and others), insecticides are still the primary means of Colorado potato beetle control in most areas. Insecticide resistance may vary greatly among local populations of Colorado potato beetle (Heim et al. 1990, Tisler & Zehnder 1990). Therefore, it is important to have knowledge of the resistance status of the target Colorado potato beetle population before appropriate management decisions are made. Insecticide resistance monitoring programs may be used to detect resistance in a pest population, thereby avoiding further selection for resistance and insecticide control failures (Roush & Miller 1986, Croft 1990). Such programs must incorporate sampling methods which permit accurate estimation of the true resistance status of the pest population, but should not be cost prohibitive.

Recently, a resistance bioassay for Colorado potato beetle larvae was developed (Heim et al. 1990) which utilizes an insecticide-treated filter paper disk (inside a Petri dish) as the sample unit. Egg masses are placed on the treated paper disks (one egg mass per disk) and larval mortality is determined 24 h after hatch. Additional studies were done to relate bioassay results with insecticide efficacy in the field and to develop a binomial resistance classification scheme (French & Kennedy 1992). This Colorado potato beetle resistance bioassay has been implemented in North Carolina and may gain commercial use in other areas. However, it is not known whether factors such as variability in mortality among individual larvae, or the time of sampling (relative to the potato beetle generation present or previous insecticide sprays) may affect bioassay results. Our study was conducted to investigate the variation in Colorado potato beetle mortality and the costs associated with different sample sizes in the filter paper bioassay, and to identify a sample size which results in the least sample variability with an acceptable cost. In addition, we performed separate field studies to determine whether the Colorado potato beetle generation sampled, or previous insecticide application, affected results of the bioassay.

## Materials and Methods.

**Experiments to Determine Insect Sample Size.** Colorado potato beetle egg masses were collected from three potato fields (AmesI, Belote, Kellam) in 1989. Egg masses were collected from 12-16 locations (approximately 50 egg masses per location) per field during the period of peak oviposition of overwintered adults (mid-May to mid-June). Egg masses were returned to the laboratory where excess foliage around each egg mass was removed. A previous insecticide resistance survey demonstrated that potato beetle populations from these areas differed in susceptibility to carbamate, organophosphate, and pyrethroid insecticides (Tisler &

Zehnder 1990). Egg masses from all collection sites per field were pooled and held in an insect rearing room at 27 C and 16:8 (L:D) until hatch. Neonate larvae were placed on fresh, untreated potato foliage and allowed to feed for 24 h before treatment.

Colorado potato beetle larvae from each field were tested using a filter paper bioassay (Heim et al. 1990). It was necessary to determine a diagnostic insecticide concentration for each field for use with the bioassay because of variation in the potato beetle population response to insecticides. Three insecticides commonly used in Virginia for Colorado potato beetle control were tested: azinphosmethyl (Guthion 2S, Mobay Corp., Kansas City, Mo.), oxamyl (Vydate L, Dupont Co., Wilmington, Del.), and permethrin (Ambush 2E, ICI Americas, Wilmington, Del.). The diagnostic concentrations of formulated insecticide in acetone were determined by probit analysis (SAS Institute 1985) of dosage mortality data from tests with five concentrations per insecticides (N=20 larvae per concentration, 10 larvae per filter paper disk). The lowest concentration produced no mortality and the highest > 90% mortality. Preliminary experiments demonstrated that an $LC_{90}$ was the most effective diagnostic insecticide concentration for use in the bioassay because lower concentrations often resulted in insignificant mortality. Larvae used in the diagnostic concentration test were from egg masses collected from 12-16 locations per field.

The filter paper disks (50 per field per insecticide; #P5, 5.5 cm, Fisher Scientific, Pittsburgh, Pa.) were treated with 0.5 ml of the diagnostic insecticide concentration in acetone, allowed to dry, and placed in a 5.5 cm glass Petri dish. Ten first instar larvae were placed on each filter paper disk using a sable-hair brush.

Dishes were covered with Parafilm and placed in a dark environmental chamber at 27 °C and 65% RH. Mortality was evaluated at 24 h and larvae were considered dead if they did not respond to probes with the brush.

Three factors were considered when calculating the cost (Cs) of processing samples in the filter paper bioassay; average time required to apply insecticide (with pipet) to filter paper disks (Tf), time required to place larvae (10 first instars per disk) on filter paper disks (Tl), and time required to evaluate larval mortality (Tm). Tf, Tl, and Tm values were the average times per sample size calculated from procedures with 50 filter paper disks, replicated seven times. One individual performed all experiments for the cost analysis experiments.

Processing costs were calculated for various sample sizes using the formula Cs = Nf (Tf + Tl + Tm), where Nf = the number of filter paper disks treated. The time spent collecting egg masses was not included in the analysis because the time cost for egg mass sampling was approximately equivalent for the smallest and largest sample sizes (Nf). Relative variation (RV = [SE/x] 100) in larval mortality in the bioassay was calculated separately for each field population and insecticide treatment. Overall efficiency of processing each sample size was determined using a relative net precision formula (RNP = [1/(Cs)(Rv)] 100) (Pedigo et al. 1972).

Relative net precision (RNP) was chosen as a criteria for determining the optimal sample size because the calculated RNP value for each sample size reflects both the variability in mean larval mortality and the cost of processing the samples. RNP is a mathematical representation of sample cost-effectiveness because the number of samples which result in the lowest multiplication product between the relative variation (RV) and cost (Cs) will generate the highest RNP value (an inverse relationship usually exists between sample variation and cost).

**Experiments to Determine Temporal Variation in Bioassay Results.** 'Superior' potato seed pieces were planted on 3 April 1990 at the Eastern Shore Agricultural Experiment Station, Painter, Va. in 12-row plots (7.7 m long) with 0.91 m row spacing. Each plot was assigned to one of four insecticide treatments (azinphosmethyl, 0.42 kg [AI]/ha; oxamyl, 0.56 kg [AI]/ha; permethrin, 0.17 kg [AI]/ha; and an untreated control) and arranged in a randomized complete block design with three replications. Treatment plots were separated by at least 10 m of bare ground to prevent insecticide spray drift between plots. Insecticide-treated plots were sprayed twice during the season using a tractor-mounted sprayer (374 liters/ha spray volume, 7.0 kg/cm$^2$ pressure). Colorado potato beetle egg masses were collected three times from random locations in each treatment plot: 1) just before spray (eggs from overwintered females, first generation larvae; 2) 7 d after the first spray (eggs from overwintered females, first generation larvae); 3) 6 d after the second spray (eggs from first generation females, second generation larvae).

Before insecticide application, diagnostic dosages for each insecticide were determined (as described previously) in bioassays with first instars from pooled egg masses collected from several locations in each treatment block. Filter paper bioassays (described previously) with the appropriate diagnostic concentration were performed on 40 larvae per insecticide treatment block per sample date (N = 120 larvae per treatment and date).

To further investigate Colorado potato beetle generation effects on bioassay results, egg masses were collected on two dates from 16 different sites in a commercial potato field (AmesII) in 1990. After determining diagnostic concentrations for each insecticide on larvae pooled over all field sites, filter paper bioassays were performed on 40 first instars sampled per field site and collection date (N = 640 larvae) for each of the three insecticides. The first egg mass collection in the AmesII field was made during the period of peak oviposition by overwintered potato beetle females (mid-May) and the second collection was made during the period of oviposition by first-generation females (late June). The AmesII field was sprayed three times during the 1990 growing season with *Bacillus thuringiensis* var. *tenebrionis* (Foil OF, Ecogen Inc., Langhorne, Pa.) and not with synthetic insecticides. Mean mortality and standard error values were determined for each insecticide and collection date and a t-test or Ryan's Q-test analysis (SAS Institute 1985) was done to determine differences in mean Colorado potato beetle mortality between collection dates and larval generations.

## Results and Discussion

**Experiments to Determine Insect Sample Size.** Mean time per filter paper disk to conduct the filter paper bioassay was 2.29 ± 0.46 min. Placing larvae on the filter paper disks (Tl) was the most time-consuming step, and required an average of 1.41 ± 0.26 min per disk. Application of the diagnostic insecticide concentration to the filter paper (Td) required the least time of all steps, an average of 0.32 ± 0.06 min per disk. Mean time required to determine larval mortality (Tm) was 0.56 ± 0.14 min per disk.

Sample size did not greatly affect mean larval mortality in the filter paper bioassay. Mean mortality differences between the 2 and 50 filter paper disk sample sizes (Nf) (20-500 larvae) ranged between 0.8 to 14% for azinphosmethyl (Table 1, 3 fields), 19-22% for oxamyl (Table 2, 2 fields) and 0.6-14% for permethrin (Table 3, 3 fields) (percentages were calculated by dividing the difference in mean mortality values obtained for the 2 and 50 disk sample sizes by the 50 sample mortality mean, then multiplying by 100).

The level of mortality exhibited by Colorado potato beetle larvae to the diagnostic concentration varied among fields. This is shown in Table 1, where mean percentage mortality in the azinphosmethyl bioassay for all sample sizes averaged 35% in the Ames field, 99% in the Belote field, and 88% in the Kellam field. However, the level of mortality did not affect the relationship between sample size and the variation and cost parameters. In all insecticide bioassays, standard error (SE) and relative variation (RV) values generally decreased with increasing sample size (Nf) (Tables 1-3). Processing time (Cs) increased dramatically with increasing sample size. The greatest sampling efficiency, indicated by the highest relative net precision (RNP) values, was achieved with all insecticides using a 2-5 filter paper disk sample size (20-50 larvae).

**Experiments to Determine Temporal Variation in Bioassay Results.** Bioassays of Colorado potato beetle larvae from the commercial potato field (AmesII) resulted in differences in larval mortality between generations (Table 4). First generation larvae from eggs laid by overwintered adults were significantly ($P \leq 0.05$) more susceptible to all three insecticides in the bioassay than were second generation larvae from eggs laid by the summer generation of adults.

Trends in insecticide susceptibility were not as clearly defined in the experiment station study (Table 5). In this experiment, first generation larvae from insecticide-treated and untreated control plots were bioassayed before and after the first spray application. First generation larvae sampled after spray application in the azinphosmethyl and oxamyl-treated plots exhibited significantly ($P \leq 0.05$) lower mortality in bioassays with azinphosmethyl and oxamyl, respectively. Permethrin spray application did not affect first generation larval mortality in the permethrin bioassay. An unexpected result was that the second sample of first generation larvae from the untreated plots were significantly ($P \leq 0.05$) less susceptible to oxamyl and permethrin than were larvae from the first sample. Therefore, it is not known whether previous insecticide application was a factor in the differential insecticide susceptibility observed between collections of first generation larvae in the insecticide-treated plots. Although trends in mortality were not always consistent among first generation larvae, second generation larvae exhibited significantly lower mortality than first generation larvae in most bioassays on samples from insecticide-treated and control plots.

In summary, these results indicate that from 2-5 filter paper disks (20-50 larvae) would be the most cost-effective sample size for use with the Colorado potato beetle filter paper insecticide bioassay. This determination is based on the variation in mortality among individual larvae in the bioassay (which decreases with increasing sample size), and the cost of processing the samples (which increases with increasing sample size). Because a large decrease in the relative variation in mortality was often associated with an increase in sample size from 2 to 5 filter paper disks, we recommend that samples of 5 disks (50 larvae) be used in the bioassay. This is a practical sample size, given the low processing cost (11.45 min, Tables 1-3), and should be acceptable to growers and pest management practitioners.

The time when Colorado potato beetle samples are taken may also affect results of the bioassay. The data from the experiment station study (Table 5) did not offer strong evidence that bioassay mortality was affected by previous insecticide application. However, larval mortality in the bioassay was often significantly different between sample collection dates. In the AmesII field, where no synthetic insecticides were applied, first generation larvae exhibited from 1.4 to 4.8 times greater susceptibility in the bioassay compared with second generation larvae, depending on the insecticide tested (Table 4). Therefore, at least one bioassay should be performed per target Colorado potato beetle generation because initial results may not accurately represent the level of insecticide susceptibility of subsequent generations. If multiple insecticide applications per field are necessary, it may be prudent to conduct a bioassay following each spray to assess the level of insecticide susceptibility of the surviving Colorado potato beetle population.

Table 1. Mean no. dead (y), standard error (SE), processing time (Cs), relative variation (Rv), and relative net precision values (RNP) for first instar Colorado potato beetle exposed to azinphosmethyl in a filter paper bioassay.

| Field | Nf[a] | y | SE | Cs[b] | Rv | RNP |
|---|---|---|---|---|---|---|
| Ames | 2 | 3.00 | 1.41 | 4.82 | 47.0 | 0.44 |
| | 5 | 4.20 | 0.80 | 12.05 | 19.05 | 0.44 |
| | 10 | 3.10 | 0.72 | 24.10 | 23.2 | 0.18 |
| | 20 | 3.40 | 0.47 | 48.20 | 13.82 | 0.15 |
| | 30 | 3.50 | 0.34 | 72.30 | 9.71 | 0.13 |
| | 40 | 3.60 | 0.28 | 96.40 | 7.78 | 0.13 |
| | 50 | 3.54 | 0.25 | 120.50 | 7.06 | 0.12 |
| Belote | 2 | 10.00 | 0.00 | 4.82 | 0.00 | 0.00 |
| | 5 | 9.80 | 0.20 | 12.05 | 2.04 | 4.07 |
| | 10 | 9.80 | 0.13 | 24.10 | 1.33 | 3.12 |
| | 20 | 9.90 | 0.07 | 48.20 | 0.71 | 2.92 |
| | 30 | 9.87 | 0.06 | 72.30 | 0.61 | 2.27 |
| | 40 | 9.90 | 0.05 | 96.40 | 0.51 | 2.03 |
| | 50 | 9.92 | 0.04 | 120.50 | 0.40 | 2.07 |

[a]Nf = number of filter paper disks treated, 10 larvae per disk.

[b]Cs = time in minutes.

$LC_{90}$ values in ml insecticide/50 ml acetone (95% CI): Ames = 2.15 (0.94-3.05); Belote = 1.93 (1.50-2.30); Kellam = 1.07 (0.80-1.81).

Table 1, Continued

| Field | Nf[a] | y | SE | Cs[b] | Rv | RNP |
|---|---|---|---|---|---|---|
| Kellam | 2 | 9.50 | 0.71 | 4.82 | 7.47 | 2.78 |
| | 5 | 9.20 | 0.20 | 12.05 | 2.17 | 3.82 |
| | 10 | 8.70 | 0.40 | 24.10 | 4.60 | 0.90 |
| | 20 | 8.60 | 0.28 | 48.20 | 3.26 | 0.64 |
| | 30 | 8.37 | 0.25 | 72.30 | 2.99 | 0.46 |
| | 40 | 8.63 | 0.21 | 96.40 | 2.43 | 0.43 |
| | 50 | 8.62 | 0.20 | 120.50 | 2.32 | 0.36 |

[a]Nf = number of filter paper disks treated, 10 larvae per disk

[b]Cs = time in minutes

$LC_{90}$ values in ml insecticide/50 ml acetone (95% CI): Ames = 2.15 (0.94-3.05); Belote = 1.93 (1.50-2.30); Kellam = 1.07 (0.80-1.81).

Table 2. Mean no. dead (y), standard error (SE), processing time (Cs), relative variation (Rv), and relative net precision values (RNP) for first instar Colorado potato beetle exposed to oxamyl in a filter paper bioassay.

| Field | Nf[a] | y | SE | Cs[b] | Rv | RNP |
|---|---|---|---|---|---|---|
| Belote | 2 | 9.50 | 0.71 | 6.28 | 7.47 | 2.13 |
| | 5 | 8.80 | 0.58 | 15.70 | 6.59 | 0.97 |
| | 10 | 8.90 | 0.40 | 31.40 | 4.49 | 0.71 |
| | 20 | 8.70 | 0.26 | 62.80 | 2.99 | 0.53 |
| | 30 | 8.47 | 0.24 | 94.20 | 2.86 | 0.37 |
| | 40 | 8.38 | 0.25 | 125.60 | 2.98 | 0.34 |
| | 50 | 7.96 | 0.27 | 157.00 | 3.39 | 0.19 |
| Kellam | 2 | 6.00 | 2.83 | 6.28 | 47.17 | 2.12 |
| | 5 | 5.20 | 0.86 | 15.70 | 16.54 | 0.39 |
| | 10 | 6.00 | 0.52 | 31.40 | 8.67 | 0.37 |
| | 20 | 6.85 | 0.37 | 62.80 | 5.40 | 0.29 |
| | 30 | 7.30 | 0.30 | 94.20 | 4.11 | 0.26 |
| | 40 | 7.28 | 0.27 | 125.60 | 3.71 | 0.21 |
| | 50 | 7.36 | 0.22 | 157.00 | 2.99 | 0.21 |

[a]Nf = number of filter paper disks treated, 10 larvae per disk

[b]Cs = time in minutes

$LC_{90}$ values in ml insecticide/50 ml acetone (95% CI): Belote = 1.13 (0.86-1.87); Kellam = 2.49 (1.47-22.03).

Table 3. Mean no. dead (y), standard error (SE), processing time (Cs), relative variation (Rv), and relative net precision values (RNP) for first instar Colorado potato beetle exposed to permethrin in a filter paper bioassay.

| Field | Nf[a] | y | SE | Cs[b] | Rv | RNP |
|---|---|---|---|---|---|---|
| Ames | 2 | 8.50 | 0.71 | 4.64 | 8.35 | 2.59 |
| | 5 | 8.40 | 0.24 | 11.60 | 2.86 | 3.01 |
| | 10 | 8.30 | 0.21 | 23.20 | 2.53 | 1.70 |
| | 20 | 7.80 | 0.33 | 46.40 | 4.23 | 0.31 |
| | 30 | 8.00 | 0.27 | 69.60 | 3.38 | 0.43 |
| | 40 | 8.00 | 0.22 | 92.80 | 2.75 | 0.39 |
| | 50 | 7.92 | 0.21 | 116.00 | 2.65 | 0.33 |
| Belote | 2 | 9.00 | 1.41 | 4.64 | 15.67 | 1.38 |
| | 5 | 8.20 | 0.49 | 11.60 | 5.98 | 1.44 |
| | 10 | 7.80 | 0.33 | 23.20 | 4.23 | 1.02 |
| | 20 | 7.35 | 0.22 | 46.40 | 2.99 | 0.72 |
| | 30 | 7.47 | 0.26 | 69.60 | 3.48 | 0.62 |
| | 40 | 7.95 | 0.25 | 92.80 | 3.14 | 0.34 |
| | 50 | 8.16 | 0.21 | 116.00 | 2.57 | 0.34 |

[a]Nf = number of filter paper disks treated, 10 larvae per disk

[b]Cs = time in minutes

$LC_{90}$ values in ml insecticide/50 ml acetone (95% CI): Ames = 0.61 (0.00-4.21); Belote = 0.47 (0.32-1.05); Kellam = 1.37 (0.00-3.69).

Table 3, Continued

| Field | Nf[a] | y | SE | Cs[b] | Rv | RNP |
|---|---|---|---|---|---|---|
| Kellam | 2 | 9.00 | 0.00 | 4.64 | 0.00 | 0.00 |
| | 5 | 9.00 | 0.32 | 11.60 | 3.56 | 2.42 |
| | 10 | 9.20 | 0.25 | 28.20 | 2.72 | 1.58 |
| | 20 | 9.00 | 0.24 | 46.40 | 2.67 | 0.81 |
| | 30 | 8.90 | 0.20 | 69.60 | 2.25 | 0.64 |
| | 40 | 9.15 | 0.22 | 92.80 | 2.40 | 0.45 |
| | 50 | 8.94 | 0.24 | 116.00 | 2.68 | 0.32 |

[a]Nf = number of filter paper disks treated, 10 larvae per disk

[b]Cs = time in minutes

$LC_{90}$ values in ml insecticide/50 ml acetone (95% CI): Ames = 0.61 (0.00-4.21); Belote = 0.47 (0.32-1.05); Kellam = 1.37 (0.00-3.69).

Table 4. Influence of Colorado potato beetle generation on larval mortality in filter paper insecticide bioassay.

| Insecticide | $LC_{90}$[a] (95% CI) | Larval generation | Mean no. dead ± SEM[b] |
|---|---|---|---|
| Azinphosmethyl | 0.26 | 1 | 9.03 ± 0.15 a |
| | (0.13-0.78) | 2 | 1.89 ± 0.28 b |
| Oxamyl | 0.64 | 1 | 3.93 ± 0.43 a |
| | (0.23-3.25) | 2 | 2.80 ± 0.33 b |
| Permethrin | 1.44 | 1 | 3.46 ± 0.39 a |
| | (0.15-18.98) | 2 | 1.57 ± 0.28 b |

[a] Diagnostic insecticide concentration (ml insecticide/50 ml acetone.

[b]Means followed by different letters are significantly different ($P \leq 0.05$); t-test analysis.

Table 5. Influence of previous insecticide spray and Colorado potato beetle generation on larval mortality in a filter paper bioassay, Eastern Shore Agricultural Experiment Station, Painter, Va.

| Insecticide treatment | No. insecticide sprays | Larval generation sampled | Mean no. dead larvae/filter paper disk[a] | | |
|---|---|---|---|---|---|
| | | | AM[b] | O | P |
| AM | 0 | 1 | 6.91 ± 1.12 a | 3.33 ± 0.78 a | 2.58 ± 0.63 a |
| | 1 | 1 | 4.66 ± 0.99 b | 3.58 ± 1.15 a | 3.50 ± 0.77 a |
| | 2 | 2 | 0.75 ± 0.28 c | 1.50 ± 0.52 b | 5.08 ± 1.08 b |
| O | 0 | 1 | 9.50 ± 0.19 a | 8.92 ± 0.50 a | 6.91 ± 0.52 a |
| | 1 | 1 | 2.75 ± 0.77 b | 2.50 ± 1.02 b | 4.66 ± 0.77 b |
| | 2 | 2 | 0.66 ± 0.18 c | 2.25 ± 0.98 b | 7.50 ± 0.83 a |
| P | 0 | 1 | 6.83 ± 1.44 a | 4.50 ± 0.88 a | 5.50 ± 1.10 a |
| | 1 | 1 | 7.41 ± 0.78 a | 5.16 ± 1.12 a | 5.08 ± 0.96 a |
| | 2 | 2 | 1.33 ± 0.51 b | 0.91 ± 0.19 b | 5.67 ± 0.96 a |
| C | 0 | 1 | 7.08 ± 1.19 a | 5.91 ± 1.11 a | 4.67 ± 0.76 a |
| | 0 | 1 | 7.83 ± 0.77 a | 1.08 ± 0.36 b | 2.17 ± 0.46 b |
| | 0 | 2 | 1.66 ± 0.58 b | 2.16 ± 0.72 b | 6.50 ± 1.02a |

[a]N=10 larvae/filter paper disk. Means within the same column by treatment are not significantly different ($p < 0.05$), Ryan's Q-test.

[b]AM=Azinphosmethyl [LC90=0.12, 95% CI (0.06-0.31)]; O=Oxamyl [LC90=1.90, 95% CI (0.28-36.16)];P=Permethrin [LC90=1.27, 95% CI (0.52-6.80); C=Control, no insecticides sprayed.

## Literature Cited

Croft, B.A. 1990. Developing a philosophy and program of pesticide resistance management. pp. 277-296. **In** R.T. Roush & B.E. Tabashnik (eds.), Pesticide resistance in arthropods. Chapman & Hall, New York.

Ferro, D.N. 1985. Pest status and control strategies of Colorado potato beetle. pp. 1-8. **In** D.N. Ferro and R.H. Voss (eds.) Proceedings of the Symposium on the Colorado Potato Beetle, XVII International Congress on Entomology. Mass. Agric. Exp. Stn. Res. Bull. #704.

Forgash, A.J. 1985. Insecticide resistance in the Colorado potato beetle. pp. 33-53. **In** D.N. Ferro and R.H. Voss (eds.) Proceedings of the Symposium on the Colorado Potato Beetle, XVII International Congress on Entomology. Mass. Agric. Exp. Stn. Res. Bull. #704.

French, N.M. & G.G. Kennedy. 1992. Insecticide resistance monitoring technique and novel decision aid for managing Colorado potato beetle (Coleoptera:Chrysomelidae) populations in North Carolina. J. Econ. Entomol. (In Press).

Gauthier, R.L., R.N. Hoffmaster, & M. Semel. 1981. History of Colorado potato beetle control. pp. 13-33. **In** J.H. Lashomb and R. Casagrande (eds.) Advances in Potato Pest Management. Hutchenson Ross, PA.

Heim, D.C., G.G. Kennedy, & J.W. VanDuyn. 1990. Survey of insecticide resistance among North Carolina Colorado potato beetle (Coleoptera:Chrysomelidae) populations. J. Econ. Entomol. 83: 1229-1235.

Leach, S.S., W.E. Fry, R.T. Jones, R. Loria, R.H. Storch, R.D. Sweet, J.P. Tette, G.B. White, & R.J. White. 1986. Integrated systems for managing potatoes in the northeast. Maine Agric. Exp. Stn. Bull. 116.

Pedigo, L.P., G.L. Lentz, J.D. Stone, & D.F. Cox. 1972. Green cloverworm populations in Iowa soybean with special reference to sampling procedure. J. Econ. Entomol. 65: 414-421.

Roush, R.T. & G.L. Miller 1986. Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol. 79:293-298.

Roush, R.T., C.W. Hoy, D.N. Ferro, & W.M. Tingey. 1990. Insecticide resistance in the Colorado potato beetle (Coleoptera:Chrysomelidae): influence of crop rotation and insecticide use. J. Econ. Entomol. 83: 315-319.

SAS Institute. 1985. Sas User's Guide: Statistics. SAS Institute, Cary, NC.

Sawicki, R.M. & I. Denholm. 1987. Management of resistance to pesticides in cotton pests. Tropical Pest Management 33: 262-272.

Tisler, A.M. & G.W. Zehnder. 1990. Insecticide resistance in the Colorado potato beetle (Coleoptera:Chrysomelidae) on the Eastern Shore of Virginia. J. Econ. Entomol. 83: 666-671.

Wright, R.J., D.P. Kain, & D. Moyer. 1987. Development and implementation of an extension IPM program for Long Island. Bull. Entomol. Soc. Am. 33: 239-245.

Zehnder, G.W. & J.J. Linduska. 1990. Colorado potato beetle (Coleoptera:Chrysomelidae) population development and effects on yield of potato with and without straw mulch. J. Econ. Entomol. 83: 1982-1987.

# PIES: A Computerized Approach to Insecticide Management for Colorado Potato Beetle [*Leptinotarsa decemlineata* (Say)]

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the primary insect pest of potato, *Solanum tuberosum* L., in the eastern U.S. Frequent applications of insecticides throughout the growing season has resulted in the development of Colorado potato beetle resistance to most classes of insecticides (Gauthier et al. 1981, Ferro 1985, Forgash 1985), In some eastern U.S. potato growing regions, insecticide failures and total crop loss have been associated with Colorado potato beetle insecticide resistance.

Management programs, designed to reduce selection pressure for insecticide resistance are being developed. Treatment thresholds, based on the number of Colorado potato beetles per potato stem, have been an established as part of an integrated management program for potato growers in Long Island, N.Y. (Halseth et al. 1987, Wright et al. 1987) The effect of defoliation levels on potato growth stage and yield have been examined for possible incorporation into Colorado potato beetle decision making programs (Cranshaw & Radcliffe 1980, Hare 1980, Logan & Casagrande 1980, Wellik et al. 1981, Ferro et al. 1983, Shields & Wyman 1984, Zehnder & Evanylo 1988, Dripps & Smilowitz 1989, Zehnder & Evanylo 1989). Also important to Colorado potato beetle management is a basic understanding of Colorado potato beetle biology, crop morphology, and pesticides.

The use of computer technology and specifically expert systems, as decision making tools for Colorado potato beetle management may enable researchers, growers, and other agricultural professionals to obtain and disseminate information on Colorado potato beetle management in a timely and cost effective manner.

A wide variety of expert systems have been developed for insect management in agricultural cropping systems (Roach et al. 1985, Lemmon 1986, Palmer 1986, Beck & Jones 1987, Roach et al. 1987, Batchelor et al. 1989, Heinemann et al. 1989). Expert systems are computer programs designed to carry out tasks usually performed by human experts (such as making insecticide recommendations based on current field conditions, weather predictions, etc.) and commonly use chains of rules to move from the user's input to a conclusion. Expert systems store rule sequences in memory, which can be accessed to provide the user with information on the logic used by the system to reach a particular decision (Parsay & Chignell 1988, Plant & Stone 1991). Rule-based systems have several advantages over other information providing computer programs, including, the acceptance and manipulation of non-numeric information, the use of incomplete or uncertain information in the decision making process, and the use of heuristic knowledge ("rule-of-thumb") (Plant & Stone).

PIES (potato insect expert system) was developed to incorporate current expertise on Colorado potato beetle management into a computerized form which growers could access for Colorado potato beetle control information. Data for the PIES knowledge base was obtained from the Eastern Shore Agricultural Experiment Station (ESAES), Painter, Va. and the system was field tested during the 1990 growing season.

## Materials and Methods

**Model Development.** Proper application and timing of insecticides are crucial aspects of Colorado potato beetle control in potatoes. While extension publications, such as Virginia's Pest Management Guide (Va. Coop. Ext. Serv. 1990), provide information on currently labeled insecticides for Colorado potato beetle control, they often lack decision-making criteria for insecticide application. Consequently, growers frequently rely on intuition, past experience, or a neighbor's advice when making treatment decisions for Colorado potato beetle, instead of sound pest management practices. PIES was developed to provide consistent recommendations for Colorado potato beetle control in Va., and target PIES users include, Va. potato growers, extension personnel, scouts, or other persons who may be required to make insecticide application decisions for Colorado potato beetle control.

The PIES program was created using VP-Expert, an expert system shell which runs on a PC compatible microcomputer. VP-Expert is a rule-based expert system, storing knowledge in IF-THEN rules and backward chaining inferencing to evaluate the rules and user input to draw specific recommendations (Paperback Software International 1987).

**PIES Model.** PIES is divided into two major decision pathways (Figure 1), a *Bt* path and an insecticide path, based on the Colorado potato beetle lifestage present in the field when the program is executed. Data collected on the Eastern Shore of Va. has demonstrated that one specific Colorado potato beetle lifestage predominates throughout a field, and that treatment decisions may be made based on that lifestage (Zehnder 1986).

If small larvae (< 0.5 cm or 0.25 in) are present, the *Bt* pathway is used. Timing of *Bacillus thuringiensis* (*Bt*) applications is crucial if Colorado potato beetle control is to be achieved (Zehnder & Gelernter 1989). Therefore, percent egg hatch is used as a second criteria to evaluate conditions within the field, before a final recommendation is made. Zehnder (pers. comm.) indicated optimal timing for *Bt* occurred when peak egg hatch (> 30% of the egg masses present in the field hatched) occurred. PIES uses 30% egg hatch as a pivotal treatment threshold. If the user indicates peak hatch has occurred, a *Bt* spray recommendation is made. However, if < 30% egg hatch has occurred, the user is provided with information on how to monitor for peak egg hatch and no application recommendation is made.

When large larvae (> 0.5 cm or 0.25 in) or adults are present, the insecticide pathway is used and results in the recommendation of a synthetic insecticide for Colorado potato beetle control, if appropriate. Potato growth stage and percent defoliation are important components of the insecticide pathway. Potato growth has been divided into three stages, emergence to pre-bloom, pre-bloom to post-bloom, and post-bloom to harvest, based on research by Zehnder & Evanylo (1988, 1989). Defoliation thresholds represent a conservative estimate of the maximum amount of Colorado potato beetle damage potatoes can sustain at a particular growth stage with no significant ($P \leq 0.05$) yield loss (Zehnder & Evanylo 1989, Zehnder pers. comm.). Additionally, previous insecticide applications, history of insecticide control failures, and expected high temperature also aid in the final spray recommendation. When field conditions fail to meet criteria for an insecticide spray recommendation, the system provides the user with information on sampling for continued monitoring of field conditions.

Early versions of PIES were updated as more complete data was available from field research. A number of parameters were revised including a reduction in number of potato growth stages from five to three, reassignment of Colorado potato beetle lifestage designations, the addition of a separate *Bt* recommendation and the inclusion of insecticide resistance sampling procedures. Program validation consisted of comparing the expert system's recommendations with those of the experts under hypothetical situations.

**Model Evaluation.** Defoliation thresholds for PIES were evaluated during the 1990 potato growing season at the Eastern Shore Agricultural Experiment Station (ESAES), Painter, Va. Seed pieces were planted on 03 April 1990 in two 24-row plots (15.2 m long) with 0.91 row spacing in four locations at ESAES. Each plot was assigned to one of two management strategies: conventional, based on Cornell recommendations (Halseth et al. 1987); or expert system, based on PIES recommendations. Additionally, two of the fields (1 & 2) were rotated fields, having been planted in small grains the previous year, and two of the fields (3 & 4) were non-rotated, having been planted in potatoes.

Weekly stem counts were made during the potato season in both plots. Counts were made by walking a V-shaped pattern through each plot, thoroughly inspecting 20 stems, and recording all Colorado potato beetles found (Zehnder et al. 1990). Additionally, a visual estimate of defoliation was made in the expert system plots by walking a V-shaped pattern through each plot and assigning 20 stems a rating of 0 to 13, with 0 = no defoliation and 13 = 100 % defoliation, based on a weighted scale for defoliation estimation (Little & Hills 1978).

Commercially available insecticides were applied to each plot at the manufacturer's recommended rate using a tractor-mounted sprayer (449 liter/ha, 7.0 kg/cm$^2$ pressure) when called for by plot management strategy (Table 1). Conventional plots were sprayed when Colorado potato beetle counts exceeded 4.0 small larvae (< 0.5 cm), 1.5 large larvae (> 0.5 cm) or 0.5 adults per stem (Halseth et al. 1987, Wright et al. 1987). Colorado potato beetle life stage present, plant growth stage, defoliation level and expected daily high temperature were used as treatment criterion for the expert system plots. Potato tubers were dug 19 July 1990 from six rows per plot, graded and weighed.

Means and standard errors were calculated for Colorado potato beetle counts and compared using the general linear model and Ryan's Q-Test (SAS User's Guide 1985). Means and standard errors for Colorado potato beetle defoliation were also calculated and compared using the general linear model and Ryan's Q-Test (SAS User's Guide 1985). Defoliation ratings were transformed to percent defoliation after analysis. Additionally, means and standard errors for tuber yields were calculated and compared using the general linear model and Ryan's Q-Test (SAS User's Guide 1985).

## Results and Discussion

**Model Implementation.** The memory and storage requirements of PIES (512 K and 350 K, respectively) are easily handled by most PC compatible computers. The program will fit on a double density diskette, run on a color or black and white monitor and does not require additional hardware or software.

PIES is well suited for on-farm use, either in an office setting or taken to the field on a portable computer.

As a qualitative evaluation of the PIES implementation, a number of non-experts were asked to run the program. Persons who tested the program (summer help, entomology students, etc.), indicated it was easy to use. Users who have little computer experience may require initial training on using arrow keys to select question responses and on overall program operation. Running the program takes 1 to 3 min per field.

PIES required less intensive field scouting in order to reach a treatment conclusion than did the conventional treatment. Threshold counts for the conventional management approach required thorough search of the potato stem, and a careful count of all Colorado potato beetle lifestages present on that particular stem, and was repeated 20 times per plot per sample time. Defoliation thresholds require only a visual examination of a stem to determine the amount of damage incurred by Colorado potato beetle, which is less time consuming and eliminates much of the bending, searching and counting required for the conventional treatment decision.

**Model Evaluation.** Insecticide sprays were required in all field plots for control of Colorado potato beetles during the 1990 potato season (Table 1). The PIES treatment regime required at least one less insecticide application throughout the growing season per field when compared with conventional treatment regimes.

The number of small larvae, large larvae and adult Colorado potato beetle per potato stem fluctuated throughout the growing season in all fields (Figure 2). An insecticide was applied when any lifestage exceeded the threshold level (4 small larvae, 0.5 large larvae, 1.5 adults per stem). The total (all lifestages) number of Colorado potato beetles per stem was significantly ($P \leq 0.05$) greater in the PIES treatment when compared with the conventional treatment in all fields (Table 2). Although the mean number of small larvae were not significantly ($P \leq 0.05$) different between treatments, there were, on average, twice as many large larvae and adults present in the PIES treatment. Higher numbers of Colorado potato beetles in PIES plots are likely due to the reduced number of insecticide applications. During the first four sampling periods (51, 58, 65 and 70 days after planting) PIES plots were sprayed, on average, 1.5 times, compared with 3.75 insecticide applications in conventional plots.

Mean percent defoliation per stem generally increased as the potato season progressed (Figure 3). Rotated fields required two fewer sprays for the PIES treatment (compared to PIES treatment, non-rotated fields) and reduced the total number of sprays by 50% when compared to rotated conventionally treated fields. Additionally, field rotation in combination with the PIES treatment reduced the number of early season (up to 70 days after planting) sprays to zero.

The use of PIES for Colorado potato beetle insecticide application decision making reduced the number of sprays needed throughout the growing season for Colorado potato beetle control by 39%, while not significantly ($P \leq 0.05$) reducing tuber yields (Table 3). In addition to reduction in number of insecticide sprays needed for Colorado potato beetle control, use of PIES by commercial potato growers may also encourage growers to increase the amount of time spent monitoring fields for Colorado potato beetle and other pests. Heinemann et al.

(1989) reported that more than 80% of the participants in their expert system studies indicated use of the expert system stimulated them to more closely monitor their orchards and 50% made some changes in production practices based on recommendations of the expert system.

PIES was developed for use in potato fields on the Eastern Shore of Va. Although region specific, the program could be adapted for other potato growing regions. Future PIES research focusing on evaluation of the system by potato growers, evaluation of the defoliation thresholds in large plots and incorporation of other pest problems (e.g. other insects, weeds, etc.) is necessary to insure maximum benefit from this expert system.

Table 1. Insecticide spray schedule for conventional and PIES plots in four fields at ESAES.

| | Rotated fields | | | | Nonrotated fields | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | |
| Days After Planting | ES[a] | Conv | ES | Conv | ES | Conv | ES | Conv |
| 51 | --[b] | A | -- | A | A | A | A | A |
| 58 | -- | -- | -- | A | -- | A | A | A |
| 65 | -- | A | -- | A | -- | A | -- | A |
| 70 | -- | A | -- | -- | A | A | -- | A |
| 77 | VF | VF | VF | VF | VF | -- | VF | -- |
| 82 | -- | -- | -- | -- | -- | -- | -- | -- |
| 84 | --[b] | -- | VF | VF | -- | VF | -- | VF |
| 86 | VF | VF | -- | -- | VF | -- | VF | -- |
| 89[c] | -- | -- | -- | -- | -- | -- | -- | -- |
| 92 | VFK | VFK | VFK | VFK | VFK | VFK | VFK | VFK |
| 96[c] | -- | -- | -- | -- | -- | -- | -- | -- |

[a]Conv=Threshold counts decision scheme, ES=PIES decision scheme.

[b]--=no spray treatment
A=esfenvalerate (Asana XL, 1.75 g [AI]/ha, DuPont, Wilmington, Del.)
VF=oxamyl + carbofuran (Vydate 2L, 0.56 kg [AI]/ha or 1.12 kg [AI]/ha, DuPont, Wilmington, Del.; Furadan 4L, 1.1 kg [AI]/ha, FMC Corp., Princeton, N.J.)
VFK=oxamyl + carbofuran + lambdacyhalothrin + PBO (Karate, 44.4 g [AI]/ha, ICI Americas, Inc., Wilmington, Del.; PBO, piperonyl butoxide 200 ml/ha).

Table 2. Mean number of Colorado potato beetle per potato stem in conventional and PIES plots at ESAES.

| Field | Treatment[a] | Mean No. Colorado potato beetles ± SEM[b] Small larvae | Large larvae | Adults |
|---|---|---|---|---|
| 1 | Conv | 1.14 ± 0.32a | 0.81 ± 0.11a | 0.55 ± 0.08a |
| | ES | 1.31 ± 0.30a | 2.04 ± 0.28b | 0.50 ± 0.07a |
| 2 | Conv | 1.07 ± 0.24a | 1.13 ± 0.18a | 0.75 ± 0.11a |
| | ES | 1.13 ± 0.29a | 2.39 ± 0.37b | 1.24 ± 0.12b |
| 3 | Conv | 0.96 ± 0.26a | 0.78 ± 0.12a | 0.54 ± 0.07a |
| | ES | 0.95 ± 0.23a | 2.42 ± 0.35b | 1.01 ± 0.08b |
| 4 | Conv | 1.04 ± 0.25a | 1.24 ± 0.20a | 1.08 ± 0.18a |
| | ES | 0.83 ± 0.19a | 2.64 ± 0.29b | 1.45 ± 0.13b |

[a]Conv=Threshold counts decision scheme, ES=PIES decision scheme.

[b]Means within column by field followed by the same letter are not significantly different at the $\underline{P} \leq 0.05$ level.

[c]Small larvae (< 0.05 cm); Large larvae (> 0.05 cm).

Table 3. Comparison of mean tuber yield and number of insecticide applications for conventional and expert system plots at ESAES.

| Plot[a] | Mean Tuber Yield (kg/ha)[b] Grade A | Grade B | Mean No. Insecticide Sprays[b] |
|---|---|---|---|
| Conv | 162.4 ± 27.2a | 28.4 ± 3.6a | 6.0 ± 0.00a |
| ES | 149.7 ± 30.3a | 28.1 ± 3.1a | 3.7 ± 0.67b |

[a]Conv = Threshold counts decision scheme, ES = PIES decision scheme.

[b]Means within columns followed by the same letter are not significantly different at the $\underline{P} \leq 0.05$ level.

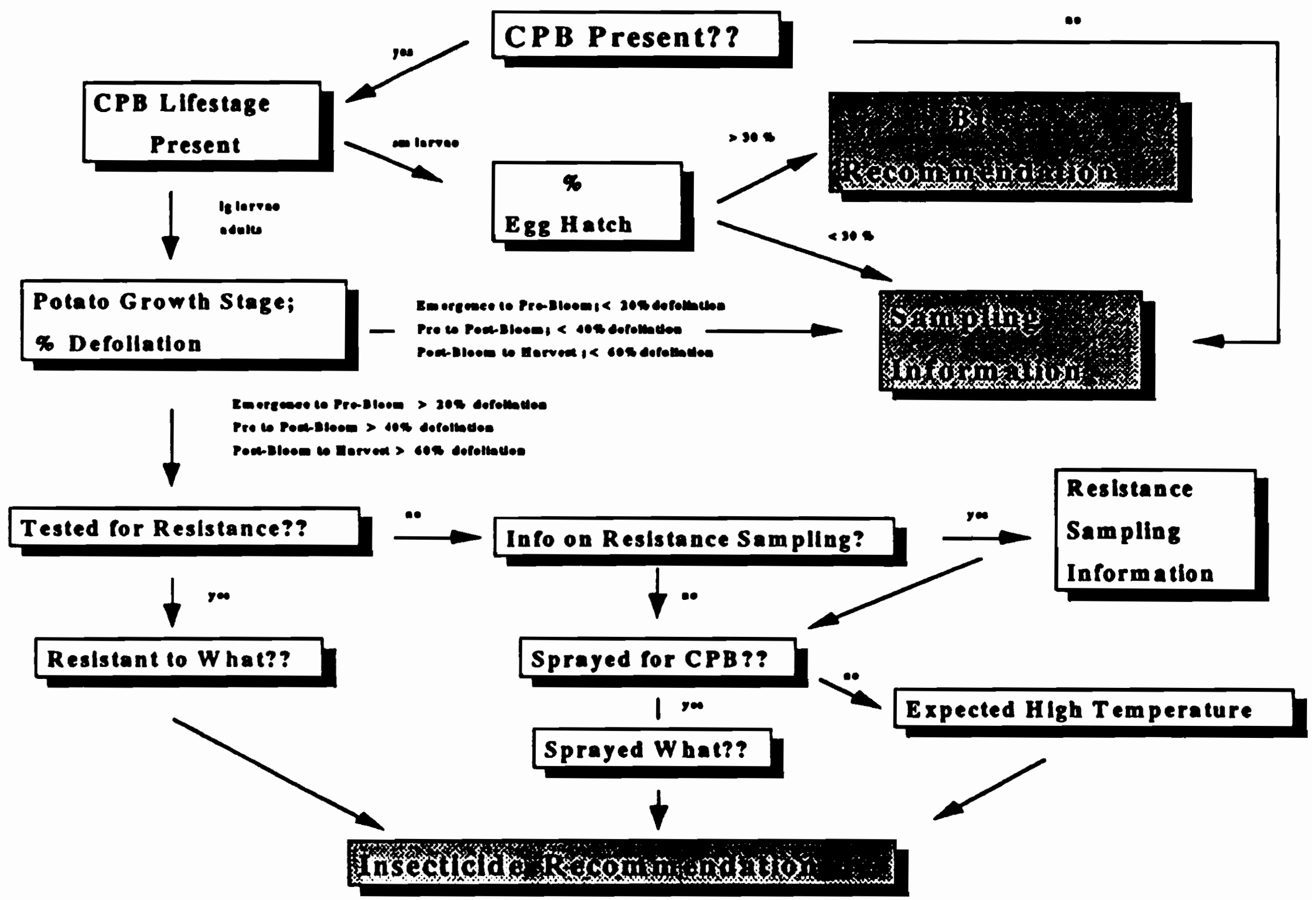


Figure 1. Flow chart for the Colorado potato beetle insect expert system.

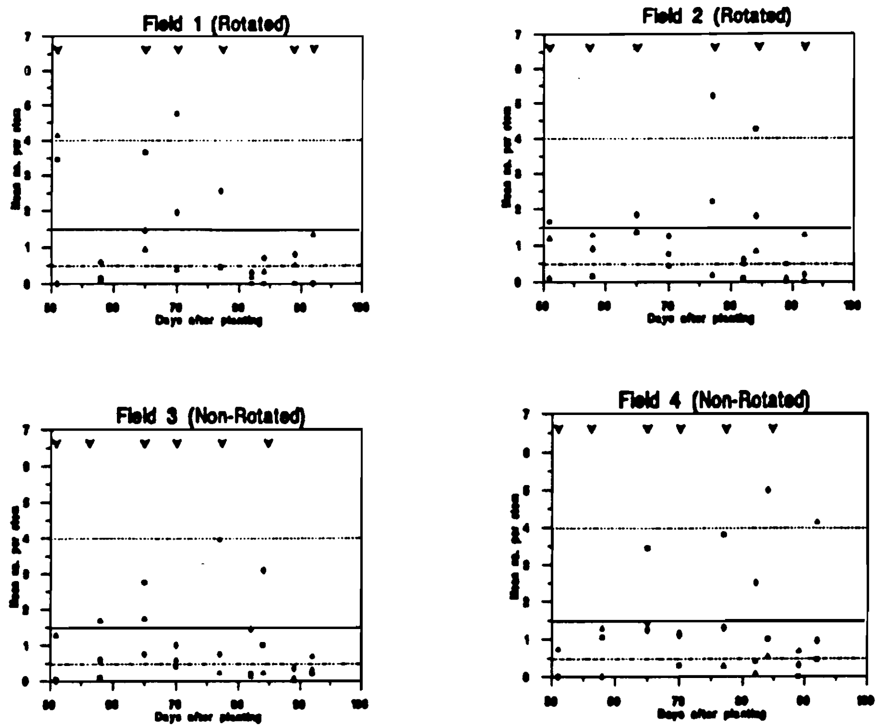


Figure 2. Mean per stem Colorado potato beetle counts (▪small larvae, ♦ large larvae, ▲ adults), threshold levels (---small larvae, — large larvae, -•- -adults), and insecticide applications (▾) for conventional treatments in fields at ESAES.

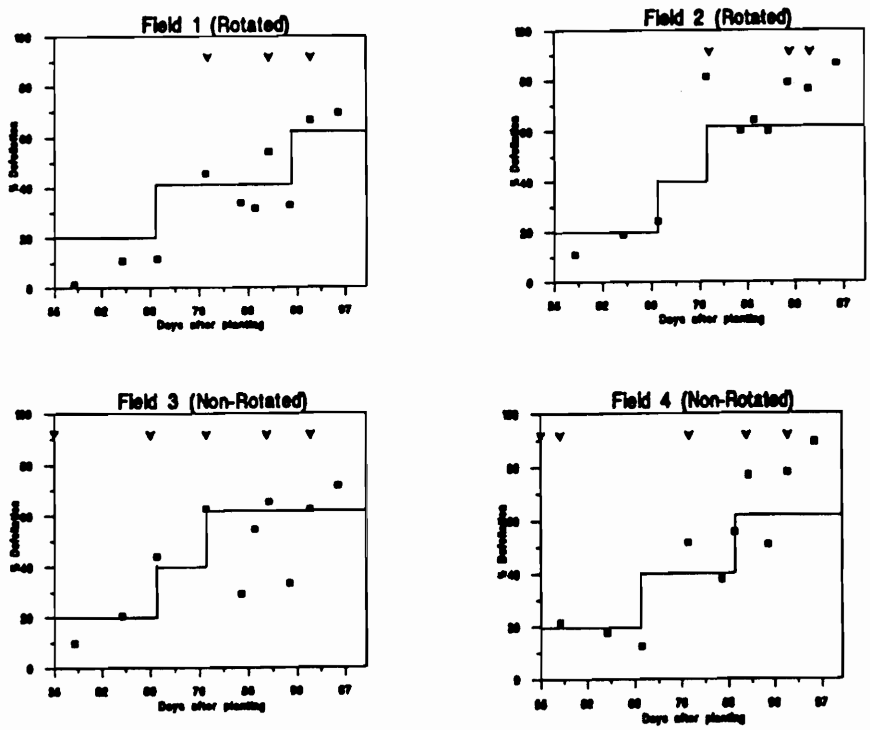


Figure 3. Mean percent defoliation per stem (■). insecticide applications (▾), and threshold levels in PIES treatments in fields at ESAES.

## References Cited

Batchelor, W.D., R.W. McClendon, J.W. Jones & D.B. Adams. 1989. An expert simulation system for soybean insect pest management. Am. Soc. Agric. Eng. 32: 355-342.

Beck, H.B. & P. Jones. 1987. SOYBUG: an expert system for soybean insect pest management. Paper #89-4502. Am. Soc. Agric. Eng. St. Joseph, MI.

Crenshaw, W.S. & E.B. Radcliffe. 1980. Effect of defoliation on yield of potatoes. J. Econ. Entomol. 73: 131-134.

Dripps, J.E. & Z. Smilowitz. Growth analysis of potato plants damaged by Colorado potato beetle (Coleoptera:Chrysomelidae) at different plant growth stages. Environ. Entomol. 18: 854-867.

Ferro, D.N., B.J. Morzuch & D. Margolies. 1983. Crop loss assessment of the Colorado potato beetle (Coleoptera:Chrysomelidae) on potatoes in western Massachusetts. J. Econ. Entomol. 76: 349-356.

Halseth, D.E., J.B. Sieczka, W.M. Tingey, & T.A. Zitter. 1987. Cornell recommendations for commercial potato production. New York State College of Agriculture and Life Science, Cornell University, Ithica.

Hare, J.D. 1980. Impact of defoliation by the Colorado potato beetle on potato yields. J. Econ. Entomol. 73: 369-373.

Heinemann, P.H., E.G. Rajotte, J.W. Travis, & T. Bowser. 1989. An expert system for apple orchard management. Paper #89-7038. Am. Soc. Agric. Eng., St. Joseph, MI.

Lashomb, J.H. & Y. Ng. 1984. Colonization by Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera:Chrysomelidae), in rotated and non-rotated potato fields. Environ. Entomol. 13: 1352-1356.

Lemmon, H. 1986. Comax: an expert system for cotton crop management. Science 233: 29-33.

Little, T.M. & F.J. Hills. 1978. Agricultural experimentation: design and analysis. Wiley, New York.

Logan, P.A. & R.A. Casagrande. 1980. Predicting Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] density and potato yield loss. Environ. Entomol. 9: 659-663.

Palmer, R.G. 1986. How expert systems can improve crop production. Agric. Eng. 67: 28-29.

Paperback Software International. 1987. VP-Expert. Berkeley, CA.

Parsaye, K. & M. Chignell. 1988. Expert Systems for Experts. John Wiley & Sons, Inc., N.Y.

Plant, R.W. & N.D. Stone. 1991. Knowledge-Based Systems in Agriculture. McGraw-Hill, Inc., N.Y.

Roach, J.W., Virkar, R.S., M.J. Weaver & C.R. Drake. 1985. POMME: a computer-based consultation system for apple orchard management using Prolog. Expert Systems 2: 56-68.

Roach, J.W., Virkar, R.S., C.R. Drake & M.J. Weaver. 1987. An expert system for helping apple growers. Computers and Electronics in Agric. 2: 97-108.

SAS Institute. 1985. SAS User's Guide: Statistics. SAS Institute, Carey, NC.

Shields, E.J. & J.A. Wyman. Effect of defoliation at specific growth stages on potato yields. J. Econ. Entomol. 77: 1194-1199.

Virginia Cooperative Extension Services. 1990. Commercial Vegetable Production Recommendations for Virginia. Pub. # 456-426.

Wellik, M.J., J.E. Slosser & R.D. Kirby. 1981. Effects of simulated insect defoliation on potatoes. Am. Potato J. 58: 627-633.

Wright, R.J. 1984. Evaluation of crop rotation for control of Colorado potato beetle (Coleoptera:Chrysomelidae) in commercial potato fields on Long Island. J. Econ. Entomol. 77: 1254-1259.

Wright, R.J., D.P. Kain, & D.D. Moyer. 1987. Development and implementation of an extension IPM program for Long Island. Bull. Entomol. Soc. Am. 54: 239-245.

Zehnder, G.W. & G.K. Evanylo. 1988. Influence of Colorado potato beetle sampling counts and plant defoliation on potato tuber production. Am. Potato J. 65: 725-736.

Zehnder, G.W. & G.K. Evanylo. 1989. Influence of extent and timing of Colorado potato beetle (Coleoptera:Chrysomelidae) defoliation on potato tuber production in Eastern Virginia. J. Econ. Entomol. 82: 1249-1255.

Zehnder, G.W. & W.D. Gelernter. 1989. Activity of the M-ONE formulation of a new strain of *Bacillus thuringiensis* against the Colorado potato beetle (Coleoptera:Chrysomelidae): relationship between susceptibility and insect life stage. J. Econ. Entomol. 82: 756-761.

Zehnder, G.W., D.M. Kolodny-Hirsch, & J.L. Linduska. 1990. Evaluation of various potato plant sampling units for cost-effective sampling of Colorado potato beetle. J. Econ. Entomol. 83: 428-433.

Appendix 1. Programming code for PIES (Potato Insect Expert System) written using VPEXPERT, a programming code by Paperback Software.

```
bkcolor=1;
runtime;
autoquery;
endoff;

actions

    color=14
    wopen 1,2,3,18,70,5
    active 1

    display"
        WELCOME TO PIES: Potato Insect Expert System!


  PIES is designed to make insecticide recommendations for Colorado
  potato beetle control in commercial potato fields.

  You will be asked questions about Colorado potato beetles in your
  potato field.  To answer these questions:

      * Use the arrow keys to highlight your response.
      * Press return to select that response.



            Press any key to begin PIES.~"

    cls

    wclose 1

    color=14
    find treatment
;
rule no_cpb
  if cpb = no
  then disp = sampling_info
;

rule yes_cpb
  if cpb = yes
  then knowledge = bt
```

```
;

rule life_stage_choices
  if life_stage = eggs_hatching or
     life_stage = small_larvae
  then knowledge = bt
  else knowledge = insecticide
;

rule bt_knowledge_base
   if knowledge = bt and
      life_stage = eggs_hatching and
      hatch >= 30
   then disp = bt_spray_display
;

rule bt_spray_display
   if disp = bt_spray_display
   then treatment = bt_spray

   cls
   wopen 1,2,5,18,70,5
   active 1
   display "
 PIES recommends the use of Bt insecticides for
 Colorado potato beetle control.  Bt insecticides are considered
 environmentally safe and may help retard the development
 of Colorado potato beetle insecticide resistance.  However, Bt
 insecticides must be applied when Colorado potato beetle larvae
 are small and within 6 days of peak egg hatch.



 A second spray application may be made 5-7 days after the initial
 application.  Subsequent Bt insecticide sprays may be made at
 7-10 day intervals, to control hatching larvae.  Discontinue Bt
 insecticide sprays when surviving larvae are larger than 1/4 inch.


              Press any key to continue.~"
   wclose 1
   cls

   color=30
   wopen 2,5,9,10,50,1
   display "
              NOTE:


  Larval reduction may not be noticeable
       for 48 to 72 hours after
```

```
      Bt insecticide application.~"

    cls
    wclose 2

;

rule bt_knowledge_2
    if knowledge = bt and
      life_stage = eggs_hatching and
      hatch < 30
      then disp = sampling_info
;

rule sampling_display
    if disp = sampling_info
    then treatment = sampling_display

    cls
    wopen 1,2,3,18,70,5
    active 1
    display "
    PIES recommends you conduct a field sampling program to
  help you detect when your peak Colorado potato beetle egg hatch
                    will occur.

It is important to determine peak egg hatch because Bt insecticides
              can be used when larvae are small.

    * Bt insecticides are considered environmentally safe.

    * Bt insecticides can help retard the development of
      Colorado potato beetle insecticide resistance.

    * Bt insecticide sprays are most effective if applied
      when Colorado potato beetle larvae are less than
      1/4 inch in length.


                Press any key to continue.~"
    wclose 1
    cls


    wopen 3,2,3,18,70,5
    active 3
    display "
  Proper timing of the first Bt spray may be determined
                by two methods:
```

```
    1. Flag at least 10 egg masses in different locations
       in your field.  Check these egg masses daily.  When
       3 of the 10 egg masses have hatched, apply a Bt spray
       within 2-4 days.


    2. Collect 2-5 leaves containing egg masses from different
       locations in your field.  Bring the leaves inside and
       place on moist paper towels.  Check eggs daily.  When
       1 of 3, or 2 of 5 have hatched, apply a Bt spray within
       3-5 days.


     Press enter twice to return to beginning of program.~"
   cls
   wclose 3
;

rule bt_knowledge_3
   if knowledge = bt and
      life_stage = small_larvae
   then disp = bt_spray_display
;

rule plnts_stg_1
   if plnt_stg = emerge_to_pre-bloom and
      plnt_defol < 18
   then disp = no_spray_consult
;


rule plnts_stg_1a
   if plnt_stg = emerge_to_pre-bloom and
      plnt_defol >= 18
   then outcome = spray
;

rule plnts_stg_2
   if plnt_stg = pre_to_post-bloom and
      plnt_defol < 39
   then disp = no_spray_consult
;

rule plnts_stg_2a
   if plnt_stg = pre_to_post-bloom and
      plnt_defol >= 39
   then outcome = spray
;

rule plnts_stg_3
   if plnt_stg = postbloom_to_harvest and
```

```
      plnt_defol < 63
    then disp = no_spray_consult
;

rule plnts_stg_3a
    if plnt_stg = postbloom_to_harvest and
      plnt_defol >= 63
    then outcome = spray
;

rule no_spray
    if disp = no_spray_consult
    then treatment = consult_info

    cls
    wopen 1,2,3,18,70,5
    active 1
    display "
  Although your potatoes have sustained some Colorado
  potato beetle feeding damage, it is not severe enough
  cause tuber yield reductions.


  An insecticide application is not recommended at this time.
  However, you should continue to monitor the level of
  defoliation caused by Colorado potato beetles.


  If defoliation levels increase, please consult this program
  again for an updated recommendation.


      Press enter twice to return to beginning of program.~"
    cls
    wclose 1
    color=14
;

rule resist_testing
    if outcome=spray and
      resistance=yes
    then continue=yes
    else continue=no
;

rule use_pyrethroids
    if continue=yes and
      resist=carbamates or
      resist=organophosphates or
      resist=carb_and_ops
    then disp=pyrethroids
```

```
;

rule use_carbamates
    if continue=yes and
       resist=pyrethroids or
       resist=organophosphates or
       resist=ops_and_pyreth
    then disp=carbamates
;

rule use_ops
    if continue=yes and
       resist=carbmates or
       resist=pyrethroids or
       resist=carb_and_pyreth
    then disp=ops
;

rule use_mixture
    if continue=yes and
       resist=all_classes_tested
    then disp=tank_mix
;
rule resist_not_tested_1
    if continue=no and
       bioassay=no
    then bioassay_consult=no
;

rule resist_not_tested_2
   if continue=no and
      bioassay=yes
   then bioassay_consult=yes
   cls
   wopen 1,2,3,18,70,5
   active 1
   display "
  You will need the following supplies to conduct a
  Colorado potato beetle insecticide resistance bioassay:

     * Five 2 inch diameter filter paper disks and glass
       Petri dishes per insecticide tested.

     * Field rate of insecticides to be tested

     * Small paint brush and plastic wrap.

     * 50 newly hatched Colorado potato beetle larvae
       collected from the field from egg masses brought
       indoors to hatch (5 egg masses collected from
       different locations in the field) for each insecticide
```

```
      being tested.

                Press any key to continue.~"

    cls
    display "
   Apply 0.5 ml of insecticide to be tested on five
   filter paper disks.  Allow disks to dry until just damp
   and place 10 larvae on each disk using a small paint brush.

   Cover Petri dishes with plastic wrap and allow them to sit
   for 24 hours.

   Remove plastic wrap and count the number of dead larvae.  If
   fewer than 7 larvae on average per dish, consider that Colorado
   potato beetle population to be resistant to the material
   being tested.


                Press any key to continue.~"
    cls
    wclose 1
    color=14
;


rule resist_not_tested_1
    if bioassay_consult=no and
      spray=no and
      temp= less_than_80
    then disp=pyrethroids
;

rule resist_not_tested_1a
    if bioassay_consult=no and
      spray=no and
      temp=greater_than_80
    then disp=carbamates
;

rule resist_unknown_1
    if bioassay_consult=no and
      spray=yes and
      chem_type=carbamates or
      chem_type=ops or
      chem_type=carb_and_ops
    then disp=pyrethroids
;

rule resist_unknown_2
    if bioassay_consult=no and
```

```
        spray=yes and
        chem_type=carbamates or
        chem_type=pyrethroids or
        chem_type=carb_and_pyreth
    then disp=ops
;

rule resist_unknown_3
    if bioassay_consult=no and
        spray=yes and
        chem_type=ops or
        chem_type=pyreth or
        chem_type=ops_and_pyreth
    then disp=carbamates
;

rule resist_unknown_4
    if bioassay_consult=no and
        spray=yes and
        chem_type=all_classes_tested
    then disp=disclaimer
;

rule bioassay_1
    if bioassay_consult=yes and
        spray=yes and
        chem_type=carbamates or
        chem_type=pyrethroids or
        chem_type=carb_and_pyreth
    then disp=ops
;

rule bioassay_2
    if bioassay_consult=yes and
        spray=yes and
        chem_type=ops or
        chem_type=pyrethroids or
        chem_type=ops_and_pyreth
    then disp=carbamates
;

rule bioassay_3
    if bioassay_consult=yes and
        spray=yes and
        chem_type=carbamates or
        chem_type=ops or
        chem_type=carb_and_ops
    then disp=pyrethroids
;

rule bioassay_4
```

```
    if bioassay_consult=yes and
       spray=yes and
       chem_type=all_classes_tested
    then disp=disclaimer
;
rule bioassay_5
    if bioassay_consult=yes and
       spray=no and
       temp=less_than_80
    then disp=pyrethroids
;

rule bioassay_6
    if bioassay_consult=yes and
       spray=no and
       temp=greater_than_80
    then disp=carbamates
;

rule disclaimer
    if disp=disclaimer
    then treatment=questionable
    display " you have real problems"
;

rule recommend_pyrethroids
    if disp=pyrethroids
    then treatment=pyreths
    cls
    wopen 1,2,5,18,70,5
    active 1
    display "
  PIES recommends the use of pyrethroid insecticides for
  Colorado potato beetle control.

  The following insecticides are registered for Colorado
  potato beetle control:

    * Ambush 3E       1/3 to 1 2/3 pts/A
    * Asana XL        3/4 to 1 1/2 pts/A
    * Pounce 3.2 EC   1/2 to 1     pt/A

  The effectiveness of pyrethroid insecticides may be
  enhanced by tank-mixing with 1/2 to 1 pt/A of PBO
  (piperonyl butoxide).


         Press any key to continue.~"
    cls
    wclose 1
```

```
    color=30
    wopen 2,5,9,10,50,1
    display "


      As with any insecticide, be sure
       to read, understand and follow
              the label.~"

    cls
    wclose 2
;

rule recommend_carbamates
    if disp=carbamates
    then treatment=carbs
    display "carbamates "

    cls
    wopen 1,2,5,18,70,5
    active 1
    display "


 PIES recommends the use of carbamate insecticides for
 Colorado potato beetle control.

 The following are registered for Colorado
 potato beetle control:


    * Furadan 4F          1 to 2     pts/A
    * Imidan 50WP              2     lbs/A
    * Thiodan 3E      1 1/3 to 2 2/3 pts/A
    * Vydate 2L           1 to 4     pts/A



          Press any key to continue.~"

    cls
    wclose 1

    color=30
    wopen 2,5,9,10,50,1
    display "


      As with any insecticide, be sure
       to read, understand and follow
              the label.~"
```

```
    cls
    wclose 2
;

rule recommend_ops
    if disp=ops
    then treatment=organos

    cls
    wopen 1,2,5,18,70,5
    active 1
    display "
  PIES recommends the use of organophosphate
  insecticides for Colorado potato beetle control.

  The following are registered for Colorado
  potato beetle control:



    * Guthion 2S       1.5 pts/A




         Press any key to continue.~"

    cls
    wclose 1

    color=30
    wopen 2,5,9,10,50,1
    display "


      As with any insecticide, be sure
       to read, understand and follow
               the label.~"

    cls
    wclose 2
;

rule recommend_tank_mix
    if disp=tank_mix
    then treatment=tank
    display "tank mix "

    cls
    wopen 1,2,5,18,70,5
```

```
    active 1
    display "
  PIES recommends the use of a tank-mix of insecticides for
  Colorado potato beetle control.

  The following can be tank mixed for Colorado
  potato beetle control:



    * Vydate 2L (1pt/A) and Furadan 4F (1pt/A)

    * Vydate 2L (1pt/A) and Furadan 4F (1pt/A)
           and parathion (1/2 pt/A)


          Press any key to continue.~"

    cls
    wclose 1

    color=30
    wopen 2,5,9,10,50,1
    display "


      As with any insecticide, be sure
       to read, understand and follow
              the label.~"

    cls
    wclose 2
;


ask cpb: "Are Colorado potato beetle populations present in
your potato field?";
choices cpb:  yes,
         no;

ask life_stage: "Which of the following best describes the CPB in your field
today?";
choices life_stage:  eggs_hatching,
               small_larvae,
               large_larvae,
               adults;

ask hatch: "What percentage of the egg masses in your field
have hatched?";
choices hatch: 0-29,
         30_or_more;
```

```
ask plnt_stg: "What growth stage are your potatoes in?";
choices plnt_stg: emerge_to_pre-bloom,
            pre_to_post-bloom,
            postbloom_to_harvest;

ask plnt_defol: "What is the average percent defoliation in your field?";
choices plnt_defol: 0-17,
             18-62,
             63-90,
             91-100;

ask temp: "What is today's expected high temperature?";
choices temp: less_than_80,
         greater_than_80;

ask spray: "Have you sprayed this field to control Colorado
potato beetles this season?";
choices spray: yes,
         no;

ask chem_type: "Which insecticides have you sprayed with";
choices chem_type: Carbamates,
            Organophosphates,
            Pyrethroids,
            Carb_and_Ops,
            Carb_and_Pyreth,
            Ops_and_Pyreth,
            All_Classes_Tested;

ask resistance: "Have you tested Colorado potato beetles
in this field for insecticide resistance?";
choices resistance: yes,
             no;

ask bioassay: "Would you like information on how to conduct
a Colorado potato beetle insecticide bioassay?";
choices bioassay: yes,
            no;

ask resist: "What materials were your Colorado potato beetles
resistant too?";
choices resist: Carbamates,
          Organophosphates,
          Pyrethroids,
          Carb_and_OPs,
          Carb_and_Pyreth,
          OPs_and_Pyreth,
          All_Classes_Tested;
```

## Cold Tolerance Mechanisms in the Colorado Potato Beetle, *Leptinotarsa decemlineata* (Say).

Cold-hardiness is the ability to tolerate prolonged exposure to temperatures below the melting point of body fluids (Zachariassen 1985). Animals, including insects, utilize two strategies for survival under cold temperature conditions. They may avoid freezing by supercooling body fluids, or may develop tolerance to the freezing of body tissues. Several biochemical and physiological mechanisms form the basis of these strategies and include, an increase or decrease in the amount and the distribution of ice nucleating agents, an increase in production and concentration of low-molecular-weight solutes like polyhydric alcohols (polyols), and the production of proteinaceous thermal hysterisis factors (Salt 1965, Mansingh & Smallman 1972, Duman 1977, Duman 1979, Zachariassen 1980, Duman et al. 1982, Zachariassen et al. 1982, Duman & Horwath 1983, Nevan et al. 1989).

A number of insect orders (Coleoptera, Collembola, Dictyoptera, Diptera, Hymenoptera, and Lepidoptera) have been reported to be cold-tolerant (Mansingh & Smallman 1972, Rains & Dimock 1978, Duman 1979, Block & Zettel 1980, Zachariassen 1980, Hamilton et al. 1985) and maybe classified as freeze-sensitive or freeze-tolerant based on the strategy utilized for supercooling [a supercooled system remains unfrozen at temperatures below its melting point (Zachariassen 1985)]. Freeze-susceptible (also referred to as freeze-sensitive) insects lack the ability to tolerate ice formation in body fluids, while freeze-tolerant insects are able to withstand ice formation in body fluids at temperatures equal to or below their supercooling capacity [supercooling capacity is the difference between supercooling point and melting point (Zachariassen 1985)]. Freeze-tolerance or susceptibility in Coleoptera appears to be species specific (Mail & Salt 1933, Rains & Dimock 1978, Zachariassen 1980, Zachariassen et al. 1982, Elsey 1988, Gustin & Wilde 1984, Zachariassen 1985, Bennett & Lee 1989, Elsey 1989).

Little is known about the mechanisms of Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] cold tolerance. The adult enters diapause in the fall and overwinters 20 to 25 cm below the soil surface (Chittenden 1907). The infestation potential of the spring potato crop by Colorado potato beetle is dependent on the survival and reproductive ability of adults overwintered from the previous fall. Factors such as soil type, snow or mulch cover, and the soil depth at which the insect diapauses may affect its ability to withstand low temperatures [Mail 1930, Mail & Salt 1933, Wymen 1991 (pers. comm.)]. Additionally, polyols, hemolymph proteins and other substances (sugars, free amino acids, inorganic ions, etc.) present in the hemolymph may play a role in Colorado potato beetle cold-hardiness.

Polyols, including glycerol, sorbitol, mannitol, ribitol, threitol and erythritol have been found to occur in the hemolymph of freeze-tolerant insects (Mansingh & Smallman 1972, Block & Zettel 1980, Zachariassen 1980, Hamilton et al. 1985). Additionally, inositol has been reported in the hemolymph of Coleoptera from the Chrysomelidae and Coccinellidae families (Hoshikawa 1981). Polyols are thought to play a role in the stabilization of protein structures, maintenance membrane integrity and prevention of extreme osmotic fluctuations (Baust 1982, Zachariassen 1980).

This study was conducted to determine the supercooling capacity of Colorado potato beetles from selected potato growing regions throughout North America. Hemolymph samples were also analyzed to tentatively identify polyols which may aid in the cold-hardiness of the Colorado potato beetle.

## Materials and Methods

**Samples.** Adult Colorado potato beetles were collected from potatoes in the fall (1990) and placed in field cages at the Eastern Shore Agricultural Experiment Station, Painter, Virginia, where they entered the soil for diapause. Beetles were extracted from the soil monthly from January to March, 1991. Once the soil had been disturbed under a field cage, subsequent samples were taken from another area. Additionally, adult Colorado potato beetles were hand collected after spring emergence (April, 1991). Beetles were placed in mailing tubes, then in coolers containing frozen blue ice and sent by overnight mail to Blacksburg, Virginia. Upon arrival, beetles were refrigerated at 7 $^{o}C$ for 24-36 h before processing. When removed from the refrigerator, viability was assessed and adults were sexed.

Three additional populations were examined: Presque Isle, Maine; Yakima, Washington; and Charlottetown, Prince Edward Island, Canada. Beetles from Maine and Washington were caged in the fall in containers of soil, allowed to diapause and were maintained outside for the winter. Beetles from Canada were collected in late summer and allowed to diapause in boxes containing soil and peatmoss. Once diapaused, beetles were placed in a cooler at 4 $^{o}C$ until shipment. In February, 1991, beetles were placed in mailing tubes, then in coolers containing frozen blue ice, and sent by overnight mail to Blacksburg, Virginia. Upon arrival, beetles were handled as described above.

**Supercooling Point Determination** - The whole body supercooling point was determined monthly (January - April, 1991) for ten male and ten female Colorado potato beetles (20 total per collection date) collected from Painter, Virginia. Two 0.015 mm copper-constantan thermocouples (Omega Engineering, Inc. Stamford, Conn.) were externally attached to each beetle with thermal grease (zinc oxide in oil emersion suspension). One thermocouple was placed on the dorsal abdomen under the elytra, the other on the ventral abdomen just below the thorax. After securing the thermocouples, the beetle was placed on its dorsal side and sandwiched between two aluminium blocks held at ambient room temperature (approximately 25-27 °C). The blocks containing the Colorado potato beetle were covered with Styrofoam for insulation (except on the bottom) to minimize the area being cooled and to help insure more even cooling of the insect and placed on a cold plate (Stir-Kool SK-31, Thermoelectronics Unlimited, Inc.). The temperature was lowered 0.6 °C/min. until the supercooling point was observed. A sudden rise in temperature due to the release of the latent heat of fusion indicated when supercooling occurred (Zachariassen 1980). Temperature was monitored using a software program (STC GYY 1.1) developed by Omega Engineering Inc. (Stamford, Conn.).

The supercooling point of hemolymph was also determined utilizing a similar procedure. Hemolymph was collected from individual beetles by pricking the dorsal thorax above the right prothoracic coxa with a pin and drawing 1 *u*l of hemolymph into a glass tube by capillary action. Each hemolymph sample was placed on top of a 0.015 mm copper-constantan thermocouple attached to a glass microscope slide. The slide was sandwiched between aluminium blocks held at ambient room temperature (approximately 25-27 °C). The blocks were covered with Styrofoam for insulation and placed on a cold plate. The temperature was lowered 0.6 °C/min until the supercooling point was observed. Beetles used to determine whole body and hemolymph supercooling points were not the same individuals.

Supercooling points for Colorado potato beetles from Canada, Maine and Washington were determined as described above. Beetles were not sexed prior to testing, as preliminary data from the Painter, Virginia population indicated no significant differences between male and female supercooling points (Table 1).

Means and standard errors for temperature data collected from Colorado potato beetle whole body and hemolymph supercooling experiments were calculated. Results were compared using the general linear model and Ryan's Q-Test for mean separation (SAS User's Guide 1985).

**Polyol Analysis.** Colorado potato beetle hemolymph was analyzed for seven polyols [arabitol, dulcitol, inositol, mannitol, ribitol (adonitol), sorbitol, xylitol] which have been found in insects and are thought to play a role in cold tolerance (Mansingh & Smallman 1972, Block & Zettel 1980, Zachariassen 1980, Hoshikawa 1981, Hamilton et al. 1985).

High performance thin-layer chromatography (HPTLC) was used to separate polyols from other hemolymph components (Hoshikawa 1981, Hamilton 1985, Baumgartner et al. 1986). Samples of hemolymph (1 *u*l) were obtained from individual Colorado potato beetles for each sampling date and location as described above and were dissolved in 10 *u*l of 95% ethanol and frozen.

Silica gel HPTLC plates (Merck Silica Gel 60, VWR Scientific, Research Triangle Park, N.C.) were washed in a 100% methanol solution and heated at 120 °C for 60 min to activate the silica gel. Hemolymph samples were removed from the freezer and centrifuged for 30 s at 9000 rpm. Supernatent samples (1 *u*l) were spotted onto the silica gel plates (5 mm spacing on plates). Polyol standards were also spotted onto the plates. Three solvent systems [acetone:butanol:water (4:1:0.5, v/v/v), water:acetic acid:propanol (6:11:83, v/v/v) and butanol:acetic acid:water (4:1:4, v/v/v)] were used to develop silica gel plates containing hemolymph samples and polyol standards (Hoshikawa 1981, Hamilton 1985, Baumgartner et al. 1986). Additional solvent systems containing varying combinations and ratios of butanol, acetic acid, water, propanol, acetone, pyridine, proprionic acid, acetonitrile and chloroform were examined and produced poor or no separation of polyol standards. A solution of 50% concentrated sulfuric acid in ethanol was applied to the silica gel plates and the plates were placed in an oven at 120 °C for 45-60 min, or until spots were detected. Spots appearing on plates in the sample lanes were visually evaluated. A plate scanner (Camag TLC Scanner II, Muttenz, Switzerland) and integrator (Camag SP4270) was used to identify samples developed in acetone:butanol:water (4:1:0.5, v/v/v). $R_f$-values calculated from polyol standards were compared with $R_f$-values generated by sample data for identification of polyols present.

## Results and Discussion

**Supercooling.** Whole body supercooling points of Colorado potato beetles collected from Painter, Virginia varied approximately 3 to 5 $^{\circ}$C throughout a four month period (-7.99 $\pm$ 0.93, -10.53 $\pm$ 0.65, -7.49 $\pm$ 0.63, and -5.45 $\pm$ 0.58 $^{\circ}$C for January, February, March, and April, respectively) (Figure 1). Hemolymph supercooling points determined for Colorado potato beetles collected from Painter, Virginia from January to April, 1991, varied approximately 1 to 3 $^{\circ}$C throughout the collection period (-16.92 $\pm$ 0.92, -13.03 $\pm$ 1.16, -12.99 $\pm$ 0.70 and -16.12 $\pm$ 0.63 $^{\circ}$C for January, February, March, and April, respectively) (Figure 1).

Hemolymph supercooling points differed significantly ($P \leq 0.05$) from the whole body supercooling points, and by as much as 10.7 $^{\circ}$C (April). Differences between the whole body and hemolymph supercooling points observed between Colorado potato beetle from Painter, Virginia may be attributed to several factors. Gut and malpighian tubule contents, as well as cooling rate and volume cooled, may affect supercooling point (Salt 1965, Duman 1982, Zachariassen 1985). Impurities and waste products found within the insect gut and excretory structures may initiate ice nucleation before the supercooling point of hemolymph is reached and result in a higher whole body supercooling temperature (Salt 1953, Somme 1982). Additionally, thermocouples attached to the external surfaces of the insect reflect more rapid freezing of extremities (legs, wings, antennae) and may also yield a higher whole body supercooling point (Salt 1965, Zachariassen 1985).

Comparisons of whole body and hemolymph supercooling points among four overwintered Colorado potato beetle populations collected in February 1991, indicated Virginia Colorado potato beetles supercooled at significantly ($P \leq 0.05$)

lower temperatures (-10.53 ± 4.12, -13.03 ± 1.16 °C for whole body and hemolymph, respectively) than Colorado potato beetles from Maine (-5.92 ± 3.40 °C, whole body), Washington (-4.50 ± 3.56, -7.88 ± 0.85 °C for whole body and hemolymph, respectively), and Canada (-5.36 ± 0.83, -7.87 ± 0.34 °C for whole body and hemolymph, respectively) (Figure 2).

Supercooling points of Colorado potato beetle from Maine, Washington and Canada indicated Colorado potato beetle was a freeze-tolerant insect species. Zachariassen (1980) reported that a number of freeze-tolerant beetles supercooled between -7.5 to -13.5 °C, whereas freeze-sensitive beetles supercooled at -20 °C or below. Supercooling points of Colorado potato beetles examined from the northeastern and western United States, as well as from eastern Canada, are well within the temperature range of freeze-tolerant species. Supercooled Colorado potato beetles regained what appeared to be normal activity (ability to walk, antennal movements, grooming behavior, etc.) after their supercooling point was reached and they were held at room temperature (25 to 27 °C). This indicated the point at which supercooling occurred was not the lower lethal limit for survival.

Surprisingly, Colorado potato beetles collected from Virginia supercooled at temperatures significantly ($P \leq 0.05$) lower than the Maine, Washington and Canada populations. Virginia Colorado potato beetles appear to be utilizing a different mechanism of cold tolerance and are probably cold-sensitive.

Shifts in supercooling points from high sub-zero temperatures (freeze-tolerance) to temperatures below -20 °C (freeze-susceptibility) have been reported and appear to be affected by climatic changes and population differences (Horwath & Duman 1984), but are not well understood. Colorado potato beetles are believed to have originated from populations in the Rocky Mountains (Jacques 1988) and exhibit low genetic diversity (Zehnder et al. 1992). Virginia winter conditions are not extremely harsh (mean winter low of approximately 5 °C) and the sandy soil in the potato growing region permits Colorado potato beetle to diapause 20 to 25 cm below the soil surface, providing additional protection. Production and maintenance of ice nucleating proteins, high concentrations of polyols, and other possible mechanisms for freeze-tolerance is metabolically costly for individuals within a population which are not likely to encounter conditions warranting freeze-protection, and it seems plausible that through selection, the Virginia Colorado potato beetle populations adopted other mechanisms of cold tolerance which better may be better adapted for Virginia. Colorado potato beetle populations (Maine, Washington, Canada) which endure different winter conditions may have maintained freeze-tolerance mechanisms. Functionally, either mode of cold adaptation (freeze-tolerance or freeze-susceptibility) has proved successful for Colorado potato beetle survival.

**Polyol Analysis.** Inositol and xylitol were identified in the hemolymph of all Colorado potato beetle populations examined in this study (Table 2) and are likely to play some role in cold-hardiness. Polyols have been implicated as cryoprotective agents, help to stabilize lipid membranes, and counter-act osmotic shrinkage associated with controlled freezing (Zachariassen 1985). The scope of this study does not permit speculation on the role of inositol and xylitol in Colorado potato beetle, as quantification of polyols and correlation between supercooling point and polyol concentration was not examined.

Colorado potato beetle overwintering mechanisms are complex and not well understood, but are essential for the survival of the species. Continued investigation of Colorado potato beetle supercooling capacity, role of polyols in Colorado potato beetle cold-hardiness, and investigation of other possible mechanisms used for cold tolerance is necessary to better understand the mechanisms of Colorado potato beetle cold-hardiness which may also aid in the development of alternative Colorado potato beetle control mechanisms.

Table 1. Mean whole body and hemolymph supercooling points (in °C) for overwintered female and male Colorado potato beetles (Virginia).

| | | Mean Supercooling Point (in °C) ± SEM[a] | |
|---|---|---|---|
| Sampling Date | Sex | Whole Body | Hemolymph |
| January | Female | -7.11 ± 1.13 | -16.37 ± 0.93 |
| | Male | -8.86 ± 1.48 | -18.79 ± 0.64 |
| February | Female | -11.53 ± 0.83 | -16.20 ± 1.43 |
| | Male | - 9.54 ± 0.97 | -13.85 ± 1.39 |

[a]Means within the same column by date are not significantly different ($P \leq 0.05$).

Table 2. Comparison of $R_f$-values of polyols and components of Colorado potato beetle hemolymph (N=5) on silica gel HPTLC plates using three solvent systems and sulfuric acid charring for detection.

| | Solvent System[a] | | |
|---|---|---|---|
| Polyol | 1 | 2 | 3 |
| Arabitol | 0.50 | 0.41 | 0.30 |
| CPB[b] | -- | -- | -- |
| Dulcitol | 0.26 | 0.28 | 0.22 |
| CPB | -- | -- | -- |
| Inositol | 0.07 | 0.07 | 0.09 |
| CPB | 0.07 | 0.07 | 0.09 |
| Mannitol | 0.33 | 0.32 | 0.23 |
| CPB | -- | -- | -- |
| Ribitol | 0.58 | 0.45 | 0.32 |
| CPB | -- | -- | -- |
| Sorbitol | 0.28 | 0.29 | 0.21 |
| CPB | -- | -- | -- |
| Xylitol | 0.43 | 0.36 | 0.29 |
| CPB | 0.43 | 0.36 | 0.29 |

[a]1=acetone:butanol:water [4:1:0.05 (v/v/v)];

2=water:acetic acid:propanol [6:11:83 (v/v/v)];

3=butanol:acetic acid:water [4:1:4 (v/v/v)].

[b]CPB=Colorado potato beetle; --=Not Detected.

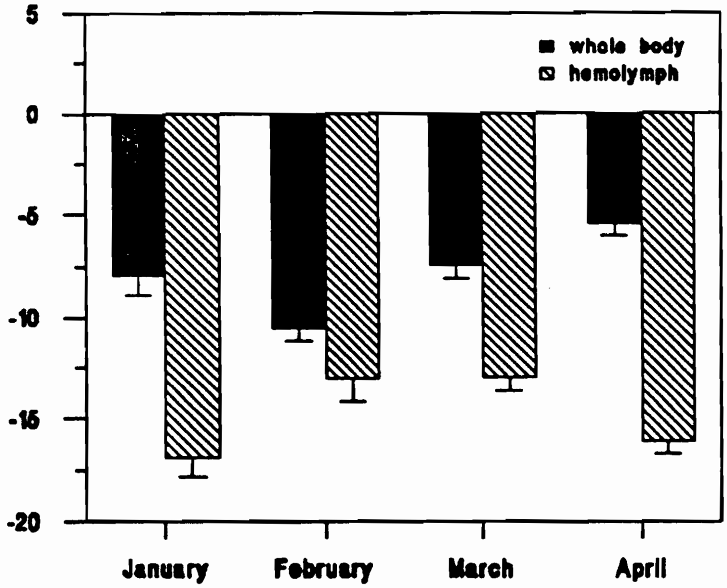


Figure 1. Whole body and hemolymph supercooling points (in °C) for Colorado potato beetle collected from Painter, Va.

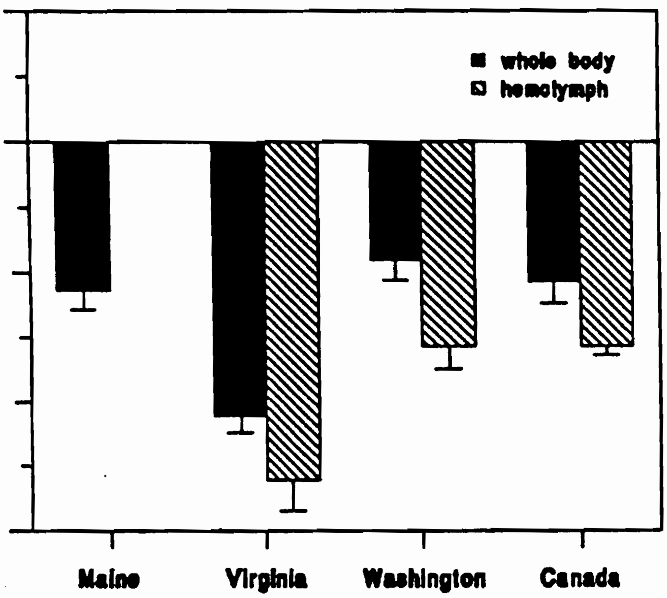


Figure 2. Whole body and hemolymph supercooling points (in °C) for four populations of Colorado potato beetle collected in February, 1991.

## Literature Cited

Baumgartner, S., R. Genner-Ritzmann, J. Hass, R. Amado, & H. Neukom. 1986. Isolation and identification of cyclitols in carob pods (*Ceratonia siliqua* L.). J. Agric. Food Chem. 34: 827-829.

Baust, J.G. 1982. Environmental triggers to cold hardening. Comp. Biochem. Physiol. 73A: 563-570.

Bennett, L.E & R.E. Lee. 1989. Simulated winter to summer transition in diapausing adults of the lady beetle (*Hippodamia convergens*): Supercooling point is no indicative of cold-hardiness. Physiol. Entomol. 14: 361-367.

Block, W. and Jurg Zettel. 1980. Cold-hardiness of some alpine Collembola. Ecol. Entomol. 5: 1-9.

Chittenden, F.H. 1907. The Colorado potato beetle. U.S.Dept.Agric. Circ. #87.

Duman, J.G. 1977. Variations in macromolecular antifreeze levels in larvae of the darkling beetle, *Mercantha contracta*. J. Exp. Zool. 201: 85-92.

Duman, J.G. 1979. Thermal-hysteresis-factors in overwintering insects. J. Insect Physiol. 25: 805-810.

Duman, J.G. 1982. Insect antifreezes and ice-nucleating agents. Cryobiology 19: 613-627.

Duman, J.G., K.L. Horwath, A. Tomchaney, & J.L. Patterson. 1982. Antifreeze agents in terrestrial arthropods. Comp. Biochem. Physiol. 73A: 545-555.

Duman, J.G. & K.L. Horwath. 1983. The role of hemolymph proteins in the cold tolerance of insects. Annu. Rev. Physiol. 45: 261-270.

Elsey, K.D. 1988. Cucumber beetle seasonality in Coastal South Carolina. Environ. Entomol. 17: 496-502.

Elsey, K.D. 1989. Cold tolerance of adult spotted and banded cucumber beetles (Coleoptera:Chrysomelidae). Environ. Entomol. 18: 1112-1116.

Gustin, R.D. & G.E. Wilde. 1985. Cold-hardiness of western corn rootworm (Coleoptera:Chrysomelidae) eggs from northern and southern zones of the corn belt. J. Kans. Entomol. Soc. 57: 722-725.

Hamilton, R.L. 1985. Cold acclimation of the wood cockroach *Cryptocercus punctulatus* (Scudder) (Dictyoptera: Cryptocercidae). Thesis. Virginia Polytechnic Institute and State University, Blacksburg, Va.

Hamilton, R.L., D.E. Mullins, & D.M. Orcutt. 1985. Freezing-tolerance in the woodroach *Cryptocercus punctulatus* (Scudder). Experientia 41: 1535-1537.

Horworth, K.L. & J.G. Duman. 1984. Yearly variations in the over-wintering mechanisms of the cold-hardy beetle *Pendroides canadensis*. Physiol Zool. 57: 40-45.

Hoshikawa, K. 1981. Accumulation of inositol by hibernating adults of coccinellid and chrysomelid beetles. Low Temp. Sci. Ser. B 39: 45-48.

Mail, G.A. 1930. Winter soil temperatures and their relationship to subterranean insect survival. J. Agric. Res. 41: 571-592.

Mail, G.A. & R.W. Salt. 1933. Temperature as a possible limiting factor in the northern spread of the Colorado potato beetle. J. Econ. Entomol. 26: 1068-1075.

Mansingh, A. & B.N. Smallman. 1972. Variation in polyhydric alcohol in relation to diapause and cold-hardiness in the larvae of *Isia isabella*. J. Insect Physiol. 18: 1565-1571.

Nevan, L.G., J.G. Duman, M.G. Low, L.C. Sehl, & F.J. Castell. 1989. Purification and characterization of an insect lipoprotein ice nucleator: evidence for importance of phosphatidylinositol and apoliprotein in the ice nucleator activity. J. Comp. Physiol. B 159: 71-82.

Rains, T.D. & R.V Dimock, Jr. 1978. Seasonal variation in cold-hardiness of the beetle *Popilus disjunctus*. J. Insect Physiol. 24: 551-554.

Salt, R.W. 1953. The influence of food on cold-hardiness of insects. Can. Entomol. 85: 261-269.

Salt, R.W. 1965. Factors influencing nucleation in supercooled insects. Can. J. Zool. 44: 117-132.

SAS Users Guide. 1985. SAS Institute. Carey, NC.

Somme, L. 1982. Supercooling and winter survival in terrestrial arthropods. Comp. Biochem. Physiol. 73A 519-543.

Zachariassen, K.E. 1980. The role of polyols and nucleating agents in cold-hardy beetles. J. Comp. Physiol. 140: 227-234.

Zachariassen, K.E., J.G. Baust, & R.E. Lee, Jr. 1982. A method for quantative determination of ice nucleating agents in insect hemolymph. Cryobiology 19: 180-184.

Zachariassen, K.E. 1985. Physiology of cold tolerance in insects. Physiol. Rev. 65: 799-832.

Zehnder, G.W., L. Sandall, A.M. Tisler, & T.O. Powers. 1992. Mitochondrial DNA diversity among seventeen geographic populations of *Leptinotarsa decemlineata* (Coleoptera:Chrysomelidae). Ann. Entomol. Soc. Am. (In Press).

## Conclusions

Data from the within-field variation study indicated Colorado potato beetle mortality in the filter paper bioassay does not vary greatly among different locations. A single bioassay (of Colorado potato beetles from one location per field) yielded at least a 0.50 probability (> 0.90 in six of the eight fields) of estimating the mean mortality response (within 95% confidence limits) of Colorado potato beetles from all field locations. In the "worst case scenario", where approximately 50% of the field blocks yielded overlapping mortality confidence intervals, the probability of sampling Colorado potato beetles from one location which differs in mean mortality ($P \leq 0.05$) from Colorado potato beetles in another location within the same field was 0.50 (7.5/15, N=16 blocks; 15 possible block pair comparisons). The probability decreases as the number of locations sampled increases: two locations = 0.27, ([7.5/15][7/14]); three locations = 0.12, ([7.5/15][7/14][6.5/13]).

The sample size used in an insecticide resistance bioassay should be determined based on the purpose of the bioassay and the precision required (Halliday & Burnham 1990). A bioassay to detect rare resistant individuals in a pest population requires a greater number of samples (Roush & Miller 1986) than one in which a diagnostic concentration is used to prevent increased selection for resistance and future control failures. Based on the number of bioassay samples North Carolina potato growers and field scouts are willing to process, French & Kennedy (1992) recommend that the Colorado filter paper bioassay be implemented using one egg mass per treated filter paper disk as the sample unit (larval mortality is determined 24 h after hatch), with five sample units per field. The results of this study are compatible with these recommendations, given that the mean larval mortality most often did not differ significantly ($P \leq 0.05$) between field blocks. However, because our data demonstrate that the probability of making an inaccurate estimate of the mortality response of the entire Colorado potato beetle field population decreases as the number of field locations sampled increases, we recommend that the egg mass or larval samples in the bioassay be collected from different sites within each field.

The sample size and temporal variation study results indicated that from 2-5 filter paper disks (20-50 larvae) would be the most cost-effective sample size for use with the Colorado potato beetle filter paper insecticide bioassay. This determination is based on the variation in mortality among individual larvae in the bioassay (which decreases with increasing sample size), and the cost of processing the samples (which increases with increasing sample size). Because a large decrease in the relative variation in mortality was often associated with an increase in sample size from 2 to 5 filter paper disks, we recommend that samples of 5 disks (50 larvae) be used in the bioassay. This is a practical sample size, given the low processing cost (11.45 min, Tables 1-3), and should be acceptable to growers and pest management practitioners.

The time when Colorado potato beetle samples are taken may also affect results of the bioassay. The data from the experiment station study did not offer strong evidence that bioassay mortality was affected by previous insecticide application. However, larval mortality in the bioassay was often significantly different between sample collection dates. In the AmesII field, where no synthetic insecticides were applied, first generation larvae exhibited from 1.4 to 4.8 times greater susceptibility in the bioassay compared with second generation larvae, depending on the insecticide tested. Therefore, at least one bioassay should be performed per target Colorado potato beetle generation because initial results may not accurately represent the level of insecticide susceptibility of subsequent generations. If multiple insecticide applications per field are necessary, it may be prudent to conduct a bioassay following each spray to assess the level of insecticide susceptibility of the surviving Colorado potato beetle population.

The use of PIES for Colorado potato beetle insecticide application decision making reduced the number of sprays needed throughout the growing season for Colorado potato beetle control by 39%, while not significantly ($P \leq 0.05$) reducing tuber yields (Table 3). In addition to reduction in number of insecticide sprays needed for Colorado potato beetle control, use of PIES by commercial potato growers may also encourage growers to increase the amount of time spent monitoring fields for Colorado potato beetle and other pests. Heinemann et al. (1989) reported that more than 80% of the participants in their expert system studies indicated use of the expert system stimulated them to more closely monitor their orchards and 50% made some changes in production practices based on recommendations of the expert system.

PIES was developed for use in potato fields on the Eastern Shore of Va. Although region specific, the program could be adapted for other potato growing regions. Future PIES research focusing on evaluation of the system by potato growers, evaluation of the defoliation thresholds in large plots and incorporation of other pest problems (e.g. other insects, weeds, etc.) is necessary to insure maximum benefit from this expert system.

Supercooling points of Colorado potato beetle from Maine, Washington and Canada indicated Colorado potato beetle was a freeze-tolerant insect species. Zachariassen (1980) reported that a number of freeze-tolerant beetles supercooled between -7.5 to -13.5 °C, whereas freeze-sensitive beetles supercooled at -20 °C or below. Supercooling points of Colorado potato beetles examined from the northeastern and western United States, as well as from eastern Canada, are well within the temperature range of freeze-tolerant species. Supercooled Colorado potato beetles regained what appeared to be normal activity (ability to walk, antennal movements, grooming behavior, etc.) after their supercooling point was reached and they were held at room temperature (25 to 27 °C). This indicated the point at which supercooling occurred was not the lower lethal limit for survival.

Surprisingly, Colorado potato beetles collected from Virginia supercooled at temperatures significantly ($P \leq 0.05$) lower than the Maine, Washington and Canada populations. Virginia Colorado potato beetles appear to be utilizing a different mechanism of cold tolerance and are probably cold-sensitive.

Inositol and xylitol were identified in the hemolymph of all Colorado potato beetle populations examined in this study (Table 2) and are likely to play some role in cold-hardiness. Polyols have been implicated as cryoprotective agents, help to stabilize lipid membranes, and counter-act osmotic shrinkage associated with controlled freezing (Zachariassen 1985). The scope of this study does not permit speculation on the role of inositol and xylitol in Colorado potato beetle, as quantification of polyols and correlation between supercooling point and polyol concentration was not examined.

Colorado potato beetle overwintering mechanisms are complex and not well understood, but are essential for the survival of the species. Continued investigation of Colorado potato beetle supercooling capacity, role of polyols in Colorado potato beetle cold-hardiness, and investigation of other possible mechanisms used for cold tolerance is necessary to better understand the mechanisms of Colorado potato beetle cold-hardiness which may also aid in the development of alternative Colorado potato beetle control mechanisms.

# Vita

Anne Marie Tisler is the eldest of five children and only daughter of John M. Tisler and Mary Anne (Morgan) Tisler. She was born on March 31, 1964 in Fairview Park, Ohio, moved to Fairfax County, Virginia in 1966, and has resided in the Commonwealth since 1966.

Anne attended Fairfax County Public Schools from 1968 to 1982 (K-12 grade). Upon graduation, she went to Mary Washington College in Fredericksburg, Va. and completed a Bachelor of Science degree in Biology in May, 1986. While at Mary Washington, she was introduced to invertebrate zoology by Dr. William Pinschmidt, and subsequently, entomology by Dr. Joella Killian. After a mix-up in transcripts [her brother Andrew's transcripts (a Physics major at Mary Washington College) were sent to several graduate schools], she was admitted as a student at Virginia Polytechnic Institute and State University (Virginia Tech) to pursue a Master of Science Degree in Entomology.

Anne began working with Dr. John Smith and the southern corn rootworm in peanuts at the Tidewater Experiment Station in Holland, Va., and after a rocky start, she changed her major professor and research project and finished her Master's degree under Dr. Geoffrey Zehnder at the Eastern Shore Agricultural Experiment Station, Painter, Va. in December 1988. Anne continued researching Colorado potato beetle at Virginia Tech under Dr. Geoffrey Zehnder and completed her doctoral degree in June 1991.

While at the Eastern Shore Agricultural Experiment Station, Anne met her fiance, William Vencill, who completed his doctoral degree in Weed Science in

December 1988, and is an assistant professor in the Agronomy Department at the University of Georgia. They will be married on July 20, 1991.

In addition to collecting insects, Anne is an avid quilter, and enjoys sewing, cooking, and reading. She and Bill will be living in Athens, Ga.

Anne Marie Tisler