Introduction

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is a major insect pest of crucifer vegetables (*Brassica* sp.) including cabbage, broccoli, cauliflower, collards, kale, kohlrabi, Chinese cabbage, and Brussels sprouts worldwide. Collards, a staple crucifer crop in Virginia and other southern states, are particularly vulnerable to economic losses because *P. xylostella* strongly prefer the crop over other crucifers, and because the leaves, which are fed upon by *P. xylostella* larvae, are the marketable portion of the crop. Two important reasons for the heightened pest status of *P. xylostella* in many regions of the world are lack of effective natural enemies and insecticide resistance.

The overall goal of my research is to better understand *P. xylostella*, its primary natural enemies, and their susceptibilities to insecticides in Virginia in order to develop an economically and environmentally sound integrated pest management program in collards. My specific objectives include the following: 1) to conduct ecological lifetable studies of *P. xylostella* in order to identify its key biotic mortality factors in Virginia; 2) to assess the current susceptibility of field-collected *P. xylostella* to some of the most commonly-used insecticides as well as some novel insecticide chemistries; 3) to assess the susceptibility of the key natural enemies of *P. xylostella* to insecticides; and 4) to evaluate the field efficacy of these insecticides for control of *P. xylostella* and other pests in collards.

Chapter One

Literature review of *Plutella xylostella*

Thorough reviews of the biology, ecology, and management of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) can be found in Harcourt (1957), Talekar and Shelton (1993), and Capinera (2001). These references are excellent starting points for learning about this important insect pest.

Origin and distribution

Plutella xylostella is not native to the U.S., and is believed to have originated in either the Mediterranean region (Talekar and Shelton 1993) or in southern Africa. This assessment is based on the rich and diverse fauna of parasitoids of *P.* xylostella present in those regions as well as the large number of indigenous *Brassica* species and arrhenotokous form of its parasitoids (Kfir 1998). *P. xylostella* was first observed in North America in 1854, in Illinois, but quickly spread across the continent (Capinera 2001). Today, this insect occurs wherever crucifers are grown and is believed to be the most universally distributed of all Lepidoptera (Talekar and Shelton 1993).

Host range

Plutella xylostella larvae only feed on plants in the family Cruciferae, which contain mustard oil and their glucosides (Hillyer and Thorsteinson 1971). Nayar and Thorsteinson (1963) determined that the glucosides sinigrin, sinalbin, and glucocheirolin produced by crucifer plants are important to stimulate *P. xylostella* feeding. Virtually all cruciferous vegetables are attacked by *P. xylostella* including cabbage, broccoli, cauliflower, collards, kale, kohlrabi, mustard, radish, turnip, Chinese cabbage, watercress, and Brussels sprouts (Capinera 2001). *P. xylostella* also feeds on many different cruciferous weeds, including yellow rocket, *Barbarea vulgaris*; shepherdspurse, *Capsella bursa-pastoris*; pepperweed, *Lepidium* spp.; and wild mustards, *Brassica* spp., which serve as

important alternate hosts for the pest, especially in spring before crucifer vegetable crops are planted (Talekar and Shelton 1993, Capinera 2001).

Pest status

Plutella xylostella has become the most destructive insect pest of crucifer vegetables worldwide, and annual costs for managing it are estimated at $1 billion (Talekar 1992, Talekar and Shelton 1993). The absence of effective natural enemies and insecticide resistance are believed to be the major cause of the *P. xylostella* pest status in most parts of the world (Lim 1986, Talekar and Shelton 1993). In 1997 in California alone, there was an outbreak of diamondback moth that resulted in crop losses in broccoli and other crucifers estimated to be more than $6 million (Sances 1997, Shelton et al. 2000). In Virginia, most of the commercial crucifer production is fresh-market cabbage, broccoli, and collards. Collards are particularly vulnerable to serious economic losses because *P. xylostella* has a strong preference for the crop (Harcourt 1957, Mitchell et al. 2000), and because the leaves, which *P. xylostella* larvae feed on, are the marketable portion of the crop.

Life history

In the southeastern U.S., *P. xylostella* breeds continuously and there are from 12 to 15 generations per year (Capinera 2001). In eastern Virginia, all life stages of *P. xylostella* may be found at practically any given time of the year (T. P. Kuhar, *personal communication*). Development rate is dependent upon temperature, and typically one life cycle is completed in about 30 days (Harcourt 1957). It is not known for sure whether any of the lifestages of *P*. *xylostella* diapause or hibernate (Talekar and Shelton 1993).

Adult stage. The adult is a slender, grayish-brown moth about 6 mm long with pronounced antennae. The moth is marked with a broad, cream- or light-brown colored band along the back that is sometimes constricted to form one or more light-colored diamonds on the back (especially in males), which is the basis for the common name of this insect (Capinera 2001). Adults can live up to 7 or 8

weeks, but usually the life span is around 2 weeks (Harcourt 1957). Adults normally rest on the host plant during the day and become more active just before dusk, when most mating and egg laying occurs (Harcourt 1957). Adults feed on flower nectar. Sex ratio of adults in the field is roughly 50:50, and most mating typically occurs on the day of emergence. Female fecundity ranges from 18 to 356, with an average of 159 eggs per female (Harcourt 1957). Fecundity is highest in the earliest generations of the year, and gradually drops off as the season progresses (Capinera 2001).

Egg stage. *P. xylostella* eggs are oval, flattened, approximately 0.44 mm long by 0.26 mm wide, and yellow or pale green in color. Eggs are deposited singly or in small groups of 2-8 eggs usually in depressions on the surface of foliage, or sometimes on other parts of the plant (Capinera 2001). Lasota and Kok (1989) found that approximately 50% more eggs were laid on the upper surfaces of cabbage leaves than on the lower surfaces. Egg incubation period in the field ranges from 4 to 8 days, averaging 5.6 days (Harcourt 1957).

Larval stage*.* The larval stage of *P. xylostella* includes four instars and typically requires from 16 to 20 days to complete development (Harcourt 1957). Average development time is about four to five days for each instar (Capinera 2001). First instars are tiny (1.7 mm long) and colorless to cream colored with a dark head capsule. After egg hatch, they crawl to the undersides of leaves, bore through the epidermis and mine the spongy mesophyll tissue. Subsequent instars are green in color and reach a maximum length of 11.2 mm. The body bears relatively few hairs. Most hairs are short in length and marked by the presence of small white patches (Capinera 2001). Larval body form tapers at both ends, and a pair of prolegs protrudes from the posterior end, forming a “V” shape. Later instars consume leaf tissue from the undersides of leaves, chewing irregular patches, but often leaving the top epidermal layer and leaf veins, which creates a window-like appearance to the injury. When disturbed, larvae often wriggle violently moving backward and spin down on a strand of silk (Capinera 2001).

Pupal stage. When fully grown, the prepupal larva constructs a loose silk cocoon, usually formed on the lower or outer leaves of the host plant. After a couple days of quiescence, pupation occurs inside the cocoon. The pupa is yellow and approximately 7 to 9 mm long (Capinera 2001). The pupal stage requires from 5 to 15 days (average 8.5 days) to complete development.

Damage

Damage is caused by larval feeding, or by the presence of insects (usually larvae) contaminating produce. Although they are small relative to other lepidopteran pests (such as cabbage looper, *Trichoplusia ni*, or imported cabbageworm, *Pieris rapae*) densities of *P. xylostella* larvae can reach levels that result in total destruction of leaves. For crops such as broccoli, the presence of larvae in florets can result in the total rejection of the produce (Capinera 2001).

Sampling

Plutella xylostella populations are usually monitored by visual counts of larvae or damage on plants. In some states, treatment is recommended if populations exceed 0.3 larva per plant (Kirby and Slosser 1984). In Florida and Georgia, treatment is recommended when one or more leaf-feeding holes are found per plant (Capinera 2001). In Virginia, treatment is recommended if >20% of plants have at least one larvae preheading, and if >5% of plants have at least one larva from heading to harvest (Bratsch et al. 2005). Harcourt (1961) recommended a minimum sample size of 40 to 50 plants for reliable estimates of *P. xylostella* densities in cabbage.

Cultural control strategies

Crop rotation. Because *P. xylostella* has a narrow host range (crucifers only), elimination of the host by crop rotation can reduce its population levels and subsequent damage. Mandatory crucifer-free periods have been undertaken as a control strategy for *P. xylostella* in regions of Mexico and Australia (Sayyed et al. 2002). However, this tactic is often not feasible in most commercial

vegetable-producing areas because of commercial demand for crucifer vegetables and/or the high price received for such crops.

Intercropping. Many studies have shown that vegetational diversity in the form of intercropping can result in reduced pest densities. This strategy works in the following two ways: (1) the insect is less likely to find its host plant due to visual and chemical interference; and (2) the insect is more likely to leave the host patch because of frequent encounters with non-host plants (Asman et al. 2001). Intercropping cabbage with tomatoes, garlic, dill, or clover has been shown to reduce the density of *P. xylostella* on cabbage plants (Buranday and Raros 1975, Dover 1986, Talekar et al. 1986). However, large-scale experiments to test the repellency of these plants in the field have yielded inconsistent results (Latheef and Irwin 1979, Ivey and Johnson 1998). Asman et al. (2001) demonstrated that cabbage intercropped with high clover densities received significantly fewer eggs of *P. xylostella* compared with cabbage monocultures.

Trap cropping. Because collard plants are more attractive to *P. xylostella* than other crucifer vegetables (Harcourt 1957, Mitchell et al. 1997a), planting collards in field peripheries may be an effective tactic to manage *P. xylostella* in crops such as cabbage or broccoli. Mitchell et al. (2000) showed that densities of *P. xylostella* never exceeded the action threshold of 0.3 larva/plant in cabbage fields that were surrounded by collards, but did exceed threshold in three out of nine fields of conventional monoculture cabbage. Moreover, the numbers of insecticide applications were greatly reduced in cabbage surrounded by collards compared with conventional cabbage, but with no reduction in marketable yield.

Resistant varieties

Crucifer crops differ somewhat in their susceptibility to attack by *P. xylostella*. Mustards, turnip, and kohlrabi are among the most resistant crucifers (Capinera 2001). The presence of leaf wax is another major component of resistance. Plants with glossy leaf waxes apparently elicit non-acceptance behavior in *P. xylostella* neonate larvae, which result in their failure to successfully establish on

these plants (Eigenbrode and Shelton 1990). Larvae spend more time searching and less time feeding on glossy varieties. Glossy plants also may enhance predation of *P. xylostella* because of the increased searching rather than tunneling or mining by early instars, and because of improved mobility of several predators, which do not move well on normal-wax crucifer plants (Eigenbrode and Trumble 1994, Eigenbrode et al. 1995).

Mating disruption

The sex pheromone emitted by female *P. xylostella* has been identified as a three component mixture containing (*Z*)-11-hexadecenal, (*Z*)-11-hexadecenyl acetate, and (*Z*)-11-hexedecenyl alcohol, and is commercially available (Chisholm et al. 1979). High concentrations of the pheromone have been used for mating disruption of *P. xylostella* in cabbage fields in Japan, but the strategy is not cost effective (Talekar and Shelton 1993). In the U.S., successful control of *P. xylostella* was achieved with mating disruption pheromones supplemented with some insecticide applications (Mitchell et al. 1997b). However, Schroeder et al. (2000) reported that mating disruption of *P. xylostella* was not effective even under very controlled conditions.

Biological control

Plutella xylostella eggs, larvae, pupae, and adults are attacked by numerous natural enemies, which in many instances help to maintain populations below damaging levels. In fact, pest outbreaks are often attributed to lack of effective natural enemies in a specific region or disruption of these natural enemies (Talekar and Shelton 1993).

***Parasitoids*.** Numerous studies suggest that parasitoids play an essential role in the natural control of *P. xylostella* (Harcourt 1960, Pimentel 1961, Talekar and Shelton 1993). Goodwin (1979) reported over 90 species of parasitoids attacking *P. xylostella* worldwide, but only about 60 species were important in Australia. Lim (1986) reported 6 species of parasitoids attacking eggs, 38 attacking larvae, and 13 attacking pupae of *P. xylostella*. Egg parasitoids

belonging to the genus *Trichogramma* are present in relatively small numbers and do not contribute much to the population regulation of *P. xylostella* (Talekar and Shelton 1993). Larval parasitoids are the most predominate and most effective. Worldwide, the most efficacious larval parasitoids belong to two major genera, *Diadegma* and *Cotesia* (=*Apanteles*) (Lim 1986). In North America, *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) and *Microplites plutellae* (Muesebeck) (Hymenoptera: Braconidae) are dominant species of parasitoids of *P. xylostella,* particularly in the northern U.S. (Pimental 1961, Harcourt 1963, Putnam 1968, Oatman and Platner 1969, Kok 2004). *Diadegma insulare* emerges from the host after the latter has spun its cocoon, and spins its own cocoon within; whereas, *M. plutellae* emerges from the still active host larva, and spins a brown-colored cocoon, less conspicuous than that of *D. insulare* (Pimentel 1961). From different regions in North America, researchers have reported parasitization rates from *D. insulare* ranging from 35 to 72% (Harcourt 1960, 1963, Oatman and Platner 1969, Mitchell et al. 1997a). In southwestern Virginia, Lasota and Kok (1986) reported *D. insulare* parasitization ranging from 46 to 69% in pesticide-free cabbage. Even higher rates of parasitism have been reported in eastern Virginia (T. P. Kuhar, *unpublished data*). *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) is another important parasitoid of *P. xylostella* (Latheef and Irwin 1983). It is a gregarious larval-pupal parasitoid that is well adapted to the high temperature conditions that are typical of the southeastern U.S. (Fitton and Walker 1992, Talekar and Hu 1996).

***Predators*.** Ullyet (1947) reported that predatory arthropods are an important source of *P. xylostella* mortality and include various staphylinids, vespids, syrphids, chrysopids, hemerobiids, anthocorids, and spiders. Further, Muckenfuss and Shepard (1994) estimated that predators kill an average of 90% of *P. xylostella* 1st instars. Birds also prey on *P. xylostella* larvae and pupae (Harcourt 1960).

***Entomopathogenic nematodes*.** *Steinernema carpocapsae* has been the most effective nematode tested against *P. xylostella* causing up to 100% mortality of larvae after 6 hours of exposure and 40% mortality of pupae

(Ratnasinghe and Hague 1998). A foliar application of infective juveniles of *S. carpocapsae* caused 98% mortality of *P. xylostella* larvae in the field (Lello et al. 1996). Further research with other nematode species and with ways to improve the economic feasibility of this control strategy should be pursued.

***Entomopathogenic fungi*.** *Zoophthora* (=*Erynia*) *radicans* Brefeld (Zygomycetes: Entomophthorales) has been identified as an important pathogen of *P. xylostella* causing epizootics in populations all over the world (Furlong and Pell 1996). In England, the fungus is being commercially developed as a pest management tool for *P. xylostella* (Furlong et al. 1995). Vandenberg et al. (1998) have reported the potential of *Beauveria bassiana* to control *P. xylostella*, and Shelton et al. (1998) have shown that later instars of *P. xylostella* appeared to be more susceptible to *B. bassiana* than earlier instars.

***Virus*.** Kadir et al. (1999) indicate that granulosis virus was highly infective against *P. xylostella* and further suggested that it could be developed as a selective microbial pesticide. The nuclear polyhedrosis virus of alfalfa looper, *Autographa californica* (AcMNPV) and closely related genomic variants such as *Galleria mellonella* (MNPV) are also infective to *P. xylostella* and may have potential as a control agent (Farrar and Ridgway 1999).

Chemical control

Historically, the control of *P. xylostella* has centered on conventional insecticides. General use patterns of insecticides vary widely over geographic locations and decades, and have been greatly influenced by the development of insecticide resistance and to novel insecticide products becoming available on the market. *Plutella xylostella* has developed resistance to almost every class of insecticide used against it (Shelton and Wyman 1990, Talekar and Shelton 1993, Shelton et al. 1993a, Shelton et al. 2000). It was one of the first agricultural pests in the world to develop resistance to DDT (Ankersmith 1953, Johnson 1953), and later to the microbial insecticide *Bacillus thuringiensis* (Berl.) (Kirsch and Schmutterer 1988, Tabashnik et al. 1990). According to Sayyed et al. (2000a, 2000b), *P. xylostella* remains as the only insect species that has developed resistance in the

field to *Bacillus thuringiensis,* and this has occurred in the South Pacific as well as in North and South America (Tabashnik 1994, Imai and Mori 1999, Diaz-Gomez et al. 2000, Liu et al. 2000, Sayyed et al. 2000a, Shelton and Wyman 1990). Georghiou (1981) reported *P. xylostella* resistance to 36 insecticides in 14 countries. In many regions of the world, the traditional classes of insecticides: organophosphates, carbamates, organochlorines, pyrethroids, and some botanicals no longer control *P. xylostella* (Tabashnik et al. 1990, Talekar and Shelton 1993). In certain populations from North America, *P. xylostella* was reported to be more than 100 fold resistant to the pyrethroid, permethrin, and the carbamate, methomyl, and more than 200 fold to *B. thuringiensis* (Shelton and Wyman 1990). Shelton et al. (1993b) found up to 461 fold resistance ratio to *B. thuringiensis* subsp. *kurstaki* in some populations. The levels and types of insecticide resistance in *P. xylostella* populations in Virginia are not currently known. Control measures for *P*. *xylostella* and other pests in crucifers have typically involved the prophylactic use of insecticides applied on a 7-to10 day schedule, with little regard for pest population level (Lasota and Kok 1986). This management approach certainly favors the development of resistance. Some frequently-used insecticides in Virginia crucifer crops have included methamidophos, methomyl, fenvalerate, permethrin, and *Bt* formulations (Lasota and Kok 1986). However, in recent years several novel insecticide chemistries have been developed and are registered for use in crucifer crops including avermectins, neonicotinoids, spinosyns, pyrazolines, and various insect growth regulators.

The overall efficacy of these insecticides to control *P. xylostella* in collards in Virginia as well as their toxicity to natural enemies has not been thoroughly tested.

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Chapter Two

Life tables of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) on collards in two regions of Virginia

Diamondback moth, *Plutella xylostella* (L.) has become the most important insect pest of crucifer crops worldwide because of insecticide resistance and lack of effective natural enemies (Talekar and Shelton 1993). The insect is particularly damaging to collards (*Brassica oleracea* L. a*cephala* group) because of a strong host preference for the crop over other crucifers (Harcourt 1957, Mitchell et al. 1997), and because larval feeding causes direct damage to leaves, which are the marketable portion of the crop. Development of a sustainable integrated pest management program for *P. xylostella* on collards requires a strong understanding of the ecological processes governing the population dynamics of this pest.

Life tables provide an ecological tool to measure survivorship, mortality and mortality factors of an organism under natural conditions (Morris and Miller 1954, Harcourt 1969). Multiple sets of life table data can be analyzed to identify key mortality factors or critical life stages or periods, which can increase our understanding of the dynamics of an insect population and at the same time reveal the most opportune periods for management (Harcourt 1969, Varley and Gradwell 1970, Southwood 1978, Bellows et al. 1992).

Life table studies of *P. xylostella* have been conducted in several different countries (Harcourt 1969, Keinmeesuke et al. 1991, Syed and Abro 2003, Furlong et al. 2004). Some of these studies were limited to laboratory conditions (Syed and Abro 2003), or emphasize only specific mortality factors (Keinmeesuke et al. 1991). Harcourt (1969) conducted a thorough life table study of *P. xylostella* in Canada, and Furlong et al. (2004) did likewise in Australia. However, the natural enemy fauna attacking *P. xylostella* varies widely across geographic locations, as does the impact of abiotic factors such as climate.

In order to better understand the ecology and mortality factors of the pest in Virginia, I conducted life table studies of *P. xylostella* on collards in two climatologically distinct locations: Painter, on the Eastern Shore, and Blacksburg, in the southwestern ridge and valley region of the state.

Materials and methods

One method for constructing a life table is called an "age-specific" or horizontal life table (Southwood 1978). Data are collected that represent the fate of a real group or cohort, typically a generation of individuals whose number and mortalities are determined over the course of time for each of a series of stages. This type of life table can also be developed by artificially releasing a known quantity of cohorts and following their fate over time (Bellows et al. 1992). I took this approach for studying *P. xylostella* on collards in Virginia.

Field plots. Field experiments were conducted simultaneously in two locations of Virginia in 2003 and 2004. The first location was the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) near Painter (75^{o} 49' W, 37^{o} 35' N; elevation ≈12 m) on the Delmarva Peninsula. The second location was the Virginia Tech Kentland Research Farm (80^{o} 25' W, 37^{o} 14' N; elevation ≈640 m), near Blacksburg at the New River Valley. Weather stations were maintained at both research farms to record daily minimum and maximum temperature as well as precipitation data (Appendix 1-4). In both years, four separate 400-m^2 plots of collards variety 'Vates' were planted in late July at both locations. Collards were grown on 0.6-m row centers at a density of ~10 plants per row meter, and maintained according to recommended production practices in Virginia (Bratsch et al. 2005), except that no insecticides were applied.

Sampling. In early September, 40 plants in each plot were marked with flags and any surrounding plants within a 0.5-m radius were removed. Marked plants were picked clean of any natural *P. xylostella* life stages. On the underside of the largest leaf of each marked plant, I fastened a 5-cm foil strip containing 20 to 30 freshly-laid *P. xylostella* eggs from Benzon Research Inc.

(Carlisle, PA). After 7 days, the foil strips were retrieved from the field and assessed percentage egg hatch and parasitization by examining the eggs under a dissecting scope in the lab. Parasitized eggs were assumed to have turned black in color (Flanders 1937). Every three to five days, starting after egg hatch until final pupation, plants were carefully sampled for *P. xylostella* neonates (1st instars), small larvae (2nd instars), large larvae (3rd and 4th instars), and pupae. Also, any arthropod predators that were observed on plants were identified to the best taxonomic level possible without disturbing or removing the specimens. After a couple of days in the field, any *P. xylostella* pupae and their cocoons were collected from plants and held in the lab at 27 ± 3 °C, 40 to 70% RH, and a photoperiod of 12:12 (L:D) to assess percentage parasitization, mortality, or adult emergence. Any parasitoids that were found were reared to adult stage in Petri dishes and sent to the USDA Systematic Entomology Laboratory, Beltsville, Maryland for identification. In each year, the same experiment was repeated with another batch of eggs in October using the same collard plots at both locations. Voucher specimens were deposited at the collection of the department of Entomology at VPI and SU.

Life tables. Life tables were developed following the parameters and column headings of Morris and Miller (1954) and Harcourt (1969) where:
x is the different life stage intervals
lx is the number of individuals entering *x* over an entire generation
dxf is the mortality factor
dx is the number of individuals dying from various mortality factors (*dxf*)
100*qx* is % mortality
k is mortality calculated from the difference between logarithms of *lx* before and after the action of *dxf.*
Plutella xylostella life stages (*x*) were similar to those used by Harcourt (1969); eggs, neonates, small larvae, large larvae, pupae, and adults (Fig. 2.1 a-e).

Statistical Analyses. Differences in mortalities between locations were analyzed separately by year using one-way ANOVA (SAS Institute 1999). All proportion data were transformed [arcsine(sqrt(x)] before analysis. Pearson

correlation test was used to associate neonates and small larval mortalities with rainfall data using Statistix 8.0 (Analytical Software 2003).

Results

2003. In 2003, total mortality of *P. xylostella* (from egg to adult stage) averaged between 99.0 and 99.6% at both locations (Table 2.1). Mortality from eggs not hatching was significantly lower at the Eastern Shore (22.3%) than the New River Valley (69.7%) ($F = 57.9$; df = 3; $P < 0.0048$). No egg parasitoids were found at either location. Mortality during neonate establishment averaged between 39 and 56% at both locations and was not significantly different ($F = 0.04$; df = 3; $P = 0.8575$). Mortality of small larvae disappearing averaged 71.4% at the Eastern Shore and 61.2% at the New River Valley ($F = 12.46$; df = 3; $P < 0.038$). In contrast, mortality of large larvae disappearing averaged 51.1% at the Eastern Shore and was significant lower than the 75.2% at the New River Valley ($F = 21.59$; df = 3; $P < 0.019$). Pupal mortality averaged 22.9% at the Eastern Shore compared with 62.5% at the New River Valley ($F = 43.7$; df = 3; $P < 0.0001$).

Three species of parasitoids were found in 2003: 1) *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) (Fig. 2.1 f), a solitary larval parasitoid that emerged from the *P. xylostella* pre-pupal stage inside the cocoon; 2) *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) (Fig. 2.1 g), a gregarious parasitoid that emerged from *P. xylostella* pupae (averaging 8.7 adults/host); and 3) *Microplitis plutellae* (Muesebeck) (Hymenoptera: Braconidae) (Fig. 2.1 h), a solitary parasitoid that emerged from the 4th instar of *P. xylostella*. Parasitization by *D. insulare* averaged 26.1% at the Eastern Shore and was lower (9.2%) in the New River Valley ($F = 26.38$; df = 3; $P < 0.001$). *Oomyzus sokolowskii* was found only at the Eastern Shore, where it parasitized an average of 15.0% of pupae. *Microplitis plutellae,* on the other hand, was found only in the New River Valley where it parasitized only a few (0.9%) of larvae.

Rainfall was high in 2003 at both locations, and likely contributed to disappearance of neonates, small larvae and perhaps large larvae (Fig. 2.2).

Notable rainfall peaks occurred soon after egg hatch at both locations, and intermittently during the larval stages.

2004. In 2004, total mortality of *P. xylostella* averaged 98.0 and 99.5% at the Eastern Shore and at the New River Valley, respectively (Table 2.2). Mortality from eggs not hatching averaged 21.1% at the Eastern Shore and, as in 2003, was again significantly higher (41.1%) than the New River Valley (F = 33.06; df = 3; $P < 0.001$). Mortality during neonate establishment was higher at the Eastern Shore (82.8%) than the New River Valley (37.1%) (F =9.08; df = 3; $P < 0.0021$). Mortality of small larvae disappearing averaged 31.1% at the Eastern Shore and was significant lower than the 71.6% at the New River Valley (F = 25.87; df = 3; $P < 0.0001$). Mortality of large larvae disappearing averaged 46.4% at the Eastern Shore and was significant lower than the 85.9% at the New River Valley (F = 20.49; df = 3; $P < 0.0001$). Pupal mortality averaged 34% at the Eastern Shore and was significantly higher than the 9.5% averaged at the New River Valley (F = 8.46; df = 3; $P < 0.0027$).

The same three species of parasitoids that were found in 2003 were found in 2004. Parasitization by *D. insulare* averaged 13.5% at the Eastern Shore and was lower (5.1%) than the New River Valley (F = 4.78; df = 3; $P < 0.0204$). *Oomyzus sokolowskii* was again found only at the Eastern Shore, where it parasitized an average of 11.3% of pupae and, *M. plutellae* was again found only at the New River Valley where it parasitized 4.0% of larvae.

Rainfall was again high in 2004 at both locations, and likely contributed to disappearance of *P. xylostella* immature stages (Fig. 2.2). Combining data across locations and years into a regression analysis of mortality of neonates and small larvae and rainfall (mm) that occurred between sampling periods explained about 58% of the variation in mortality (Fig. 2.3; r^2 = 0.58; $P < 0.0003$).

Discussion

A number of abiotic and biotic mortality factors can affect the natural intra-generation population dynamics of *Plutella xylostella* (Harcourt 1969, Keinmeesuke et al. 1991, Syed and Abro 2003, Furlong et al. 2004). In my

study, at two different locations in Virginia, *P. xylostella* suffered high total mortality (98-100%) from egg to adult stage. Similar results were found by Harcourt (1969) in Ontario, Canada. The relative contributions of each of the individual mortality factors, however, can vary. In my study, *P. xylostella* egg mortality (from not hatching) was higher at the New River Valley (41 to 70%) than the Eastern Shore (21 to 22.3%) in both years. Lower temperatures at the New River Valley, particularly at night and in late fall probably contributed to this difference. Mortality from disappearance of neonates, small larvae, and large larvae was variable across locations and years in my study, but was one of the biggest mortality factors overall. Furlong et al. (2004) also found that larval disappearance was the main mortality factor of *P. xylostella* on cabbage in Queensland, Australia. Although it is impossible to determine for sure, some biotic and abiotic factors that may have contributed to the disappearance of *P. xylostella* life stages during my study include: rainfall, predation, dispersal, and disease. Rainfall was high in both years and undoubtedly contributed substantially to larval disappearance through physical displacement, neonate drowning, or epizootics of pathogens. Harcourt (1969, 1986) indicated that small larval mortality of *P. xylostella* was directly related to the amount of rainfall that occurs during this life stage interval explaining 60% (r^2 = 0.598) of its variation. A similar regression analysis of the data in my study revealed similar results, with rainfall explaining 58% of the variability in small larval disappearance.

Although the actual role of predation in my study was not known, some arthropod predators that were observed on the collard plants included: spiders (Araneae); the lady bug *Coleomegilla maculata* (Coccinellidae); syrphid larvae (Syrphidae); green lacewing larvae, *Chrysoperla* spp. (Chysopidae); big-eyed bugs, *Geocoris* spp. (Lygaeidae); and assassin bugs (Reduviidae). Ullyet (1947) reported that predatory arthropods are an important source of *P. xylostella* mortality and include various staphylinids, vespids, syrphids, chrysopids, hemerobiids, anthocorids, and spiders. Further, Muckenfuss and Shepard (1994) estimated that predators kill an average of 90% of *P. xylostella* 1st instars.

Many studies suggest that parasitoids play an important role in the natural control of *P. xylostella* (Harcourt 1960, Pimentel 1961, Talekar and Shelton 1993). In my study in Virginia, *D. insulare* was the dominant parasitoid at both locations, parasitizing from 5 to 27% of larvae. Others have reported *D. insulare* as a dominant species in North America (Pimental 1961, Harcourt 1963, Putnam 1968, Oatman and Platner 1969, Kok 2004). In southwestern Virginia, Lasota and Kok (1986) reported *D. insulare* parasitization ranging from 46 to 69%. Another parasitoid found in my study only at the Eastern Shore location was *O. sokolowskii,* which parasitized 11 to 15% of *P. xylostella* pupae. This gregarious larval-pupal parasitoid appears to be better adapted to the high temperature conditions that are typical of the southeastern U.S. (Fitton and Walker 1992, Talekar and Hu 1996). The only other parasitoid found in my study was *M. plutellae*, which was only found at the New River Valley, and which only parasitized relatively few larvae. However, *M. plutellae* is considered one of the dominant species of parasitoids of *P. xylostella* in the U.S. (Pimental 1961, Harcourt 1963, Putnam 1968, Oatman and Platner 1969, Kok 2004).

In summary, the results of my life table study on *P. xylostella* showed that there are many natural mortality factors impinging on populations of this pest in collards. For sustainable management of this important pest, these mortality factors should be included in or not disrupted by other control practices in an integrated pest management program.

Summary. In fall 2003 and 2004, life table studies of *Plutella xylostella* were conducted in two regions of Virginia, the Eastern Shore and the New River Valley in the Appalachian Mountains. In both years and locations, four separate field plots of collards were inoculated with *P. xylostella* eggs and sampled regularly until final pupation of the cohort. *Plutella xylostella* had a high mortality from egg to adult stage (98-100%). Egg mortality (from not hatching) was higher in the New River Valley (41 to 70%) than the Eastern Shore (21 to 23%) in both years. No egg parasitoids were found at either location. Mortality from disappearance of neonates, small larvae, and large larvae was the highest mortality factor overall. Some abiotic and biotic factors that may have

contributed to the disappearance of *P. xylostella* life stages included rainfall, predation, parasitism, dispersal, and disease. Rainfall was high in both years and contributed significantly to larval disappearance through physical displacement, neonate drowning, or epizootics of pathogens. Some arthropod predators that were observed on the collard plants included: spiders (Araneae); lady beetles, *Coleomegilla maculata* (Coccinellidae); syrphid larvae (Syrphidae); *Chrysoperla* spp. (Chysopidae); *Geocoris* spp. (Lygaeidae); and ambush bugs (Reduviidae). *Diadegma insulare* was the dominant parasitoid at both locations, parasitizing from 5 to 27% of larvae. *Oomyzus sokolowskii,* which parasitized 11 to 15% of *P. xylostella* pupae, was found only on the Eastern Shore. *Microplitis plutellae* was only found in the New River Valley, and parasitized relatively few larvae.

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Table 2.1. Composite life tables of *Plutella xylostella* on collards at two locations in Virginia in late summer and fall 2003 (numbers represent a mean of eight plots).

Eastern Shore, Painter, VA

x	***lx***	***dxf***	***dx***	**100*qx***	***k***
Eggs deposited	88.3 (85.1-91.5) (±2.2) [a]	did not hatch	19.6	22.3	0.252
Neonates	68.6 (64.3-73) (±3.1)	did not establish	38.2	55.7	0.815
small larvae	30.4 (25.2-35.6) (±3.6)	loss of small larvae	21.6	71.4	1.245
large larvae	8.7 (7.2-10.3) (±1.1)	*D. insulare*	2.3	26.1	0.302
		loss of large larvae	4.5	51.1	1.179
pupa	1.9 (1.4-2.5) (±0.3)	*O. sokolowskii*	0.2	15.0	0.162
		unknown causes	0.8	40.0	0.425
moth	0.9 (0.7-1.2) (±0.2)				
Total					4.380

Virginia Tech Kentland Research Farm, at the New River Valley, VA

x	***lx***	***dxf***	***dx***	**100*qx***	***k***
Eggs deposited	153.4 (148.9-157.9) (±3.18) [a]	did not hatch	106.8	69.7	1.193
Neonates	46.5 (23.8-69.3) (±16.1)	did not establish	18.5	39.7	0.507
small larvae	28.1 (11.5-44.6) (±11.7)	loss of small larvae	17.2	61.2	0.950
large larvae	10.8 (8.1-13.6) (±1.9)	*D. insulare*	1.0	9.2	0.096
		M. plutellae	0.1	0.9	0.011
		loss of large larvae	8.2	75.2	1.812
pupa	1.5 (1.2-1.9) (±0.2)	unknown causes	1.0	62.5	0.980
moth	0.6 (0.5-0.6) (±0.04)				
Total					5.548

[a] range and se

Table 2.2. Composite life tables of *Plutella xylostella* on collards at two locations in Virginia in late summer and fall 2004 (numbers represent a mean of eight plots).

Eastern Shore, Painter, VA

x	***lx***	***dxf***	***dx***	**100*qx***	***k***
Eggs deposited	68.6 (35.6-101.7) (±23.3) [a]	did not hatch	10.7	21.1	0.248
Neonates	57.9 (24-91.8) (±23.9)	did not establish	49.5	82.8	1.794
small larvae	8.5 (5.1-11.8) (±2.3)	loss of small larvae	3.2	31.1	0.398
large larvae	5.3 (4.3-6.3) (±0.7)	*D. insulare*	0.7	13.5	0.145
		loss of large larvae	2.5	46.4	0.771
pupa	2.1 (1.8-2.3) (±0.17)	*O. sokolowskii*	0.2	11.3	0.123
		unknown causes	0.5	22.9	0.296
moth	1.4 (1.2-1.5) (±0.1)				
Total					3.775

Virginia Tech Kentland Research Farm, at the New River Valley, VA

x	***lx***	***dxf***	***dx***	**100*qx***	***k***
Eggs deposited	95.2 (79.8-110.5) (±10.8) [a]	did not hatch	39.2	41.1	0.530
Neonates	56.0 (35.6-76.4) (±14.4)	did not establish	20.8	37.1	0.464
small larvae	35.2 (7.2-63.2) (±19.7)	loss of small larvae	25.2	71.6	1.258
large larvae	10.0 (1.5-18.4) (±5.9)	*D. insulare*	0.5	5.1	0.052
		M. plutellae	0.4	4.0	0.043
		loss of large larvae	8.6	85.9	1.515
pupa	0.5 (0.05-0.95) (±0.3)	unknown causes	0.1	9.5	0.105
moth	0.5 (0.02-0.87) (±0.3)				
Total					3.968

[a] range and se

(a) *P. xylostella* adult

(b) *P. xylostella* eggs

(c) *P. xylostella* neonate larva

(d) *P. xylostella* large larva and feeding injury

(e) *P. xylostella* pupal cocoon

(f) *Diadegma insulare* adult and pupa

(g) *Oomyzus sokolowskii* adults emerging from *P. xylostella* pupa.

(h) *Microplitis plutellae* adult and pupa

Fig. 2.1. *Plutella xylostella* life stages (a)-(e), and its parasitoids (f)-(h) on collards in Virginia.

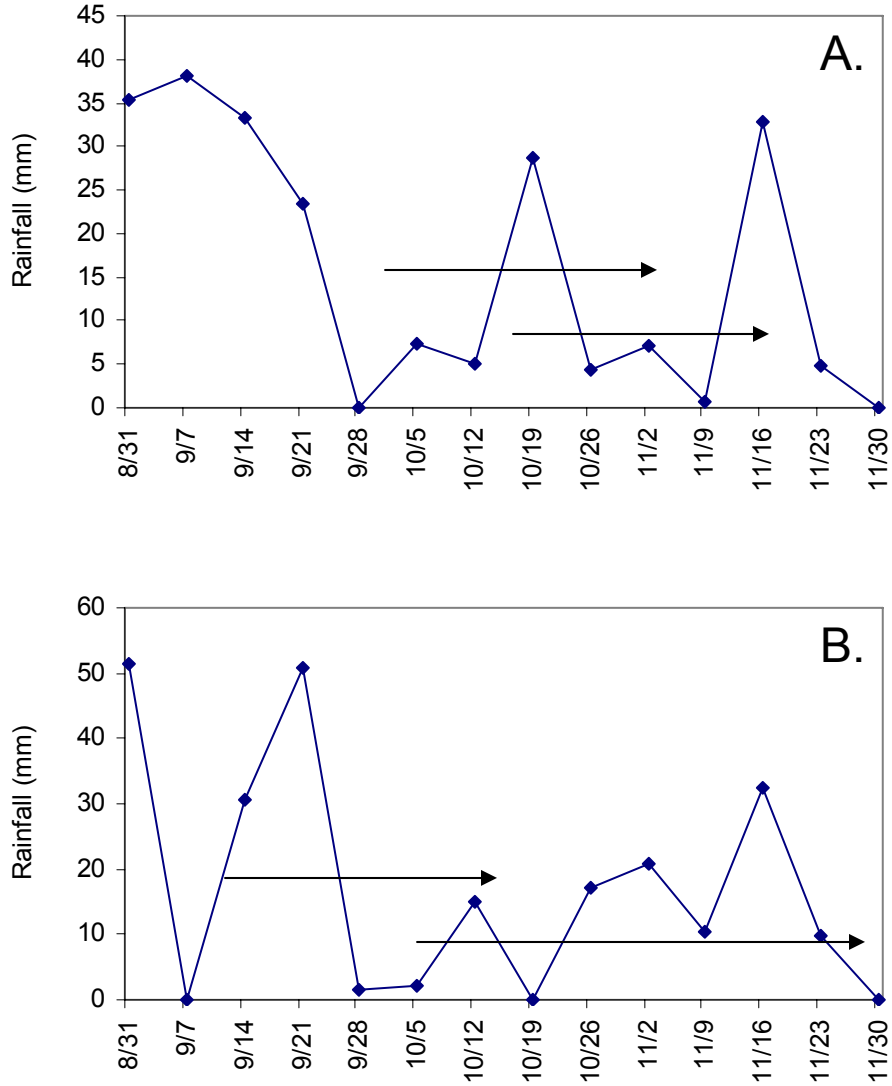

Fig. 2.2. Precipitation over time in 2003 at the Eastern Shore, VA (A) and Kentland Research Farm near Blacksburg, at the New River Valley, VA (B). Arrows represent the period between egg stage and final large larvae during two *Plutella xylostella* life tables sampling periods.

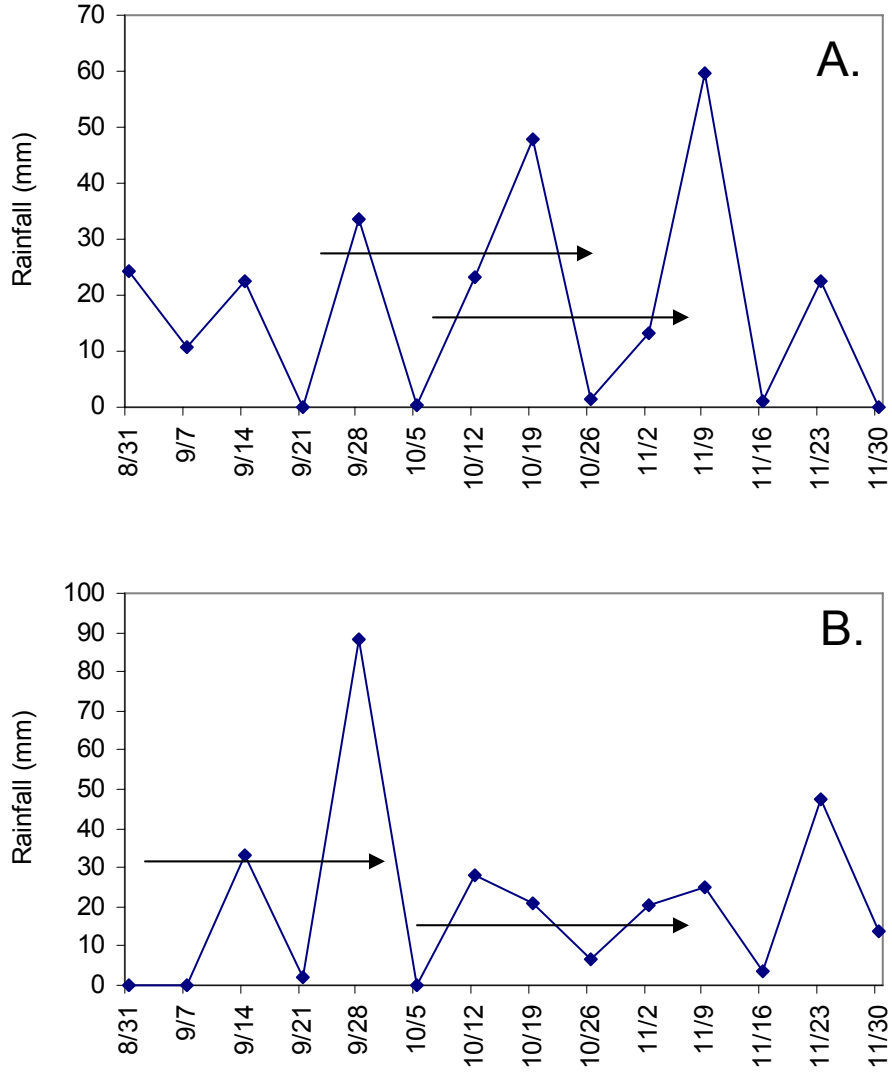

Fig. 2.3. Precipitation over time in 2004 at the Eastern Shore, VA (A) and Kentland Research Farm near Blacksburg, at the New River Valley, VA (B). Arrows represent the period between egg stage and final large larvae during two *Plutella xylostella* life tables sampling periods.

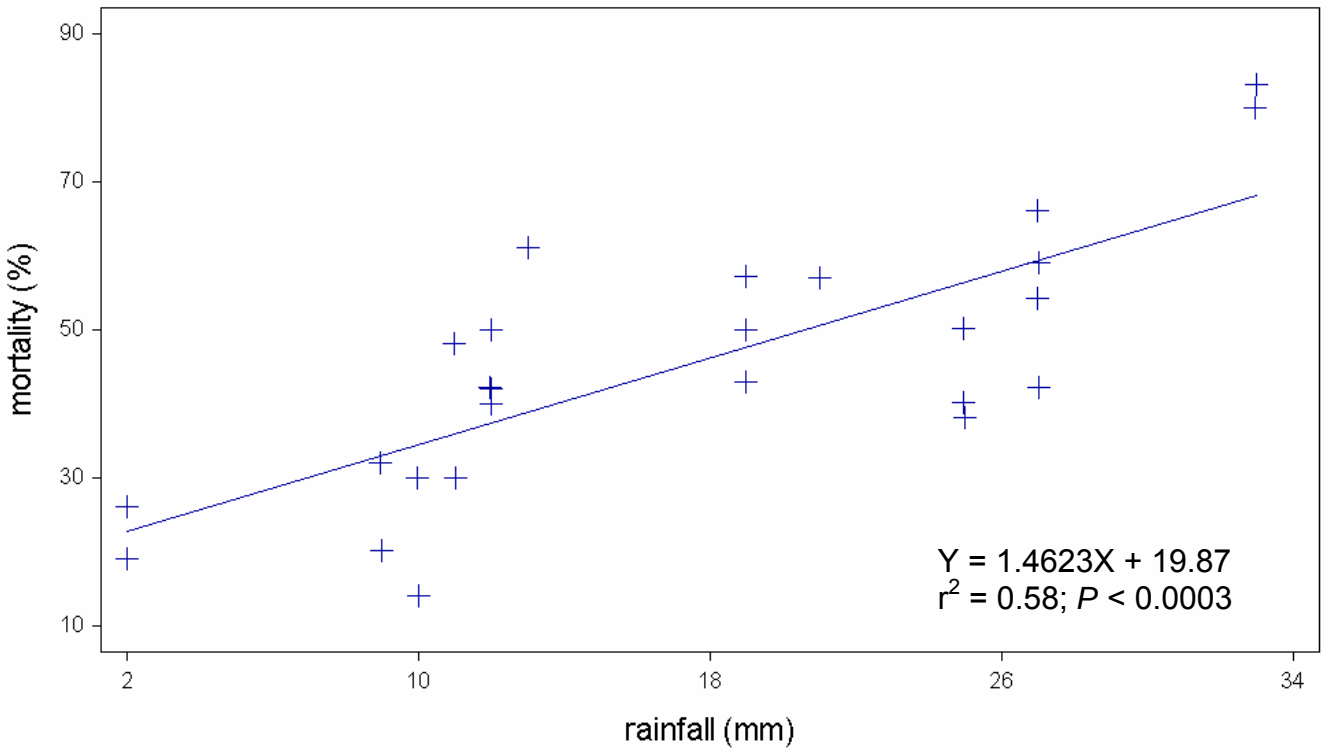

Fig. 2.4. Regression analysis of mortality of *Plutella xylostella* neonates and small larvae and rainfall (mm) that occurred between sampling periods during life table studies conducted in Virginia; 2003-2004.

Chapter Three

Insecticide susceptibility of field-collected *Plutella xylostella* from Virginia

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a major insect pest of crucifer crops worldwide (Talekar and Shelton 1993). Some populations of the pest have developed resistance to almost every class of insecticide used against it (Diaz-Gomez et al. 2000, Shelton et al. 2000). For instance, Georghiou (1981) reported *P. xylostella* resistance to 36 insecticides in 14 countries. In North America, some populations of *P. xylostella* were reported to be more than 100 fold resistant to the pyrethroid, permethrin, and the carbamate, methomyl, and more than 400 fold to the microbial insecticide, *Bacillus thuringiensis* (Shelton and Wyman 1990, Shelton et al. 1993a). Shelton et al. (1993a) found up to 461 fold resistance ratio of *B. thuringiensis* subsp. *kurstaki* in some populations of *P*. *xylostella* in the U.S. Insecticide resistance patterns in *P. xylostella* populations appear to be localized geographically (Tabashnik et al. 1987, Shelton and Wyman 1990, Shelton et al. 2000).

The levels and types of insecticide resistance in *P. xylostella* populations from Virginia are not currently known. Historically, control measures for *P*. *xylostella* and other pests in commercial cabbage, broccoli, and collards in Virginia have involved multiple applications of insecticides applied on a 7-to10 day schedule, with little regard for pest population level (Lasota and Kok 1986). In the 1980's, the frequently-used insecticides on crucifer crops included the organophosphate, methamidophos, the carbamate, methomyl, the pyrethroids, fenvalerate and permethrin, and *B. thuringiensis* (Lasota and Kok 1986). Many of these insecticides, or very similar ones from the same classes of chemistry, are still used today on crucifer crops in Virginia (Bratsch et al. 2005). However, since the mid-1990's, several novel insecticide chemistries have been developed and are registered for use in crucifer crops including avermectins, spinosyns, pyrazolines, neonicotinoids, and various insect growth regulators. The purpose of this study was to assess the current susceptibility of a *P. xylostella* field

population from the Eastern Shore of Virginia to some of the most commonly-used insecticides as well as some novel insecticide chemistries and compare these susceptibility levels with those of a known susceptible strain of *P. xylostella*.

Materials and Methods

***Plutella xylostella* field population**. Several hundred larvae and pupae of *P. xylostella* were collected ~weekly during July and August of 2003 and 2004 from collards (*Brassica oleracea* L. acephala group, variety 'Vates') located on the Eastern Shore of Virginia, the major commercial vegetable region in the state. Larvae and pupae were initially quarantined in separate containers to remove any parasitized individuals, and then all unparasitized *P. xylostella* pupae were used to initiate a laboratory colony. Insects were maintained on potted collard plants inside of screen cages (24 x 24 x 24 cm) in a rearing room at 27 ± 3 ^{o}C, 40 to 70% RH, and a photoperiod of 14:10 (L:D). *Plutella xylostella* adults were fed with 10% sugar solution in distilled water. Bioassays were conducted using 2nd instars of the first laboratory generation of *P. xylostella*.

***Plutella xylostella* susceptible population.** An insecticide-susceptible *P. xylostella* colony (>80 generations) was acquired from Benzon Research® (Carlisle, PA) and reared on artificial diet number F9441B (Bioserv Inc., Frenchtown, NJ) at 27 ± 2 ^{o}C, 40 to 70% RH, and a photoperiod of 14:10 (L:D). As with the field-collected population of *P. xylostella,* all bioassays were conducted using 2nd instars.

Insecticides. Eleven different commercial insecticides were assayed using serial dilutions of the lowest recommended field application rate on crucifer crops (Table 3.1). Insecticides were diluted in a volume of distilled water proportional to a typical field spray volume of 355 liters per ha. Four to eight concentrations of each insecticide were prepared in serial dilutions including a control of distilled water. To improve uniform coverage over the leaf surface, a spreader-sticker, Latron B-1956® (Loveland Industries Inc., Greeley, CO), was

added to each insecticide solution and the control at a concentration of 0.25% vol:vol.

Toxicity bioassays. For each insecticide, bioassay experiments were replicated a minimum of four times and a maximum of seven times depending upon the number of *P. xylostella* larvae available at the time of assaying. The toxicity bioassay utilized a leaf dip method similar to that used by Shelton et al. (1993a, 1993b, 2000). Leaf disks of 8.5 cm diameter were cut from the outer layers of cabbage heads (not including wrapper leaves). Leaf disks were dipped for 10 seconds in each concentration, held vertically to allow excess solution to drip off, and placed in a drying rack in a fume exhaust hood to air dry for 2 hr. Leaf disks were then placed individually into 9 cm diameter plastic Petri dishes along with approximately 10 *P. xylostella* 2^{nd} instars. Petri dishes were maintained at 27 ± 2^{o}C, 40 to 70% RH, and a photoperiod of 14:10 (L:D). Mortality of the 2^{nd} instars was determined after 48 h of exposure for esfenvalerate, indoxacarb, spinosad, methomyl, acephate, and emamectin benzoate; 72 h for acetamiprid, *B. thuringiensis*, novaluron, and methoxyfenozide; and 96 h for azadirachtin. Larvae were considered dead if they did not move when prodded.

Statistical Analyses. The dose-mortality for each insecticide concentration was estimated using probit analysis in Polo Plus (LeOra Software 2002). Control mortality, which averaged 9.2% (range 0 - 14%), was corrected for by using Abbott's formula (Abbott 1925) for each probit analysis. The LC_{50} and the corresponding 95% fiducial limits (FL) were estimated for each insecticide tested on *P. xylostella* populations. The LC_{50} and the corresponding 95% FL are the criteria used to compare insecticide susceptibilities between *P. xylostella* populations (Tabashnik et al. 1990, Kobayashi et al. 1992, Shelton et al. 1993a, Zhao et al. 2002, Liu et al. 2003). The response curves of two populations to a particular insecticide were considered different if their corresponding 95% FL did not overlap (Shelton et al. 1993b). When the response to one insecticide was different, the tolerance ratio (TR) was calculated by dividing the LC_{50} of the field population by the corresponding LC_{50} of the

susceptible population. The more neutral term 'TR' was used rather than 'RR' (Resistance Ratio) because of the latter's potentially unfounded implications (Shelton et al. 2000).

Results

Based on non-overlap of the 95% FL of the $LC_{50,}$ significant differences in toxicity response to esfenvalerate, acetamiprid, indoxacarb, methoxyfenozide, methomyl, and acephate were found between the Eastern Shore population and the susceptible population of *P. xylostella* (Table 3.2). The largest difference in toxicity occurred with esfenvalerate, where the LC_{50} of the field population was 15.009 mg AI/liter compared with 0.008 mg AI/liter for the susceptible population, representing a tolerance ratio of 1876. For acetamiprid the LC_{50} of the field population was 131.538 mg AI/liter compared with 0.944 mg AI/liter for the susceptible population, a tolerance ratio of 139. The tolerance ratios for methomyl, methoxyfenozide, indoxacarb, and acephate were 32, 26, 19, and 8, respectively. No differences in insecticide susceptibility between the *P. xylostella* field population and the susceptible population were found for *B. thuringiensis,* novaluron, azadirachtin, emamectin benzoate, and spinosad (Table 3.2). However, for spinosad, the overlap of the 95% FL was minimal, and the toxicity resistance ratio with respect to the susceptible population was near 10x. Thus, although not statistically significant, the tolerance ratio to spinosad should be monitored in the Virginia *P. xylostella* population.

Discussion

Insecticide resistance in *P. xylostella* is a major concern worldwide (Georghiou 1981, Talekar 1992, Diaz-Gomez et al. 2000, Shelton et al. 2000). Although the relationship between laboratory bioassay and insecticide field efficacy is not always straightforward, bioassay resistance results are valuable in that they indicate potential problems before full-scale field failures occur (ffrench-Constant and Roush 1990, Tabashnik et al. 1990). Bioassay data alone,

however, cannot predict the development of field resistance (Tabashnik 1994). In my study, *P. xylostella* collected from the Eastern Shore of Virginia, showed significant tolerance levels to esfenvalerate, acetamiprid, methomyl, methoxyfenozide, indoxacarb, and acephate compared with a susceptible strain of *P. xylostella*. The highest tolerance ratio (1,876 fold) was to esfenvalerate, a commonly-used pyrethroid. High tolerance ratios to pyrethroids have also been found in many other populations of *P. xylostella* in the U.S. (Shelton et al. 1993a, Liu et al. 2003), and it has been suggested that past selection by older insecticides such as DDT, cyclodienes, and organophosphates have caused some cross-resistance to pyrethroids (Tabashnik et al. 1987). *Plutella xylostella* from the Eastern Shore of Virginia also had a high tolerance ratio (139 fold) to acetamiprid. This was surprising because acetamiprid has not yet been registered on crucifer crops in the U.S. Also, selection pressure to the neonicotinoid class of insecticides should be extremely low in *P. xylostella* because they are a relatively new class of pesticide, are typically not applied for control of lepidopteran pests, and have not been used often on crucifer crops in Virginia. However, Tomizawa and Casida (2003) suggest that cross-resistance between neonicotinoids and nicotinoids may be associated with evolutionary selection for nicotine tolerance, over expression of *CYP450* oxidative enzymes, and other mechanisms.

The susceptibility of *P. xylostella* to other neonicotinoids such as imidacloprid and thiamethoxam as well as the physiological mechanisms behind this potential resistance should be investigated in future studies. Although statistically significant, the tolerance ratios exhibited by the field-collected *P. xylostella* to acephate, indoxacarb, methoxyfenozide, and methomyl were relatively low (ranging from 8 to 32). Shelton et al. (1993a) suggest that because no standards define problematic resistance levels for diamondback moth, resistance ratios close to 10 cannot be attributed to the insecticide toxicity itself, but may be the impact of other factors such as variability in field population response to insecticides or experimental procedures. Resistance to carbamates and organophosphates is very common in *P. xylostella* populations around the

world (Shelton et al. 1993a). The levels of tolerance to methomyl (32x) and acephate (8x) exhibited by the Eastern Shore population of *P. xylostella,* suggest a conservative use of these insecticides to avoid increase of resistance by *P. xylostella* in the future.

No signs of tolerance in *P. xylostella* were found to *B. thuringiensis,* novaluron, azadirachtin, emamectin benzoate, and spinosad. In Virginia, these insecticides currently appear to be excellent insecticide resistance management tools for *P. xylostella*, and are all IPM-compatible products with reduced impact on natural enemies. However, *P. xylostella* has developed resistance to some of these (or similar) insecticides in other regions. For instance, toxicity ratios of 1641 and 1125 for *B*. *thuringiensis* products were reported by Shelton et al. (1993b) and Perez and Shelton (1996). Iqbal and Wright (1997) reported toxicity ratios of 357 for teflubenzuron, and 190 for abamectin. Sayyed and Wright (2004) reported toxicity ratios of 1170 to spinosad and 2840 to *B. thuringiensis*. A toxicity ratio of 6422 for spinosad in Hawaii after ~2.5 years of use of this insecticide was reported by Zhao et al. (2002). Because *P. xylostella* has already shown the ability to develop resistance to some of these novel insecticides, continued monitoring of *P. xylostella* field populations to insecticide susceptibility should be done, particularly in those instances when field failures have been reported.

Summary. In 2004, insecticide toxicity bioassays were conducted on a field population of *Plutella xylostella* from the Eastern Shore and a susceptible lab population in order to determine LC50 levels and resistance levels (ratios) to several different insecticides. Bioassays were conducted on 2nd instars of *P. xylostella* using cabbage leaf-dips. Based on non-overlap of the 95% fiducial limits of the $LC_{50,}$ *P. xylostella* from the Eastern Shore showed significant tolerance levels to esfenvalerate (1,876 fold), acetamiprid (139 fold), methomyl (32 fold), methoxyfenozide (26 fold), indoxacarb (19 fold), and acephate (8 fold) compared with the susceptible population of *P. xylostella*. Although not statistically significant, the tolerance ratio to spinosad was 10 fold in the Eastern Shore population. No significant levels of tolerance in *P. xylostella* were found to

B. thuringiensis, novaluron, azadirachtin, and emamectin benzoate. In Virginia, these insecticides currently appear to be excellent insecticide resistance management tools for *P. xylostella*, and are all IPM-compatible products with reduced impact on natural enemies.

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Table 3.1. Insecticides tested in laboratory toxicity assays conducted on a field-collected population of *Plutella xylostella* from the Eastern Shore of Virginia and a susceptible strain of *P. xylostella*, 2003 and 2004.

Insecticide (ai)	**Product name (manufacturer)**	**Insecticide Class**	**Recommended field application rate (kg [ai]/ha)**
Acephate	Orthene 97 (Valent BioScience Corp. Libertyville, IL)	Organophosphate	1.087
Acetamiprid	Assail 70WP (Cerexagri, Inc., King of Prusia, PA)	Neonicotinoid	0.084
Azadirachtin	Neemix 4.5EC (Certis USA L.L.C., Columbia, MD)	Botanical – Neem extract	0.011
Bacillus thuringiensis subsp. *kurstaki* strain HD-1	DiPel DF (Valent BioScience Corp. Libertyville, IL)	Microbial	0.605
Emamectin benzoate	Proclaim 5WDG (Syngenta Crop Protection Inc., Greensboro, NC)	Avermectin	0.008
Esfenvalerate	Asana XL (E. I. du Pont de Nemours and Co., Wilmington, DE)	Pyrethroid	0.032
Indoxacarb	Avaunt 30WG (E. I. du Pont de Nemours and Co., Wilmington, DE)	Pyrazoline	0.072
Methomyl	Lannate LV (E. I. du Pont de Nemours and Co., Wilmington, DE)	Carbamate	0.504
Methoxyfenozide	Intrepid 2F (Dow AgroSciences LLC, Indianapolis, IN)	Insect growth regulator	0.112
Novaluron	Rimon 0.83EC (Crompton Corporation, Middlebury, CT)	Insect growth regulator	0.087
Spinosad	SpinTor 2SC (Dow AgroSciences LLC, Indianapolis, IN)	Spinosyn	0.026

Table 3.2. Laboratory susceptibility to insecticides of 2nd instar *Plutella xylostella* collected from a field population at the Eastern Shore, Virginia and a known susceptible population.

Population	Insecticide	n	LC_{50}[a]	95% FL[a]	Slope ± SE	x^2 (df)	TR[b]
Painter	esfenvalerate	250	15.009	2.203-44.211	1.18 ± 0.17	6.99 (3)	1876
Susceptible	esfenvalerate	200	0.008	0.001-0.020	0.76 ± 0.15	0.63 (3)	
Painter	acetamiprid	320	131.53	70.346-191.394	1.45 ± 0.31	2.16 (4)	139
Susceptible	acetamiprid	200	0.944	0.131-7.828	0.30 ± 0.09	0.91 (3)	
Painter	methomyl	250	621.14	464.536-770.36	3.05 ± 0.63	2.07 (4)	32
Susceptible	methomyl	250	19.159	5.631-111.227	0.43 ± 0.10	0.88 (3)	
Painter	methoxyfenozide	300	144.12	75.212-230.805	1.28 ± 0.23	1.21 (4)	26
Susceptible	methoxyfenozide	200	5.414	1.976-20.565	0.58 ± 0.11	1.01 (3)	
Painter	indoxacarb	390	4.563	1.354-10.572	1.32 ± 0.13	21.46 (6)	19
Susceptible	indoxacarb	240	0.235	0.074-0.660	0.51 ± 0.08	0.15 (3)	
Painter	acephate	300	133.94	67.417-201.815	1.91 ± 0.27	6.22 (5)	8
Susceptible	acephate	200	16.708	8.583-34.526	0.73 ± 0.11	1.61 (3)	
Painter	*B. thuringiensis*	720	1.976	0.330-7.499	0.98 ± 0.08	13.84 (3)	
Susceptible	*B. thuringiensis*	380	0.613	0.137-1.629	0.43 ± 0.07	0.39 (3)	
Painter	novaluron	200	2.098	0.581-10.934	0.41 ± 0.10	1.92 (3)	
Susceptible	novaluron	800	1.142	0.003-6.614	0.57 ± 0.06	30.71 (6)	
Painter	azadirachtin	220	22.353	12.410-30.545	2.57 ± 0.88	0.42 (3)	
Susceptible	azadirachtin	360	9.826	1.836-26.497	0.98 ± 0.19	8.26 (5)	
Painter	emamectin benzoate	680	0.018	0.003-0.050	0.86 ± 0.08	19.94 (5)	
Susceptible	emamectin benzoate	200	0.009	0.002-0.018	1.08 ± 0.23	1.25 (3)	
Painter	spinosad	510	0.213	0.079-0.429	1.13 ± 0.13	8.11 (5)	
Susceptible	spinosad	240	0.022	0.001-0.112	0.73 ± 0.11	4.85 (3)	

[a] mg AI/l
[b] TR, tolerance ratio = LC_{50} of field population-Painter, VA/ LC_{50} of susceptible population

Chapter Four

Susceptibility of two diamondback moth parasitoids, *Diadegma insulare* (Cresson) and *Oomyzus sokolowskii* (Kurdjumov), to selected commercial insecticides

Hymenopteran parasitoids can play an important role in the population regulation of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), one of the most destructive pests of crucifer crops throughout the world (Talekar and Shelton 1993). More than 90 species of parasitoids have been reported attacking *P. xylostella* (Harcourt 1960, Goodwin 1979, Lim 1986, Ooi 1992, Kok 2004, Rowell et al. 2005). The two primary parasitoid species of *P. xylostella* in Virginia and much of the southeastern U.S. are *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) and *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) (Latheef and Irwin 1983, Chamberlin and Kok 1986, Lasota and Kok 1986, Cordero and Kuhar 2004, Kahn et al. 2004). *Diadegma insulare* is a solitary larval parasitoid that can cause up to 70% larval mortality of *P. xylostella* in a given season (Lasota and Kok 1986, T. P. Kuhar *unpublished data*). It has multiple generations per year and its population dynamics are highly synchronized with its host (Harcourt 1960). *Oomyzus sokolowskii* is a gregarious larval-pupal parasitoid that is well adapted to the high temperature conditions that are typical of the southeastern U.S. (Fitton and Walker 1992, Talekar and Hu 1996). In Virginia, this species appears to be more prominent later in the season and in the eastern half of the state where elevations are lower and temperatures are warmer (Chapter 2 of this dissertation).

Despite the contribution of the aforementioned parasitoids along with other natural enemies, commercial growers of crucifer crops in Virginia still typically apply insecticides usually on a 7-to10 day schedule. Many commonly-used insecticides include organophosphates, carbamates, and pyrethroids (Lasota and Kok 1986), which are broad-spectrum toxicants that also cause mortality to natural enemies, especially adult parasitoids (Hill and Foster 2000, Xu et al.

2001a, 2001b, 2004, Haseeb et al. 2005). More recently, the microbial insecticide, *Bacillus thuringiensis* Berliner var. *kurstaki* has been incorporated into spray programs for crucifer crops, and it is generally only toxic to lepidopteran pests and safe on natural enemies (Idris and Grafius 1993, Ulpah and Kok 1996, Hill and Foster 2000, Xu et al. 2004). However, *B.t. kurstaki* insecticides do not control any non-lepidopteran pests such as harlequin bugs, cabbage aphids, and flea beetles, which also may reach damaging levels in crucifers. Also, insecticide resistance to *B.t. kurstaki* has developed in some populations of *P. xylostella* (Tabashnik et al. 1990, Shelton et al. 1993a, 1993b). Thus, *B.t.* products are not always the proper choice for pest management in crucifers.

In recent years, several novel insecticides including avermectins, spinosyns, pyrazolines, neonicotinoids, and insect growth regulators have been registered for use on crucifer crops. Many of these insecticides are efficacious against diamondback moth and other pests on crucifers (Kuhar and Speese 2002, Kuhar et al. 2003, Liu et al. 2003, Chapter 5 of this dissertation), and also claim to be safe on beneficial arthropods (Dow AgroSciences 1999, Novartis Crop Protection 1999, E. I. du Pont de Nemours and Company 2001). The latter claim is not always true and the overall impact of these insecticides on parasitoids such as *D. insulare* and *O. sokolowskii* is not thoroughly understood (Hill and Foster 2000, 2003, Haseeb et al. 2004, Shi et al. 2004, Xu et al. 2004). Thus, the purpose of this study was to determine the susceptibility of *D. insulare* and *O. sokolowskii* adults to several conventional insecticides as well as some novel insecticides that are registered on crucifer crops. This information will be useful for selecting the most appropriate insecticides to use in an integrated pest management program for crucifer crops in Virginia.

Materials and Methods

Field collection and rearing of *Diadegma insulare* and *Oomyzus sokolowskii*.

From July to October 2004, several thousand larvae and pupae of *P. xylostella* were collected from an untreated crop of collards (*Brassica oleracea* L. acephala group, cultivar 'Vates') located at the Eastern Shore Agricultural Research and Extension Center at the Eastern Shore, Virginia. *Plutella xylostella* larvae were placed on potted collard plants inside screen cages (24 x 24 x 24 cm) in a rearing room at 27 ± 3 ^{o}C, 40 to 70% RH and a photoperiod of 14:10 (L:D). Plants were checked daily for the presence of *P. xylostella* net-like cocoons. Within these cocoons, any *P. xylostella* pupae that were parasitized by *O. sokolowskii*, or pupae of *D. insulare* were collected and placed inside inflated transparent plastic bags (3.78 l, 26.8 cm by 27.9 cm; Hefty, Pactiv Co., Lake Forest, IL) along with cotton balls moistened with 10% sucrose solution. Approximately 15 specimens of each species were placed in a single bag. After parasitoid adults emerged they were allowed to feed for approximately 6 to12 h before being used in bioassays.

Insecticides. Commercial insecticides were assayed using serial dilutions of the lowest recommended field application rate on crucifer crops. Five insecticides were tested on *D. insulare* including: esfenvalerate 0.032 kg [AI]/ha (Asana 0.66 XL; E. I. du Pont de Nemours and Co., Wilmington, DE); acetamiprid 0.084 kg [AI]/ha (Assail 70 WP, Cerexagri, Inc., King of Prusia, PA); indoxacarb 0.072 kg [AI]/ha (Avaunt 30WG; E. I. du Pont de Nemours, Wilmington, DE); methomyl 0.504 kg [AI]/ha (Lannate 2.4 LV; E. I. du Pont de Nemours and Co., Wilmington, DE); and spinosad 0.026 kg [AI]/ha (SpinTor 2SC, Dow AgroSciences LLC, Indianapolis, IN). Eight insecticides were tested on *O. sokolowskii* including the five previously mentioned and the following: methoxyfenozide 0.112 kg [AI]/ha (Intrepid 2F; Dow AgroSciences LLC, Indianapolis, IN); acephate 1.087 kg [AI]/ha (Orthene® 97 P; Valent USA Corporation, Walnut Creek, CA); and emamectin benzoate 0.008 kg [AI]/ha (Proclaim® 5SG, Syngenta Crop Protection Inc., Greensboro, NC). More insecticides were tested on *O. sokolowskii* because of the greater number of parasitoid adults available.

All insecticides were diluted in a volume of distilled water proportional to a typical field spray volume of 355 liters per ha (Bratsch et al. 2005). Four to six concentrations of each insecticide were prepared in serial dilutions including a control of distilled water. To improve uniform coverage over the leaf surface, a spreader-sticker, Latron B-1956® (Loveland Industries Inc., Greeley, CO), was added to each insecticide solution and the control at a concentration of 0.25% vol:vol (Bratsch et al. 2005).

Toxicity bioassays. For each insecticide, bioassay experiments were replicated four – six times depending upon the number of parasitoid adults available at the time of assaying. Preliminary bioassays were conducted on small groups of parasitoids so that an appropriate range of insecticide concentrations (10–90% mortality range) could be selected for a full scale test. The toxicity bioassay was a leaf dip method similar to that used by Shelton et al. (1993a, 1993b). Leaf disks of 8.5 cm diameter were cut from the outer layers of cabbage heads (not including wrapper leaves), dipped for 10 seconds in each concentration, held vertically to allow excess solution to drip off, and placed in a drying rack in a fume exhaust hood to air dry for 2 h. They were then placed individually in 9-cm diameter plastic Petri dishes. The inflated bags containing parasitoid adults were chilled for a few seconds in a freezer to allow handling with an aspirator. Approximately 12 adults (unsexed) of *D. insulare* or *O. sokolowskii* were added to each dish and placed in a rearing room at 27 ± 3 ^{o}C, 40 to 70% RH, and a photoperiod of 14:10 (L:D). Mortality was determined after 24, 48 and 72 h of exposure. Parasitoid adults were considered dead if they were found upside down and not moving or if they did not move when prodded with a brush probe.

Statistical Analyses. Proportion mortality data after 24, 48 and 72 h of exposure were analyzed using ANOVA and treatment means separated by Fisher's protected LSD at $P < 0.05$ (SAS Institute 1999). To stabilize variances, proportion data were transformed [arcsin sqrt (x + 0.001)] before analysis. Untransformed data are presented in the results. The dose-mortality of each insecticide concentration was estimated using probit analysis in Polo Plus (LeOra

Software 2002). Control mortality, which averaged 12% (range 0 – 16%), was corrected for by using Abbott's formula (Abbott 1925) for each probit analysis. The LC_{50}, the corresponding 95% fiducial limits (FL) and slope were estimated for each insecticide.

Results

Diadegma insulare. At the field rate concentration, there was a significant effect of insecticide treatment on proportion mortality of *D. insulare* adults after exposure for 24 hours ($F = 4.18$; df = 5, 12; $P < 0.0197$), 48 hours ($F = 88.9$; df = 5, 12; $P < 0.0001$), and 72 h ($F = 96.0$; df = 5, 12; $P < 0.0001$). At 24 h, all insecticides except acetamiprid caused an average of about 80% mortality of *D. insulare* adults, which was significantly higher than the untreated control mortality of 8% (Fig. 4.1 A). Acetamiprid caused an average of almost 50% mortality and was not different from the control or any of the other insecticides. At 48 h, all of the insecticides except acetamiprid resulted in 100% mortality. Acetamiprid caused an average of about 64% mortality at 48 h and 77% mortality after 72 h, which was lower than all other insecticides, but higher than the untreated control.

At a concentration of 1% of that of the field rate, there was a significant effect of insecticide treatment on proportion mortality of *D. insulare* adults only after exposure for 48 h ($F = 4.21$; df = 5, 12; $P < 0.0193$) and 72 hours ($F = 26.0$; df = 5, 12; $P < 0.0001$). At 48 h, all insecticide treatments averaged from 36 to 60% mortality, which was higher than the untreated control mortality of 10% (Fig. 4.1 B). No differences in mortality occurred among insecticides. However, at 72 h after exposure, indoxacarb caused 100% mortality, which was highest among all insecticides, and was followed by esfenvalerate and spinosad, which averaged 80 and 67%, respectively. Mortality due to acetamiprid and methomyl averaged 53 and 50%, respectively, which was still higher than the untreated control (13.3%).

The LC_{50} values and 95% FL for all of the insecticides tested on *D. insulare* adults are shown in Table 4.1. Spinosad (after 48 h of exposure) had

the lowest LC_{50} value (0.346 mg AI/l), which was about 3 × more toxic than indoxacarb (after 48 h of exposure) or esfenvalerate (after 48 h of exposure), and >70 × more toxic than acetamiprid (after 72 h of exposure) or methomyl (after 48 h of exposure).

Oomyzus sokolowskii. As with *D. insulare*, at the full field rate concentration, there was a significant effect of insecticide treatment on proportion mortality of *O. sokolowskii* adults after exposure for 24 h (F = 9.25; df = 8, 32; P < 0.0001), 48 h (F = 60.4; df = 8, 32; P < 0.0001), and 72 h (F = 54.5; df = 8, 32; P < 0.0001). At 24 h, mortality of *O. sokolowskii* adults ranged from 32 to 69% among the insecticide treatments, which did not differ from one another, but were all significantly higher than the untreated control (Fig. 4.2 A). At 48 h after exposure, mortality was highest (averaging 95-100%) for acephate, esfenvalerate, methomyl, indoxacarb, and spinosad, followed by emamectin benzoate (85%), then acetamiprid (73%). Methoxyfenozide had the lowest mortality (38% at 48 h and 62% at 72 h) among insecticide treatments, but was still significantly higher than the untreated control.

At a concentration of 1% of the field rate, there was a significant effect of insecticide treatment on mortality of *O. sokolowskii* adults after exposure for 24 h (F = 15.3; df = 8, 31; P < 0.0001), 48 h (F = 19.6; df = 8, 31; P < 0.0001), and 72 h (F = 42.4; df = 8, 31; P < 0.0001). At 24 h, mortality of *O. sokolowskii* adults was highest for indoxacarb and acephate (63 and 58%, respectively), followed by the remaining insecticide treatments, which were all higher than the untreated control (5.8%) (Fig. 4.2 B). At 48 h after exposure, mortality varied markedly among treatments. Acephate had the highest mortality (91%), followed by indoxacarb, methomyl, and esfenvalerate (57-73%). Spinosad caused the lowest mortality (20%), which was not significantly different from the untreated control (9.8%). At 72 h, indoxacarb and acephate had the highest mortality (96-98%), followed by methomyl (72%), then the others. Spinosad again had the lowest mortality (30%), which was significantly different from the untreated control (14%).

The LC_{50} values and 95% FL for all of the insecticides tested on *O. sokolowskii* adults are shown in Table 4.2. Indoxacarb (after 48 h of exposure) and esfenvalerate (after 48 h of exposure) had the lowest LC_{50} values (0.466 and 0.599 mg AI/l, respectively), which were >20 × more toxic than methomyl (after 48 h of exposure), and >58 × more toxic than acetamiprid (after 72 h of exposure) or methoxyfenozide (after 72 h of exposure).

Discussion

All of the insecticides tested in this study were toxic to the adult stage of *D. insulare* and *O. sokolowskii,* the primary parasitoids of *P. xylostella* in Virginia. However, it should be noted that the untreated controls in this study did not correct for effects of inert ingredients, which are not always completely benign (J. R. Bloomquist, *personal communication*). The broad-spectrum toxicity of organophosphates, carbamates, and pyrethroids has been well documented by previous researchers, and thus, it is not surprising that in my study, field rate concentrations of esfenvalerate (pyrethroid), methomyl (carbamate), and acephate (organophosphate) resulted in 100% mortality of adult parasitoids after 72 h of exposure. Idris and Grafius (1993) reported that esfenvalerate and methomyl at 1000 mg AI per liter in a leaf dip assay killed 100% of *D. insulare* adults after 24 h. Shi et al. (2004) reported 93% mortality of *O. sokolowskii* adults after 24 h exposure to methomyl residue. Moreover, Xu et al. (2004) found that the pyrethroid λ-cyhalothrin caused 100% mortality and Hill and Foster (2000) found that the pyrethroid permethrin and the carbamate carbaryl caused 90-100% mortality of *D. insulare* adults in contact bioassays after 24 h.

Some of the newer insecticides that have been registered on vegetable crops such as spinosad, indoxacarb and emamectin benzoate have been shown to be relatively safe on predacious hemipterans, mites, coccinellids, lacewings and some parasitoids (Ruberson and Tillman 1999, Studebaker and Kring 1999, Scholz and Zalucki 2000, Elzen 2001). However, the results of my study showed that all three of these compounds, at their field rate concentrations, resulted in 100% mortality to either *D. insulare* or *O. sokolowskii* or both after 72 h of

exposure. Moreover, LC_{50} values for all of these insecticides were only a fraction (< 2%) of the actual field rate concentration. Other researchers have found similar results. Hill and Foster (2000) found that the field rate of spinosad killed 100% of *D. insulare* adults after only 8 h of exposure, and Xu et al. (2004) found that spinosad and indoxacarb caused 100% mortality of *D. insulare* adults in contact bioassays. Shi et al. (2004) reported that avermectin was extremely harmful to *O. sokolowskii* adults producing up to 100% mortality. Similarly, Haseeb et al. (2005) found that emamectin benzoate killed 100% of adults of *O. sokolowskii* and *Diadegma semiclausum* after 24 h. Moreover, Haseeb et al. (2004) found that spinosad and indoxacarb caused high adult mortality to *Cotesia plutellae* (Kurdjumov), another parasitoid of *P. xylostella*. Further, Pietrantonio and Benedict (1999) and Ruberson and Tillman (1999) found that spinosad residues on cotton leaves were toxic to the parasitoid adults of *C. plutellae* and highly toxic to the egg parasitoid, *Trichogramma pretiosum*.

Neonicotinoid insecticides are relatively narrow-spectrum toxicants and are typically used to control beetles and sucking insects. In my study, the neonicotinoid, acetamiprid, was generally less toxic than the other insecticides, but still killed 77% of *D. insulare* adults and 91% of *O. sokolowskii* adults after 72 h of exposure. These data represent some of the first published information on acetamiprid toxicity to *D. insulare* or *O. sokolowskii*. However, Hill and Foster (2000) found that the field rate of imidacloprid, another neonicotinoid insecticide, killed nearly 100% of *D. insulare* adults after 24 h of exposure.

In contrast to the aforementioned insecticides, insect growth regulators (IGRs) such as chlorfluazuron, flufenoxuron, teflubenzuron, and tebufenozide, which target immature insects, have been shown to have relatively low toxicity to adult parasitoids (Hill and Foster 2000, Haseeb et al. 2005). In my study, however, the IGR, methoxyfenozide, though considerably less toxic than the other insecticides based on LC_{50} levels, still resulted in substantial (62%) mortality of *O. sokolowskii* adults after 72 h. Unfortunately, I was unable to test the toxicity of this insecticide to *D. insulare* because of lack of specimens. Adult parasitoid toxicity due to IGRs has been reported before; for instance, Shi et al.

(2004) found that the IGR chlorfluazuron was more toxic to adults of *C. plutellae* than *O. sokolowskii*.

Although all of the insecticides tested in my study were toxic to *D. insulare* and *O. sokolowskii* in lab assays, field studies need to be conducted to better understand the compatibility of the products with natural enemies. Relatively rapid degradation of surface residues in the field would definitely improve the compatibility potential with natural enemies. This would likely be the case with spinosad (Anonymous 1977 cited by Tomkins et al. 1999, Viktorov et al. 2002, EPA 2002) and emamectin benzoate degradation by photolysis (SEPA 1999, Viktorov et al. 2002).

Future studies should address the potential for behavioral avoidance to selected insecticides by these parasitoids. Hill and Foster (2000) found that spinosad was extremely toxic to *D. insulare* in leaf dip assays; however, in field experiments, they found that *D. insulare* parasitism of *P. xylostella* larvae in spinosad-sprayed plots was four times higher than that in permethrin-sprayed plots (Hill and Foster 2003).

Summary. In summer and fall 2004, insecticide toxicity bioassays were conducted on adults of *Diadegma insulare* and *Oomyzus sokolowskii*, two important parasitoids of *P. xylostella* in Virginia. The toxicity bioassay utilized a cabbage leaf dip method treated with different concentrations of various insecticides. All of the insecticides tested in this study including spinosad, indoxacarb, esfenvalerate, methomyl, acetamiprid, acephate, emamectin benzoate, and methoxyfenozide were toxic to the adult stage of *D. insulare* or *O. sokolowskii* or both. The broad-spectrum insecticides esfenvalerate, methomyl, and acephate as well as the more IPM-compatible insecticides, spinosad, indoxacarb, and emamectin benzoate at their field rate concentrations resulted in 100% mortality to either *D. insulare* or *O. sokolowskii* or both after 72 h of exposure. Moreover, LC_{50} values for all of these insecticides were only a fraction (< 2%) of the actual field rate concentration. The neonicotinoid, acetamiprid, was less toxic than the other insecticides, but still killed 77% of *D. insulare* adults and 91% of *O. sokolowskii* adults after 72 h of exposure. The insect growth regulator,

methoxyfenozide, though considerably less toxic than the other insecticides based on LC_{50} levels, still resulted in substantial (62%) mortality of *O. sokolowskii* adults after 72 h. Future studies should investigate toxicity of these insecticides to immature stages of the parasitoids, as well as toxicity of residues after exposure in the field for certain time intervals.

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Table 4.1. Toxicity of selected insecticides to *Diadegma insulare* adults from the Eastern Shore, VA.

Insecticide	**n**	**LC_{50}[a]**	**95% FL[a]**	**Slope ±SE**	**Proportion of LC_{50} to the field spray concentration[b]**
spinosad	111	0.346	0.034-0.904	1.344 ± 0.453	0.002
indoxacarb	91	1.052	0.048-4.465	0.672 ± 0.209	0.002
esfenvalerate	91	1.259	1.191-4.059	0.940 ± 0.260	0.006
methomyl	86	25.857	1.790-90.215	0.950 ± 0.310	0.007
acetamiprid	110	23.930	2.252-446.927	0.449 ± 0.159	0.041

[a] mg AI/l

[b]based on lowest recommended rate on crucifer crops (Bratsch et al. 2005) and a spray volume of 355 liters per hectare.

Table 4.2. Toxicity of selected insecticides to *Oomyzus sokolowskii* adults from the Eastern Shore, VA.

Insecticide	**n**	**LC_{50}[a]**	**95% FL[a]**	**Slope ± SE**	**Proportion of LC_{50} to the field spray concentration[b]**
acephate	196	7.869	1.872-15.675	1.911 ± 0.518	0.001
indoxacarb	222	0.466	0.001-4.945	0.530 ± 0.092	0.001
esfenvalerate	310	0.599	0.009-3.209	0.743 ± 0.115	0.003
methomyl	179	12.198	3.920-26.305	1.209 ± 0.243	0.003
emamectin benzoate	414	1.083	0.303-2.565	0.666 ± 0.110	0.019
spinosad	191	4.938	2.593-8.348	1.358 ± 0.221	0.027
acetamiprid	258	35.183	1.524-114.896	0.637 ± 0.202	0.060
methoxyfenozide	251	47.203	3.416-184.964	0.262 ± 0.074	0.061

[a] mg AI/l

[b]based on lowest recommended rate on crucifer crops (Bratsch et al. 2005) and a spray volume of 355 liters per hectare.

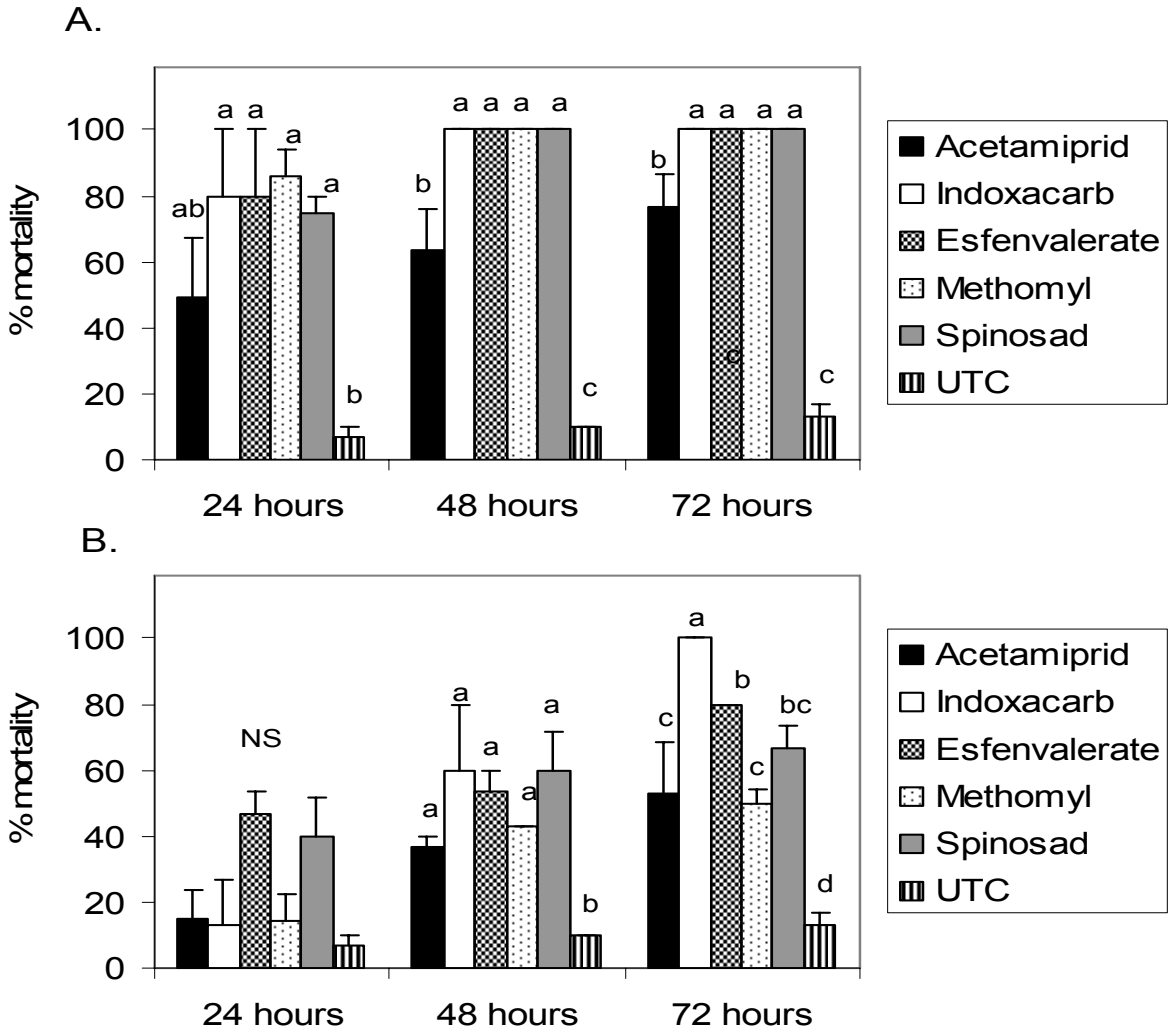

Fig. 4.1. Mortality of *Diadegma insulare* (Cresson) adults after leaf-dip assays with field rate concentrations (A) and 1% of field rate concentrations (B) of various insecticides. Columns within a group with a letter in common are not significantly different according to Fisher's protected LSD at $P < 0.05$.

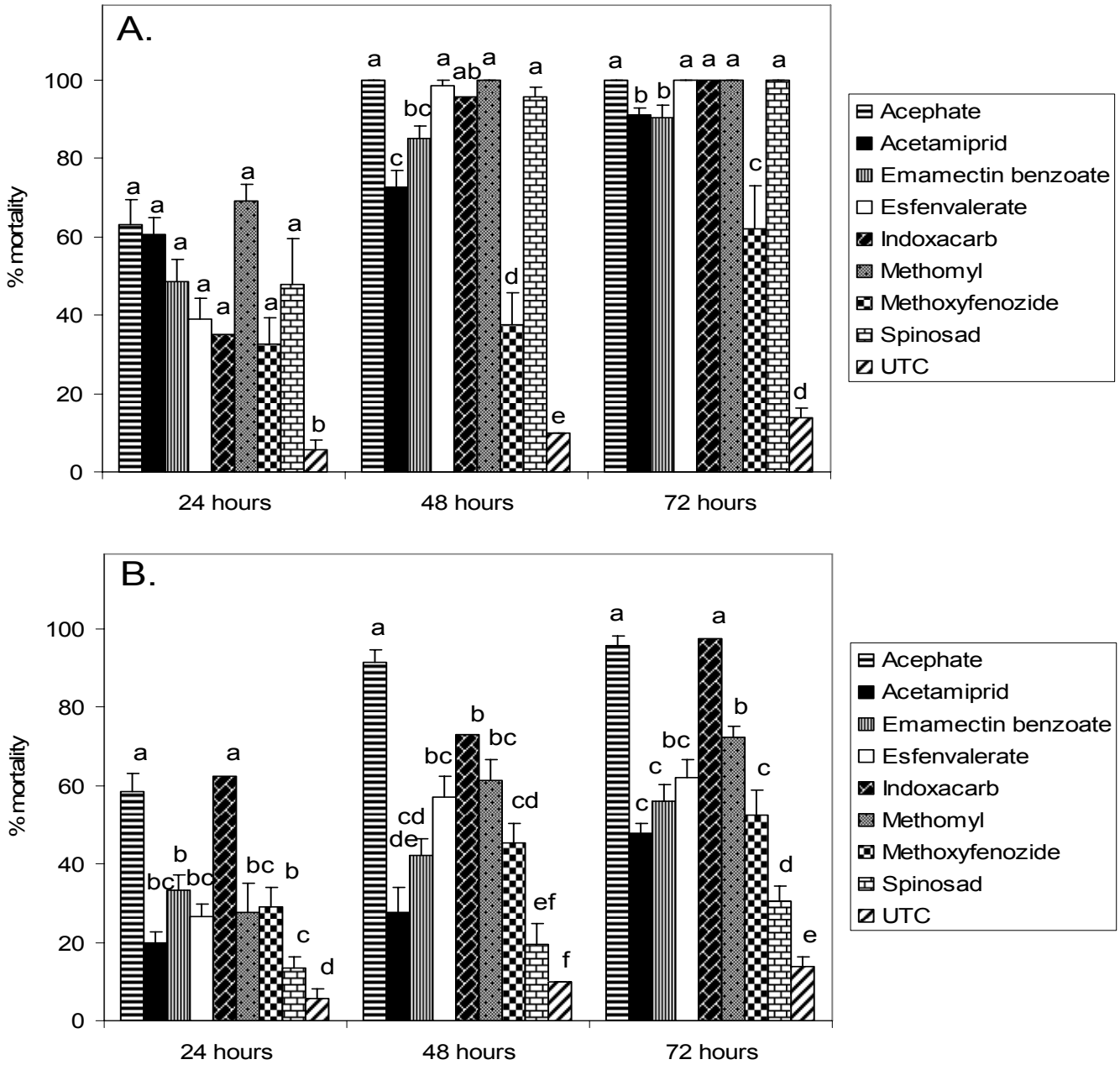

Fig. 4.2. Mortality of *Oomyzus sokolowskii* (Kurdjumov) adults after leaf-dip assays with field rate concentrations (A) and 1% of field rate concentrations (B) of various insecticides. Columns within a group with a letter in common are not significantly different according to Fisher's protected LSD at $P < 0.05$.

Chapter Five

Field Efficacy of Insecticides for Control of Lepidopteran Pests on Collards in Virginia

Collards (*Brassica oleracea* L. acephala group) are a popular cruciferous vegetable grown in the southeastern U.S., and are highly-attractive to diamondback moth, *Plutella xylostella* (L.), a major pest of cruciferous crops worldwide (Talekar and Shelton 1993). Diamondback moth larvae feed on leaves, which for collards are the marketable portion of the crop. Although some minor feeding injury is acceptable for local marketability, the presence of live insects on leaves or defoliation levels exceeding 10% render the crop unmarketable (Fig. 5.1).

Natural enemies, particularly hymenopteran parasitoids, can play an important role in reducing lepidopteran pest populations (Latheef and Irwin 1983, Chamberlin and Kok 1986, Mitchell et al. 1997, Kok 2004), but insecticide applications are typically needed for economic pest control. The efficacy of many conventional insecticides such as organophosphates, carbamates, pyrethroids, and even *Bacillus thuringiensis* (*Bt*) may vary considerably from region to region due to resistance levels in diamondback moth (Shelton et al. 1993a and b, Liu 1999, Gonzalez-Cabrera et al. 2001, Mohan and Gujar 2003). Moreover, many integrated pest management (IPM)-compatible insecticides that have been registered on vegetable crops in the U.S. in recent years such as indoxacarb, methoxyfenozide, spinosad, emamectin benzoate, azadirachtin, novaluron, and acetamiprid have not been thoroughly tested for efficacy in collards. In addition, in order to be truly effective in collards, an insecticide must control not only diamondback moth, but other pests that may damage the leaves. Based on previous studies (Latheef and Irwin 1983, Chamberlin and Kok 1986, Lasota and Kok 1989, Kuhar and Speese 2002, Kuhar et al. 2003), the following lepidopteran species may attack collards in Virginia: imported cabbageworm, *Pieris rapae* (L.) (Pieridae); cabbage looper, *Trichoplusia ni* (Hübner) (Noctuidae); fall armyworm,

Spodoptera frugiperda (J. E. Smith) (Noctuidae); corn earworm, *Helicoverpa zea* (Boddie) (Noctuidae); cabbage webworm, *Hellula rogatalis* (Hulst) (Pyralidae); beet armyworm, *Spodoptera exigua* (Hübner) (Noctuidae); cross-striped cabbageworm, *Evergestis rimosalis* (Guenée) (Pyralidae), and saltmarsh caterpillar, *Estigmene acrea* (Drury) (Arctiidae) (Fig. 5.2). The rank of species importance generally varies with latitude (Kok 2004). The objective of this study was to assess the current field efficacy of several conventional broad-spectrum insecticidal products as well as several newer IPM-compatible products for lepidopteran pest control in collards in Virginia.

Materials and Methods

2003 efficacy trials. In 2003, a field experiment was conducted at two locations, Virginia Tech Kentland Research Farm (80^{o} 25' W, 37^{o} 14' N; elevation ≈640 m), near Blacksburg, VA and the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) (75^{o} 49' W, 37^{o} 35' N; elevation ≈12 m) near Painter, VA. Collards (variety 'Vates') were direct seeded at a rate of10 plants/m on 0.9-m row centers on 23 July at Kentland and on 22 August at ESAREC. The experiment consisted of 11 insecticide treatments each representing a different insecticide class (Table 5.1) plus an untreated check arranged in a randomized complete block and replicated 5 times. Each individual plot consisted of a single 6-m row flanked on both sides by an untreated guard row. Treatments were applied using a gas-pressurized sprayer that delivered 355 l of spray/ha at a pressure of 2.81 kg/cm^2 through a boom, with one hollow cone nozzle oriented over the center of the row and two hollow cone drop nozzles oriented to the sides of the row. Latron B-1956® spreader sticker was added to each treatment and the untreated check at 0.25% v/v of spray. Plants were inspected weekly for the presence of insect pests. When ~50% of the plants had at least one lepidopteran larva, insecticide treatments were applied. Two foliar applications (17 and 30 September) were made at Kentland and a single foliar application (24 October) was made at the ESAREC.

To evaluate efficacy, any live species of lepidopteran larvae were counted on 20 leaves per plot approximately 5 to 8 days after each application. Also, on 11 November (ESAREC) and 31 October (Kentland), 20 leaves per plot were harvested and rated by eye as marketable (no insects present and <10% injury) or unmarketable (≥ 10% defoliation and/or the presence of a larva) (Fig. 5.1).

2004 efficacy trials. In 2004, the experiment was repeated three more times at the same two locations described previously. The experimental design and methods were the same as in 2003, with the exception of the following details: collards were planted on 4 June at ESAREC-1, on 26 July at Kentland, and on 10 August at ESAREC-2; insecticides were applied on 14 and 29 July (ESAREC-1), 14 September and 5 October (Kentland), and 14 November (ESAREC-2); lepidopteran larvae were sampled on 19 July and 4 August at ESAREC-1, on 22 September and 13 October at Kentland, and on 19 and 21 November at ESAREC-2; plots were harvested and rated for damage on 4 August at ESAREC-1, 13 October at Kentland, and 21 November at ESAREC-2.

Statistical Analyses. Data including lepidopteran larval density and proportion of marketable collard leaves at harvest were analyzed using ANOVA and means separated by Fisher's protected LSD at $P \leq 0.05$ (SAS Institute 1999). To stabilize variances, proportion data were transformed [arcsin sqrt (x + 0.001)] before analysis, however, actual percentages are presented in the results.

Results

2003 efficacy trials. At Kentland, total lepidopteran pest pressure on collards was high and averaged more than 8 larvae per 20 leaves in the untreated control plots (Table 5.2). *Pieris rapae* was the dominant species observed (67% of the total larvae) with *P. xylostella* (31%) and a mix of other lepidopteran species comprising the rest. At five days after treatment, there was a significant treatment effect on densities of *P. rapae* ($F = 3.59$; df = 4, 40; $P < 0.0018$) and total lepidopteran larvae ($F = 5.82$; df = 4, 40; $P < 0.0001$), but not *P. xylostella* ($F = 1.44$; df = 4, 40; $P = 0.1996$). The untreated control had the

highest overall density of lepidopteran larvae, but was not significantly different than azadirachtin. Methomyl, acephate, and esfenvalerate had the fewest total lepidopteran larvae. Insecticide treatment also had a significant effect on the percentage of marketable leaves at harvest (F = 3.61; df = 4, 40; P < 0.0031). All treatments had a higher percentage of marketable leaves than the untreated control except azadirachtin and *Bt kurstaki* (Table 5.2).

At the ESAREC, the overall density of lepidopteran larvae on collards was lower than Kentland, and *P. xylostella* accounted for the majority (70%) of lepidopteran larvae observed (Table 5.3). Other species included: *P. rapae, S. frugiperda*, and *S. exigua*. There was a significant treatment effect on densities of *P. xylostella* (F = 5.63; df = 4, 40; P < 0.00001) and total lepidopteran larvae (F = 6.38; df = 4, 40; P < 0.00001). The untreated control averaged 3.8 larvae/20 leaves, which was significantly more than all insecticide treatments (Table 5.3). Several of the treatments including emamectin benzoate, esfenvalerate, indoxacarb, methoxyfenozide and spinosad had no live larvae present on leaves. Insecticide treatment also had a significant effect on the percentage of marketable leaves at harvest (F = 3.55; df = 4, 40; P < 0.0022). All treatments had a higher percentage of marketable leaves than the untreated control, and esfenvalerate had a higher percentage than azadirachtin.

2004 efficacy trials. In 2004 at Kentland, a low density of lepidopteran pests was found on collards, averaging only 1.4 total lepidopteran larvae/20 leaves. Approximately 42% of larvae were *P. xylostella*, and the rest consisted of a broad mix of species including *T. ni*, *P. rapae*, *S. frugiperda*, *H. zea*, and *E. rimosalis* (Table 5.4). Despite the low density, there was a significant treatment effect on densities of *T. ni* (F = 6.0; df = 4, 44; P < 0.0001) and total number of live lepidopteran larvae (F = 2.51; df = 4, 44; P < 0.0151). Though densities were relatively low, the untreated control had significantly more *T. ni* larvae than all of the insecticide treatments, and significantly more total lepidopteran larvae than all treatments except azadirachtin, esfenvalerate, acetamiprid, and methomyl (Table 5.4). Insecticide treatment did not have a significant effect on the percentage of marketable leaves at harvest (F = 1.41; df = 4, 44; P = 0.2024).

At the ESAREC, a moderately high density of lepidopteran larvae was found on collards in the early-planted experiment (ESAREC-1). Species complex was comprised of *T. ni* (52.3%) and *P. xylostella* (31.3%), with a few *P. rapae* (8.4%) and other species. There was no significant treatment effect on densities of *P. xylostella, T. ni*, or total lepidopteran larvae; however, numeric differences were apparent (Table 5.5). The azadirachtin, *Bt kurstaki*, acetamiprid, and esfenvalerate treatments had similar numbers of total lepidopteran larvae as the untreated control. Insecticide treatment did have a significant effect on the percentage of marketable leaves at harvest ($F = 2.66$; df = 4, 44; $P < 0.0106$). Indoxacarb averaged 97% marketable leaves and was the only treatment that was higher than the untreated control, which averaged 78% marketable leaves. The same aforementioned insecticide treatments that had similar larval numbers as the untreated control also had similar percentages of marketable leaves (59 to 80%).

At the ESAREC-2, lepidopteran pest pressure was moderately high and was comprised almost exclusively *P. xylostella* (97%) (Table 5.6). There was a significant treatment effect on densities of *P. xylostella* ($F = 2.98$; df = 4, 44; $P < 0.0075$) and total lepidopteran larvae ($F = 3.06$; df = 4, 44; $P < 0.0063$). The untreated control had the highest larval density (5.8 larvae/20 leaves), but only methomyl, emamectin benzoate and esfenvalerate had significantly fewer live larvae than the untreated control (Table 5.6). Insecticide treatment did not have a significant effect on the percentage of marketable leaves at harvest ($F = 1.07$; df = 4, 44; $P = 0.4065$).

Discussion

A wide range of insecticides is registered for use on cruciferous vegetables in the U.S. Our experiments in Virginia showed that not all insecticides (applied at their lowest-labeled rates) provided the same efficacy against lepidopteran larvae on collards. The most efficacious insecticides over the five experiments included acephate, emamectin benzoate, esfenvalerate, methomyl, methoxyfenozide, novaluron, indoxacarb, and spinosad. These

insecticides were followed in relative efficacy by *Bt kurstaki*, acetamiprid, and azadirachtin, which provided relatively inconsistent control of lepidopteran larvae over the experiments.

Interestingly, two of the oldest insecticides used by growers in Virginia, the organophosphate, acephate, and the carbamate, methomyl, are currently still two of the most efficacious for lepidopteran pest control in collards. Acephate and methomyl are broad-spectrum toxicants that also control other insect pests such as harlequin bugs, flea beetles, and cabbage aphids. However, these insecticides also kill natural enemies such as insect predators and parasitoids, which can play an important role in regulating certain lepidopteran pest and aphid populations (Latheef and Irwin 1983, Chamberlin and Kok 1986, Mitchell et al. 1997, Kok 2004).

A sound integrated pest management program in collards should include pest monitoring, minimizing insecticide applications whenever possible, and if chemical control is necessary, applying reduced risk or IPM-compatible products. The most efficacious insecticide options in collards that have reduced toxicity to natural enemies include indoxacarb, spinosad, novaluron, emamectin benzoate, and methoxyfenozide (Ruberson and Tillman 1999, Studebaker and Kring 1999, Scholz and Zalucki 2000, Elzen 2001). However, spinosad (Ruberson and Tillman 1999, Hill and Foster 2000, Haseeb et al. 2004, Xu et al. 2004), indoxacarb (Haseeb et al. 2004, Xu et al. 2004), and emamectin benzoate (Haseeb et al. 2005) have been shown to be toxic to a wide range of parasitic hymenoptera. However, relatively rapid degradation of surface residues in the field probably improves the compatibility potential of these insecticides with natural enemies. The IPM-compatible insecticides, *Bt* subsp. *kurstaki*, acetamiprid, and azadirachtin have shown efficacy against lepidopteran pests of crucifers in other studies (Leskovar and Boales 1996, Liu 1999), but were inconsistent in their performance in our experiments.

Summary. Control of lepidopteran pests on collards (*Brassica oleracea* L. acephala group) can be challenging because the leaves are the marketable portion of the crop and because of insecticide resistance problems in

diamondback moth, *Plutella xylostella* (L.). In 2003 and 2004 in two locations of Virginia, field efficacy tests were conducted on several conventional standard insecticides as well as several new and more IPM-compatible insecticides (applied at their lowest-labeled rates) for control of lepidopteran pests in collards. Lepidopteran pest species included the following (in relative order of abundance): *P. xylostella*, *Pieris rapae* (L.), *Trichoplusia ni* (Hübner), *Spodoptera frugiperda* (J. E. Smith), *Estigmene acrea* (Drury), *S. exigua* (Hübner), *Helicoverpa zea* (Boddie), *Evergestis rimosalis* (Guenée), and *Hellula rogatalis* (Hulst). The most efficacious insecticides over the five experiments included acephate, emamectin benzoate, esfenvalerate, methomyl, methoxyfenozide, novaluron, indoxacarb, and spinosad. Although acetamiprid, *Bacillus thuringiensis* subsp. *kurstaki*, and azadirachtin have shown efficacy against lepidopteran pests in other studies, they were inconsistent in their performance in these experiments. Insecticide options that provide reliable control of the suite of lepidopteran pests that attack collards in Virginia, and that are relatively less toxic to natural enemies and thus can fit well into integrated pest management programs include indoxacarb, spinosad, novaluron, emamectin benzoate, and methoxyfenozide.

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Table 5.1. Insecticides tested in efficacy trials on collards in Virginia, 2003-2004.

Insecticide (ai)	**Product name (manufacturer)**	**Insecticide Class**
Acephate	Orthene 97 (Valent)	Organophosphate
Acetamiprid	Assail 70WP (Cerexagri)	Neonicotinoid
Azadirachtin	Neemix 4.5EC (Certis USA)	Botanical – Neem extract
Bacillus thuringiensis subsp. *kurstaki* strain HD-1	DiPel DF (Valent)	Microbial
Emamectin benzoate	Proclaim 5WDG (Syngenta)	Avermectin
Esfenvalerate	Asana XL (DuPont)	Pyrethroid
Indoxacarb	Avaunt 30WG (DuPont)	Pyrazoline
Methomyl	Lannate LV (DuPont)	Carbamate
Methoxyfenozide	Intrepid 2F (Dow AgroSciences)	Insect growth regulator
Novaluron	Rimon 0.83EC (Crompton)	Insect growth regulator
Spinosad	SpinTor 2SC (Dow AgroSciences)	Spinosyns

Table 5.2. Density of lepidopteran larvae and percentage marketable leaves (5 days after treatment) on collards after lowest-labeled rate applications of insecticides in field efficacy trials conducted at Kentland Farm near Blacksburg, VA, 2003.

Treatment	Rate (kg [ai]/ha)	Mean no. live larvae/20 leaves			% of leaves marketable (Rating 1 or 2) at harvest
		Plutella xylostella	*Pieris rapae*	Total lepidoptera	
Acephate	1.087	0.2	0.8 bcd	1.6 d	88.0 ab
Acetamiprid	0.084	1.0	1.4 bcd	3.2 bc	79.0 bc
Azadirachtin	0.011	1.8	3.2 a	5.4 ab	68.0 cd
Bt subsp. *kurstaki* strain HD-1	0.605	0.8	1.0 bcd	2.0 cd	70.0 bcd
Emamectin benzoate	0.008	1.0	0.8 cd	2.0 cd	78.0 bc
Esfenvalerate	0.032	0.8	0.8 d	1.8 d	84.0 ab
Indoxacarb	0.072	0.8	2.0 abc	3.0 cd	83.0 ab
Methomyl	0.504	0.2	0.8 cd	1.2 d	94.0 a
Methoxyfenozide	0.112	0.8	2.2 ab	3.6 bc	76.0 bc
Spinosad	0.026	0.8	1.4 bcd	2.4 cd	88.0 ab
UTC	-	2.4	3.4 a	8.2 a	55.0 d

Means in a column with a letter in common are not significant ($P > 0.05$, Fisher's protected LSD).

Table 5.3. Density of lepidopteran larvae and percentage marketable leaves (~ 7 days after treatment) on collards after lowest-labeled rate applications of insecticides in field efficacy trials conducted in Painter, VA, 2003.

Treatment	Rate (kg [ai]/ha)	Mean no. live larvae/20 leaves		% of leaves marketable (Rating 1 or 2) at harvest
		Plutella xylostella	Total lepidoptera	
Acephate	1.087	0.2 b	0.2 c	97.0 ab
Acetamiprid	0.084	0.4 b	0.4 bc	93.0 ab
Azadirachtin	0.011	0.4 b	0.4 bc	83.0 b
Bt subsp. *kurstaki* strain HD-1	0.605	0.4 b	1.2 b	94.0 ab
Emamectin benzoate	0.008	0.0 b	0.0 c	97.0 ab
Esfenvalerate	0.032	0.0 b	0.0 c	99.0 a
Indoxacarb	0.072	0.0 b	0.0 c	98.0 ab
Methomyl	0.504	0.4 b	0.6 bc	96.0 ab
Methoxyfenozide	0.112	0.0 b	0.0 c	93.0 ab
Spinosad	0.026	0.0 b	0.0 c	94.0 ab
UTC	-	3.2 a	3.8 a	76.0 c

Means in a column with a letter in common are not significant ($P > 0.05$, Fisher's protected LSD).

Table 5.4. Density of lepidopteran larvae and percentage marketable leaves (8 days after treatment) on collards after lowest-labeled rate applications of insecticides in field efficacy trials conducted at Kentland Farm near Blacksburg, VA, 2004.

Treatment	Rate (kg [ai]/ha)	Mean no. live larvae/20 leaves				% of leaves marketable (Rating 1 or 2) at harvest
		Plutella xylostella	*Trichoplusia ni*	*Pieris rapae*	Total lepidoptera	
Acephate	1.087	0.0	0.0 b	0.0	0.0 d	100.0 a
Acetamiprid	0.084	0.4	0.0 b	0.0	0.6 abcd	88.0 a
Azadirachtin	0.011	0.6	0.0 b	0.0	1.0 abc	89.0 a
Bt subsp. *kurstaki* strain HD-1	0.605	0.0	0.0 b	0.0	0.0 d	97.0 a
Emamectin benzoate	0.008	0.2	0.0 b	0.0	0.2 cd	99.0 a
Esfenvalerate	0.032	1.0	0.0 b	0.2	1.2 abc	84.0 a
Indoxacarb	0.072	0.0	0.0 b	0.0	0.0 d	100.0 a
Methomyl	0.504	0.4	0.0 b	0.2	0.6 abcd	97.0 a
Methoxyfenozide	0.112	0.4	0.0 b	0.0	0.4 bcd	98.0 a
Novaluron	0.087	0.0	0.0 b	0.4	0.4 bcd	97.0 a
Spinosad	0.026	0.0	0.0 b	0.0	0.0 d	99.0 a
UTC	-	0.6	0.6 a	0.0	1.4 a	88.0 a

Means in a column with a letter in common are not significant ($P > 0.05$, Fisher's protected LSD).

Table 5.5. Density of lepidopteran larvae and percentage marketable leaves (6 days after treatment) on collards after lowest-labeled rate applications of insecticides in field efficacy trials conducted at ESAREC-1 in Painter, VA, in July, 2004.

Treatment	Rate (kg [ai]/ha)	Mean no. live larvae/20 leaves			% of leaves marketable (Rating 1 or 2) at harvest
		Plutella xylostella	*Trichoplusia ni*	Total lepidoptera	
Acephate	1.087	0.4	0.6	1.0	86.0 abc
Acetamiprid	0.084	1.0	2.0	4.4	59.0 d
Azadirachtin	0.011	0.6	3.2	4.4	72.0 bcd
Bt subsp. *kurstaki* strain HD-1	0.605	1.6	1.2	4.8	70.0 cd
Emamectin benzoate	0.008	1.0	1.2	2.2	84.0 abc
Esfenvalerate	0.032	0.6	2.2	3.0	80.0 abc
Indoxacarb	0.072	0.2	0.8	1.4	97.0 a
Methomyl	0.504	1.0	1.6	2.6	75.0 bcd
Methoxyfenozide	0.112	0.0	0.4	1.4	84.0 abc
Novaluron	0.087	0.0	0.8	1.6	89.0 ab
Spinosad	0.026	1.0	1.0	2.2	84.0 abc
UTC	-	1.6	2.0	4.2	78.0 bc

Means in a column with a letter in common are not significant ($P > 0.05$, Fisher's protected LSD).

Table 5.6. Density of lepidopteran larvae and percentage marketable leaves (~ 7 days after treatment) on collards after lowest-labeled rate applications of insecticides in field efficacy trials conducted at ESAREC-2 in Painter, VA, in November, 2004.

Treatment	Rate (kg [ai]/ha)	Mean no. live larvae/20 leaves		% of leaves marketable (Rating 1 or 2) at harvest
		Plutella xylostella	Total lepidoptera	
Acephate	1.087	3.0 ab	3.0 abc	97.5 a
Acetamiprid	0.084	5.3 a	5.5 a	90.0 a
Azadirachtin	0.011	5.0 a	5.3 ab	81.0 a
Bt subsp. *kurstaki* strain HD-1	0.605	4.5 a	4.5 abc	82.5 a
Emamectin benzoate	0.008	2.5 ab	2.5 bc	80.0 a
Esfenvalerate	0.032	1.8 bc	2.0 cd	92.5 a
Indoxacarb	0.072	3.0 ab	3.3 abc	90.0 a
Methomyl	0.504	0.3 c	0.3 d	92.5 a
Methoxyfenozide	0.112	5.0 a	5.0 ab	81.4 a
Novaluron	0.087	3.8 ab	3.8 abc	95.0 a
Spinosad	0.026	2.5 ab	2.5 abc	90.0 a
UTC	-	5.8 a	5.8 a	80.0 a

Means in a column with a letter in common are not significant ($P > 0.05$, Fisher's protected LSD).

A. marketable collards leaves **B. unmarketable collards leaves**

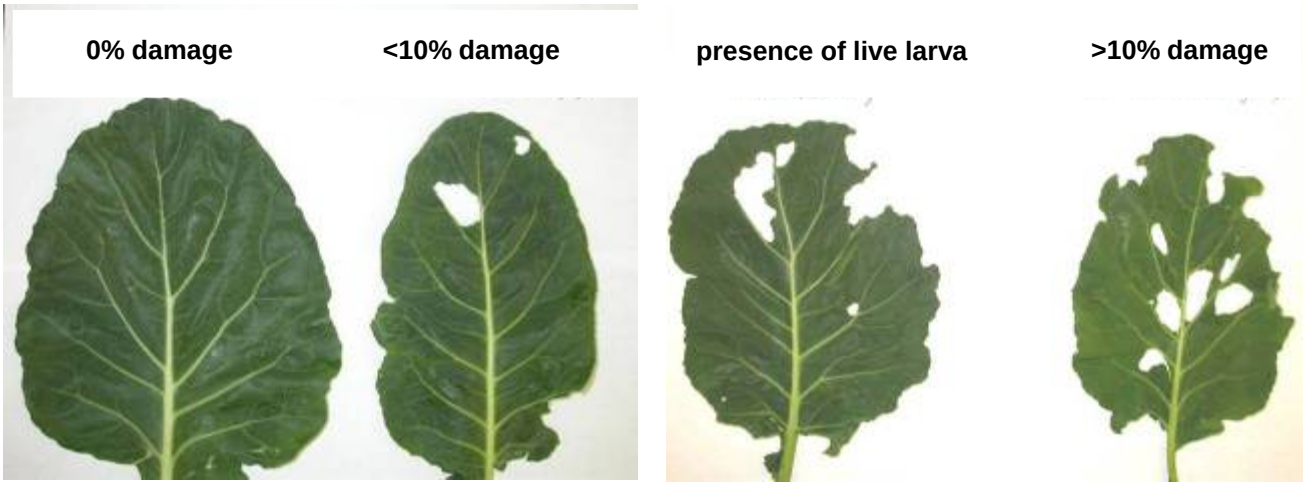

Fig. 5.1. Examples of marketable (A) and unmarketable (B) collards leaves based on local market acceptability on the Eastern Shore of Virginia.

(a) Diamondback moth, *Plutella xylostella* (L.) (Plutellidae) ≈ 0.8 cm

(b) Imported cabbage worm, *Pieris rapae* (L.) (Pieridae) ≈ 2.2 cm

(c) Cabbage looper, *Trichoplusia ni* (Hübner) (Noctuidae) ≈ 2.5 cm

(d) Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Noctuidae) ≈ 2.7 cm

(e) Saltmarsh caterpillar, *Estigmene acrea* (Drury) (Arctiidae) ≈ 2.5 cm

(f) Beet armyworm, *Spodoptera exigua* (Hübner) (Noctuidae) ≈ 1.9 cm

(g) Corn earworm, *Helicoverpa zea* (Boddie) (Noctuidae) ≈ 2.9 cm

(h) Cross-striped cabbageworm, *Evergestis rimosalis* (Guenée) (Pyralidae) ≈2 cm

Fig. 5.2. Lepidopteran pests (a)-(h) on collards in Virginia.

Chapter Six

Conclusions

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most damaging insect pests of crucifer vegetables (*Brassica* sp.) in the world. In Virginia, collards are particularly vulnerable to this pest because the leaves, which *P. xylostella* larvae feed on, are the marketable portion of the crop, and because the pest has a strong preference for the crop over other crucifers. Based on life table studies that I conducted in two regions of Virginia, most (98 to 99%) of *P. xylostella* will perish from natural causes before reaching the adult stage. Mortality factors can vary from year to year and across the state. Larval disappearance from rainfall, predators, disease, parasitoids, or dispersal plays a major role in the overall mortality of *P. xylostella*. Two important parasitoid species in Virginia are *Diadegma insulare* and *Oomyzus sokolowskii,* which parasitize up to 27% and 15% of larvae and pupae, respectively. These parasitoids as well as other natural enemies such as generalist arthropod predators should be conserved in a sound integrated pest management program for *P. xylostella*.

Based on insecticide toxicity bioassays that I conducted on a field population of *P. xylostella* from the Eastern Shore, the pest has significant tolerance levels to the insecticides esfenvalerate, acetamiprid, methomyl, methoxyfenozide, indoxacarb, spinosad, and acephate compared with a susceptible lab population of *P. xylostella*. The highest resistance level (1,876 fold) was to the pyrethroid, esfenvalerate. On the other hand, there were little to no signs of insecticide tolerance to *B. thuringiensis,* novaluron, azadirachtin, or emamectin benzoate. Future bioassays on insecticide susceptibility of *P. xylostella* are needed to continue monitoring resistance levels in the population as well as to monitor new chemicals that are registered for control. Bioassays are relatively cheap and easy to conduct and can give an early indication of potential insecticide resistance problems.

Other toxicity bioassays that I conducted revealed that spinosad, indoxacarb, esfenvalerate, methomyl, acetamiprid, acephate, emamectin benzoate, and methoxyfenozide were toxic to the adult stage of *D. insulare* or *O. sokolowskii* or both. The broad-spectrum insecticides esfenvalerate, methomyl, and acephate as well as the more IPM-compatible insecticides, spinosad, indoxacarb, and emamectin benzoate at their field rate concentrations resulted in 100% mortality to either *D. insulare* or *O. sokolowskii* or both after 72 h of exposure. Moreover, LC_{50} values for all of these insecticides were only a fraction (< 2%) of the actual field rate concentration. The neonicotinoid, acetamiprid, was less toxic than the other insecticides, but still killed 77% of *D. insulare* adults and 91% of *O. sokolowskii* adults after 72 h of exposure. The insect growth regulator, methoxyfenozide, though considerably less toxic than the other insecticides based on LC_{50} levels, still resulted in substantial (62%) mortality of *O. sokolowskii* adults after 72 h. Future studies should investigate toxicity of these insecticides to immature stages of the parasitoids, as well as toxicity of residues after exposure in the field for certain time intervals.

Regardless of what toxicity levels to insecticides are shown in the laboratory, growers are only going to use products that are efficacious in the field to not only *P. xylostella*, but other pests that attack the crucifer crop. Therefore, I conducted field efficacy tests at two locations in Virginia of several conventional insecticides as well as several new and more IPM-compatible insecticides (applied at their lowest-labeled rates) for control of lepidopteran pests in collards. The most efficacious insecticides included acephate, emamectin benzoate, esfenvalerate, methomyl, methoxyfenozide, novaluron, indoxacarb, and spinosad. These insecticides were followed in relative efficacy by *Bt kurstaki*, acetamiprid, and azadirachtin, which provided relatively inconsistent control of lepidopteran larvae in my experiments.

A sound integrated pest management program in collards should probably incorporate the more narrow-spectrum insecticides, such as novaluron, indoxacarb, spinosad, emamectin benzoate, methoxyfenozide, and *Bacillus thuringiensis*. These products are efficacious in the field, and although most that

were tested were toxic to parasitoid adults, these insecticides have been shown to be considerably less toxic to arthropod predators than older insecticides such as pyrethroids, organophosphates, or carbamates.

Appendix 1. Rainfall (mm) and temperature (°C) at Kentland Research Farm, Montgomery Co., at the New River Valley, VA, 2003.

August				September				October				November			
	Julian days	mm	°C		Julian days	mm	°C		Julian days	mm	°C		Julian days	mm	°C
1	213	1.27	22.1	1	244	18.80	22.4	1	274		9.1	1	305		11.5
2	214		22.7	2	245	0.25	22.5	2	275		5.2	2	306		12.7
3	215	1.02	21.9	3	246	14.73	21.4	3	276		4.3	3	307		11.7
4	216		22.2	4	247	17.53	19.7	4	277		9.4	4	308		12.5
5	217	10.67	20.5	5	248	0.25	18.5	5	278		12	5	309	0.25	17.3
6	218		20.5	6	249		15.9	6	279		12	6	310	20.32	16.6
7	219	12.19	20.6	7	250		15.5	7	280		13	7	311	20.57	12.5
8	220	38.10	20	8	251		17.7	8	281		14	8	312		6.5
9	221	0.51	20.6	9	252		16.4	9	282		16	9	313		1.4
10	222	1.27	20.1	10	253		17.3	10	283	1.78	16	10	314		4
11	223	0.25	21.4	11	254		15.7	11	284	0.25	16	11	315		15.3
12	224	11.68	20.9	12	255		15	12	285		15	12	316	0.51	16.8
13	225	0.25	21.9	13	256		18.9	13	286		17	13	317	3.30	6.6
14	226	7.11	23.1	14	257		21.1	14	287		17	14	318		2.2
15	227		24.3	15	258	2.54	18.9	15	288	13.21	11	15	319		2.7
16	228	13.97	22.7	16	259	0.25	15.1	16	289		9.6	16	320	3.56	7.6
17	229		22.5	17	260		14.2	17	290		7.6	17	321	3.05	10.1
18	230		20.7	18	261	24.13	14.5	18	291	1.78	9.8	18	322		11.1
19	231		21.2	19	262	3.81	18.7	19	292		9.5	19	323	1.02	14.1
20	232		21.9	20	263		18.4	20	293		10	20	324	31.24	8.3
21	233	2.29	22.6	21	264		18.8	21	294		14	21	325		6.2
22	234	0.25	23.1	22	265	23.88	18	22	295		11	22	326		5.9
23	235		22.1	23	266	0.76	17.3	23	296		7.6	23	327		6.8
24	236		20.8	24	267	0.25	13.6	24	297		5.9	24	328	0.25	6.5
25	237		21.4	25	268		15.8	25	298		7.9	25	329	1.27	0.3
26	238		22.7	26	269		17.5	26	299		9.9	26	330		2
27	239		23.7	27	270	23.62	16.2	27	300	4.32	10	27	331		6.8
28	240		22.6	28	271	2.29	14	28	301	11.94	5.3	28	332	1.02	9.3
29	241	0.51	23.3	29	272		9.5	29	302		8.8	29	333	7.37	-1.9
30	242		23	30	273	1.52	8.3	30	303	1.02	8.4	30	334		2.8
31	243	6.35	21.9					31	304		11		335		

Appendix 2. Rainfall (mm) and temperature (°C) at Kentland Research Farm, at the New River Valley, VA, 2004.

August				September				October				November			
	Julian days	mm	°C		Julian days	mm	°C		Julian days	mm	°C		Julian days	mm	°C
1	214	18.80	23.3	1	245		18.7	1	275		17	1	306		15.7
2	215	1.78	23.4	2	246		18.3	2	276	1.78	16	2	307		16.3
3	216		22.8	3	247		18.4	3	277	0.25	15	3	308		17.5
4	217	0.76	22.7	4	248		18.7	4	278	0.51	14	4	309	20.57	9.5
5	218	12.95	19.6	5	249		18.6	5	279		13	5	310		8.5
6	219		16	6	250		19.7	6	280		13	6	311		6.6
7	220		15.5	7	251		20.2	7	281		11	7	312		8.7
8	221		16.7	8	252		21.1	8	282		11	8	313		9.5
9	222		18.4	9	253		19.9	9	283		13	9	314		3.9
10	223		19.6	10	254		18.1	10	284		15	10	315		3.1
11	224		20.1	11	255		17.8	11	285	0.25	10	11	316		5.4
12	225	9.65	18.5	12	256		18.9	12	286		10	12	317	24.89	9.6
13	226		17.6	13	257	0.25	19.1	13	287	26.92	13	13	318		7.8
14	227		15.5	14	258		17.8	14	288	0.76	13	14	319		1.1
15	228		17.2	15	259		171	15	289		11	15	320		1.4
16	229		18.7	16	260		19.7	16	290	0.25	9.2	16	321		4.6
17	230		19.3	17	261	33.02	20.8	17	291		9.4	17	322		8.9
18	231	0.25	20.9	18	262	0.25	15.5	18	292	0.25	7.6	18	323	1.02	12.3
19	232		21.3	19	263		13.9	19	293	8.64	16	19	324	0.25	13.1
20	233		21.4	20	264		10.7	20	294	2.54	14	20	325	2.29	12.4
21	234	17.78	20.5	21	265	1.02	13.2	21	295	2.54	13	21	326		10.7
22	235		17.5	22	266		16.1	22	296	0.25	12	22	327		11
23	236		20	23	267		17.1	23	297	0.76	9.8	23	328	14.99	11.3
24	237		20.6	24	268	0.25	16.4	24	298	6.10	12	24	329	29.46	11.6
25	238		21.1	25	269		17.8	25	299	0.25	13	25	330		6.2
26	239		19.3	26	270		17.4	26	300		12	26	331		1.1
27	240		21.4	27	271	1.02	16.2	27	301	5.84	12	27	332	2.79	1.7
28	241		22.3	28	272	85.85	18.9	28	302	0.25	12	28	333		5.7
29	242		21.6	29	273		17.5	29	303	0.51	14	29	334		3.9
30	243		22	30	274		16.4	30	304	0.25	17	30	335	13.97	5.8
31	244		21.2					31	305		19				

Appendix 3. Rainfall (mm) and temperature (°C) at the Eastern Shore Research and Education Center, Painter, VA, 2003.

August				September				October				November			
	Julian days	mm	°C		Julian days	mm	°C		Julian days	mm	°C		Julian days	mm	°C
1	213	16.51	25.8	1	244	0.51	25.3	1	274		19.4	1	305		21.1
2	214	5.59	26.7	2	245		28.1	2	275		16.7	2	306		20.6
3	215	6.10	25.6	3	246		26.4	3	276		14.4	3	307		21.7
4	216		25.6	4	247	14.22	26.1	4	277		22.2	4	308		22.8
5	217	30.23	25.0	5	248	20.57	23.6	5	278		19.4	5	309		23.9
6	218	4.32	23.6	6	249		20.6	6	279		19.4	6	310	2.79	21.7
7	219	15.24	23.6	7	250		19.4	7	280		20.6	7	311	4.32	15.0
8	220	1.52	24.4	8	251		20.0	8	281		22.8	8	312		10.0
9	221		23.3	9	252	2.03	21.9	9	282		22.2	9	313		7.8
10	222	2.54	25.0	10	253		19.7	10	283	1.02	20.0	10	314		10.6
11	223	14.99	23.6	11	254		18.3	11	284	3.30	16.7	11	315		16.7
12	224		25.3	12	255	25.40	20.6	12	285		23.9	12	316	0.25	17.2
13	225		25.6	13	256	10.67	23.6	13	286		20.6	13	317		10.6
14	226		26.9	14	257		22.8	14	287		20.0	14	318		9.4
15	227		28.1	15	258		25.0	15	288	9.65	20.0	15	319		10.6
16	228		28.6	16	259		23.3	16	289		18.9	16	320	0.51	10.6
17	229	37.34	25.3	17	260		19.7	17	290		21.7	17	321	0.51	13.9
18	230		25.3	18	261	33.27	21.7	18	291	1.27	15.0	18	322		14.4
19	231		23.3	19	262		25.3	19	292		20.0	19	323	4.06	17.8
20	232		22.8	20	263		23.9	20	293		19.4	20	324	28.19	12.8
21	233		25.3	21	264		23.6	21	294		23.9	21	325		20.0
22	234		27.8	22	265		23.1	22	295		16.1	22	326		13.9
23	235	2.03	27.2	23	266	8.89	21.9	23	296	0.25	11.1	23	327		14.4
24	236		22.5	24	267		19.7	24	297		12.8	24	328	0.51	16.7
25	237		21.9	25	268		20.6	25	298		17.2	25	329		6.1
26	238		26.9	26	269		21.9	26	299	10.92	18.9	26	330		10.0
27	239		28.3	27	270		22.8	27	300	1.78	19.4	27	331		11.7
28	240	7.37	27.8	28	271	14.48	23.3	28	301	13.46	11.1	28	332	0.51	18.3
29	241	1.52	28.1	29	272		19.2	29	302	74.93	14.4	29	333	3.81	7.2
30	242		29.2	30	273		14.4	30	303		17.2	30	334		12.2
31	243	27.69	26.7					31	304		18.3		335		

Appendix 4. Rainfall (mm) and temperature (°C) at the Eastern Shore Research and Education Center, Painter, VA, 2004.

August				September				October				November			
	Julian days	mm	°C		Julian days	mm	°C		Julian days	mm	°C		Julian days	mm	°C
1	214	18.80	26.7	1	245		24.2	1	275		19.7	1	306		18.3
2	215	20.32	26.4	2	246		21.7	2	276	23.88	20.0	2	307		16.9
3	216	39.88	25.0	3	247		21.9	3	277	0.51	20.8	3	308		19.2
4	217	0.25	26.1	4	248		24.4	4	278		18.1	4	309	13.21	11.9
5	218	0.76	26.9	5	249		21.9	5	279	0.25	17.2	5	310		14.2
6	219	7.87	20.3	6	250	1.78	23.1	6	280		12.5	6	311		8.3
7	220		18.6	7	251	0.51	25.3	7	281		13.6	7	312		13.1
8	221		20.3	8	252		24.4	8	282		16.4	8	313		15.0
9	222		23.6	9	253	10.16	25.8	9	283		16.7	9	314		5.3
10	223		25.0	10	254		23.3	10	284		18.3	10	315		5.0
11	224		25.0	11	255		22.8	11	285		15.8	11	316		8.1
12	225		25.0	12	256		20.3	12	286		11.1	12	317	10.92	10.8
13	226	14.48	23.9	13	257		20.8	13	287	6.10	12.8	13	318	48.77	10.3
14	227	22.61	23.6	14	258		20.0	14	288	9.65	14.4	14	319		4.2
15	228	47.75	20.8	15	259	11.18	22.8	15	289	3.30	15.8	15	320		6.1
16	229	15.75	22.8	16	260	0.25	23.3	16	290	4.06	15.0	16	321		7.8
17	230		21.7	17	261		23.9	17	291		12.2	17	322		8.3
18	231		23.9	18	262	10.16	23.1	18	292		11.4	18	323	0.51	8.9
19	232	0.51	26.4	19	263	1.02	16.1	19	293	7.87	18.9	19	324		12.8
20	233		28.1	20	264		15.8	20	294	38.86	18.6	20	325	0.51	12.2
21	234	3.30	28.1	21	265		16.4	21	295		15.3	21	326		12.5
22	235	12.45	21.7	22	266		20.8	22	296		13.3	22	327		11.4
23	236		20.6	23	267		21.1	23	297		12.5	23	328	1.78	12.8
24	237		22.5	24	268		20.0	24	298	1.27	10.6	24	329	1.02	14.4
25	238		22.2	25	269		18.3	25	299		11.7	25	330	1.78	16.1
26	239		23.3	26	270		18.3	26	300		12.5	26	331		6.9
27	240		23.9	27	271		20.0	27	301		11.9	27	332		5.6
28	241		24.2	28	272	3.00	23.6	28	302		12.2	28	333	18.03	14.7
29	242		24.4	29	273	6.35	21.4	29	303	1.27	12.5	29	334		9.4
30	243	18.03	26.1	30	274		21.1	30	304	0.25	18.1	30	335		6.9
31	244	22.61	26.1					31	305		21.7				

Vita

Roberto Jose Cordero Alonso was born on February 3, 1965 in Managua, Nicaragua. He is the son of Roberto Cordero and Emelina Alonso and has three sisters and one brother. He is married to Dione Coty and they have a son, Roberto Jr. Roberto attended the Panamerican School of Agriculture, Zamorano, Honduras where he earned an Engineer degree in Agriculture in 1989 under the direction of Dr. Ronald D. Cave. He worked for several years at Zamorano with Dr. Cave, and on bananas pest control in Ecuador. Later, he earned a M.S. in entomology at Mississippi State University, Starkville, Mississippi under the direction of Dr. Henry N. Pitre in 1999. In August 2002, he started a Ph.D. program in entomology at Virginia Polytechnic Institute and State University under the direction of Dr. Thomas P. Kuhar.