

## **Abstract**

BORCHERT, DANIEL MICHAEL. Oriental fruit moth phenology in North Carolina apples and ecdysone agonist activity on oriental fruit moth and codling moth. (Under the direction of James F. Walgenbach and George G. Kennedy).

Oriental fruit moth, *Grapholita molesta* (Busck) and codling moth, *Cydia pomonella* (L.), are internal fruit pests of apple. Codling moth is recognized as a pest of apple, but until the last several years, oriental fruit moth was considered primarily a pest of peaches.

Elimination or reduction of organophosphate insecticides and their replacement with reduced-risk insecticides has created the need to evaluate the activity and efficacy of these newly introduced insecticides. The purpose of this research was to evaluate the activity of the ecdysone agonists tebufenozide and methoxyfenozide for codling moth and oriental fruit moth and to develop management strategies for early season control of the two pests.

Methoxyfenozide had greater activity than tebufenozide on codling moth and oriental fruit moth eggs and was active for at least 28 d. Residue breakdown of the two ecdysone agonists was similar, with 60 and 80% decline at 14 and 28 d after application, respectively. Effects of sublethal exposure to methoxyfenozide on the population dynamics of oriental fruit moth was studied in single tree cages and laboratory studies. Population differences were observed between treated and untreated field cages, but could not be directly attributed to sublethal effects of methoxyfenozide; no sublethal effects were observed in laboratory studies. The phenology of oriental fruit moth was studied using egg sampling and pheromone traps to increase the knowledge of the pest in apple. Oriental fruit moth eggs were found on cluster leaves early and on fruit late in the season, predominantly on the top leaf surface and calyx end of the fruit. A degree-day model for oriental fruit moth oviposition was developed and

the start of second-generation oviposition was predicted to occur at 507 DD (7.2 C base temp) after peak trap catch. Spray timing of methoxyfenozide was varied for control of codling moth and oriental fruit moth; two applications applied at 21-d spray intervals provided high levels of control for both pests.

**Oriental Fruit Moth Phenology in North Carolina Apples and Ecdysone  
Agonist Activity on Oriental Fruit Moth and Codling Moth**

by  
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**Approved By:**

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## **Dedication**

This dissertation first and foremost is dedicated to my wonderful wife Kristen Mary Borchert, who dutifully endured the long periods of my absence during research in Fletcher, as well as the relentless barrage of me talking about my research. Kristen's support and love over the entire process from deciding to return to graduate school, to the time apart and finally the push to the finish, has made a difficult journey not only bearable, but wonderful and impossible without her.

The next dedication is to my family (both sides) and friends, especially Rob, Jennifer and Bev, who have always provided love and support through my studies and time here in North Carolina.

The final dedication is to Rick Weires who was always a true role model and friend.

## **Biography**

Daniel Michael Borchert, the youngest of seven children, was born October 16, 1969 in Newburgh, New York to Anne Louise and Robert Alfred Borchert Sr. Growing up on the family fruit farm in Marlboro, New York, Dan's interest in apples grew. Dr. Richard Weires and Dr. Richard Straub turned a summer job as a research assistant in a fruit entomology lab into a desire to study entomology for a living. Following graduation from Marlboro Central High School in 1988, Dan attended Cornell University where he earned a Bachelor of Science degree with a concentration in Plant Science in 1992. Dan then entered a Master's program at North Carolina State University studying pheromone-mediated mating disruption of tufted apple bud moth under Dr. James Walgenbach in 1992. In 1994, Dan married Kristen Mary Prosser, and the two have resided in Morrisville since 1996. In 1995, Dan began work as a research technician in the Biological Control Laboratory under Dr. David Orr. Dan found writing a thesis and working a full time job to be a slow process and finally received his Master of Science degree in 1997. The desire to obtain a Ph.D. never left Dan, and in 1999 he returned to graduate school again under Dr. Walgenbach. Following degree completion, Dan and Kristen will continue to reside in Morrisville with Gus, Belle and Emmett. They are expecting their first child in July.

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## Table of Contents

	Page
LIST OF TABLES . . . . .	viii
LIST OF FIGURES . . . . .	ix
CHAPTER 1: TOXICITY AND RESIDUAL ACTIVITY OF METHOXYFENOZIDE AND TEBUFENOZIDE TO CODLING MOTH AND ORIENTAL FRUIT MOTH . . .	1
Abstract . . . . .	2
Introduction . . . . .	3
Materials and Methods . . . . .	5
Dose Response Bioassays . . . . .	5
Residual Activity Studies . . . . .	6
1999 . . . . .	7
2000 . . . . .	7
2001 . . . . .	8
Apple Leaf and Fruit Expansion . . . . .	10
Results . . . . .	10
Dose Response Bioassays . . . . .	10
Residual Activity Studies . . . . .	11
1999 . . . . .	11
2000 . . . . .	11
2001. . . . .	13
Apple Leaf and Fruit Expansion . . . . .	15
Discussion . . . . .	16
References Cited . . . . .	20
Chapter 1 Figures and Table . . . . .	22
CHAPTER 2: SUBLETHAL EFFECTS OF METHOXYFENOZIDE ON ORIENTAL FRUIT MOTH . . . . .	30
Abstract . . . . .	31
Introduction . . . . .	33
Materials and Methods . . . . .	35
2001 Cage Study . . . . .	35
2002 Cage Study . . . . .	37
Laboratory Studies . . . . .	38
Results . . . . .	39
2001 Cage Study . . . . .	39



2002 Cage Study . . . . .	39
Laboratory Studies . . . . .	40
Discussion . . . . .	41
References Cited . . . . .	43
Chapter 2 Figures . . . . .	45

### CHAPTER 3: ORIENTAL FRUIT MOTH PHENOLOGY AND MANAGEMENT WITH METHOXYFENOZIDE IN NORTH CAROLINA APPLES. . . . . 50

Abstract. . . . .	51
Introduction . . . . .	52
Materials and Methods . . . . .	53
Egg Survey . . . . .	53
Model Development . . . . .	54
Predicted Exposure of Oriental Fruit Moth. . . . .	56
Optimization of Treatment Timing Study. . . . .	56
Results and Discussion . . . . .	59
Seasonal Trap and Egg Patterns . . . . .	59
Model Development . . . . .	63
Predicted Exposure of Oriental Fruit Moth. . . . .	65
Optimization of Treatment Timing Study. . . . .	67
References Cited . . . . .	69
Chapter 3 Figures and Table . . . . .	71

### APPENDIX . . . . . 87

## List of Tables

### Chapter 1

1.1 Dose Response Statistics . . . . .	29
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### Chapter 3

3.1 ANOVA Statistics for Oriental Fruit Moth and Codling Moth Eggs Sampling. . . .	82
3.2 Regression Statistics for Predicted versus Observed Percent Oviposition by Generation . . . . . .	83
3.3 Percentage of Predicted Exposure of Second Generation Oriental Fruit Moth Eggs to Methoxyfenozide . . . . .	85

## List of Figures

### Chapter 1

1.1 Residue breakdown of methoxyfenozide and tebufenozide and percentage of codling moth egg mortality in 1999 . . . . .	22
1.2 Residue breakdown of methoxyfenozide and tebufenozide and number of entries per codling moth egg in 2000. . . . .	23
1.3 Residue breakdown of methoxyfenozide and tebufenozide and number of entries per codling moth and oriental fruit moth egg in 2000. . . . .	24
1.4 Residue breakdown of one versus two applications of methoxyfenozide applied at two volumes and number of entries per codling moth and oriental fruit moth egg in 2001. . . . .	25
1.5 Percentage of fruit damage for internal feeding lepidoptera in 2001. . . . .	26
1.6 Season long leaf surface area measurements 1999 and 2000. . . . .	27
1.7 Season long fruit surface area measurements 1999 and 2000. . . . .	28

### Chapter 2

2.1 Number of oriental fruit moth eggs and percentage of infested vegetative shoots by generation in single tree cages in 2001. . . . .	45
2.2 Percentage of fruit damage and number of larvae recovered from single tree cages in 2001. . . . .	46
2.3 Number of oriental fruit moth eggs and percentage of infested vegetative shoots by generation in single tree cages in 2002. . . . .	47
2.4 Percentage of fruit damage and number of larvae recovered from single tree cages in 2002. . . . .	48
2.5 Developmental and oviposition parameters for oriental fruit moth reared on methoxyfenozide treated diet. . . . .	49

## Chapter 3

3.1 Predicted methoxyfenozide decline for three different application timings. . . . .	71
3.2 Pheromone trap catch and number of eggs for oriental fruit moth and codling moth from four abandoned orchards in 2000. . . . .	72
3.3 Pheromone trap catch and number of eggs for oriental fruit moth and codling moth from four abandoned orchards in 2001. . . . .	73
3.4 Categorized percentage of oriental fruit moth and codling moth eggs found in four abandoned orchards in 2000 and 2001. . . . .	74
3.5 Seasonal percentage of oriental fruit moth and codling moth eggs found on fruit versus leaves averaged over 2000 and 2001. . . . .	75
3.6 Proportion of oriental fruit moth eggs per generation in four abandoned orchards in 2000 and 2001. . . . .	76
3.7 Cumulative number of oriental fruit moth eggs per generation in four abandoned orchards in 2000. . . . .	77
3.8 Cumulative number of oriental fruit moth eggs per generation in four abandoned orchards in 2001. . . . .	78
3.9 Cumulative number of oriental fruit moth and codling moth eggs per generation in Lamb Mt. orchard in 2000 and 2001. . . . .	79
3.10 Cumulative number of oriental fruit moth and codling moth eggs per generation in Oates orchard in 2000 and 2001. . . . .	80
3.11 Percentage of fruit damage and number of larvae found in optimization of timing treatments in 2002. . . . .	81
Appendix. . . . .	86
A 1. Predicted versus observed percentage of cumulative oriental fruit moth oviposition at Lamb Mt. and Oates for four generations in 2000. . . . .	87
A 2. Predicted versus observed percentage of cumulative oriental fruit moth oviposition at Barnwell and Henderson for four generations in 2000. . . . .	88

A 3. Predicted versus observed percentage of cumulative oriental fruit moth oviposition at Lamb Mt. and Oates for four generations in 2001. . . . .	89
A 4. Predicted versus observed percentage of cumulative oriental fruit moth oviposition at Station and Fruitland for four generations in 2000. . . . .	90
A 5. Predicted versus observed percentage of cumulative oriental fruit moth oviposition for four orchards combined for four generations in 2000 and 2001. . . . .	91
A 6. Predicted versus observed percentage of cumulative oriental fruit moth oviposition for four orchards for four generations combined over 2000 and 2001 . . . . .	92

## **Chapter 1**

### **Toxicity and Residual Activity of Methoxyfenozide and Tebufenozide to Codling Moth and Oriental Fruit Moth**

### Abstract

A series of studies was conducted to examine the residual activity and toxicity of the ecdysone agonists tebufenozide and methoxyfenozide to codling moth, *Cydia pomonella* (L.), and oriental fruit moth, *Grapholitha molesta* (Busck), in North Carolina apple systems. Methoxyfenozide exhibited greater activity than tebufenozide against codling moth eggs in dose-response bioassays, with a 4.5- and 5.3-fold lower LC<sub>50</sub> value to eggs laid on fruit treated before or after oviposition, respectively. Oriental fruit moth eggs were 57- and 12-fold less sensitive to methoxyfenozide compared with codling moth eggs on fruit treated before and after oviposition, respectively. Methoxyfenozide was effective in reducing larval entries of both codling moth and oriental fruit moth in field residual activity bioassays, exhibiting activity for at least 28 days after application. Residue breakdown on fruit was approximately 80% at 28 d after treatment for both methoxyfenozide and tebufenozide, with the most rapid residue decline (60%) occurring during the first 14 d after application. Two applications of methoxyfenozide applied at 14-d intervals provided better canopy coverage and higher residue levels than one application. Spray volume (683 versus 2,057 liters/ha) did not affect the efficacy of methoxyfenozide. Leaf and fruit expansion during the season was measured to determine potential plant-growth dilution effects on residual activity. There was very little increase in leaf expansion after mid May, but fruit expansion was described by a second order polynomial regression. Implications for codling moth and oriental fruit moth managed programs are discussed.

## Introduction

The codling moth, *Cydia pomonella* (L.), and oriental fruit moth, *Grapholitha molesta* (Busck), are important internal fruit-feeding pests on apple in North Carolina. While codling moth has long been recognized worldwide as an important pest of apple, walnut and pear (Barnes 1991), oriental fruit moth has only recently emerged as a key pest of apple. Historically, oriental fruit moth has been of greatest importance on peaches and plums, and damage to apples occurred primarily when they were grown in close proximity to the aforementioned crops (Rothschild and Vickers, 1991). In recent years there has been an increased incidence of oriental fruit moth damage to apple in the eastern United States. (Felland and Hull, 1998, Bergh and Engleman, 2001). Hence, integrated pest management programs previously focused on codling moth must now also account for oriental fruit moth.

Broad-spectrum organophosphate insecticides have been used successfully to control codling moth and oriental fruit moth for more than 30 years. More recently, however, organophosphate-resistant populations of both insects have developed throughout North America (Bush et al. 1993, Knight et al. 1994, Varela et al. 1993, Dunley and Welter 2000, Pree et al. 1998, Kanga et al 1999, Usmani and Shearer 2001). In addition, implementation of the Food Quality Protection Act (1996) has resulted in the loss of a number of important organophosphate insecticide registrations on apple. Consequently, alternative control strategies are needed. Bisacylhydrazine is a new class of insect growth regulating insecticides that have exhibited excellent potential for management of codling moth (Pons et al. 1999, Knight, 2000), but their efficacy against oriental fruit moth is less clear.



Bisacylhydrazines have a friendly toxicological profile, with low mammalian toxicity and high insect specificity, affecting only lepidopteran insects with minimal activity against most beneficial insects (Dhadialla et al. 1998). Bisacylhydrazines are agonists of 20-hydroxyecdysone, the hormone in insects that initiates molting during metamorphosis. Ecdysone agonists stimulate initiation of the molting process, but they prevent completion of molting because they persist in the hemolymph and prevent the release of eclosion hormone. (Retnakaran et al. 2001). Dhadialla et al (1998) has provided a detailed review of the bisacylhydrazine class of insecticides. Tebufenozide, the first bisacylhydrazine registered for use in apple, is toxic to codling moth eggs and larvae (Pons et al. 1999, Knight 2000). Methoxyfenozide is an analog of tebufenozide that is active at lower doses and against a broader range of lepidopteran pests compared with tebufenozide; it also exhibits a high degree of safety to non-target insects (Carlson et al. 2001).

The concomitant occurrence of codling moth and oriental fruit moth in many North Carolina apple orchards requires that management programs account for both insects. While use patterns of tebufenozide and methoxyfenozide have been developed for management of codling moth (Knight 2000), their use in managing oriental fruit moth is less clear. The objectives of this study were to examine the relative toxicity, residual breakdown, and length of residual activity of tebufenozide and methoxyfenozide to codling moth and oriental fruit moth, and to examine seasonal apple leaf and fruit expansion to help determine the role of plant-growth dilute effects on residual activity.

## Materials and Methods

**Dose-response bioassays.** Dose-response bioassays were conducted to determine the toxicity of tebufenozide and methoxyfenozide to codling moth and oriental fruit moth eggs. Laboratory colonies of both insects were continuously reared on artificial diet (Yokohama et al. 1987). The codling moth colony was initiated with larvae collected from a commercial apple orchard in Henderson County, NC, in 1998, and the oriental fruit moth colony was obtained from Rutgers University in 1999. For each species, 10-15 male and female pupae were placed into an oviposition bucket, which consisted of a 4-liter plastic bucket lined with cheesecloth and supplied with a sucrose and water source; the sucrose concentration was 5 and 10% for oriental fruit moth and codling moth, respectively. Oviposition buckets were placed in growth chambers at 25°C, 50-60% RH, and a photoperiod of 16:8 (L:D) h. Moths were allowed to develop and mate for 2-3 d before non-sprayed field-collected 'Golden Delicious' apples were placed in the buckets. A minimum of four concentrations per replicate of tebufenozide and methoxyfenozide (Confirm 2F<sup>®</sup> and Intrepid 80 WP<sup>®</sup>, respectively, Rohm and Haas, Spring House, PA) were prepared from serial dilutions using tap water. Triton B-1956 (Rohm and Haas, Spring House, PA) was added at 0.05% to all solutions. Each insecticide was tested against eggs laid on apples before (post oviposition treatment) and after (pre-oviposition treatment) dipping fruit into insecticide solutions. For pre-oviposition treatments, fruit were dipped into solutions for 5 s, removed and allowed to air dry for 2 h, and then placed into oviposition buckets overnight for approximately 14 h. Fruit were then removed from oviposition buckets, eggs counted, and fruit held in sealed plastic buckets for 7 d. Eggs were then examined to determine percentage of hatch. For

post-oviposition treatments, non-treated fruit were placed into oviposition buckets overnight and fruit were then removed and dipped into solutions for 5 seconds and allowed to air dry. Fruit were then handled as previously described. Each concentration was replicated a minimum of three times. Probit analysis was conducted on the dose-response data using Polo PC (LeOra Software 1987).

**Residual Activity Studies.** To aid in determining the residual activity of tebufenozide and methoxyfenozide on apple fruit, chemical analysis and bioassays were conducted on various days after applying insecticides in the field from 1999 to 2001. To determine the concentration of chemical on fruit in the experiments described below (1999 – 2001 studies), samples of four or five fruit per plot were collected arbitrarily from the tree canopy, placed in plastic bags and returned to the laboratory for processing. Height and width measurements (0.1 mm) of each fruit were obtained with a digital micrometer to estimate the surface area of fruit. Surface area was estimated by assuming that each fruit was a sphere, and then calculating the surface area of a sphere ( $4\pi r^2$ ), using the average of the height and width as the diameter. Surface area for all fruit per sample were calculated and combined for sample surface area. Fruit were then returned to the plastic bag and a 50:50 mixture of HPLC grade methanol and distilled water were added at a ratio of 1 ml of solution for every  $2.7 \text{ cm}^2$  of surface area. The bag was shaken for 2 minutes to dislodge residue and the rinsate was collected. Samples were filtered (0.2  $\mu\text{m}$  Gelman Acrodisc) and analyzed with HPLC (Supelcosil LC18 column, Supelco, Bellefonte, PA, 5 ODS-3 250 by 3.0 mm, 5 $\mu\text{m}$  with a 0.6 ml/min flow rate and 235 nm and 210 nm UV detector) for tebufenozide and methoxyfenozide, respectively.

*1999 Studies.* This study was conducted at the Mountain Horticultural Crops Research Station, Fletcher, NC using 'Golden Delicious' apples. Three replicates each of two rates of methoxyfenozide (0.28, 0.14 kg [AI]/ ha) (Intrepid 80WP), one rate of tebufenozide (0.28 kg [AI]/ ha) (Confirm 2F), and a non-treated control were arranged in a randomized complete block design. All treatments were sprayed with an airblast sprayer delivering 990 liters/ha. Each plot consisted of four or five trees, and fruit for bioassays were collected from the middle trees of each plot. Samples of fruit were collected on 0, 7, 14, 21, and 28 d after treatment, and analyzed for residue as described above. An additional five fruit per plot were harvested on each sample date and used in bioassays to determine the residual activity of treatments against codling moth eggs. The bioassay samples were exposed to adult moths as described above for pre-oviposition treatment dose-response bioassays. Percentage of egg hatch data was collected 7 d after oviposition and analyzed using PROC GLM ANOVA with a protected LSD (SAS 1999).

*2000 Studies.* Field studies were conducted on 'Golden Delicious' apples at the Mountain Horticultural Crops Research Station, Fletcher NC. Three plots were used in the study, and each plot consisted of five trees with the three center trees used as individual replicates. Confirm 2F and Intrepid 2F were applied at 0.28 kg [AI]/ha with an airblast sprayer at 990 liters/ha. Treatments consisted of two applications of each insecticide, plus a non-treated control (water only). Treatments were applied on 31 May and 14 June. Another application of treatments was applied on 14 August, 61 days after the 14 June applications. Three fruit were collected from each replicate tree for a total of nine fruit per treatment. Samples were collected on the day of first application and at 7-d intervals up to 28 d after the

14 June application. Fruit were exposed to codling moth adults in oviposition buckets in the same manner as described in the pre-oviposition treatment dose-response bioassay study. In addition to examining percentage of egg hatch on fruit 7 d after oviposition, the number of larval entries into fruit were counted, and expressed as larval entries per egg. Fruit bioassays were also conducted later in the season after the applications on 14 August. Pre-treatment samples were collected from all treatments on 13 August, and post-treatment samples were collected on 0, 7, 14, 21, and 28 d after application. For the 14 August application evaluation, two sets of three fruit each were collected from each replicate, one three fruit sample was exposed to ovipositing codling moth and the other sample to ovipositing oriental fruit moth. Percentage of egg hatch and number of larval entries were counted 7 d after oviposition. Data were analyzed using PROC GLM ANOVA with a protected LSD (SAS 1999).

*2001 Studies.* This study was conducted in a commercial apple orchard in Lincoln County, NC, with a history of high codling moth and oriental fruit moth populations. The experimental block consisted of a 20-yr old mixed block of 'Delicious' and 'Golden Delicious' trees with an estimated tree-row-volume of 2552 liters/ ha. Plots were 0.09 ha. (3 rows x 9 trees), and were arranged in a randomized complete block design, with three replications. In addition to evaluating the one versus two applications of methoxyfenozide (0.25 kg [AI]/ha), each rate of insecticide was applied at either 682 or 2057 liters/ha. Additional treatments consisted of two applications of azinphosmethyl (Guthion 50W<sup>®</sup>, Bayer CropScience, Research Triangle Park, NC)) at 1.12 kg [AI]/ha and 2057 liters/ha, and a non-treated control. Applications were timed for the first generation of codling moth, with the first

application made on 9 May (about 156 DD after codling moth biofix and 360 DD after OFM biofix). For treatments receiving two applications, the first and second applications were made on 9 and 23 May (about 294 DD after codling moth biofix and 536 DD after OFM biofix), respectively. Fruit were selected arbitrarily from the center five trees of each plot on 9 and 23 May, and 6 and 19 June, to monitor residue levels and the biological activity of treatments. Samples for residue and laboratory bioassay were collected from four positions within the tree canopy: low outside (LO), low inside (LI), high outside (HO) and high inside (HI). High-canopy samples were from the upper half of the tree, and inside samples were in close proximity to the center of the tree. Fourteen fruit from each replicate and position were collected arbitrarily, with 2-3 fruit collected from each of the five sample trees (from the fourteen fruit collected, eight fruit were separated and used for residue analysis, two fruit were exposed to oriental fruit moth adults and two fruit were exposed to codling moth adults for laboratory bioassays, with the remaining two fruit discarded). Residue samples were processed and analyzed as described previously. Bioassay samples were placed into oviposition buckets in the same manner as in pre-oviposition treatment dose-response experiments. Percentage of egg hatch and number of larval entries per egg were examined on bioassay fruit 7 d after oviposition. Fruit damage assessments in plots were conducted once per week until the beginning of the second codling moth generation, by examining 100 fruit selected arbitrarily per replicate (25 fruit per position) on each sample date. At the end of the first codling moth generation, 200 fruit per replicate (50 fruit per position) were examined for damage. All bioassay data from residual activity studies were analyzed with PROC GLM (SAS Institute 1999), with a protected LSD used for means separation.

**Apple leaf and fruit expansion.** Studies were conducted at the Mountain Horticultural Research Station in 1999 and 2000. Ten sites were established for both 'Delicious' and 'Golden Delicious' trees. Each site consisted of four to five trees with the center tree used for data collection. For each field-measurement tree, five non-fruiting and five fruiting "clusters" were marked with flagging tape. Beginning in early May of each year (between the petal fall and first cover spray period) and continuing at weekly intervals through the end of September, leaves were counted on each cluster. Fruit height and width were measured with a digital caliper to calculate fruit surface area. Leaves from two non-fruiting and two fruiting clusters were collected from one of the non-marked trees at each site. Leaves were counted and leaf surface area was measured using a Dias II image analyzer (Decagon Devices, Pullman, WA) to obtain mean leaf size. Leaf area of the field structures was estimated from the laboratory measurements. Data were plotted as date versus surface area. Second order polynomial trend lines were fit to the data.

## **Results**

**Dose-Response Bioassays.** Codling moth eggs were 4- to 5-fold more sensitive to methoxyfenozide compared with tebufenozide, and both insecticides were more active when applied to fruit before versus after eggs were deposited on fruit (Table 1.1). For example, the  $LC_{50}$  value for methoxyfenozide against codling moth eggs was 1.58 ppm when fruit were treated before oviposition, but increased to 18.79 ppm when apples were dipped into insecticide after eggs were deposited on fruit. In addition, methoxyfenozide was much more active against codling moth eggs compared with oriental fruit moth eggs;  $LC_{50}$  values for

oriental fruit moth were approximately 57- and 12-fold higher compared with codling moth on fruit treated before and after oviposition, respectively.

**1999 Residual Studies.** Tebufenozide residues on fruit declined more rapidly than those of methoxyfenozide, with reductions of 86 and 55%, respectively, by 21 d after treatment (Figure 1.1A). Although codling moth egg mortality was quite variable, mortality was generally higher on methoxyfenozide- compared with tebufenozide-treated fruit (Figure 1.1B). Egg mortality in the methoxyfenozide treatment was significantly different from the control at 0, 7 and 14 d (For 0-d  $F = 8.56$ ,  $df = 3, 6$ ,  $P = 0.01$ ; for 7-d  $F = 8.90$ ;  $df = 3, 6$ ,  $P = 0.01$ ; for 14-d  $F = 17.60$ ,  $df = 3, 6$ ,  $P = 0.002$ ), respectively. Also, the decline of residues observed on fruit did not appear to be linked to a decline in biological activity as measured by egg mortality. While methoxyfenozide residues declined by  $\approx 50\%$  at 21 d after treatment, there was no apparent decline in egg mortality during this time period. This suggests that even small amounts of residues present after 21 d exhibited activity against codling moth eggs.

**2000 Residual Studies.** Methoxyfenozide residue declined by approximately 60% 13 days after the first application, but tebufenozide residue levels remained stable (Figure 1.2A). However, tebufenozide residue levels were inexplicably low after the first application. Following the second application on day 14, residue levels of both tebufenozide and methoxyfenozide increased to  $\approx 0.7 \text{ ug/cm}^2$ . Variation within samples was reduced after the second application, suggesting that a second application provided more uniform canopy coverage. Residue decline was similar for both methoxyfenozide and tebufenozide after the second application, with a period of rapid decline followed by a reduced rate of decline.



Long field persistence of both tebufenozide and methoxyfenozide was evident with approximately 15% of the residues remaining 28 d after the second application.

Methoxyfenozide exhibited greater activity against eggs of codling moth compared with tebufenozide in the bioassays with field-treated fruit, which supports the findings of the dose-response bioassays. Methoxyfenozide had significantly fewer larval entries per egg compared with the control at 0, 7, 14, 21, 28, 35, and 42 d after treatment (Figure 1.2B) (statistics for 1, 7, 14, 21, 28, 35 and 42 d after treatment were  $F = 9.19$ ,  $df = 2, 4$ ,  $P = 0.03$ ;  $F = 23.87$ ,  $df = 2, 4$ ,  $P < 0.01$ ;  $F = 31.83$ ,  $df = 2, 4$ ,  $P < 0.01$ ;  $F = 20.96$ ,  $df = 2, 4$ ,  $P < 0.01$ ;  $F = 66.6$ ,  $df = 2, 4$ ,  $P < 0.001$ ;  $F = 14.91$ ,  $df = 2, 4$ ,  $P = 0.02$ ; and  $F = 25.62$ ,  $df = 2, 4$ ,  $P < 0.01$ ; respectively). Larval entries in nontreated and tebufenozide-treated fruit did not significantly differ at 0, 7 and 35 d after treatment, when residue levels were  $\leq 0.2 \mu\text{g}/\text{cm}^2$ .

Residue levels of tebufenozide and methoxyfenozide on 13 August, or 55 d after the 14 June application, averaged  $0.05 \mu\text{g}/\text{cm}^2$ . (Figure 1.3A). Following application on 14 August, the rate of residue decline of both methoxyfenozide and tebufenozide was considerably slower when compared with the rate of decline observed in other residual activity studies (Figures 1.1A, 1.2A and 1.4A), all of which were applied earlier in the season (i.e., May and June). At 28 d after treatment, 80 and 54 % of the initial residues of methoxyfenozide and tebufenozide, respectively, still remained. The average residue level over the 28-d period was  $0.47$  and  $0.37 \mu\text{g}/\text{cm}^2$  for methoxyfenozide and tebufenozide, respectively.

Methoxyfenozide-treated fruit had fewer codling moth larval entries compared with the control or tebufenozide in the pre-application bioassay on 13 August, but treatment means were not significantly different ( $F = 2.91$ ,  $df = 2, 4$ ,  $P = 0.20$ ). The number of codling moth larval entries per egg for both methoxyfenozide and tebufenozide were significantly lower compared with the control at 7, 21 and 28 d after treatment (for 7 d  $F = 93.97$ ,  $df = 2, 4$ ,  $P < 0.001$ ; for 21 d  $F = 20.88$ ,  $df = 2, 4$ ,  $P = 0.008$ ; and for 28 d  $F = 19.84$ ,  $df = 2, 4$ ,  $P = 0.008$ ), but not significantly different on 0 and 14 d after treatment (for 0 d  $F = 5.16$ ,  $df = 2, 4$ ,  $P = 0.08$ ; for 14 d  $F = 3.43$ ,  $df = 2, 4$ ,  $P = 0.14$ ). Treatment means for larval entries per egg were 0.11, 0.06, and 0.43 for tebufenozide, methoxyfenozide and the control, respectively.

The number of oriental fruit moth larval entries per egg was lower on methoxyfenozide- compared with tebufenozide-treated fruit on all sample dates (Figure 1.3C). Methoxyfenozide and tebufenozide did not differ significantly from the control at 0 d after treatment ( $F = 3.35$ ,  $df = 2, 4$ ,  $P = 0.14$ ). Methoxyfenozide had significantly fewer larval entries per egg compared with the control at 7, 14, 21 and 28 d after treatment (statistics for 7, 14, 21 and 28 d were  $F = 7.88$ ,  $df = 2, 4$ ,  $P = 0.04$ ;  $F = 12.78$ ,  $df = 2, 4$ ,  $P = 0.02$ ;  $F = 17.09$ ,  $df = 2, 4$ ,  $P = 0.01$ ; and  $F = 7.13$ ,  $df = 2, 4$ ,  $P = 0.048$ , respectively). Larval entries on tebufenozide-treated fruit were significantly lower compared with the control only at 14 d. Tebufenozide was clearly more effective in reducing codling moth compared with oriental fruit moth larval entries into fruit.

*2001 Residual Study.* Residue decline of methoxyfenozide on fruit was similar for both spray volumes, but residue levels were consistently higher in the lower (683 liters/ha) compared with higher (2,057 liters/ha) application volumes (Figure 1.4A). Methoxyfenozide

residues decline after the first application at both 2,057 and 683 liters/ha declined by  $\approx 40$  and 80% at 14 and 28 d after application, respectively. The rate of residue decline was much slower for the remaining 14 d through 19 June, at which time residue levels averaged  $<0.05 \mu\text{g}/\text{cm}^2$ .

In those treatments receiving two applications of methoxyfenozide, residue levels increased three-fold following the second application on 23 May. Residue declined  $>60\%$  14 d later on 6 June, but the rate of decline slowed during the next 14 days, for a total reduction of  $\approx 72\%$  on 19 June.

The number of codling moth larval entries on control fruit was relatively high and constant throughout the experiment (Figure 1.4B). Codling moth larval entries were significantly lower compared with the control for all insecticide treatments on 9 and 23 May and 6 June ( $F=86.07$ ;  $\text{df}=3,24$ ;  $P<0.001$ ;  $F=23.08$ ;  $\text{df}=5,36$ ;  $P<0.001$ ;  $F=16.24$ ;  $\text{df}=5,36$ ;  $P<0.001$ ; respectively). Larval entries in the two-application treatments of methoxyfenozide at 683 and 2057 liters/ha were significantly lower compared with the control on 19 June ( $F=7.94$ ;  $\text{df}=5,36$ ;  $P<0.001$ ), but the other insecticide treatments did not differ from the control. Although two applications of methoxyfenozide numerically reduced larval entries more than one application, there was no significant difference between 683 and 2057 liters/ha.

Overall, the number of oriental fruit moth larval entries was reduced for all insecticides for the duration of the study, but the large amount of variation did not always result in statistical differences among insecticide treatments and the control (Figure 1.4C). Larval entries were significantly reduced in all treatments compared with the control on 23

May and 6 June ( $F=1.80$ ;  $df=5,36$ ;  $P=0.15$ ;  $F=2.00$ ;  $df=5,36$ ;  $P=0.10$ ; respectively).

Treatments receiving two applications of methoxyfenozide at 683 and 2057 liters/ha and the one application at 2057 liters/ha were significantly lower compared with the control on 19 June ( $F=3.54$ ;  $df=5,36$ ;  $P=0.01$ ). Oriental fruit moth data were not as consistent as those of the codling moth data, and this may have been due to a relatively high percentage of infertile eggs laid by moths. Based on low oriental fruit moth egg hatch rates in the control, it is assumed that fruit may have been introduced into oviposition chambers before sufficient mating took place, which resulted in infertile eggs being laid. Hence, the main interactions of interest, application number and spray volume, were not as apparent with oriental fruit moth compared with codling moth, because number of larval entries were similar among all treatments. There was no consistent position x treatment interaction observed during the study.

There were no significant differences among treatments in fruit damage on 16 May, or one wk after the first application of materials ( $F=1.22$ ;  $df=5,10$ ;  $P=0.37$ ) (Figure 1.5). Damage present at this time was likely due to first generation oriental fruit moth larvae, which typically would have been controlled with an insecticide applied at petal fall, but was omitted from this study. All treatments significantly reduced fruit damage below that of the control at the end of the first codling moth generation on 19 June ( $F=24.71$ ;  $df=5,10$ ;  $P<0.001$ ), but there was no difference among insecticide treatments, in which damage ranged from 1.3 to 3.7%, compared with 20% in the control.

**Leaf and Fruit Growth.** Total leaf area of fruited clusters for 'Golden Delicious' and 'Delicious' changed very little between early May and late September of 1999 and 2000

(Figure 1.6). The change in leaf area over time for ‘Delicious’ clusters in 2000 was best fit by a polynomial regression ( $y = -0.0045x^2 + 329.39x - 6e+6$ ,  $r^2 = 0.83$ ). However, the fit of remaining data sets to regression models was poor. While these data suggest that there was little if any change in total foliage during the season, this should not be interpreted as implying that new leaves were not generated during the season. Indeed, new leaves were produced during the season, but the loss of some older leaves resulted in negligible change in total leaf area.

In contrast to leaf expansion, fruit expansion was very consistent and predictable for ‘Delicious’ and ‘Golden Delicious’ in 1999 and 2000. Fruit expanded at a steady, almost linear rate for much of the season, with a typical increase of 8-10 cm<sup>2</sup> per week. Second order polynomial regression equations fit the fruit expansion data sets very well, with  $r^2$  values  $\geq 95\%$  for all regressions. These data did not follow the sigmoid, three-stage growth model described by Pratt (1988), because our sampling was initiated after the initial slow period of fruit expansion.

## Discussion

Tebufenozide and methoxyfenozide both demonstrated high levels of activity against codling moth eggs, and subsequently reduced larval entries into fruit. The higher activity of methoxyfenozide compared with tebufenozide was evident in that LC<sub>50</sub> values for eggs on fruit were about 5-fold lower for methoxyfenozide. Although methoxyfenozide was also toxic to oriental fruit moth eggs, its activity was less than that against codling moth eggs. Our results with tebufenozide are consistent with those of Pons et al. (1999) and Knight

(2000), who both showed that tebufenozide was effective against egg and larval stages of codling moth. However, tebufenozide was considerably more toxic to codling moth eggs oviposited on fruit dipped in insecticide solutions in our studies compared with Pons et al. (1999), who obtained an  $LC_{50}$  value of 117.47 ppm compared to our value of 7.05 ppm. The type of fruit may have accounted for these differences. In preliminary studies, when we used fruit stored at 32°C for 4 to 5 months, the  $LC_{50}$  to codling moth eggs was approximately 200-fold higher compared with fruit harvested from trees in July ( $LC_{50}$  for tebufenozide applied before oviposition on stored fruit was 1,397.73 ppm compared with 7.05 ppm on fruit harvested from trees).

Pons et al. (1999) reported a 30-fold increase in the  $LC_{50}$  value of tebufenozide to codling moth eggs on leaves treated after versus before, oviposition. We observed a similar occurrence for both tebufenozide and methoxyfenozide on fruit; i.e.,  $LC_{50}$  values were 12- to 13-fold higher on fruit dipped after versus before oviposition. The same occurrence was also observed with oriental fruit moth eggs, in which  $LC_{50}$  values of 91.4 and 237.6 ppm on fruit treated before and after oviposition, respectively. In contrast, Knight (2000) did not detect differences in codling moth egg mortality on leaves treated with tebufenozide before or after oviposition. However, Knight (2000) also reported that application of tebufenozide shortly after biofix (19-77 degree days) was more effective compared with later applications. It appears that eggs are more sensitive to tebufenozide and methoxyfenozide residues on surfaces treated before oviposition, suggesting that uptake of the chemical through the bottom of the egg chorion may be an important mode of entry. The top surface of the egg chorion may repel or reduce the uptake of much of the insecticide when applied topically. In

the laboratory, the egg surface was not exposed to environmental conditions present in the field that may affect hardening of the chorion, such as variations in temperature, humidity, sunlight, leaf abrasion, or dust. These conditions may affect the permeability of the upper egg chorion, allowing greater uptake or penetration of insecticide.

The relatively long residual activity of tebufenozide and methoxyfenozide on fruit was apparent in the various field trials. In two out of three tebufenozide experiments and three out of four methoxyfenozide experiments, we observed a decline in residue levels of approximately 80% by 28 days after application, which was similar to results with tebufenozide on apple leaves in Washington (Knight 2000). However, we did not observe this level of decline after the 14 August application in 2000, when at 28 d after application residues declined by only about 44 and 17% for tebufenozide and methoxyfenozide, respectively. The reduced rate of insecticide breakdown in August compared to May and June may have been due to the fact that fruit were approaching maturity at this time, when the rate of fruit expansion was declining compared to May and June. It should be noted that plant growth dilution effects would be expected to be of less concern on leaves compared with fruit, because very little if any leaf expansion of fruiting clusters was observed after early May. The increased activity of methoxyfenozide compared with tebufenozide observed in laboratory bioassays was also evident in field studies, where methoxyfenozide was more effective in reducing both codling moth and oriental fruit moth larval entries into fruit. This greatly improves the opportunities of using this ecdysone agonist in IPM programs where these two insects occur concomitantly. However, the different mode of action of methoxyfenozide compared to organophosphate insecticides may require use patterns

different from those developed for organophosphate insecticides, which have contact activity to all life stages. The efficacy and long residual activity of methoxyfenozide against eggs of codling moth and oriental fruit moth suggests that insecticide should be applied before egg laying begins. This is in contrast to the use patterns of organophosphate insecticides for which application timing is directed at peak moth flight (Riedl and Croft, 1978).

The long residual activity of methoxyfenozide provides a useful tool for management of both codling moth and oriental fruit moth, but this long residual activity also raises concerns about resistance management. Incidences of cross-resistance between azinphosmethyl and tebufenozide has been reported for codling moth (Knight et al. 2001) and obliquebanded leafroller (Waldstein and Reissig, 2000). Resistance management strategies should be implemented that reduce multiple-generation exposure to low residue levels of either methoxyfenozide and/or azinphosmethyl. The inclusion of new chemistry insecticides and/or mating disruption would aid in minimizing the potential for methoxyfenozide resistance.

These series of studies provide important pieces of information that can be used to improve management of codling moth and oriental fruit moth. The higher activity of methoxyfenozide compared with tebufenozide to both codling moth and oriental fruit moth makes this insecticide an ideal option in orchards where both insects require control. Applications of methoxyfenozide at earlier degree-day timings than previously used for organophosphates should ensure the presence of residue before oviposition and reduce residue levels affecting subsequent generations.



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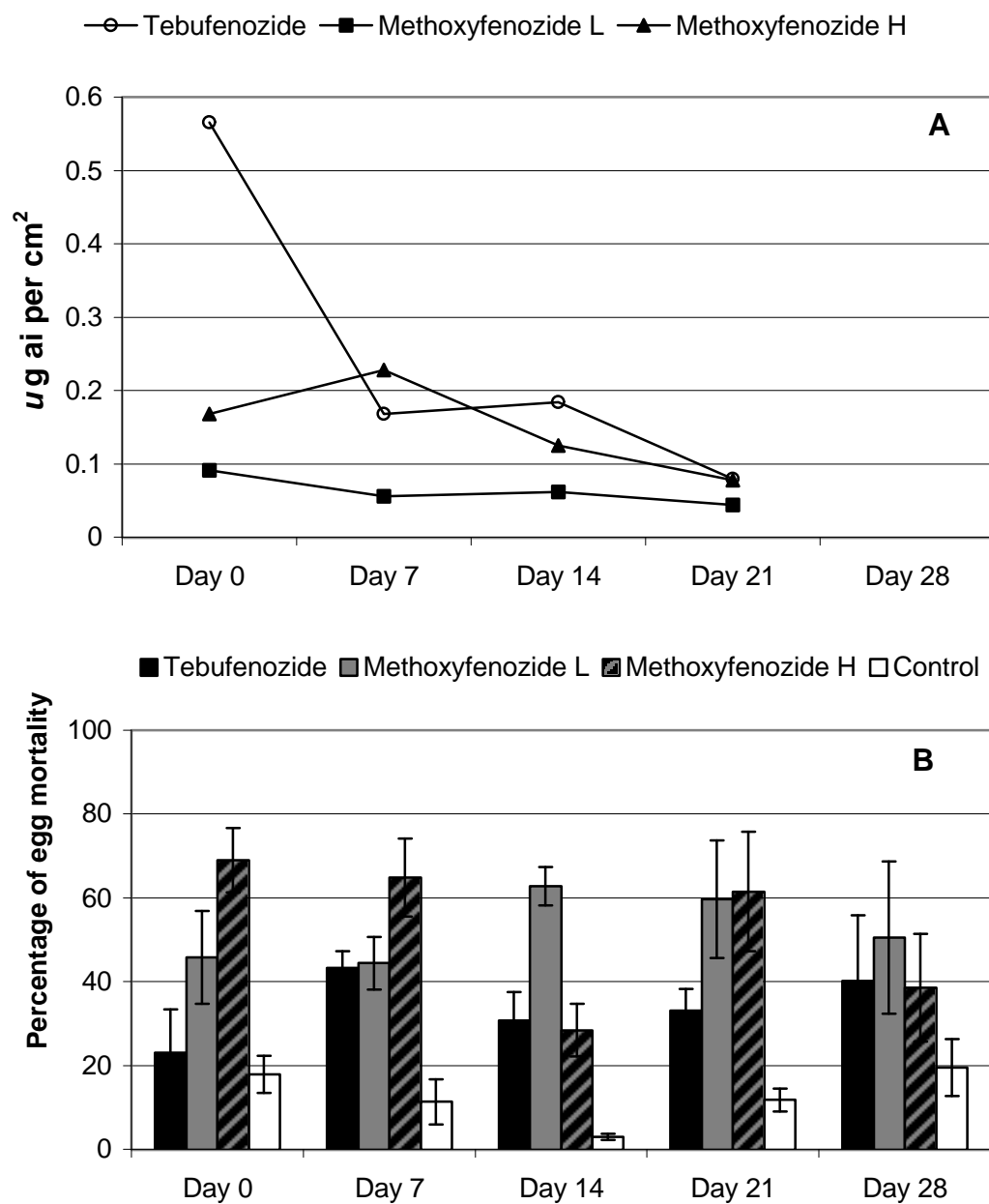


Figure 1.1 Mean tebufenozide and methoxyfenozide residues on fruit (A) and codling moth (B) percentage of egg mortality ( $\pm$  SEM) on fruit harvested from trees sprayed with one application of tebufenozide (0.28 kg[ai]/ha) or methoxyfenozide L (0.14 kg[ai]/ha) or H (0.28 kg[ai]/ha) on Day 0 (12 July). Henderson County, NC. 1999.

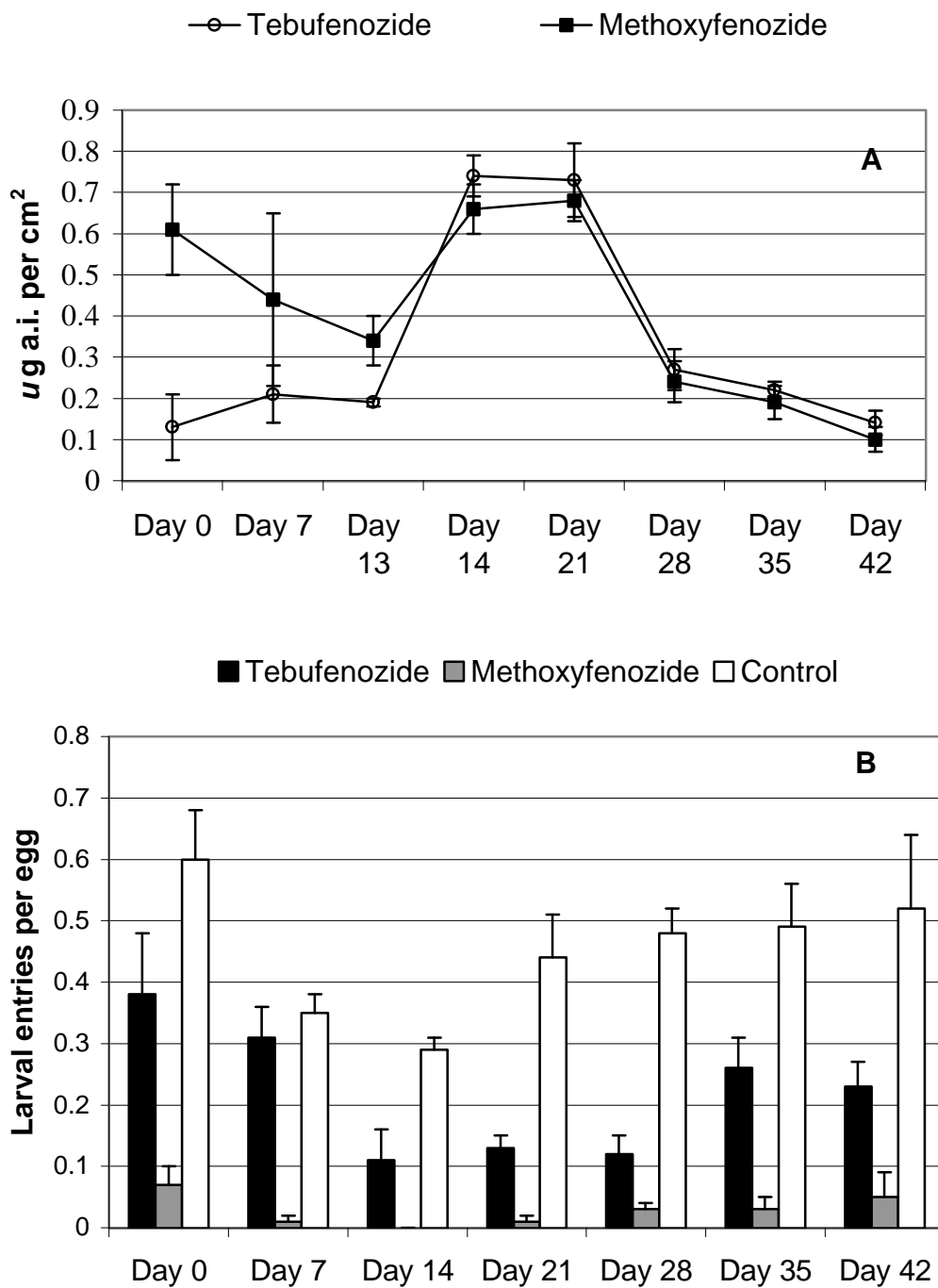


Figure 1.2. Mean (+/- SEM) tebufenozide and methoxyfenozide residues on fruit (A) and codling moth (B) larval entries per oviposited egg into fruit harvested from trees sprayed with two applications of tebufenozide or methoxyfenozide at 0.28 kg[ai]/ha on Day 0 and 14 (May 31 and June 14). Henderson County, NC. 2000.

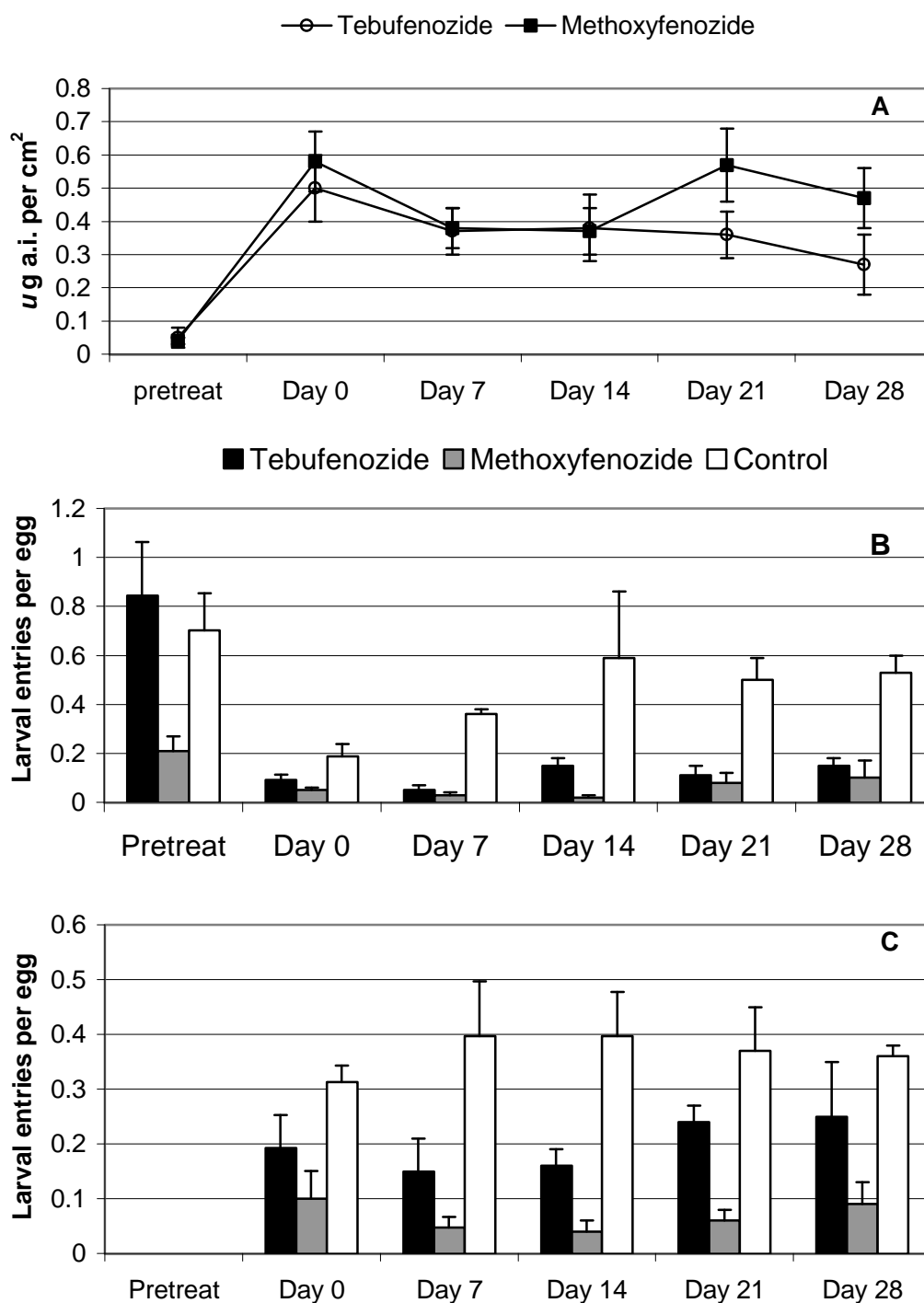


Figure 1.3. Mean (+/- SEM) tebufenozide and methoxyfenozide residues on fruit (A) and codling moth (B) and Oriental fruit moth (C) larval entries per oviposited egg into fruit harvested from trees sprayed with one application of tebufenozide or methoxyfenozide (0.28 kg[ai]/ha) at 990 liters/ha on Day 0 (August 14). Henderson County, NC. 2000.

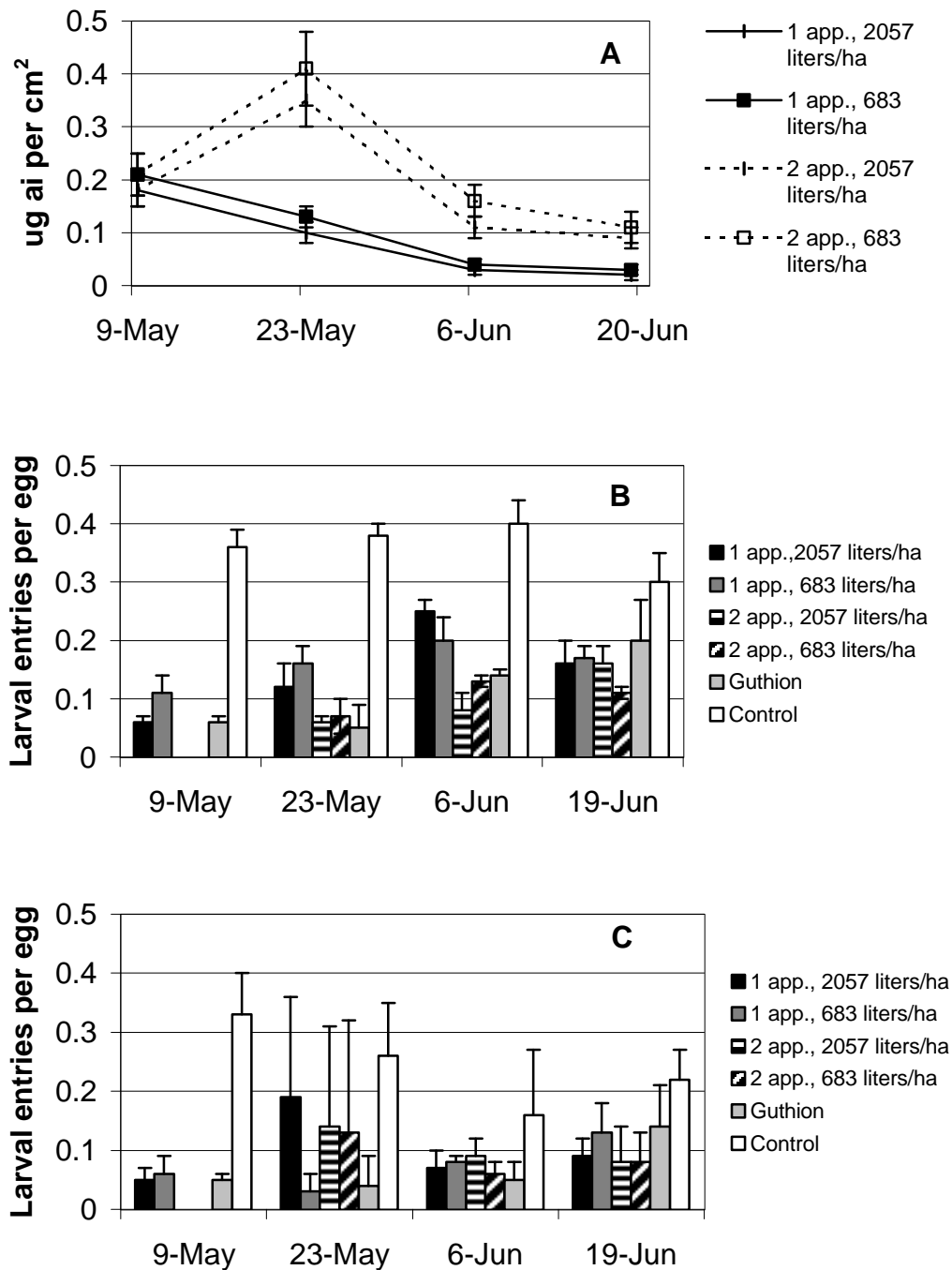


Figure 1.4. Mean ( $\pm$  SEM) methoxyfenozide residue on fruit (A) and codling moth (B) and Oriental fruit moth (C) larval entries into fruit harvested from trees sprayed with one (9 May) or two (9 and 23 May) applications of methoxyfenozide (0.25 kg[a.i.]/ha) at 2057 or 683 liters/ha, or two applications (9 and 23 May) of azinphosmethyl (1.12 kg[AI]/ha) at 2057 liters/ha. Lincoln County, NC. 2001.

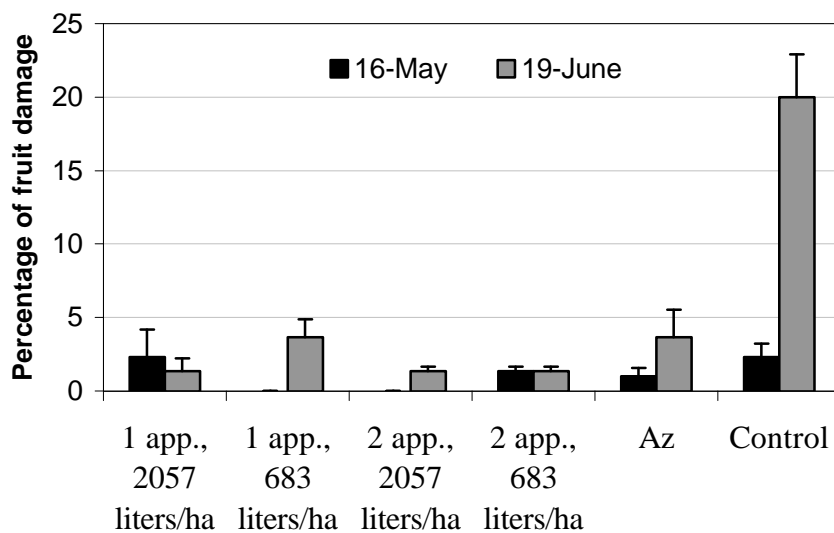


Figure 1.5. Mean (+/- SEM) percentage of fruit damage (16 May and 19 June) for early season internal fruit feeding lepidopteran complex on treatments receiving one or two applications of methoxyfenozide (0.25 kg [ai]/ha at 2057 liters/ha or 683 liters/ha, or two applications of azinphosmethyl (Az)(1.12 kg [ai]/ha) at 2057 liters/ha and an untreated control. Lincoln County, NC. 2001.

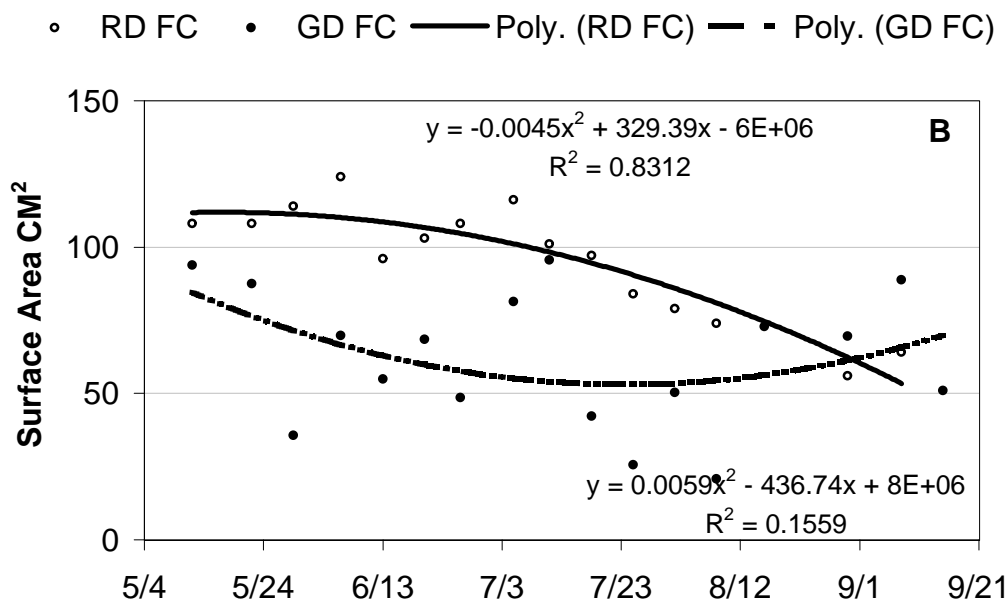
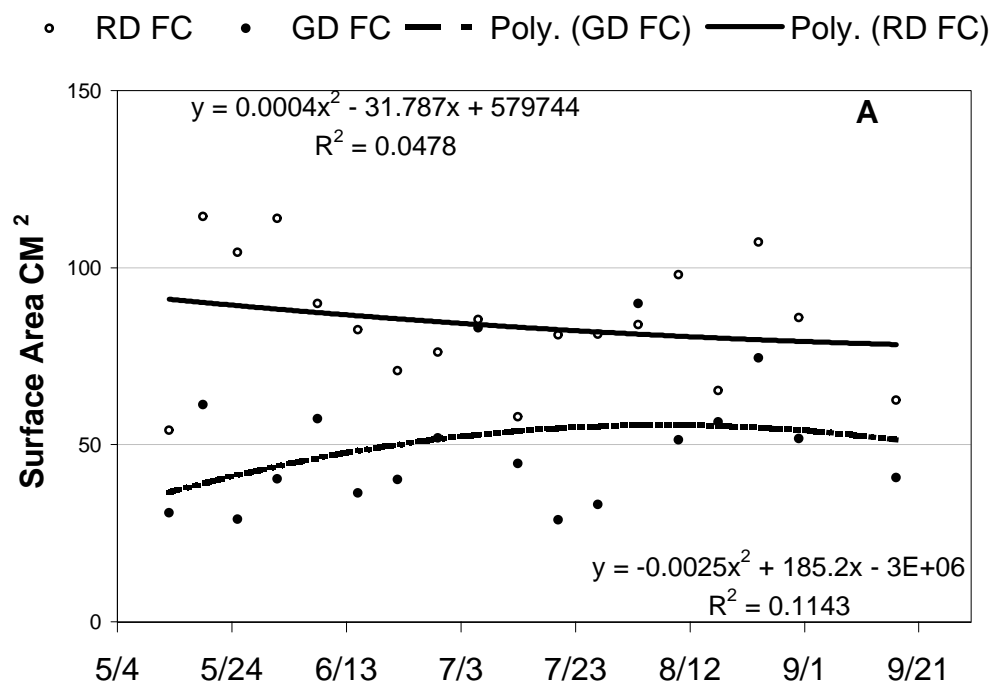


Figure 1.6. Season long surface area measurements of fruited cluster apple leaves for 'Delicious' and 'Golden Delicious' in 1999 (A) and 2000 (B). Henderson County, NC. 1999 and 2000.



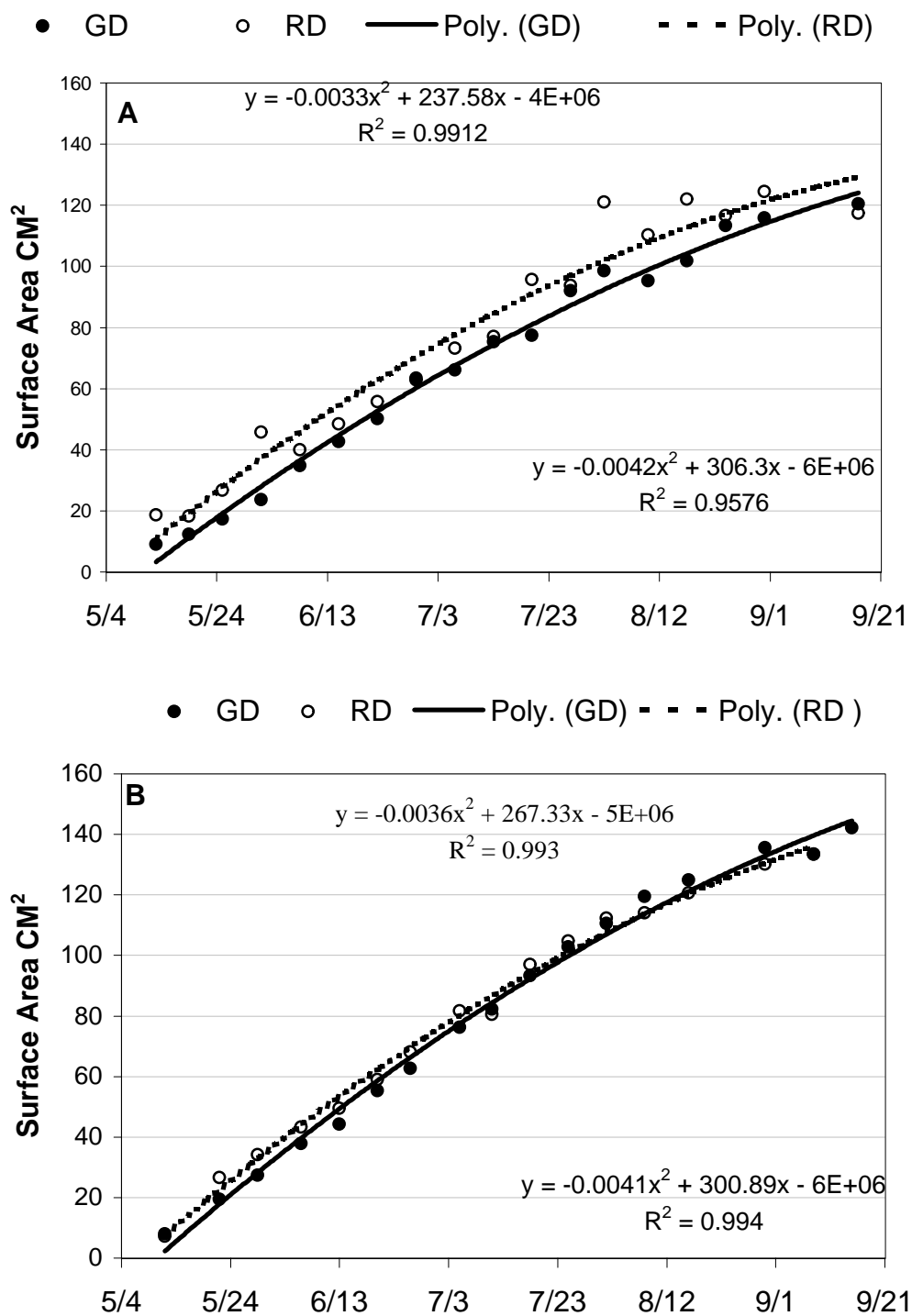


Figure 1.7. Season long surface area measurements of 'Delicious' and 'Golden Delicious' fruit in 1999 (A) and 2000 (B). Henderson County, NC. 1999 and 2000.

Table 1.1. Dose-response statistics for exposure of codling moth and oriental fruit moth eggs to tebufenozide and methoxyfenozide on apple fruit.

Insecticide	Species	<i>n</i>	Slope $\pm$ SE	LC <sub>50</sub> (95% FL)	$\chi^2$
Fruit treated before oviposition					
Tebufenozide	Codling moth	11,191	0.95 $\pm$ 0.04	7.05 (3.06-12.08)	80.31
Methoxyfenozide	Codling moth	12,610	1.02 $\pm$ 0.02	1.58 (1.24-1.97)	16.21
Methoxyfenozide	Oriental fruit moth	8,253	0.98 $\pm$ 0.06	91.39 (38.83-378.82)	11.69
Fruit treated after oviposition					
Tebufenozide	Codling moth	5,053	0.95 $\pm$ 0.05	100.44 (24.26-263.73)	59.33
Methoxyfenozide	Codling moth	8,093	0.50 $\pm$ 0.02	18.79 (1.62-168.12) <sup>a</sup>	309.31
Methoxyfenozide	Oriental fruit moth	8,879	0.67 $\pm$ 0.02	237.63 (109.47-551.45)	119.13

<sup>a</sup> 90 % FL

## **Chapter 2**

### **Sublethal Effects of Methoxyfenozide on Oriental Fruit Moth**

### Abstract

Sublethal effects of the ecdysone agonist methoxyfenozide were examined in oriental fruit moth, *Grapholita molesta* (Busck), through laboratory and field studies. In laboratory studies, oriental fruit moth larvae reared on diet amended with 0.1 ppm methoxyfenozide developed at the same rate as larvae reared on untreated diet, and paired moths reared as larvae from the same treated or untreated diets exhibited similar fecundity and fertility. Population growth differences over multiple generations were used to examine sublethal effects of methoxyfenozide on population dynamics in the field. Multiple single tree cages were established in an apple orchard trees not treated and trees treated with two applications of methoxyfenozide (70 g [AI]/ha). Cages were infested at a single time point with virgin male and female oriental fruit moth adults and population growth was evaluated by egg counts, shoot infestation, fruit damage and larval counts over a 12-wk period. Significantly fewer eggs, percentage of damaged fruit and larvae were found in the methoxyfenozide treatment compared with the control in 2001. Observed population differences may have been a result of direct mortality to eggs and larvae of the first generation rather than sublethal effects. In 2002, no differences in were observed among treatments, but a heavy rain event shortly after early infestation impacted the experiment. A late moth-release treatment was tested in 2002 to examine the effects of methoxyfenozide residual remaining from the initial applications. Significantly fewer eggs were found in the methoxyfenozide treatment compared with the control, but there were no differences between treatments for shoot infestation, percentage of damaged fruit or larval populations. Based on laboratory results (where no sublethal effects were observed) and the potential for direct mortality of methoxyfenozide on the first generation as the cause of reduction of subsequent generations,

it was concluded that there are minimal if any sublethal effects on the population dynamics of oriental fruit moth.

## Introduction

Organophosphate insecticides have been an important component of apple IPM programs for more than 40 years (Croft and Bode 1983), particularly against internal-feeding Lepidoptera, such as the codling moth, *Cydia pomonella* (L.). Implementation of the Food Quality Protection Act (Anonymous 1996) has resulted in the loss of a number of organophosphates on apples, and additional regulatory actions are expected in the future. Consequently, new insecticides with novel modes of action are being developed for use in apple systems.

Among the new group of insecticides used in apples are insect growth regulators that affect the hormonal or physiological development of insects. The bisaclyhdrazine class of insect growth regulators are ecdysone agonists that mimic the steroid 20-hydroxyecdysone, the molting hormone in insects (Dhadialla et al. 1998). The normal molting process during the larval stage of development is initiated with the release of prothoracicotropic hormone (PTTH) from neurosecretory cells in the brain, which stimulates the release of ecdysone from the prothoracic gland into the insect hemolymph (Evans 1984). The presence of high titers of ecdysone initiates an up regulation of genes expressed in the molting process, and when ecdysone is cleared or removed from the hemolymph there is a down regulation expression of genes that leads to ecdysis and the completion of molting (Retnarkaran et al. 2001). Ecdysone agonists trigger initiation of the molting process but the persistence of agonists in hemolymph prevent the completion of the molting process, which results in a precocious and incomplete molt and subsequent death. Tebufenozide and methoxyfenozide are two bisaclyhdrazine insecticides that are lepidopteran-specific in activity and are used to manage a diversity of lepidopteran larvae in several crops (Carlson et al. 2001).

Insect growth regulators are typically slower acting compared with neurotoxic insecticides and generally do not kill the adult stage, but exposure may result in various changes within the insect itself. For example, exposure of insects to sublethal doses of insecticides may result in behavioral or reproductive alterations and subsequent reduction in offspring production (Haynes, 1988). Bisaclyhdrazine compounds have been shown to reduce oviposition in a number of insects (Smaghe and Degheele 1992, 1994). Laboratory studies have demonstrated that exposure of adult codling moth to tebufenozide and methoxyfenozide residues reduced adult fecundity and fertility (Sun and Barrett 1999, Knight 2000). Knight (2000) also observed an 80 and 75 % reduction in number of offspring, primarily due to reduced egg hatch, when female codling moth were placed in sleeve cages on vegetative apple shoots treated with tebufenozide 14 and 21 d after application, respectively. Pons et al. (1999) reported that longevity of adult codling moths was not affected by topical exposure to tebufenozide. Biddinger and Hull (1999) also reported that tufted apple bud moth, *Platynota idaeusalis* (Walker), larval development was not affected when reared on tebufenozide-treated diet (equivalent of LC<sub>25</sub> dose), but fecundity of emerging females was reduced compared to non-treated individuals. Similar results have been observed with redbanded leafroller, *Argyrotaenia velutinana* (Walker), and obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Sun et al. 2000).

The oriental fruit moth, *Grapholita molesta* (Busck), has recently emerged as a serious pest of apple in the eastern United States (Felling and Hull 1994, Bergh and Engleman 2001). Methoxyfenozide has been shown to be toxic to oriental fruit moth eggs and larvae (Chapter 1), but sublethal effects have not been investigated. Considering the long residual activity of methoxyfenozide (Chapter 1) and the multiple generations of

oriental fruit moth that occur on apple, sublethal effects could significantly impact oriental fruit moth populations on apple. Reported here are the results of laboratory and field studies designed to detect potential sublethal effects of methoxyfenozide on oriental fruit moth.

## **Materials and Methods**

Oriental fruit moths used in all experiments were obtained from a laboratory colony from Rutgers University in 1999, and were continuously reared on artificial diet (Yokohama et al. 1987).

**2001 Cage Studies.** To examine the occurrence of potential sublethal effects of methoxyfenozide on an initial cohort and subsequent generations of oriental fruit moth, a field cage study was conducted at the Mountain Horticultural Crops Research Station, Fletcher, NC. Cages were placed over individual trees (14-yr-old spur 'Delicious') which averaged 4 m tall x 3 m wide. A total of 10 trees were used in the study; five trees treated with methoxyfenozide and five control trees each. The methoxyfenozide treated trees received two applications of methoxyfenozide (Intrepid 2F<sup>®</sup>, Dow AgroSciences, Indianapolis, IN) at 70.5 g a.i./ha applied at 990 L per hectare, on 10 and 24 May. The recommended application rate of methoxyfenozide is much higher (0.24-0.28 kg [AI]/ha), but a reduced rate was used to simulate low residue levels that may be encountered by oriental fruit moth in the field. In addition, our interest was in subsequent generations, so low rates were used to ensure survival of the initial cohort. No other insecticides were applied to trees, although fungicides were applied at 14-d intervals.



After the 24 May application, cages were erected over the five methoxyfenozide-treated and five non-treated trees. Cages were constructed from gray fiberglass screening placed over an internal metal frame. The frame consisted of five support legs made of 19 mm galvanized electrical conduit pipe, joined at the top with a steel weather-resistant electrical box, and stabilized at the bottom by burying the legs 0.3 m into the ground. The frame was 4.6-m tall and 3.0-m wide on each side. Each cage consisted of eight sections of screening 5.5-m long and 1.83 m-wide joined by sewing with a heavy-duty 100 % nylon thread. The cage was placed over the frame and the bottom edge was secured with rocks or sandbags.

Moths were released into cages on 4 June (50 virgin females and males) and on 8 June (100 virgin females and males). The adult moths were previously sexed as pupae and maintained in separate 4-liter plastic containers to prevent mating before release into the cage. Moths were released at dusk to reduce potential heat stress and to promote mating.

Fruit samples were collected from three sites in each treatment on 12 June to quantify the residue level of methoxyfenozide. Residue samples were processed and analyzed in the same manner as described in chapter 1.

*Data collection.* Every two weeks from 12 June to 5 September, all caged trees were examined as completely as possible for number of eggs, infested vegetative shoots, and number of damaged fruit. When eggs were located, a permanent marker was used to circle the eggs to prevent duplicate counting. Infested shoots and fruit were similarly marked or flagged. At harvest on 5 September, all fruit in the cages were collected and examined for the presence of larvae and type of injury. Egg data were analyzed using a PROC GLM repeated measures analysis (SAS Institute 1999). Fruit data were analyzed using a PROC GLM ANOVA. Oriental fruit moth degree-day accumulations were calculated from the

initial release date through 5 September, using a lower and upper base of 7.2 and 32.7° C, respectively, based on the sine-wave method (Baskerville and Emin 1969). The degree-day base temperature limits were the same as used for development of the PETE model for oriental fruit moth (Croft et al. 1980). Temperature data used were collected from a weather station located approximately 0.5 km from the study site. Degree-day accumulations were used to assign data collected from each sample date to the first (0 – 530 DD), second (531 – 1060 DD) and third (>1060 DD) generations.

**2002 Cage Study.** In 2002, the study was expanded to determine if treatment effects observed in 2001 during second and third oriental fruit moth generations were due to mortality or sublethal effects on the initial cohort released into cages (which contributed to lower second and third generation populations), or if declining methoxyfenozide residues were directly affecting later generations. To differentiate these effects, two additional treatments were established in which the release of moths was delayed until the second generation period. The study was conducted in the same block of trees as in 2001.

Methoxyfenozide-treated (70.5 g[AI]/ha of Intrepid 2F) trees were sprayed on 15 and 29 May, after which cages were erected over 12 trees, six treated with methoxyfenozide and six non-treated. In three methoxyfenozide-treated and non-treated cages each, 100 moths (50 virgin females and males) were released at dusk on 12 June. In the six remaining cages (three methoxyfenozide-treated and three non-treated trees) the release of moths was delayed until 23 July (610 DD after the first release). Data were collected in the same manner as in 2001, beginning 14 days after initial moth release and continuing until 30 September, when fruit were harvested. Early release cages had three generations of oriental fruit moth, while the late release cages had two generations. Data were analyzed in the same manner as 2001.

**Laboratory Studies.** To help explain results observed in field cage studies, experiments were conducted to examine the effects of prolonged exposure of oriental fruit moth larvae to low levels of methoxyfenozide residues. Four oriental fruit moth neonates were placed into 30 ml plastic diet cups with approximately 10 ml of lima bean based artificial diet (Yokohama et al 1987) that contained 0.0 (untreated) or 0.1 ppm methoxyfenozide incorporated into the diet (equivalent to  $LC_{25}$  value). Diet cups were capped with a paper lid and a plastic cap to reduce desiccation. Larvae were held at 25°C and 70% RH with a 16:8 L:D h cycle throughout the experiment. Starting at 10 d post infestation, and continuing each day thereafter, diet cups were examined for pupae. When pupation was observed, length of larval development was recorded, and pupae were removed from the diet cup, identified to sex, and weighed. Pupae were held until adult eclosion, and the length of pupal development was recorded. One female and two male moths of similar ages, reared on the same diet treatment, were combined in a small oviposition chamber consisting of a 1 liter plastic cup that was lined with cheesecloth. Sucrose and water solutions, along with waxed paper strips for oviposition, were placed in cups. Beginning 2 d after moths were placed into cups and each day thereafter, wax strips were collected until moths died. Waxed paper strips onto which moths oviposited were collected at 2-d intervals, number of eggs counted, and then placed in 25-ml test tubes and incubated at 27°C, 90% RH, and 16:8 L:D h. After 7 d, eggs were observed to determine the number hatching. Data were analyzed using a t-test.

## Results

**2001 Cage Studies.** Methoxyfenozide residue levels on fruit from treated trees averaged  $0.04 (\pm 0.005) \text{ } \mu\text{g/cm}^2$  on 12 June, approximately 7 d after initial moth release. The number of oriental fruit moth eggs was significantly higher in the control cages compared with methoxyfenozide cages over the three generations ( $F = 13.30$ ;  $df = 1,4$ ;  $P = 0.02$ ) (Figure 2.1A). The most dramatic increase in egg numbers was observed in the control cages between the second and third generations. There was a significant generation effect ( $F = 6.01$ ;  $df = 2,8$ ;  $P = 0.025$ ) and a generation x treatment interaction ( $F = 4.62$ ;  $df = 2,8$ ;  $P = 0.05$ ). The total mean number of eggs in the control and methoxyfenozide treatments over all three generations was 572.4 and 116.2, respectively. The percentage of infested shoots was higher on trees in the control cages versus methoxyfenozide cages, but these differences were not significant ( $F = 1.84$ ;  $df = 1,4$ ;  $P = 0.25$ ) (Figure 2.1B). Percentage of fruit damage at the end of the third generation was significantly higher on trees in the control cages ( $70.9 \pm 3.51\%$ ) compared with methoxyfenozide cages ( $10.4 \pm 3.49\%$ ) ( $F = 130.44$ ;  $df = 1,4$ ;  $P < 0.001$ ) (Figure 2.2). In addition, significantly more larvae were collected from control cage fruit compared with methoxyfenozide cage fruit, with  $29.4 \pm 10.32$  and  $1.0 \pm 0.63$  larvae per cage, respectively ( $F = 7.51$ ;  $df = 1,4$ ;  $P = 0.052$ ) (Figure 2.2 B).

**2002 Cage Studies.** A severe thunderstorm that produced 11.5 cm of rainfall on 26 June severely impacted the early cohort of oriental fruit moth released into cages. Consequently, there were no significant differences between early release treatments in number of eggs ( $F = 2.32$ ;  $df = 1,2$ ;  $P = 0.27$ ) (Figure 2.3A), percentage of shoots infested ( $F = 0.01$ ;  $df = 1,2$ ;  $P = 0.92$ ) (Figure 2.3B), percentage of fruit infested ( $F = 0.28$ ;  $df = 1,2$ ;  $P = 0.65$ ) (Figure 2.4A), or number of larvae ( $F = 4.00$ ;  $df = 1,2$ ;  $P = 0.18$ ) (Figure 2.4B).

However, there were significant differences between the late release treatments of methoxyfenozide and control cages for cumulative number of eggs for both generations ( $F = 30.01$ ;  $df = 1,2$ ;  $P = 0.03$ ), but there was no generation x treatment interaction ( $F = 0.08$ ;  $df = 1,2$ ;  $P = 0.80$ ). Although the percentage of infested shoots was higher in control versus methoxyfenozide treatments of the late release, these differences were not significant ( $F = 3.54$ ;  $df = 1,2$ ;  $P = 0.20$ ). In addition, there were no differences between late release treatments in percentage of infested fruit ( $F = 0.00$ ;  $df = 1,2$ ;  $P = 0.96$ ), or number of larvae ( $F = 0.00$ ;  $df = 1,2$ ;  $P = 1.00$ ).

**Laboratory Studies.** Larval and pupal development of oriental fruit moth reared on methoxyfenozide amended diet did not differ from the control treatment. While larval developmental length on methoxyfenozide diet was slightly shorter compared with non-treated diet, the opposite occurred with pupae (Fig. 2.5A), and total development length did not differ between treatments for both sexes combined ( $t = 0.86$ ;  $df = 1,109$ ;  $P = 0.41$ ) or for males ( $t = 0.19$ ,  $df = 1,69$ ,  $P = 0.85$ ) or females ( $t = 1.35$ ,  $df = 1,45$ ,  $P = 0.18$ ). Pupae from the methoxyfenozide treatment weighed slightly less compared with the control; differences were significant for male pupae ( $t = 3.07$ ,  $df = 1,55$ ,  $P = 0.003$ ), but not for female pupae ( $t = 1.42$ ;  $df = 1,49$ ;  $P = 0.16$ ). When pooled across treatments, mean weight of females ( $13.7 \pm \text{SEM mg}$ ) was significantly higher than males ( $10.5 \pm \text{SEM mg}$ ) ( $t = 12.55$ ;  $df = 1,124$ ;  $P < 0.001$ ).

The percentage of individuals that completed development to the adult stage when reared on methoxyfenozide-treated and non-treated diet was 60 and 80%, respectively, or a mortality rate of 25% on methoxyfenozide diet. When male and female moths from methoxyfenozide and non-treated diet were paired in oviposition studies, there was no

significant difference in number of eggs per female ( $t = 0.57$ ,  $df = 1,38$ ,  $P = 0.57$ ) or percentage of eggs that hatched ( $t = 1.76$ ,  $df = 1, 38$ ,  $P = 0.09$ ). The mean number of eggs per female reared on methoxyfenozide and non-treated diet were 66.4 and 57.6, respectively (Fig. 2.5B). In addition, there was no difference in the number of egg-laying days for females reared on methoxyfenozide and non-treated diet ( $t = 0.23$ ;  $df = 1,38$ ;  $P = 0.83$ ).

## Discussion

Several studies have demonstrated sublethal effects of tebufenozide or methoxyfenozide to a number of tortricid species (Biddinger and Hull 1999, Knight 2000, Sun and Barrett 1999, and Sun et al. 1999), manifested as reductions in fecundity and/or fertility. In laboratory studies examining the development and fecundity of oriental fruit moth reared on methoxyfenozide-amended diet, we were unable to detect any sublethal effects due to larval exposure. Long-term continuous exposure of oriental fruit moth adults to methoxyfenozide residue was not examined, but may provide additional information related to sublethal effects.

When conducting field-cage studies, our hypothesis was that sublethal effects of methoxyfenozide on oriental fruit moth would be expressed as a reduction in the rate of population increase on trees sprayed with methoxyfenozide compared with control trees. Based on results in 2001, the hypothesis appeared true, because there were large differences observed between populations of oriental fruit moth on trees treated and not treated with methoxyfenozide. There were significant differences in the number of eggs, number larvae recovered, and the percentage of fruit damaged. These differences between the two

treatments were quite large, as was necessary to show significance with the small degrees of freedom in the study, but it is not possible to attribute differences to sublethal effects alone.

It is probable that oriental fruit moth eggs and/or emerging larvae produced by adults released into methoxyfenozide treated cages died as a result of exposure to methoxyfenozide residue. This initial mortality of the first generation would obviously reduce the size of subsequent generations compared with control cages. Based on egg sampling data from abandoned orchards, oriental fruit moth requires multiple generations to build to large numbers (Chapter 3). Hence, it is unlikely that there was sufficient time during our cage study for populations to rebound to the same level as in control cages. Unfortunately, due to the large rainfall event on 26 June 2002, we were unable to reproduce the results from 2001 or demonstrate initial mortality as the main cause of population differences. Although there were lower egg numbers in the late release methoxyfenozide cages the shoot infestation and fruit damage data did not differ from control cages.

In laboratory studies, we were unable to detect sublethal effects when larvae were reared on diet amended with methoxyfenozide equivalent to the  $LC_{25}$  dose, which further suggests that sublethal effects may not be an important factor influencing population dynamics under field conditions.

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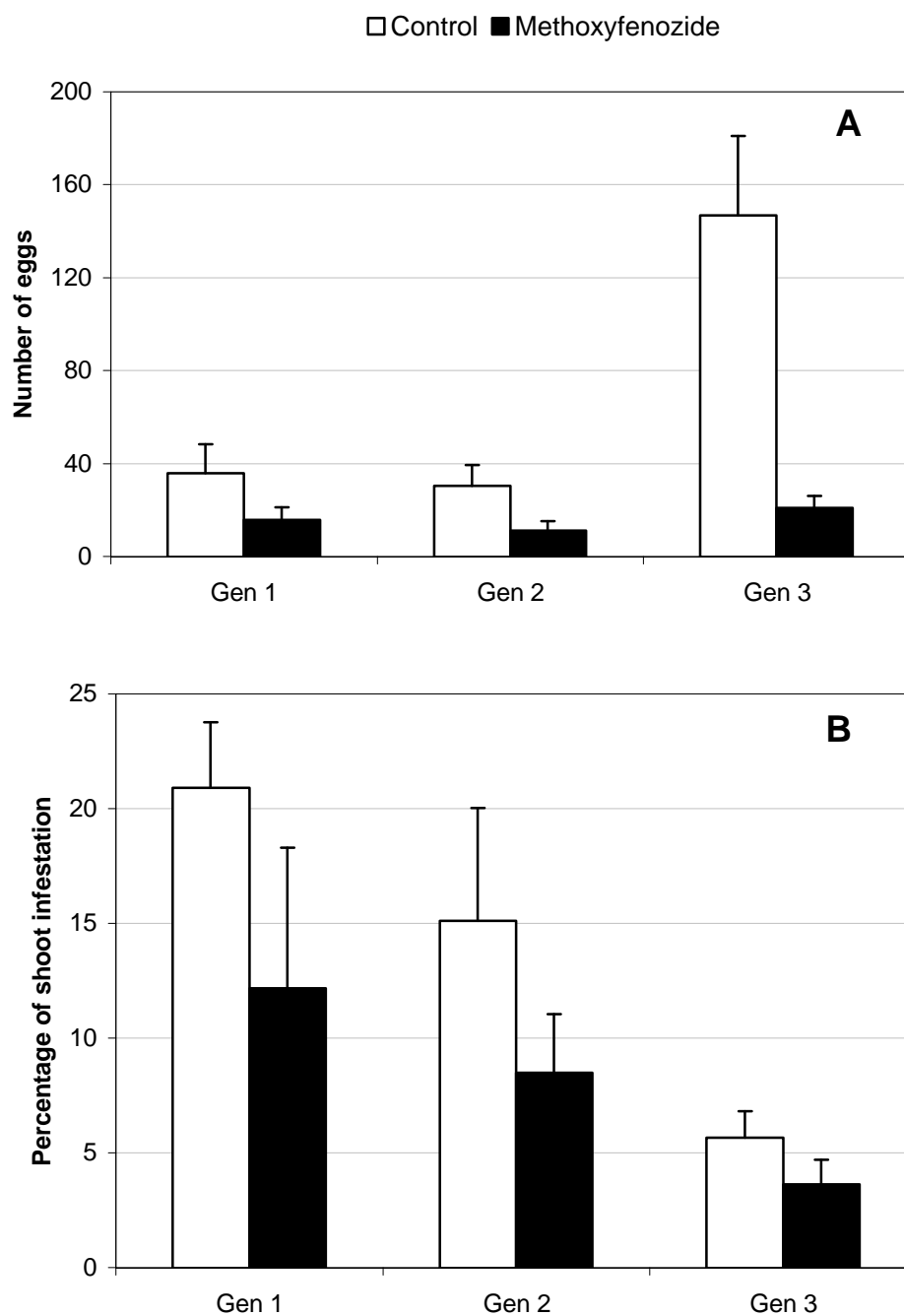


Figure 2.1. Mean ( $\pm$  SEM) oriental fruit moth eggs per generation (A) and percentage of vegetative shoot infestation (B) in single tree cages treated with two applications of methoxyfenozide (70g [ai]/ha) or nontreated prior to infestation with adult moths for the first generation. Henderson County, NC. 2001.

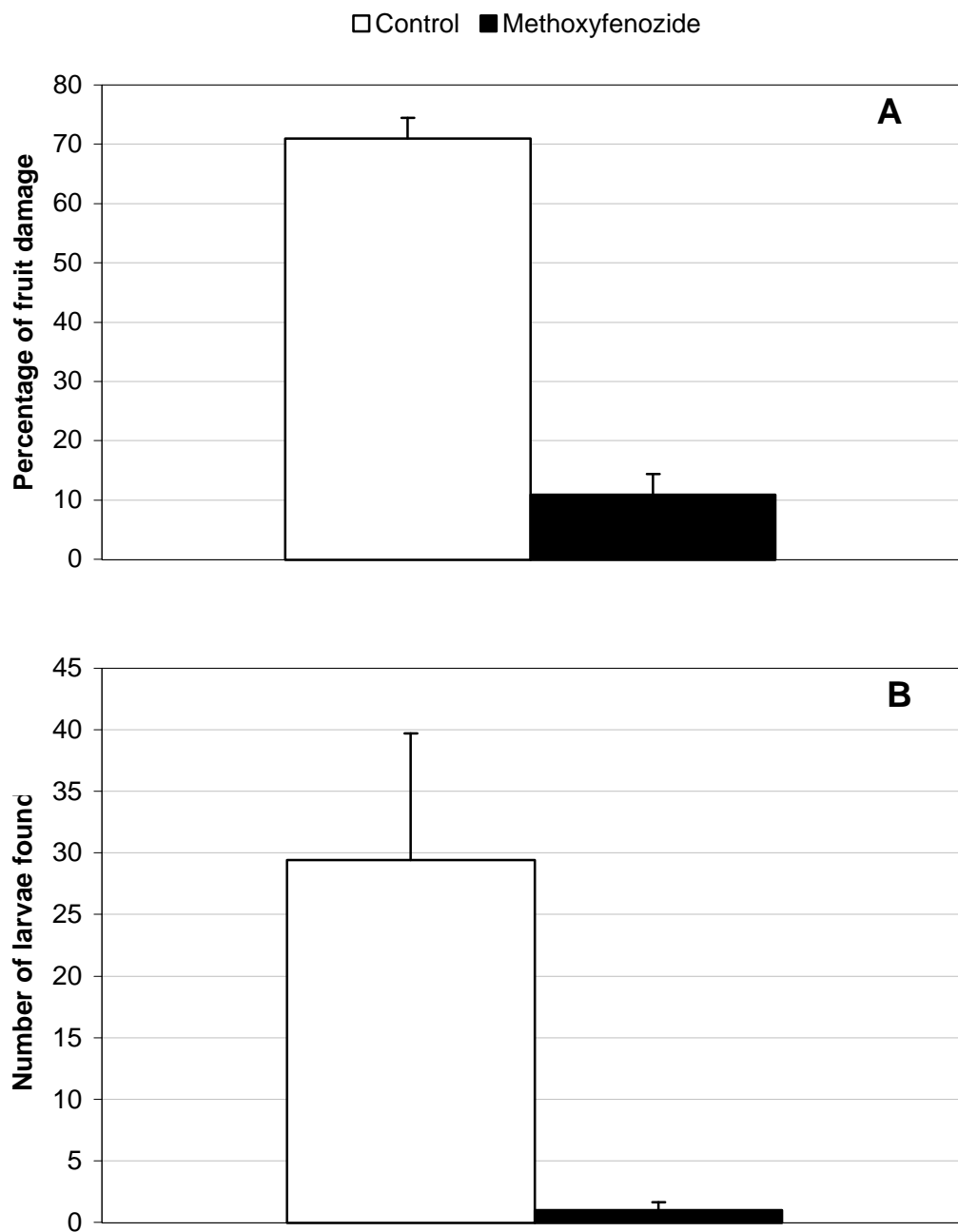


Figure 2.2. Mean ( $\pm$  SEM) percentage of fruit damage (A) and number of larvae collected from fruit (B) in single tree cages treated with two applications of methoxyfenozide (70 g [AI]/ha) or untreated, and infested one time with oriental fruit moth adults and allowed to complete 3 generations within cage. Henderson County 2001.

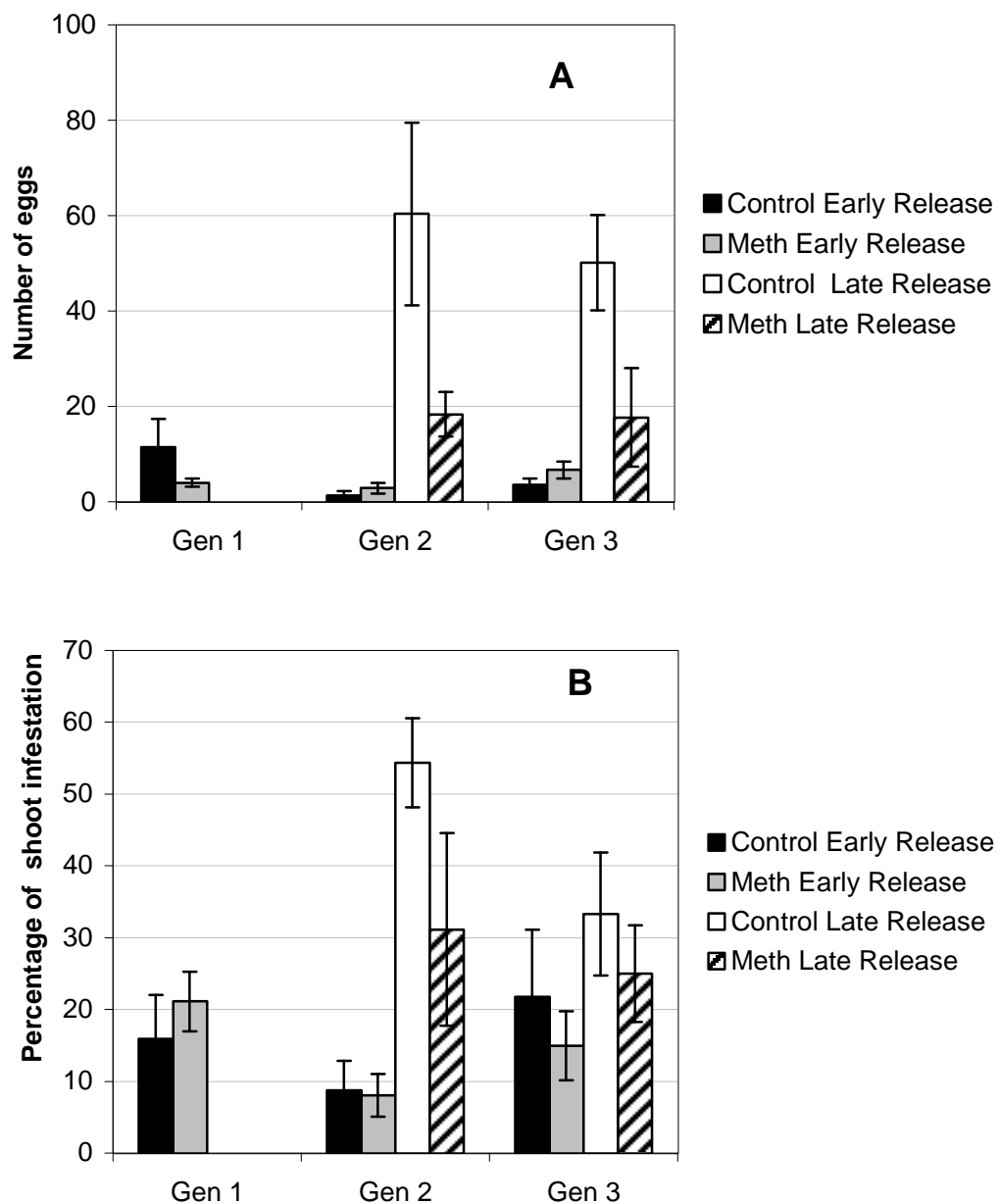


Figure 2.3. Mean ( $\pm$  SEM) oriental fruit moth eggs (A) and percentage of vegetative shoot infestation (B) per generation in single tree cages treated with two applications of methoxyfenozide (70 g [ai]/ha) or untreated prior to infestation with adult moths for the first generation.

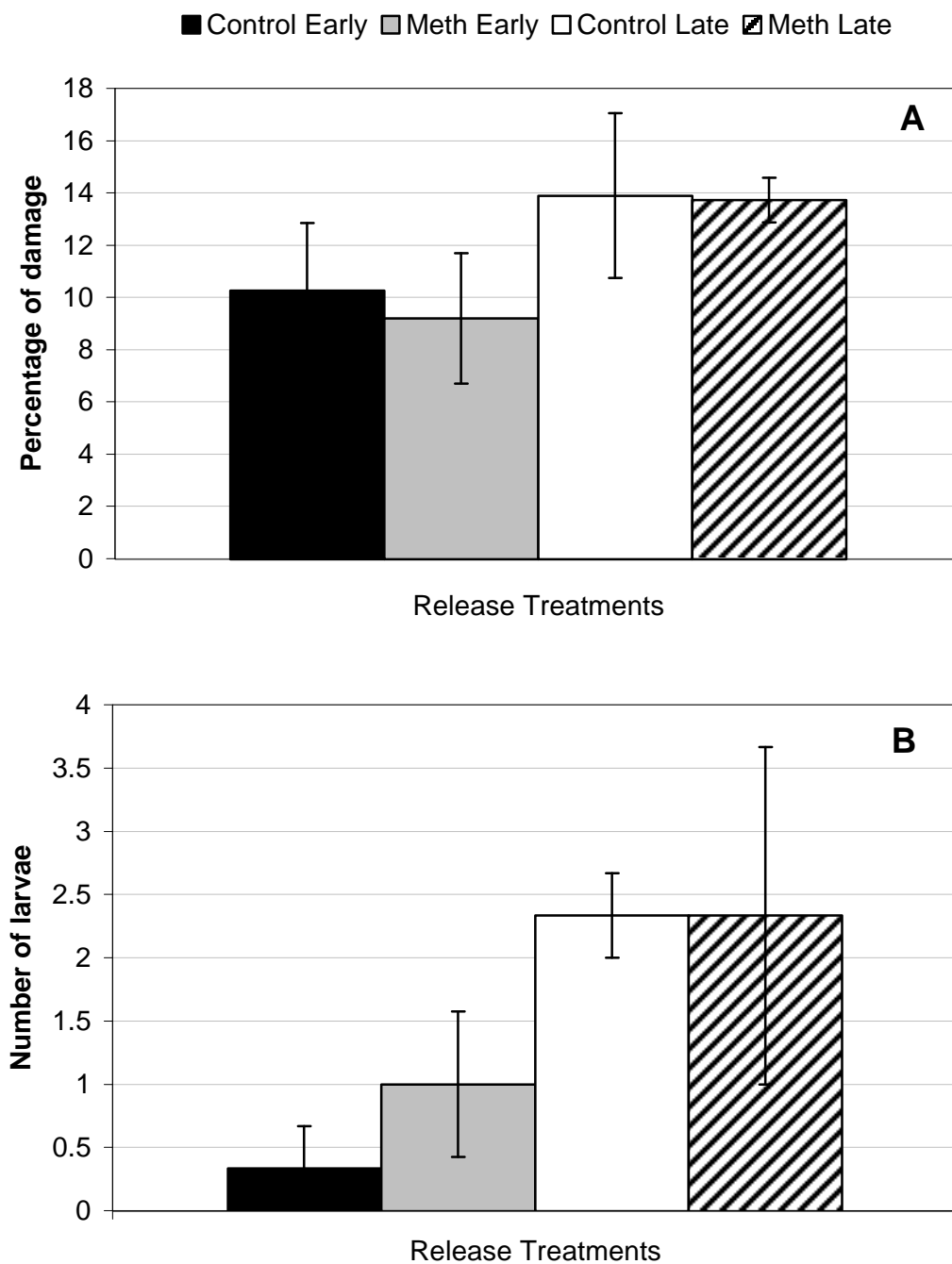


Figure 2.4. Mean ( $\pm$  SEM) percentage of fruit damage (A) and number of larvae recovered (B) in single tree cages infested with Oriental fruit moth for three generations (Early) or two generations (Late) treated with two applications of methoxyfenozide (70 g [AI]/ha) prior to early infestation. Henderson County, NC. 2002

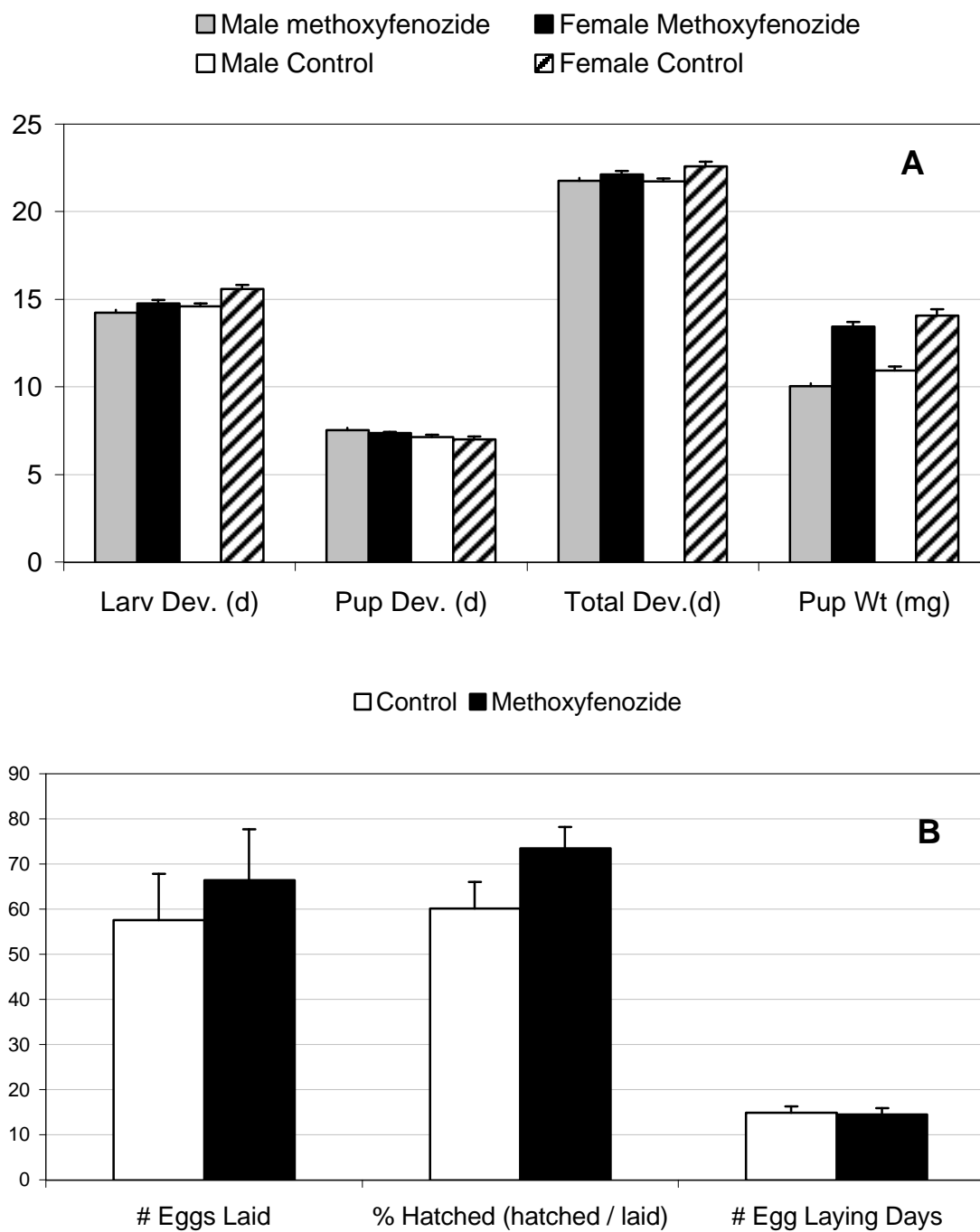


Figure 2.5. Mean (+/-SEM) developmental measurements (A) of oriental fruit moth larvae and pupae reared on diet incorporated with methoxyfenozide (0.1 ppm) or untreated diet and reproductive measurements (B) of paired oriental fruit moth adults reared on methoxyfenozide treated diet or untreated diet. Henderson County, NC. 2002.

## **Chapter 3**

### **Oriental Fruit Moth Phenology and Management with Methoxyfenozide in North Carolina Apples**

### **Abstract**

The phenology of oriental fruit moth, *Grapholita molesta* (Busck), on apple in North Carolina was studied using pheromone traps and egg sampling in abandoned and commercial orchards in 2000 and 2001, with subsequent development of an oviposition degree-day model and management studies in relation to codling moth, *Cydia pomonella* (L.), phenology. Oriental fruit moth eggs were found in greater numbers on leaves early and on fruit later in the growing season, on the top versus the bottom of the leaf surface, and on the calyx area versus the side or stem end of the fruit. A degree-day model to predict oriental fruit moth oviposition was developed based on temperature accumulations from peak moth trap capture of the first (overwintering) generation, using 7.2° C and 32.2° C as the temperature limits. The model predicted four ovipositing generations of oriental fruit moth with the second beginning 507 DD after peak moth catch. Using predictions of the oriental fruit moth and codling moth degree-day oviposition models, an experiment was conducted to determine the level of second-generation oriental fruit moth control with methoxyfenozide applied under different scenarios for first generation codling moth. Methoxyfenozide was equally effective in managing codling moth and oriental fruit moth for all treatment timings.



## Introduction

Insect phenology models are important tools used in apple pest management programs to accurately time insecticide applications to sensitive periods of insect development. The codling moth, *Cydia pomonella* (L.), phenology model, originally developed in Michigan (Riedl et al. 1976), is one of the most widely used models in apple systems; albeit, adjustments are made for geographic location and voltinism. The development of this model used data from pheromone trap catches, moth emergence from overwintering pupae, and oviposition in several abandoned orchards to determine degree-day accumulations from a biologically important event or "biofix." A testament to the effectiveness and simplicity of the codling moth model is demonstrated by its continued use almost 30 years later.

A phenology model for oriental fruit moth, *Grapholita molesta* (Busck), was developed in Michigan as part of the predictive extension timing estimator (PETE) program (Welch et al 1978), and validated on apple in Michigan and peaches in California (Croft et al. 1980). The degree-day based model used oriental fruit moth developmental parameters (7.2°C lower and 32.7°C upper threshold temperature) initially reported by Peterson and Hauessler (1930) and modified by Croft et al (1980). The use of the oriental fruit moth model in apple has been limited, because this insect has traditionally been a minor pest of apple (Rothschild and Vickers 1991).

In recent years, oriental fruit moth has developed into a serious pest of apple in eastern North America (Felland and Hull 1996, Bergh and Engleman 2001). A survey of rejected processing apple loads from mid-Atlantic states in 2001 showed that 77% of 248 rejections were due to the presence of oriental fruit moth larvae, while 17% were rejected due

to codling moth (Bergh and Engleman 2001). While the reason for the increased incidence of oriental fruit moth in this region is unknown, the increased use of narrow-spectrum insecticides and/or development of insecticide resistance may be contributing factors (Shearer and Usmani 2001).

The increased importance of oriental fruit moth in North Carolina has complicated the overall management of tortricid pests in apples. The concomitant occurrence of codling moth and oriental fruit moth have made it necessary to devise management systems that account for both pests. Methoxyfenozide is an insect growth regulator with insecticidal activity on multiple stages of codling moth and oriental fruit moth, and its long residual activity make it an ideal tool for managing this complex of internal-feeding lepidopteran pests (Chapter 1). The objective of this study was to examine the oviposition phenology of oriental fruit moth in relation to the codling moth, and to develop and validate a degree-day based oviposition model for managing early season populations of this pest complex on apple.

## **Material and Methods**

**Egg Survey.** Preliminary studies were conducted in 1999 to develop sampling methods for oriental fruit moth and codling moth eggs on apple. Based on the findings of this work, surveys were conducted in four abandoned and one commercial orchard in 2000 and 2001. Wing-style pheromone traps used to monitor oriental fruit moth and codling moth males were set up in early and late March, respectively, and monitored at weekly intervals throughout the season. Pheromone lures (Trece Inc., Salinas, CA) were replaced at four-week intervals. Temperature data in sample orchards were obtained with Hobo loggers

(Onset, Proccasset, MA) or Watchdog weather stations (Model 900ET, Spectrum Technologies, Plainfield, IL) that were located in orchards and downloaded once per week. At each orchard, samples were collected weekly from 10 sampling sites. To avoid defruiting or defoliating trees with our destructive sampling method, each sampling site consisted of four trees sampled in a 4-week rotation. Sampling from a single tree per week was done to reduce sample collection time, which was considerable in abandoned orchards due to large tree size and difficult sampling conditions. Beginning in late April (full bloom-petal fall) and continuing weekly until October, ten fruit clusters from both the upper and lower half of one tree per sample site were removed and returned to the laboratory, where leaves and fruit were examined for eggs under lighted magnifying lenses. Several parameters were recorded when an egg was detected on a fruit cluster; including species determination (oriental fruit moth or codling moth), stage of development (fresh, hatched or parasitized), substrate (leaf or fruit), and position on leaf (top or bottom) or fruit (stem, side or calyx). When fresh eggs were found, they were excised from the substrate, surface disinfected in a 0.0628% NaOCl solution for five s, placed in a 30 ml plastic cup containing 10 ml of lima bean diet (Yokohama et al. 1987), and held at 25°C 16:8 L:D h until adults emerged and individuals could be identified to species. Egg data were transformed with square root and analyzed with PROC GLM and protected Least Significant Difference (SAS 1999).

**Model Development.** A model predicting oriental fruit moth oviposition was tested using pheromone trap catches, oviposition data and degree-day (DD) information, with the key point for prediction designated as the first oviposition by second-generation moths. The basis for the model was the developmental time required for oriental fruit moth to complete a generation (563 DD) as determined by Croft et al. (1980). Degree-day accumulations were

calculated beginning at the peak catch of first (overwintering) generation moths in pheromone traps, which is defined as Biofix 2 by Riedl et al. (1976), using the sinus-wave method (Baskerville and Emin 1969) and a lower and upper base temperature of 7.2 and 32.2°C, respectively (Croft et al. 1980). It was assumed that 563 DD were required for completion of one generation of oriental fruit moth, which included two pre-oviposition periods (preoviposition period for overwintering and second generation) (Croft et al. 1980). Peak trap catch of a generation has been described to represent 50 % emergence of the population (Riedl et al. 1976); therefore, the model was adjusted to predict the start of oviposition for the second generation by multiplying the degree day generation time (563 DD) by 0.9 (R. E. Stinner, personal communication). Hence, an accumulation of 507 DD from peak trap catch represented the hypothesized beginning of oviposition for the second generation of oriental fruit moth. To estimate the start of oviposition for the third and fourth generations, 535 DD and 1070 DD, the requirements for one and two generations to complete development, respectively, were added to 507 DD. Eggs detected at each sampling site were separated into first, second, third and fourth generations based on the degree-day value for each generation. Degree-day values were plotted against the observed cumulative percentage of oviposition per generation for each location and year, and linear regression trendlines were fit to the data. The line equations were then used to obtain predicted values of cumulative percentage of oviposition using the same degree-day measurements as the observed. Predicted values were plotted versus observed values and regression lines were calculated with resultant line equations and residual sum of squares ( $r^2$ ).

**Predicted Exposure of Oriental Fruit Moth.** Pheromone trap data and degree-day data from 10 orchards in 2002 were used to predict the percentage of second-generation oriental fruit moth eggs exposed to lethal methoxyfenozide residues when hypothetical applications of methoxyfenozide were applied for control of first generation codling moth. Initial methoxyfenozide application timing was based on the size of first generation codling moth populations. For low codling moth populations ( $< 7$  moths per trap per wk), it was assumed that an application of methoxyfenozide was applied at 167-195 DD after codling moth biofix and a second application was either not applied (no spray), or applied 14 or 21 d later. For high codling moth populations, methoxyfenozide applications were assumed to be made at 111-139 DD and 14 or 21 days later. The date of hypothetical applications were based on the Monday of the week that the degree-day requirement was reached, not on the specific degree-day date, as trap and temperature data were collected once per week on Monday. Date of first oviposition of second-generation oriental fruit moth (507 DD from peak trap catch) was determined for each location, and comparisons between hypothetical application timings were made. Methoxyfenozide has residual activity of  $\approx 28$  d under North Carolina conditions (Chapter 1), and predicted control assumptions were made based on the number of days from application to the initiation of second generation oviposition. The average length of second-generation oriental fruit moth oviposition at the 10 locations in 2002 was 35.7 d. The percentage of second generation oriental fruit moth eggs exposed to methoxyfenozide residue was estimated based on the number of wk with sufficient residue divided by the number of wk for completion of oviposition for each generation.

**Optimization of Treatment Timing Study.** Our objective in developing an oriental fruit moth oviposition model was not to create a model for targeting oriental fruit moth alone,

but to predict the extent of second generation oriental fruit moth control achieved when insecticides were applied for control of first generation codling moth. The concomitant occurrence of codling moth and oriental fruit moth in North Carolina apples has created the need to develop management guidelines that include both insects. The efficacy and long residual activity of methoxyfenozide against both insects makes this an especially effective tool for managing this insect complex. In North Carolina, the first generation of oriental fruit moth is typically controlled with a single insecticide applied at petal fall for a diversity of pests (Walgenbach et al. 2002). The next one to three insecticide applications are directed at the first codling moth generation, and the timing of the spray applications are based on the codling moth degree-day model (Riedl et al. 1976, Walgenbach et al. 2002). In North Carolina, methoxyfenozide is commonly used for first generation codling moth control, and due to its long residual activity also provides control of first generation tufted apple bud moth, *Platynota idaeusalis* (Walker). The low toxicity of methoxyfenozide to beneficial arthropods (Carlson 2001) also makes this a desirable management option for first generation codling.

To evaluate the utility of the oriental fruit moth model in determining the extent to which methoxyfenozide controls second generation oriental fruit moth populations when applied for first generation codling moth, field studies were conducted at two locations; the Mountain Horticultural Crops Research Station (MHCRS, Fletcher, NC), and a recently abandoned orchard in Fruitland, NC. At each location, pheromone traps were used to monitor codling moth and oriental fruit moth flight as described previously. Degree-day accumulations for codling moth were initiated when biofix, defined as the first sustained trap catch, was achieved. Hobo<sup>®</sup> temperature data loggers were placed in each orchard and data

were downloaded weekly for degree-day calculations. Four treatments and a non-treated control were arranged in a randomized complete block design with four replicates. At the abandoned site, plots consisted of five 'Delicious' trees within a single row, with samples taken arbitrarily from the middle three trees. Non-treated border rows separated replicate rows to minimize the impact of drift on treatment effects. At the MHCRS, each plot consisted of four to five 'Golden Delicious' trees in a single row, with an adjacent 'Delicious' row that received treatment applications, but was not sampled. There was no insecticide treatment applied at petal fall in the abandoned site, but there was a petal fall insecticide application at MHCRS. At both study sites, treatments consisted of three different application timings of methoxyfenozide (0.21 kg[AI]/ha Intrepid 2F<sup>®</sup>, DowAgrosciences, Indianapolis, IN), a single application timing of azinphosmethyl (1.12 kg[AI]/ha Guthion 50WP<sup>®</sup>, Bayer, Kansas City, MO) and a non-treated control. Application timing treatment of methoxyfenozide consisted of 1) first application at 111 DD after biofix (15 May) and the second 14 d later (29 May), 2) first application at 111 DD (15 May) and the second 21 d later (5 June), 3) first application at 195 DD (20 May) and the second 21 d later (10 June). Azinphosmethyl was applied at 111 DD (15 May) and 14 d later (29 May). The rationale for the selection of these different application scenarios is based on the following: timing 1 was based on the prediction of ensuring methoxyfenozide residue throughout oviposition of the first codling moth generation, but would not cover the latter part of second generation oriental fruit moth oviposition; timing 2 was based on the prediction of ensuring methoxyfenozide residue for the entire oviposition period of first generation codling moth and second generation oriental fruit moth; and timing 3 was based on the prediction of applying methoxyfenozide after initiation of first generation codling moth oviposition, but would

cover the entire oviposition period of second generation oriental fruit moth. The predictions are illustrated in Fig. 3.1. Fruit damage assessments were conducted on 9 and 25 June, and 9 July by observing 200 arbitrarily selected fruit per plot. All damaged fruit were collected, and larvae extracted from fruit and identified to species. Data were analyzed using PROC GLM and protected LSD separation (SAS 1999).

## **Results and Discussion**

**Seasonal trap and egg patterns.** Pheromone trap catches of oriental fruit moth in abandoned orchards in 2000 and 2001 were typical of those observed in North Carolina apple orchards; i.e., large numbers of moths were caught during the first (overwintering) generation and relatively low trap catches during the remainder of the season (Figs. 3.2A and 3.3A). Codling moth pheromone trap catches were generally lower than those of oriental fruit moth, and trap catches did not exhibit large fluctuations in numbers (Figs. 3.2B and 3.3B). The average weekly trap catch for first generation oriental fruit moth was 145 and 46 in 2000 and 2001, respectively. The average weekly trap catch the remainder of the season was 17 and 5 in 2000 and 2001, respectively. Oriental fruit moth eggs were generally found in low numbers early in the season, with the number of eggs increasing as the season progressed and the highest numbers occurring during August and September. The large number of oriental fruit moth males caught in pheromone traps during the first generation versus subsequent generations has been an area of interest for several years. Oriental fruit moth eggs were found in the greatest number late in the season and may account for the relatively large moth catch in the spring, but there may be other factors that contribute to the large size of the overwintering generation. An increased proportion of oriental fruit moth larvae entering



diapause for each successive generation within a season has been reported, and decreasing day length is the primary factor for diapause initiation (Peterson and Haeussler 1930, Rothschild and Vickers 1991, Sari 1970). Diapausing larvae from two or three generations may become synchronized to emerge as the first (overwintering) generation in the spring. Despite the large number of moths captured in the first generation, egg numbers were very low compared with later generations. The low egg numbers associated with the first generation may be due to a fitness cost associated with diapause. Phillips and Proctor (1969) reported reduced fecundity of females following emergence from overwintering conditions, and Deseö and Saringer (1975) reported that laboratory eggs and neonates exposed to short day lengths also led to reduced female fecundity. It is possible large numbers of larvae are required to enter diapause to overcome the associated fitness cost. Alternatively, oriental fruit moth has a wide host range (Rothschild and Vickers 1991), and it is possible that eggs were laid on plants other than apple during the first generation, such as multiflora rose, then moved to apple in subsequent generations.

*Oriental fruit moth oviposition.* The total number of oriental fruit moth eggs found in abandoned orchards in 2000 and 2001 were 2,252 and 2,158, respectively. The mean ( $\pm$ SEM) number of eggs per orchard was 563 ( $\pm$  204) in 2000 and 540 ( $\pm$  154) in 2001. The majority of oriental fruit moth eggs detected were hatched (80 and 90%) in 2000 and 2001, respectively (Fig. 3.4A). There was no significant difference between the total number of eggs found on leaves versus fruit (Table 3.1). However, the preference of oriental fruit moth to oviposit on leaves and fruit changed during the season, with more eggs found on leaves early in the season and on fruit later in the season, which resulted in a significant leaf vs fruit x week interaction (Fig. 3.5a). Among oriental fruit moth eggs found on leaves,

significantly more were found on the upper versus lower leaf surface. This was consistent throughout the season, with no significant top vs bottom leaf x week interaction detected. Of the oriental fruit moth eggs found on fruit, significantly more were found on the calyx end compared to the side or stem end, and this preference for the calyx end became more pronounced as the season progressed, with a significant egg position x week interaction. Throughout the season there was no significant difference in number of oriental fruit moth eggs found on fruit clusters collected from the upper or lower canopy of trees, and there was no significant upper canopy vs lower canopy x week interaction. It is important to note that there were no significant treatment x year interactions detected for leaves versus fruit comparison, leaf top or bottom comparison, egg position on fruit or canopy height preference, which indicates that relationships were consistent between the two years. The number of oriental fruit moth eggs found in conventional orchards in 2000 and 2001 were very low, 36 and 13, respectively, but the categorical distribution of eggs on fruit and leaves was similar to those observed in abandoned orchards. The location of oriental fruit moth eggs on apple leaves in our study is consistent with that observed by Peterson and Haeussler (1930), who reported that eggs were often laid on the upper leaf surface. The apparent high affinity for oviposition on fruited clusters by oriental fruit moth is similar to that reported for codling moth (Jackson 1979). The switch in oviposition site from leaves to fruit as the season progressed and the higher percentage of eggs found near the calyx end of the fruit have not been previously reported on apple. Egg deposition near the calyx end may be highly beneficial to the emerging oriental fruit moth larvae, because larval entry into the fruit through the calyx opening reduces the potential for exposure to insecticides on the fruit surface, therefore increasing potential larval survival. This data provides useful information

for development of egg sampling methods for oriental fruit moth by enabling egg sampling to be conducted on particular structures at different time periods; i.e., top of fruit cluster leaves during first generation oviposition and the calyx end of fruit during third and fourth generation oviposition.

*Codling moth oviposition.* The total number of codling moth eggs found in abandoned orchards in 2000 and 2001 were 484 and 460, with a mean ( $\pm$ SEM) of 121 ( $\pm$  49.5) and 115 ( $\pm$  61.9), respectively. The large majority of codling moth eggs found were hatched, with 80 and 95 % in 2000 and 2001, respectively (Fig. 3.4B). Significantly more codling moth eggs were found on leaves compared with fruit (Table 3.1), but, as with oriental fruit moth, there was a significant leaf or fruit x week interaction and more eggs were found on fruit late in the season (Fig. 3.5B). Among codling moth eggs found on leaves, significantly more were found on the top versus bottom leaf surface, and there was no significant top vs bottom leaf x week interaction. On fruit, there were no significant differences between the number of codling moth eggs found on the stem, side or calyx end, and there was no significant egg position on fruit x week interaction. There was no significant difference in the number of codling moth eggs found on fruited clusters from the upper or lower portions of the canopy, and there was no significant upper vs lower canopy x week interaction. Similar to the results for oriental fruit moth, there were no significant treatment x year interactions for codling moth eggs found on the top versus bottom of leaves, position of egg on fruit, or number of eggs found on fruit clusters high or low in the canopy. However, there was a significant treatment x year interaction for codling moth eggs found on leaves or fruit, but year was not significantly different ( $F = 0.09$ ,  $df = 1, 259$ ,  $P = 0.76$ ). Examination of data by individual year showed that significantly more codling moth eggs

were found on leaves versus fruit in 2000 ( $F = 50.82$ ,  $df = 1, 131$ ,  $P < 0.0001$ ), but not in 2001 ( $F = .032$ ,  $df = 1, 128$ ,  $P = 0.58$ ). Codling moth oviposition in apple has been widely examined, most recently by Jackson (1979). The pattern of oviposition observed in abandoned apple orchards in North Carolina was similar to that reported by Jackson (1979), with the main difference being that we observed a higher proportion of eggs laid directly on fruit, and a higher proportion of eggs found on fruit versus leaves as the season progressed. However, the overall number of codling moth eggs found in our study was not large and may not be reflective of oviposition behavior observed with higher populations.

**Model Development.** Oriental fruit moth egg data from the four abandoned orchards in 2000 and 2001 were assigned to the first, second, third and fourth generation based on the degree-day model using peak first generation pheromone trap catch as biofix. When the cumulative proportion of eggs per generation was plotted against degree-day accumulations from peak first generation pheromone trap catch, the oviposition pattern observed in the four abandoned orchards was quite similar in 2000 and 2001 (Fig. 3.6). Regression statistics for predicted versus observed cumulative percentage of each oriental fruit moth generation in each orchard in 2000 and 2001 are presented in Table 3.2. In general there was a good fit between observed and predicted for all four generations. However, the variation among orchards in the slope and y-intercept values increased, and  $r^2$  values decreased with succeeding generations. For example, slopes for the first generation ranged from only 0.98 - 1.01, the y-intercepts from -0.69 to +0.69 and  $r^2$  values from 0.84 to 0.99. In contrast, for the fourth generation the slopes ranged from 0.73 to 1.02, the y-intercepts from -18.27 to +24.73, and  $r^2$  values from 0.57 to 0.97. From the regression equations it is apparent that with each successive generation, the fit of the data to the model declined. However, this reduction

in model accuracy was not unexpected, because errors such as temperature measurements, hatched eggs remaining on trees, and overlapping of generations are cumulative (Peterson and Haeussler 1930, Schoene et al. 1937). Regardless of the errors and other difficulties that caused the model predictions to deviate from observed values later in the season, the slopes and y-intercepts were still within reasonable limits.

In all orchards there were four complete ovipositing generations of oriental fruit moth, and number of eggs per generation increased with each succeeding generation (Fig. 3.7 and 3.8). The number of eggs per generation averaged across years were averaged 55, 107, 134 and 272 for generations 1, 2, 3 and 4, respectively. An exception to this trend occurred in the Lamb Mountain orchard in 2000, where egg numbers were high for all generations, particularly the second (Fig. 3.7B). Egg sampling did not extend into October, because the majority of fruit in the orchards had dropped off trees by this time, but it is probable that eggs from a fifth generation were laid.

In the Lamb Mountain and Oates orchards, where there were relatively high numbers of both codling moth and oriental fruit moth eggs, it was possible to compare the timing of oviposition for both species over two years and locations. In three of the four cases examined, ovipositing generations of oriental fruit moth and codling moth appeared in an alternating timeframe. Oriental fruit moth initiated oviposition before codling moth. Typically two generations of oriental fruit moth partially overlapped each of the three codling moth generations (Figs. 3.9 and 3.10). However, at Lamb Mountain in 2001 (Fig. 3.9B), the pattern of oviposition for oriental fruit moth and codling moth appeared to be quite well synchronized.

**Predicted Exposure of Oriental Fruit Moth.** In the 10 orchards used to predict the percentage of second generation oriental fruit moth eggs that were exposed to methoxyfenozide residues when applications were timed for codling moth, five were classified as having low and six as having high populations of codling moth. One orchard, Lancaster, had intermediate levels of codling moth pheromone trap captures, and was therefore examined with both exposure criteria. The Lancaster and MHCRS orchards were the same locations where the optimization of treatment timing studies were conducted. Our assumption was that methoxyfenozide had residual activity of 28 d (Chapter 1). The percentage of second generation oriental fruit moth eggs exposed to methoxyfenozide residues was estimated based on number of weeks of residue (4) divided by the number of weeks for second generation oriental fruit moth to complete oviposition. The average length of oviposition by second-generation oriental fruit moth at the 10 locations in 2002 was 35.7 d. The minus (-) and plus (+) signs indicate that the last methoxyfenozide application was made before or after initiation of second generation oviposition, respectively. The absence of + or - indicates that the application was made when oviposition started.

In orchards with low codling moth populations, three methoxyfenozide-application options were tested: 1) only one application at 166-194 codling moth DD; 2) two applications, the first at 166-194 and the second 14 d later; and 3) two applications, the first at 166-194 and the second 21 d later. Under the scenario with only one application (option 1), the average percentage of the second-generation oriental fruit moth eggs exposed to methoxyfenozide residue was -34% (Table 3.3). Under the scenario with a second application made 14 d after the first (option 2), the average percent of second-generation oriental fruit moth eggs exposed to methoxyfenozide residue was -71%. When the second application was

made 21 d after the first application (option 3), the average percentage of second-generation oriental fruit moth eggs exposed to methoxyfenozide residue was +90%. While application timing options 2 and 3 may be equally effective for controlling codling moth and oriental fruit moth, application of methoxyfenozide after the start of oriental fruit moth oviposition, as observed in option 3, would be expected to be less efficacious and less desirable. This is because methoxyfenozide is more toxic to oriental fruit moth (and codling moth) eggs when eggs are laid on top of residue compared with topical application to eggs (Chapter 1). In addition, this later application would likely lead to exposure of second generation codling moth and third generation oriental fruit moth to methoxyfenozide residues, which may contribute to the more rapid development of resistant populations.

In orchards with high codling moth populations, two methoxyfenozide application options were tested: 1) two applications of methoxyfenozide, the first at 111-139 codling moth DD and the second 14 d later; and 2) two applications of methoxyfenozide, the first at 111-139 codling moth DD and the second 21 d later. Under option 1, the average percentage of second-generation oriental fruit moth eggs exposed to methoxyfenozide residue was -30%. Under option 2, the average percent of second-generation oriental fruit moth eggs exposed to methoxyfenozide residue was -50%. These predictions for high populations of codling moth indicate that extending the spray interval to 28 d would increase the percentage of second-generation oriental fruit moth egg exposed to methoxyfenozide residue to an average of -70%. It is important to note that this length of spray interval has not been tested for efficacy in the field, but is only a prediction. Although not field evaluated, this spray regimen is not without logic, as it would essentially be applying a single application of methoxyfenozide for

codling moth control and a single application for oriental fruit moth control. Further field testing of this prediction would provide additional information to determine its feasibility.

**Optimization of Treatment Timing Study.** Timing of the initial spray applications in treatments timed for 111 DD after codling moth biofix was delayed until 139 DD due to unseasonably warm weather and poor spraying conditions. Also, initial application of methoxyfenozide scheduled at 194 DD was applied at 177 DD after codling moth biofix. Fruit damage was very low at the Mountain Horticultural Crops Research Station site, where only one damaged fruit was observed in all of the treatments. However, populations were relatively high at the abandoned site, where the level of fruit damage and the number of larvae found in fruit were low in all treatments except the control (Fig. 3.10). Percentage fruit damage in the control was significantly higher compared with the methoxyfenozide and azinphosmethyl treatments all sample dates (for 9 June  $F = 87.67$ ;  $df = 4, 12$ ;  $P < 0.001$ ; for 25 June  $F = 11.43$ ;  $df = 4, 12$ ;  $P = < 0.001$ ; for 9 July  $F = 186.80$ ;  $df = 4, 12$ ;  $P < 0.001$ ), and there were no differences in damage among the insecticide treatments. The number of larvae found in damaged fruit was also significantly higher in the control compared with insecticide treatments for all dates (for 9 June  $F = 5.75$ ;  $df = 4, 12$ ;  $P = 0.008$ ; for 25 June  $F = 9.62$ ;  $df = 4, 12$ ;  $P = 0.001$ ; for 9 July  $F = 6.38$ ;  $df = 4, 12$ ;  $P = 0.005$ ). Except for one codling moth larva found in the control, all larvae were identified as oriental fruit moth. The predicted percentage of 2<sup>nd</sup> generation oriental fruit moth eggs exposed to lethal levels of methoxyfenozide residue at Lancaster for the three treatments were - 60, 80 and +100 %, for 139-14, 139-21 and 177-21, respectively. Although the range in predicted percentage of exposure was fairly large, the fruit damage and number of larvae found were similar. The delay of the initial application in the early treatments (from 111 to 139 DD) probably



accounted for the presence of sufficient methoxyfenozide residue to control second-generation oriental fruit moth, which contributed to the lack of differences among treatments.

From the predicted exposure values and the treatment timing optimization study, it is concluded that two well-timed applications of methoxyfenozide can provide control of both first generation codling moth and second-generation oriental fruit moth. Application timing of methoxyfenozide can be adjusted for codling moth population density while reducing the number of applications to avoid multiple generation exposure, and thus the potential for resistance development. Methoxyfenozide is a valuable insecticide for management of the lepidopteran pest complex in apple, and these studies have demonstrated that it is flexible in terms of application timing to enhance its activity against multiple pests. Although our evaluation of spray optimization was based on the use of methoxyfenozide, it is possible to use this model with other insecticides as long as the residual activity of the product is known.

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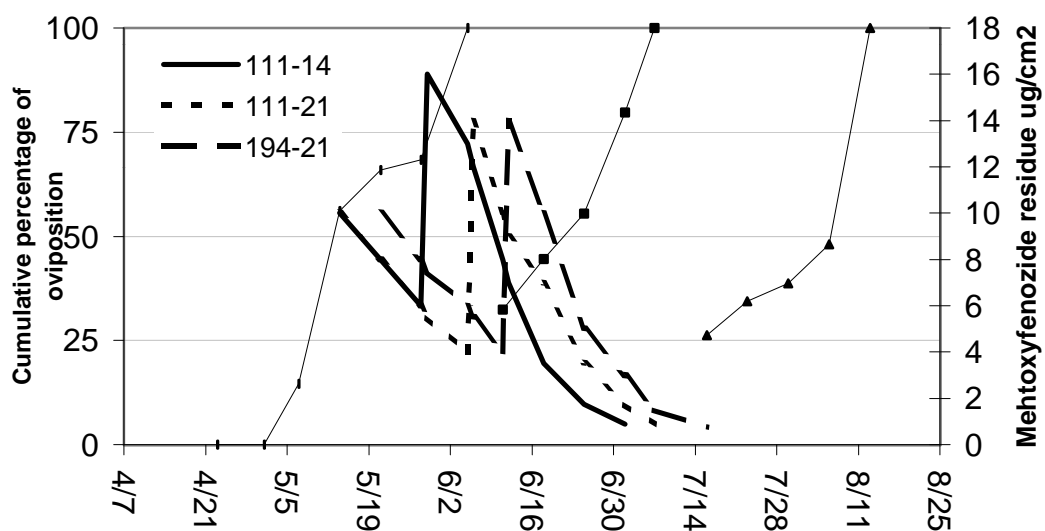


Figure 3.1. Methoxyfenozide residue decline resulting from different application timings (initial application at either 111 DD or 194 DD after codling moth biofix, and the second application either 14 or 21 d later) in relation to predicted cumulative percentage of eggs oviposited by first, second and third generation oriental fruit moth populations.

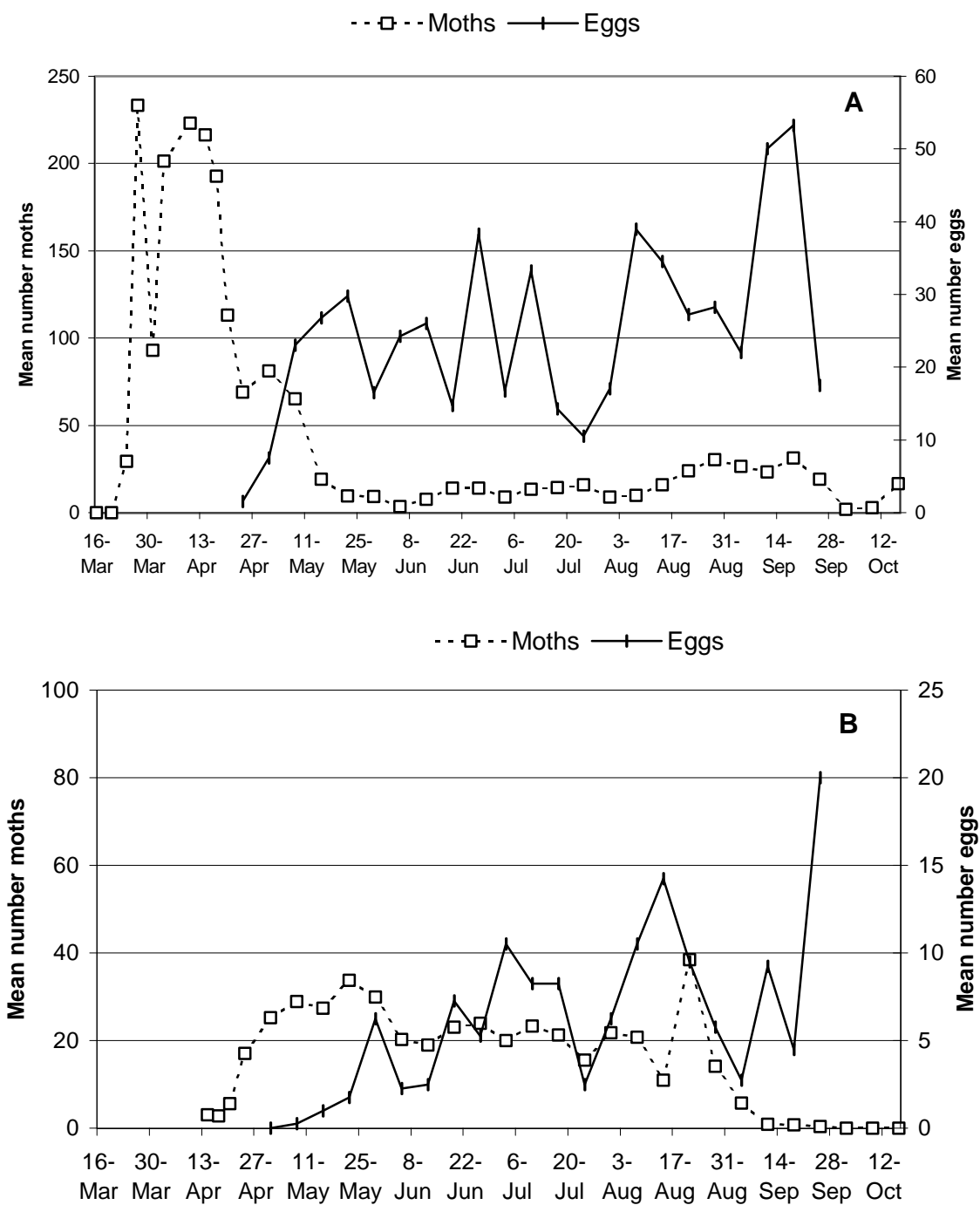


Figure 3.2. Mean pheromone trap catch and number of eggs for oriental fruit moth (A) and codling moth (B) from four abandoned apple orchards. Henderson County, NC. 2000

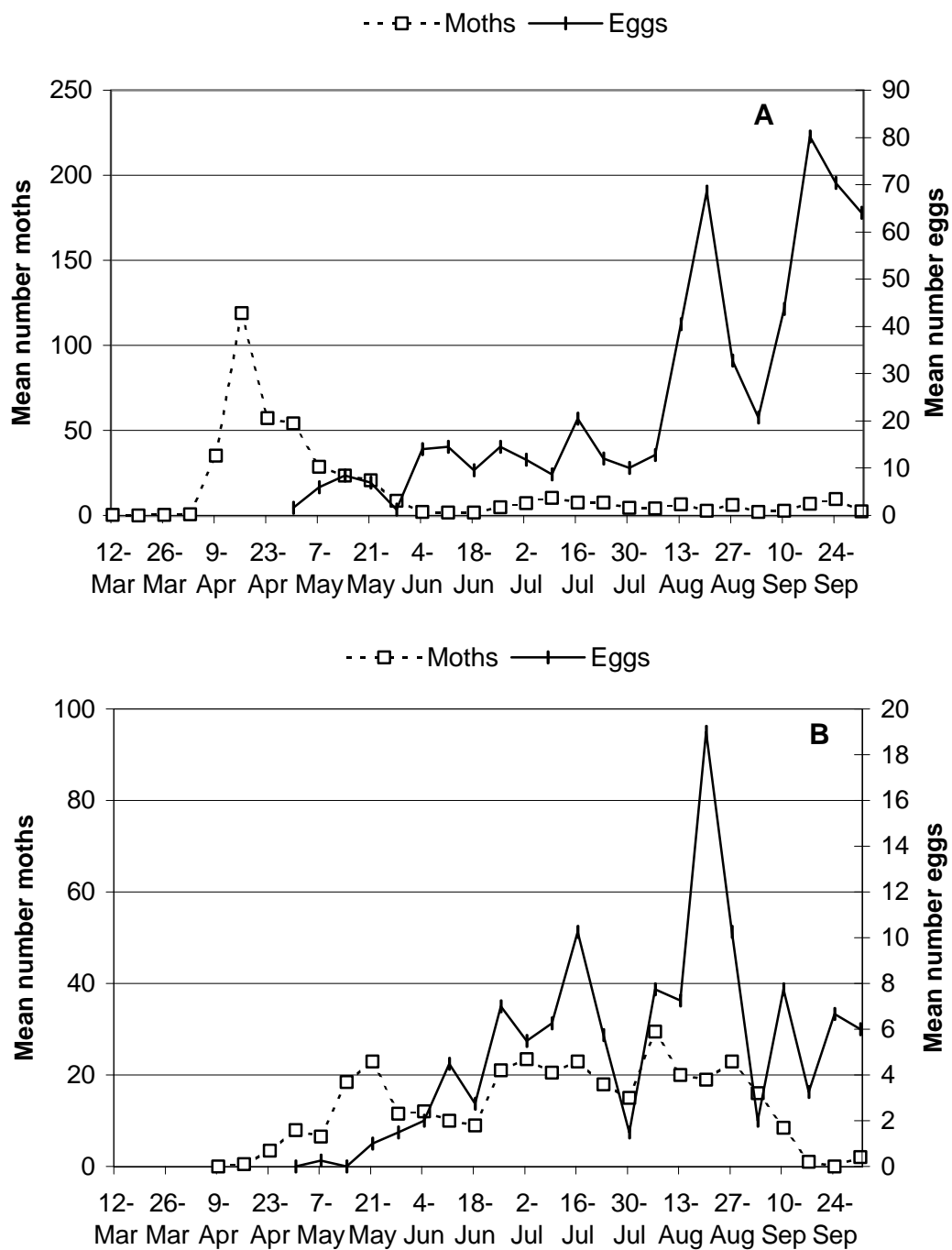


Figure 3.3. Mean pheromone trap catch and number of eggs for oriental fruit moth (A) and codling moth (B) from four abandoned apple orchards. Henderson County, NC. 2001

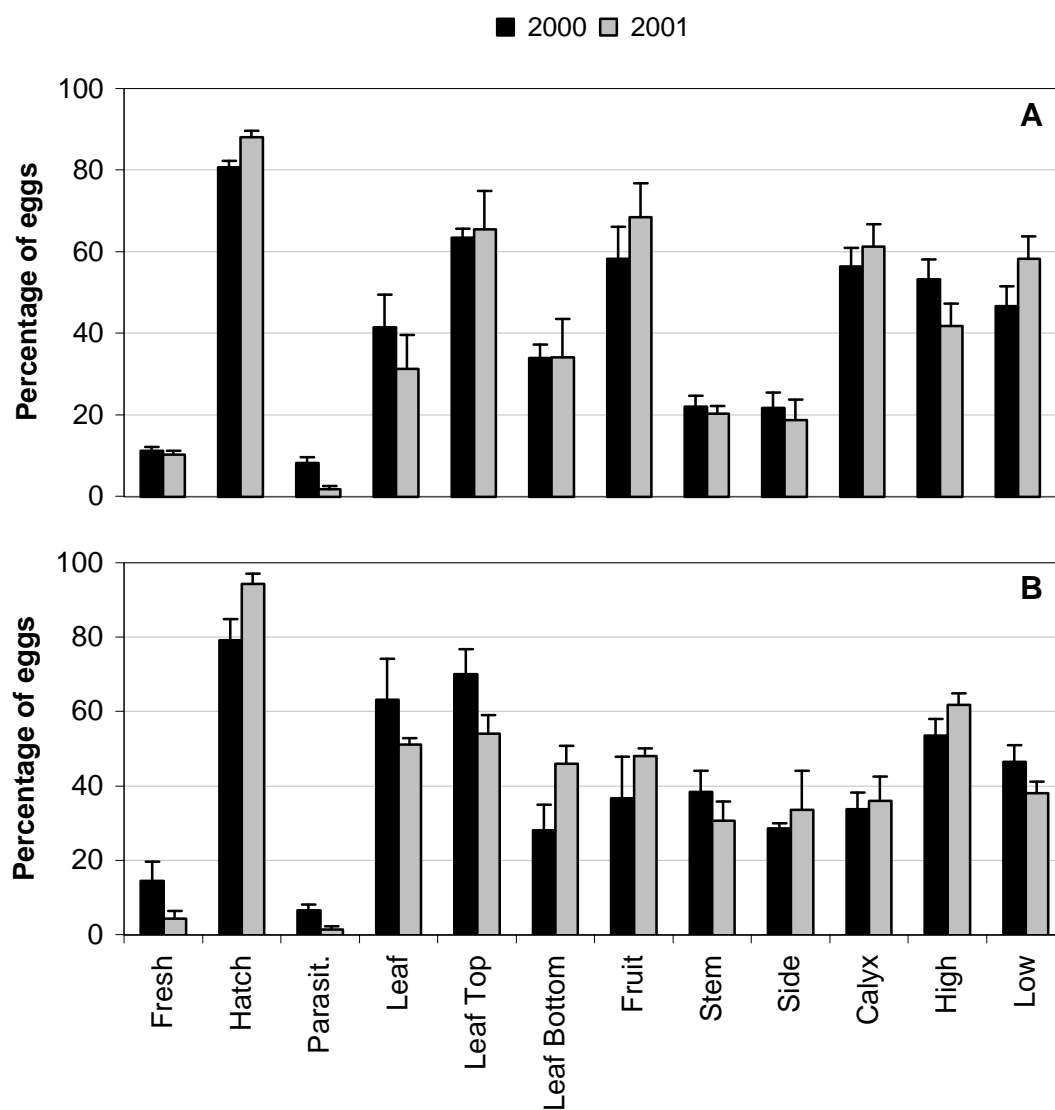


Figure 3.4. Mean ( $\pm$  SEM) percentage of oriental fruit moth (A) and codling moth (B) eggs from abandoned orchards separated into categories for 2000 and 2001. Henderson County, NC. 2000 and 2001

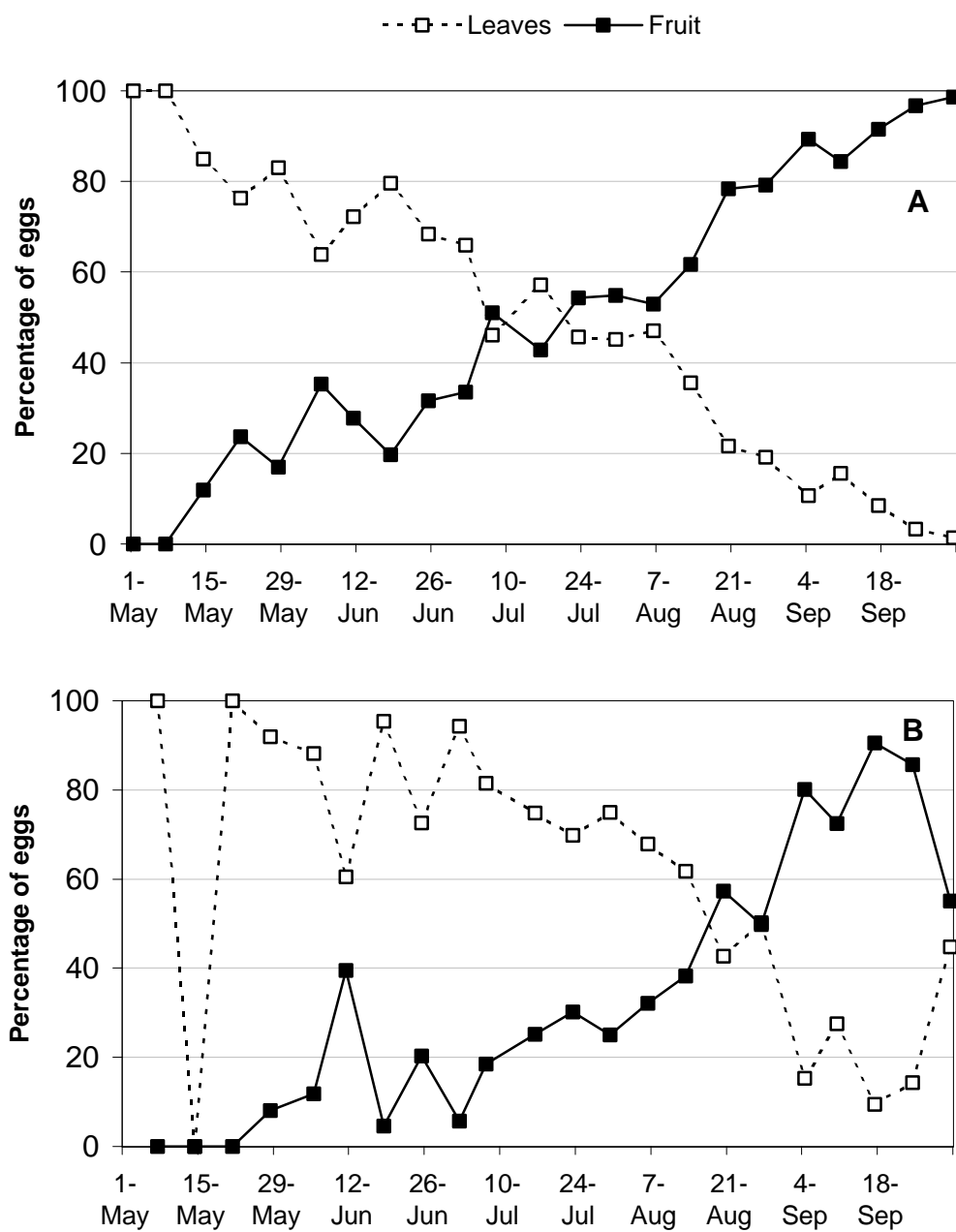


Figure 3.5. Percentage of oriental fruit moth (A) and codling moth (B) eggs found on apple leaves or fruit in abandoned orchards averaged over two years. Henderson County, NC. 2000 and 2001



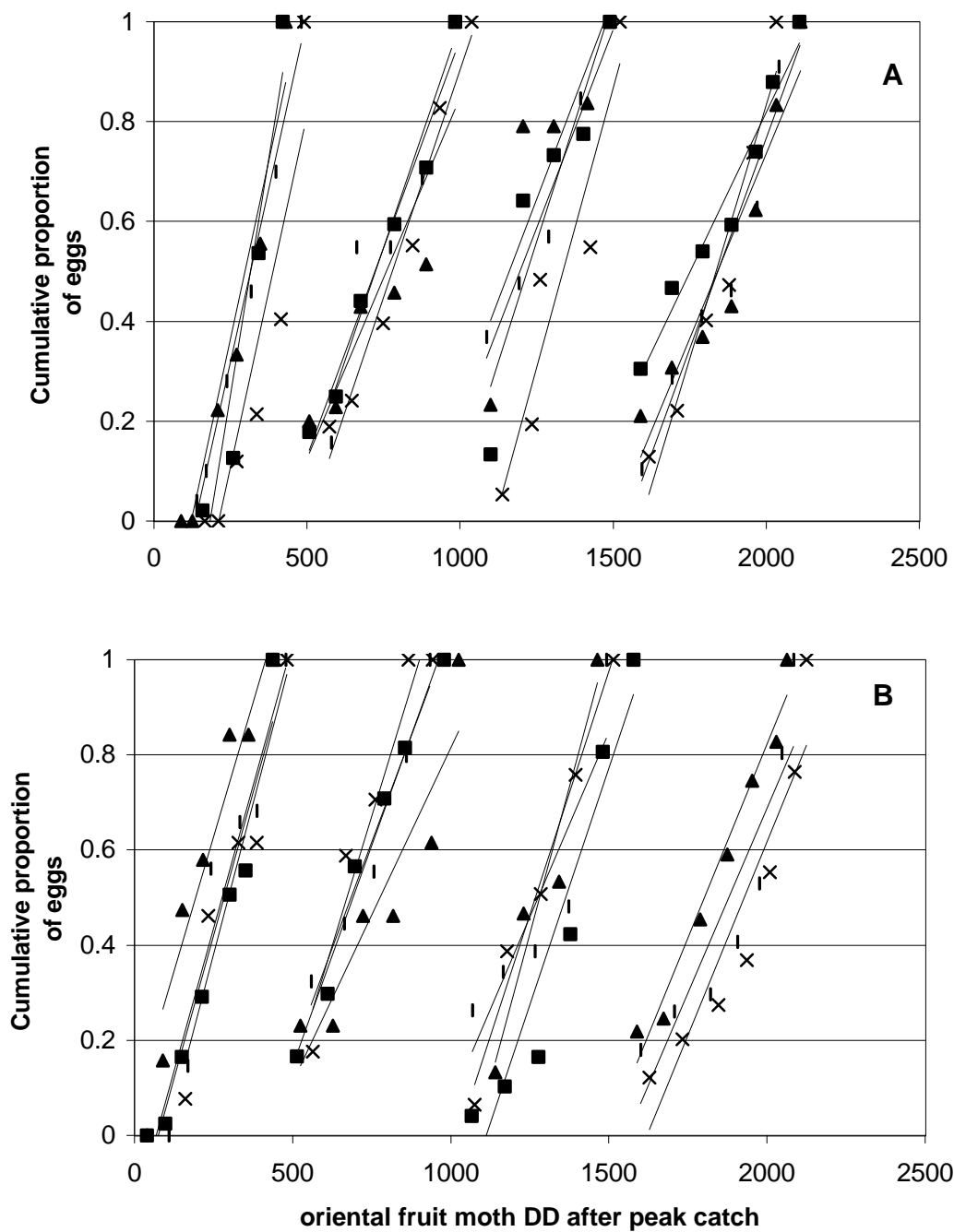


Figure 3.6. Cumulative proportion of oriental fruit moth oviposition in apple by generation with linear trendlines applied for 2000 (A) and 2001 (B) from four abandoned orchards. Henderson County NC. 2000 and 2001.

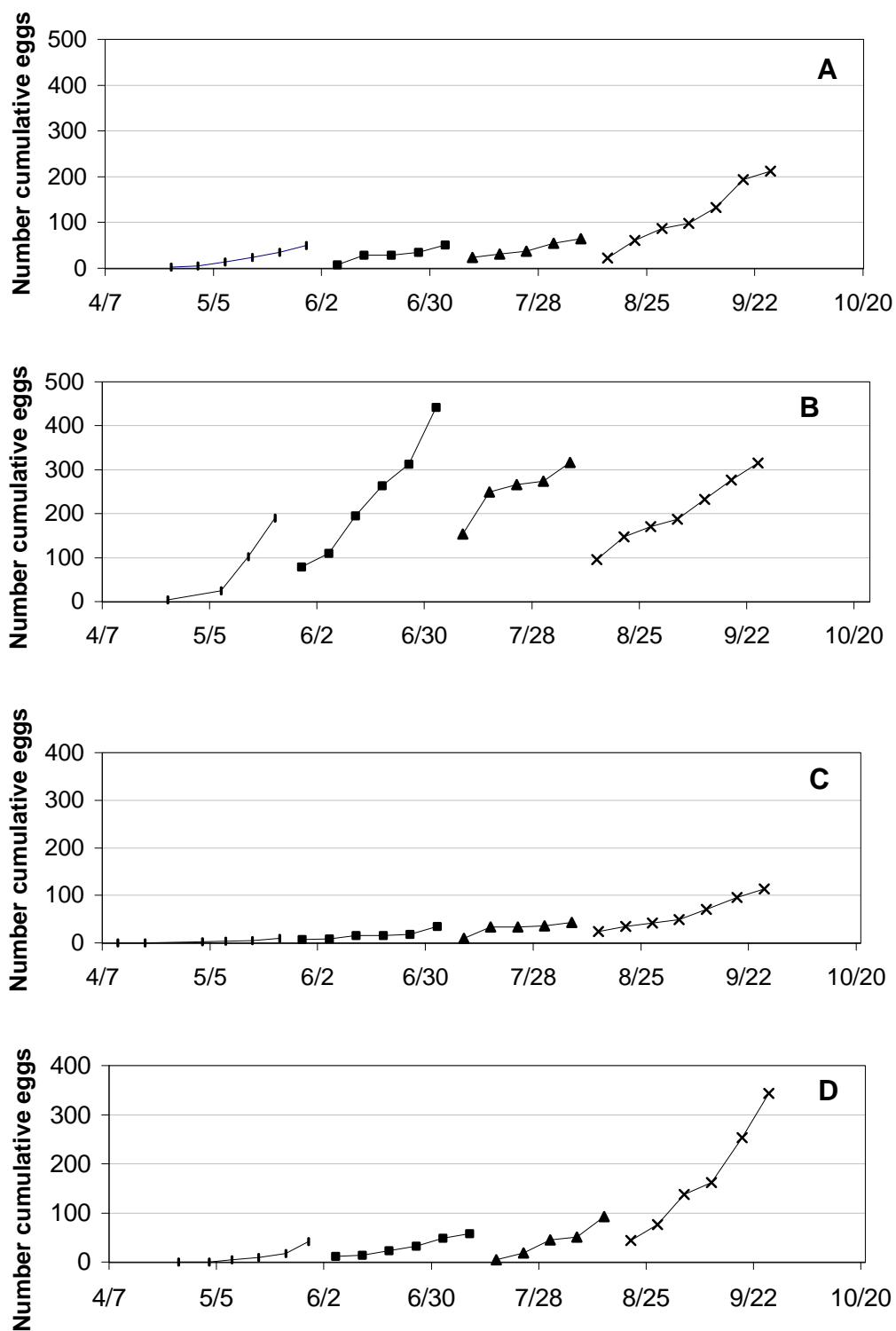


Figure 3.7. Cumulative number of oriental fruit moth eggs found per generation in Oates (A), Lamb Mt (B), Barnwell (C), and Henderson (D) abandoned orchards. Henderson County, NC. 2000.

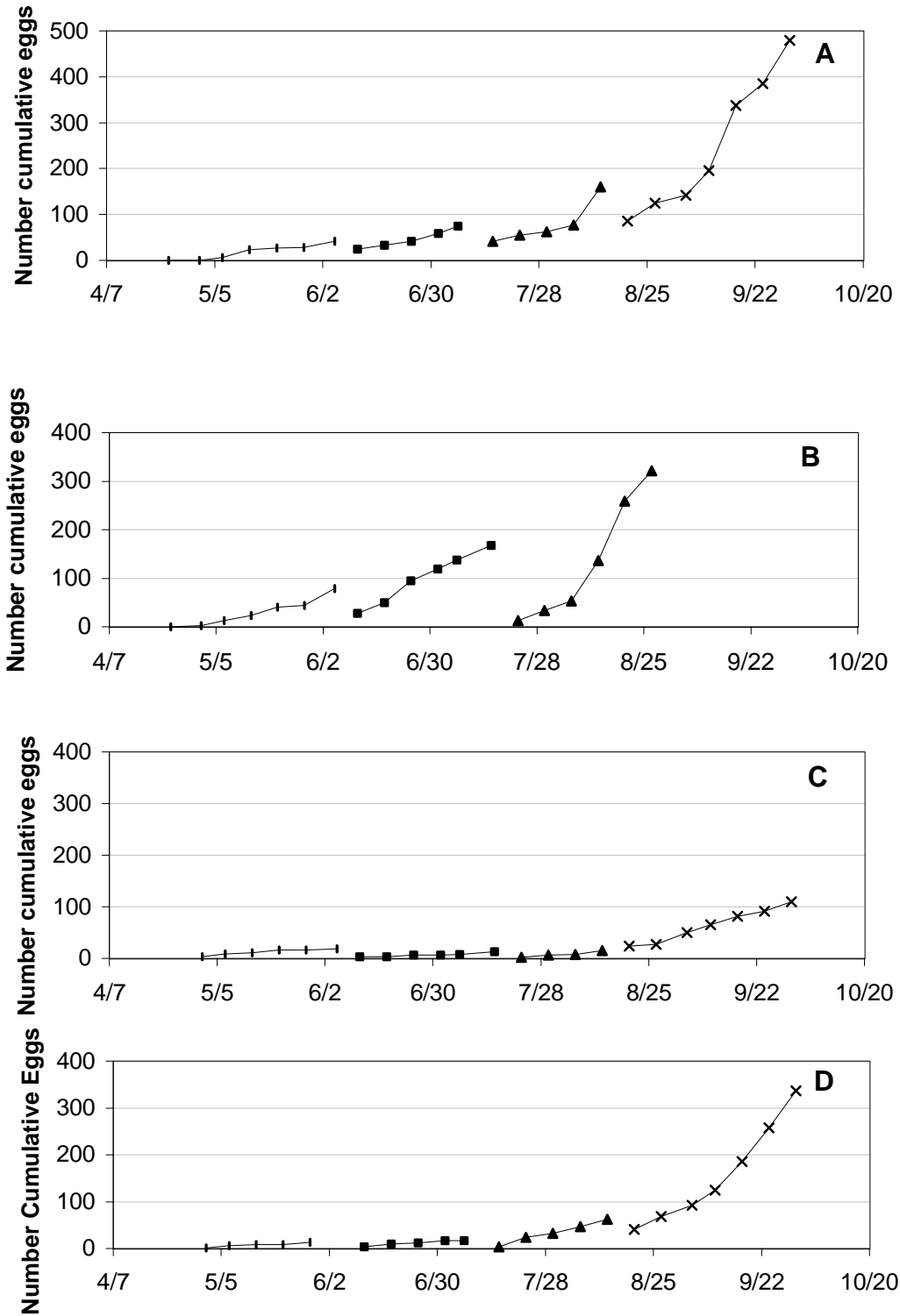


Figure 3.8. Cumulative number of oriental fruit moth eggs found per generation in Oates (A), Lamb Mt.(B), Station (C) and Fruitland (D) abandoned orchards. Henderson County, NC. 2001.

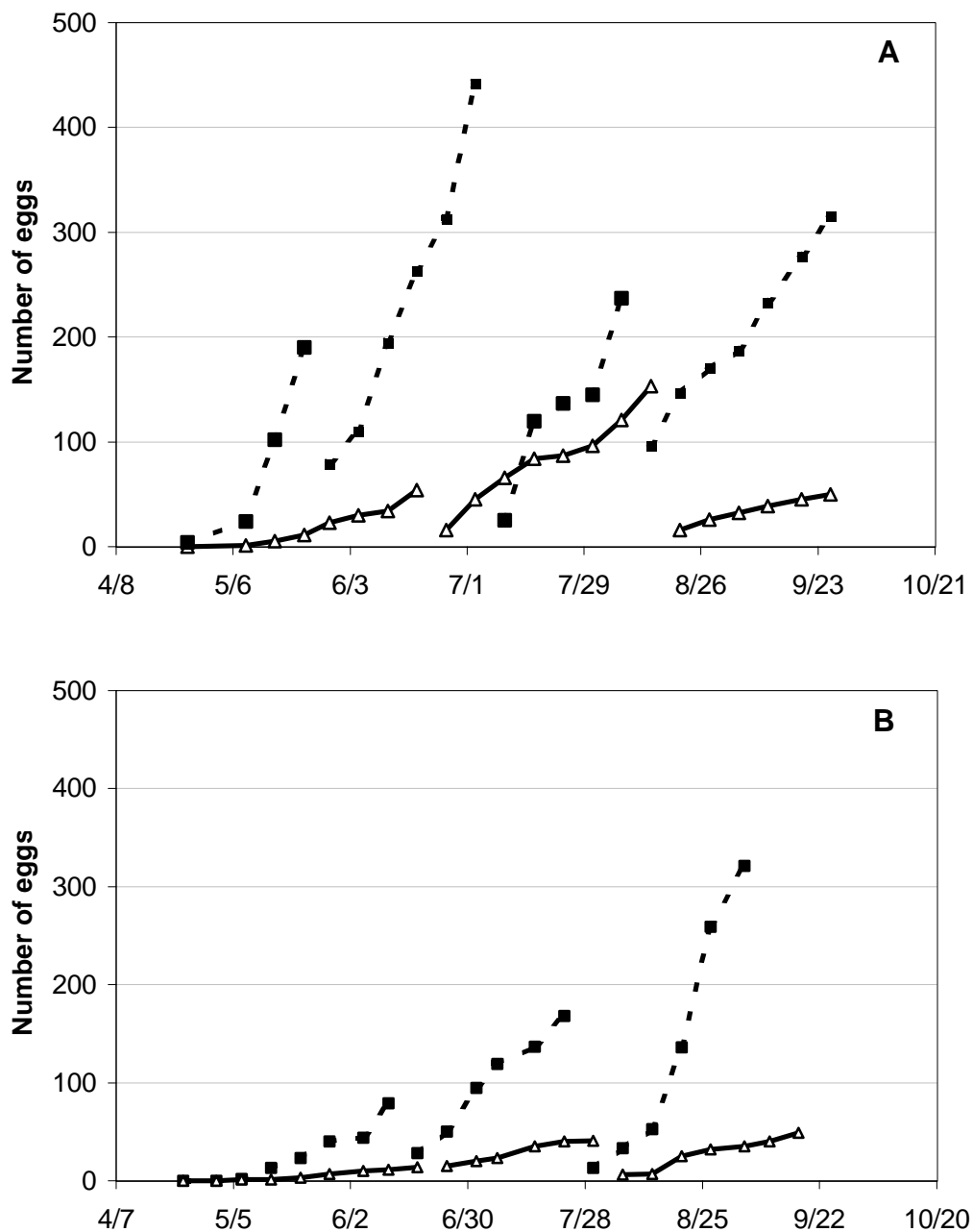


Figure 3.9. Oriental fruit moth (dashed lines) and codling moth (solid lines) egg numbers by generation at Lamb Mt. abandoned orchard site in 2000 (A) and 2001 (B). Henderson County, NC. 2000 and 2001.

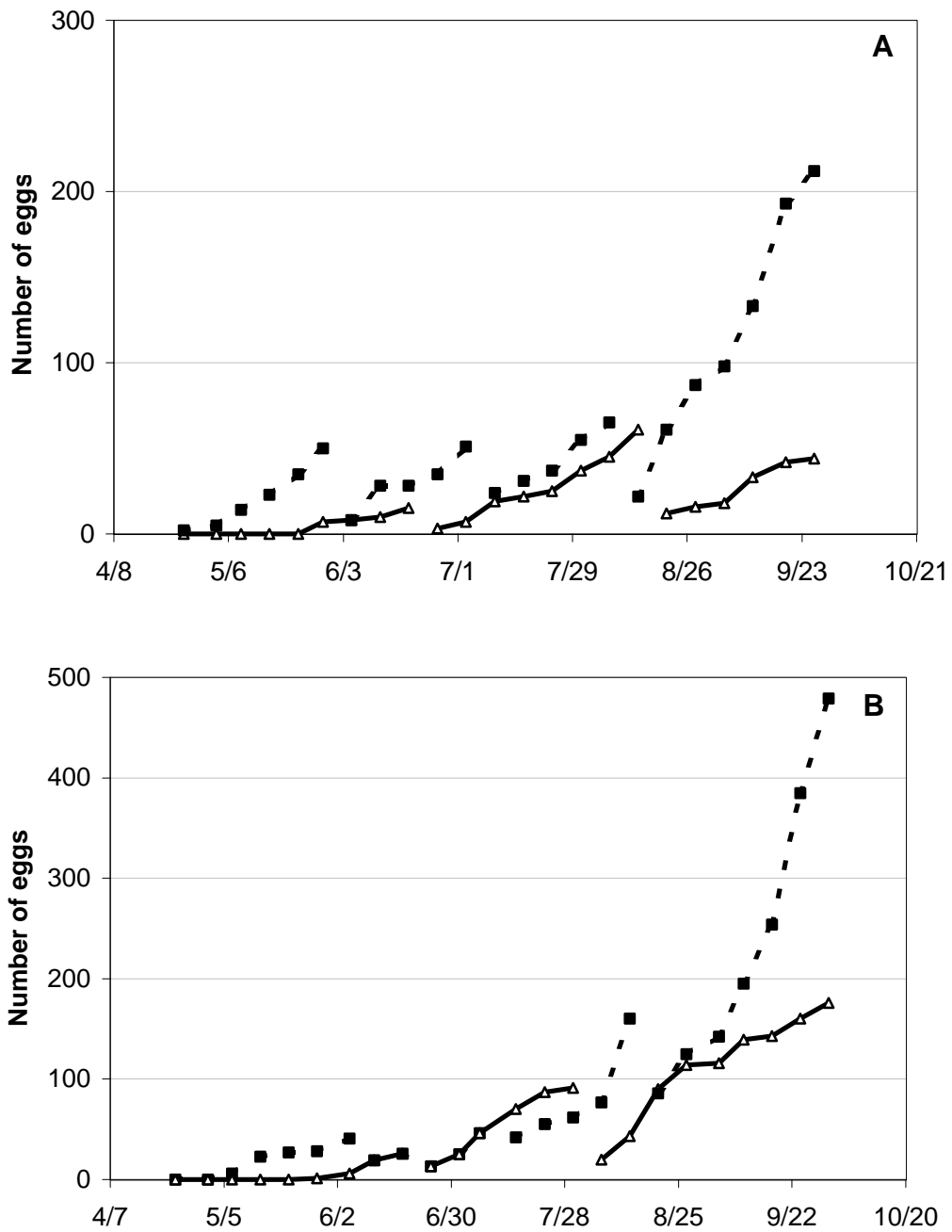


Figure 3.10. Oriental fruit moth (dashed lines) and codling moth (solid lines) egg numbers by generation at Oates abandoned orchard site in 2000 (A) and 2001 (B). Henderson County, NC. 2000 and 2001.

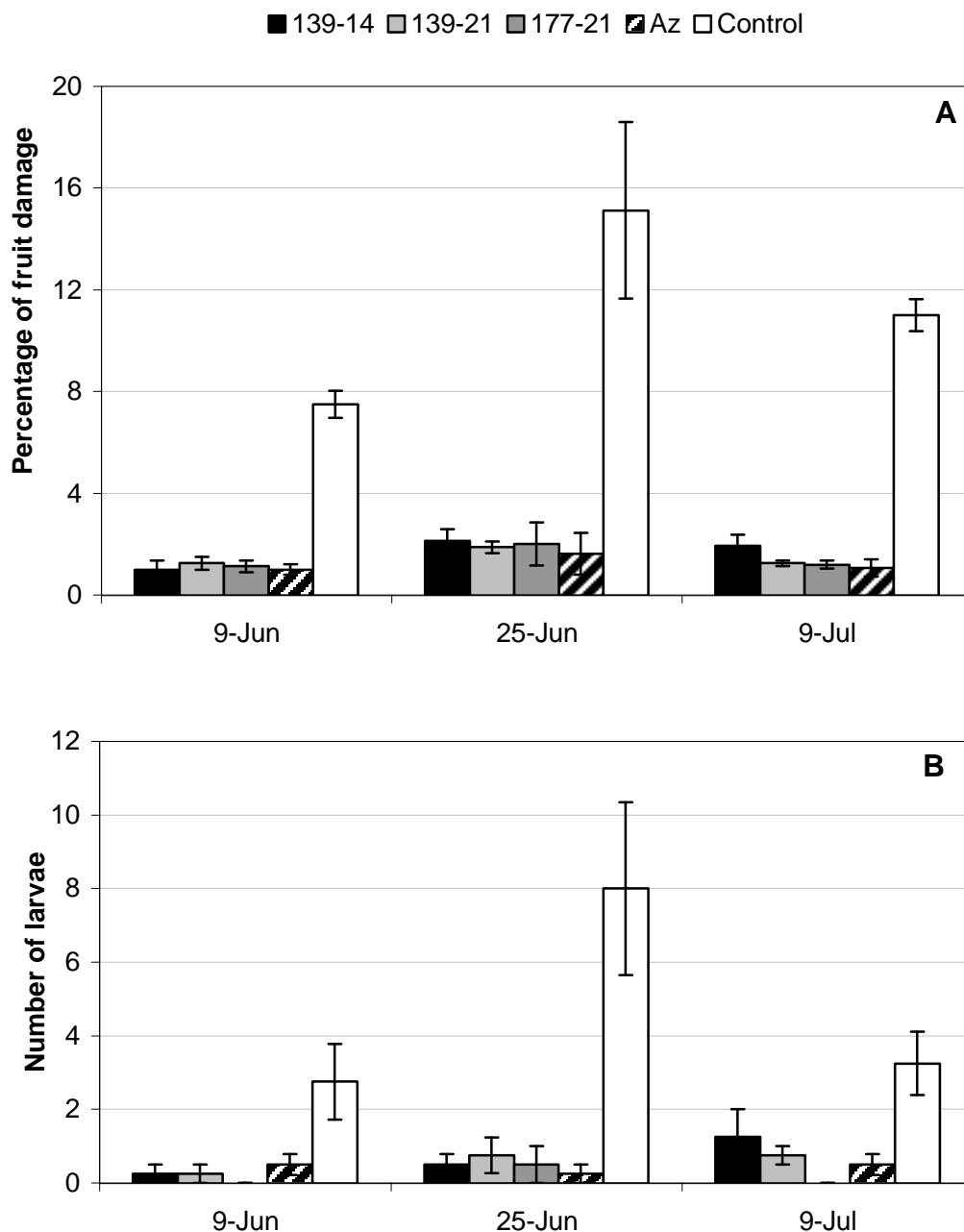


Figure 3.11. Mean ( $\pm$  SEM) percentage of fruit damage (A) and number of larvae (B) for dates during the first generation of codling moth and second generation of oriental fruit moth in plots treated with two applications of methoxyfenozide (0.24 kg [AI]/ha) at 139 DD after codling moth biofix (May 15) and 14 (139-14) or 21 (139-21) days later, 179 DD and 21 days later (179-21), or azinphosmethyl (Az) (2.24 kg [AI]/ha) at 139 DD and 14 days later or an untreated control. Henderson County, NC. 2002.

**Table 3.1 ANOVA repeated measures statistics for categorical comparisons of oriental fruit moth and codling moth eggs sampled from abandoned orchards in Henderson County, NC, in 2000 and 2001**

Treatment	<i>F</i>	df	<i>P</i>
Oriental fruit moth			
Leaf vs. Fruit	2.26	1, 254	0.13
Leaf vs. Fruit x Week	9.01	22, 254	< 0.001
Leaf vs. Fruit x Year	2.38	1, 254	0.12
Leaf top vs. Leaf bottom	22.15	1, 256	< 0.001
Leaf top vs. Leaf bottom x Week	0.44	22, 256	0.99
Leaf top vs. Leaf bottom x Year	2.80	1, 256	0.10
Egg position on fruit	53.18	2, 384	< 0.001
Egg position on fruit x Week	3.73	44, 384	< 0.001
Egg position on fruit x Year	1.45	2, 384	0.24
Upper canopy vs Lower canopy	0.04	1, 256	0.84
U. canopy vs L. canopy x Week	0.34	22, 256	0.99
U. canopy vs L. canopy x Year	1.73	1, 256	0.19
Codling moth			
Leaf vs. Fruit	21.86	1, 259	< 0.001
Leaf vs. Fruit x Week	3.26	22, 259	< 0.001
Leaf vs. Fruit x Year	15.98	1, 259	< 0.001
Leaf top vs. Leaf bottom	9.59	1, 255	0.002
Leaf top vs. Leaf bottom x Week	0.80	22, 256	0.73
Leaf top vs. Leaf bottom x Year	3.60	1, 255	0.06
Egg position on fruit	0.64	2, 387	0.53
Egg position on fruit x Week	1.31	44, 387	0.10
Egg position on fruit x Year	0.87	1, 387	0.42
Upper canopy vs Lower canopy	1.18	1, 258	0.13
U. canopy vs L. canopy x Week	0.52	22, 258	0.97
U. canopy vs L. canopy x Year	1.18	1, 258	0.26

**Table 3.2 Regression statistics for predicted versus observed percentage of cumulative oriental fruit moth oviposition for each of four generations in abandoned apple orchards in Henderson County, NC, in 2000 and 2001.**

Orchard	Generation	Year	<i>n</i>	Regression Equation	$r^2$
Lamb Mt.	1	2000	4	$y = 0.99x - 0.69$	0.91
Oates	1	2000	6	$y = 0.99x - 0.56$	0.99
Barnwell	1	2000	6	$y = 1.01x + 0.33$	0.95
Henderson	1	2000	6	$y = 1.01x + 0.69$	0.84
Combined	1	2000	22	$y = 0.99x + 0.16$	0.92
Lamb Mt.	1	2001	7	$y = 1.00x + 0.05$	0.95
Oates	1	2001	7	$y = 1.00x + 0.02$	0.94
Station	1	2001	6	$y = 0.98x + 0.11$	0.93
Fruitland	1	2001	5	$y = 1.01x + 0.31$	0.92
Combined	1	2001	25	$y = 1.00x + 0.19$	0.94
Combined		2000			
	1	2001	47	$y = 1.00x + 0.17$	0.94
Lamb Mt.	2	2000	6	$y = 0.98x - 1.41$	0.97
Oates	2	2000	5	$y = 1.00x - 0.17$	0.88
Barnwell	2	2000	6	$y = 1.03x + 1.80$	0.82
Henderson	2	2000	6	$y = 1.01x + 1.16$	0.97
Combined	2	2000	23	$y = 0.99x + 1.07$	0.91
Lamb Mt.	2	2001	6	$y = 0.98x - 1.81$	0.98
Oates	2	2001	5	$y = 0.99x - 0.38$	0.96
Station	2	2001	6	$y = 1.01x + 0.32$	0.86
Fruitland	2	2001	5	$y = 0.99x - 1.05$	0.93
Combined	2	2001	22	$y = 0.98x - 0.13$	0.94
Combined		2000			
	2	2001	45	$y = 0.98x + 0.75$	0.93



**Table 3.2 Continued.**

Orchard	Generation	Year	<i>n</i>	Line Equation	$r^2$
Lamb Mt.	3	2000	5	$y = 1.01x + 2.39$	0.86
Oates	3	2000	5	$y = 1.00x - 0.54$	0.97
Barnwell	3	2000	5	$y = 1.00x + 0.66$	0.75
Henderson	3	2000	5	$y = 1.01x + 3.36$	0.89
Combined	3	2000	20	$y = 0.99x + 2.57$	0.88
Lamb Mt.	3	2001	6	$y = 1.00x - 0.32$	0.92
Oates	3	2001	5	$y = 0.97x - 4.15$	0.79
Station	3	2001	5	$y = 1.02x + 4.81$	0.99
Fruitland	3	2001	4	$y = 0.99x - 3.02$	0.94
Combined	3	2001	20	$y = 0.98x - 0.01$	0.90
Combined		2000			
	3	2001	40	$y = 0.99x + 0.86$	0.89
Lamb Mt.	4	2000	7	$y = 0.99x - 2.24$	0.96
Oates	4	2000	7	$y = 0.99x - 2.16$	0.95
Barnwell	4	2000	7	$y = 0.99x - 3.05$	0.91
Henderson	4	2000	6	$y = 1.02x + 5.78$	0.94
Combined	4	2000	27	$y = 0.96x + 1.35$	0.92
Lamb Mt.	4	2001		--	--
Oates	4	2001	7	$y = 0.97x - 6.03$	0.83
Station	4	2001	7	$y = 1.02x + 24.73$	0.97
Fruitland	4	2001	7	$y = 1.02x - 18.27$	0.87
Combined	4	2001	21	$y = 0.73x + 14.11$	0.57
Combined		2000			
	4	2001	48	$y = 0.85x + 7.65$	0.75

Table 3.3. Predicted percentage of second generation oriental fruit moth exposed to lethal methoxyfenozide residue based on hypothetical application timings estimated from pheromone trap catch and degree day accumulation for codling moth and oriental fruit moth from ten orchards in North Carolina. The minus, plus, or no sign in front of the predicted percentage is used to indicate application timing before, after, or at the initiation of second generation oriental fruit moth, respectively. 2002.

Orchards	Initial Spray Date	Second Application Interval		
Low Population				
		<u>No Spray</u>	<u>14 d</u>	<u>21 d</u>
Lancaster	5/20	- 40 %	80 %	+ 100 %
Ranch	5/27	- 40 %	80 %	+ 100 %
Reed	5/27	- 40 %	80 %	+ 100 %
McCraw	5/20	- 33 %	67 %	+ 83 %
Airport	5/13	- 17 %	- 50 %	67 %
Average		- 34 %	- 71 %	+ 90 %
High Population				
			<u>14 d</u>	<u>21 d</u>
Lancaster	5/06		- 40 %	- 60 %
Staton	4/22		- 20 %	- 40 %
Brown	4/29		- 0 %	- 20 %
MHCRS	5/13		80 %	+ 100 %
Davis	4/22		- 40 %	- 60 %
Lynch	4/22		- 0 %	- 20 %
Average			- 30 %	- 50 %

## **Appendix**

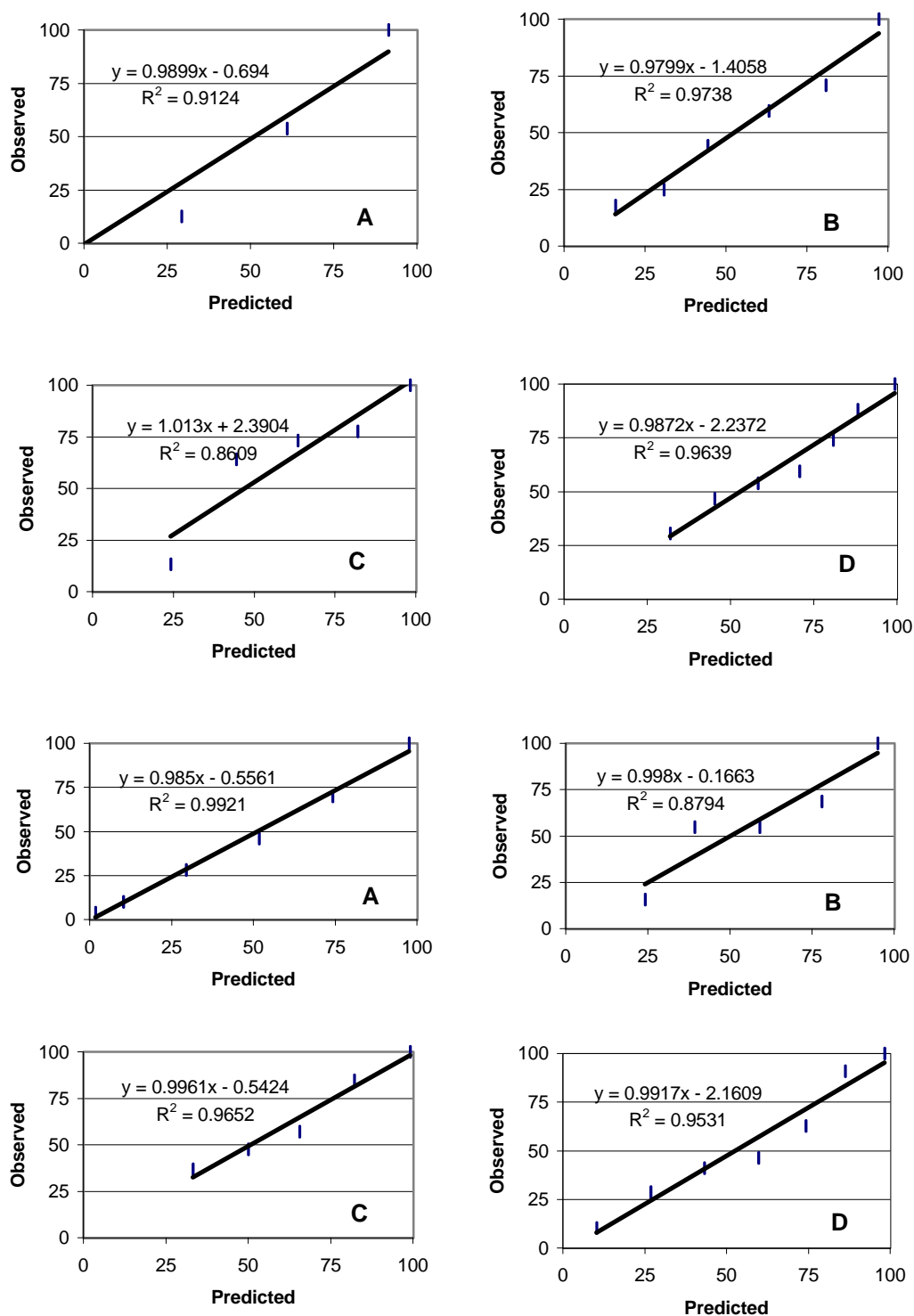


Figure A1. Predicted versus observed percentage of cumulative oriental fruit moth oviposition at Lamb Mt. and Oates abandoned apple orchards for first (A), second (B), third (C) and fourth (D) generations in 2000, respectively. Henderson Co. NC.

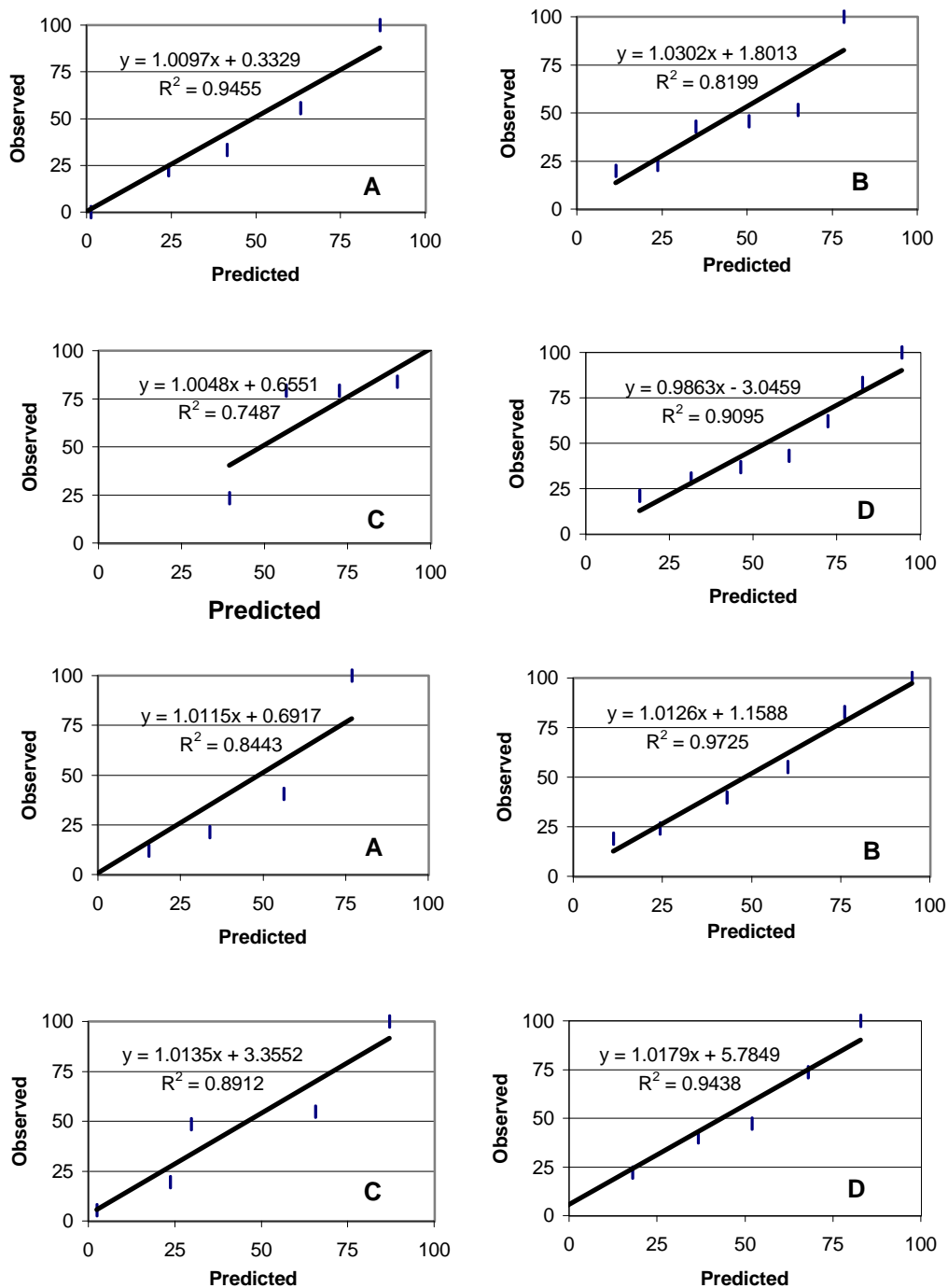


Figure A 2. Predicted versus observed percentage of cumulative oriental fruit moth oviposition at Barnwell and Henderson abandoned apple orchard for first (A), second (B), third (C) and fourth (D) generations 2000, respectively. Henderson Co. NC.

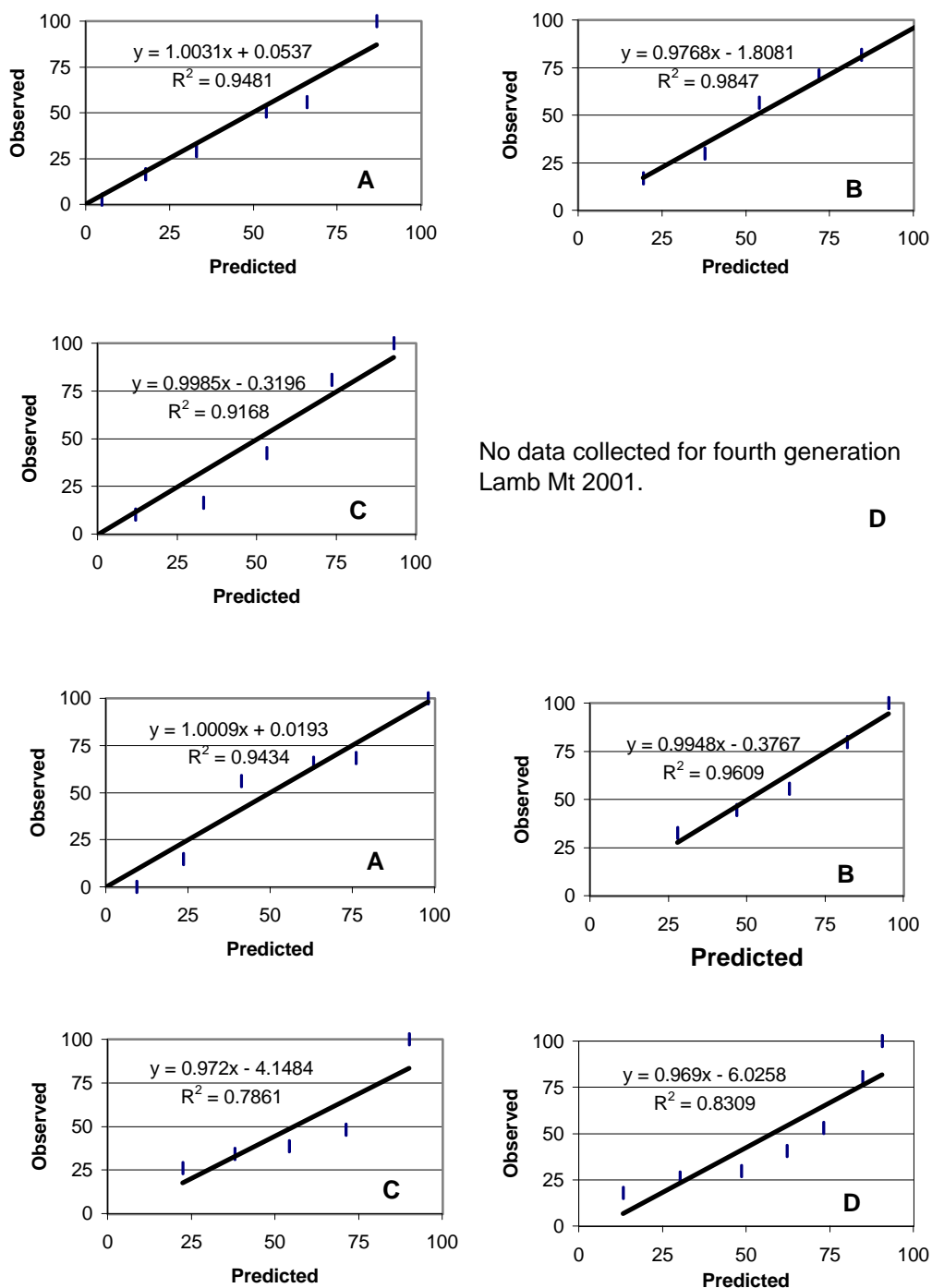


Figure A 3. Predicted versus observed percentage of cumulative oriental fruit moth oviposition at Lamb Mt. and Oates abandoned apple orchards for first (A), second (B), third (C) and fourth (D) generations in 2001, respectively. Henderson Co. NC.

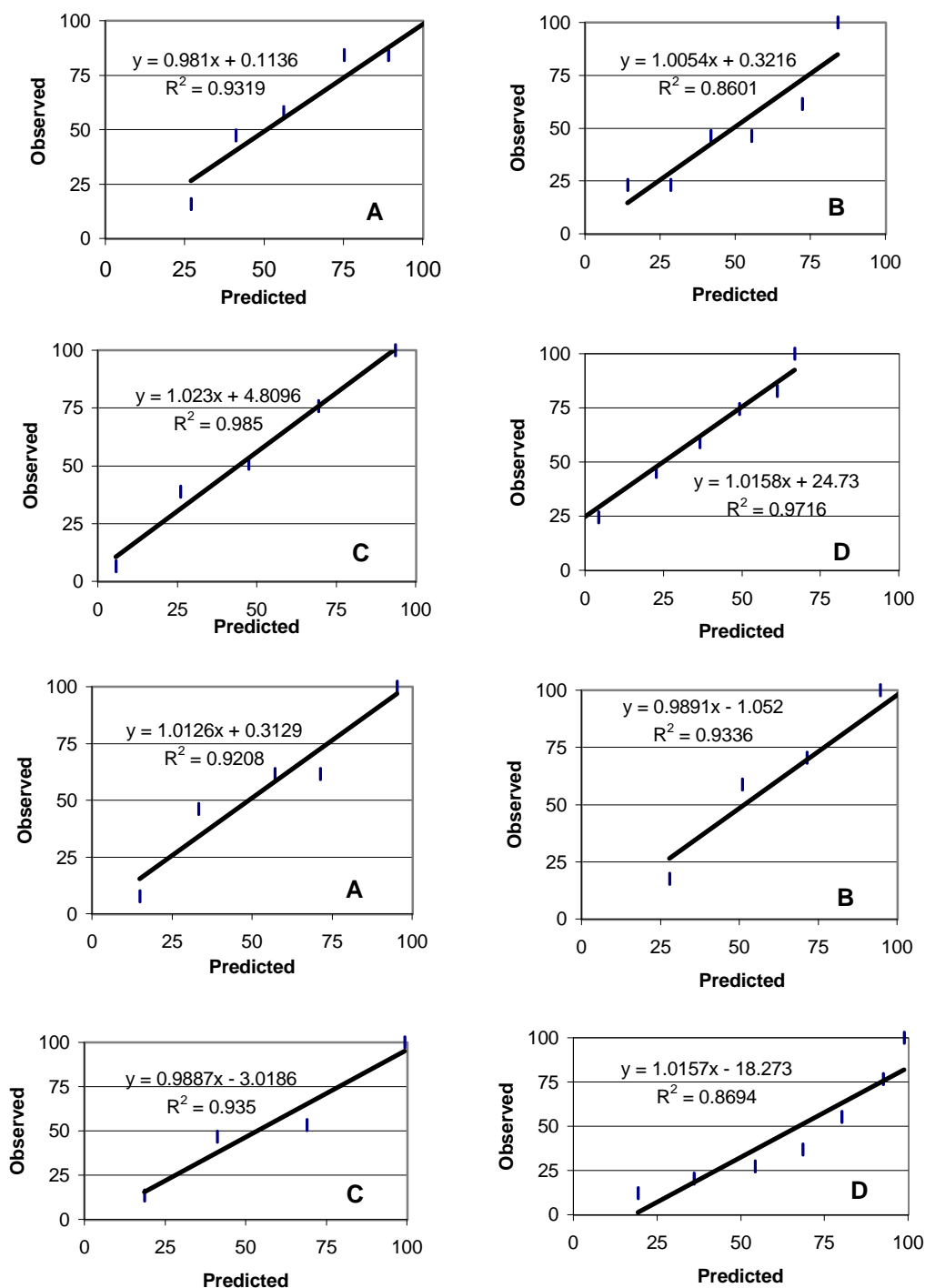


Figure A 4. Predicted versus observed percentage of cumulative oriental fruit moth oviposition at Station and Fruitland abandoned apple orchards for first (A), second (B), third (C) and fourth (D) generations in 2001, respectively. Henderson Co. NC.

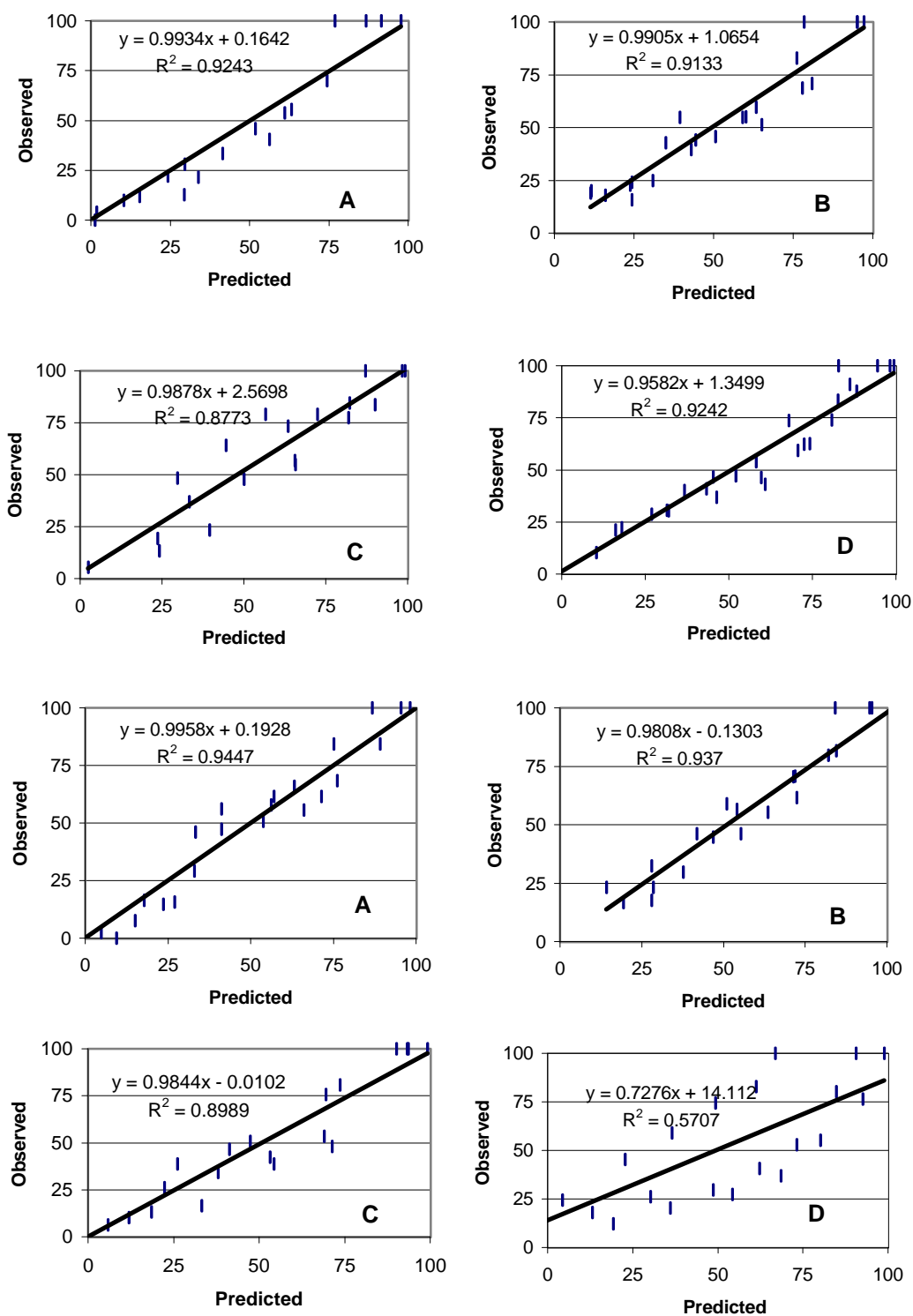


Figure A 5. Predicted versus observed percentage of cumulative oriental fruit moth oviposition for first (A), second (B), third (C) and fourth (D) generations from four abandoned orchards in Henderson Co. 2000 and 2001, respectively.



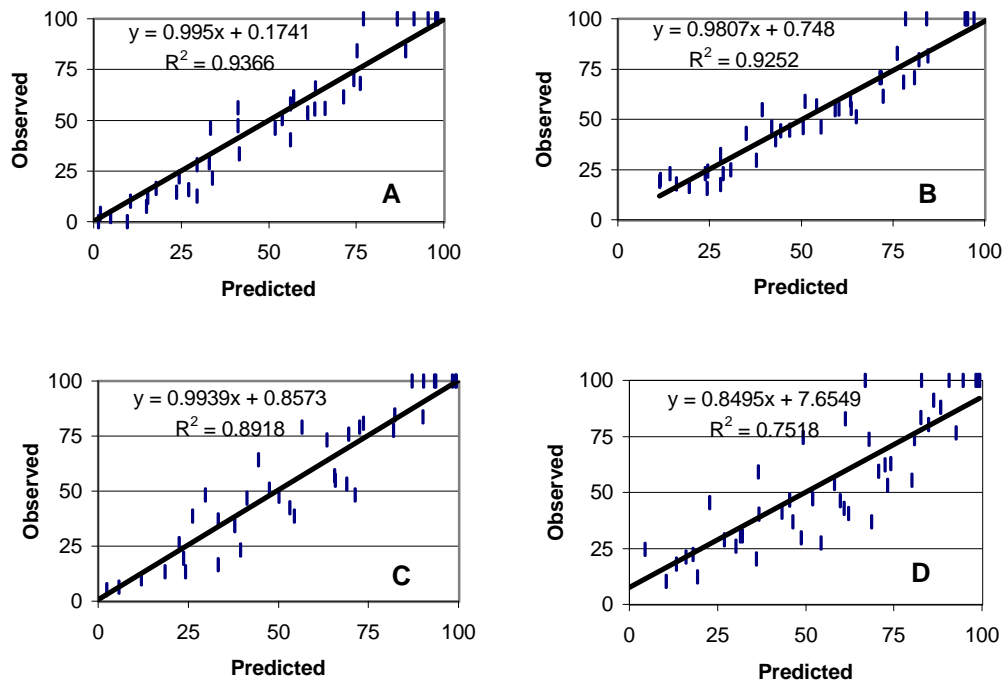


Figure A 6. Predicted versus observed percentage of cumulative oriental fruit moth oviposition for first (A), second (B), third (C) and fourth (D) generations from four abandoned orchards in Henderson Co combined over 2000 and 2001.