ABSTRACT

ABNEY, MARK RAY. Population Dynamics of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) in a Host-Species Rich Agroecosystem: Implications for Insecticide Resistance Management. (Under the direction of Clyde E. Sorenson and Fred Gould)

A three-year evaluation of crop host utilization by the tobacco budworm, *Heliothis virescens* (F.), was conducted from 2001 to 2003 in the central coastal plain of North Carolina. Weekly monitoring of commercial tobacco and non-Bt cotton fields revealed spatial and temporal patterns of host use, but showed that tobacco budworm may be produced in tobacco throughout the growing season. Small plot trials conducted in 2002 and 2003 demonstrated a strong oviposition preference of tobacco budworm for tobacco when located adjacent to plantings of alternate crop hosts. Moths collected in pheromone traps placed up to a quarter mile from primary sources of tobacco budworm production demonstrate sufficient short-range movement by adult insects to facilitate mating of individuals produced in distant cotton and tobacco fields.

The bodies of individual moths were analyzed for the presence of a cotton-specific analyte, gossypol, using high-pressure liquid chromatography–mass spectrometry/mass spectrometry (HPLC-MSMS). In a validation study, 100% of moths reared in the laboratory on cotton tested positive for the presence of gossypol, while 81% of moths reared on non-cotton hosts were determined to be negative for gossypol. This technique provides a conservative estimate of tobacco budworm production on hosts other than cotton. Analysis of feral moths revealed that <10% of tobacco budworms collected in pheromone traps in the central coastal plain of North Carolina contained gossypol.

Isotope ratio mass spectrometry (IRMS) analysis of $^{13}C/^{12}C$ ratios of moths reared on four crop-plant species and two common weed species revealed a range of $\delta^{13}C$ values within that expected for plants utilizing the C3 photosynthetic pathway. No significant differences in mean $\delta^{13}C$ values were detected between moths reared on any of the host plant species. Vegetative and reproductive tissues from potential host plants utilized in the study also could not be separated to species. Feral tobacco budworm moths collected over three years were found to have carbon isotope ratios consistent with those having fed on C3 plants. The average $\delta^{13}C$ value of feral insects collected within a single year appeared to be closely associated with the total accumulated rainfall for the months May through August. Results demonstrate that within the range of C3 host plants tested, no unique carbon isotope signature exists that would enable a reliable determination of natal origin of feral tobacco budworm with current IRMS technology.

Dose-mortality studies were conducted to determine the susceptibility of two strains of tobacco budworm collected in North Carolina in 2004 to the pyrethroid insecticide cypermethrin. LD_{50} values were 4 and 9 times greater for tobacco budworms collected in June and August respectively than for a susceptible laboratory strain. Results are similar to those observed in states where control failures due to pyrethroid resistance have been reported.

Foliar applications of three pyrethroid insecticides were made to flue-cured tobacco and compared with *Bacillus thuringiensis* (Bt) bait and sprays of acephate and spinosad for control of the tobacco budworm in 2001, 2002, and 2003. Lambda-cyhalothrin, cyfluthrin, and bifenthrin provided significant control of tobacco budworm when compared to untreated checks in all three years of the study; however, they were generally less efficacious than the other insecticides tested. The level of control among the pyrethroids differed significantly within years and was inconsistent from year to year. Damage ratings averaged over pyrethroid treatments were 54% lower than the untreated control in 2001 and 80% lower in 2003. Pyrethroid treatments had no impact on yield of cured leaf in 2001 or 2003.

POPULATION DYNAMICS OF *HELIOTHIS VIRESCENS* (F.) (LEPIDOPTERA: NOCTUIDAE) IN A HOST-SPECIES RICH AGROECOSYSTEM: IMPLICATIONS FOR INSECTICIDE RESISTANCE MANAGEMENT

by

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BIOGRAPHY

Mark Ray Abney was born on October 23, 1975, and he is the son of Mr. and Mrs. Monty Ray Abney. He was raised in Cochran, Georgia (pop. 4,455), a beautiful small town in the central part of the state, with his older sister Doreen. As a youth, Mark enjoyed the outdoors and at a very early age became especially fond of the pursuit of fish with hook and line (an obsession that plagues him yet today). Growing up in the rural South, Mark developed an interest in agriculture that was cultivated by his parents' love for gardening and his work experience on the farm. Following graduation from Bleckley County High School in 1994, he enrolled at Middle Georgia College where he received his Associate of Science degree in biological sciences in 1996. Mark transferred to the University of Georgia in the fall of 1996 and in 1998 received his Bachelor of Science degree in Crop Science.

Mark began his graduate work in the Entomology Department at the University of Georgia in 1998 under the direction of Dr. John Ruberson. While conducting his research at the UGA Coastal Plains Experiment Station in Tifton, GA, he met his future wife, the lovely Miss Billie Jo Royal. Mark received his Master of Science degree in Entomology in 2000 after completing his research focusing on the impact of natural enemies on cotton aphid populations in South Georgia cotton production systems.

Mark was accepted into the Entomology Department at North Carolina State University in 2001 where he began work on his doctorate degree under the direction of Dr. Clyde Sorenson and Dr. Fred Gould. His research included studies of defined aspects of tobacco budworm population dynamics that were of particular concern for insecticide resistance management in North Carolina. In July of 2004, Mark and Billie Jo were married and have since lived together happily in a tiny one-room apartment. Mark currently enjoys his career as an entomologist but has not lost sight of his ambition (published originally under his senior picture in the 1994 Bleckley County High School yearbook) to be paid well to fish.

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I would also like to thank my parents for their unfailing support through all my years of growing up and pursuing my education. Words certainly cannot express my gratitude for all they have done, especially their efforts “to raise me right.” Finally, I wish to thank my wife, Billie Jo, for her untiring support and patience. Her understanding, encouragement, and love through the years have been priceless, and for that I will be forever in her debt.

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INTRODUCTION

The tobacco budworm, *Heliothis virescens* (F.), has long been regarded as a pest of tobacco and is a very serious pest of cotton in the United States (Johnson 1979, Williams 2004). The larvae of this insect feed directly on the harvestable portion of both crops and may reduce cotton yields if not controlled. Though the impact on tobacco yield has recently come into question (unpublished data), under some circumstances, tobacco budworm infestations have been shown to decrease yield and quality of cured leaf (Johnson 1979). Chemical control of *H. virescens* has been complicated over the years by the development of resistance in the insect to many classes of insecticides (Sparks et al. 1993). Resistance originally developed in tobacco budworm to the chlorinated hydrocarbons (Lingren and Bryan 1965), and control failures have since been reported following applications of certain carbamate and organophosphate insecticides (Sparks 1981). Pyrethroid insecticides first became commercially available in the late 1970's, and they are generally efficacious and cost effective tools for the management of many insect pests in many crops. While initially very effective at controlling heliothine pests in cotton, the development of resistance by the tobacco budworm to pyrethroids in the mid-to-late 1980’s had a major impact on cotton insect pest management in many parts of the United States (Luttrell et al. 1987, Leonard et al. 1988, Campanhola and Plapp 1989, Elzen et al. 1992). North Carolina is unique in that the appearance of resistance in the tobacco budworm has occurred more slowly here than in other states (Sparks et al. 1993). Nevertheless, pyrethroid resistance management continues to be a concern in North Carolina because of the importance of this class of insecticides for controlling the corn earworm, *Helicoverpa zea* (Boddie), in cotton, and the increased potential for resistance evolution that comes with the recent labeling of pyrethroids for use in flue-cured tobacco.

The introduction and subsequent widespread adoption of transgenic cotton varieties that produce on or more toxins derived from the bacterium *Bacillus thuringiensis* have drastically altered control tactics employed for heliothine pests in cotton. Insecticide applications made to control the corn earworm/tobacco budworm complex decreased from an average of 2.4 per season in 1995 (before the commercial release of Bt varieties) to 0.76 per season in 2003 (53% of U.S. cotton hectares planted to Bt varieties) (Williams 1996, 2004, Adamczyk and Burris 2004). The efficacy of Bt cotton against the tobacco budworm has in large measure alleviated much of the concern over pyrethroid resistance in the insect and taken a great deal of attention away from pyrethroid resistance management in the crop. The refinement of resistance management strategies for transgenic Bt crops has become the major focus of current insecticide resistance management (IRM) research.

The focus of the research presented here is the identification and quantification of biological, ecological, and environmental factors that might impact the rate of insecticide resistance development by *H. virescens* in North Carolina. The distribution, abundance, phenology, and utilization of host plants, the presence and frequency of resistance genes, and the selection pressure that tobacco budworm populations are likely to experience must all be determined. It is critical that comprehensive, research-based resistance management plans be implemented in North Carolina in order to delay or prevent the development of resistance by the tobacco budworm to established and future novel insecticide chemistries.

The ecological characteristics of an insect population in a given region play an integral role in determining the response of that population to insecticide pressure. The tobacco budworm is a highly mobile insect that is found throughout much of the Western Hemisphere with greatest abundance in tropical regions. Though present in North Carolina on an annual basis, the severity of tobacco budworm infestations on crop hosts in the state varies from year to year. The exact number of generations of *H. virescens* that occurs each year is thought to fluctuate, but four generations are common. Larval development may progress through a variable number of instars from four to six depending on climatic and nutritional factors. Development times from egg to adult depend on time of year and range from 38.2 days (May-June) to 33.6 days (July-August). Larvae pupating after mid September typically overwinter and emerge the following spring. Adult females deposit eggs singly on the reproductive and vegetative structures of host plants and normally produce 300-500 eggs in a lifetime. A complete review of tobacco budworm biology in North Carolina is presented in Neunzig 1969.

The tobacco budworm is an oligophagous insect capable of developing on a number of cultivated and wild host plants, though its host range varies from region to region (Barber 1937, Neunzig 1969, Sudbrink and Grant 1995). The primary cultivated hosts in North Carolina are tobacco and cotton, and the insect is known to develop on a number of wild host plant species in the state. Nevertheless, reliable production estimates for the insect do not exist for either cultivated or wild hosts. The availability of cotton suitable for tobacco budworm development has declined in recent years with the advent of new insect management tools; since their commercial introduction in 1996, transgenic Bt cotton varieties have made up of an increasing proportion of the cotton acreage in North Carolina. Approximately 75% of all cotton acres in the state in 2003 were planted to Bt varieties (Williams 2004, NC Department of Agriculture 2005). These plants represent a dead end for *H. virescens* development because of the high toxicity of Bt to the tobacco budworm. The impact of widespread planting of Bt cotton on the overall tobacco budworm population is unknown. Soybeans are common in North Carolina and are capable of supporting *H. virescens* development (Sheck and Gould 1993, Deitz et al.1976), but relatively few individuals are found in soybeans in the field (Deitz et al.1976). The extent to which cotton, soybeans, peanuts, and wild hosts contribute to overall tobacco budworm numbers in the state is likely to be small compared to the impact of tobacco. Nevertheless, these plants may be of importance as refuge areas for tobacco budworm development as they are, with the exception of the inherently toxic Bt cotton varieties, infrequently treated with insecticides specifically for *H. virescens* control.

Understanding the mechanisms of insecticide resistance and managing that resistance in pest populations have become key concerns for scientists and pest control practitioners. Georghiou (1972) identified two key factors determining the development of resistance in an insect population: The existence of resistance genes and sufficient selection pressure to concentrate these genes in the genome of the population. Determining the presence and frequency of insecticide resistance genes in North Carolina populations of tobacco budworm and monitoring the changes in frequency of those genes are significant aspects of resistance management that will be addressed in this study. Likewise, the potential selection pressure placed on populations of the insect will be examined. Currently, the tobacco budworm is considered an economic pest of tobacco in North Carolina. In the past, infestations were controlled primarily with organophosphate insecticides, but toady, organophosphates, spinosad, Bt products, and a pyrethroid are all used. Populations of tobacco budworm reach economic thresholds only sporadically in cotton in the state, and these infestations have historically been controlled largely with pyrethroid insecticides. The North Carolina Cooperative Extension Service currently discourages the use of pyrethroids for early season heliothine infestations in cotton (Bacheler 2003). The recommendation to use alternate chemistries against early generations of heliothines is an attempt to preserve susceptibility in the later, historically more damaging generations. The separation of insecticide classes used in tobacco and cotton may be at least partly responsible for the apparent delay in resistance to pyrethroids seen in North Carolina populations of tobacco budworm. Novel insecticides are being introduced to the market for control of lepidopterous pests in both cotton and tobacco. Dual registration of new as well as older insecticides for tobacco budworm on both crops (i.e. spinosad and pyrethroids) could significantly increase the selection pressure imposed upon populations of the insect.

Nearly three hundred thousand hectares of cotton and more than sixty thousand hectares of tobacco were harvested in North Carolina in 2004. These two crops represent a major nutrient resource for *H. virescens* production. Given the abundance of cultivated host plants and the relatively low tolerance for insect damage in both cotton and tobacco, the development of resistance by the tobacco budworm to insecticides in North Carolina has surprisingly been much slower and less pronounced than in many other cotton producing states. Reasons for this phenomenon are not fully understood but may be linked to the structural differences associated with the North Carolina agroecosystem. Pyrethroid resistance developed in the Mid-South due to repeated use of pyrethroids on successive generations of budworms in cotton crops within the growing season. In North

Carolina, budworms have typically been exposed to materials like pyrethroids during only one generation annually. The only significant exposure to pyrethroids experienced by tobacco budworms in North Carolina before 2004 occurred in late July-early August when pyrethroid applications were made to cotton for control of the cotton bollworm. Today, the pyrethroid lambda cyhalothrin is also labeled for use in flue-cure tobacco, although the tobacco hectarage treated with this material annually has not been quantified.

It has been shown that tobacco is a preferred host of *H. virescens* (Waldvogel and Gould 1990), and more than 60% of the flue-cured tobacco produced in the United States is grown in North Carolina (NC Department of Agriculture 2005). The presence of an abundant preferred host in the state may effectively limit oviposition of *H. virescens* in cotton thus reducing the need for chemical control in that crop. Ramaswamy et al. (1987) proposed that damaging populations of tobacco budworm occur in cotton only because of the abundance of the plants in what they termed a "no choice" situation. Waldvogel and Gould (1990) found cotton to the least attractive of six potential host plant species in caged oviposition studies with four strains of *H. virescens*. While alternative hosts may be rare in many cotton producing states, they often abound in North Carolina, thus minimizing the impact of the “no choice” scenario and reducing the number of *H. virescens* developing in cotton. Nevertheless, the incidence and severity of tobacco budworm infestations in cotton do not vary substantially between areas of North Carolina where tobacco and cotton are grown in close proximity and those areas where cotton is grown in the absence of tobacco culture (Bacheler personal communication). Understanding how tobacco budworm populations are structured and how they utilize available hosts in the agricultural landscape is key for developing insecticide resistance management plans. The tobacco budworm population in North Carolina may be comprised of a single inter breeding population with the oviposition site for an individual female determined primarily by the proximity of any one of a number of suitable hosts. An alternative hypothesis is that tobacco budworm populations exist in the state as more than one potentially inter-breeding but more or less genetically distinct host specific races.

The importance of wild and cultivated host plants other than cotton and tobacco to overall population dynamics of tobacco budworm in North Carolina is unclear. While tobacco almost certainly acts as the major host in terms of annual production of individual insects, other plant hosts may be critical for maintaining *H. virescens* populations early and/or late in the growing season (assuming a single interbreeding population). Additionally, these alternate hosts may serve as natural refuge areas for *H. virescens* production that may prove invaluable in the management of resistance to insecticides. A study conducted in Georgia in the 1930's identified fourteen wild host plant species for the tobacco budworm (Barber 1937), and many of these plants, while not reported as hosts in North Carolina, are present in the state. A survey of potential host plants in eastern North Carolina revealed surprisingly few *H. virescens* on only a limited number of wild plant species; *Linaria canadensis* (toad flax) and *Rhexia spp.* (deergrass) were observed to be of primary importance (Neunzig 1963). Neunzig (1969) reported the seasonal occurrence of tobacco budworm on tobacco, cotton, toadflax, and deergrass in North Carolina in 1959 and 1960. Early season oviposition was distributed between tobacco and toadflax, as neither plant was sufficiently abundant and/or attractive to receive a majority of the oviposition pressure. During the period of tobacco flowering almost all *H. virescens* eggs were deposited on tobacco, and only a few were seen in wild hosts or cotton. Deergrass became a potentially important host plant later in the season (mid-late August), but tobacco continued to receive a large proportion of the *H. virescens* eggs.

The studies described here focus on pyrethroid and Bt insecticide resistance management, but the biological and environmental data that will be examined will be useful in developing resistance management plans for any new synthetic insecticides. The ultimate goal of this project is to enable informed decisions about the use of new insecticides in tobacco and cotton to prevent or delay the development of resistance to these materials in the tobacco budworm. The short-term objective of these studies is to quantify certain behavioral and physiological characteristics of the tobacco budworm; these data will be used to help refine current IRM strategies for the pest. The specific objectives are: 1) assess the population flow of tobacco budworms through agronomic hosts in areas where both tobacco and cotton are components of the agroecosystem; 2) develop an analytical tool that will enable the identification of the natal host origin of an individual adult tobacco budworm; 3) determine the current susceptibility of NC populations of tobacco budworm to pyrethroid insecticides; 4) evaluate the population structure of North Carolina populations of *H. virescens*: i.e. do host specific races exist?; and 5) assess the efficacy of new insecticides against the tobacco budworm in tobacco. The assimilation and application of the findings from these studies have the potential to increase the useful life of insecticides by lowering the speed at which resistance develops in a tobacco budworm population.

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Alternate Crop Hosts as Resistance Management Refuges for Tobacco Budworm, *Heliothis virescens*, (Lepidoptera: Noctuidae) in North Carolina.

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Abstract

A three-year evaluation of crop host utilization by the tobacco budworm, *Heliothis virescens* (F.), was conducted from 2001 to 2003 in the central coastal plain of NC. Weekly monitoring of commercial tobacco and non-Bt cotton fields revealed spatial and temporal patterns of host use, and demonstrated that tobacco budworm may be produced in tobacco throughout the growing season. Small plot trials conducted in 2002 and 2003 demonstrated a strong oviposition preference of tobacco budworm for tobacco when located adjacent to plantings of alternate crop hosts. Moths collected in pheromone traps placed up to 0.40 km from primary sources of tobacco budworm production demonstrate sufficient short-range movement by adult insects to facilitate mating of individuals produced in cotton and tobacco fields. Results of this study indicate that tobacco may serve as an important refuge for both Bt transgenic plants and conventional insecticide resistance management in NC.

The tobacco budworm, *Heliothis virescens* (F.), has long been regarded as a pest of tobacco and it is a serious pest of non-Bt cotton in the United States (Johnson 1979, Williams 2004). Contributing to its pest status in cotton, a long history of insecticide resistance has been documented for this insect beginning with the chlorinated hydrocarbons and followed by the carbamates, organophosphates and pyrethroids (Sparks et al. 1993). In the last decade transgenic cotton varieties expressing Bt (*Bacillus thuringiensis*) endotoxins have revolutionized tobacco budworm control across the U.S. Cotton Belt, and to date no evidence of field resistance to Bt toxins has been found. Nevertheless, resistance management for both conventional and transgenic insecticide technologies remains a key concern for this important pest.

The development of resistance by the tobacco budworm to insecticides in North Carolina has been much slower and less pronounced than in other cotton producing states (Sparks et al. 1993). Reasons for this phenomenon are not fully understood but are probably linked to the structural differences associated with the North Carolina agroecosystem. The presence of an abundant preferred host (tobacco) in the state may effectively limit oviposition of *H. virescens* in cotton for much of the season, thus reducing the need for chemical control in that crop. Ramaswamy et al. (1987) proposed that damaging populations of tobacco budworm occur in cotton only because of the abundance of the plants in what they termed a "no choice" situation. While alternative hosts may be rare in many cotton producing states, they often abound in North Carolina, thus minimizing the impact of the “no choice” scenario and reducing the number of *H. virescens* developing in cotton. In addition to tobacco, soybean is common in North Carolina and is capable of supporting tobacco budworm development (Sheck and Gould

1993, Deitz et al.1976), though densities of tobacco budworm in soybean fields are generally low (Deitz et al.1976). The insect's utilization of peanut and wild hosts is poorly understood, and yet constitutes a potentially large source of production. In 2003, Jackson et al. provided evidence of significant production of bollworms, *Helicoverpa zea*, from non-cotton crop refuges in NC. The extent to which cotton, soybean, peanut, and wild hosts contribute to overall tobacco budworm numbers in the state is unknown. Nevertheless, these hosts may be important as refuge areas for tobacco budworm development as they are, with the exception of the inherent toxicity of Bt cotton varieties, infrequently treated with insecticides for lepidopteran pests.

The refuge concept of insecticide resistance management (IRM) has become a major component of current resistance management strategies for heliothine pests of cotton (Tabashnik et al. 2003). North Carolina's agroecosystems are typically characterized by a rich diversity of potential host plants (both cultivated and non-cultivated), and these hosts may serve as unintentional yet valuable refuges for resistance management. The work described herein was designed to determine the role of alternate crop hosts as possible refuges for insecticide resistance management of tobacco budworm. The specific objectives were 1) to determine the spatial and temporal utilization of commercial cotton and tobacco by *H. virescens* in the central coastal plain of NC and 2) to estimate the production of *H. virescens* from tobacco relative to three other crop hosts (cotton, peanut, and soybean) grown in adjacent small plots.

Materials and Methods

Work was conducted in 2001, 2002, and 2003 to quantify the seasonal distribution of tobacco budworm larvae within a mixed tobacco/cotton agroecosystem in the central coastal plain of North Carolina. Each year 10 tobacco fields (11 in 2001) and 6 non-Bt cotton fields (5 in 2001) from commercial farming operations were selected from a three county area including parts of Pitt, Wilson, and Edgecombe Counties. Tobacco fields were sampled for heliothine larvae twice weekly beginning in late May and continuing until stalk destruction in September. Cotton fields were sampled twice weekly from mid-June until plants were no longer suitable for larval development in mid-September. One hundred whole tobacco/cotton plants were randomly selected and examined in each field on each sampling date. Heliothine larvae were collected and placed in vials containing 75% ethanol pending identification to species. Larvae were transported to the laboratory where they were measured and identified; species determinations were based on larval keys presented in Neunzig (1969).

An investigation was initiated in Pitt County NC in 2003 to determine if intercrop movement by adult tobacco budworm was sufficient to facilitate mating of moths produced in "refuge crops" with moths that had been selected with insecticides. A study site that provided an isolated patch of preferred hosts (at least 0.8 km from another crop host) was selected. Potential crop hosts of *H. virescens* present at the study site were limited to one soybean field adjacent to two tobacco fields; the balance of cropland at the site was planted to Bt cotton varieties. Ten modified Harstack wire-cone pheromone traps were arranged along a line running east to west and placed at distances from zero to 0.4 km from the tobacco and soybean fields. Traps were deployed on 3 July and were removed from the field on 17 September 2003. Moths were collected from the traps weekly and transported to the lab where they were counted.

Because routine insect management practices on a commercial farm may interfere with the inherent suitability of a crop for tobacco budworm oviposition and larval development, a small plot experiment was conducted in 2002 and 2003 at NC State University's Central Crops Research Station in Clayton NC. This test was designed to evaluate peanut, soybean, and cotton as alternate hosts for *H. virescens* in a more controlled, insecticide free environment. Tobacco, cotton, peanut, and soybean were planted in 0.04 hectare plots in a randomized complete block design with four replicates. Production practices for all crops were conducted according to recommendations from the North Carolina Cooperative Extension Service. Tobacco budworm sampling was initiated in May of both years and continued until the crops were no longer suitable for larval development. Forty whole plants from the center four rows of each eight-row tobacco plot were sampled for the presence of larvae. Heliothine larvae were sampled in cotton and soybean plots by examining 4 row meters from the middle four rows of each eight-row plot. Sampling in peanut was conducted by taking fifteen sweeps through the center four rows of each plot with a 15" diameter sweep net. Crop phenology was recorded and plots were sampled weekly for presence of heliothine larvae. Sub-samples of larvae collected from each plot were transported to the laboratory for species identification. Data were subjected to one-way ANOVA, and means were separated ($P<0.5$) using student's t test in SAS JMP version 5.1 (SAS Institute Inc. 2002).

Results and Discussion

Tobacco budworm larvae were consistently present in commercial tobacco fields from early June until late July in all years of the study. Early sampling in 2003 revealed densities >700 larvae per hectare in May, and larvae were recovered from tobacco as late as 18 August in both 2001 and 2003. Two distinct peaks in larval density were evident in each year (Figure 1). The early season peak was associated with rapidly growing vegetative tobacco, while the second peak corresponded with emergence of reproductive structures in the tobacco in late June. Tobacco budworm larvae were also observed in post-harvest fields in September feeding on tobacco regrowth (though no formal samples were taken). Cultural practices employed by tobacco growers will have a significant impact on the amount of tobacco budworm production that occurs annually in the crop. Harvest and stalk destruction was complete on most of the sample sites by mid August in all years; an increase in larval density on 25 August 2003 was the result of an infestation in a single field of late maturing tobacco. The rapid decline in tobacco budworm density following early season peaks was the result of acephate applications (Orthene 97PE at 0.42 kg AI per hectare) made for control of the insect. Larval numbers in tobacco were reduced significantly after topping (flower bud removal) in late June and early July in all years. While tobacco stalks in this study were destroyed immediately following the final harvest, it is not uncommon to see stalks left standing for some time after harvest with a significant amount of regrowth. Though the proportion of fields left standing after harvest is unknown, the potential for substantial tobacco budworm production from regrowth in these fields does exist. These results indicate that tobacco grown in NC may contribute significantly to tobacco budworm production in as many as three generations annually depending on cultural practices and may thus provide an extended seasonal refuge for Bt transgenes and possibly conventional insecticide technologies respective to cotton.

Tobacco budworm larvae were rare in cotton fields in 2001 (Figure 2), and total heliothine larval densities never exceeded 5% infested plants (approximately 6000 per hectare) in any of the fields sampled throughout the season. Only five of the heliothine larvae collected in cotton fields in all of 2001were found to be *H. virescens.* Conservative insecticide treatment thresholds employed by producers contributed to the low numbers of larvae observed in cotton and tobacco in 2001. No tobacco budworm larvae were recovered from cotton in 2002. Cotton in the sample area in 2002 was severely drought stressed and was likely unfavorable for oviposition for much of the growing season. Tobacco budworm larvae were recovered from cotton fields in 2003. While the proportion of infested plants was never greater than 2% of those sampled, the estimated number of larvae per hectare did approach 3000 in August; this result was due largely to the influence of two fields with relatively heavy heliothine infestations that went untreated. Tobacco budworm densities in all other fields were reduced by pyrethroid applications directed primarily at bollworm, *Helicoverpa zea* (Boddie). Only 16% of tobacco budworm larvae collected from cotton in 2003 were >15mm, and 87% of those larger than 15mm were collected in the two untreated fields. Peak abundance of tobacco budworm larvae in cotton in August corresponds with a rapid decline in the suitability of tobacco for larval development (Fitt 1989). Results show that *H. virescens* larvae may be present in cotton and tobacco throughout the growing season in North Carolina, though temporal differences in peak seasonal occurrence exist for each crop. Temporal differences in tobacco budworm host utilization may impact the effectiveness of tobacco as a refuge; continuing research is focused on resolving this issue. While the actual production of adults from a specific host cannot be determined from these data, the rare occurrence of late instar larvae in cotton suggests only limited production of *H. virescens* from this crop, especially when treated with insecticides for heliothine control. All cotton fields included in this study were planted to non-Bt varieties and were part of an EPA mandated refuge requirement (80% Bt/20% non-Bt option) for planting Bt cotton. The lack of tobacco budworm production from cotton refuges could have serious implications for resistance management if alternate crop host refuges are unavailable.

Small fields and high crop diversity generally characterize the agroecosystem of North Carolina's central coastal plain. Pheromone trap catches of tobacco budworm moths in 2003 at distances up to 0.4 km away from any known crop host indicate that short-range, inter-crop movements by this insect do occur. This inter-crop movement should facilitate mating of individuals produced in a "refuge crop" with those produced in crops treated with insecticides or in transgenic, insecticide producing crops, fulfilling a key assumption of the refuge concept of IRM. (A "refuge crop" is defined here as any crop in which tobacco budworms may be produced without exposure to a particular insecticide technology.)

Results from small plot alternate host studies indicate a strong oviposition preference by tobacco budworm females for tobacco over the other crops tested. Tobacco budworm densities in untreated tobacco plots approached 10,000 larvae per hectare in two separate peaks occurring in late June and early August 2002 (Figure 3). Larval densities in tobacco were significantly greater than zero on 14 of 19 sampling dates in 2002. Tobacco budworm densities in tobacco were lower in 2003 than 2002, though timing of peak infestations was similar. Larval densities were significantly greater than zero on 8 of 13 sample dates in 2003. The abundance of *H. virescens* larvae in non-tobacco crop hosts was minimal in both years. Tobacco budworm larvae were collected from cotton on three sample dates (22 and 25 July and 2 August) in 2002. Mean larval density on each date was estimated to be 262 insects per hectare, but these means were not significantly greater than zero. Less than 10% of total heliothine larvae observed in cotton plots in 2002 were identified as *H. virescens*; the remainder of the larvae collected was *H. zea*. No tobacco budworm larvae were recovered from cotton in 2003. These results support the idea that tobacco budworm utilization of cotton may be determined largely by the proximity of a more attractive host. No tobacco budworm larvae were collected from soybean or peanut plots on any of the sampling dates in either year. This result was consistent with the work of Deitz et al. (1976) who found little tobacco budworm production from soybean in NC. The importance of peanut for tobacco budworm production remains unclear, but results here indicate limited utilization of the crop when tobacco is nearby. Nevertheless, high densities of tobacco budworm larvae have been observed in peanut fields in areas of North Carolina where peanut is typically planted (Jackson R.E. personal communication). Further research is needed to elucidate the role of peanut and wild hosts as refuges for tobacco budworm development in NC.

The presence of tobacco appears to dictate both spatial and temporal occurrence of tobacco budworm in NC agroecosystems. The close association between this pest and its preferred host is likely responsible for the minimal tobacco budworm problem experienced in cotton in the state. Tobacco may also play an important role in slowing the development of insecticide resistance in NC populations of tobacco budworm by providing a refuge for production of individuals susceptible to insecticides. Prior to 2003, pyrethroid insecticides were not labeled for use in tobacco, and during that time the crop provided a significant pyrethroid free refuge for tobacco budworm production. With the labeling of lambda cyhalothrin for use in tobacco in 2003, the utility of tobacco as a refuge for pyrethroid resistance management may have been compromised. A survey of tobacco growers revealed that foliar formulations of Bt insecticides were used on 25% of the tobacco acres in North Carolina in 2003 (unpublished data). Foliar Bt applications in tobacco primarily target infestations of hornworm species, *Manduca* spp., which generally occur after topping (Southern, P.S. personal communication). The impact of these applications on the effectiveness of tobacco as a natural refuge for Bt resistance management is unknown. Nevertheless, the data presented here suggest that tobacco budworm larvae are likely to be present in tobacco fields at the time foliar Bt applications are made. July through August is likely to be the most critical time for tobacco budworm production from Bt resistance refuges as the density of larvae in cotton generally peaks at this time. In spite of the use of foliar Bt insecticides in tobacco, the crop likely serves as an important refuge for Bt resistance management of tobacco budworm in North Carolina, and may continue to serve as a refuge for conventional insecticides if cultural practices including future insecticide use patterns permit.

Acknowledgements

The authors wish to thank grower-cooperators Glenn and Barbara Smith and Alan Stephenson, David Nichols, Billie Jo Royal, Andrew Shaffer, and Annette Crandall for assistance in data collection. We also express sincere gratitude to the NSF Center for Integrated Pest Management and the North Carolina Tobacco Foundation, Inc. for partial funding of this research.

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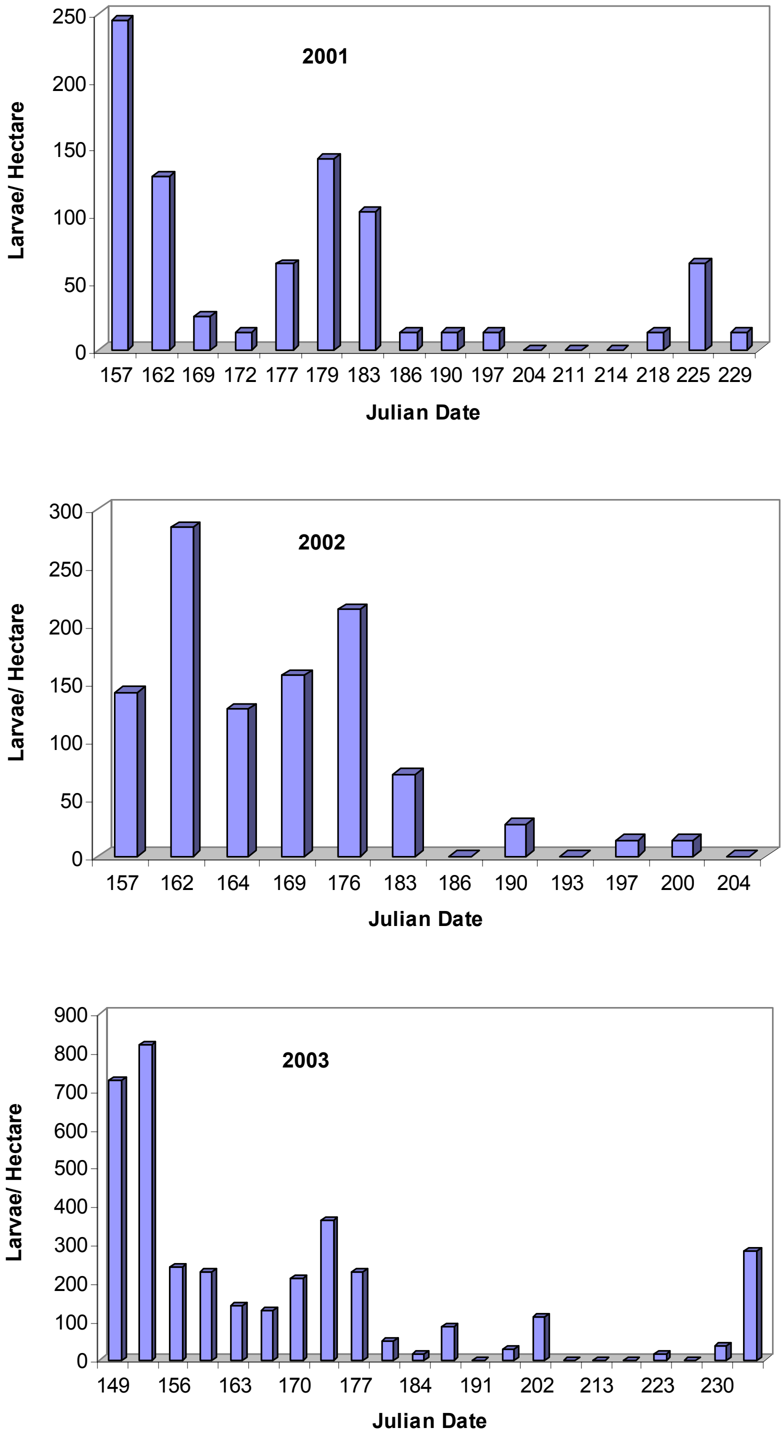


Figure 1. Estimated mean density of *Heliothis virescens* larvae (>5mm length) per hectare by sampling date in commercial tobacco fields in the central coastal plain of North Carolina in 2001, 2002, and 2003.

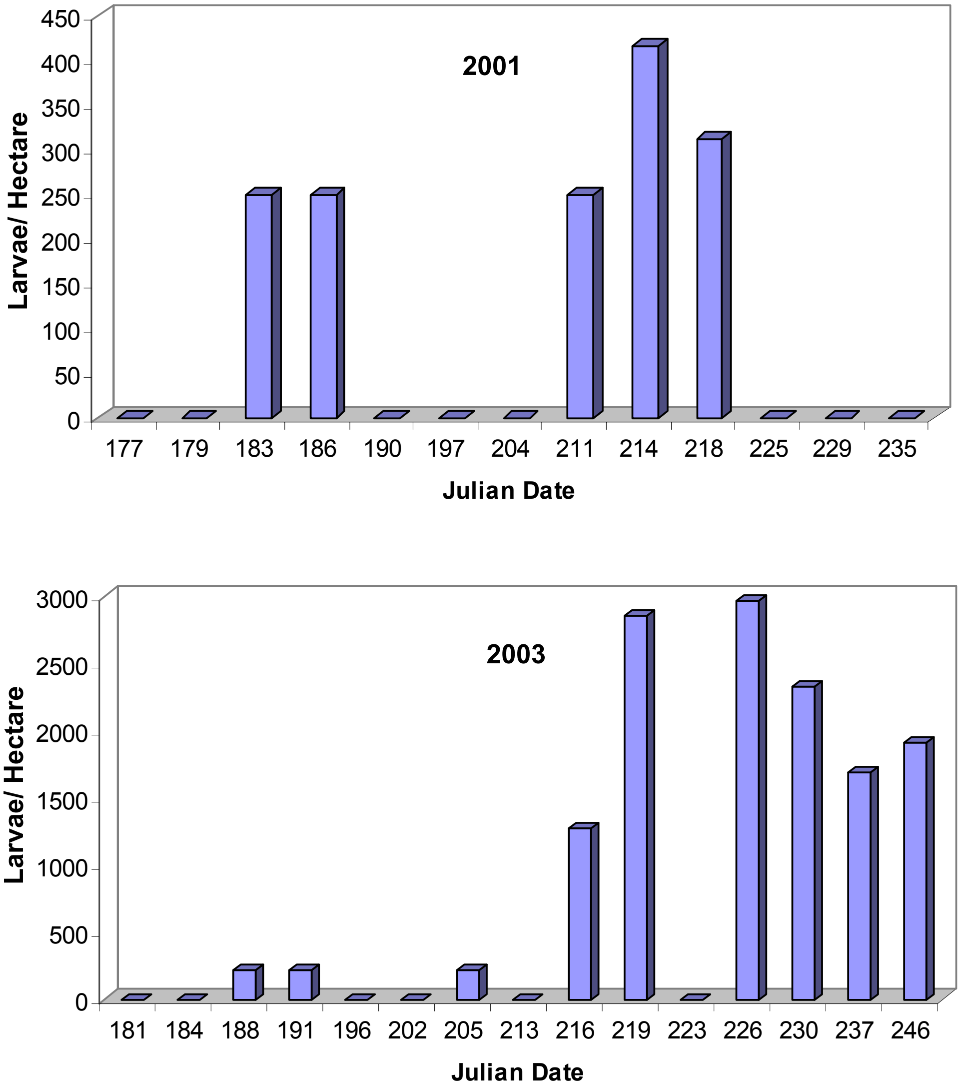
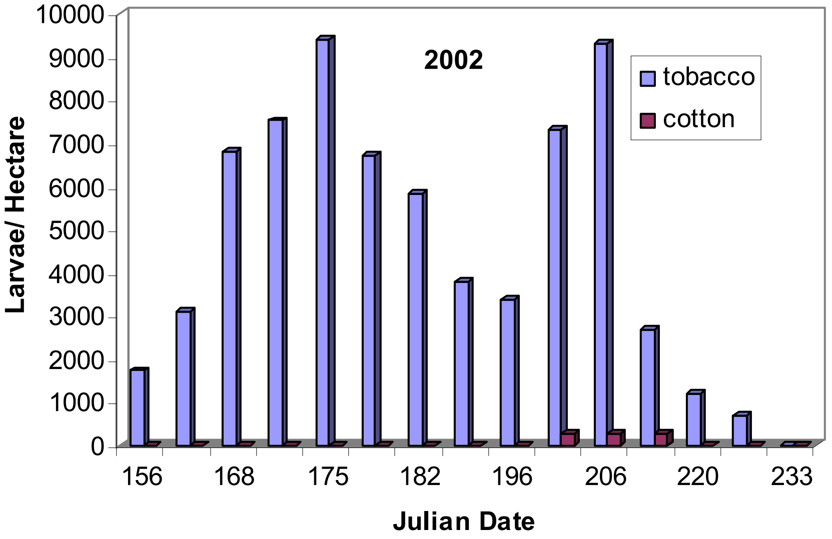


Figure 2. Estimated mean density of *Heliothis virescens* larvae (>5mm length) per hectare by sampling date in commercial cotton fields in the central coastal plain of North Carolina in 2001, and 2003.Though sampling was conducted, no *H. virescens* larvae were collected from cotton in 2002.



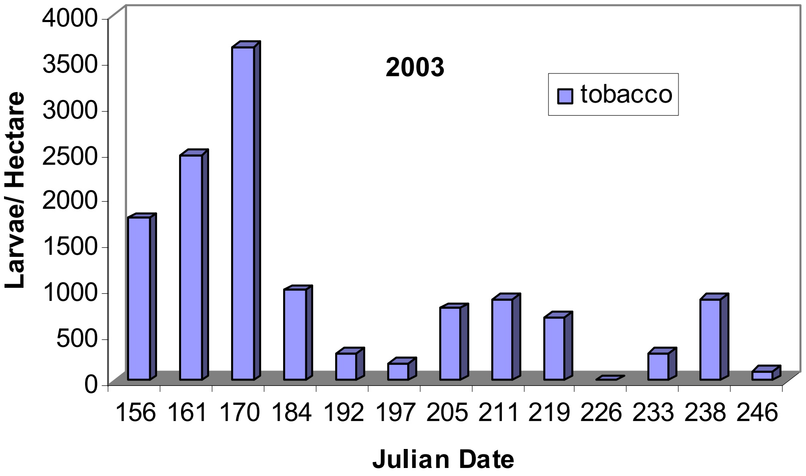


Figure 3. Mean estimated density of *Heliothis virescens* larvae per hectare in small plots of alternate crop hosts by sample date in the central piedmont of NC in 2002 and 2003. (No *H. virescens* larvae were recovered from any crop other than tobacco in 2003.)

Abney et al.: Natal host origins of
Heliothis virescens

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Assessing Production of *Heliothis virescens* (Lepidoptera: Noctuidae) from Cotton Using a Plant Derived Compound and the Implications of Alternate Host Use for Insecticide Resistance Management

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Abstract

Empirical data quantifying host utilization by the tobacco budworm, *Heliothis virescens* (F.), a multivoltine, oligophagous pest of cotton, have been lacking. We used a novel technique to assess the proportion of the adult tobacco budworm population present in North Carolina in July and August 2004 that developed on cotton. The bodies of individual moths were analyzed for the presence of a cotton-specific analyte, gossypol, using high-pressure liquid chromatography–mass spectrometry/mass spectrometry (HPLC-MSMS). In a blind validation study, 100% of moths reared in the laboratory on cotton tested positive for the presence of gossypol. For moths reared on non-cotton hosts, 81% were determined to be negative for gossypol. Results show that this technique will provide a conservative estimate of alternate crop host contribution to tobacco budworm populations as it tends to overestimate production from cotton. Analysis of feral moths revealed that <10% of tobacco budworms collected in pheromone traps in the central coastal plain of North Carolina contained gossypol. This result supports the hypothesis that non-cotton alternate hosts play an important role in the annual production of tobacco budworm in the state and may serve as significant refuges for Bt resistance management.

Commercial introduction of transgenic cotton varieties containing genes that code for the expression of insecticidal Bt proteins prompted the US Environmental Protection Agency (EPA) to mandate the use of insecticide resistance management (IRM) strategies by cotton producers. The basic tenet of the IRM plan developed for heliothine pests is the high dose refuge strategy: (i.e. the use of transgenic varieties producing a high dose of toxin combined with non-Bt cotton refuges to insure the production of individuals possessing insecticide susceptible genes). Since empirical data pertaining to the risk of insecticide resistance evolution to this new technology were limited at the time of introduction, resistance management plans were constructed using models based on empirical knowledge of agroecosystems and the target insects' biology, supplemented as necessary by theoretical information.

Researchers have been working to untangle two of the more confounding and controversial biological questions related to heliothine resistance management in cotton. First, can non-Bt alternate hosts provide adequate "natural refuge" to delay resistance evolution, and second how does one quantify seasonal production of the target insect from various alternate hosts? Workers utilizing isotope ratio mass spectrometry (IRMS) to differentiate between corn earworm moths, *Helicoverpa zea* (Boddie), that developed on C_3 vs. C_4 hosts recently determined that much smaller than expected proportions of the corn earworm populations in Louisiana, Texas, and North Carolina develop annually on cotton (Gould et al. 2002, R. E. Jackson unpublished data). Abney et al. (2005) explored the utility of IRMS analysis for elucidating the natal host origins of the tobacco budworm but found that host plants within the C_3 photosynthetic pathway could not reliably be distinguished from one another on the basis of carbon isotope composition.

PCR fingerprinting revealed high gene flow between populations of *Helicoverpa armigera* (Hübner) collected from various host plants in China (Tan et al. 2001), and Wu et al. (2002), further showed that mating between *H. armigera* moths from Bt cotton and alternate hosts was possible due to overlapping adult emergence. While these findings indicate that interbreeding between individuals developing on alternate hosts is likely, they do not quantify the level of production from specific hosts. Studies monitoring heliothine larval densities on crops have been conducted and provide some indication of host utilization (Wu et al. 2002, Jackson et al. 2004, Abney et al. 2004), but do not directly address the ultimate issue of adult production. By exhuming heliothine pupae in the fall and comparing densities to spring pheromone trap catch data, Schneider (2003) was able to determine that the proportion of the overwintering tobacco budworm population that developed in cotton fields in Mississippi was less than 2%.

Though data now exists for *H. zea* and partial data exists for *H. armigera* and *H. virescens*, to date no reliable, cost effective method of determining the season long production of tobacco budworm from its possible alternate hosts has been identified. The ability to detect compounds unique to a specific host plant or group of related host plants in an adult tobacco budworm would be a valuable tool for those seeking to quantify seasonal production of the insect from its range of potential host plants. As the sensitivity of detection technology increases, so does the likelihood of identifying a compound that may be useful as a biological marker. Cotton and tobacco, two important cultivated hosts of the tobacco budworm in North Carolina, contain likely candidate compounds that might serve as biological markers. Gossypol is a polyphenolic binaphthyl aldehyde produced by plants in the genus *Gossypium*, including commercial cotton varieties.

Previous efforts to determine the fate of ingested gossypol within the tobacco budworm failed to detect the compound in larvae after they were a fed a diet into which it had been incorporated; this result indicated complete gossypol metabolism by the insect (Parrot et al. 1983). Nevertheless, we hypothesized that some level of gossypol or a derivative thereof would be detectable using modern analytical techniques in tobacco budworm moths reared on cotton. Attempts to use enzyme linked immunosorbant assays (ELISA) to detect gossypol in tobacco budworm moths reared on known hosts provided unreliable results (unpublished data).

We report here on a new technique utilizing liquid chromatographic analysis linked to mass spectrometry to detect a derivative of gossypol in the tobacco budworm. The results confirm our hypothesis that a gossypol derivative can be detected in tobacco budworm moths and further support the idea that only a small fraction of the tobacco budworm population in North Carolina's central coastal plain develops annually on cotton. This study provides important data for the continuing refinement of Bt and conventional IRM strategies for the tobacco budworm.

Materials and Methods

Analytical Technique. The bodies of individual tobacco budworm moths were analyzed for the presence of a cotton-specific analyte, gossypol, using high-pressure liquid chromatography – mass spectrometry/mass spectrometry (HPLC-MSMS). The accuracy of the technique was tested in blind validation studies by analyzing tobacco budworm moths reared on known hosts in the laboratory. Tobacco budworm larvae were obtained from a laboratory colony maintained at the NC State University insectary (Raleigh, NC). Neonate larvae were placed on excised terminal leaf tissue from cotton, tobacco, soybean, and peanut in 473-ml plastic cups. Holes were punched into the lids of the cups for ventilation, and a moistened filter paper disk was placed in each cup to maintain humidity. Second instar larvae reared on cotton, soybean, and peanut were transferred to bolls, pods, and naked seeds respectively and reared to pupation. Larvae reared on tobacco were maintained on leaf tissue throughout their development. Host tissue was replaced as needed and was collected from the field (cotton, soybean, and peanut) or from plants established for that purpose in the greenhouse at NC State University (tobacco). Pupae were removed from feeding cups and placed in 30ml diet cups; upon eclosion, moths were frozen and stored at -10C pending analysis.

Feral Moth Collection. Wire cone pheromone traps (modified after Harstack et al. 1979) were placed on the borders of commercial cotton and tobacco fields in 2004 in the central coastal plain of North Carolina. The counties selected for trapping were composed of large plantings of soybean and Bt cotton varieties with a lesser area planted to non-Bt cotton, tobacco, and peanut (Fig. 1). A total of 15 traps were located in Wilson, Pitt, and Edgecombe Counties, and an additional 10 traps were placed in Lenoir County. The traps were baited with Luretape® tobacco budworm lure, Z-11-hexadecenal and Z-9-tetradecenal (Hercon® Environmental, Emigsville, PA), and lures were replaced at 14-day intervals. Moths were removed from the traps weekly beginning in April and continuing through September. All moths were transported to the laboratory at NC State University where they were counted and stored in a freezer at –10°C pending shipment to a laboratory at Monsanto Corporation for gossypol analysis. Three traps per county were selected from Lenoir, Wilson, and Pitt Counties, and a random subsample of 10 moths for each of four collection dates was made from each trap for analysis of gossypol content.

Results

Technique Validation. In validation studies, 100% of moths reared in the laboratory on cotton (n=118) tested positive for the presence of gossypol. For moths reared on non-cotton hosts, 81% of those analyzed were found to be negative for gossypol. The technique yielded no false negatives and only a limited number of false positives for gossypol content.

Feral Moth Analysis. Low numbers of tobacco budworm moths were common in pheromone traps until late May; the increase in trap catches at this time likely corresponds to the emergence of the second in-season generation of tobacco budworm generally thought to develop on tobacco. Peak pheromone trap catches occurred between 21 July and 25 August (Fig. 2). This is also a time period, due to host plant phenology and the life history of the pest, that some percentage of the total moth population in North Carolina is expected to have been produced on cotton. Chemical analysis of 350 moths collected during the period of peak trap catch revealed only 4% of the individuals contained gossypol. Gossypol was detected in less than 2% of the moths collected prior to 19 August supporting the belief that tobacco budworm production from cotton before July is minimal. The greatest proportion of moths testing positive for gossypol (<8%) was collected during the period 12-19 August (Fig. 3).

Discussion

Although the natal host origin of individual tobacco budworm moths cannot presently be resolved beyond the level of "cotton vs. all others", the data presented here represent the first empirical measure of seasonal *H. virescens* production from cotton. The results support the hypothesis that only a small proportion of tobacco budworm production in North Carolina occurs annually in cotton, and this finding should provide valuable information for updating and refining resistance management strategies for the tobacco budworm in Bt cotton. Studies have provided evidence that a mandatory conventional cotton refuge for the corn earworm is not needed in Louisiana, Texas, and North Carolina due to the abundance of "natural refuges" in these states (Gould et al. 2002, Jackson et al. 2004). Using carbon isotope ratios to distinguish the natal host origin of corn earworm moths collected in the field, it was shown that in these states corn provided a much larger than expected refuge for insect development. The results presented here suggest that a similar scenario may be true for the tobacco budworm in North Carolina, as 96% of the moths tested in our study appear to have developed on non-cotton hosts. Nevertheless, there are important questions that must be addressed before one can say unequivocally that a structured non-Bt cotton refuge is unnecessary in the state.

Tobacco is the preferred host of *H. virescens*, and because of its wide spatial distribution and temporally extended period of suitability as a host, it may serve as an excellent refuge for management of Bt resistance in transgenic cotton in North Carolina. Tobacco is grown in all but 12 of the cotton producing counties in the state, and though most attractive as an oviposition site at flowering, research has shown that tobacco plants are capable of supporting tobacco budworm development over an extended period (Abney et al. 2004). Tobacco budworm larvae are often observed feeding on seedling tobacco soon after transplant, and axilar shoots on mature plants regularly support infestations as late as September. The land area planted in tobacco in North Carolina has decreased by more than 40% in the last 15 years, and the impact of changes in federal farm policy regarding tobacco in 2005 is still unclear. Should tobacco plantings continue to decline in the future, the effectiveness of the natural Bt refuge could be compromised. Work is currently underway to develop a reliable technique for the detection of nicotine or its derivatives in tobacco budworm moths that developed on tobacco. Quantifying the contribution of tobacco to the seasonal production of tobacco budworm populations is an important next step in ensuring that the Bt resistance management strategies mandated for North Carolina cotton producers are both effective and scientifically sound.

Another consideration that must be weighed when evaluating the size of the natural refuge is the use of insecticides targeting *H. virescens* in flue-cured tobacco. The extent to which tobacco budworm is controlled annually could certainly impact the crop's role as a refuge. Not only does the production of adult insects decrease with increases in insecticide use, but the use of Bt insecticides also increases selection pressure which could speed resistance evolution. Foliar formulations of Bt insecticides were estimated to have been used on 25% of the tobacco acres in North Carolina in 2003 (unpublished data). This represents an increase from previous years in which bait and foliar Bt formulations were applied on 13.4% of all flue-cured tobacco grown in North Carolina in 1989, 5% in 1996, and 15% in 1998 (S. J. Toth Jr., personal communication). Remedial action is typically taken against the tobacco budworm only prior to bloom as feeding by the insect after flower buds are removed generally does not result in economic damage. Recent studies have shown that in North Carolina even very high, early season infestations rarely lead to economic loss in flue-cured tobacco indicating that the current threshold (10% of plants infested) is too low (Juba, MS Thesis 2000). Raising the economic threshold could increase the production of tobacco budworm from flue-cured tobacco and concomitantly reduce the exposure of the insect to Bt toxins in foliar applications thereby increasing the crop's usefulness as a natural refuge for management of Bt resistance evolution.

This research proves that plant derived chemical compounds can be detected in the adult tobacco budworm and that these compounds can be used as biological markers to determine natal host plant origin. The results reported here provide evidence that the current non-Bt cotton refuge required by the EPA may not be necessary in North Carolina. The data indicate that there is substantial tobacco budworm production from non-cotton hosts in the state, and that these alternate hosts may provide a sufficient refuge to delay resistance evolution in populations of the insect. The contribution of individual host plant species other than cotton to seasonal production of the tobacco budworm cannot yet be determined, though it is believed that tobacco may play an important role. Continuing research will focus on identifying new marker compounds from other host species of the tobacco budworm with a particular emphasis on tobacco, and on quantifying annual production from these hosts.

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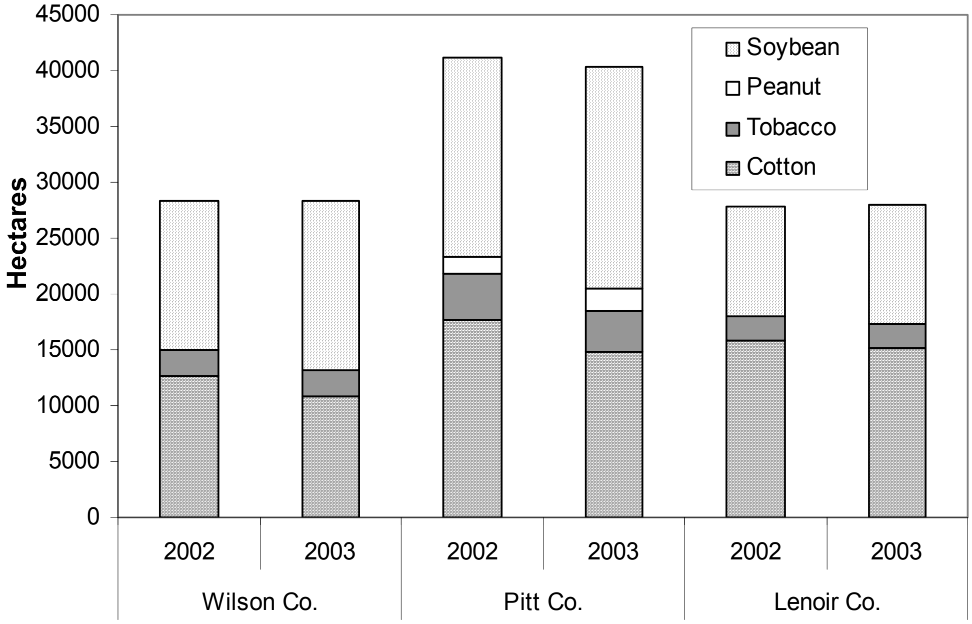

Figure 1. Total production area in 2002 and 2003 of four potential crop hosts of tobacco budworm in counties from which moths were collected for gossypol content analysis in 2004 (2004 production data were unavailable at the time of writing).

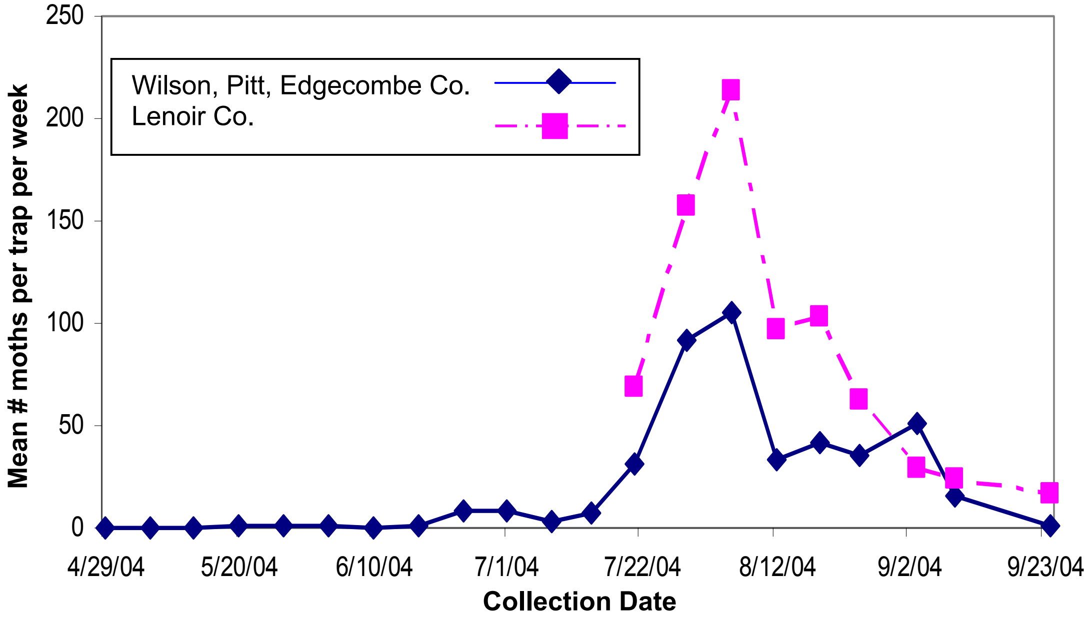

Figure 2. Mean number of tobacco budworm moths collected per trap at seven day intervals in two study regions in North Carolina in 2004.

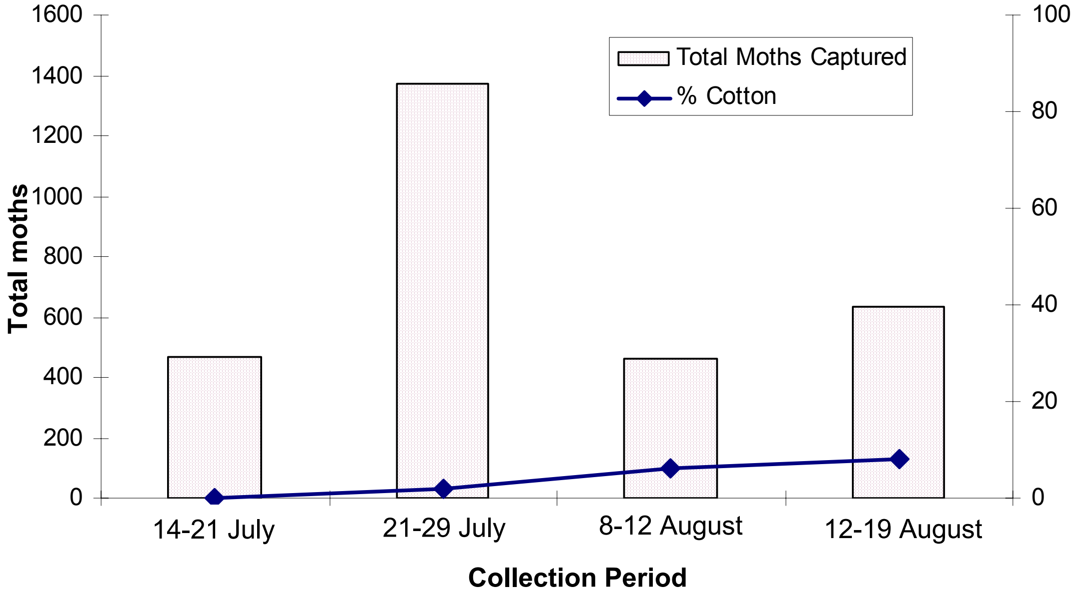

Figure 3. Total number of tobacco budworm moths collected in pheromone traps (n=9, collection interval=1 week) in the central coastal plain of North Carolina and percent of moths tested containing gossypol.

Abney et al.: Natal host origin
of *Heliothis virescens*

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Assessing the Utility of Stable Carbon Isotopes for Determining Natal Host Origins of Tobacco Budworm, *Heliothis virescens*, (Lepidoptera: Noctuidae) in a Host Species Rich Agro-ecosystem

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Abstract

This study was conducted to investigate whether stable carbon isotope ($^{13}C/^{12}C$) ratios in the wings of tobacco budworm, *Heliothis virescens* (F) might serve as a diagnostic tool for elucidating the natal host origin of this economically important pest. Isotope ratio mass spectrometry (IRMS) analysis of $^{13}C/^{12}C$ ratios of moths reared on four crop-plant species and two common weed species revealed a range of $\delta^{13}C$ values within that expected for plants utilizing the C3 photosynthetic pathway. No significant differences in mean $\delta^{13}C$ values were detected between moths reared on any of the host plant species. Analysis of vegetative and reproductive tissues from the plants utilized in the study likewise resulted in $\delta^{13}C$ values that could not be separated statistically on the basis of plant species. Feral tobacco budworm moths collected over three years were found to have carbon isotope ratios consistent with those having fed on C3 plants. The average $\delta^{13}C$ value of feral insects collected within a single year appeared to be closely associated with the total accumulated rainfall for the months May through August. Results demonstrate that within the range of C3 host plants tested, no unique carbon isotope signature exists that would enable a reliable determination of natal origin of feral tobacco budworm with current IRMS technology.

The looming specter of Bt resistant tobacco budworm, *Heliothis virescens* (F), populations is an ongoing concern for cotton producers and IPM practitioners throughout the U.S. Cotton Belt. This potentially serious pest of both cotton and tobacco has a long history of developing resistance to conventional insecticides, and while not yet reported in the field, resistance to Bt toxins has been shown in laboratory selected tobacco budworm strains (Gould et al. 1992). Because of the economic and environmental significance of transgenic Bt cotton, the US EPA mandates the use of specific resistance management strategies in an effort to prolong the life of this important technology. The high dose/refuge strategy currently employed for Bt resistance management of heliothines in cotton is based on a number of assumptions including the level of adult insect production from non-Bt refuge areas. The development of an effective insecticide resistance management strategy requires extensive knowledge of the biology of the insect and the environment in which it exists, and quantifying the seasonal utilization of host plant species by the tobacco budworm is currently an important area of investigation. Sufficient tobacco budworm production in existing weedy or non-selected alternate crop hosts could eliminate the need for the planting of costly non-Bt cotton refuges currently required by EPA's heliothine resistance management strategy.

An oligophagous insect, the tobacco budworm's pattern of host utilization varies with the appearance and phenology of suitable weedy and cultivated plant species within a growing season and from one geographic region to the next. In the Midsouth, weedy plant species serve as hosts for the first generation of *H. virescens*, while subsequent generations are more likely to be associated with cotton, the insect's predominate crop host in the region. Similarly, first generation tobacco budworms in the Southeast develop almost exclusively on weeds; however, a greater variety and abundance of host plants occurring throughout the growing season in this region reduces the importance of cotton for later generations (Abney et al. 2004). Investigations in North Carolina in the 1960's found old field toadflax, Carolina geranium, and tobacco to be significant resources for early season tobacco budworm development (Neunzig 1969). Later tobacco budworm generations in North Carolina can be found on tobacco, cotton, and peanut, and, to a lesser extent, soybean. Additionally, a number of weedy plant species, most notably members of the genus *Rhexia*, may be locally abundant through the summer months and serve as an important source of *H. virescens* production. The spatial and temporal diversity of the tobacco budworm's host suite in North Carolina cotton production regions may reduce the impact and therefore the necessity of non-Bt cotton refuges in the state.

Isotopes are atoms of the same element with an equal number of protons and electrons but varying numbers of neutrons. Naturally occurring stable isotopes can be used as biological markers as they often exist in predictable ranges in particular tissue types. Two stable isotopes of carbon exist in the environment. The overwhelming majority of carbon (98.98%) exists as ^{12}C while only a small proportion (1.11%) exists as ^{13}C. Variation in the ratio of ^{13}C to ^{12}C can be measured via isotope ratio mass spectrometry (IRMS) and is reported as "δC" or parts per thousand deviation from a recognized standard (Dawson and Brooks 2001, Pate 2001). For example, plants utilizing the C3 and C4 photosynthetic pathways have distinct non-overlapping ranges of stable carbon isotope ratios. Because isotopes present in a plant are acquired by an herbivore when it feeds upon the plant's tissues, the carbon isotope composition of the herbivore can be used to link it to its host plant. IRMS is used to measure the relative abundance of specific isotopes in a sample of given material, and this technology has recently been utilized to broadly identify the natal host origin (C3 vs. C4 host plants) of field collected corn earworm, *Helicoverpa zea*, adults (Gould et al. 2002). While differences in the stable carbon isotope ratios of C3 vs. C4 plants have been well established, it is not known whether plant species within a single photosynthetic pathway can be distinguished on the basis of carbon isotope composition. This research project was designed to determine the utility of isotope ratio mass spectrometry for identifying the natal host origins of field collected tobacco budworm moths.

Materials and Methods

Insects. *Heliothis virescens* used in all experiments reported here were obtained from a laboratory colony (strain YDK) maintained at NC State University.

Controlled Rearing Study: Greenhouse Experiment. A preliminary greenhouse study was conducted to determine if differences in carbon isotope ratios between tobacco budworm adults reared on cotton and those reared on tobacco were sufficient to warrant further investigation. Cotton variety DP 50 and flue-cured tobacco variety K-326 were planted into commercial media (Metromix 200®) in 6 inch diameter sterilized clay pots in a greenhouse at NC State University on 14 February 2001. Plants were hand watered daily until introduction of tobacco budworm larvae. Three neonate tobacco budworm larvae were placed on the upper 1/3 of each of 10 cotton and tobacco plants on 17 May. Immediately following infestation, plants were transferred to water filled moats constructed on greenhouse benches. Moats provided continuous water thereby reducing the chance of disturbing larvae with overhead watering and also prevented larval movement from plant to plant on a single bench. The larvae were allowed to feed uninterrupted until just prior to pupation at which time final instar larvae were removed from plants and transported to the lab to complete development on excised tissue from the respective host plant. A total of 7 and 11 moths were reared from cotton and tobacco, respectively. Adults were frozen shortly after eclosion and stored at -5°C until preparation for analysis of carbon isotope composition. All tissue samples were analyzed at the Stable Isotope Mass Spectrometry Facility in the Department of Soil Science located at NC State University. Insect samples were prepared by removing the right forewing of each tobacco budworm to be analyzed and placing it in a tin combustion capsule. The samples were analyzed using a CE Elantech NA 2500 elemental analyzer coupled to a Therma Finnigan DELTA plus mass spectrometer via a Conflo II open split interface.

Host Plant Tissue Collection. Tests were conducted to determine whether several common plant hosts of the tobacco budworm in North Carolina could be distinguished on the basis of their carbon isotope composition. Cotton (*Gossypium hirsutum*), soybean, (*Glycine max*), peanut (*Arachis hypogea*), tobacco (*Nicotiana tobaccum*), old field toadflax (*Linaria canadensis*), and Carolina geranium (*Geranium carolinianum*) were collected at various developmental stages and multiple locations in North Carolina. Cotton was obtained from Wilson and Johnston Counties on 21 July and from Martin County on 29 July and 20 August. Soybean and peanut samples were acquired from Johnston County, NC on 2 and 17 September and 2 and 23 September, respectively. Tobacco was collected on 24 June and 29 July from Martin County and on 1 and 29 July from Johnston County. Old field toadflax was collected from sites in

Edgecombe and Lenoir Counties on 30 April and from Johnston County on 1 May. Carolina geranium was obtained from Edgecombe and Lenoir Counties on 30 April. On each sample date at each location, tissue was collected from 10 individual plants of a single species and combined in a resealable plastic storage bag. Tissue samples were transported to NC State University and stored at -10° C until processed for analysis. Plant samples were dried in a laboratory oven at 43°C for 8 days and then ground to a fine powder. Approximately 1.4 mg of homogenized ground plant tissue were placed in a tin combustion capsule for each plant sample examined. Plant tissue was analyzed using the same technique described earlier for moth wings.

Controlled Rearing Study: Field Experiment. Within a single plant species, carbon isotope composition may be influenced by a number of factors including photosynthetic rate, moisture availability, and the plant structure tested. For isotope analysis to provide useful insight into natal host origin, the ratio of stable carbon isotopes conferred to tobacco budworms feeding on a single host plant species must remain consistent over a wide geographic region within a referenced period of time. A field study was initiated to test the hypothesis that tobacco budworms reared on a particular host species would have similar stable carbon isotope composition regardless of location of origin or plant structure fed upon. Tobacco budworm larvae were reared on four species of crop hosts and two weed host species in 2003.

Tobacco budworm larvae were reared in the lab on field collected old field toad flax and Carolina geranium from Johnston and Wake Co, NC respectively. Plants were cut at ground level, placed in distilled water, and transported to the lab where they were rinsed in tap water, allowed to air dry, and then placed singly into #50 water pics

(Aquapic®, Syndicates Sales Inc., Kokomo, IN) containing distilled water. Neonate tobacco budworm larvae were placed individually on plants using a size 0 camel hair paint brush. The plants were secured via water pics in a polystyrene foam base and placed in a 20x20x40cm Plexiglas® box with two 400cm^2 screen covered openings for ventilation. Larvae were transferred to fresh plant material as needed, and distilled water was added to water pics as necessary. Prepupae were collected from the plants and transferred to 60 x 15mm plastic petri dishes for pupation. Following adult eclosion, wings from individual insects were prepared as previously described for greenhouse reared insects and subjected to IRMS analysis (n=8 per host species).

Tobacco, cotton, soybean, and peanuts were planted in small test plots at the Central Crops Research Station in Clayton, NC, and cotton and tobacco were planted in small plots on a private research farm in Martin County, NC. Sleeve-type field cages were constructed from polypropylene floating row cover (Gardens Alive®, Lawrenceburg, IN) and placed over individual plants of each host species; neonate larvae (strain YDK) were placed directly on plant tissue within the cages using a size 0 camel hair paint brush. Tobacco budworms were reared on tobacco in early and late July and on cotton in mid July and mid August; the insects were reared on soybean and peanut in late August. Cages were sealed at the bottom by securing the polypropylene material around a stalk or branch with plastic coated twist wire. The tops of cages were folded over twice and held fast with two #3 gem clips. Cages were monitored twice each week, and larvae were transported to the lab just prior to pupation. Pupae were held in individually labeled 60 x 15mm plastic petri dishes until adult eclosion at which time moths were frozen and then prepared for isotope analysis as previously described.

Feral Insect Collection. Tobacco budworm adults were evaluated to determine whether the ranges of carbon isotope ratios found in feral populations were consistent with the host plants tested and/or moths from controlled rearing studies. Male tobacco budworm moths were collected in Harstack-type pheromone traps in North Carolina each summer from 2001 to 2003 in the central coastal plain counties of Greene, Pitt, Edgecombe, and Wilson. Moths were removed from the traps twice weekly and transported to NC State University where they were stored at 0°C. A random subsample of ten moths per year for each year of the study was collected and prepared for analysis of stable carbon isotope composition.

Climate Data. Cumulative rainfall data for the three years of the study were obtained from the State Climate Office of North Carolina's NC climate retrieval and observations network of the southeast (NC CRONOS) database (Anonymous 2004).

Results

Controlled Rearing Study: Greenhouse Experiment. The $\delta^{13}C$ values of tobacco budworm moths reared on tobacco in the greenhouse differed from and did not overlap the $\delta^{13}C$ values of moths reared on cotton. The $\delta^{13}C$ value of moths reared on tobacco (mean $\delta^{13}C = -29.89‰$, SE = 0.18) was significantly lower than value for moths reared on cotton (mean $\delta^{13}C = -26.21‰$, SE = 0.14) at $\alpha = 0.05$.

Host Plant Tissue Analysis. The $\delta^{13}C$ values of the six host plant species tested were consistent with those expected for plants utilizing the C3 photosynthetic pathway; however, there was considerable overlap in the range of values obtained for each species (Table 1). The $\delta^{13}C$ values of a single tissue type from the same plant species varied by collection date for peanut, by location for Carolina geranium, and by both collection date and location for cotton and tobacco. Variation was seen in the $\delta^{13}C$ values recorded for different tissue types (vegetative vs. reproductive tissue) of the same species for cotton, tobacco, and soybean; tissue types were not evaluated separately for peanut, Carolina geranium, or old field toadflax.

Controlled Rearing Study: Field Experiment. IRMS analysis of wings from laboratory and field reared tobacco budworm revealed $\delta^{13}C$ values that were within the range expected for insects feeding on C3 plants. The data collected from insects reared in the field on cotton and tobacco did not corroborate the findings of earlier greenhouse experiments. While insects reared in the field on cotton had a $\delta^{13}C$ value (mean $\delta^{13}C$ = -26.55, SE = 0.17) similar to those reared in the greenhouse, *H. virescens* reared on tobacco in the field were consistently less depleted in ^{13}C (mean $\delta^{13}C$ = -26.16‰, SE = 0.13) than those reared on tobacco in the greenhouse. Additionally, the range of mean $\delta^{13}C$ values obtained from moths reared on tobacco and cotton at different locations and dates were shown to overlap (-26.81‰ to -25.36‰ and -27.70‰ to -25.64‰ respectively) (Table 2). The $\delta^{13}C$ values of tobacco budworms reared on Carolina geranium and old field toadflax were also very similar (mean $\delta^{13}C$ = -28.59‰, SE = 0.13 and -28.07‰, SE = 0.13 respectively). Peanut and soybean reared insects (mean $\delta^{13}C$ = -28.00‰, SE = 0.37 and -26.47‰, SE = 0.41, respectively) likewise could not be separated from those reared on other hosts based on these data.

Feral Insect Analysis. The $\delta^{13}C$ values observed for feral *H. virescens* adults (Table 2) were similar to those seen in the controlled rearing experiment conducted in the field and were consistent with larval development on C3 hosts. Significant variation in carbon isotope composition was observed between years. Differences in $\delta^{13}C$ values from year to year appear to be correlated with rainfall amounts during the growing season. Tobacco budworm moths collected during 2003, the year with greatest in-season precipitation, were the most depleted in ^{13}C (mean $\delta^{13}C$ = -26.80‰, SE = 0.34). Moths collected during the droughty summer of 2002 contained the greatest proportion of ^{13}C (mean $\delta^{13}C$ = -24.95‰, SE = 0.60), while tobacco budworms collected in 2001 were found to have intermediate levels of ^{13}C (mean $\delta^{13}C$ = -25.54‰, SE = 0.52).

Discussion

Though preliminary greenhouse studies indicated IRMS might provide a useful tool for elucidating the natal host origin of tobacco budworm, further investigation revealed that environmental variation is apparently too great to enable separation of C3 host plants on the basis of stable carbon isotope ratios. Analysis of plant tissue collected in the field as well as analysis of tobacco budworm moths reared on those plants resulted in a wide range of $\delta^{13}C$ values for both plants and insects that could not be separated on the basis of host plant species. Variation observed in the amount of ^{13}C present in a specific tissue type among plants of a single species and the resulting variation in the herbivore is likely due in part to differences in moisture availability as the plants grow. The stomata of a plant growing in conditions of adequate moisture are expected to remain open more than those of a plant growing under moisture stress. This in turn leads to a greater depletion of ^{13}C in plants with ample moisture compared to those under stress. By nature, plant species that have higher water use efficiencies may be expected to have different ratios of stable carbon isotopes than their less efficient relatives. This phenomenon could provide a mechanism for separating plant species utilizing the C3 photosynthetic pathway on the basis of their carbon isotope composition if differences in water use efficiencies between species are sufficiently great. However, in these studies we were unable to differentiate between plants species using IRMS as the plant to plant variation within a specific species at different locations and times proved to be quite large. The very narrow, non overlapping range of $\delta^{13}C$ values seen in insects reared in the greenhouse on both cotton and tobacco may be explained by the continuous excess water provided to the host plants.

The analysis of feral male tobacco budworm moths collected in pheromone traps over three years provided further evidence that stable carbon isotope composition is dependant upon the moisture available to the host plant. The mean $\delta^{13}C$ value of moths varied by year, and the level of depletion of ^{13}C was directly related to the amount of rainfall recorded during the growing season in each year. Though variation was observed between years, the range of $\delta^{13}C$ values of moths collected in all years was consistent with feeding on hosts utilizing the C3 photosynthetic pathway. The $\delta^{13}C$ value of several individual moths collected in 2001 and 2002 was higher than any of the $\delta^{13}C$ values observed for plant tissue or insects from the controlled rearing experiments in 2003. This result could indicate utilization of a host plant(s) by tobacco budworm populations in these years that was/were not included in our studies. Given that there were no insect samples tested in 2003 with similarly elevated ^{13}C levels, it is more likely that results in 2001 and 2002 are due to variation in carbon isotope composition caused by differences in moisture availability.

We can conclude from our data, that within the range of C3 host plants tested, no unique carbon isotope signature exists that would enable a reliable determination of the natal origin of feral tobacco budworm with current IRMS technology. The data do provide empirical evidence that tobacco budworm populations are not developing on plants species that utilize the C4 photosynthetic pathway. Future studies should investigate other techniques for determining host origin including the search for secondary plant metabolites unique to a specific host species that might serve as biological markers within the insect.

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Table 1. Mean (SE) and Range of δ 13C values for four crop and two weed hosts of tobacco budworm collected at multiple locations and dates in 2003.

Plant Tested	**Date of Collection**	**Collection Location**	**Structures Tested**	**Mean δ 13C(SE)**	**δ 13C range (‰)**
Tobacco	6/24/2003	Martin Co., NC	Leaves	-26.92 (0.15)	-27.4 to -26.5
	7/1/2003	Johnston Co., NC	Leaves		
	7/21/2003	Johnston Co., NC	Flowers		
	7/29/2003	Martin Co., NC	Leaves		
	7/29/2003	Martin Co., NC	Flowers		
Cotton	7/21/2003	Wilson Co., NC	Leaves	-28.35 (0.45)	-29.1 to -27.1
	7/21/2003	Johnston Co., NC	Leaves		
	7/21/2003	Johnston Co., NC	Large Squares		
	7/29/2003	Martin Co., NC	Leaves		
Toadflax	4/30/2003	Edgecombe Co., NC	Whole plant	-29.30 (0.06)	-29.5 to -29.1
	4/30/2003	Lenoir Co., NC	Whole plant		
	5/1/2003	Johnston Co., NC	Whole plant		
C. Geranium	4/30/2003	Edgecombe Co., NC	Whole plant	-28.65 (0.45)	-29.1 to -28.2
	4/30/2003	Lenoir Co., NC	Whole plant		
Peanut	9/2/2003	Johnston Co., NC	Leaves	-27.35 (0.35)	-27.7 to -26.9
	9/17/2003	Johnston Co., NC	Leaves		
Soybean	9/2/2003	Johnston Co., NC	Leaves/Stems	-27.20 (0.90)	-28.1 to -26.3
	9/23/2003	Johnston Co., NC	Pods		

Table 2. Mean (SE) and range of δ 13C values for tobacco budworm moths reared on four crop hosts and two weedy hosts at different locations and dates in 2003.

Host Plant	**Location**	**Date**	**Mean δ 13C(SE)**	**δ 13C range (‰)**
Cotton	CCRS	29 July	-26.30 (0.32)	-27.71 to -25.17
Cotton	CCRS	20 August	-25.64 (0.07)	-26.01 to -25.22
Cotton	Wayne Co.	29 July	-27.70 (0.23)	-28.88 to -26.56
Cotton	Wayne Co.	20 August	-26.47 (0.24)	-27.61 to -25.16
Tobacco	CCRS	1 July	-26.70 (0.09)	-27.04 to -26.10
Tobacco	CCRS	25 July	-25.37 (0.15)	-26.01 to -24.85
Tobacco	Wayne Co.	1 July	-26.81 (0.12)	-27.46 to -26.15
Tobacco	Wayne Co.	29 July	-25.75 (0.32)	-27.37 to -24.59
Soybean	CCRS	20 August	-26.95 (0.41)	-28.09 to -26.14
Soybean	CCRS	27 August	-25.51 (0.39)	-25.89 to -25.12
Peanut	CCRS	27 August	-28.12 (0.30)	-28. 96 to -26.16
Carolina Geranium	Wake Co.	17 April	-28.59 (0.13)	-29. 26 to -28.19
Old Field Toadflax	CCRS	22 April	-28.07 (0.13)	-28.65 to -27.60

Abney et al.: Pyrethroid Susceptibility
In *H. virescens*

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Dose-Mortality Response of North Carolina Strains of Tobacco Budworm (Lepidoptera: Noctuidae) to Cypermethrin

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Abstract

Dose-mortality studies were conducted to determine the susceptibility of two strains of tobacco budworm, *Heliothis virescens*, collected in North Carolina in 2004 to the pyrethroid insecticide cypermethrin. LD_{50} values were 4 and 9 times greater for tobacco budworms collected in June and August respectively than for a susceptible laboratory strain. Results in these investigations are similar to those observed in states where control failures due to pyrethroid resistance have been reported. Implications for future resistance management are discussed.

Since its introduction in the late 1970's, the pyrethroid class of insecticides has generally provided an efficacious and cost effective tool for the management of numerous insect pests in a wide range of cropping systems. Cotton producers in the 1980's came to rely on pyrethroids as a main line of defense against the cotton bollworm, *Helicoverpa zea*, and tobacco budworm, *Heliothis virescens*, two of the most serious pests of that crop. The development of resistance by the tobacco budworm to pyrethroids in the mid-to-late 1980's had a major impact on cotton insect pest management in many parts of the United States. Prior to the commercial release of transgenic Bt cotton varieties, growers in much of the U.S. Cotton Belt faced the daunting challenge of managing pyrethroid resistant tobacco budworm populations with a dwindling arsenal of effective materials, and pyrethroid resistance management became one of the most important issues facing entomologists in cotton.

As resistance to pyrethroids by the tobacco budworm spread across the Cotton Belt in the 1980's and early 90's, no published reports existed of resistance or decreased efficacy in North Carolina (Sparks et al. 1993). At the turn of the twenty-first century, pyrethroids still played a significant role in bollworm management in NC cotton. The importance of this class of chemistry continues today with the need to overspray Bt cotton varieties for control of *H. zea* and secondary bug pests and the recent labeling of pyrethroids for use in tobacco. Even before their commercial release, resistance to pyrethroids (linked to cross resistance to methyl parathion) was seen in California populations of tobacco budworm (Twine and Reynolds 1980), and elevated LD_{50}'s were detected in the Mid-south before control failures were observed in the field (Luttrel et al. 1987). There have been sporadic reports of control failures associated with the use of pyrethroids in NC cotton since their introduction (J.R. Bradley, personal communication); nevertheless, because the insect is a minor pest in cotton in the state, there have been no empirical studies to test the susceptibility of tobacco budworm populations.

North Carolina's agroecosystem as it pertains to tobacco budworm habitat has generally been characterized by a diversity of both cultivated and wild host species, and this diversity may have contributed to the delayed resistance evolution in the pest (Abney et al. 2004). However, the structure of the state's agroecosystem has changed considerably in recent decades. Cotton production area has increased dramatically since the early 1980's; however, the recent introduction and widespread adoption by growers of transgenic Bt cotton varieties have turned much of North Carolina's cotton into an effective trap crop for tobacco budworm. Changes in federal farm policies have and will continue to result in adjustments to peanut and tobacco plantings, and in 2004 the US Environmental Protection Agency (EPA) granted registration for the use of pyrethroids in flue-cured tobacco. The implications of these changes for tobacco budworm population dynamics, pyrethroid susceptibility, and insecticide resistance management in general are unknown.

The efficacy of Bt toxins against the tobacco budworm has in large measure alleviated much of the concern and taken a great deal of focus away from pyrethroid resistance management; nevertheless, this class of chemistry may continue to fill an important pest management niche in North Carolina. Monitoring the level of susceptibility of a target organism is a key component of an effective resistance management strategy. In the case of tobacco budworm in North Carolina, there are no historical data regarding pyrethroid susceptibility nor is there currently a system for monitoring pyrethroid resistance. This study was conducted to determine the pyrethroid susceptibility of two tobacco budworm strains established from tobacco fields in North Carolina in 2004.

Materials and Methods

Insects. Two laboratory strains of tobacco budworm were established from insects collected in North Carolina in 2004. Eggs were obtained from commercial tobacco fields in Wilson and Pitt Counties on 16 June 2004 (strain GS04) and from Edgecombe County on 6 August 2004 (strain EC04). On each collection date, blooms and leaves of tobacco plants containing eggs were removed from the field and transported to the lab; eggs were removed by soaking the tobacco tissue in a 0.05% sodium hypochlorite solution for 10-15 minutes. Eggs were held in a rearing chamber at 28 C and 70% relative humidity until eclosion at which time neonate larvae were transferred to 30 ml plastic diet cups (3 larvae per cup) containing approximately 10 ml of artificial diet (n=200 for GS04 and n= 300 for EC04). Larvae were reared at 28C and 70% relative humidity and a photoperiod of L:D 14:10. Fourth instar larvae from field collected eggs were examined to verify species; all species other than *H. virescens* were discarded (>99% of developing larvae from both collection dates were tobacco budworm). Pupae were removed from diet cups and placed in 2 liter plastic ice cream buckets for adult eclosion, mating, and oviposition; adults were fed a 10% sucrose solution. A pyrethroid susceptible reference strain (NRA_6) was obtained from North Carolina State University where it has been maintained for over 250 generations without exposure to insecticides.

Bioassay Procedure. Pyrethroid dose-mortality studies were conducted according to guidelines established by the ESA's standard test method for determining resistance to insecticides in *Heliothis* (Anonymous 1970). Neonate larvae from each strain were placed individually into 30ml plastic diet cups containing approximately 15ml of artificial diet, and held in a growth chamber at 28C, 70-75% relative humidity and a photoperiod of L:D 14:10. Assays were initiated when larvae reached third instar (approximately 9 days after being placed on diet) and mean larval weight was 30mg.

A single trial was conducted for each strain evaluated with six doses per trial and a minimum of 12 insects per dose. Tests were conducted using the F2 generation of strain EC04 (n= 216) and the F3 generation of the GS04 strain (n=360). Cypermethrin (98% pure) was obtained from Chem Service Inc. (Westchester, PA) and was dissolved in acetone to create a new stock solution for each replicate of each trial. Without removing them from the diet, insects were treated on the dorsal thorax with 1µl aliquots of acetone alone (control) or one of five concentrations of cypermethrin dissolved in acetone. After treatment, larvae were returned to the growth chamber and mortality was assessed at 24, 48 and 72 hrs.

Larvae were considered dead if they failed to move any part of their bodies within 10 seconds of being prodded with a blunt probe. Results were subjected to probit analysis using SAS version 8.0 software. Differences in pyrethroid susceptibility among strains were considered significant if the 95% confidence intervals for the LD_{50}'s did not overlap.

Results and Discussion

While control failures have not occurred with great frequency in North Carolina, researchers in other states identified reduced pyrethroid susceptibility in *H. virescens* before control failures were reported in the field (Davis et al. 1977, Crowder et al. 1979, Brown et al. 1982). The LD_{50} estimates obtained from dose-mortality regressions in this study indicate that tobacco budworm populations in North Carolina may be less susceptible to pyrethroids than previously thought. The LD_{50} for cypermethrin of strain GS04 was 4 fold greater than the susceptible laboratory strain, and strain EC04 was found to have a 9 fold increase in LD_{50} compared to the susceptible strain (Table 1). Additionally, tobacco budworm resistance to pyrethroids has been shown to decrease after the first laboratory generation (Martinez-Carillo and Reynolds 1983, Staetz 1985); thus our results, based on bioassays of the F2 and F3 generations, may overestimate the actual susceptibility of the insects in the field. Luttrel et al. (1987) reported LD_{50} values for tobacco budworm strains established from fields in Mississippi where control failures were observed that were lower than those seen in North Carolina in 2004. Though reported here for the first time for North Carolina, the observed increase in the LD_{50} of tobacco budworms collected later in the season is similar to the phenomenon reported in other regions of the cotton belt where resistance to pyrethroids has been identified, and susceptibility levels decrease as the season progresses (Campanhola and Plapp 1989, Plapp et al. 1990, Cook et al. 2002).

Because historical data are not available for North Carolina, it is unclear whether the high LD_{50} values obtained in this study are indicative of an area wide increase in resistance level, a spatially and/or temporally restricted decrease in susceptibility, or simply a consistently high tolerance to pyrethroids. If these results represent an overall increase in resistance in the population, the source of the resistance is unclear. It is currently believed that only a small portion of the total tobacco budworm population develops annually on cotton in North Carolina, and the selection pressure of pyrethroids in tobacco prior to 2004 was probably low given that any application in that crop would have been illegal. Research has shown that *H. virescens* is capable of sustained flights of at least 300 km per night when conditions are favorable (Westbrook et al. 1990). Migration has been implicated in the rapid spread of resistance observed in the Midsouth in the 1980's (Plapp et al. 1990); however, no data exist that would document annual migration patterns of the tobacco budworm into North Carolina. Cross resistance between pyrethroids and the organophosphate insecticide methyl parathion has been demonstrated (Crowder et al. 1979, Twine and Reynolds 1980). While methyl parathion has not been used on tobacco in over ten years, the possibility that a similar mechanism of cross resistance is responsible for the elevated LD_{50}'s observed in this study cannot be dismissed.

Regardless of the mechanism(s) responsible for the low susceptibility to pyrethroids observed in the North Carolina tobacco budworm strains we tested, the presence of resistance or tolerance may foreshadow future control failures if this class of insecticides is not used wisely. The widespread use of pyrethroids in tobacco (*H. virescens*' preferred host) could amplify the selection pressure placed on tobacco budworm populations considerably. While it is believed that *H. virescens* has historically been exposed to pyrethroids in North Carolina only in a single generation annually, the use of the insecticide in tobacco could increase the number of generations selected to two or three per season. Current labeling of pyrethroids in flue-cured tobacco prohibits applications within a 40 day pre-harvest interval, and this stipulation should limit exposure primarily to second generation tobacco budworms. Nevertheless, caution should be taken to ensure that selection of multiple generations within a single growing season, a key driver of resistance evolution, is minimized. The identification of heliothine larvae in non Bt cotton, and the use of alternative chemistries when tobacco budworm infestations occur would limit the selection pressure placed on the population and help guard against costly control failures.

Our findings show that susceptibility to pyrethroids in the tobacco budworm strains tested from North Carolina is similar to that observed in pyrethroid resistant populations in the Midsouth. The uncommon occurrence of control failures in North Carolina cotton may reflect a generally low annual frequency of tobacco budworm infestation in that crop rather than an appreciable level of susceptibility.

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Table 1. Dose-mortality response of two strains of *H. virescens* larvae[a] collected in North Carolina in 2004 and a susceptible laboratory strain to cypermethrin in topical bioassays.

Strain	*n*[b]	Slope ± SEM	LD_{50} (95% CL)[C]	LD_{90} (95% CL)[C]
GS04	360	1.274 ± 0.186	0.433 (0.220-0.619)	2.157 (1.770-2.826)
EC04	216	1.445 ± 0.283	0.897 (0.684-1.231)	2.417 (1.869-3.613)
NRA_6	294	30.900 ± 3.713	0.097 (0.084-0.113)	0.168 (0.145-0.203)

[a] Third instar (30 mg)

[b] Number of larvae tested

[C] Dosages are expressed as micrograms of insecticide per larva.

Abney et al.: Pyrethroid efficacy against
Heliothis virescens in flue-cured tobacco

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Efficacy of Pyrethroid Insecticides for Control of the Tobacco Budworm, *Heliothis virescens*, (Lepidoptera: Noctuidae) in North Carolina Flue-Cured Tobacco

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Abstract

Foliar applications of three pyrethroid insecticides were made to flue-cured tobacco and compared with *Bacillus thuringiensis* (Bt) bait and sprays of acephate and spinosad for control of the tobacco budworm, *Heliothis virescens* (F.) in 2001, 2002, and 2003. Lambda cyhalothrin, cyfluthrin, and bifenthrin provided significant control of tobacco budworm when compared to untreated checks in all three years of the study; however, they were generally less efficacious than the other insecticides tested. The level of control among the pyrethroids differed significantly within years but was inconsistent from year to year. The severity of tobacco budworm feeding damage was recorded for individual plants in each treatment, and damage averaged over pyrethroid treatments was 54.17% lower than the untreated control in 2001 and 79.84% lower in 2003. Pyrethroid treatments had little impact on yield of cured leaf in 2001 or 2003.

Since the late 1970's, pyrethroid insecticides have been widely used in many cropping systems to control a broad spectrum of insect pests. Despite its use on other crops, this class of insecticide was not registered for use in flue-cured tobacco until 2003. Early evaluations of pyrethroid efficacy against the tobacco budworm, *Heliothis virescens* (F.), in flue-cured tobacco revealed that control was acceptable though generally not as good as that obtained with alternative insecticides (Tappan et al. 1982). Concerns about issues other than efficacy against heliothines were also associated with pyrethroids, making their use in tobacco problematic. First, populations of a potentially serious economic pest of tobacco, the tobacco aphid, *Myzus persicae* (Sulzer), increased following applications of pyrethroids in some field trials (Mistric and Clark 1979). Second, the potential for pyrethroid residues to persist on cured leaf at unacceptable levels presented a major threat to the marketability of the crop (Tappan et al. 1982). Finally, pyrethroids have been an important component of heliothine management in cotton since their introduction. The potential for more rapid evolution of resistance in the tobacco budworm and corn earworm, *Helicoverpa zea* (Boddie), to pyrethroid insecticides should they be widely used in tobacco continues to be a serious concern.

Lambda-cyhalothrin, a third generation pyrethroid, was registered for use on flue-cured tobacco by the US Environmental Protection Agency (EPA) in 2003. In anticipation of this registration, we conducted a three year study beginning in 2001 to evaluate the efficacy of lambda-cyhalothrin and two other commonly used pyrethroids, bifenthrin and cyfluthrin, against the tobacco budworm. The data presented here indiciate that the susceptibility of tobacco budworm populations to pyrethroid insecticides may be too low to justify their use in flue-cured tobacco.

Materials and Methods

Foliar spray applications of lambda-cyhalothrin (Karate Z 2EC), bifenthrin (Capture 2EC), and cyfluthrin (Baythroid 2EC) were tested on flue-cured tobacco (variety K 326) at the Central Crops Research Station, Clayton, NC from 2001 to 2003. The efficacy of these materials against the tobacco budworm was evaluated and compared to three insecticides commonly used in tobacco (*Bacillus thuringiensis* (Bt) bait (Dipel 10G), acephate (Orthene 97PE), and spinosad (Tracer 4 SC)).

All experiments were arranged in a randomized complete block design with seven treatments and four replicates. Plots consisted of four rows of 22 plants each in 2001 and 2003 and two rows of 22 plants each in 2002. Test plots were transplanted using a two-row tractor drawn transplanter; rows were 1.14 m on center with a 0.56 m within row plant spacing. Tobacco was transplanted on 27 April 2001, 23 April 2002, and 28 April 2003. All cultural practices with the exception of tobacco budworm management were carried out by Central Crops Research Station personnel according to the North Carolina Cooperative Extension Service's recommendations for flue-cured tobacco production. Tobacco budworm larvae were counted in rows two and three of each four row plot in 2001 and 2003, and plots were examined at least once per week beginning four weeks after transplant. Treatments were applied when the mean larval density in one or more treatments surpassed the published economic threshold of 10% infested plants. All foliar spray applications were made using a CO_2 powered backpack sprayer fitted with a single, solid cone nozzle (Model # D2-33, Spraying Systems Co®, Wheaton, IL) centered over the row delivering 118.3 liters per hectare at 413.7 kPa; Bt bait was hand applied by directly placing the product (0.8 gm/plant) in the terminal bud of each plant. Larval counts were made at 3 and 10 days after treatment (DAT) to assess treatment effects. Tobacco budworm feeding damage was rated on a scale of 0 to 4 (n= 40 plants per plot) at 14 DAT in 2001 and 2003. The damage rating scale is as follows: 0= no damage, 1= holes<1 cm, 2= holes from 1-2.5 cm, 3= holes >2.5 cm on multiple leaves, 4= apical bud destroyed. In 2001 and 2003, plots were harvested individually according to standard cultural practices for flue-cured tobacco. Harvested leaf was cured and weighed to determine yield.

Methods were altered in 2002 because of very low tobacco budworm numbers in test plots early in the season. On 1 July 2002, 64 rows of flue-cured tobacco (variety K 326) were cut back to 15 cm above ground level, and individual plants were allowed to regrow from a single axilar shoot. Plots were two rows wide with 22 plants per row; all evaluations were made on 30 plants per plot (15 plants per row). Following cutback, tobacco plants were fertilized as necessary to maintain vigor, and counts were made weekly to determine the presence of tobacco budworm larvae. Treatments were applied according to the protocols given above. Damage rating and yield data were not collected in 2002 because ratoon crop yields are inherently variable.

All insect count data were square root transformed and subjected to analysis of variance in SAS JMP version 5.1 (SAS Institute Inc. 2002). When significant treatment differences were indicated ($P<0.10$), means were separated using Student's *t* test ($\alpha=0.05$). Untransformed data are reported here.

Results and Discussion

Insecticides had a significant effect on the mean abundance of tobacco budworm larvae 3 days after treatment (DAT) ($F = 2.76$, df = 6, 21, $P = 0.03$) and 10 DAT ($F = 14.07$, df = 6, 21, $P < 0.0001$) in 2001, a highly significant effect 4 DAT ($F = 29.44$, df = 6, 21, $P < 0.0001$) and 11 DAT ($F = 60.61$, df = 6, 21, $P < 0.0001$) in 2002, and 3 DAT ($F = 14.60$, df = 6, 21, $P < 0.0001$) and 10 DAT ($F = 11.20$, df = 6, 21, $P < 0.0001$) in 2003. Each of the pyrethroids evaluated significantly reduced numbers of tobacco budworm larvae by 10 DAT compared to the untreated control in all three years of the study (Tables 1, 2, and 3). While treatment means for the pyrethroids were generally lower on the second post application evaluation date than the first, in two cases (bifenthrin treatment in 2002 and lambda cyhalothrin treatment in 2003) larval densities increased during this time. This result indicates that these materials may not be sufficiently persistent on flue-cured tobacco to prevent even short term reinfestation by tobacco budworm. Acephate and spinosad provided significantly greater control of tobacco budworm than did cyfluthrin and bifenthrin at 10 DAT in 2001 and significantly greater control than cyfluthrin, bifenthrin, and lambda cyhalothrin at both 4 and 11 DAT 2002. Efficacy of acephate and spinosad was again significantly greater than the three pyrethroids 10 DAT in 2003. Additionally, mean larval densities never increased between post application sampling dates in acephate or spinosad treatments. Researchers have noted that controlling tobacco budworm in flue-cured tobacco in hot/dry conditions can be difficult when the insect is protected from insecticides within the tightly closed bud (Lampert and Southern 1987). The high levels of tobacco budworm control obtained in acephate and spinosad treatments in all three years indicate that neither temperature nor drought were likely factors contributing to poor pyrethroid efficacy in our studies. The relative efficacy of pyrethroids compared to alternative insecticides in this study is consistent with findings of early research conducted on the first generation pyrethroids fenvalerate and permethrin in flue-cured tobacco (Tappen et al. 1982).

None of the pyrethroids tested reduced mean tobacco budworm densities below the economic threshold in 2002. Nevertheless, the percent reduction in larval density averaged across all three pyrethroids was 70% in 2002, midway between the 59% and 85% reductions observed in 2001 and 2003 respectively. In areas where pyrethroid resistant tobacco budworm populations occur, susceptibility of the insect to pyrethroids has been shown to decrease with each generation within a season (Campanhola and Plapp 1989, Plapp et al. 1990, Cook et al. 2002). Dose-mortality studies conducted with tobacco budworm collected from commercial tobacco fields in North Carolina yielded similar findings; a 2-fold decrease in pyrethroid susceptibility was observed in insects collected in August versus June (Abney 2005). The efficacy tests in 2001 and 2003 targeted second generation tobacco budworm, while the insect population treated in 2002 would have been made up of mostly third generation larvae. However, because multiple generations were not tested within a single season in this study, it is impossible to draw conclusions concerning potential changes in susceptibility from one generation to the next based on these data.

Tobacco budworm damage ratings were low for all treatments including the controls in 2001 and 2003. Nevertheless pyrethroid treatments did significantly reduce the mean damage rating compared to the control in 2001 (F=3.73, df = 6, 21, P= 0.007) and 2003 (F= 28.54, df = 6, 21, P<0.0001). Reductions in damage ratings did not translate into differences in yield of cured leaf. In 2001, the untreated control produced significantly higher yield of cured leaf than the Bt bait and the cyfluthrin treatments and numerically higher yields than all other treatments. There were no significant differences between treatments for yield in 2003.

The results presented here suggest that while pyrethroids may provide some control of tobacco budworm, there are currently more efficacious materials available for use in flue-cured tobacco. Pyrethroids are often used for controlling corn earworm, *Helicoverpa zea* (Boddie), infestations in North Carolina cotton. However, because of the potential for insecticide resistance evolution, the North Carolina Cooperative Extension Service currently advises against the use of pyrethroids for early season heliothine control in cotton (Bacheler 2003). The use of pyrethroids in tobacco will likely increase the selection pressure on the corn earworm (which also develops on tobacco) and the tobacco budworm thereby elevating the potential for the evolution of resistance in these pests. Current US Environmental Protection Agency (EPA) restrictions preventing the use of pyrethroids within a 40 day pre-harvest interval in flue-cured tobacco will likely prevent unacceptable levels of pyrethroid residues on cured leaf. These restrictions are not likely to appreciably reduce selection pressure on heliothines, as pyrethroid applications will still be made to multiple generations annually. In addition to concerns about insecticide resistance development and the low toxicity to tobacco budworm relative to other insecticides, the inability to detect a relationship between tobacco budworm damage and yield of cured leaf raises questions about the validity of the current economic threshold in the crop. Studies have recently shown that early season heliothine infestations rarely lead to economic loss in flue-cured tobacco in North Carolina (Juba, MS Thesis 2000). Given the potential problems associated with their use, we feel that the application of pyrethroid insecticides in flue-cured tobacco should be approached with caution.

Acknowledgements

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Table 1. Efficacy of three pyrethroid and three standard insecticides for control of feral tobacco budworm larvae in flue-cured tobacco in 2001 at 3 and 10 days after treatment.

Treatment	Rate (kg of AI/ha)	No. Budworm larvae per 40 plants*			Damage rating
		PRE	3 DAT	10 DAT	
Bt Bait	0.80 gm/plant	2.5	0.8 (0.48) cd	0 (0) d	0.21 (0.14) b
Acephate	0.84	3.8	1.3 (0.63) bcd	0 (0) d	0.16 (0.07) b
Cyfluthrin	0.028	4.5	3.75 (1.11) ab	2.8 (0.85) b	0.18 (0.09) b
Lambda cyhalothrin	0.028	4.0	2.3 (0.48) abcd	1.0 (0.58) bcd	0.14 (0.05) b
Bifenthrin	0.045	3.5	3.3 (1.25) abc	1.3 (0.63) bc	0.07 (0.04) b
Spinosad	0.05	4.5	1.75 (0.85) bcd	0 (0.50) cd	0.11 (0.06) b
Check	-	4.0	4.8 (1.11) a	10.0 (1.08) a	0.73 (0.23) a

Means followed by (SEM).
Means within a column followed by the same letter are not significantly different ($\alpha = 0.05$).

Table 2. Efficacy of three pyrethroid and three standard insecticides for control of feral tobacco budworm larvae in flue-cured tobacco in 2002 at 4 and 11 days after treatment.

Treatment	Rate (kg of AI/ha)	No. Budworm larvae per 30 plants*		
		PRE	4 DAT	11 DAT
Bt Bait	0.80 gm/plant	23.5	2.3 (0.75) d	5.8 (1.65) cd
Acephate	0.84	28.3	0.3 (0.25) e	0.3 (0.25) e
Cyfluthrin	0.028	27.8	6.0 (0.71) c	4.0 (0.71) d
Lambda cyhalothrin	0.028	26.8	12.3 (2.72) b	11.3 (1.65) b
Bifenthrin	0.045	22.0	3.0 (0.91) cd	7.5 (0.65) bc
Spinosad	0.05	26.3	0 (0) e	0.3 (0.25) e
Check	-	28.8	25.8 (4.97) a	21.8 (1.03) a

Means followed by (SEM).

Means within a column followed by the same letter are not significantly different (α = 0.05).

Table 3. Efficacy of three pyrethroid and three standard insecticides for control of feral tobacco budworm larvae in flue-cured tobacco in 2003 at 3 and 10 days after treatment.

Treatment	Rate (kg of AI/ha)	No. Budworm larvae per 40 plants*			Damage rating
		PRE	3 DAT	10 DAT	
Bt Bait	0.80 gm/plant	10.0	0 (0) d	1.5 (0.87) b	0.09 (0.04) bc
Acephate	0.84	10.8	1.5 (0.65) bc	1.0 (0.41) b	0.22 (0.04) b
Cyfluthrin	0.028	9.5	1.8 (0.63) b	1.3 (0.63) b	0.23 (0.07) b
Lambda cyhalothrin	0.028	10.5	0.5 (0.50) bcd	2.3 (0.48) b	0.19 (0.03) b
Bifenthrin	0.045	9.8	0.8 (0.25) bcd	0.8 (0.25) bc	0.10 (0.04) bc
Spinosad	0.05	9.5	0.3 (0.25) cd	0 (0) c	0.02 (0.01) c
Check	-	9.0	12.8 (2.93) a	9.5 (2.02) a	0.86 (0.01) a

Means followed by (SEM).
Means within a column followed by the same letter are not significantly different ($\alpha = 0.05$).

APPENDIX

Abney et al.: Management of *Phthorimaea operculella* in flue-cured tobacco

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Effect of Application Timing on Efficacy of Selected Insecticides against *Phthorimaea operculella* (Lepidoptera: Gelechiidae) in North Carolina Flue-Cured Tobacco

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Abstract

A field study was conducted in 2004 to determine the relative efficacy of six foliar insecticides at different application timings against the potato tuberworm, *Phthorimaea operculella* (Zeller), in flue-cured tobacco. While none of the compounds tested resulted in complete control, two early insecticide treatments made at 6 and 10 days after initial infestation generally provided numerically superior control compared to the same treatments made at 10 and 14 days after initial infestation. Lambda cyhalothrin applied at 6 and 10 days after infestation was the only material tested that significantly reduced the number of plants with active mines relative to the control. Though preliminary, these data provide an essential starting point for developing rational and effective recommendations for management of *P. operculella* in North Carolina.

The potato tuberworm, *Phthorimaea operculella* (Zeller), commonly called the tobacco splitworm by tobacco workers, is an occasional but potentially serious pest of flue-cured tobacco in North Carolina. Tobacco splitworm larvae create feeding tunnels or mines within the leaves and stems of tobacco plants and cause damage both by reducing the yield and the quality of cured leaf. Widespread damaging populations of splitworms were documented across the state's flue-cured tobacco production region for the first time in 2002 (Southern 2003, personal communication). There are currently no insecticides labeled for *P. operculella* control in flue-cured tobacco, and the North Carolina Cooperative Extension Service publishes no official recommendations for splitworm management. Cultural practices that promote avoidance of pest infestations have been and continue to be the primary methods for dealing with *P. operculella* (Collins and Hawks 1993). For established populations, rapid harvest and curing of infested leaves is recommended for reducing damage levels (Semtner 2003). Though the incidence of infestations returned to normal levels in 2003, the importance of developing an economic threshold and management plan for tobacco splitworm remains. A great deal of research is necessary to develop and validate such a threshold. An evaluation of insecticide efficacy, however, can be conducted relatively inexpensively over a short period of time and provides growers with much needed information when heavy pest pressure occurs. This study was designed to determine the effect of application timing on the relative efficacy of six foliar insecticides against *Phthorimaea operculella* in North Carolina flue-cured tobacco.

Materials and Methods

Insects. *Phthorimaea operculella* used in this study were obtained from a colony maintained at NC State University. The colony was established using late instar larvae collected from tobacco fields at two locations on 4 August and one location on 12 August 2004 in Halifax County NC and at one location on 6 August 2004 in Edgecombe County NC. Insects from the first laboratory generation were employed in this study; adult voucher specimens of P. operculella have been placed in the NC State University Entomology Museum.

Experimental Design. The study was arranged in a randomized complete block design with 13 treatments and four replications. Two treatment groups were established to evaluate the efficacy of six insecticide compounds currently labeled for use in tobacco at different application timings. Plots in treatment group I (TGI) received foliar applications at 6 and 10 days after initial infestation, while plots in treatment group II (TGII) received foliar applications at 10 and 14 days after initial infestation. The following materials were evaluated: 1. emamectin benzoate (Denim 0.16 EC at 0.59 liters/hectare), 2. Bt (Dipel ES at 2.37 liters/hectare), 3. methomyl (Lannate 2.4 LV at 0.71 liters/hectare), 4. acephate (Orthene 97 PE at 16 oz/0.4 hectares), 5. spinosad (Tracer 4 F at 0.06 liters/hectare), 6. lambda cyhalothrin (Warrior 1 CS at 0.09 liters/hectare). Application rates were determined based on the recommended rates for each material for other lepidopteran pests in flue-cured tobacco.

Treatments were applied to plots in TGI on 2 and 6 September and to plots in TGII on 6 and 10 September. All applications were made using a CO_2 powered backpack sprayer fitted with a single Spraying Systems D2-33 nozzle centered over the row delivering 118.3 liters per hectare at 413.7 kPa.

Cultural Practices. Flue-cured tobacco variety K-326 was transplanted on 21 April 2004 at NC State University's Central Crops Research Station in Clayton, NC using a tractor drawn two-row transplanter. Plots consisted of two rows (1.14 m on center) 13.7 meters long each containing 22 plants (0.56 m within row spacing). Experiment station personnel managed the crop until the early button stage (appearance of primary reproductive bud) according to the North Carolina Cooperative Extension Service's recommendations for flue-cured tobacco production. On 25 June tobacco stalks were cut back to six inches above ground level, and the plants were allowed to grow from a single sucker. Following cutback, tobacco plants were fertilized as necessary to maintain vigor, and three foliar Bt insecticide applications (Dipel ES, Valent Biosciences Corp., Libertyville, IL) were made to control tobacco budworm and tobacco hornworm infestations. Tobacco plants were topped on 31 August, and maleic hydrazide (Sucker Stuff 60 WS, Drexel Chemical Company, Memphis, TN) was applied on 1 September to prevent excess sucker (axilar shoot) growth.

Artificial Infestation and Monitoring. Female *P. operculella* in culture oviposited on paper towels affixed to the openings of the glass jars in which they were held. Paper towels containing eggs were removed from oviposition jars daily, placed in resealable plastic storage bags, and kept in an incubator at 16.5°C to slow development. On 27 August (once approximately 5000 eggs had been accumulated), the paper towels were removed from the incubator and cut into approximately 1.5cm squares containing 5 to 10 eggs each. The squares were affixed egg side up to one end of a 2.5 x 6.5cm index card using rubber cement and then transported from the laboratory to the study site. Index cards were attached to the underside of one leaf in the middle third of seven randomly selected tobacco plants in the first row of each two row plot. Cards were placed at the edges of the leaves with the egg side up and directly in contact with the leaf surface; each card was held in place with a single large paper clip.

Test plots were examined and small splitworm mines were observed in leaves containing egg cards on 31 August; infestation of plants in the study by feral tobacco splitworms was extremely rare. The number of plants with active mines in each plot was determined prior to the initiation of insecticide treatments on 2 September. Counts were subsequently made on 10 and 14 September to determine the efficacy of the materials tested. No attempt was made to determine the actual number of *P. operculella* larvae per plant as time resources would not permit doing so accurately without the use of destructive sampling.

Statistical Analysis. All data were subjected to analysis of variance and means ($P<0.05$) were separated with Student's t test in SAS JMP version 5.1 (SAS Institute Inc. 2002).

Results

The success rate of artificial placement of eggs in the test plots measured by the establishment of at least one actively tunneling splitworm larva per leaf was 56% averaged over all treatments. Evaluation of the number of plants with active tobacco splitworm mines revealed no statistical differences between any of the treatments prior to insecticide application. At 4 days after the second application in TGI, only lambda cyhalothrin had significantly reduced the number of active mines compared to the control (Table 1). There were no statistically significant differences between any of the treatments within TGII, nor were there any differences between the treatments in TGII and the control at 4 days after the first application (Table 1). On 13 September, 8 days following the second application to TGI and 4 days following the second application to TGII, lambda cyhalothrin in TGI continued to be the only compound in either treatment group to significantly reduce the number of plants containing active mines compared to the control. Though not statistically significant, all of the materials tested in both treatment groups reduced the number of plants containing active mines compared to the control by 4 days after the second application. The majority of splitworms remaining in test plots on 13 September were observed to be large, late instar larvae. Counts were terminated at this time (14 days after initial infestation) as a reduction in the mean number of plants with active mines in the control treatment indicated that many larvae had vacated leaf mines presumably to pupate in the soil.

Discussion

The tobacco splitworm has become a late season pest of flue-cured tobacco in North Carolina, and it is often associated with dry weather occurring in July and August (prior to 2002, this pest was more commonly seen in the early season). While effective at reducing the number of active splitworm mines in the current study, the use of lambda cyhalothrin on flue-cured tobacco is restricted to no later than 40 days prior to first harvest. This constraint renders the material useless against all but very early season *P. operculella* infestations. Similarly, a 14-day pre-harvest interval exists for emamectin benzoate thereby limiting its potential utility for splitworm management.

Two insecticide applications at 6 and 10 days after initial infestation were shown to be slightly more effective at controlling splitworms than treatments made and 10 and

14 days after infestation. This result indicates that careful late season observation of tobacco fields is necessary by consultants and farmers in order to detect the very small mines created by early instar larvae. However, the only way suppressive action against early instar *P. operculella* larvae can be justified is if economic thresholds for the pest are established.

The tobacco splitworm presents a number of very complex challenges to those seeking to control it; not the least of these is a lack of knowledge of the pest's life history and potential for economic damage. Tobacco splitworm feeding behavior, characterized by mining within the leaf or stem tissue, provides the insect with a refuge from non-systemic insecticides. Splitworms are generally most abundant on lower leaves in flue-cured tobacco fields further compounding the difficulty of targeting the pest with foliar sprays. Additional research is needed to determine the impact of various application methods, spray volumes and rates, and the developmental stage of the insect on the efficacy of the materials tested.

Despite its potential to cause serious economic losses in flue-cured tobacco very little has been published concerning the biology or management of the tobacco splitworm in North Carolina. Because of the capricious nature of splitworm infestations in tobacco from year to year, obtaining meaningful data from research projects in North Carolina can be difficult. This work demonstrates the feasibility of conducting field studies using tobacco that has been artificially infested with laboratory reared *P. operculella*. Once established in tobacco leaves the feeding behavior of individuals from laboratory culture and the damage they produce cannot be distinguished from that of feral splitworms observed in natural infestations (personal observation). The relatively low success rate

(56%) for establishing larvae on plants in the current study was due largely to an inconsistency in the placement of eggs on individual leaves; eggs must be placed in direct contact with the leaf surface to minimize the likelihood of desiccation and or predation. The authors feel that since this problem has been identified, much higher infestation success rates can be obtained in future studies. The ability to efficiently infest tobacco fields with *P. operculella* opens the door for more far-reaching research projects designed not only to discover efficacious control methods, but more importantly to elucidate important life history characteristics and to establish economic thresholds for the tobacco splitworm in North Carolina.

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Table 1. Effect of insecticide treatment on mean number of active *Phthorimaea operculella* mines on tobacco plants in 2004.

Treatment	Formulation	Application Dates	Mean Number of Plants with Active Mines		
			2 Sept	10 Sept	13 Sept
1. Emamectin benzoate	Denim .16 EC	2, 6 Sept	4.0	1.5 ab	0.5 ab
2. Bt	Dipel ES	2, 6 Sept	3.3	2.0 ab	1.5 ab
3. Methomyl	Lannate 2.4 LV	2, 6 Sept	4.5	2.0 ab	1.3 ab
4. Acephate	Orthene 97 PE	2, 6 Sept	4.3	1.3 ab	0.5 ab
5. Spinosad	Tracer 4F	2, 6 Sept	5.3	3.8 ab	1.8 ab
6. Lambda cyhalothrin	Warrior 1 CS	2, 6 Sept	3.0	0.5 b	0.0 b
7. Emamectin benzoate	Denim .16 EC	6, 10 Sept	4.3	3.3 a	2.0 ab
8. Bt	Dipel ES	6, 10 Sept	3.8	3.5 a	2.0 ab
9. Methomyl	Lannate 2.4 LV	6, 10 Sept	4.0	3.8 a	1.3 ab
10. Acephate	Orthene 97 PE	6, 10 Sept	3.8	3.8 a	1.8 ab
11. Spinosad	Tracer 4F	6, 10 Sept	2.8	3.8 a	0.8 ab
12. Lambda cyhalothrin	Warrior 1 CS	6, 10 Sept	4.0	3.3 a	1.0 ab
13. Control			4.0	4.0 a	2.8 a

Means within a row followed by the same letter are not significantly different (α=0.05)