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ALTERNATE CROP HOSTS AS RESISTANCE MANAGEMENT REFUGES FOR TOBACCO BUDWORM IN NC

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Abstract

A three-year evaluation of crop host utilization by the tobacco budworm (TBW), *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, but showed that TBW may be produced in tobacco throughout the growing season. Small plot trials conducted in 2002 and 2003 demonstrated a strong oviposition preference of TBW 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 TBW production demonstrate sufficient short-range movement by adult budworms to facilitate mating of individuals produced in distant 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.

Introduction

The tobacco budworm, *Heliothis virescens* (F.), is considered a pest of tobacco and continues to be a serious pest of cotton in the United States. Contributing to its pest status, a long history of insecticide resistance has been documented for the tobacco budworm 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* endotoxins have revolutionized TBW 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, soybeans are common in North Carolina and are capable of supporting budworm development (Sheck and Gould 1993, Dietz et al.1976), though densities of TBW in soybean fields are generally low (Dietz et al.1976). Budworm utilization of peanuts and wild hosts is only poorly understood and yet constitutes a potentially large source of TBW 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, soybeans, peanuts, 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 for *H. virescens*.

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 of the current study were 1) to determine the spatial and temporal utilization of commercial cotton and tobacco by the TBW in the central coastal plain of NC and 2) to quantify the production of TBW 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 a commercial farming operation were selected from a three county area consisting of Pitt, Wilson, and Edgecombe Counties. Tobacco fields were sampled for tobacco budworm presence 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 tobacco budworm 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; determinations were based on larval keys presented in Neunzig (1969). A modified Harstack wire-cone pheromone trap placed at each field site was used to monitor adult budworm activity. Moths were collected from pheromone traps twice weekly and taken to the laboratory where they were stored in a freezer at 0°C.

A study was initiated in Pitt County NC in 2003 to determine if intercrop movement by adult TBW 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 ½ mile from another crop host) was selected. Potential crop hosts of TBW 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 in an east west pattern at selected distances from zero to one-quarter mile 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 for counting.

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 study was conducted in 2002 and 2003 at NC State University's Central Crops Research Station in Clayton NC. This test was designed to evaluate peanuts, soybeans, and cotton as alternate hosts for *H. virescens* in a more controlled, insecticide free environment. Tobacco, cotton, peanuts, and soybeans were planted in one-tenth acre 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. TBW 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 two rows of each eight-row tobacco plot were sampled for the presence of larvae. Tobacco budworms were sampled in cotton and soybean plots by examining 4 row meters from the middle four rows of each eight-row plot. Sampling in peanuts 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 tobacco budworm larvae. Sub-samples of larvae collected from each plot were transported to the laboratory for species identification.

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 >200 larvae per acre in May, and budworms 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. Budworm larvae were also observed in post-harvest fields in September feeding on tobacco regrowth (though no formal samples were taken). Harvest and stalk destruction was complete on most of the sample sites by mid August in all years; an increase in budworm density on 25 August 2003 was the result of an infestation in a single field of late maturing tobacco. The rapid decline in budworm density following early season peaks was the result of acephate applications (Orthene 97PE at 0.37 lbs AI per acre) made for budworm control. Larval numbers in tobacco were reduced significantly after topping (flower bud removal) in late June and early July in all years. These results indicate that tobacco grown in NC may contribute significantly to tobacco budworm production in as many as three generations annually and may thus provide an extended seasonal refuge for *Bt* transgenes and possibly conventional insecticide technologies.

Tobacco budworm larvae were rare in cotton fields prior to late July in 2001 (Figure 2), and total heliothine larval densities never exceeded 5% infested plants in any of the fields sampled throughout the season. Few late instar larvae of either heliothine species were found in cotton in 2001 indicating low survival in that crop. Conservative insecticide treatment thresholds employed by producers contributed to the low numbers of late instar larvae observed in both crops 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. 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 acre did approach 700 in August; this result was due largely to the influence of two untreated fields with relatively heavy infestations. Budworm densities in all other fields were reduced by pyrethroid applications directed primarily at bollworms. Few late instar larvae were recovered from cotton in 2003 except in the two previously mentioned untreated fields. Peak abundance of budworm larvae in cotton in August corresponds with a rapid decline in the suitability of tobacco for larval development. 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. The impact of temporal differences in TBW host utilization on the effectiveness of tobacco as a refuge is currently unknown, but 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 TBW from this crop. All cotton fields included in this study were planted to non-*Bt* varieties and were part of a mandated refuge requirement (80% *Bt*/20%

non-*Bt* option) for planting *Bt* cotton. The lack of 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 one-quarter mile away from any known 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, 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 4,000 larvae per acre in two separate peaks occurring in late June and early August 2002 (Figure3). Budworm densities in tobacco were lower in 2003 than 2002, though timing of peak infestations was similar. Production of *H. virescens* larvae in non-tobacco crop hosts was minimal. Tobacco budworm larvae were collected from cotton on three sample dates on 22 and 25 July and 2 August 2002; larval densities on each date were estimated to be 250 insects per acre. 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 previous work showing little tobacco budworm production from soybeans in NC. The importance of peanuts 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 certain areas of North Carolina (Jackson 2003, personal communication). Further research is needed to elucidate the role of peanuts and wild hosts as refuges for TBW development in NC.

The presence of tobacco culture 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 has provided a significant 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. Tobacco 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 future use patterns of pyrethroids and other insecticide classes permit.

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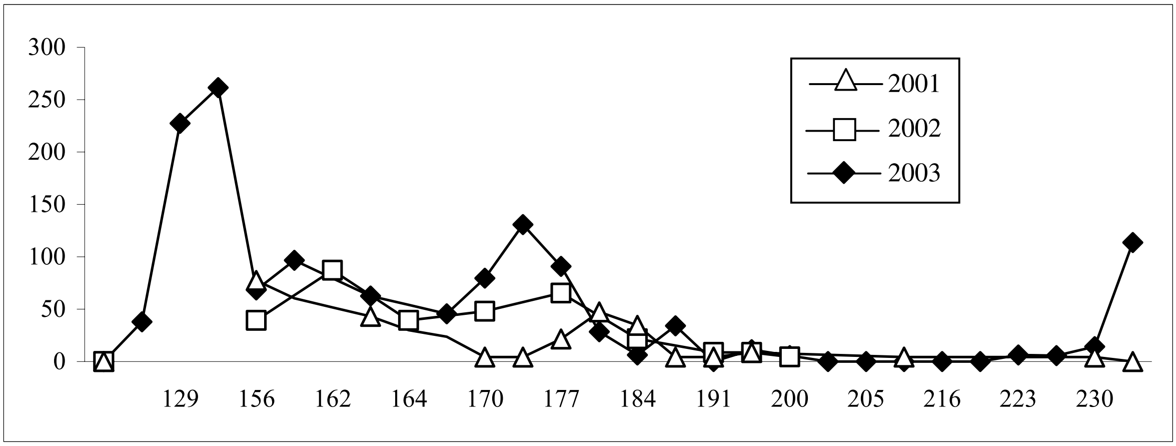

Figure 1. Estimated density of *Heliothis virescens* larvae (all developmental stages) per acre by sample date (Julian) in commercial tobacco in the central coastal plain of NC in 2001, 2002, and 2003.

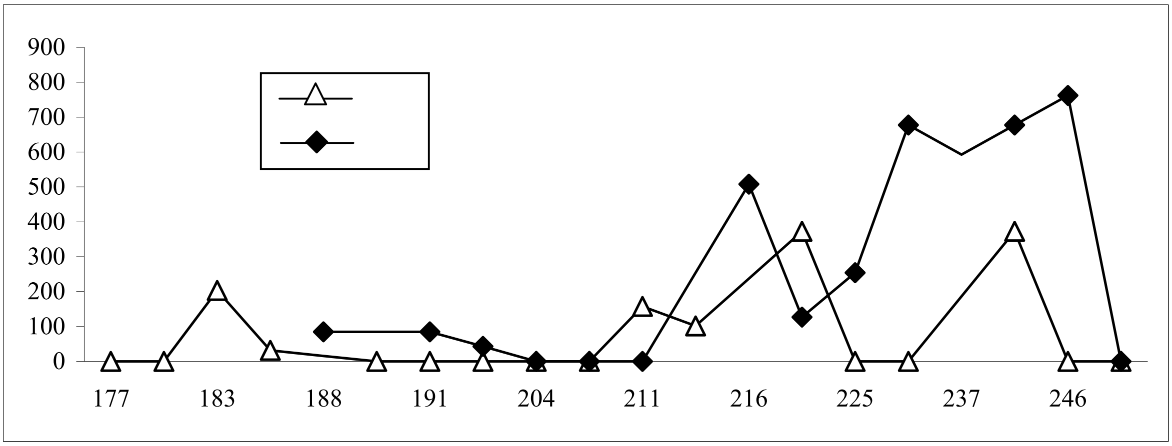

Figure 2. Estimated density of *Heliothis virescens* larvae (all developmental stages) per acre by sample date (Julian) in commercial cotton in the central coastal plain of NC in 2001 and 2003. (No *H. virescens* larvae were recovered from commercial cotton fields in 2002.)

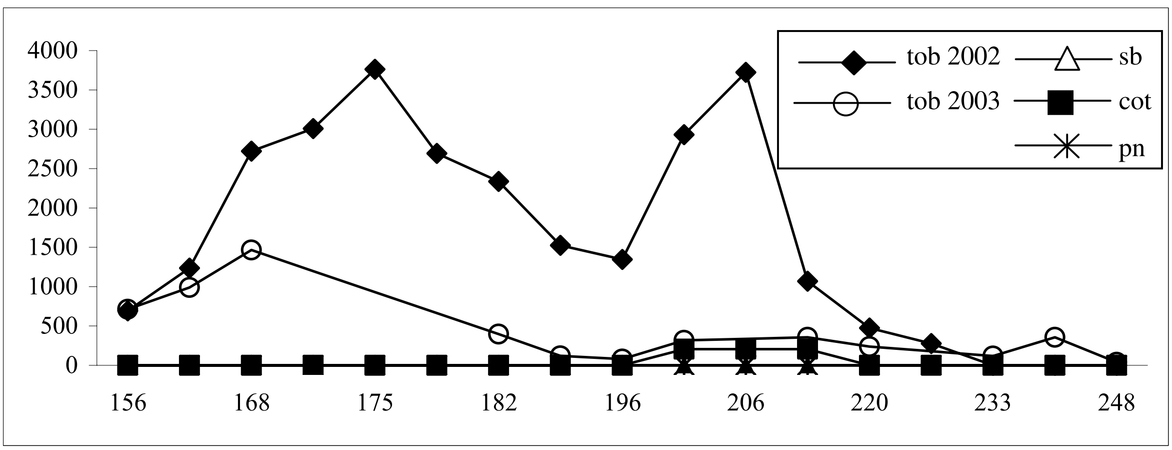

Figure 3. Mean estimated number of *H. virescens* larvae per acre in small plot evaluations of alternate crop hosts by sample date (Julian) in the central piedmont of NC in 2002. Legend key: tob=tobacco, cot=cotton, sb=soybeans, pn= peanuts. (No *H. virescens* larvae were recovered from any crop other than tobacco in 2003.)