

ARTHROPOD MANAGEMENT

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

Mark R. Abney*, Clyde E. Sorenson, and J.R. Bradley, Jr.

ABSTRACT

A three-year evaluation of host crop use 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, and 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. Results of this study indicate that flue-cured tobacco is an important host of *H. virescens* in North Carolina. The crop may also serve as a refuge for tobacco budworm from insecticide selection and play a critical role in insecticide resistance management in North Carolina.

The tobacco budworm [*Heliothis virescens* (F.)] has long been regarded as a pest of tobacco (*Nicotiana tabacum* L.), and is a serious pest of cotton (*Gossypium hirsutum* L.) in the United States (Johnson, 1979; Williams, 2004). Contributing to its pest status in cotton is a long history of insecticide resistance beginning with the chlorinated hydrocarbons (Lingren and Bryan, 1965) and followed by the carbamates, organophosphates, and pyrethroids (Sparks et al., 1993). In the last decade, transgenic cotton cultivars expressing *Bacillus*

thuringiensis endotoxins (Bt) have revolutionized tobacco budworm control across the U.S. Cotton Belt, and 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 occurred more slowly and has been 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, which reduces 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, which minimizes the impact of the “no choice” scenario and reduces the number of *H. virescens* developing in cotton. In addition to tobacco, soybean [*Glycine max* L. (Merr.)] is common in North Carolina and is capable of supporting tobacco budworm development (Sheck and Gould, 1993; Deitz et al., 1976), although densities of tobacco budworm in soybean fields are generally low (Deitz et al., 1976). The insect’s use of peanut (*Arachis hypogaea* L.) and wild hosts is poorly understood, but they constitute a potentially large source of *H. virescens*. Jackson et al. (2003) provided evidence of significant production of bollworms [*Helicoverpa zea* (Boddie)] from non-cotton crop refuges in North Carolina. The extent to which cotton, soybean, peanut, and wild hosts contribute to overall tobacco budworm numbers in the state is unknown. These hosts may be important as refuge areas for tobacco budworm development,

M.R. Abney, C.E. Sorenson, and J.R. Bradley, Jr., North Carolina State University, Department of Entomology, Research Annex West A, Box 7630, Raleigh, NC 27695
*Corresponding author: mrabney2@ncsu.edu

because, with the exception of the inherent toxicity of Bt cotton cultivars, they are 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 (EPA, 1998; Gould, 1998). Agroecosystems in North Carolina 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. This research 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 use of commercial cotton and tobacco by *H. virescens* in the central coastal plain of North Carolina 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

Research 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. Ten tobacco fields (11 in 2001) and 6 non-Bt cotton fields (5 in 2001) from a commercial farming operation were selected each year 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, and transported to the laboratory where they were measured and identified to species. Species 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 populations of *H. virescens*. Moths were collected from pheromone traps twice weekly and taken to the laboratory where they were stored in a freezer at -10 °C until they could be counted.

An investigation was initiated in Pitt County in 2003 to determine if inter-crop movement by adult tobacco budworm was sufficient to facilitate the mating of moths produced in refuge crops with moths produced in crops where selection by insecticides is likely. 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* at the study site were limited to one soybean field adjacent to two tobacco fields. The balance of the cropland at the site was planted to Bt cotton cultivars. Ten modified Harstack wire-cone pheromone traps were arranged along a line running east to west and placed at selected 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 Sept. 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 experiment was conducted in 2002 and 2003 at Central Crops Research Station in Clayton, North Carolina. 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 ha plots in a randomized complete block design with four replicates. Production practices for each crop were conducted according to recommendations from the North Carolina Cooperative Extension Service (North Carolina Cooperative Extension, 2006). 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 two rows of each eight-row tobacco plot were sampled for the presence of larvae. Heliothine larvae were sampled in cotton and soybean plots from the middle four rows of each eight-row plot. Four meters of row were sampled in each plot using a 1-m shake cloth. Fruiting structures on cotton plants in the sample area were examined following shake sampling to determine the presence of larvae not dislodged in shake cloth samples. Sampling in peanut was conducted by taking 15 sweeps through the center four rows of each plot with a 5.9-cm-diameter sweep net. Crop phenology was recorded and plots were sampled weekly for the presence of heliothine 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 greater than 200 larvae per 0.4 ha in May, and larvae were recovered from tobacco as late as 18 Aug (Julian day 230) in both 2001 and 2003. Two distinct peaks in larval density were evident in each year (Fig. 1). The early season peak was associated with rapidly growing vegetative tobacco, and the second peak corresponded with emergence of reproductive structures. Tobacco budworm larvae were also observed in fields after harvest in September feeding on tobacco regrowth, although actual samples were not taken. Harvest and stalk destruction was completed on most of the sample sites by mid-August in all years. An increase in larval density on 25 Aug. 2003 (Julian day 237) 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 made for control of the insect. Larval numbers in tobacco were reduced significantly after flower bud removal in late June and early July in all years. These results indicate that tobacco grown in North Carolina may contribute significantly to tobacco budworm production with as many as three generations annually, and may provide an extended seasonal refuge for tobacco budworm, if pyrethroid insecticide and Bt transgene use in the crop is limited.

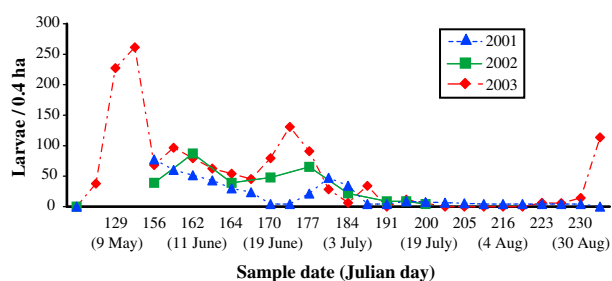


Fig. 1. Estimated density of *Heliothis virescens* larvae (instars grouped by date) per 0.4 hectares by sample date (Julian) in commercial tobacco in the central coastal plain of North Carolina in 2001, 2002, and 2003.

Tobacco budworm larvae were rare in cotton fields prior to late July in 2001 (Fig. 2), and total heliothine larval densities never exceeded 5% infested plants in any of the fields sampled throughout the season. In 2001, few late instar larvae of either heliothine species were found in cotton indicating low survival in that crop. Conservative insecticide treatment thresholds

employed by producers contributed to the low numbers of late instar 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 0.4 ha did approach 700 in August. This result was largely because of the influence of two untreated cotton fields with relatively heavy infestations. Tobacco budworm densities in all other fields were reduced by pyrethroid applications directed primarily at bollworm [*Helicoverpa zea* (Boddie)]. Few late instar larvae were recovered from cotton in 2003 except in the two previously mentioned 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 larvae of *H. virescens* may be present in cotton and tobacco throughout the growing season in North Carolina, although temporal differences in peak seasonal occurrence exist for each crop. Temporal differences in tobacco budworm host use 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. All cotton fields included in this study were planted to non-Bt cultivars 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 not available.

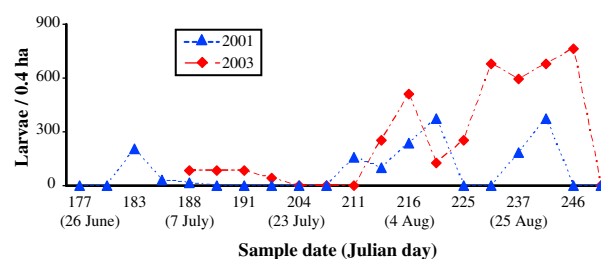


Fig. 2. Estimated density of *Heliothis virescens* larvae (all developmental stages) per 0.4 hectares by sample date (Julian) in commercial cotton in the central coastal plain of North Carolina in 2001 and 2003. (No *H. virescens* larvae were recovered from commercial cotton fields in 2002.)

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. Averaged across the season, a mean of 24 moths per one week sample period was collected from the pheromone trap located at the greatest distance from a known crop host (0.4 km). A mean of 40 moths per sample period was collected in the pheromone trap located directly adjacent to a preferred host (tobacco). These data suggest sufficient adult movement to 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.) No conclusions can be drawn from these data concerning movement of tobacco budworm moths at distances greater than 0.4 km.

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 0.4 ha in two separate peaks occurring in late June and early August 2002 (Fig. 3). Tobacco 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 Aug. 2002 (Julian days 203, 206, and 214); larval densities on each date were estimated to be 250 insects per 0.4 ha. 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 oviposition in cotton may be determined largely by the proximity of a more attractive host. Given the choice between cotton and tobacco, tobacco budworm females in the current study oviposited preferentially on tobacco. 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 North Carolina. The importance of peanut for tobacco budworm production remains unclear, but results here indicate limited use 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 2003, personal communication). Further research is needed to elucidate the role of peanut and wild hosts as refuges for tobacco budworm development in North Carolina.

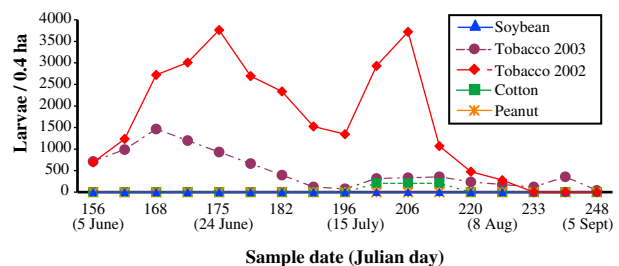


Fig. 3. Mean estimated number of *H. virescens* larvae per 0.4 hectares in small plot evaluations of alternate crop hosts by sample date (Julian) in the central piedmont of North Carolina in 2002.

Tobacco appears to play an important role in both the spatial and temporal occurrence of tobacco budworm in North Carolina agroecosystems. The close association between this pest and its preferred agricultural host is likely responsible for the low tobacco budworm pressure experienced in cotton in the state. Tobacco may also play an important role in slowing the development of insecticide resistance in populations of tobacco budworm in North Carolina 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 use of tobacco as a refuge for pyrethroid resistance management may have been compromised. Tobacco likely serves as an important refuge from insecticide selection for tobacco budworm in North Carolina and may help to delay the development of resistance to insecticides.

ACKNOWLEDGEMENTS

The authors wish to thank grower-cooperators Glenn and Barbara Smith, and Alan Stephenson, David Nichols, Billie Jo Abney, 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.

REFERENCES

- Deitz, L.L., J.W. Van Duyn, J.R. Bradley Jr., R.L. Rabb, W.M. Brooks, and R.E. Stinner. 1976. A guide to the identification and biology of soybean arthropods in North Carolina. Tech. Bull. 238. North Carolina Agric. Res. Serv., Raleigh.
- Fitt, G.P. 1989. The ecology of *Heliothis* species in relation to agroecosystems. *Ann. Rev. Entomol.* 34: 17-52.
- Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: Integrating pest genetics and ecology. *Annu. Rev. Entomol.* 43: 701-726.
- Jackson, R.E., J.R. Bradley Jr., and J.W. Van Duyn. 2003. Quantification of *Helicoverpa zea* in eastern North Carolina crop environments: Implications for *B.t.* resistance management. p.1017-1021. *In Proc. Beltwide Cotton Conf.*, Nashville, TN. 6-10 Jan. 2003. Natl. Cotton Counc. Am., Memphis, TN
- Johnson, A.W. 1979. Tobacco budworm damage to flue-cured tobacco at different plant growth stages. *J. Econ. Entomol.* 72: 602-605.
- Lingren, P.D. and D.E. Bryan. 1965. Dosage-mortality data on the bollworm, *Heliothis zea*, and the tobacco budworm, *Heliothis virescens*, in Oklahoma. *J. Econ. Entomol.* 58: 14-18.
- North Carolina Cooperative Extension. 2006. Publications for Field Crops. Available online at <http://www.ces.ncsu.edu/Publications/fieldcrops.php> (verified 16 Jan. 2007)
- Neunzig, H.H. 1969. The biology of the tobacco budworm and the corn earworm in North Carolina. Tech. Bull. 196. North Carolina Agric. Exp. Stn., Raleigh.
- Ramaswamy, S.B., W.K. Ma, and G.T. Baker. 1987. Sensory cues and receptors for oviposition by *Heliothis virescens*. *Entomol. Exp. Appl.* 43: 159-168.
- Sheck, A.L., and F. Gould. 1993. The genetic basis of host range in *Heliothis virescens*: larval survival and growth. *Entomol. Exp. Appl.* 69: 157-172.
- Sparks, T.C., J.B. Graves, and B.R. Leonard. 1993. Insecticide resistance and the tobacco budworm: Past, present, and future. *Rev. Pestic. Toxicol.* 2: 149-183.
- U.S. Environmental Protection Agency (EPA). 1998. The Environmental Protection Agency's white paper on *Bt* plant-pesticide resistance management. EPA Publication 739-S-98-001 U.S. E.P.A, Biopesticides and Pollution Prevention Division.
- Williams, M.R. 2004. Cotton insect loss estimates-2003. p. 1249-1257. *In Proc. Beltwide Cotton Conf.*, San Antonio, TX. 5-9 Jan. 2004. Natl. Cotton Counc. Am., Memphis, TN.