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To the Graduate Council:

I am submitting herewith a thesis written by Douglas Scot Bidlack entitled "Incidence and species composition of Heliothis spp. and their parasitoids on selected tobacco breeding lines and cultivars in Eastern Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Jerome F. Grant, Major Professor

We have read this thesis and recommend its acceptance:

Charles Pless, Robert Miller

Accepted for the Council: Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

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INCIDENCE AND SPECIES COMPOSITION OF <u>HELIOTHIS</u> SPP. AND THEIR PARASITOIDS ON SELECTED TOBACCO BREEDING LINES AND CULTIVARS IN EASTERN TENNESSEE

Presented for the Master of Science Degree The University of Tennessee, Knoxville

A Thesis

Douglas Scot Bidlack

August 1989

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ii

ABSTRACT

Alternative insect pest management tactics, such as host plant resistance and biological control, have recently received more research emphasis due to the current concern over pesticide residue and contamination problems. In addition, several insect species have been shown to be resistant to several commonly used pesticides, which poses a problem in control of these pest species. Little information is available on the influence of breeding lines/cultivars of tobacco on populations of Heliothis species, as well as on their larval parasitoid species. Therefore, a two-year study was initiated to better understand this host plant-pest-natural enemy interaction. The objectives of this study were to: 1) determine the species composition and seasonal incidence of Heliothis on tobacco, 2) determine the seasonal parasitism levels and species composition of larval parasitoids on tobacco, 3) evaluate the influence of selected tobacco breeding lines/cultivars on populations of Heliothis, and 4) evaluate the influence of selected tobacco breeding lines/cultivars on populations of parasitoids of larvae of Heliothis.

As many as three generations of <u>Heliothis</u> larvae may have occurred on tobacco, with <u>Heliothis</u> <u>virescens</u>, the tobacco budworm, comprising ca. 83% of the <u>Heliothis</u> spp. collected. <u>Heliothis</u> <u>zea</u>, the corn earworm, comprised the

iii

remaining 17% of the <u>Heliothis</u> larvae collected from tobacco. <u>Campoletis sonorensis</u>, <u>Cardiochiles nigriceps</u>, <u>Microplitis croceipes</u>, <u>Archytas marmoratus</u> and other tachinid species (possibly all <u>Winthemia rufopicta</u>) were found to parasitize ca. 25% of the <u>Heliothis</u> larvae collected from tobacco in eastern Tennessee. <u>Campoletis</u> <u>sonorensis</u>, present throughout the entire tobacco-growing season, was the most important parasitoid species, accounting for ca. 90% of all parasitism. In 1987, <u>Archytas</u> <u>marmoratus</u> and several other tachinid species were present late in the season and accounted for ca. 5% of the total parasitism of <u>Heliothis</u> larvae. In 1988, however, tachinid species accounted for ca. 0.3% of the parasitoid complex.

Populations of <u>Heliothis</u> larvae were influenced by the duvane diterpenes secreted from the leaf surfaces of selected breeding lines/cultivars of tobacco. Those breeding lines/cultivars that secreted high levels of duvane diterpenes also had high levels of <u>Heliothis</u> larvae. Early (mid-June to late July) in the tobacco-growing season, densities of <u>Heliothis</u> larvae were ca. three times (Knoxville, 1988) to ca. six times (Knoxville, 1987) greater on the tobaccos that secreted high levels of duvane diterpenes ("sticky") than on those that secreted low levels of duvane diterpenes ("non-sticky"). In general, parasitism levels were lower on the "non-sticky" (especially TI 1112) lines than on the "sticky" lines of tobacco.

iv

TABLE OF CONTENTS

CHAPTE	R		PAGE
I.	LITER	ATURE REVIEW	1
II.	MATER	ATALS AND METHODS	17
III.	RESUL	TS AND DISCUSSION	22
	i.	HELIOTHIS INCIDENCE AND COMPOSITION	22
	ii.	PARASITOID INCIDENCE AND SPECIES COMPOSITION	23
	iii.	INFLUENCE OF SELECTED TOBACCO ENTRIES ON <u>HELIOTHIS</u> AND PARASITOID SPECIES	42
IV.	CONCL	USIONS	64
REFER	ENCES	CITED	67
VITA.			74

LIST OF TABLES

÷.,

FIGU	URE	PAGE
1.	Seasonal incidence of <u>Heliothis</u> lar Knoxville and Greeneville, 1987	cvae on tobacco,
2.	Seasonal incidence of <u>Heliothis</u> lar Knoxville and Greeneville, 1988	cvae on tobacco,
3.	Species composition of <u>Heliothis</u> la at Knoxville and Greeneville, 1987	
4.	Seasonal incidence of parasitoid sp from <u>Heliothis</u> collected from tobac and Greeneville, 1987 and 1988	co at Knoxville
5.	Percent parasitism and parasitoid s composition of <u>Heliothis</u> larvae col tobacco, Knoxville, 1987	Llected from
6.	Percent parasitism and parasitoid s composition of <u>Heliothis</u> larvae co tobacco, Greeneville, 1987	Llected from
7.	Percent parasitism and parasitoid s composition of <u>Heliothis</u> larvae co tobacco, Knoxville, 1988	Llected from
8.	Percent parasitism and parasitoid s composition of <u>Heliothis</u> larvae co tobacco, Greeneville, 1988	Llected from
9.	Percent parasitism by <u>Campoletis</u> so <u>Heliothis</u> larvae collected from tob and Greeneville, 1987	Dacco, Knoxville
10.	Percent parasitism by <u>Campoletis</u> so <u>Heliothis</u> larvae collected from tob and Greeneville, 1988	Dacco, Knoxville
11.	Percent parasitism by <u>Cardiochiles</u> <u>Heliothis</u> larvae collected from tob Knoxville and Greeneville combined, 1988	pacco at 1987 and
12.	Percent parasitism by <u>Microplitis</u> <u>Heliothis</u> larvae collected from to 1987	Dacco, Knoxville,

13.	Percent parasitism by all tachinid species of <u>Heliothis</u> larvae collected from tobacco, Knoxville and Greeneville, 198744
14.	Concentrations of duvane-diterpenes on selected tobacco entries, Greeneville, 198747
15.	Concentrations of duvane-diterpenes on selected tobacco entries, Greeneville, 198849
16.	Densities of <u>Heliothis</u> larvae on selected tobacco entries, Knoxville and Greeneville, 1987 and 1988
17.	Densities of <u>Heliothis</u> larvae on selected tobacco entries early in the season (late June to late July), Knoxville and Greeneville, 198753
18.	Seasonal incidence of <u>Heliothis</u> larvae on "sticky" and "non-sticky" tobacco entries, Knoxville, 1987
19.	Seasonal incidence of <u>Heliothis</u> larvae on "sticky" and "non-sticky" tobacco entries, Greeneville, 1987
20.	Seasonal incidence of <u>Heliothis</u> larvae on "sticky" and "non-sticky" tobacco entries, Knoxville, 1988
21.	Seasonal incidence of <u>Heliothis</u> larvae on "sticky" and "non-sticky" tobacco entries, Greeneville, 1988

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CHAPTER I

LITERATURE REVIEW

The largest family in the Lepidoptera is the owlet or noctuid (Noctuidae) moth family, consisting of ca. 20,000 species worldwide and ca. 2,900 species in North America (Covell 1984). Of the noctuid moth genera, Heliothis Ochsenheimer may contain the most important agricultural pest species in the world (King et al. 1981). The two economically important species of <u>Heliothis</u> in the United States are <u>Heliothis zea</u> (Boddie), the corn earworm, bollworm or tomato fruitworm, and <u>Heliothis virescens</u> (Fabricius), the tobacco budworm.

Adult <u>H</u>. <u>virescens</u> and <u>H</u>. <u>zea</u> are, as are most noctuids, nocturnal and have stout, hairy bodies, moderately long labial palps and simple antennae (Covell 1984 and Borror et al. 1981). Adult <u>H</u>. <u>virescens</u> are generally pale green with slanted, nearly parallel, dark olive or dark brown bands on each forewing. The forewings of adult <u>H</u>. <u>zea</u> are yellowish tan with a single, dark reniform spot near the center and variable reddish brown, olive green or gray markings and shading. <u>Heliothis virescens</u> and <u>H</u>. <u>zea</u> are both medium-sized moths; <u>H</u>. <u>virescens</u> has a wingspan from 27 to 38mm and <u>H</u>. <u>zea</u> has a wingspan from 32 to 45mm (Covell 1984 and Hunt 1983).

Female Heliothis lay an average of 1,000 eggs, singly on their host plant (Hunt 1983). Eggs of both species are about 0.6mm in diameter, subspherical with a flattened base and white when first deposited, but develop reddish brown bands prior to hatching. Newly-emerged larvae (ca. 1.5mm long) are yellowish white with brown heads. Fully-grown larvae are moderately hairy with pale longitudinal stripes and scattered black spots and may vary from greenish yellow to tan to reddish brown to black. The larval stage of both species of Heliothis consists of five or six instars. Larval developmental time is ca. 23 days. Last-instar larvae burrow 5 to 10cm into the soil and pupate. Pupae are initially shiny and reddish brown but become dark brown just before adult emergence. In North Carolina, Heliothis virescens pupae enter diapause in September and adults emerge the following year from late April to early May. Heliothis zea pupae enter diapause in August and adults emerge the following year from early May to early June. Both species have three to four generations per year in North Carolina (Hunt 1983).

Both <u>H</u>. <u>virescens</u> and <u>H</u>. <u>zea</u> have similar distributions throughout the Western Hemisphere from Canada in the North to Argentina in the South (Hunt 1983 and King et al. 1981). In the United States both of these species are abundant in the Southeast, becoming less important north and west of this region (King et al. 1981).

<u>Heliothis zea</u> infests more than 100 species of plants and occurs on at least 17 cultivated plant species in the South (Hunt 1983). These include alfalfa, bean, chrysanthemum, corn, cotton, geranium, gladiolus, okra, peanut, pea, sorghum, soybean, strawberry, sweet pepper, sweet potato, tobacco and tomato. Corn is the preferred host, but this species is also destructive to cotton. <u>Heliothis zea</u> is also found on a wide variety of wild hosts, such as toadflax (Hunt 1983). <u>Heliothis virescens</u> has a narrower host range than <u>H</u>. <u>zea</u>. The only cultivated crops in the South which this species infests are tobacco, cotton and soybeans, with tobacco as the most important host crop (Hunt 1983). Some common wild hosts of <u>H</u>. <u>virescens</u> include species of deergrass, toadflax, beggarweed, groundcherry, geranium and ageratum (Hunt 1983).

In the United States damage (crop loss + control costs) to cultivated crops and vegetables caused by <u>H</u>. <u>virescens</u> and <u>H</u>. <u>zea</u> is estimated to be greater than one billion dollars annually (King et al. 1981). In 1987, about 93% of the total value (1.8 billion dollars) of tobacco in the United States was produced in the Southeast, where these two species of <u>Heliothis</u> are the most damaging insect pests of tobacco (USDA 1987). For example, damage to tobacco by <u>Heliothis</u> in Georgia was estimated to be \$ 6,796,000 during 1986 (Douce and Suber 1983). Most of the damage to tobacco is believed to be caused by H. virescens. About 90% of the

<u>Heliothis</u> larvae found on tobacco were identified as <u>H</u>. <u>virescens</u> in North Carolina (Hunt 1983). Although both species of <u>Heliothis</u> may feed on mature tobacco leaves, this type of feeding causes little damage to the plant. Most of the damage to tobacco occurs when <u>Heliothis</u> larvae feed upon the vegetative buds of the plant, causing large holes to develop in the leaves as the plant matures (Hunt 1983). <u>Heliothis</u> larvae may also bore into the midribs or stalks of tobacco and cause sucker growth by prematurely topping plants. The economic threshold for <u>Heliothis</u> on tobacco is reached when five or more of 50 plants are infested with larvae of any size before buttoning (Hunt 1983). After the plants have buttoned, damage by <u>Heliothis</u> larvae is slight.

Tobacco growers rely primarily on chemical and cultural control tactics to suppress populations of <u>Heliothis</u>. The insecticides commonly used for control of <u>Heliothis</u> include organophosphates (e.g., acephate, methidathione and monocrotophos), chlorinated hydrocarbons (e.g., endosulfan) and carbamates (e.g., carbofuran and methomyl) (Schneider et al. 1987). Cultural methods include topping plants, control of suckers, destruction of stalks after harvest, and plowing during the fall and winter (Hunt 1983). When used together, chemical insecticides and cultural methods are effective in suppressing populations of <u>Heliothis</u> on tobacco. The use of chemical insecticides can, however, have some disadvantages, such as development of resistance to chemical insecticides

by <u>Heliothis</u>, environmental contamination and harmful effects on natural enemy populations and wildlife, as well as human health hazards. The use of chlorinated insecticides in controlling <u>Heliothis</u> has been especially effected by environmental problems. Chlorinated hydrocarbons are no longer commonly used because of their long term persistence in the environment (Schneider et al. 1987).

Host plant resistance, an alternative management tactic, is the inheritable property that enables a plant to inhibit the growth of pest populations or to recover from injury caused by poulations that were not inhibited to grow (Metcalf and Luckman 1982). There are several advantages to the use of insect-resistant cultivars in a pest management system. Host plant resistance is specific, safe, long lasting, residue free and is an easy and economical method for the control of insect pests (Schneider et al. 1987). Implementation of host plant resistance may not eliminate the need for insecticides, but it may reduce the number of applications and/or the application rate, and it can be effectively integrated into a management program that uses insecticides.

Because of interest in tobacco breeding lines/cultivars resistant to <u>Heliothis</u>, several workers have initiated research in this area. Burk and Stewart (1971) evaluated a

large number of <u>Nicotiana</u> species for resistance to <u>Heliothis</u>. Although several resistant species of <u>Nicotiana</u> were identified by Burk and Stewart (1971), Chaplin and Burk (1970) suggested that resistance to <u>Heliothis</u> should be evaluated in <u>Nicotiana tabacum</u> Linnaeus because the transfer of germplasm from one species of <u>Nicotiana</u> to another is difficult. Some cultivars of flue-cured tobacco were found to be less susceptible to damage by <u>Heliothis</u> than others (Girardeau 1968). Girardeau et al. (1973) described some of the possible causes of susceptibility to <u>Heliothis</u> in certain commercial cultivars of tobacco. Chaplin et al. (1976) found that tobacco introduction (TI) 1112 exhibited high resistance to damage by <u>Heliothis</u> in greenhouse and field tests, and suggested that resistant cultivars of tobacco could be developed.

Resistance to green peach aphid, <u>Myzus persicae</u> (Sulzer), was reportedly due to alkaloids, such as nicotine, nornicotine and anabasine, secreted by glandular trichomes of several <u>Nicotiana</u> species (Thurston et al. 1966). Nicotine levels, however, were later found to have no relationship with alate or apterous green peach aphid populations (Thurston et al. 1977) and neither alkaloid composition nor total alkaloid levels were found to be related to resistance of TI 1112 to <u>Heliothis</u>. The trichomes of TI 1112 are simple (unbranched) or branched without glandular heads or visible exudates whereas most

tobacco cultivars have simple or branched trichomes with glandular heads and exudates (Johnson et al. 1985). Elsey and Chaplin (1978) speculated that the resistance of TI 1112 to <u>Heliothis</u> may be due to the lack of secretions from the non-glandular headed trichomes. Other tobacco types, such as TI 1024 and TI 1462, have simple or branched trichomes with glandular heads that produce little or no exudates (Johnson et al. 1985).

The major cuticular components of several tobacco cultivars have been identified as alpha- and beta-4,8,13duvatriene-1,3- diols and C25-C36 paraffinic hydrocarbons (Severson et al. 1982). All of the tobacco cultivars that they tested had similar levels of the hydrocarbons, but the relative duvatrienediol levels were directly correlated with trichome exudate levels. Low levels of duvatrienediols were also found to be associated with lower levels of Heliothis infestations. Johnson and Severson (1984) and Severson et al. (1984) found that the major cuticular components of tobacco consist primarily of diterpenes (duvanes and labdanes), hydrocarbons and sucrose esters. However, Severson et al. (1984) also identified a series of free fatty alcohols, of which docosanol was the most important, and high molecular weight wax esters as minor components in tobacco cuticular waxes.

Many flue-cured and burley tobaccos produce mainly the duvane diterpenes; whereas, some oriental and cigar tobaccos produce primarily the labdane diterpenes. Other types of tobacco produce a combination of both duvanes and labdanes (Jackson et al. 1986 and Severson et al. 1984). Tobaccos that produce mainly duvane diterpenes yield a mixture of alpha- and beta-4,8,13-duvatriene- 1,3-diols and alpha- and beta-4,8,13-duvatriene-1-ols. The concentration of duvatrienediols are generally at least 100 times that of the duvatriene-ols in the young leaves of commercial American tobacco cultivars. (122)-labda-12,14-diene- 8alpha-ol (cisabienol) and (13E)-labda-13-ene-8alpha, 15diol are the major diterpenes found as cuticular components of tobaccos that produce labdane diterpenes (Severson et al. 1984).

Tobaccos with very low levels of alpha- and betaduvatrienediols and alpha- and beta-duvatriene-ols were reported to have correspondingly low populations of <u>Heliothis</u> (Johnson and Severson 1984). The number of <u>Heliothis</u> larvae increased with an increase in either of these diterpenes. Other tobacco types classified as resistant to <u>Heliothis</u> had high sucrose ester levels and high duvatrienediol levels.

Significantly (p=0.01) fewer <u>Heliothis</u> eggs were laid on TI 1112 than on two flue-cured varieties (NC 2326 and NC 95) in field tests (Elsey and Chaplin 1978). This resistance was attributed to ovipositional nonpreference that was

associated with the lack of glandular trichomes of TI 1112 that secrete sticky exudates. In cage tests, Jackson et al. (1983) confirmed that ovipositional nonpreference is the mechanism of resistance of TI 1112 to <u>Heliothis</u>. In nochoice tests, more than twice as many eggs were laid by <u>Heliothis</u> on NC 2326, a typical sticky exudate- secreting, flue-cured tobacco, than on TI 1112, and when provided a choice in screened-cage ovipositional tests, 74 to 91% of the eggs were laid on NC 2326. Jackson et al. (1984, 1986) determined that each of the duvane diterpenes, when sprayed on TI 1112 plants, significantly (p=0.05) increased oviposition by <u>Heliothis</u>. Hydrocarbons, labdane diterpenes, fatty alcohols and wax esters, however, did not stimulate egg laying by Heliothis when sprayed on TI 1112 plants.

TI 165, as well as several similar tobaccos (TI 163, TI 168 and TI 170), has trichomes that secrete sticky exudates and has exhibited larval mortality. The mechanism of <u>Heliothis</u> resistance for TI 165 is believed to be larval antibiosis due to the high levels of sucrose esters and/or the very high levels of duvatrienediols produced on the cuticular surface of these tobacco plants (Johnson and Severson 1984). Other tobaccos have exhibited <u>Heliothis</u> resistance and some of these tobaccos have different leaf surface chemistries than either TI 1112 or TI 165. Therefore, other mechanisms of resistance to Heliothis may

exist for some of these types of tobacco (Johnson and Severson 1984).

Biological control, another alternative to insecticides, is compatible with host plant resistance, as well as with cultural control. In biological control, predators, parasitoids and pathogens are utilized to manage pest populations by either increasing native natural enemy populations through conservation, augmentation and attraction, or by releasing foreign natural enemies (classical biological control) (Huffaker 1971).

Parasitoids may be the most important group of natural enemies that attack Heliothis on tobacco. Several researchers have reported larval parasitism rates greater than 50% (Grayson 1944; Johnson and Manley 1982 and Wene 1943). Egg parasitoids, however, are not considered as important as larval parasitoids in suppressing populations of lepidopterous pests such as Heliothis or tobacco hornworm, Manduca sexta (Linnaeus), on tobacco. Sticky exudates present on most commercial tobacco cultivars deter and/or trap the small adult egg parasitoids (Rabb and Bradley 1968). Telenomus sphingus (Ashwood), an egg parasitoid of M. sexta, was found to parasitize only 15% of M. sexta eggs on TI 1298 plants even though this tobacco type secretes only non-sticky exudates, compared with 60-70% parasitism of eggs on jimson weed or tomato plants (Katanyukul and Thurston 1979). Elsey and Chaplin (1978)

reported that none of the <u>Heliothis</u> eggs that they collected from NC 95, a sticky exudate- secreting, flue-cured tobacco, was parasitized; whereas, nine <u>Heliothis</u> eggs collected from TI 1112 were parasitized by a species of <u>Trichogramma</u>.

The most important larval parasitoids of Heliothis on tobacco in the southeastern United States are the braconids Cardiochiles nigriceps Viereck and Microplitis croceipes (Cresson), the ichneumonid Campoletis sonorensis (Cameron), and the tachinids Archytas marmoratus (Townsend) and Winthemia rufopicta (Bigot) (Danks et al. 1979, Grayson 1944, Johnson and Manley 1982 and Wene 1943). In general, either C. nigriceps or C. sonorensis is the abundant parasitoid of Heliothis throughout the season on tobacco in the Southeast. This abundance is especially true early in the season when percent parasitism of Heliothis by C. nigriceps or C. sonorensis may be as high as 80 or 90% (Grayson 1944, Johnson and Manley 1982 and Wene 1943). Microplitis croceipes, on the other hand, is usually a relatively minor parasitoid of Heliothis on tobacco, but it is the most important larval parasitoid of Heliothis on cotton in Arizona (Butler 1958), Oklahoma (Bottrell et al. 1968) and Mississippi (Lewis and Brazzel 1968 and Smith et al. 1976). Although the tachinids A. marmoratus and W. rufopicta occur early in the tobacco-growing season, their densities do not become high until September and October,

when they may be the most dominant parasitoids of <u>Heliothis</u> on tobacco (Danks et al. 1979, Johnson and Manley 1982). <u>Cardiochiles nigriceps</u> and <u>M. croceipes</u> are both host specific to <u>Heliothis</u>, <u>C. sonorensis</u> prefers <u>Heliothis</u> but parasitizes several other noctuid species as well as one species of pierid, and both of the tachinids commonly parasitize noctuids other than <u>Heliothis</u> (Danks et al. 1979). Descriptions of these parasitoid species can be found in the following references: <u>C. nigriceps</u> (Chamberlin and Tenhet 1926, Lewis and Vinson 1968 and Viereck 1912), <u>M</u>. <u>croceipes</u> (Lewis 1970 and Muesebeck 1922), <u>C. sonorensis</u> (Bigornia 1956 and Wilson and Ridgway 1975), <u>A. marmoratus</u> (Curran 1928 and Townsend 1915) and <u>W. rufopicta</u> (Allen 1925, Danks 1974 and Guimaraes 1972).

<u>Cardiochiles nigriceps</u> develop only in <u>H</u>. <u>virescens</u> and <u>H</u>. <u>subflexa</u>. Although they have been shown to oviposit in <u>H</u>. <u>zea</u>, the eggs become encapsulated and die inside this host (Lewis and Vinson 1971). Eggs are laid mainly on first instar larvae, but the parasitoids usually remain as first instar larvae until the host larva pupates (Neunzig 1969). The parasitoids then develop quickly and emerge from the pupae in 4 to 5 days (Neunzig 1969). Parasitized larvae develop more slowly, consume less food, and, subsequently, are smaller than non-parasitized larvae (Neunzig 1969). In North Carolina, <u>C</u>. <u>nigriceps</u> overwinter as prepupae in their cocoons. Adults emerge in May, undergo several generations,

and enter diapause as early as August, although most may not enter diapause until September or October (Danks et al. 1979).

<u>Microplitis croceipes</u> generally parasitize second and third instar larvae and emerge from the fourth instar (Lewis and Burton 1970). Developmental time from egg to adult emergence averaged 14.5 and 28 days at 30 and 200C, respectively. Almost half of this period was in the pupal stage (Danks et al. 1979). Based on laboratory studies, <u>M</u>. <u>croceipes</u> may undergo several generations per year and overwinter as prepupae in their cocoons (Bryan et al. 1969). <u>Microplitis croceipes</u> has been collected from June to September in North Carolina (Neunzig 1969).

<u>Campoletis sonorensis</u> usually parasitize first and second instar larvae and emerge from the third instar (Schmidt 1974). Eggs hatch within 2 days of oviposition. Development through four larval instars requires 6 to 12 days, and the duration of the pupal stage is 5 to 7 days (Danks et al. 1979). <u>Campoletis sonorensis</u> has been recorded from the field from May to October, but the overwintering stage is unknown (Danks et al. 1979 and Neunzig 1969).

<u>Archytas</u> <u>marmoratus</u> does not oviposit, instead they deposit larvae onto vegetation, usually near <u>Heliothis</u> larvae. The maggots must attach to and then penetrate the

cuticle of a Heliothis larva (Hughes 1975). If a maggot does not enter a final instar larva, the maggot must repenetrate the cuticle each time the host molts. Because many of the maggots are unable to repenetrate after the host molts, the last instar larva is usually the most successfully parasitized (Hughes 1975). Winthemia rufopicta, however, oviposits white eggs onto the outside of the host, generally in the dorsal area of segments 2 to 4 of large larvae. After a few days, the maggots hatch and penetrate the host cuticle (Danks 1975). Archytas marmoratus maggots live 13 to 14 days at 21⁰C and the pupal period lasts for about 17 and 30 days at 27 and 21°C. respectively (Hughes 1975). Duration of the egg stage of W. rufopicta averaged about 3 and 4 days, larval development 5 and 7 days, and pupal development 9 and 13 days at 30 and 21⁰C, respectively (Danks 1975). The overwintering stage of A. marmoratus is unknown (Danks et al. 1979). Adults have been recorded in North Carolina in early June but are not common until the fall (Hughes and Rabb 1976). Winthemia rufopicta overwinter as fully grown maggots in the soil and emerge in April in North Carolina, and are also common in the fall (Danks 1975).

Alternative insect pest management tactics, such as host plant resistance and biological control, have recently received more research emphasis due to the current trend towards integrated pest management. The recent research in

tobacco breeding lines/cultivars resistant to insect pests at the University of Tennessee began as an extension of a tobacco breeding program established by Dr. Robert Miller for the development of tobacco plants resistant to the PVY complex (tobacco vein mottling virus, tobacco etch virus and potato virus Y) of virus diseases (Miller 1987). Although a good source (TI 1406) of resistance to the PVY complex was known, tobacco breeding lines with PVY resistance derived from TI 1406 were also highly susceptible to several insect pests (Gupton 1980, Nielson et al. 1982, Ples from TI 1406 were also highly susceptible to several insect pests (Gupton 1980, Nielson et al. 1982, Pless and Miller 1986 and Smeeton 1976). Therefore, in 1984 tobacco breeding lines/cultivars were evaluated for their tolerance to insects as well as their resistance to the PVY complex. In 1986, this research was expanded when tobacco breeding lines/cultivars known to have insect resistance (e.g., TI 1112), were incorporated into the field evaluations (Miller, personal communication).

Little information is available on the influence of exudates on populations of natural enemies on tobacco. Therefore, it is important to concentrate research efforts to better understand this host plant-pest-natural enemy interaction. In addition, little information is available on the influence of these tobacco breeding lines/cultivars on Heliothis populations, and on populations of parasitoid

species in Tennessee. The objectives of this two-year study were to: 1 determine the seasonal incidence and species composition of <u>Heliothis</u> larvae on tobacco in eastern Tennessee, 2) determine the parasitoid complex impacting on <u>Heliothis</u> larvae and their seasonal parasitism levels on tobacco, 3) evaluate the effects of selected tobacco breeding lines/cultivars on the seasonal incidence of <u>Heliothis</u> larvae and, 4) assess the influence of selected tobacco breeding lines/cultivars on the larval parasitoids of Heliothis in eastern Tennessee.

CHAPTER II

MATERIALS AND METHODS

Tobacco plots were located at two sites in eastern Tennessee (the University of Tennessee Plant and Soil Science Farm, Knoxville, and the University of Tennessee Tobacco Experiment Station, Greeneville) during 1987 and 1988. Tobacco was grown at both locations using standard agronomic practices as recommended by the University of Tennessee Extension Service. Tobacco plants, however, were not topped during the growing season or harvested at the end of the season, and no insecticides were applied so that these plants could be studied in a model system.

Plants of each tobacco breeding line/cultivar selected for evaluation during 1987 and 1988 were grown from seed in seed beds using standard agronomic practices at the Tobacco Experiment Station at Greeneville under the direction of Dr. Bob Miller. During both years of this study, tobacco breeding lines/cultivars evaluated at Knoxville and Greeneville were arranged in randomized complete block designs with four replications at each location. A replication consisted of one single-row plot (12.8m long) which contained ca. 32 tobacco plants. Plants were spaced 41cm apart within a row and the rows were spaced 107cm apart.

In 1987, ten tobacco entries, consisting of two tobacco introductions (TI 1112 and TI 1406), five breeding lines (PDJA 309, TN 86 x TI 1112, TN 86 x PDJA 309, GR 115 and GR 131) and three cultivars (TN 86, BU 21 and VA 509), were transplanted at Knoxville on June 1 and Greeneville on June 4. The pedigrees of each tobacco entry are listed in Table 1.

In 1988 eight (TI 1406, PDJA 309, TN 86, TN 86 x PDJA 309, BU 21, VA 509, GR 115 and GR 131) of the ten tobacco entries assessed in 1987 were evaluated. The other two tobacco entries (TI 1112 and TN 86 x TI 1112) were not transplanted and evaluated because of poor survival in the plant bed. These eight tobacco entries from 1987 were transplanted at both Knoxville and Greeneville on June 6 and June 3, respectively (Table 2). The pedigrees of each tobacco entry are listed in Table 2.

The cuticular leaf components of eight (TI 1112, TI 1406, PDJA 309, TN 86, TN 86 x TI 1112, TN 86 x PDJA 309, VA 509 and GR 115) of the ten tobacco entries/cultivars evaluated for <u>Heliothis</u> resistance at Greeneville in 1987 were determined on July 21 by Dr. Ray Severson, USDA, Agricultural Research Service, Tobacco Safety Research Unit, Athens, Georgia (Severson 1982). In 1988, Dr. Severson also analyzed the leaf surface chemistries of eight (TI 1406, PDJA 309, TN 86, TN 86 x PDJA 309, VA 509, GR 115, GR 131 and BU 21) tobacco entries/cultivars at Greeneville on July 11.

Entries	Pedigrees	
IN-37	(TN 86 x TI 1112)F1	
IN-38	(TN 86 x PDJA 309)F1	
IN-39	TI 1112	
IN-40	TI 1406	
IN-41	PDJA 309	
BU 21	BURLEY 21	
TN 86	TN 86	
VA 509	VA 509	
GR 115	GR 115	
GR 131	GR 131	

Table 1. Entries and pedigrees of tobacco transplanted in 1987.

Entries	Pedigrees	
IN-38	(TN 86 x PDJA 309)F1	
IN-40	TI 1406	
IN-41	PDJA 309	
BU 21	BURLEY 21	
TN 86	TN 86	
VA 509	VA 509	
GR 115	GR 115	
GR 131	GR 131	

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Table 2. Entries and pedigrees of tobacco transplanted in 1988.

Heliothis larvae were counted by visually examining each tobacco plant once each week at each location from late June to late October in 1987 and from mid-June to mid-October in 1988. At each location, Heliothis larvae were collected each week from two replications; these replications were alternated each week (e.g., replications 1 and 3 one week, replications 2 and 4 the next week). Collected larvae were individually placed in 40ml plastic cups containing a commercial corn earworm diet (Bioserv Inc.) and capped with a cardboard lid. Larvae were taken to the laboratory and measured lengthwise to the nearest millimeter, placed in an insectary (ca. 28⁰C., 14L:10D, ca. 60% R.H.) and reared until moth emergence or death caused by parasitoid, disease or other causes. In 1988, the number of tobacco plants with blooms was counted each week for each entry from late July (when tobacco plants first began blooming) to the end of August.

Data were combined for all entries during each year to determine seasonal incidence of <u>Heliothis</u> larvae on tobacco, as well as the seasonal incidence of parasitoid species. Data were subjected to an analysis of variance procedure to evaluate influence of entries on populations of <u>Heliothis</u> larvae and on levels of parasitism. When significant (p=0.05) differences were observed, Duncan's multiple range test was used to determine significant (p=0.05) differences among the means.

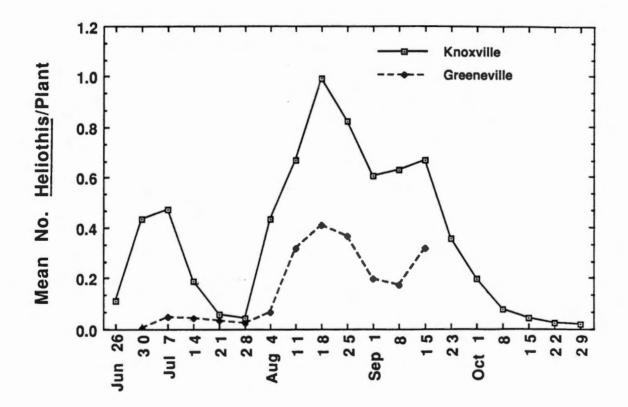
CHAPTER III

RESULTS AND DISCUSSION

i. HELIOTHIS INCIDENCE AND COMPOSITION

In 1987, <u>Heliothis</u> larvae were found in the field from late June to late October. During that time, three peaks of <u>Heliothis</u> larval densities were observed at Knoxville and Greeneville (Figure 1). The first peak occurred in early July, the second in mid-August and the third in mid-September. The greatest density (ca. 1.0 larva/plant) of <u>Heliothis</u> larvae during the season was recorded on August 18 at Knoxville. In general, densities of <u>Heliothis</u> larvae were ca. two to six times greater at Knoxville than at Greeneville. Early in the season, however, densities of <u>Heliothis</u> larvae were much higher (ca. 93 and 10 times greater on June 30 and July 7, respectively) at Knoxville than at Greeneville.

In 1988, <u>Heliothis</u> larvae were found in the field from mid-June to mid-October. During that four month-period, densities of <u>Heliothis</u> larvae peaked twice at Knoxville and Greeneville (Figure 2). Although data collected in 1987 supported the occurrence of three generations of <u>Heliothis</u> during the tobacco-growing season, data for 1988 suggested only two generations of <u>Heliothis</u> on tobacco. In 1988, densities of <u>Heliothis</u> larvae early in the season at Knoxville and Greeneville were greatly reduced from those



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Figure 1. Seasonal incidence of <u>Heliothis</u> larvae on tobacco, Knoxville and Greeneville, 1987.

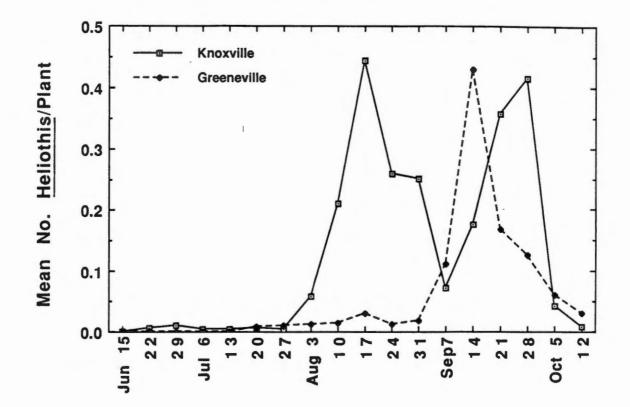


Figure 2. Seasonal incidence of <u>Heliothis</u> larvae on tobacco, Knoxville and Greeneville, 1988.

observed in 1987. In fact, densities of <u>Heliothis</u> larvae in 1988 were about 50% lower (peaking at ca. 0.44 larvae/plant on August 17 at Knoxville) than those in 1987. The low numbers of larvae present early in the season in 1988 may have been partially attributed to the drought. The dry weather which caused the surface of the ground to become hard may have effected adult <u>Heliothis</u> emergence from the soil.

Of the Heliothis larvae collected from tobacco at Knoxville and Greeneville during 1987 and 1988, ca. 83% (n=2945) of those that emerged in the laboratory were H. virescens. During the early and middle periods (mid-June to early August) of the tobacco- growing season, 80 to 100% of the Heliothis larvae collected from tobacco at both locations were H. virescens (Figure 3). By mid-September about one-half of the Heliothis larvae that emerged in the laboratory was H. virescens and the other one-half was H. zea. Later in the season (October), more than 50% of the Heliothis larvae that emerged in the laboratory was H. zea. The increase of H. zea in percent Heliothis composition that occurred later (late August to mid-October) in the season may have been due to H. zea adults moving into tobacco from their preferred host plant (corn) after the corn silks matured (Hunt 1983).

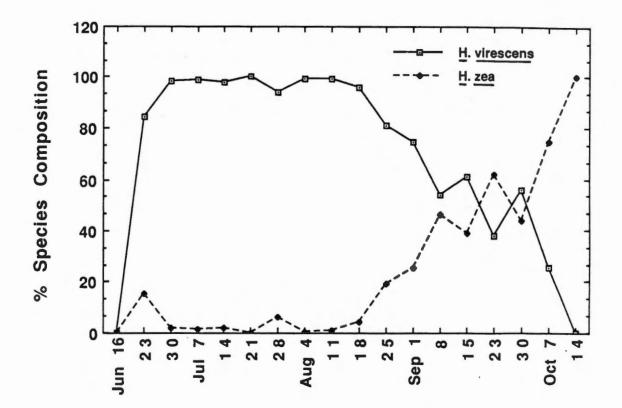


Figure 3. Species composition of <u>Heliothis</u> larvae on tobacco at Knoxville and Greeneville, 1987 and 1988.

ii. PARASITOID INCIDENCE AND SPECIES COMPOSITION

During this study, four species of parasitoids were reared from Heliothis larvae collected from tobacco. These species were Campoletis sonorensis, Cardiochiles nigriceps, Microplitis croceipes and Archytas marmoratus. In addition, several other tachinid parasitoid species (possibly Winthemia rufopicta) were reared from Heliothis larvae. The seasonal occurrence of each parasitoid species reared from Heliothis larvae collected from tobacco is provided in Figure 4. Heliothis larvae parasitized by C. sonorensis were collected from tobacco throughout the season from mid-June to late October (Figure 4). Campoletis sonorensis was the first and last species of parasitoid encountered at Knoxville and Greeneville in 1987 and 1988. Cardiochiles nigriceps was also reared from Heliothis larvae during most of the growing season (late June to late September). Microplitis croceipes, A. marmoratus and the other tachinid species were encountered in the latter part of the growing season.

In 1987, a greater percentage (ca. six times greater) of the <u>Heliothis</u> larvae collected from tobacco early in the season were parasitized by <u>C</u>. <u>sonorensis</u> than those collected later in the season at Knoxville and Greeneville (Table 3). <u>Cardiochiles nigriceps</u> parasitized less than 1% of the <u>Heliothis</u> larvae collected during early, mid or late season. No <u>Heliothis</u> larvae collected early in the season

						7
Parasitoid species	Jun	Jul	Aug	Sep	Oct	
<u>Campoletis</u> sonorensis						
<u>Cardiochiles</u> <u>nigriceps</u>						
<u>Microplitis</u> croceipes						
Archytas marmoratus						
Other tachinid species						

Month

Figure 4. Seasonal incidence of parasitoids reared from <u>Heliothis</u> collected from tobacco at Knoxville and Greeneville, 1987 and 1988.

Table 3. Percent parasitism (number parasitized) of <u>Heliothis</u> larvae collected from tobacco during early, mid and late season at Knoxville and Greeneville, 1987.

Time of Season	<u>Campoletis</u>	<u>Cardiochiles</u>	Microplitis	Archytas	Other Tachinids
Early	65.21%(298)	0.66%(3)	0.00%(0)	0.00%(0)	0.00%(0)
Mid	10.51%(168)	0.69%(11)	0.19%(3)	0.06%(1)	0.19%(3)
Late	10.51%(33)	0.64%(2)	1.91%(6)	0.64%(2)	7.64%(24)
Total	21.06%(499)	0.68%(16)	0.38%(9)	0.13%(3)	1.14%(27)

were parasitized by <u>M</u>. <u>croceipes</u>, <u>A</u>. <u>marmoratus</u> or other tachinid species. <u>Microplitis croceipes</u>, however, did parasitize less than 1% of the larvae collected in midseason and ca. 2% of those collected late in the season. <u>Archytas marmoratus</u> parasitized less than 1% of the larvae collected in mid and late season while other tachinid species (primarily <u>W</u>. <u>rufopicta</u>) parasitized less than 1% of the larvae collected in mid-season and ca. 8% of those collected late in the season.

In 1987, ca. 23% of all <u>Heliothis</u> larvae (n=3375) collected from tobacco throughout the season were parasitized. Of the parasitoid species that emerged from <u>Heliothis</u> larvae during 1987, <u>C</u>. <u>sonorensis</u> was the most abundant species and accounted for ca. 91% of all parasitoid species. <u>Cardiochiles nigriceps</u> and tachinid species other than <u>A</u>. <u>marmoratus</u> accounted for ca. 3 and 5%, respectively, of all parasitoid species (Table 3). Several other parasitoid species (<u>M</u>. <u>croceipes</u> and <u>A</u>. <u>marmoratus</u>) were present in low numbers and accounted for the remainder of the parasitoid complex.

In 1988, <u>C</u>. <u>sonorensis</u> parasitized ca. 63% (n=958) of the <u>Heliothis</u> larvae collected from tobacco at Knoxville and Greeneville during early (mid-June to late July) season (Table 4). Parasitism of <u>Heliothis</u> larvae by <u>C</u>. <u>sonorensis</u> early in the season in 1988 was similar to that observed in 1987. In mid-season (early August to early September) and

Table 4. Percent parasitism (number parasitized) of <u>Heliothis</u> larvae collected from tobacco during early, mid and late season at Knoxville and Greeneville, 1988.

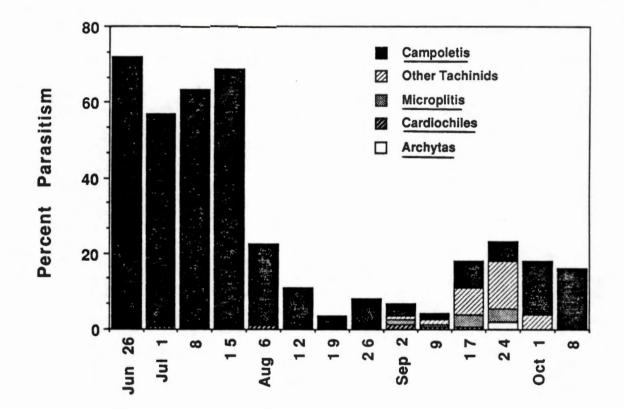
Time of Season	Campoletis	Cardiochiles	Microplitis	Archytas	Óther Tachinids
Early	62.50%(20)	3.13%(1)	0.00%(0)	0.00%(0)	0.00%(0)
Mid	23.15%(178)	1.43%(11)	0.52%(1)	0.52%(1)	0.00%(0)
Late	42.33%(91)	0.93%(2)	0.00%(0)	0.00%(0)	0.00%(0)
Total	28.35%(288)	1.38%(14)	0.10%(1)	0.10%(1)	0.00%(0)

late season (mid-September to mid-October), however, C. sonorensis parasitized ca. 23 and 42% of the Heliothis larvae, respectively. In 1988, levels of parasitism of Heliothis larvae by C. sonorensis were about twice as high in mid-season and four times as high late in the season as in 1987. The parasitism levels of C. nigriceps were also higher (ca. two times greater) in 1988 than in 1987. Approximately 3% of the Heliothis larvae collected early in the season, ca. 1% in mid-season and ca. 1% in late season were parasitized by C. nigriceps. No Heliothis larvae collected early or late in the season were parasitized by either M. croceipes or A. marmoratus. Microplitis croceipes and A. marmoratus, however, each parasitized 0.5% of the Heliothis larvae collected in mid-season. No tachinid species other than A. marmoratus were reared from Heliothis larvae collected from tobacco at any time during the season.

During 1988, percent parasitism of <u>Heliothis</u> larvae by <u>C. sonorensis</u> (ca. 28%) and <u>C. nigriceps</u> (ca. 1.4%) was greater than that recorded in 1987. However, percent parasitism of <u>Heliothis</u> larvae by <u>M. croceipes</u>, <u>A</u>. <u>marmoratus</u> and other tachinid species was lower in 1988 than in 1987. Approximately 95% of the parasitoid species during 1988 were <u>C. sonorensis</u> and most of the remaining species were <u>C. nigriceps</u>. <u>Microplitis croceipes</u> and <u>A. marmoratus</u> comprised less than 1% of the parasitoid complex in 1988. During both years of this study, <u>C. sonorensis</u> was the most

abundant parasitoid species, accounting for ca. 92% of all parasitoids reared from <u>Heliothis</u> larvae collected from tobacco.

Total percent parasitism (combined for all parasitoid species) of Heliothis larvae collected from tobacco at Knoxville (Figure 5) and Greeneville (Figure 6) from late June to early October in 1987 was highest (72.0% at Knoxville and 95.5% at Greeneville) early in the season, lowest (3.5% at Knoxville and 16.2% at Greeneville) in midseason and increased moderately (21.4% at Knoxville and 34.6% at Greeneville) late in the season. The seasonal fluctuations in percent parasitism were similar at both locations in 1987; however, levels of parasitism were generally greater at Greeneville than at Knoxville. In 1988, total percent parasitism at Knoxville (Figure 7) and Greeneville (Figure 8) from mid-June to Mid-October was also highest (100.0% at Knoxville and Greeneville) early in the season, lowest (5.6% at Knoxville and 25.0% at Greeneville) in mid-season and increased moderately (56.8% at Knoxville and 45.5% at Greeneville) late in the season (Figure 6). Parasitism levels were generally higher at Knoxville than at Greeneville early and late in the season, whereas during the middle of the season parasitism levels were higher at Greeneville. In 1988, total percent parasitism was more erratic than in 1987, especially early in the season. These erratic parasitism levels may have resulted from the low



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Figure 5. Percent parasitism and parasitoid species composition of <u>Heliothis</u> larvae collected from tobacco, Knoxville, 1987.

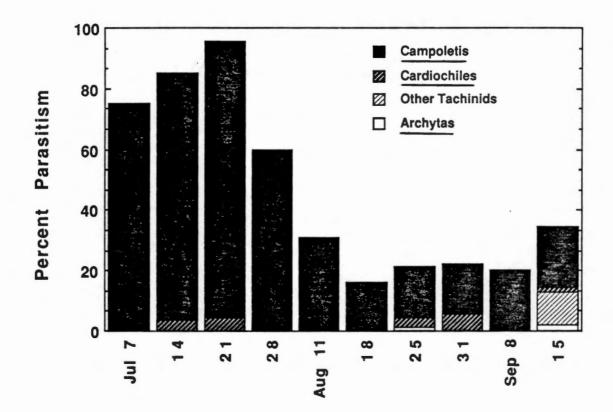
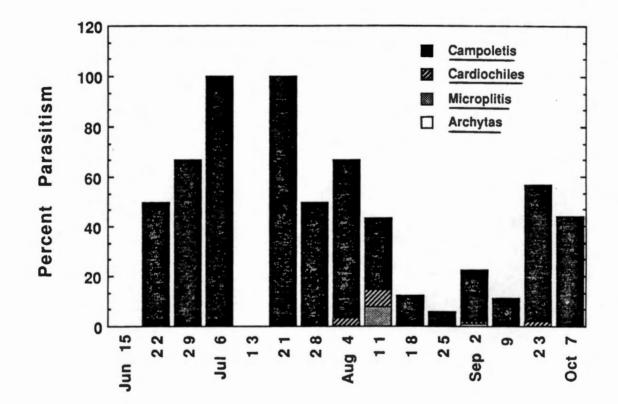


Figure 6. Percent parasitism and parasitoid species composition of <u>Heliothis</u> larvae collected from tobacco, Greeneville, 1987.



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Figure 7. Percent parasitism and parasitoid species composition of <u>Heliothis</u> larvae collected from tobacco, Knoxville, 1988.

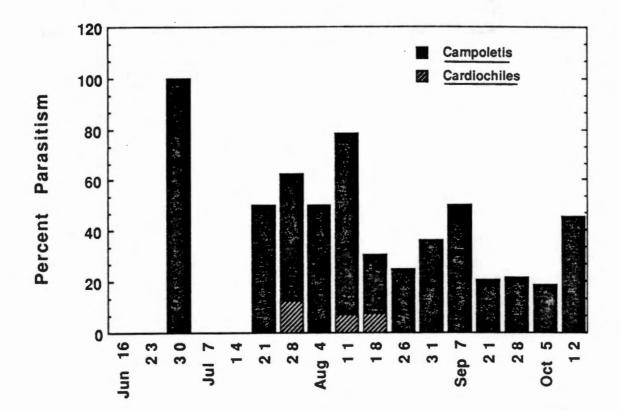


Figure 8. Percent parasitism and parasitoid species composition of <u>Heliothis</u> larvae collected from tobacco, Greeneville, 1988.

numbers of <u>Heliothis</u> larvae found on the plants early in the season (Figures 7 and 8).

In 1987, percent parasitism of Heliothis larvae by C. sonorensis was similar to the total percent parasitism by all parasitoid species until the end of August (Figures 5, 6 and 9). This similarity was not surprising since ca. 91% of all of the parasitoids encountered in 1987 were C. sonorensis (Table 3). In addition, at least 75% of the parasitoid complex encountered each week until the end of August consisted of C. sonorensis at Knoxville and Greeneville. In September, however, parasitoid species other than C. sonorensis (especially tachinid species) comprised a greater percentage of the parasitoid complex than at any other time in the season. After September at Knoxville, the parasitoid composition each week was mainly (>77%) C. sonorensis. Therefore, percent parasitism by C. sonorensis in October was again similar to the total percent parasitism by all parasitoid species at Knoxville.

In 1988, the species composition of parasitoids reared from <u>Heliothis</u> larvae during any week at Knoxville or Greeneville was always greater than 75% <u>C</u>. <u>sonorensis</u> (Figures 7, 8 and 10). As discussed earlier, <u>C</u>. <u>sonorensis</u> accounted for ca. 95% of the total parasitism during 1988 (Table 4). Therefore, the percent parasitism by <u>C</u>. <u>sonorensis</u> was similar to the total percent parasitism by all parasitoids at Knoxville and Greeneville in 1988 (Figure

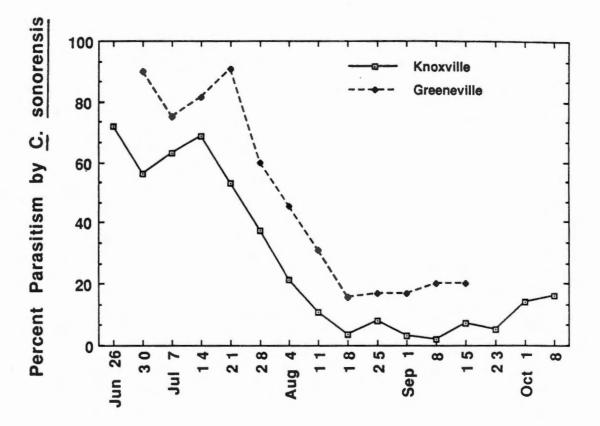
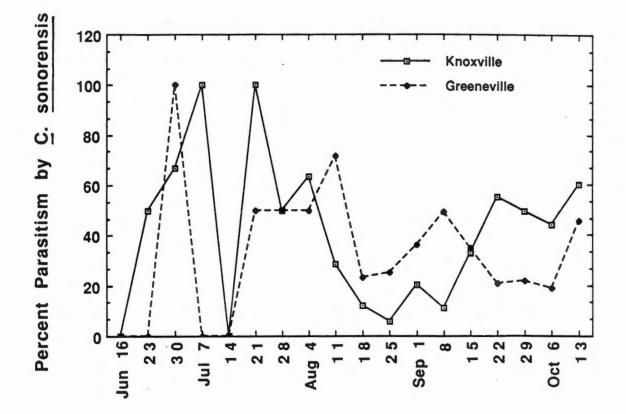


Figure 9. Percent parasitism by <u>Campoletis</u> <u>sonorensis</u> of <u>Heliothis</u> larvae collected from tobacco, Knoxville and Greeneville, 1987.



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Figure 10. Percent parasitism by <u>Campoletis</u> <u>sonorensis</u> of <u>Heliothis</u> larvae collected from tobacco, Knoxville and Greeneville, 1988.

6 and 8).

Percent parasitism of <u>Heliothis</u> larvae by <u>C</u>. <u>nigriceps</u> was highest in late July in 1987 (ca. 4.6%) and early August in 1988 (ca. 6.9%) combined for Knoxville and Greeneville (Figure 11). The maximum parasitism level by <u>M</u>. <u>croceipes</u>, however, was ca. 3.6% in late September (Figure 12). The maximum parasitism level by all tachinid parasitoid species in 1987 was ca. 14.3% in mid- September at Knoxville and ca. 12.7% in late September at Greeneville (Figure 13). As discussed earlier, parasitism of <u>Heliothis</u> larvae by tachinid species in 1988 was insignificant as only one tachinid parasitoid (<u>A</u>. <u>marmoratus</u>) was reared from a <u>Heliothis</u> larva collected on September 1 at Knoxville.

Of the <u>Heliothis</u> larvae collected from tobacco, those parasitized by <u>C</u>. <u>sonorensis</u> were smaller (x = 9.02mm) than those parasitized by any other species (Table 5). <u>Cardiochiles nigriceps</u> and <u>M</u>. <u>croceipes</u> both were reared from larger field-collected larvae than <u>C</u>. <u>sonorensis</u> (x =18.15mm and 23.63mm, respectively). <u>Archytas marmoratus</u> and the other tachinid species were reared from the largest larvae (x = 25.00mm and 27.67mm, respectively). As has already been discussed, Schmidt (1974) reported that <u>C</u>. <u>sonorensis</u> parasitized small larvae and Neunzig (1969) reported that <u>C</u>. <u>nigriceps</u> also preferred to parasitize small larvae. <u>Campoletis sonorensis</u> larvae develop quickly inside their host and kill it while it is small (Schmidt

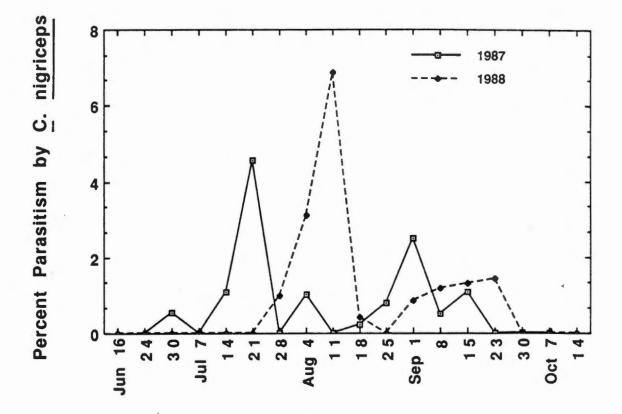


Figure 11. Percent parasitism by <u>Cardiochiles</u> <u>nigriceps</u> of <u>Heliothis</u> larvae collected from tobacco at Knoxville and Greeneville combined, 1987 and 1988.

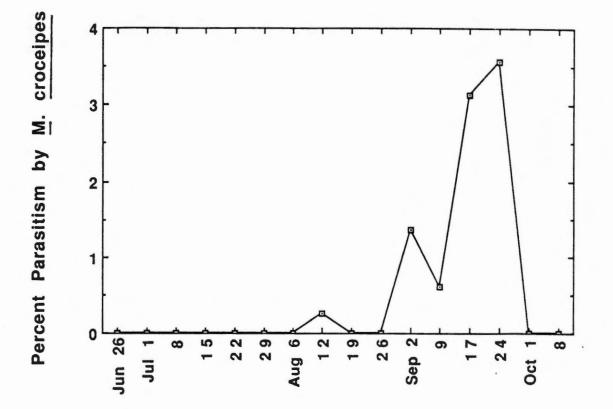


Figure 12. Percent parasitism by <u>Microplitis croceipes</u> of <u>Heliothis</u> larvae collected from tobacco, Knoxville, 1987.

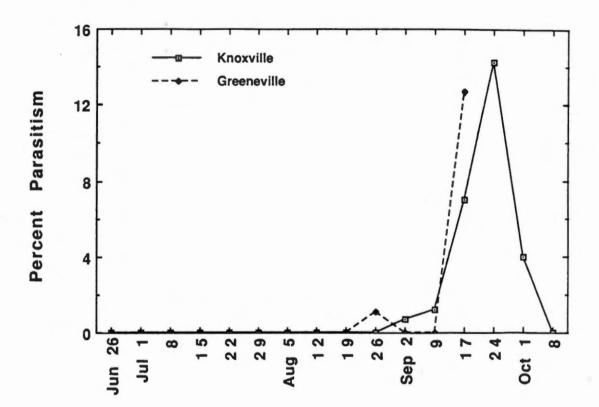


Figure 13. Percent parasitism by all tachinid species of <u>Heliothis</u> larvae collected from tobacco, Knoxville and Greeneville, 1987.

Parasitoid species	No. of larvae parasitized	Mean(+SE) (mm)	Median (mm)	Range (mm)
<u>Campoletis</u> sonorensis	1117	9.02 <u>+</u> 0.09	9	2 to 19
<u>Cardiochiles</u> <u>nigriceps</u>	27	18.15 <u>+</u> 1.40	19	4 to 33
<u>Microplitis</u> croceipes	8	23.63 <u>+</u> 2.32	24	12 to 32
<u>Archytas</u> <u>marmoratus</u>	4	25.00 <u>+</u> 3.34	24	18 to 34
Other Tachinids	27	27.67 <u>+</u> 1.31	30	14 to 37

Table 5. Lengths of field-collected <u>Heliothis</u> larvae parasitized by each parasitoid species, Knoxville and Greeneville, 1987 and 1988.

Lengths of parasitized larvae

1974), and <u>C</u>. <u>nigriceps</u> larvae usually remain in the first instar and do not develop further until their host begins to pupate (Neunzig 1969). This biological information accounts for the size discrepancy of larvae parasitized by <u>C</u>. <u>sonorensis</u> and <u>C</u>. <u>nigriceps</u> as well as the greater size range (4 to 33mm) of hosts parasitized by <u>C</u>. <u>nigriceps</u> in this study (Table 5). <u>M</u>. <u>croceipes</u> has been shown to parasitize larger larvae than those preferred by <u>C</u>. <u>sonorensis</u> and <u>C</u>. <u>nigriceps</u> (Lewis and Burton 1970). As mentioned previously, late instar <u>Heliothis</u> larvae are successfully parasitized more often by <u>A</u>. <u>marmoratus</u> because many parasitoid larvae are lost after the host molts (Hughes 1975). Other tachinid species such as <u>W</u>. <u>rufopicta</u> generally prefer to parasitize last instar larvae (Danks 1975).

iii. INFLUENCE OF SELECTED TOBACCO ENTRIES

ON HELIOTHIS AND PARASITOID SPECIES

Diol duvane diterpene levels ranged from ca. 12.3 to 68.8 micrograms/cm² and monol duvane diterpene levels ranged from ca. 0.2 to 1.2 micrograms/cm² on the five tobacco entries (TN 86 x PDJA 309 [IN 38], PDJA 309 [IN 41], VA 509, TN 86, and TN 86 x TI 1112 [IN 37]) that secreted high levels of exudates at Greeneville in 1987 (Figure 14). These duvane diterpene levels were much higher than on the three tobacco entries (TI 1112 [IN 39], TI 1406 [IN 40], GR

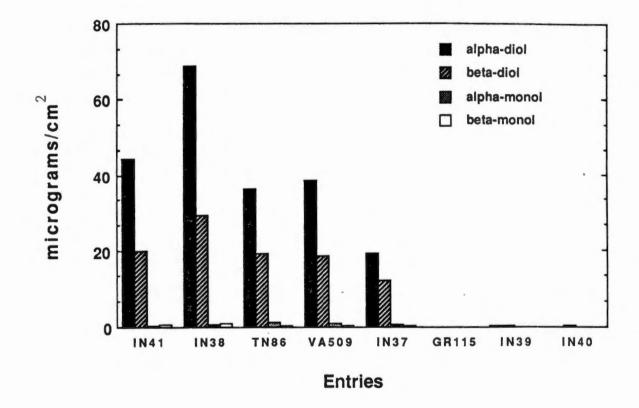
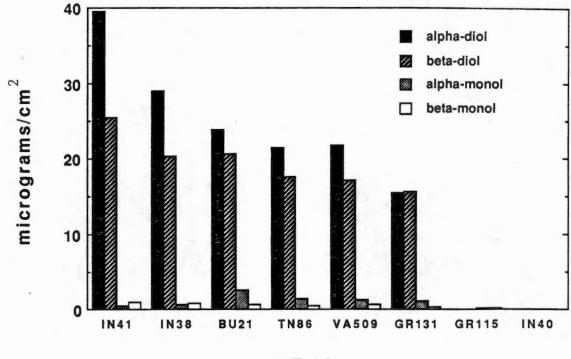
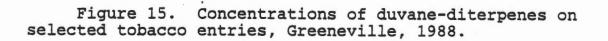


Figure 14. Concentrations of duvane-diterpenes on selected tobacco entries, Greeneville, 1987.

115) that secreted low levels of exudates at Greeneville in 1987. The levels of diol duvane diterpenes on these three low exudate secreters ranged from ca. 0.1 to 0.3 micrograms/cm² while the monol duvane diterpene levels ranged from ca. 0.01 to 0.06 micrograms/cm² at Greeneville in 1987 (Figure 14). In 1988 at Greeneville, diol duvane diterpene levels ranged from ca. 15.5 to 39.6 micrograms/ cm^2 and monol duvane diterpene levels ranged from ca. 0.3 to 2.5 micrograms/cm² on the six tobacco entries (IN 41, IN 38, BU 21, TN 86, VA 509 and GR 131) that secreted high levels of exudates, which were again much higher than levels on the two tobacco entries (IN 40 and GR 115) that secreted low levels of exudates (Figure 15). The diol duvane diterpene levels ranged from below detection limits (<0.01 $micrograms/cm^2$) to ca. 0.2 $micrograms/cm^2$, and all of the monol duvane diterpene levels were below detection limits (Figure 15). Levels of alpha- and beta-diol duvane diterpenes were higher (ca. 10 to 320 times greater in 1987 and ca. 6 to 125 times greater in 1988) than levels of alpha- and beta-monol duvane diterpenes on the tobacco entries that had high levels of duvane diterpenes (Figures 14 and 15). In 1987, IN 38 had the highest levels of alphaand beta-diol duvane diterpenes (ca. 36.3 and 19.4 micrograms/cm², respectively) as well as the highest level of total (alpha- and beta- combined) monol duvane diterpenes (ca. 1.7 micrograms/ cm^2). The maximum levels of alpha- and

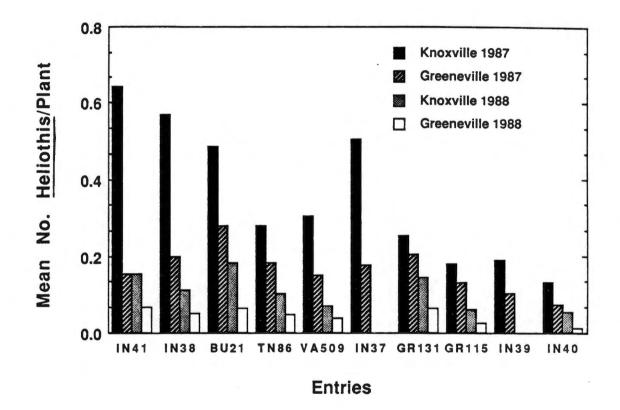






beta- duvane diterpenes were ca. 39.6 and 25.5 micrograms/cm², respectively, on IN 41 in 1988. The highest level of total monol duvane diterpenes (ca. 3.1 micrograms/cm²), however, occurred on BU 21. Of the tobacco entries that secreted high levels of diol duvane diterpenes during 1987 and 1988, IN 38 and IN 41 secreted the greatest levels, BU 21, TN 86 and VA 509 moderate levels, and IN 37 and GR 131 secreted the lowest levels.

The seven tobacco entries (IN 41, IN 38, BU 21, TN 86, VA 509, IN 37 and GR 131) with high levels of duvane diterpenes also had higher densities of Heliothis larvae than those three (GR 115, IN 39 and IN 40) with low levels of duvane diterpenes at Knoxville and Greeneville during 1987 and 1988 (Figure 16). The maximum density (ca. 0.64/plant/week) of Heliothis larvae occurred on IN 41 at Knoxville during 1987. Although IN 41 and IN 38 had the greatest densities of Heliothis larvae at Knoxville in 1987 (corresponding with their high diol duvane diterpene levels), this association was not consistent at both locations and years. Tobacco entries IN 37, IN 39, IN 40 and GR 115 had high densities of Heliothis larvae in relation to their levels of duvane diterpenes. The high densities of Heliothis larvae on these tobacco entries may be due to the early and profuse blooming of these plants, especially IN 39 (TI 1112) and IN 37 (TN 86 x TI 1112). Hardwick (1965) and Neunzig (1969) have reported that



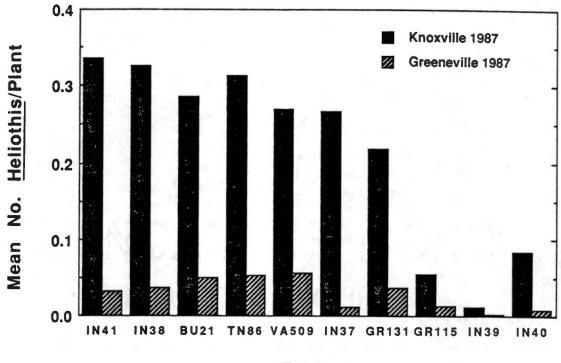
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Figure 16. Densities of <u>Heliothis</u> larvae on selected tobacco entries, Knoxville and Greeneville, 1987 and 1988.

<u>Heliothis</u> spp. prefer host plants in the flowering or fruiting stages to those plants in other stages of development. Flower buds and flowers of tobacco have been found to be preferred over tobacco leaves by <u>H</u>. <u>zea</u> and <u>H</u>. <u>virescens</u> (Johnson et al. 1975, Lingren et al. 1977 and Neunzig 1969).

The effect of tobacco flower buds and flowers on densities of <u>Heliothis</u> larvae has, however, little practical importance, because tobacco plants are topped when they begin to bloom. Therefore, the influence of tobacco entries on densities of <u>Heliothis</u> larvae early in the year (before tobacco plants began to bloom) was observed. The high duvane diterpene secreting tobacco entries supported relatively high populations of <u>Heliothis</u> larvae, whereas those entries that secreted low levels of duvane diterpenes had much lower densities of <u>Heliothis</u> larvae (Figure 17).

The seven tobacco entries (IN 41, IN 38, BU 21, TN 86, VA 509, IN 37 and GR 131) that secreted high levels of duvane diterpenes were classified as "sticky" and the three entries (GR 115, IN 39 and IN 40) that secreted low levels of duvane diterpenes were classified as "non-sticky". In 1987 at Knoxville, densities of <u>Heliothis</u> larvae were greater on the "sticky" tobacco entries than on the "nonsticky" tobacco entries throughout the growing season except on the last collecting date (October 15) (Figure 18). The difference in densities of Heliothis larvae was greatest



Entries

Figure 17. Densities of <u>Heliothis</u> larvae on selected tobacco entries early in the season (late June to late July), Knoxville and Greeneville, 1987.

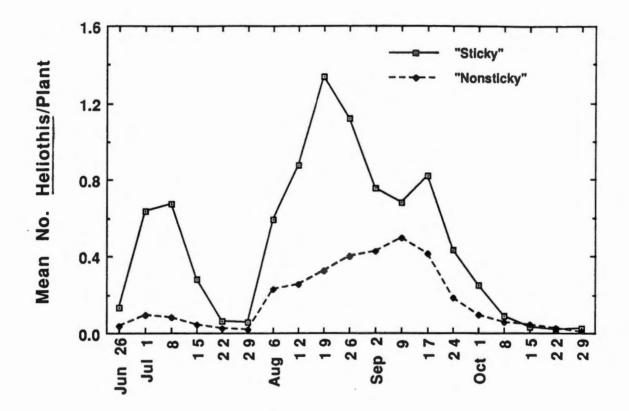


Figure 18. Seasonal incidence of <u>Heliothis</u> larvae on "sticky" and "non-sticky" tobacco entries, Knoxville, 1987.

early in the season (late June to late July), when <u>Heliothis</u> densities on "sticky" tobacco entries were as much as ca. 8 times (July 7) greater than <u>Heliothis</u> densities on "nonsticky" tobacco entries. Later in the season (early August to mid-October), the difference in <u>Heliothis</u> densities was as much as ca. 4 times (August 18) greater on the "sticky" tobacco entries than on the "non-sticky" tobacco entries. The smaller difference in <u>Heliothis</u> densities later in the season may have been due to the blooming of the tobacco plants, which began in late July and early August. At Greeneville in 1987, the results were similar; <u>Heliothis</u> densities were as much as ca. 22 times (July 14) and 3 times (August 18) greater on "sticky" tobacco entries than on "non-sticky" entries early in the season and late in the season, respectively (Figure 19).

In 1988 at Knoxville, densities of <u>Heliothis</u> larvae were also greater on the "sticky" tobacco entries (IN 38, IN 41, VA 509 and TN 86) than on the "non-sticky" entries (IN 40 and GR 115) throughout most of the season (Figure 20). <u>Heliothis</u> densities were as much as ca. 8 times (August 24) greater on "sticky" tobacco entries than on "non-sticky" entries earlier in the season (mid-June to late August) and as much as ca. 2.5 times (September 7) greater on "sticky" tobacco entries than on "non-sticky" tobacco entries later in the season (early September to mid-October). No <u>Heliothis</u> larvae were found on the "non-sticky" tobacco

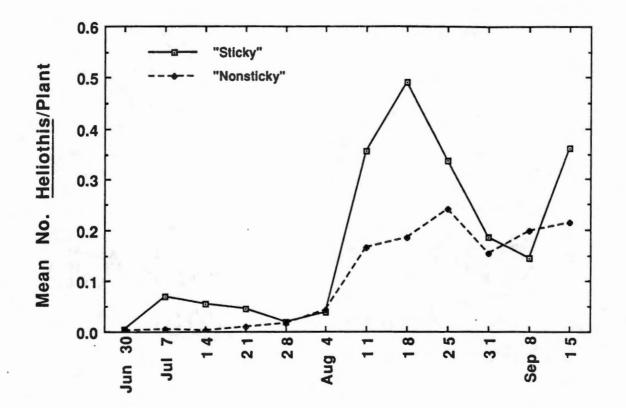
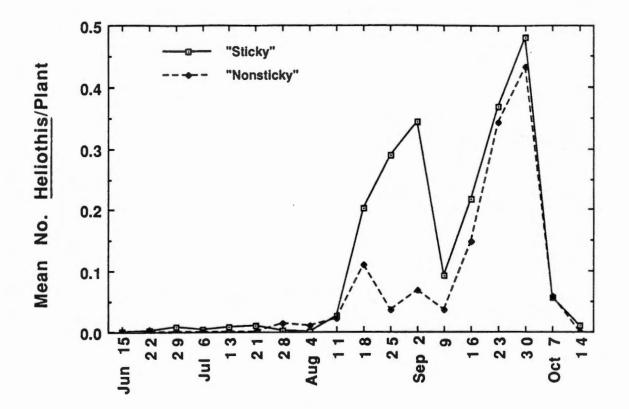
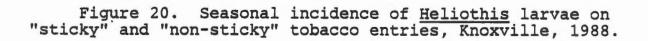


Figure 19. Seasonal incidence of <u>Heliothis</u> larvae on "sticky" and "non-sticky" tobacco entries, Greeneville, 1987.





entries and only six larvae were found on the "sticky" tobacco entries early in the season (mid-June to late July) at Greeneville in 1988 (Figure 21). From early August to mid-October, the difference in densities of <u>Heliothis</u> larvae was as much as five times (September 21) greater on "sticky" tobacco entries than on "non-sticky" entries (Figure 21).

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Tobacco entry influence on percent parasitism of <u>Heliothis</u> larvae by all parasitoid species varied greatly. Although none of the tobacco entries had consistently high parasitism levels, TI 1112 (IN 39) had the lowest parasitism levels at both Knoxville and Greeneville in 1987 (Table 6). In 1987, percent parasitism of <u>Heliothis</u> larvae collected from "sticky" tobacco entries was greater than for those collected from "non-sticky" tobacco entries at both Knoxville and Greeneville. In 1988, however, the parasitism levels were not consistently higher on either the "sticky" or "non-sticky" tobacco entries (Table 6).

Percent parasitism of <u>Heliothis</u> larvae by <u>C</u>. <u>sonorensis</u>, as discussed earlier, was similar to total percent parasitism by all parasitoid species. TI 1112 (IN 39) had the lowest (ca. 1-5%) parasitism levels by <u>C</u>. <u>sonorensis</u> at both Knoxville and Greeneville and the remaining tobacco entries had inconsistent parasitism levels at Knoxville and Greeneville during 1987 and 1988 (Table 7). Percent parasitism by <u>C</u>. <u>sonorensis</u> was also greater on "sticky" entries than on "non-sticky" entries in 1987;

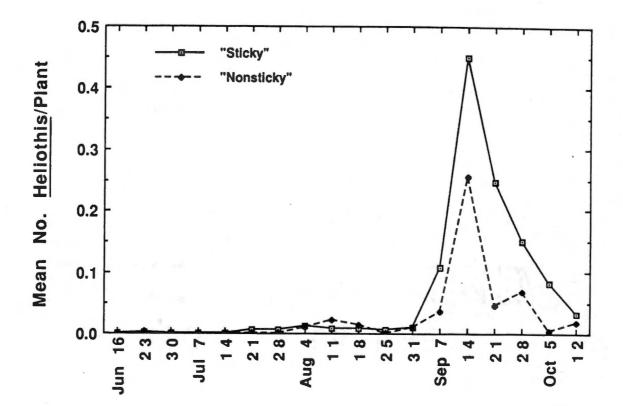


Figure 21. Seasonal incidence of <u>Heliothis</u> larvae on "sticky" and "non-sticky" tobacco entries, Greeneville, 1988.

ry ^a	Knoxville 1987	Greeneville 1987	Knoxville 1988	Greeneville 1988
1	19.44	37.93	14.93	28.13
8				17.39
				45.45
				40.00
			30.00	44.44
				50.00
			38.89	33.33
406	16.67	33.33	30.00	0.00
ckv" ^b	33,35	23.84	25.70	37.06
-sticky"	24.00	15.92	34.21	28.57
	1 8 1 6 09 7 31 15 112 406	1987 1 19.44 8 25.93 1 18.23 6 46.74 09 22.70 7 21.13 31 27.88 15 21.35 112 10.78 406 16.67 cky" ^b 33.35	1987 1987 1 19.44 37.93 8 25.93 41.10 1 18.23 41.18 6 46.74 36.47 09 22.70 26.92 7 21.13 30.00 31 27.88 16.47 15 21.35 33.33 112 10.78 10.00 406 16.67 33.33	1987 1987 1988 1 19.44 37.93 14.93 8 25.93 41.10 19.35 1 18.23 41.18 37.65 6 46.74 36.47 33.33 09 22.70 26.92 30.00 7 21.13 30.00 31 27.88 16.47 20.59 15 21.35 33.33 38.89 112 10.78 10.00 406 16.67 33.33 30.00

Table 6. Influence of tobacco entries on total percent parasitism of <u>Heliothis</u> larvae collected at Knoxville and Greeneville, 1987 and 1988.

"Sticky" entries were IN 41, IN 38, BU 21, TN 86, VA 509,

IN 37, and GR 131.

^C"Non-sticky" entries were GR 115, TI 1112, and TI 1406.

Entry ^a	Knoxville 1987	Greeneville 1987	Knoxville 1988	Greeneville 1988
IN 41	18.59	37.93	14.93	25.00
IN 38	25.25	39.73	19.35	17.39
BU 21	17.24	41.18	36.47	45.45
TN 86	44.57	34.12	33.33	40.00
VA 509	22.70	26.92	30.00	44.44
IN 37	17.53	22.00		
GR 131	26.06	11.76	20.59	50.00
GR 115	17.98	28.21	38.89	33.33
TI 1112	0.98	5.00		
TI 1406	9.26	33.33	25.00	0.00
"Sticky" ^b	22.53	29.86	25.42	36.36
"Non-sticky"	8.98	24.00	31.58	28.57

Table 7. Influence of tobacco entries on percent parasitism by Campoletis sonorensis of Heliothis larvae

^aIN 41 = PDJA 309, IN 38 = TN 86 x PDJA 309, and IN 37 = TN 86 x TI 1112.

^b"Sticky" entries were IN 41, IN 38, BU 21, TN 86, VA 509, IN 37, and GR 131.

^C"Non-sticky" entries were GR 115, TI 1112, and TI 1406.

parasitism levels were again inconsistent in 1988 (Table 7).

Early in the tobacco-growing season, tobacco entries had no apparent influence on parasitism levels by all parasitoid species or <u>C</u>. <u>sonorensis</u> in 1987. The parasitism levels ranged from ca. 42% to ca. 86% at Knoxville (for both total and <u>C</u>. <u>sonorensis</u> parasitism) and from ca. 69% to 100% (for both total and <u>C</u>. <u>sonorensis</u> parasitism) (Table 8). Parasitism levels early in the season were similar for "sticky" and "non-sticky" tobacco entries. Table 8. Influence of tobacco entries on percent parasitism by all parasitoid species and by <u>Campoletis</u> <u>sonorensis</u> of <u>Heliothis</u> larvae collected early in the season (mid-June to late July) at Knoxville and Greeneville, 1987.

	All Parasitoid Species		<u>Campoletis</u> sonorensis	
Entry ^a	Knoxville	Greeneville	Knoxville	Greeneville
	1987	1987	1987	1987
IN 41	70.59	100.00	69.12	100.00
IN 38	64.00	90.00	64.00	90.00
BU 21	52.08	82.35	52.08	82.35
TN 86	55.36	80.00	55.36	66.67
VA 509	58.82	69.23	58.82	69.23
IN 37	85.71	100.00	85.71	100.00
GR 131	66.67	88.89	66.67	88.89
GR 115	41.67	80.00	41.67	80.00
TI 1112	50.00	0.00	50.00	0.00
TI 1406	62.50	100.00	62.50	100.00
"Sticky" ^b	63.91	83.56	63.64	80.82
"Non-sticky" ^C	50.00	87.50	50.00	87.50

^aIN 41 = PDJA 309, IN 38 = TN 86 x PDJA 309, and IN 37 = TN 86 x TI 1112.

^b"Sticky" entries were IN 41, IN 38, BU 21, TN 86, VA 509, IN 37, and GR 131.

^C"Non-sticky" entries were GR 115, TI 1112, and TI 1406.

CONCLUSIONS

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Densities of Heliothis larvae observed on tobacco at Knoxville and Greeneville peaked three and two times from late June to late October in 1987 and 1988, respectively. Data collected during 1987 support the occurrence of three generations of Heliothis populations in eastern Tennessee during the tobacco-growing season; however, data for 1988 suggest only two generations of Heliothis larvae on tobacco. In 1988, the drought may have been partially responsible for the greatly reduced numbers of Heliothis larvae collected early in the season. In addition, environmental conditions (e.g., amount of rainfall and time of season it occurs) may influence Heliothis populations. Densities of Heliothis larvae were much lower in 1988 than in 1987; maximum densities in 1987 and 1988 were ca. 1.00 and 0.44 larvae/plant/week, respectively. Throughout most of the tobacco-growing season, more than 80% of the Heliothis larvae collected from tobacco were H. virescens; the remaining larvae were H. zea. By mid-September, however, about one-half of the Heliothis larvae collected from tobacco was <u>H. zea</u>, and most of the larvae collected in October were H. zea.

<u>Campoletis</u> <u>sonorensis</u>, an ichneumonid wasp, <u>C</u>. <u>nigriceps</u> and <u>M</u>. <u>croceipes</u>, both braconid wasps, and <u>A</u>. <u>marmoratus</u>, a tachinid fly, as well as several other

64

tachinid parasitoid species (possibly <u>W</u>. <u>rufopicta</u>), were all reared from <u>Heliothis</u> larvae collected from tobacco. <u>Campoletis sonorensis</u> was the most commonly encountered parasitoid of <u>Heliothis</u> larvae on tobacco in eastern Tennessee as more than 90% of the parasitoids reared from these larvae were <u>C</u>. <u>sonorensis</u> during 1987 and 1988.

Total percent parasitism of <u>Heliothis</u> larvae was high early in the season, low in mid-season and moderate late in the season. <u>Campoletis sonorensis</u> was an important parasitoid of <u>Heliothis</u> larvae throughout the season, but especially early in the season. <u>Cardiochiles nigriceps</u> was reared mainly from <u>Heliothis</u> larvae collected in early and mid-season; whereas, <u>M. croceipes</u> and the tachinid parasitoids were more abundant late in the season.

Duvane diterpenes are the major components of the leaf surface exudates secreted by burley and flue-cured tobacco grown in the United States. Several tobacco entries are known to be resistant to <u>Heliothis</u> due to the lack of duvane diterpenes secreted by these plants (Johnson and Severson 1984). For example, duvane diterpenes were previously shown to stimulate egg laying by <u>Heliothis</u> on tobacco (Elsey and Chaplin 1978, Jackson et al. 1983, Jackson et al. 1984 and Jackson et al. 1986). In this study, high densities of <u>Heliothis</u> larvae were associated with high levels of leaf surface duvane diterpenes and low densities of <u>Heliothis</u> larvae were associated with low levels of leaf surface

65

duvane diterpenes.

The sticky exudates present on most tobacco entries have been shown to influence parasitism of lepidopterous eggs. For example, these sticky exudates have been reported to deter and/or trap the egg parasitoids of <u>Manduca sexta</u> (Rabb and Bradley 1968). In addition, greater numbers of <u>Heliothis</u> eggs were parasitized on TI 1112, a tobacco breeding line that secretes low levels of sticky exudates, than on NC 95, a flue-cured tobacco that secretes relatively high levels of sticky exudates (Elsey and Chaplin 1978). Parasitoids of <u>Heliothis</u> larvae in this study, however, did not appear to be affected by high or low levels of leaf surface duvane diterpenes.

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VITA

74