ABSTRACT

BOCK, MARIAH JANE. Insect Management in Burley Tobacco in Non-traditional Areas of North Carolina. (Under the direction of Clyde E. Sorenson.)

Since the end of tobacco price supports in 2004, flue-cured tobacco growers in eastern North Carolina have been investigating new agricultural enterprises, including burley tobacco. It is unknown if the pests and diseases common to flue-cured tobacco will be equally problematic for burley tobacco growers in eastern North Carolina. Research was conducted to determine the insect and insect vectored disease pressures on burley tobacco. Studies were conducted over a two year period at the Central Crops Research Station, the Cunningham Research Station, North Carolina State University greenhouses, and in commercial tobacco fields in eastern North Carolina counties.

Incidence of tomato spotted wilt virus (TSWV), an economically important plant virus transmitted by thrips, was examined. Surveys of commercial burley tobacco fields revealed higher TSWV incidence in burley tobacco compared to flue-cured tobacco. Results from research station conducted field studies also showed TSWV incidence was higher in burley tobacco compared to flue-cured tobacco under several agronomic conditions. Tobacco plants treated with imidacloprid had lower TSWV incidence than those plants left untreated. Planting date, although variable, also had an effect on incidence of TSWV. In the greenhouse, mechanically inoculated burley tobacco generally had the highest rate of TSWV infection; infection rates decreased as plant age increased. Colored adhesive traps were used to determine the attraction of thrips to varying colors of burley, flue-cured and Maryland tobaccos. A slightly higher number of thrips were caught using burley colored traps; thus,

color is most likely only a minor contributing factor to higher TSWV incidence in burley tobacco.

The presence of *Heliothis virescens*, the hornworm complex (composed of *Manduca sexta* and *Manduca quinquemaculata*), and *Epitrix hirtipennis* in burley and flue-cured tobaccos was compared. *Heliothis virescens* numbers were greater in flue-cured tobacco compared to burley tobacco; hornworm complex larvae and *E. hirtipennis* exhibited equal presence in both tobacco types. *Heliothis virescens* numbers were higher in plots treated with imidacloprid, while *E. hirtipennis* numbers were lower in these plots. Imidacloprid use did not affect hornworm complex larval presence. Hornworm complex larvae were not affected by transplant date, while *E. hirtipennis* numbers were lower in plots assigned a late transplanting date. *Heliothis virescens* larvae numbers were affected differently by transplant date dependent on the location.

The effect of *M. sexta* larvae in open-air burley curing structures on cured burley yield was assessed. Larvae that enter the curing structure near the fourth instar caused the greatest yield loss to curing burley tobacco plants.

Insect Management in Burley Tobacco in Non-traditional Areas of North Carolina

by
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DEDICATION

For my brother, 1LT Amos Camden Riley Bock.

BIOGRAPHY

Mariah Jane Bock was born on May 6, 1985 to H. Riley and Jill Bock of New Madrid, Missouri. She attended Immaculate Conception Elementary School and New Madrid County Central High School. Growing up she enjoyed spending her summers at Camp Marymount and exploring the outdoors. In 2003 Mariah enrolled at the University of Missouri where she earned her B.S. in Plant Science with an emphasis in plant protection. She was an active member of Pi Beta Phi Sorority and the Mizzou Agronomy Club. In 2007 Mariah enrolled at North Carolina State University. Under the direction of Dr. Clyde Sorenson she began research to further the understanding of insect management in burley tobacco in non-traditional growing areas in North Carolina.

ACKNOWLEDGEMENTS

I feel extremely fortunate to have been a part of the North Carolina State University Department of Entomology. I have gained an enormous amount of knowledge and experience, and have had the opportunity to interact with an elite group of scientists. I would like to thank my advisor, Clyde E. Sorenson. He has acted as an exceptional mentor, sharing his knowledge, and field and extension experiences. I would also like to thank my committee members, Mark R. Abney and Hannah J. Burrack, who have given endless advice, ideas, and feedback. I am especially grateful to those that helped me in the field and laboratory: Alan Stephenson, Anirudh Dhammi, Jessica Lawrence, Phil Dunn, Chris Jernigan, Anna Chapman, Carol Berger, Amanda Beaudoin, Seth Hemphill, and Anna Carpenter.

I would like to thank the North Carolina Tobacco Foundation for providing the funding that made this research possible. Finally, I am thankful for my family who has been there to support me, encourage me, and always remind me no matter how far from home I am to "remember where I came from".

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INTRODUCTION

The tobacco price support program was established by the Agricultural Adjustment Act of 1933 to support tobacco growers and stabilize the price of tobacco. During the years following, changes were made to the Agricultural Adjustment Act of 1933, and the Agricultural Adjustment Act of 1938 was adopted. The program was administered by the United States Department of Agriculture (USDA) and the Farm Service Agency (FSA). The tobacco price support program consisted of market quotas and nonrecourse loans. Market quotas determined the amount of tobacco that could be produced, thus limiting the supply of tobacco and indirectly increasing the price. Market quotas also designated the land on which tobacco could be grown. Nonrecourse loans guaranteed growers a minimum price (Womach, 2005).

Since the end of the tobacco price support program in 2004, many tobacco growers in the eastern two-thirds of North Carolina, who traditionally grew flue-cured tobacco (*Nicotiana tabacum*), are investigating and pursuing new agricultural enterprises. Burley tobacco, previously confined to the far western regions of the state by the aforementioned support program, is a crop of great interest to some of these growers located in the piedmont and coastal plain. However, little is known about burley tobacco production in eastern North Carolina. It is important for growers to understand the different insect and disease pressures they might encounter when planting burley in the more temperate climate of the eastern flue-cured tobacco growing regions. The following review will briefly summarize the current understanding of pest management in flue-cured tobacco in eastern North Carolina, and indicate issues of potential concern for burley production in this region.

Flue-cured and burley tobacco differ genetically, resulting in different physical traits, such as color and thickness of leaves (Legg et al. 1977). The leaves of burley tobacco appear more yellow in color and stand in an upright position, while the leaves of flue-cured tobacco appear green in color and fall perpendicular to the plant. Previous studies have revealed differences in disease progression between burley and flue-cured types of tobacco for some soil born diseases, such as black shank and Granville wilt (Mila and Radcliff 2009).

Tomato spotted wilt virus (TSWV) is an economically important plant virus belonging to the family *Bunyaviridae* and genus *Tospovirus*. It was first identified in the United States in the middle to late 1980's (Culbreath et al. 1991), and in North Carolina tobacco in 1988 (unpublished data, NCSU Plant Disease and Insect Clinic). By 1997 TSWV had been identified in nearly every North Carolina county (Groves et al. 2002).

TSWV has a wide host range, infecting over 900 plant species in addition to tobacco (Peters 1998). In three southeastern North Carolina counties (Duplin, Onslow and Pender) mean incidence of TSWV infected plants ranged from 10 to 15 percent before the year 2000 (Groves et al. 2002). In 1999, Georgia tobacco growers experienced losses of 35 to 40 percent, although some stands experienced loss greater than 70 percent (Williams-Woodward 1999). While losses in North Carolina typically do not reach these very high levels, TSWV incidence has steadily increased since its introduction.

TSWV is transmitted mechanically by seven thrips species worldwide (Whitfield et al. 2005). *Frankliniella fusca* (Hinds), the tobacco thrips, is the most important vector of TSWV in eastern and central North Carolina, while *Frankliniella occidentalis* (Pergande), the western flower thrips, is a locally important vector in the western piedmont and

mountainous region of the state (Eckel et al. 1996). Additional thrips vectors include: *Frankliniella bispinosa* (Morgan), *Frankliniella intonsa* (Trybom), *Frankliniella schultzei* (Trybom), *Thrips tabaci* (Hinds), and *Thrips setosus* Moulton (Whitfield et al. 2005). Other thrips species may be present in tobacco fields without transmitting the virus. First stage thrips larvae obtain the virus by feeding on infected plant tissue (Van de Wetering et al. 1996). Once the thrips has acquired the virus, it remains a vector for its entire life (Ullman 1996).

Tobacco plants infected with TSWV display a range of symptoms, including wilting and yellowing of leaves, ring spots, necrotic lesions, discoloration of leaf veins, and stunting. The majority of tobacco plants infected with TSWV will eventually die. In flue-cured tobacco, susceptibility to TSWV varies with the age of the plant. Young plants, 40 to 75 days after sowing (DAS), have been shown to be more likely to develop local infections than older plants, at 95 to 100 DAS. Systemic infection gradually decreases as plant age increases, with plants at 40 DAS being most susceptible followed by those at 60 to 75 DAS, and 95 to 100 DAS (Mandal et al. 2007).

Two types of chemicals are commonly used to control the spread of TSWV in tobacco. Imidacloprid (Bayer Corp., Kansas City, MO) is a chloronicotinyl insecticide and is recommended for use on flea beetles, aphids, and other sucking insects (Elbert et al. 1990). Imidacloprid can be applied as a greenhouse float tray overspray, as a soil drench after transplanting, or as a foliar insecticide (Groves et al. 2001). Acibenzolar-S-methyl (Syngenta Crop Protection, Inc., Wilmington, DE) is a plant activator and is applied in the form of a soil drench or foliar spray. Acibenzolar-S-methyl induces a plant's natural defense mechanisms

and has antifungal, antibacterial, and antiviral activity. Past studies have shown in order to demonstrate beneficial effects, acibenzolar-S-methyl must be applied no more than seven to nine days prior to transplanting (Csinos et al. 2001).

Several insect pests also pose a threat to burley tobacco growers. *Manduca sexta* (L.), the tobacco hornworm, and *Manduca quinquemaculata* (Haw.), the tomato hornworm, compose the hornworm complex. Both are mid- to late-season defoliating pests of tobacco. In North Carolina two to three generations of the hornworm complex occur during each growing season in the eastern region of the state. Larvae typically first begin to appear in late June and early July when tobacco plants are well developed. If control measures are not implemented when thresholds are reached, larval populations can become very destructive, on occasion completely removing all leaf tissue from tobacco plants. However, if control measures are implemented in a timely manner, plants can compensate for some or all injury sustained (Kolodny-Hirsch and Harrison 1982). The threshold is reached when a single healthy one inch larva is identified per ten plants, or five parasitized larvae are identified per ten plants (Burrack 2010).

As tobacco plants mature, their tolerance to larval hornworm feeding damage increases (Kolodny-Hirsch and Harrison 1986). Plants subjected to defoliation after topping were able to completely compensate for damage that simulated feeding by one larva, while plants subjected to defoliation during the establishment stage, vegetative stage and buttoning stage were only able to partially compensate for damage, resulting in yield reductions of 16%, 26% and 26% respectively (Kolodny-Hirsch et al. 1986). (Topping is a common practice during tobacco production, and is accomplished by removing the plant's apical

flower bud, in order to divert plant energy to vegetative, not reproductive, sinks. The buttoning stage occurs before flowering when the plant has only a flower bud present at the apical meristem.) Some cultural practices can affect hornworm complex populations. For instance, high rates of nitrogen have been documented to create more succulent, dark green plants that increase production of *M. sexta* larvae over that of plants receiving only moderate levels of nitrogen (Reagan et al. 1978).

Late season infestations of the hornworm complex occurring just prior to harvest can result in eggs and small larvae on harvested leaves and plants. In eastern North Carolina larvae that remain on flue-cured leaves at harvest are killed in high temperature curing barns, therefore preventing further damage to curing tobacco leaves. Harvested burley tobacco is not subjected to high temperatures during curing, but is instead cured in open-air structures; thus hornworm complex larvae may continue to feed for an undetermined amount of time. This could be especially problematic for organic tobacco growers who wish to avoid spraying pre-harvest chemical insecticides in order to control hornworm populations.

Previous studies have identified fifth stage *M. sexta* larvae as consuming the greatest amount of leaf material, followed by the fourth, third, second and first instars (Wolcott 1937). Approximately 90 percent of total leaf material consumed by an *M. sexta* larva is eaten by the fifth instar when feeding on dark fired and burley types of tobacco (Gilmore 1938 and Jones and Thurston 1970).

Heliothis virescens, the tobacco budworm, is a well known tobacco pest found throughout the Southeastern United States. *Heliothis virescens* larvae are found in the developing apical meristem of tobacco plants. While feeding on the small, developing

leaves, larvae bore holes into and through the bud. As the injured leaves increase in size and unfurl, they appear ragged and damaged. Larval feeding may also result in premature topping, causing plant stunting and delayed plant maturity (Johnson 1979). *Heliothis virescens* larval numbers have been documented to increase as soil nutrients increase (Girardeau 1969). This is perhaps due to the ovipositing female's preference for greener, lusher plants.

Current *H. virescens* thresholds recommend implementing control when 10 percent of plants are found to be infested with *H. virescens* larvae (Burrack 2010). Yet, recent studies show damage caused by *H. virescens* has little to no effect on flue-cured tobacco yield or quality in North Carolina (Juba et al. 2007). Although growers have been informed of these findings, the presence of *H. virescens* is generally not tolerated by eastern North Carolina tobacco growers, and therefore control methods are still deployed.

Parasitoid wasps, transgenic plants modified to include toxins from *Bacillus thuringiensis*, resistant tobacco cultivars, and chemical insecticides are all proven effective methods of *H. virescens* control. Two Hymenoptera species have been identified as particularly effective parasitoids of *H. virescens*: *Campoletis sonorensis* (Cameron) and *Cardiochilies nigriceps* Viereck. Both species deposit eggs into *H. virescens* larva during the first instar; the resulting parasitoid larvae develop within the host and emerge, resulting in the death of the host larva during its third instar. *Campoletis sonorensis* must parasitize *H. virescens* within the first three to six days of the host's life, before the host larvae becomes too large.

Transgenic plants slow the development of the *H. virescens* larvae; allowing *C. sonorensis* wasps a greater window of opportunity to parasitize *H. virescens* larvae (Johnson and Gould 1992). Unlike *C. sonorensis*, *C. nigriceps* wasps have the ability to parasitize older, larger larvae (Johnson 1997). Transgenic tobacco varieties have yet to be developed for commercial release. Traditionally bred resistant tobacco cultivars sustain less feeding injury and reduce *H. virescens* growth (T. Juba, unpublished data). CU 263 and CU 370 are two resistant flue-cured lines; however due to low yield, growers do not plant either of these varieties.

Epitrix hirtipennis, the tobacco flea beetle, is another common tobacco pest in North Carolina (Metcalf and Underhill 1919). Although *E. hirtipennis* feeds on many members of the plant family Solanaceae, flue-cured tobacco appears to be a preferred host (Martin and Herzog 1987). Adult beetles cause damage by feeding on the leaves of new transplants, while soil dwelling larvae feed on the lower portion of the stem and the plant roots (Duke and Lampert 1986).

Adult *E. hirtipennis* beetles over winter in the soil and emerge in early spring. Emerging females deposit eggs onto the soil surface near tobacco plants (Martin and Herzog 1987). Under field conditions, egg incubation lasts from three to eleven days (Chamberlin et al. 1924); once eggs are mature they appear yellowish in color. Neonates immediately begin their search for food by burrowing into the soil toward the tobacco plant roots; occasionally neonates will begin feeding above ground on young leaves and the stem. Mature larvae create earthen cells in the soil for pupation and remain in the cell for five to six days. Similar to the neonates, the adult beetle emerges and also immediately begins its search for food.

Adult females begin to deposit eggs 12 to 16 days post eclosion and continue to deposit eggs until 52 to 57 days post eclosion. The average life span of *E. hirtipennis* is approximately 70 days post eclosion; although some *E. hirtipennis* live up to 161 days (Martin and Herzog 1987).

Low population densities of adult *E. hirtipennis* during the first three weeks after transplanting have been shown to have adverse effects on flue-cured tobacco; reductions in leaf area through adult feeding decreases photosynthesis, while larval feeding decreases root growth (Semtner 1984). Control of *E. hirtipennis* can be accomplished with the use of several insecticides, including the chloronicotinyl insecticide, imidacloprid (Elbert et al. 1990).

Adult tobacco flea beetles respond to color traps. Higher numbers of *E. hirtipennis* were caught using yellow sticky traps than those colored green, white, blue, or red (Dominick 1971). Semtner (1980) determined that treating tobacco with low rates of nitrogen resulted in a yellowish plant, while treatment with medium and high rates of nitrogen resulted in green plants. Higher numbers of *E. hirtipennis* were found on tobacco plants that received the low rates of nitrogen compared to those that received the medium to high rates of nitrogen.

We hypothesize that *Epitrix hirtipennis* may be more attracted to burley tobacco than flue-cured tobacco due to the yellow color of burley tobacco's leaves. H*eliothis virescens* and hornworm complex adults may prefer to deposit eggs on flue-cured tobacco rather than burley tobacco because of the greener color of flue-cured tobacco's leaves, thus resulting in higher numbers of each pest.

The studies described herein were conducted to determine if burley tobacco is more, less, or equally susceptible to common insect pests and insect-vectored diseases of flue-cured tobacco grown in eastern North Carolina. Field studies were conducted to determine the natural infection rate of TSWV in both tobacco types under field conditions in eastern North Carolina. Commercial surveys were completed to determine the occurrence of TSWV in eastern North Carolina tobacco fields. The incidence of TSWV infection in several manually inoculated tobacco types was assessed, and the attraction of feral thrips populations to the varying tobacco leaf colors was evaluated. Further studies were conducted on the presence of *E. hirtipennis*, the hornworm complex and *H. virescens* in burley and flue-cured types of tobacco under field conditions. Finally, the effects of *M. sexta* on open-air cured burley tobacco yield was measured.

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Chapter I: Incidence of Tomato Spotted Wilt Virus in Burley and Flue-cured Types of Tobacco and Thrips Color Attraction

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Following the end of tobacco price supports in 2004, many North Carolina flue-cured tobacco growers began investigating and pursuing new agricultural enterprises. Burley tobacco, which was previously confined to the far western region of the state, is a crop of great interest to some of these growers, located in the piedmont and coastal plain. However, pest and disease pressures, such as thrips (Thysanoptera: Thripidae) and the tomato spotted wilt virus they transmit, are not well understood in burley types of tobacco in these eastern areas.

Flue-cured and burley tobacco differ genetically, resulting in different physical traits, such as color and thickness of leaves (Legg et al. 1977). The leaves of burley tobacco appear more yellow in color and stand in an upright position, while the leaves of flue-cured tobacco appear green in color and fall perpendicular to the plant. Previous studies have revealed differences in disease progression between burley and flue-cured types of tobacco for some soil born diseases, such as black shank and Granville wilt (Mila and Radcliff 2009).

Tomato spotted wilt virus (TSWV) is an economically important plant virus belonging to the family *Bunyaviridae* and genus *Tospovirus*. It was first identified in the United States in the middle to late 1980's (Culbreath et al. 1991), and in North Carolina tobacco in 1988 (NCSU Plant Disease and Insect Clinic, unpublished data). By 1997 TSWV had been identified in nearly every county of North Carolina (Groves et al. 2002).

TSWV has a wide host range, infecting tobacco and over 900 other plant species (Peters 1998). In some flue-cured tobacco fields in North Carolina, TSWV has caused 30 to 50 percent loss. In three southeast North Carolina counties (Duplin, Onslow and Pender),

mean incidence of TSWV infected plants ranged from 10 to 15 percent before the year 2000 (Groves et al. 2002). Since the introduction of TSWV, incidence has steadily increased.

TSWV is transmitted mechanically by seven thrips species worldwide (Whitfield et al. 2005). The tobacco thrips, *Frankliniella fusca* (Hinds), is the most important vector of TSWV in eastern and central North Carolina, while the western flower thrips, *Frankliniella occidentalis* (Pergande), is a locally important vector in the western piedmont and mountain region of the state (Eckel et al. 1996). Other thrips species may be present in the field without transmitting the virus. First instar thrips larvae obtain the virus by feeding on infected plant tissue (Van de Wetering et al. 1996). Once the thrips has acquired the virus, it remains a vector for its entire life (Ullman 1996).

Tobacco plants infected with TSWV display a range of symptoms, including wilting and yellowing of leaves, ring spots, necrotic lesions, discoloration of leaf veins, and stunting. The majority of tobacco plants infected with TSWV will eventually die. Plant susceptibility to TSWV varies with plant age. Young plants, between 40 to 75 days after sowing (DAS), have been shown to be more likely to develop local infections than older plants, aged 95 to 100 DAS. Systemic infection gradually decreases as plants age increases, with plants aged 40 DAS being most susceptible, followed by 60 to 75 DAS, and 95 to 100 DAS (Mandal et al. 2007).

Two types of chemicals are commonly used to control the spread of TSWV in tobacco. Imidacloprid (Bayer Corp., Kansas City, MO) is a chloronicotinyl insecticide and is recommended for use on flea beetles, aphids, and other sucking insects (Elbert et al. 1990). Imidacloprid can be applied as a greenhouse float tray overspray, as a soil drench after

transplanting, or as a foliar insecticide in the field (Groves et. al 2001). Acibenzolar-S-methyl (Syngenta Crop Protection, Inc., Wilmington, DE) is a plant activator and is applied in the form of a soil drench or foliar spray. Acibenzolar-S-methyl induces a plant's natural defense mechanisms and has antifungal, antibacterial, and antiviral activity. Studies have demonstrated that acibenzolar-S-methyl must be applied no more than seven to nine days prior to transplanting to elicit the material's beneficial effects (Csinos et al. 2001).

As burley tobacco production increased in the eastern region of the state after 2004, some growers and county extension personnel noticed an apparent higher incidence of TSWV infection in burley fields compared to nearby flue-cured fields. We hypothesized that TSWV transmitting thrips may be more attracted to the yellow color of burley tobacco's leaves, therefore leading to higher TSWV incidence in burley types of tobacco. The studies reported herein were conducted to elucidate the response of burley and flue-cured tobaccos to TSWV under the growing conditions in eastern North Carolina, and to characterize the nature of any differences detected.

Materials and Methods

TSWV Incidence in Burley and Flue-cured Tobaccos

The incidence of TSWV under different agronomic conditions was examined through field studies conducted at two locations during the summers of 2008 and 2009. Field sites were located at the Cunningham Research Station in Kinston, NC and the Central Crops Research Station in Clayton, NC. The experimental design consisted of eight treatments: burley or flue-cured tobacco types, greenhouse treatment with imidacloprid or no treatment

with imidacloprid, and an early or late planting date. Each treatment was replicated four times within the field in a randomized complete block design; tobacco type was superimposed by interplanting the tobaccos as explained below.

NC 7 burley tobacco and NC 71 flue-cured tobacco varieties were used throughout all field trials. Seeds were sown in polystyrene float trays, 288 cells per tray, in greenhouses dedicated to tobacco transplant production at both locations. (It is common practice to sow tobacco seeds in the greenhouse, allowing the plants to become established seedlings before transplanting to the field.) Half of the plants of each tobacco type were pretreated with imidacloprid, while the other half remained untreated. Imidacloprid (AdmirePro®, Bayer Corp., Kansas City, MO) was applied as a soil drench to the float trays at 0.8 oz of formulated product per 1000 plants two to three days before transplanting for appropriate plots.

The field at each location consisted of sixteen plots arranged in a 4 x 4 randomized complete block (Figure 1.1). Each plot measured 16 rows wide and 15.24 meters in length. Within each plot the number of plants per row ranged from 20 to 25. At the Cunningham Research Station rows were 1.12 meters apart and plants within rows were 0.56 meters apart, while at the Central Crops Research Station between row spacing measured 1.14 meters and within row plant spacing measured 0.56 meters. Planting date and insecticide treatment were applied to the entire plot. Tobacco type was superimposed within each plot; burley and flue-cured tobacco was planted in alternating pairs of rows (i.e. two rows of burley followed by two rows of flue-cured, and so forth, for a total of eight rows of each tobacco type). This was

done to account for any uneven thrips distribution and resultant TSWV incidence that might have occurred within the field.

At each location transplanting was completed on two dates, two weeks apart. In 2008, transplanting at the Cunningham Research Station occurred on April 23 and May 8 and at the Central Crops Research Station on May 1 and May 14. In 2009, transplanting at the Cunningham Research Station occurred on April 14 and April 29 and at the Central Crops Research Station on April 27 and May 11. Tractor mounted mechanical transplanters were used during both years at both locations.

All plants in all plots were visually inspected for the presence of TSWV symptoms weekly. In 2008 surveys were conducted from May 8 to July 7; in 2009 surveys were conducted from May 12 to July 1. All plants displaying visual symptoms of TSWV at each weekly examination were recorded. In 2009 plants displaying visual symptoms of TSWV were also marked with a colored flag; this allowed us to more accurately account for plants dead from TSWV later in the season.

Statistical analyses were completed using SAS® software Version 9.1 (SAS Institute, Cary, NC). Data from the entire season at each location was pooled and subjected to an analysis of variance (ANOVA) procedure (PROC GLM); with means separation through an LSD test (LSMEANS).

TSWV Commercial Surveys

With the help of North Carolina Cooperative Extension Agents, commercial growers in the southeastern region of the state growing a tobacco type other than flue-cured were identified and contacted. Visual surveys were conducted in neighboring burley, flue-cured

and Maryland tobacco fields during the summers of 2007, 2008 and 2009 to determine the incidence of TSWV infection in commercial tobacco in eastern North Carolina. Neighboring fields were as close as several meters and as far apart as approximately three kilometers. Transplant date, tobacco variety and insecticide use information were collected from each grower when possible.

In 2007 survey sites were located in Sampson, Wilson, Johnston, and Duplin counties. A total of eight fields, four flue-cured and four burley, were surveyed. Fields in Sampson County were surveyed on July 24, while fields in Wilson, Johnson and Duplin counties were surveyed on August 1. At each field site four, 100 plant samples were randomly selected. Samples were visually surveyed, and the number of plants apparently infected with TSWV, as indicated by visual symptoms, per 100 plants was recorded.

In 2008 survey sites were located in Sampson, Wilson, Edgecombe, Johnston, and Duplin counties. A total of fifteen fields were surveyed, six flue-cured, seven burley and two Maryland. Fields in Sampson County were surveyed on July 29, fields in Edgecombe and Wilson counties were surveyed on August 1, and fields in Johnston and Duplin counties were surveyed on August 8. At each field site four, 200 plant samples were randomly selected. Samples were visually surveyed, and the number of plants apparently infected with TSWV, as indicated by visual symptoms, per 200 plants was recorded.

In 2009 survey sites were located in Sampson, Wilson, Johnston, and Duplin counties. A total of nineteen fields were surveyed, nine flue-cured and ten burley. Fields in Sampson and Wilson counties were surveyed on July 15, fields in Johnston County were surveyed on July 23, and fields in Duplin County were surveyed on July 30. At each field

site four, 200 plant samples were randomly selected. Samples were visually surveyed, and the number of plants apparently infected with TSWV as indicated by visual symptoms per 200 plants was recorded.

Statistical analyses were completed using SAS® software Version 9.1 (SAS Institute, Cary, NC). Individual site data and yearly pooled data were subjected to an analysis of variance (ANOVA) procedure and Fisher's LSD.

TSWV Greenhouse Study

Field trials and commercial surveys indicated an apparent higher TSWV incidence in field grown burley tobacco compared to flue-cured tobacco (see results below). These observations could be due to susceptibility to the virus in burley tobacco, to differential behavioral responses to the tobacco types by the vectoring thrips, or to a combination of these factors. Two greenhouse trials utilizing mechanical inoculation were conducted to determine if burley tobacco is more susceptible to TSWV than flue-cured tobacco. In the first trial, three tobacco types were selected: NC 7, SC 58 and SC 58-modified. NC 7 is a popular burley tobacco hybrid known for general disease resistance and high yield. SC 58 and SC 58-modified are nearly isogenic lines (NILs) of the flue-cured tobacco SC 58. They differ in the presence or absence of recessive alleles at the Yellow Burley 1 (Yb1) and Yellow Burley 2 (Yb2) loci that contribute to a chlorophyll deficient phenotype (Ramsey Lewis, personal communication). Thus SC 58-modified is a burley type of tobacco.

Tobacco seed of each variety was sown on August 26, September 15 and September 30 of 2008 in the greenhouse. On each date three large black plastic pots were filled with Fafard® (Fafard, Inc., Agawam, MA) soil mixture and watered until thoroughly

moist. Seeds of each tobacco type were sown by hand into the pots and covered with 0.5 cm of vermiculite. Seeded pots were protected with a covering of white cheesecloth held secure with a large rubber band to reduce the disturbance of germinating seeds during watering. The cheesecloth remained on the pots until the tobacco seedlings reached approximately 2 cm in height. When plants reached nearly 5 cm in height, twenty-five seedlings from each tobacco type were transplanted into individual pots. Plants were watered daily and fertilized using the recommended rate of a 20-20-20, water soluble fertilizer (The Scotts Company LLC, Marysville, OH) weekly.

On November 28, 2008 the plants seeded on August 26, September 15, and September 30 had reached 95, 75, and 60 days after seeding (DAS) respectively. Twenty plants, or all plants available up to 20 plants, from each tobacco type and seeding date were mechanically inoculated with TSWV obtained from an *Emilia sonchafolia* plant containing the Parker TWSV isolate.

TSWV inoculum was prepared the day of plant inoculation. First, a clean mortar and pestle were chilled in the refrigerator, and a small cooler of ice was set aside. Next, a buffer solution was prepared by weighing and combining Na_2SO_3 with L-cysteine hydrochloride in a 500 ml beaker. The beaker was covered with paraffin wax film (Parafilm M®, Alcan Inc., Menasha, WI) and placed in the cooler. Finally, three to five leaves were removed from a TSWV-infected *E. sonchafolia* plant. All but one leaf was wrapped in a moist paper towel and placed in the cooler alongside the prepared buffer. The remaining leaf was divided into several small pieces and combined with a portion of the buffer solution and small amount of

carborundum powder (Fisher Scientific, Hampton, NH) in the cooled mortar. Using the cooled pestle, the leaf tissue was ground for several minutes, creating the final inoculum.

The inoculum was applied using a cotton swab (Q-tip®, Unilever U.S., Englewood Cliffs, NJ) to the two newest fully expanded leaves of each plant. Inoculum was applied to an area approximating the size of the smallest fully expanded leaf from the 60 DAS plants, approximately 2.5 cm x 5 cm, on each treated leaf. Following inoculation, plants were misted with water to remove excess carborundum powder. Plants were then monitored daily for the following twenty-five days. The number of leaves with localized signs of infection, the number of leaves with systemic infection, and the total number of leaves per plant were recorded. (Systemic infection occurs when the virus has moved from a localized lesion into the veins of the plant.)

The second trial included four tobacco types. In addition to the original three tobacco types, TN 90 was also included in the study. Like NC 7, TN 90 is a popular burley tobacco variety planted in North Carolina. Tobacco plants were started on January 30, February 19, and March 6 of 2009. The procedures described above were used to produce transplants. On May 5 the plants seeded on January 30, February 19, and March 6 had reached 95, 75, and 60 DAS, and were ready for inoculation. From the plants seeded on January 30 only the NC 7 and TN 90 were inoculated with the virus; due to soil-borne fungal disease neither the SC 58 nor SC 58-modified of this age were healthy enough to use in the experiment. Again, plants were monitored daily for the twenty-five days following inoculation, and the number of leaves with localized signs of infection, the number of leaves with systemic infection, and the total number of leaves per plant were recorded.

After completion of the second trial, an ELISA (enzyme-linked immunosorbent assay) was performed to determine whether tobacco plants displaying no TSWV symptoms were truly virus free. Four asymptomatic plants from each of the ten treatments were randomly selected, along with three uninfected plants, which had not been inoculated, as negative controls, and three symptomatic plants as positive controls. Three small (approximately 1 cm x 1 cm) leaf samples from each plant were then collected. In the laboratory, a pre-coated plate was read at 405 nm, and the plate wells were numbered 1 through 46. All plant tissue samples were ground, and the sap from each was combined with an extraction buffer. 100 ml of the sap and extraction buffer solution was transferred to its assigned well, and the plate was stored at 4 degree Celsius in a sealed container. After 24 hours the plate was emptied, rinsed with a phosphate buffered saline- Tween (PBS- Tween), and dried thoroughly. 100 ml of ECI/anti-TSWV solution was added to each well, and the plate was allowed to stand at room temperature in the sealed container. After two hours the plate was again emptied, rinsed with PBS-Tween, and dried thoroughly. 100 ml of PNP solution was added to each well, and the plate was again allowed to stand at room temperature in the sealed container. After one hour, 1 drop of stop solution was added to each well. Well contents that turned yellow were positive for TSWV. The plate was read a final time at 405 nm.

Statistical analyses were completed using SAS® software Version 9.1 (SAS Institute, Cary, NC) on the mean number of locally infected leaves and mean number of systemic leaves per treatment over the course of the 25 days following inoculation. Data was subjected to an analysis of variance (ANOVA) procedure and Fisher's LSD.

Colored Adhesive Trap Study

Potential color preference in feral thrips populations for varying shades of green represented by different tobacco types was assessed through trapping studies. Digital photographs of healthy burley, flue-cured and Maryland tobacco leaves under ambient, clear sky conditions were taken on August 1, 2008. Color prints of swaths of leaf area from these pictures were produced on an inkjet printer (Hewlett-Packard Officejet 5510 All-in-one, Hewlett-Packard, Palo Alto, CA). The prints were then used to color match paints (Ultra Premium Semi-Gloss, Valspar Corporation, Minneapolis, MN) in the three shades (Figure 1.2) represented by the different tobacco types, at a commercial home improvement store. Fifteen wooden alightment traps were built using plywood squares measuring 30 cm by 30 cm and 44.5 cm wooden stakes. The plywood squares were connected perpendicularly to the wooden stakes using drywall screws so that the surface of the plywood lay flat. Next, the top of each plywood trap was painted with one of the three shades of green; five replications were made of each color. Large sheets of clear adhesive paper (Great Lakes IPM, Inc., Vestaburg, MI) were cut into 12 cm by 12 cm squares; the squares were then attached with double-sided tape to the center of each trap.

Two trials were conducted at the Central Crops Research Station. Traps were positioned on bare soil, near flue-cured and burley tobacco, in a 3 by 5 randomized complete block. Traps were spaced three meters apart. The adhesive squares on each trap surface were replaced and the traps were re-randomized every seven days. The initial trial began on May 28, 2009 and remained in the field for four weeks. A second trial was initiated on

September 23, 2009 and remained in the field for three weeks. Traps were given a fresh coat of paint before the second trial.

Removed adhesive squares were covered with clear plastic (Ziploc®, SC Johnson, Racine, WI) and stored in a laboratory freezer. The adhesive squares were later examined under a stereo microscope, and the number of thrips per each adhesive square was recorded. Due to time constraints, no attempt to identify thrips to species was made.

Statistical analyses were completed using SAS® software Version 9.1 (SAS Institute, Cary, NC). Data was subjected to an analysis of variance (ANOVA) procedure and Tukey's HSD when needed.

Results

TSWV Incidence in Burley and Flue-cured Tobaccos

Due to low rates of TSWV infection at the Central Crops Research Station throughout both field seasons, in addition to heavy plant damage caused by severe weather in 2009, all data analyzed in this study was generated at the Cunningham Research Station. TSWV infection at Central Crops Research Station was less than 1.6% per plot on July 15, 2008; no data was collected in 2009 due to early season weather injury.

In 2008, plots transplanted early had a higher percentage of infected plants per plot than the plots transplanted two weeks later ($F = 278.56$, $df_N = 1$, $df_D = 33$, $P < .0001$) (Figure 1.3). Plots receiving a pretreatment of imidacloprid had a lower percentage of infected plants per plot than plots not receiving imidacloprid ($F = 43.30$, $df_N = 1$, $df_D = 33$, $P < .0001$). Burley tobacco plants had a higher percentage of infected plants per plot than the flue-cured

tobacco plants ($F = 260.59$, $df_N = 1$, $df_D = 33$, $P < .0001$); infection in burley tobacco was approximately twice that of infection in flue-cured tobacco.

In 2009, plots transplanted on the late planting date had a higher percentage of infected plants per plot than plots transplanted two weeks earlier ($F = 26.40$, $df_N = 1$, $df_D = 29$, $P < .0001$). Plots receiving a pretreatment of imidacloprid had a lower percentage of infected plants per plot than plots not receiving imidacloprid ($F = 125.25$, $df_N = 1$, $df_D = 29$, $P < .0001$). Burley tobacco plants again had a higher percentage of infected plants per plot than the flue-cured ($F = 282.62$, $df_N = 1$, $df_D = 29$, $P < .0001$). Infection in burley tobacco plants was approximately twice that of infection in flue-cured tobacco plants. Overall TSWV infection was greater in 2009.

TSWV Commercial Surveys

In 2007 a significant difference was identified between mean incidence of TSWV infected burley and flue-cured tobacco at locations in Duplin County ($F = 55.05$, $df_N = 1$, $df_D = 6$, $P = 0.0003$), Johnston County ($F = 10.29$, $df_N = 1$, $df_D = 6$, $P = 0.0184$), Sampson County ($F = 9.14$, $df_N = 1$, $df_D = 6$, $P = 0.0233$), and Wilson County ($F = 10.26$, $df_N = 1$, $df_D = 10$, $P = 0.0094$). In 2008 a significant difference was identified between mean incidence of TSWV infected burley and flue-cured tobacco at locations in Sampson County ($F = 55.21$, $df_N = 1$, $df_D = 10$, $P < .0001$), Duplin County ($F = 25.22$, $df_N = 1$, $df_D = 6$, $P = .0024$), Wilson County- BBQ ($F = 19.09$, $df_N = 1$, $df_D = 6$, $P = .0047$) and Wilson County- Contentnea Creek ($F = 11.19$, $df_N = 1$, $df_D = 6$, $P = .0155$), and between mean incidence of TSWV infected burley, flue-cured and Maryland tobacco in Edgecombe County- Old ($F = 4.83$, $df_N = 2$, $df_D = 9$, $P = .0376$). A significant difference was not identified between mean incidence of

TSWV infected burley and flue-cured tobacco in Johnston County or in Edgecombe County-Young. In 2009 a significant difference was identified between mean incidence of TSWV infected burley and flue-cured tobacco at locations in Johnston County- C. Church Rd ($F = 30.38$, $df_N = 1$, $df_D = 6$, $P = .0015$), Johnston County- Raleigh Rd ($F = 18.38$, $df_N = 1$, $df_D = 6$, $P = .0052$), Johnston County- Langdon ($F = 53.57$, $df_N = 1$, $df_D = 6$, $P = .0003$), Sampson County ($F = 83.33$, $df_N = 1$, $df_D = 6$, $P < .0001$), Wilson County ($F = 35.27$, $df_N = 1$, $df_D = 6$, $P = .0010$), Wilson County- water tower ($F = 12.26$, $df_N = 1$, $df_D = 6$, $P = .0128$), Duplin County ($F = 17.47$, $df_N = 1$, $df_D = 7$, $P = .0019$), and Duplin County- Sandridge Rd ($F = 66.65$, $df_N = 1$, $df_D = 6$, $P = .0002$). A significant difference was not identified between mean incidence of TSWV infected burley and flue-cured tobacco in Wilson County- Wilco Rd (Table 1.1). Burley tobacco fields generally had a significantly higher incidence of TSWV infected plants when compared to nearby fields of flue-cured and Maryland tobaccos.

When the total infection rate for each tobacco type during each year was analyzed, a significant difference was identified between tobacco types for 2007 ($F = 13.86$, $df_N = 1$, $df_D = 34$, $P = .0007$), 2008 ($F = 11.95$, $df_N = 2$, $df_D = 61$, $P < .0001$), and 2009 ($F = 18.57$, $df_N = 1$, $df_D = 72$, $P < .0001$).

TSWV Greenhouse Study

Significant differences in mean percent localized TSWV infection were detected among treatments for the first trial ($F = 125.23$, $df_N = 4$, $df_D = 1306$, $P < .0001$). NC 7 tobacco infected at 60 DAS exhibited the highest rate of TSWV infection at approximately 50 percent (Figure 1.4). Significant differences in mean percent localized TSWV infection were also detected among treatments in the second trial ($F = 95.21$, $df_N = 5$, $df_D = 1548$, $P <$

.0001). SC 58-modified and TN 90 tobaccos infected 60 DAS exhibited the highest rates of TSWV infection at 43 to 45 percent (Figure 1.5).

Significant differences in the occurrence of systemically infected leaves were detected among treatments for the first trial ($F = 73.0$, $df_N = 4$, $df_D = 1306$, $P < .0001$). NC 7 tobacco infected at 60 DAS exhibited the highest rate of systemically infected leaves at 38 percent (Figure 1.6). Significant differences in the occurrence of systemically infected leaves were again detected among treatments in the second trial ($F = 88.94$, $df_N = 5$, $df_D = 1548$, $P < .0001$). SC 58-modified and TN 90 tobaccos infected at 60 DAS exhibited the highest rate of systemic leaves at approximately 40 percent (Figure 1.7).

ELISA results confirmed that aerial portions of plants not exhibiting TSWV-like symptoms were indeed virus free.

Colored Adhesive Trap Study

Results from trial 1 identified a significant difference ($F = 3.57$, $df_N = 2$, $df_D = 54$, $P = .0350$) between the mean number of thrips per trap for the traps painted with burley tobacco colored paint and traps painted with Maryland tobacco colored paint (Figure 1.8). No significant difference was indentified between traps painted with burley colored paint and traps painted with flue-cured colored paint, or between the traps painted with Maryland colored paint and traps painted with flue-cured colored paint. Mean number of thrips per trap color for burley, flue-cured and Maryland tobacco colored traps were 19.72, 17.32 and 10.10, respectively.

Results from trial 2 identified no significant differences among any of the colors. Mean number of thrips per trap color for burley, flue-cured and Maryland tobacco colored

traps were 4.07, 3.21, and 3.60, respectively. Burley tobacco colored traps had the highest mean number of thrips per trap for both trial 1 and trial 2.

Discussion

TSWV Incidence in Burley and Flue-cured Tobaccos

Transplant date, insecticide treatment and tobacco type affected the mean incidence of TSWV infected plants during the 2008 and 2009 growing seasons. In 2008 the late planting date had lower incidence of TSWV infection while in 2009 the early planting date had lower incidence of TSWV infection. It is suspected that by selecting a transplant date that avoids major thrips flights, incidence of TSWV infected plants can be lowered (Amanda Beaudoin, personal communication). Established, healthy plants are more likely to resist TSWV infection (Mandal et al, 2007).

Plots receiving pre-transplant treatments of imidacloprid had a lower mean incidence of TSWV infected plants. Imidacloprid continues to act as an effective means of chemical control, reducing the number of thrips feedings and the duration time of each feeding (Groves et al. 2001). TSWV incidence reductions of 50 percent were observed in some imidacloprid-treated plots (Figure 1.3)

Burley tobacco plants had a higher mean incidence of TSWV infection than flue-cured tobacco plants. On most survey dates burley tobacco had nearly twice the incidence of infection when compared to the same treatment of flue-cured tobacco plants. This could be a result of greater susceptibility of burley types of tobacco to TSWV, and/or a greater attraction of thrips to the lighter, yellowish color of burley tobacco types.

Overall TSWV incidence was greater in 2009. This could be a result of greater disease pressure, or due to more accurate TSWV assessment with the use of flags to mark dead plants. Throughout both field seasons, TSWV incidence at the Central Crops Research Station remained very low. Southeastern North Carolina apparently continues to have higher TSWV pressure than the central region of the state.

TSWV Commercial Surveys

Generally, commercial fields of burley types of tobacco had a higher mean incidence of TSWV infected plants when compared to nearby commercial fields of flue-cured and Maryland types of tobacco in 2007, 2008 and 2009. This is most likely a result of greater susceptibility of burley types of tobacco to TSWV, and/or a greater attraction of thrips to the light green, almost yellow color of burley types of tobacco's leaves.

Tobacco fields treated with chemicals containing imidacloprid or acibenzolar-S-methyl generally had lower rates of infection than fields left untreated. The highest rate of TSWV infection occurred in a field of KT 204 burley tobacco in Duplin County in 2007 at 41%. Duplin County continued to have high rates of infection in 2008 and 2009. Duplin County was the most southeastern county included in our survey, and historically has high TSWV incidence.

TSWV Greenhouse Study

Burley types of tobacco exhibited the greatest mean percent of TSWV infection and the greatest mean percent of systemic leaves when infected at 60 DAS during trial 1 and trial 2. This suggests that burley types of tobacco are more susceptible to TSWV at a young age. The type of tobacco exhibiting the greatest mean percent of TSWV infection and greatest

mean percent of systemic leaves when infected at 75 DAS varied more among trials. Tobacco types with a high mean percent of TSWV infection also had a high mean percent of systemic infection. It appears that TSWV susceptibility is related to tobacco variety.

Tobacco plants infected at a young age were more likely to exhibit symptoms of TSWV. These results concur with previous studies conducted by Mandal et al. (2007). Only a single ring spot was identified on a single NC 7 tobacco plant infected at 95 DAS, representing the only occurrence of infection at this plant age.

Higher rates of infection were achieved during the first trial of this study. Trial 1 was conducted in the spring, when greenhouse temperatures were rising, while trial 2 was conducted in the fall when greenhouse temperatures were steadily warm. The time of year each trial was conducted may have affected rates of TSWV infection.

Colored Adhesive Trap Study

Although a higher number of thrips were attracted to traps painted with burley tobacco colored paint, plant color is most likely only a minor contributing factor explaining why incidence of TSWV in burley tobacco fields is greater than incidence of TSWV in flue-cured tobacco fields. Studies show that thrips are attracted to yellow and light green (Yudin et al. 1987); the hint of yellow found in burley tobacco leaves might act on thrips preference to certain tobacco types. Perhaps, if further studies were conducted, and more data collected, a greater correlation between tobacco plant color and the number of thrips might be identified.

TSWV is an economically devastating disease in tobacco, and understanding its potential to infect burley types of tobacco is important to growers. Burley tobacco is more

likely to exhibit signs of TSWV infection than flue-cured tobacco in areas of high TSWV pressure is eastern North Carolina. To avoid high rates of TSWV infection tobacco producers should be aware of thrips flights in their area prior to deciding on a transplant date. Producers should also continue to use the recommended chemicals to reduce thrips feeding and enhance plant defenses. TSWV incidence in burley is most likely related to tobacco type. However, our results suggest that TSWV infection could be a combination of plant susceptibility and thrips attraction. Further studies should be conducted to completely understand the processes regulating this phenomenon.

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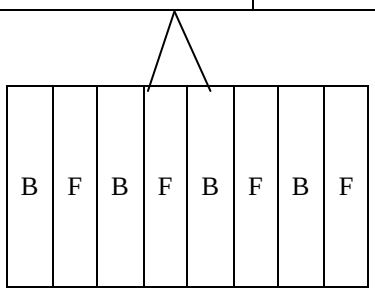
P2N	P2A	P1N	P1A
P2A	P1N	P2N	P1A
Alley			
P2A	P1N	P1A	P2N
P2N	P2A	P1N	P1A



KEY:
P1 – planting date 1
P2 – planting date 2
A – treated with imidacloprid
N – not treated with imidacloprid
B – burley tobacco
F – flue-cured tobacco

Figure 1.1. Kinston 2008 field map, showing four replications of eight treatments in a randomized complete block design.

Figure 1.2. Maryland, flue-cured, and burley leaf swatches used to create adhesive trap paint colors.

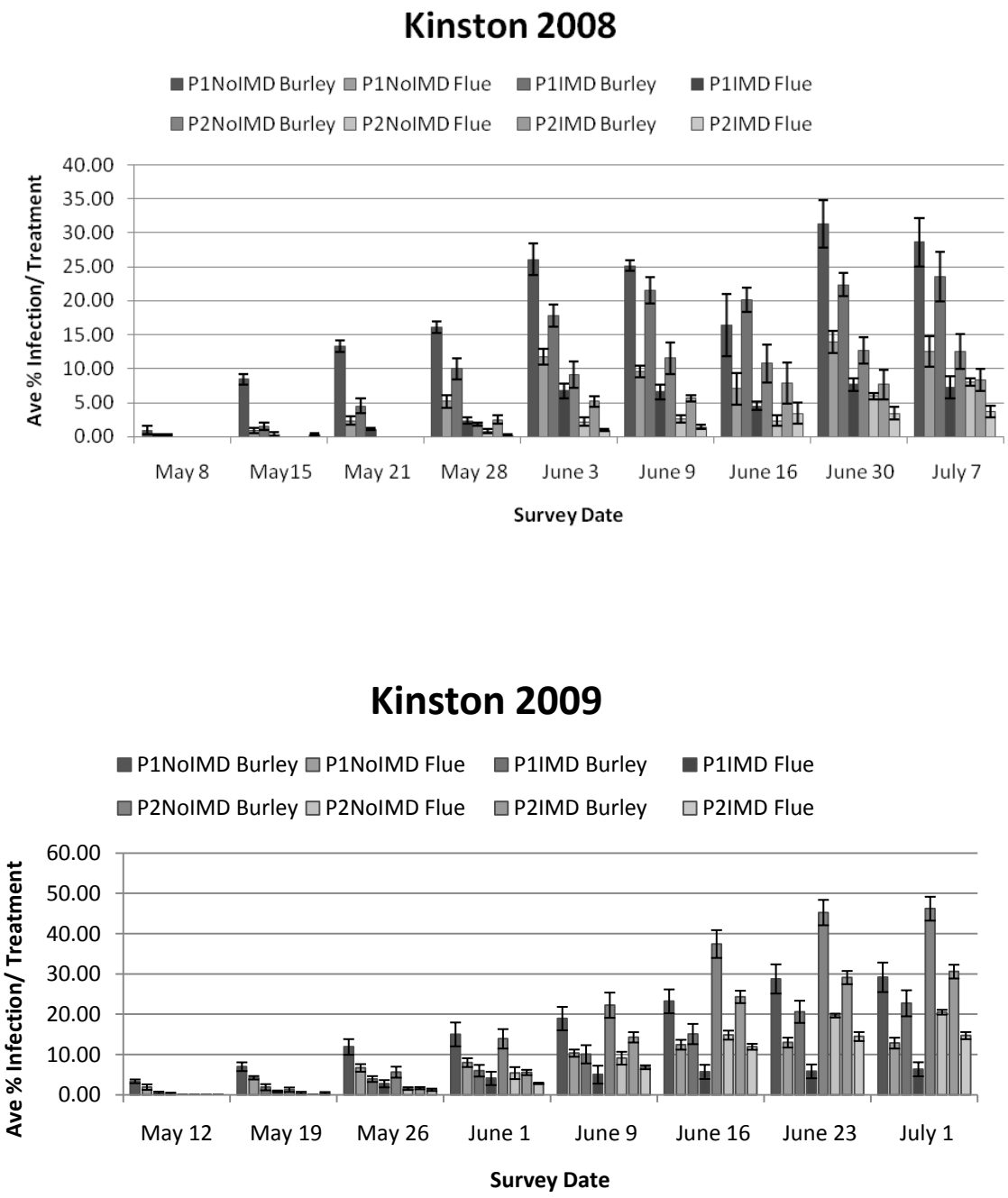


Figure 1.3. Mean ± standard error of tomato spotted wilt virus incidence by sampling date at Kinston in 2008 and 2009.

Table 1.1. Commercial survey results 2007, 2008 and 2009.

Key: AD = Admire, ACT = Actigard, PLT = Platinum, IMD = imidacloprid

Year	County	Tobacco Type	TSWV Treatment	Transplant date	Survey date	Mean % Incidence
2007	Duplin	Burley (KT204)	NONE	5/10	8/1	41.0
	Duplin	Flue-cured (NC71)	AD, ACT	4/23	8/1	7.0
	Johnston	Burley	unknown	unknown	8/1	7.0
	Johnston	Flue-cured	unknown	unknown	8/1	1.0
	Sampson	Burley	NONE	5/15	7/24	26.5
	Sampson	Flue-cured	AD	4/21	7/24	11.8
	Wilson	Burley (KY204 & NC7)	PLT on KY204	5/10 & 4/12	8/1	11.6
	Wilson	Flue-cured	unknown	unknown	8/1	2.8

Table 1.1. Continued.

Year	County	Site	Tobacco Type	TSWV Treatment	Transplant date	Survey date	Mean % Incidece
2008	Duplin		Burley	AD, ACT	unknown	8/8	12.4
	Duplin		Flue-cured	AD, ACT	unknown	8/8	3.9
	Edgecombe	Old	Burley (NC7)	PLT	4/29 -5/3	8/1	3.4
	Edgecombe	Old	Flue-cured	unknown	unknown	8/1	1.5
	Edgecombe	Old	Maryland (M609)	PLT	4/29 -5/3	8/1	1.0
	Edgecombe	Young	Burley (NC7)	PLT	~ 5/15	8/1	0.6
	Edgecombe	Young	Maryland (M609)	PLT	~ 5/15	8/1	0.5
	Johnston		Burley	unknown	unknown	8/8	0.9
	Johnston		Flue-cured	unknown	unknown	8/8	0.9
	Sampson		Burley	AD	unknown	7/29	13.3
	Sampson		Flue-cured	AD	unknown	7/29	4.0
	Wilson	Parkers BBQ	Burley	unknown	unknown	8/1	5.6
	Wilson	Parkers BBQ	Flue-cured	unknown	unknown	8/1	1.8
	Wilson	Contentnea	Burley	unknown	unknown	8/1	7.4
	Wilson	Contentnea	Flue-cured	unknown	unknown	8/1	2.8

Table 1.1. Continued.

Year	County	Site	Tobacco Type	TSWV Treatment	Transplant date	Survey date	Mean % Incidence
2009	Duplin	Sandridge Rd	Burley (KT 204)	AD, ACT	4/23	7/30	19.1
	Duplin	Sandridge Rd	Flue-cured (K326)	AD, ACT	4/20	7/30	4.0
	Duplin		Burley (KT204)	AD	4/27	7/30	29.6
	Duplin		Burley (KT204)	AD, ACT	4/27	7/30	27.3
	Duplin		Flue-cured (NC71)	AD	4/20	7/30	4.1
	Johnston	C. Church Rd	Burley	unknown	unknown	7/23	6.0
	Johnston	C. Church Rd	Flue-cured	unknown	unknown	7/23	1.5
	Johnston	Raleigh Rd	Burley	unknown	unknown	7/23	4.8
	Johnston	Raleigh Rd	Flue-cured	unknown	unknown	7/23	1.3
	Johnston	Langdon	Burley (KT204)	IMD	5/10	7/23	4.0
	Johnston	Langdon	Flue-cured	unknown	unknown	7/23	0.9
	Sampson		Burley	unknown	unknown	7/15	19.3
	Sampson		Flue-cured	unknown	unknown	7/15	6.8
	Wilson		Burley (NC7)	PLT	5/5	7/15	9.4
	Wilson		Flue-cured (CC27)	PLT	unknown	7/15	2.9
	Wilson	Water Tower	Burley (NC7)	PLT	unknown	7/15	12.4
	Wilson	Water Tower	Flue-cured (CC27)	PLT	5/1	7/15	4.5
	Wilson	Wilco Rd	Burley (NC7)	AD	5/5	7/15	5.1
	Wilson	Wilco Rd	Flue-cured (K326)	PLT	5/4	7/15	3.9

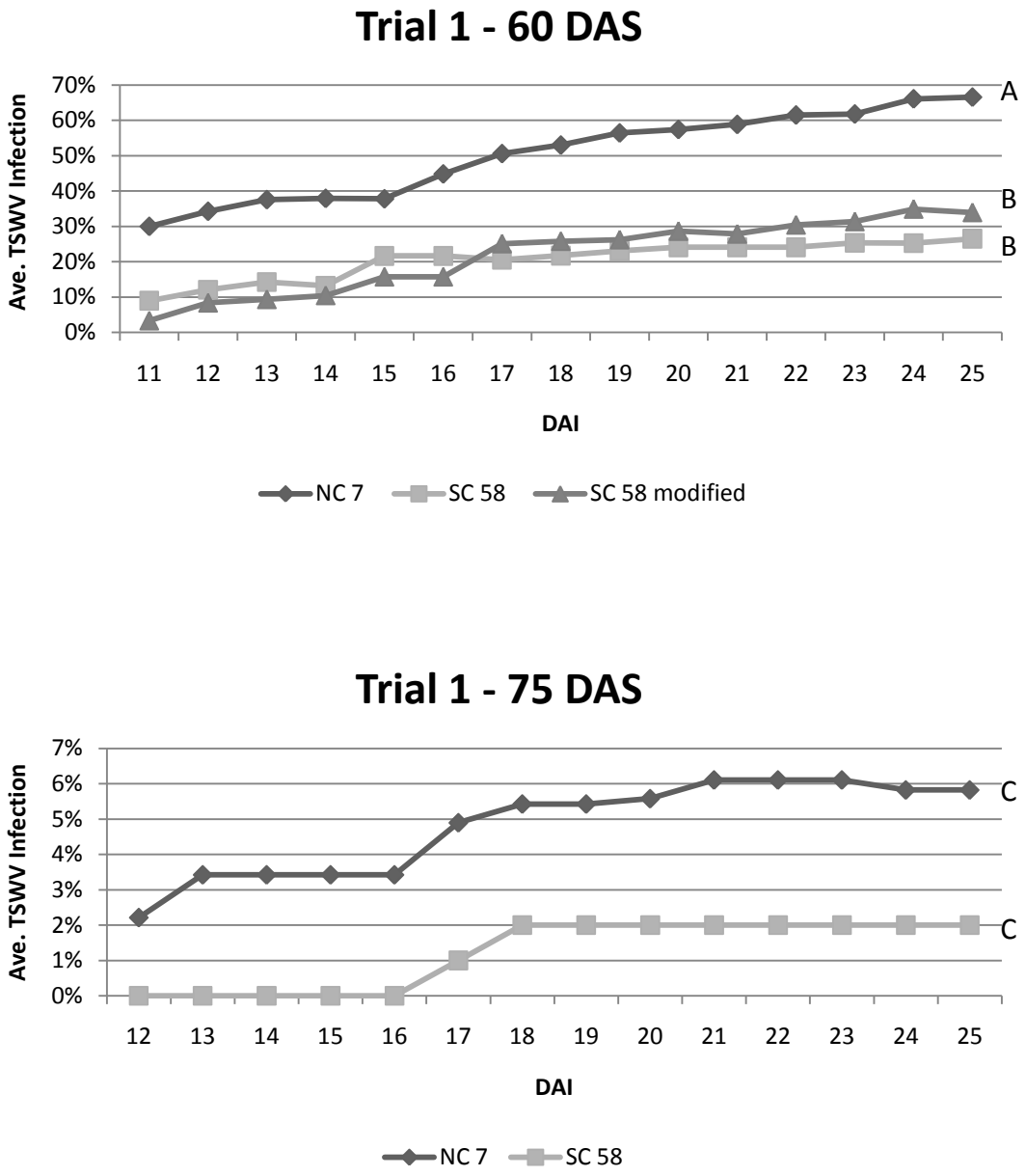


Figure 1.4. Mean percent TSWV infection for several tobacco types infected at 60 and 75 DAS. Tobacco types not graphed showed no signs of infection. Treatments followed by the same letter are not significantly different according to Fisher's LSD.

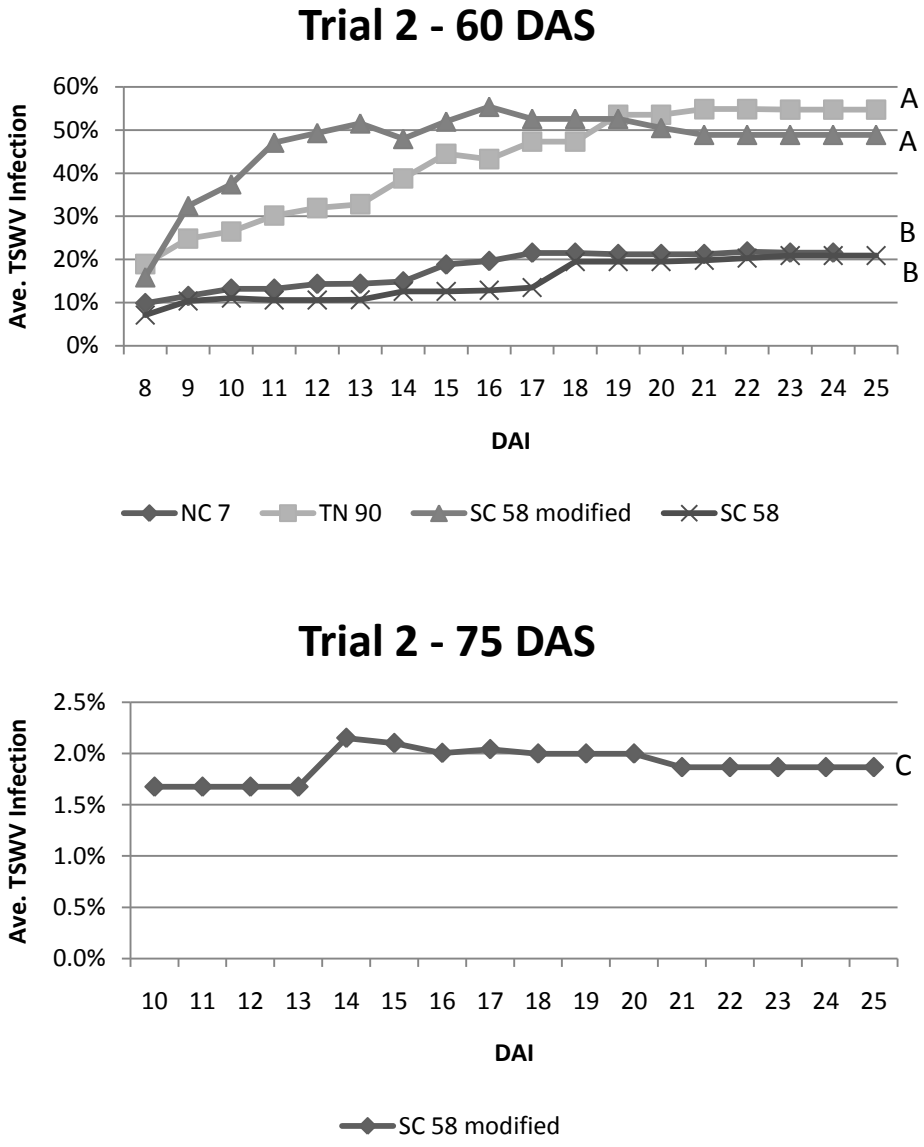


Figure 1.5. Mean percent TSWV infection for several tobacco types infected at 60 and 75 DAS. Tobacco types not graphed showed no signs of infection. Treatments followed by the same letter are not significantly different according to Fisher's LSD.

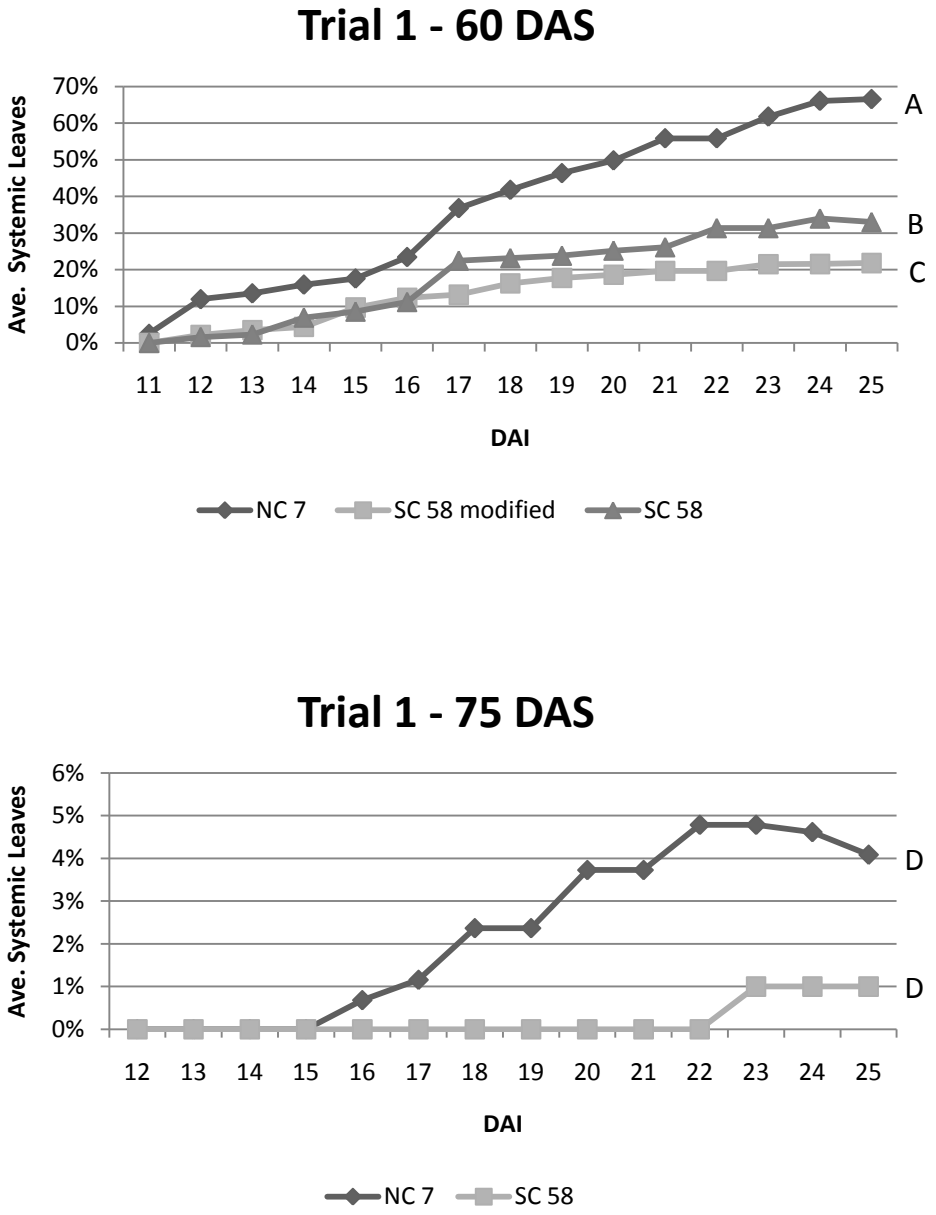


Figure 1.6. Mean percent of systemic leaves for several tobacco types infected at 60 and 75 DAS. Tobacco types not graphed showed no systemic leaves. Treatments followed by the same letter are not significantly different according to Fisher's LSD.

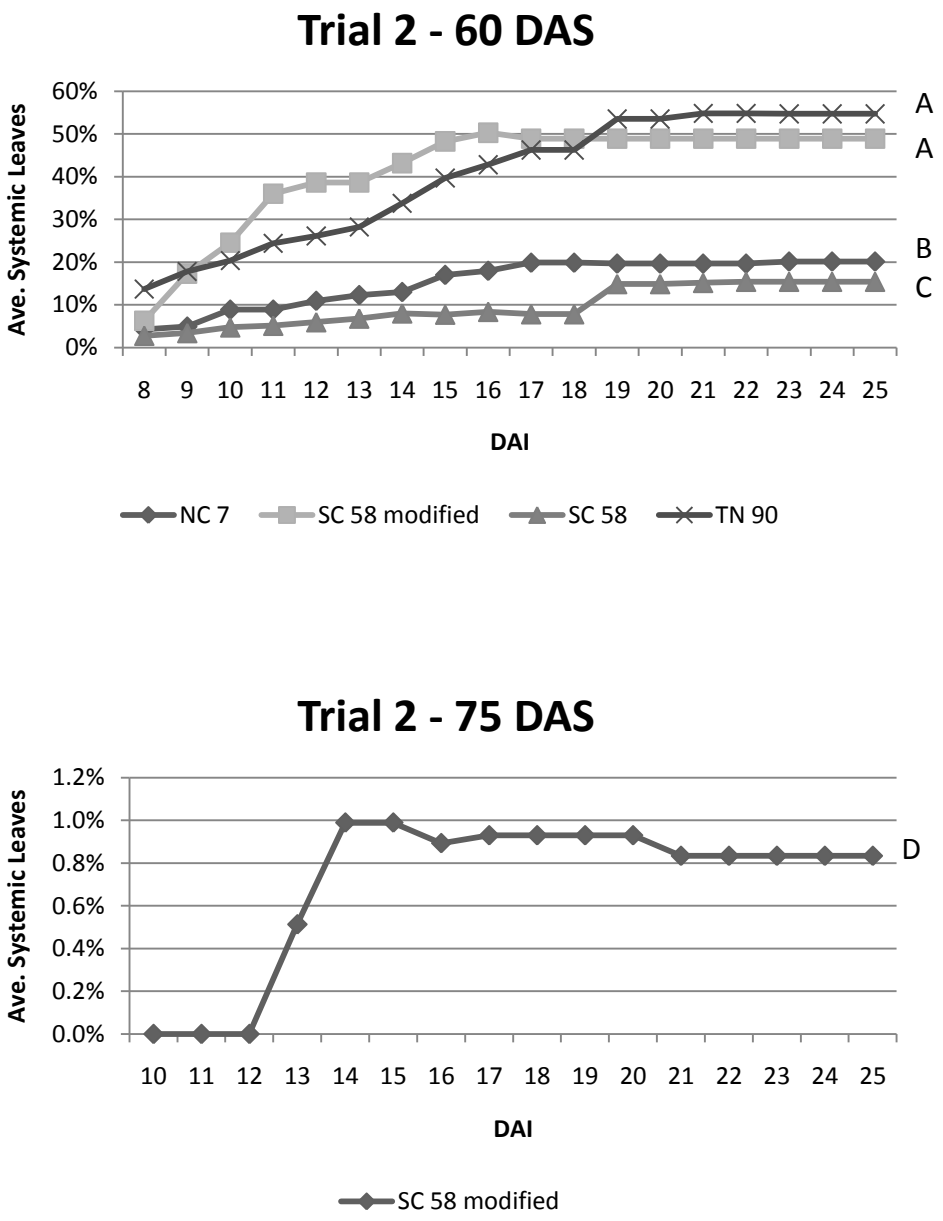


Figure 1.7. Mean percent of systemic leaves for several tobacco types infected at 60 and 75 DAS. Tobacco types not graphed showed no systemic leaves. Treatments followed by the same letter are not significantly different according to Fisher's LSD.

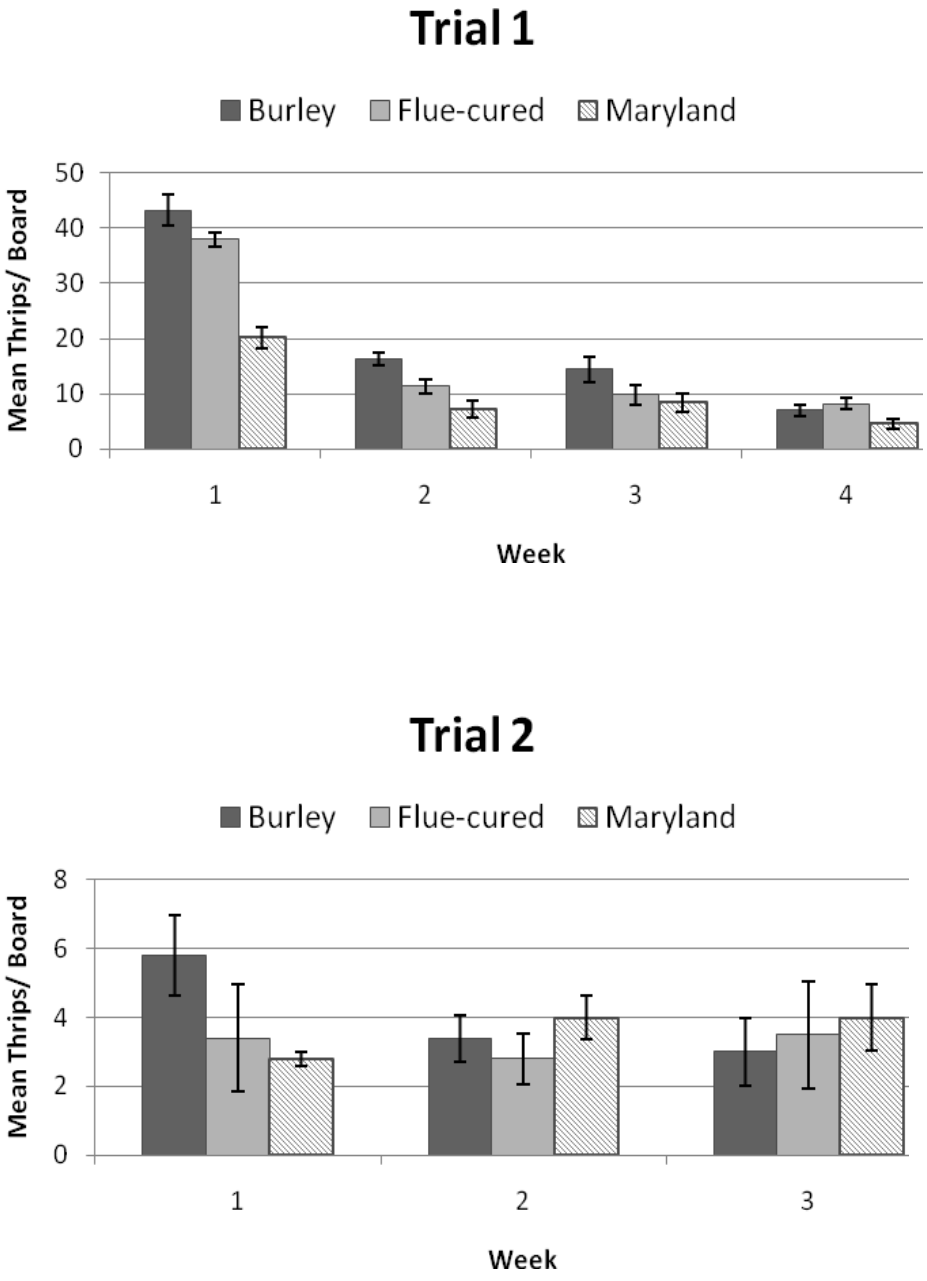


Figure 1.8. Mean ± standard error of thrips per trap by week at Clayton in 2009.

Chapter II: Presence of Three Tobacco Pests in Burley and Flue-cured Types of Tobacco in Eastern North Carolina

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Mandcua Sexta (L.), *Manduca quinquemaculata* (Haw.), *Heliothis virescens* (F.), and *Epitrix hirtipennis* (Melsheimer) are four common pests of flue-cured tobacco (*Nicotiana tabacum*) in eastern North Carolina. Methods of control have been established for each of these insect pests. Following the end of tobacco price supports in 2004, many growers who traditionally grew flue-cured tobacco began investigating and pursuing new agricultural enterprises. Burley tobacco, which was typically confined to the far western regions of the state, is a crop of great interest to some growers in the piedmont and coastal plain regions. However, pest pressures in burley types of tobacco in the eastern regions of the state are not well understood.

Manduca Sexta, the tobacco hornworm, and *Manduca quinquemaculata*, the tomato hornworm, compose the hornworm complex. Both are mid- to late-season defoliating pests of tobacco. In North Carolina two to three generations of the hornworm complex occur during each growing season. Larvae typically begin to appear in late June and early July when tobacco plants are well developed with large amounts of leaf material. In North Carolina, control measures should be taken when a single healthy one inch larva is identified per ten plants or five parasitized larvae are identified per ten plants (Burrack 2010). If control measures are not implemented when thresholds are reached, larval populations can become very destructive, completely removing all leaf tissue from the plant. However, if control measures are implemented in a timely manner, plants can compensate for some or all injury sustained (Kolodny-Hirsch and Harrison 1982).

As tobacco plants mature, tolerance to larval feeding damage increases (Kolodny-Hirsch and Harrison 1986). Plants subjected to simulated defoliation, equivalent to one *M.*

sexta larva, after topping were able to completely compensate for damage. Plants subjected to defoliation during the establishment stage, vegetative stage and buttoning stage were able to partially compensate for damage, resulting in yield reductions of 16%, 26% and 26% respectively (Kolodny-Hirsch et al. 1986). (The buttoning stage occurs before flowering when the plant has only a reproductive bud present at the apical meristem.) Some cultural practices can affect hornworm complex populations. High rates of nitrogen have been documented to create more succulent, greener plants that increase production of *M. sexta* larvae over that on plants receiving moderate levels of nitrogen (Reagan et al. 1978).

Heliothis virescens, the tobacco budworm, is a common tobacco pest found throughout the Southeastern United States. *Heliothis virescens* larvae are found in the developing apical meristem of tobacco plants. While feeding on the small, developing leaves, larvae bore holes into and through the bud. As the injured leaves increase in size and unfurl, they appear ragged and damaged. Feeding larvae may also result in premature topping, causing plant stunting and delayed plant maturity (Johnson 1979). *Heliothis virescens* larvae numbers have been documented to increase as soil nutrients increase (Girardeau 1969). This is perhaps due to the ovipositing female's preference for greener, lusher plants.

Current North Carolina Cooperative Extension *H. virescens* thresholds recommend implementing control methods when ten percent of plants are infested with *H. virescens* larvae (Burrack 2010). Yet, recent studies show feeding by *H. virescens* has little to no effect on tobacco yield or quality (Juba et al. 2007). Although growers have been informed

of these findings, the presence of *H. virescens* is generally not tolerated and therefore control methods are still deployed.

Parasitoid wasps, transgenic plants modified using *Bacillus thuringiensis*, resistant tobacco cultivars, and chemical insecticides have all proved to be effective methods of *H. virescens* control. Two Hymenoptera species have been identified as particularly effective parasitoids of *H. virescens*: *Campoletis sonorensis* (Cameron) and *Cardiochilies nigriceps* Viereck. Both species deposit eggs into *H. virescens* larvae during the first instar. Parasitoid larvae develop within the host larvae and emerge, thus resulting in the death of the host during the third instar. *Campoletis sonorensis* must parasitize *H. virescens* larvae within the first three to six days of their life, before larvae become too mature.

Transgenic plants slow the development of the *H. virescens* larvae; therefore *C. sonorensis* wasps have a greater window of opportunity to parasitize *H. virescens* larvae (Johnson and Gould 1992). Unlike *C. sonorensis*, *C. nigriceps* wasps have the ability to parasitize older, larger larvae (Johnson 1997). Transgenic tobacco plants have yet to be commercially accepted. Traditionally bred resistant tobacco cultivars sustain less feeding injury and reduce *H. virescens* growth (T. Juba, unpublished data). CU 263 and CU 370 are two resistant flue-cured lines; however due to low yield, growers do not plant either of these varieties.

Epitrix hirtipennis, the tobacco flea beetle, feeds on many members of the plant family Solananaceae. Unfortunately for North Carolina tobacco growers, flue-cured tobacco appears to be a preferred host (Martin and Herzog 1987). Adult beetles cause damage by

feeding on the leaves of new transplants, while soil-dwelling larvae feed on the lower portion of the stem and the plant roots (Duke and Lampert 1986).

Adult *E. hirtipennis* beetles over winter in the soil and emerge in early spring. Emerging females deposit eggs onto the soil surface near tobacco plants (Martin and Herzog 1987). Under field conditions, egg incubation lasts from three to eleven days (Chamberlin et al. 1924). Neonates emerge and immediately begin their search for food by burrowing into the soil toward the tobacco plant roots; occasionally larvae will begin feeding above ground on young leaves and the stem. Mature larvae create earthen cells in the soil for pupation; here they remain for five to six days. Similar to emerging neonates, the adult beetle emerges and also immediately begins its search for food. Adult females begin to deposit eggs 12 to 16 days post eclosion and continue to deposit eggs until 52 to 57 days post eclosion. The average life span of *E. hirtipennis* is approximately 70 days post eclosion; although some *E. hirtipennis* live up to 161 days (Martin and Herzog 1987).

Relatively small populations of adult *E. hirtipennis* during the first three weeks after transplanting have been shown to have adverse effects on flue-cured tobacco; reductions in leaf area by adult feeding decreases plant photosynthesis, while reduced root size from larval feeding decreases root growth, thus further decreasing plant growth potential (Semtner 1984). Control of *E. hirtipennis* can be accomplished with several insecticides, including the chloronicotinyl insecticide, imidacloprid (Elbert et al. 1990). Treatment should be deployed when four or more beetles are identified per small plant or 60 or more beetles are identified per large plant (Burrack 2010).

Adult tobacco flea beetles respond to color traps. Higher numbers of *E. hirtipennis* were caught using yellow sticky traps than those colored green, white, blue, or red (Dominick 1971). Semtner (1980) determined that tobacco treated with low rates of nitrogen resulted in yellowish plants, while treatments with medium and high rates of nitrogen resulted in greener plants. Higher numbers of *E. hirtipennis* were found on tobacco plants that received the low rates of Nitrogen compared to those that received the medium to high rates of nitrogen.

It is important for tobacco scouts, growers and researchers to fully understand the insect pest pressures on burley tobacco in eastern North Carolina. We hypothesized that *E. hirtipennis* may be more attracted to burley tobacco than flue-cured tobacco due to the yellow color of burley tobacco's leaves. *Heliothis virescens* and hornworm complex larval presence may be higher in flue-cured tobacco than burley tobacco because of the green color of flue-cured tobacco's leaves. The following studies were conducted to determine the presence of these insect pests in burley and flue-cured type tobaccos in eastern North Carolina.

Materials and Methods

Field studies were conducted during the summers of 2008 and 2009 at the Central Crops Research Station in Clayton, NC and the Cunningham Research Station in Kinston, NC to determine incidence of *H. virescens*, *E. hirtipennis*, and the hornworm complex in burley and flue-cured type tobaccos under several treatments. At both research stations each year, seeds of NC 7 burley tobacco and NC 71 flue-cured tobacco were started in greenhouse

float trays, following commercial production standards. Half of the plants of each tobacco type were pretreated with imidacloprid (AdmirePro®) while the other half remained untreated. Imidacloprid was applied as a soil drench at 0.8 oz of formulated product per 1000 plants 2-3 days before transplanting.

The experimental design was a randomized complete block, with eight treatments and four replications (Figure 2.1). Each plot was 16 rows wide and measured 15.24 meters in length. Within each plot the number of plants per row ranged from 20 to 25. At the Cunningham Research Station rows were 1.12 meters apart and plants within rows were 0.56 meters apart, while at the Central Crops Research Station between row spacing measured 1.14 meters and within row plant spacing measured 0.56 meters. Planting date and insecticide were applied to the entire plot, while tobacco type was applied within each plot. Burley and flue-cured tobacco were planted in alternating pairs of rows (i.e. two rows of burley followed by two rows of flue-cured and so forth, for a total of eight rows of each tobacco type per plot). This was done to account for any uneven thrips distribution that might have occurred within the field. (Field sites were additionally used for studies involving preference of thrips and incidence of tomato spotted wilt virus.)

Transplanting at each location took place on two dates, two weeks apart. In 2008, transplanting at the Cunningham Research Station occurred on April 23 and May 8 and at the Central Crops Research Station on May 1 and May 14. In 2009, transplanting at the Cunningham Research Station occurred on April 14 and April 29 and at the Central Crops Research Station on April 27 and May 11. A tractor mounted mechanical transplanter was used during both years at both locations.

Visual surveys were conducted once per week while feral populations of each insect were present in the field. In 2008 and 2009 the number of larvae belonging to the hornworm complex per 40 plants per tobacco type per plot were recorded, the number of *H. virescens* larvae and larval frass per 40 plants per tobacco type per plot were recorded, and the number of *E. hirtipennis* beetles and holes per five plants per tobacco type per plot were recorded at both field locations. In 2009 the Clayton field location could not be used for surveying due to heavy plant damage caused by severe weather. Larvae belonging to the hornworm complex could not be evaluated in 2009 due to very low populations until very late during the field season at both locations.

Statistical analyses were completed on the number of hornworm complex larvae, the number of *H. virescens* larvae, the number of plants with *H. virescens* frass only, the total presence of *H. virescens* (plants with larvae and/or frass), and the number of *E. hirtipennis* beetles and holes to determine the effect of the eight treatments on these insect pest populations. All statistical analyses were completed using SAS® software Version 9.1 (SAS Institute, Cary, NC). Data was subjected to an analysis of variance (ANOVA) procedure with means separation through an LSD test (LSMEANS).

Results

Hornworm Complex

In 2008, planting date was significant for the number of hornworm complex larvae at Kinston ($F = 61.48$, $df_N = 1$, $df_D = 21.9$, $P < 0.0001$) and Clayton ($F = 9.35$, $df_N = 1$, $df_D = 24.8$, $P = 0.0053$) (Figure 2.2). However, tobacco type and imidacloprid treatment were not

significant at either location. At Kinston, early planted tobacco had significantly lower numbers of larvae compared to the plots assigned the late planting date. At Clayton, treatments assigned a late planting date had significantly lower numbers of larvae compared to the treatments assigned the early planting date. The number of larvae greatly increased toward the end of July at both locations. The total number of larvae present at Clayton was much greater than the total number of larvae present at Kinston.

Heliothis virescens

Results from frass only analyses will not be discussed in detail since frass only is not a consistent predictor of *H. virescens* populations. Tobacco type was significant for *H. virescens* larvae ($F = 8.61$, $df_N = 1$, $df_D = 38.2$, $P = .0056$) and total presence ($F = 5.06$, $df_N = 1$, $df_D = 33.9$, $P = .0311$) at Clayton in 2008 (Figures 2.3 and 2.4) and for *H. virescens* larvae ($F = 11.53$, $df_N = 1$, $df_D = 6$, $P = .0146$) and total presence ($F = 16.48$, $df_N = 1$, $df_D = 25.4$, $P = .0004$) at Kinston in 2009. Planting date was significant for *H. virescens* larvae ($F = 39.37$, $df_N = 1$, $df_D = 137$, $P < .0001$) and total presence ($F= 40.42$, $df_N = 1$, $df_D = 137$, $P < .0001$) at Kinston in 2008 and significant for *H. virescens* larvae ($F = 50.06$, $df_N = 1$, $df_D = 27.4$, $P < .0001$) and total presence ($F = 119.70$, $df_N = 1$, $df_D = 25.4$, $P < .0001$) at Kinston in 2009. Imidacloprid use was significant for total presence ($F = 5.50$, $df_N = 1$, $df_D = 29.2$, $P = 0.0260$) at Clayton in 2008 and significant for *H. virescens* larvae ($F= 12.77$, $df_N = 1$, $df_D = 137$, $P = .0005$) and total presence ($F = 14.42$, $df_N = 1$, $df_D = 137$, $P = .0002$) at Kinston in 2008.

Epitrix hirtipennis

Planting date was significant for both number of holes ($F = 72.59$, $df_N = 1$, $df_D = 81$, $P < .0001$) and beetles ($F = 35.12$, $df_N = 1$, $df_D = 81$, $P < .0001$) at Clayton in 2008, the number

of holes ($F = 99.01$, $df_N = 1$, $df_D = 109$, $P < .0001$) and beetles ($F = 29.90$, $df_N = 1$, $df_D = 109$, $P < .0001$) at Kinston in 2008, and the number of holes ($F = 19.28$, $df_N = 1$, $df_D = 81$, $P < .0001$) and beetles ($F = 25.50$, $df_N = 1$, $df_D = 81$, $P < .0001$) at Kinston in 2009. Treatments assigned an early planting date had higher numbers of holes and beetles during all three trials (Figures 2.5 and 2.6). A significant difference was also identified for imidacloprid use for both the number of holes ($F = 71.55$, $df_N = 1$, $df_D = 81$, $P < .0001$) and beetles ($F = 14.22$, $df_N = 1$, $df_D = 81$, $P = .0003$) at Clayton in 2008, the number of holes ($F = 79.80$, $df_N = 1$, $df_D = 109$, $P < .0001$) and beetles ($F = 13.46$, $df_N = 1$, $df_D = 109$, $P = .0004$) at Kinston in 2008, and the number of holes ($F = 27.95$, $df_N = 1$, $df_D = 81$, $P < .0001$) and beetles ($F= 32.15$, $df_N = 1$, $df_D = 81$, $P < .0001$) at Kinston in 2009. Treatments assigned a pretreatment of imidacloprid had fewer holes and beetles during all three trials. A significant difference was not identified between tobacco types at either location for either year.

Discussion

Hornworm Complex

Planting date affected the presence of hornworm complex larval populations. However, the planting date with higher hornworm complex larval numbers varied between the two locations. Population numbers for both planting dates would have required control methods to be implemented as the season progressed. Imidacloprid use and tobacco type did not affect the presence of hornworm complex larvae.

Heliothis virescens

Results from these analyses were mixed. Planting date had an effect on the presence of *H. virescens* populations in two of three field trials and four of six statistical analyses. At Kinston in 2008, plants assigned an early planting date had higher numbers of *H. virescens* larvae. At Kinston in 2009, plants assigned a late planting date had higher numbers of *H. virescens* larvae. This is most likely due the maturity of the tobacco plants from each planting date at the time of adult *H. virescens* emergence. Tobacco type had an effect on the presence of *H. virescens* populations in two of the three field trials and four of six statistical analyses. At Clayton in 2008 and Kinston in 2009, flue-cured tobacco had higher numbers of *H. virescens* larvae, possibly a result of egg laying adults' preference for the greener flue-cured plants. Flue-cured and burley tobacco differ genetically, resulting in different physical traits such as color and thickness of leaves (Legg et al. 1977). Additional investigations to fully understand this effect are warranted.

Imidacloprid affected the presence of *H. virescens* larvae in three of six statistical analyses. The number of larvae was higher in treatments that had received pretreatments of imidacloprid at Kinston in 2008 and Clayton 2008. This may be due to the healthy conditions of plants that had received treatments of imidacloprid and thus had less *E. hirtipennis* damage than plants not receiving pretreatments of imidacloprid (see below). Perhaps, egg laying *H. virescens* prefer to deposit eggs on healthy, damage free plants. An additional explanation for this could be reduced parasitism. Studies conducted by Anirudh Dhammi (unpublished data) show that imidacloprid has a negative effect on *H. virescens*

parasitoids when foliar applications are made. Treated plots may have had less parasitism, and therefore increased *H. virescens* presence.

Epitrix hirtipennis

Planting date and imidacloprid treatment had an effect on the presence of *E. hirtipennis* beetles and the damage caused by their feeding. A higher number of holes and beetles were found on plants assigned an early planting date. Adult beetles emerged and began to feed before transplanting of the plants assigned the later planting date during both years. Beetles continued to primarily feed on the older plants, plants assigned the early planting date, for the remainder of their lifespan. Few holes and beetles were found on plants that had been pretreated with imidacloprid. Imidacloprid continues to act as an effective method of chemical control for *E. hirtipennis* beetles. These results agree with previously conducted studies by Elbert et al. (1990). *Epitrix hirtipennis* beetles are an early season pest of tobacco, present when all tobacco types were similar in size, leaf structure, and color, therefore making the plants very difficult to distinguish from one another; thus it appears *E. hirtipennis* beetles showed no preference to one tobacco type over the other.

It cannot be assumed if pest numbers are low in flue-cured tobacco fields that pest numbers will always be low in nearby burley tobacco fields and vice versa. Given that *H. virescens* numbers were higher in flue-cured tobacco, control inputs for *H. virescens* may be higher for flue-cured growers if these trends continue. However, if imidacloprid use is the cause of these higher numbers, new methods of control might be needed. Growers should also be aware of transplant timing for *E. hirtipennis* and early season hornworm complex larvae. Yield data was not collected during these studies, and the possibility for differential

yield responses between tobacco types to varying populations of these insects needs further investigation.

Acknowledgements

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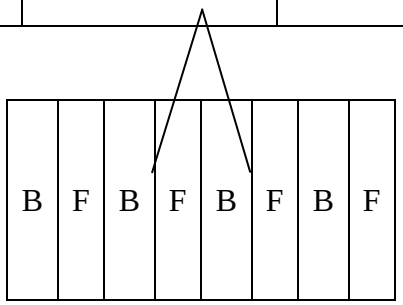
P1A	P1N	P2A	P2N
P1N	P2N	P2A	P1A
Alley			
P2A	P1N	P1A	P2N
P2N	P2A	P1N	P1A



KEY:
P1 – planting date 1
P2 – planting date 2
A – treated with imidacloprid
N – not treated with imidacloprid
B – burley tobacco
F – flue-cured tobacco

Figure 2.1. Clayton 2008 field map, showing four replications of eight treatments in a randomized complete block design.

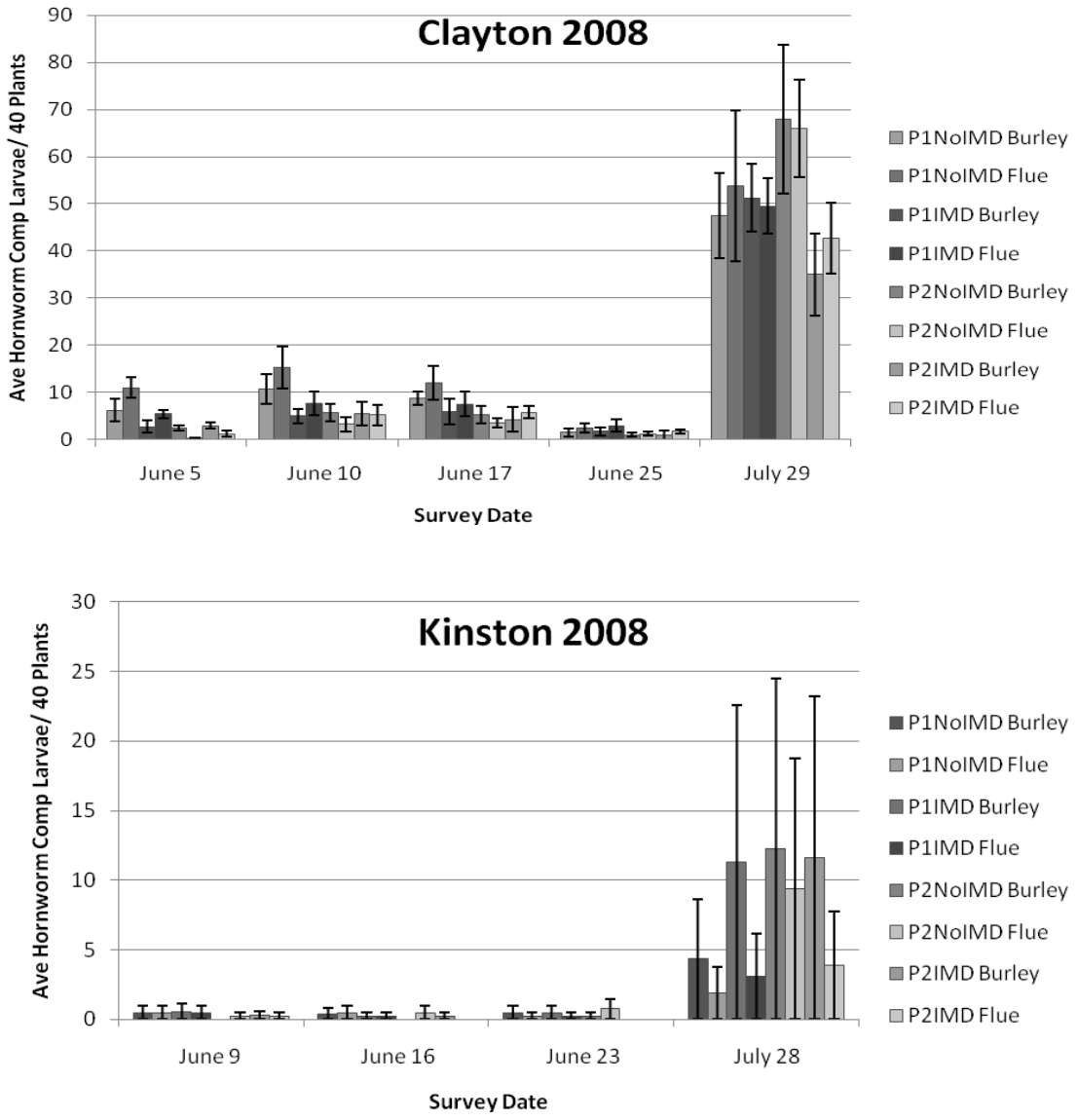


Figure 2.2. Mean ± standard error of populations of hornworm complex larvae per 40 plants by sampling date at two locations in 2008.

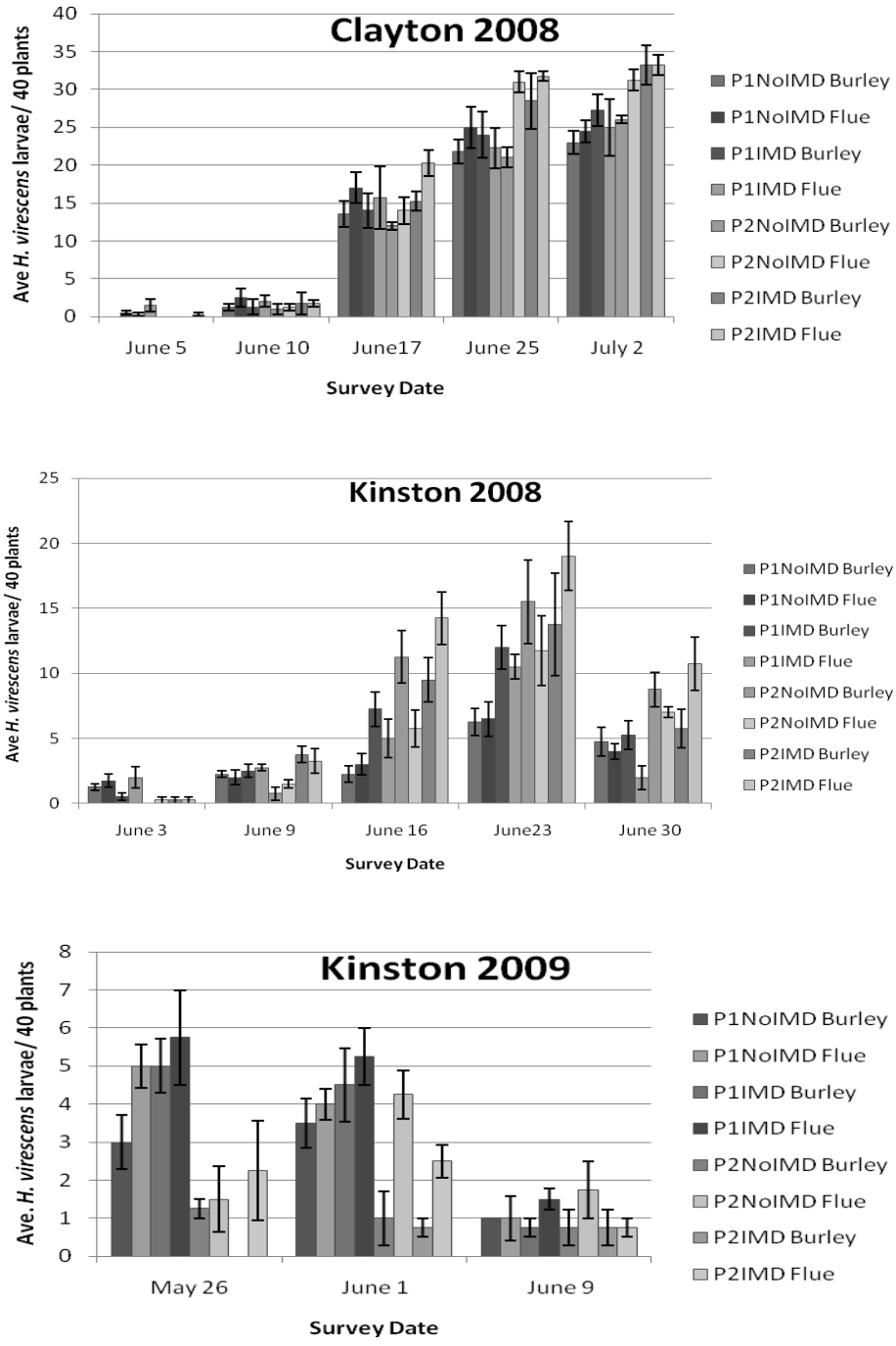


Figure 2.3. Mean ± standard error of populations of *H. virescens* larvae per 40 plants by sampling date at two locations in 2008 and one location in 2009.

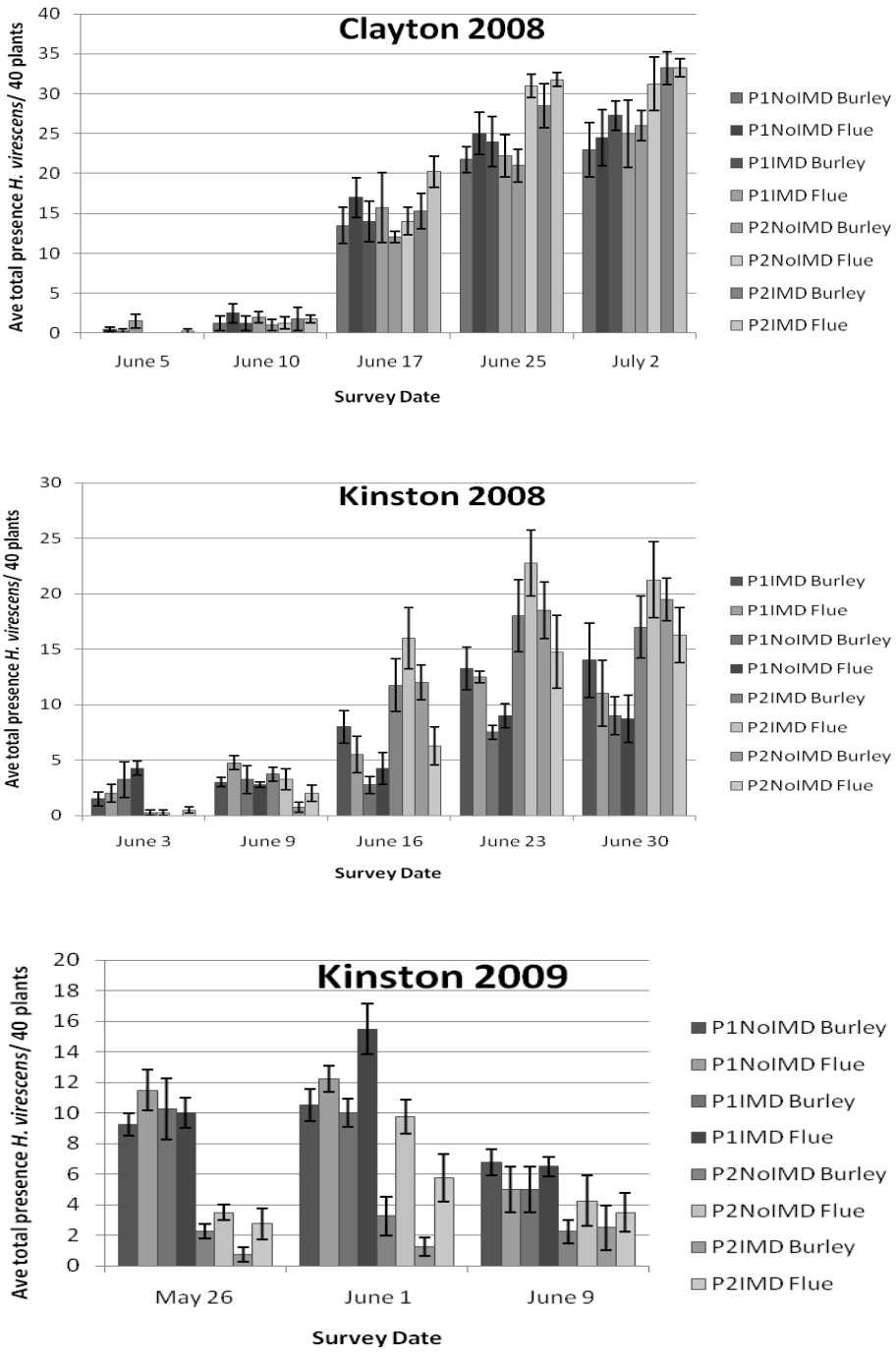


Figure 2.4. Mean ± standard error of total presence of *H. virescens* per 40 plants by sampling date at two locations in 2008 and one location in 2009.

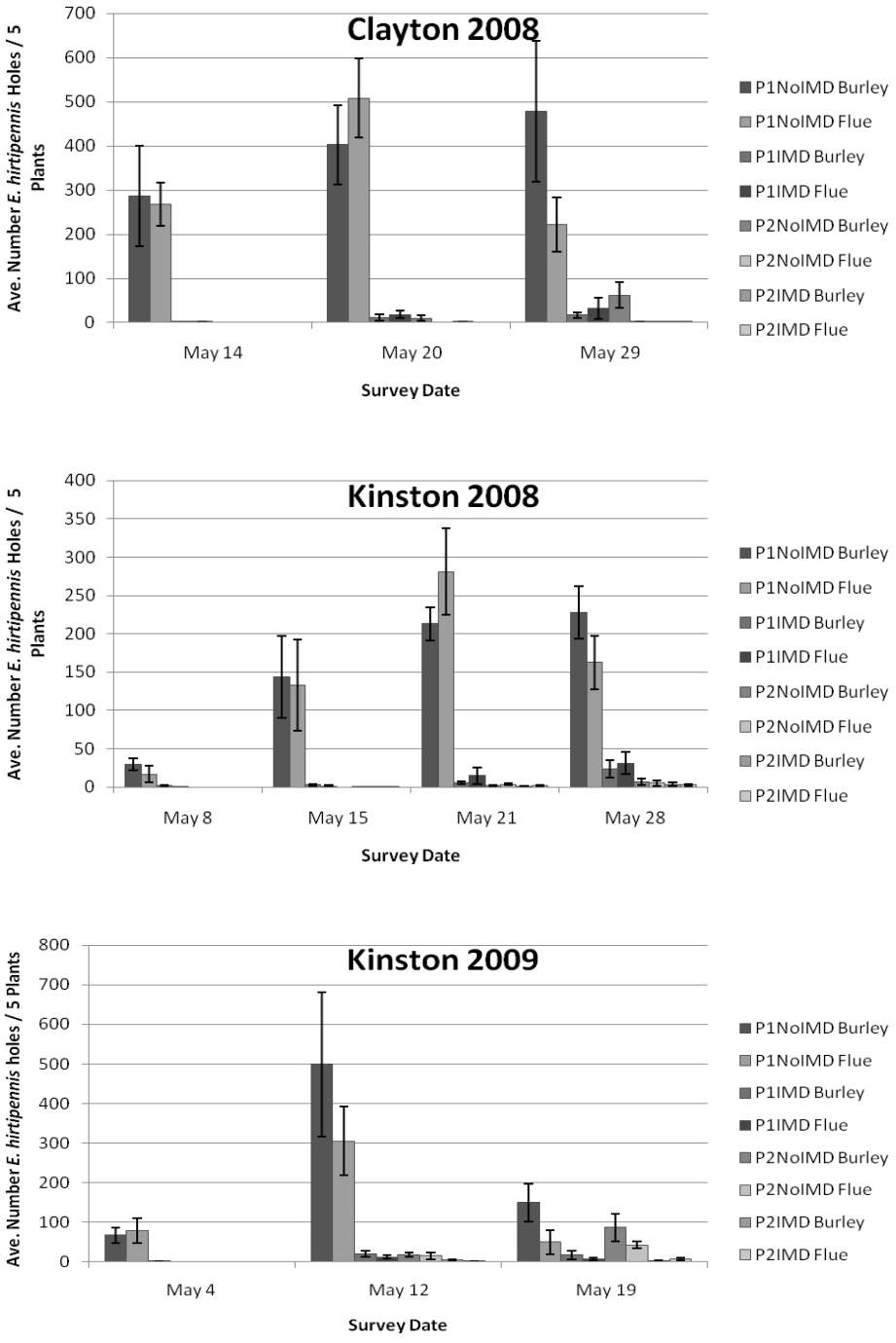


Figure 2.5. Mean ± standard error of number of holes created by *E. hirtipennis* beetles per five consecutive plants by sampling date at two locations in 2008 and one location in 2009.

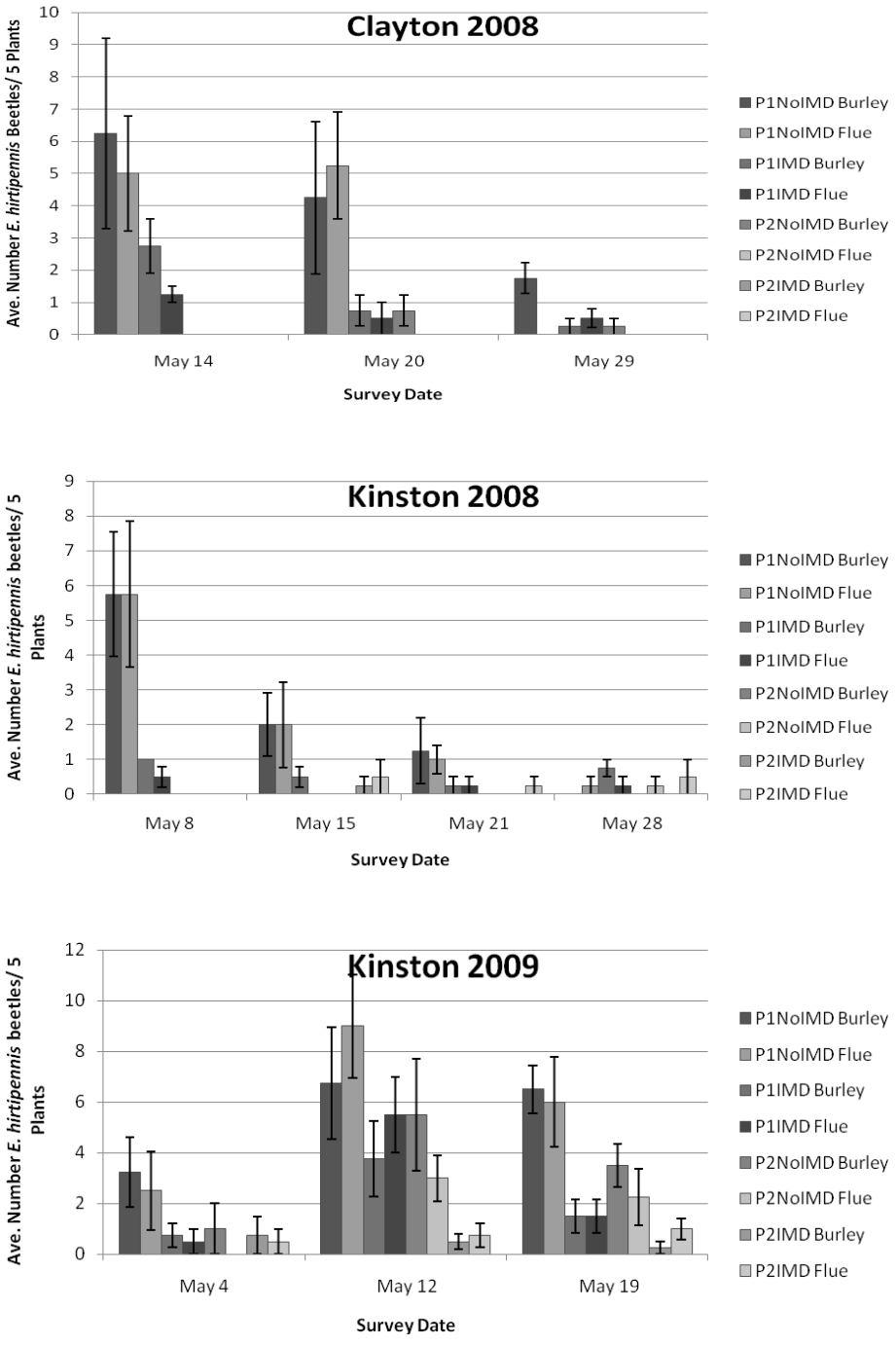


Figure 2.6. Mean ± standard error of *E. hirtipennis* beetles per five consecutive plants by sampling date at two locations in 2008 and one location in 2009.

Chapter III: Damage by the Tobacco Hornworm, *Manduca sexta*, (Lepidoptera: Sphingidae) in Open Air Burley Tobacco Curing Structures

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Manduca Sexta (L.), the tobacco hornworm, is a destructive defoliating pest of field tobacco found throughout the Southeastern United States (Madden and Chamberlin 1945). Previous studies have identified fifth stage *M. sexta* larvae as consuming the greatest amount of leaf material, followed by the fourth, third, second and first instars (Wolcott 1937). Approximately 90 percent of total leaf material is consumed by fifth stage *M. sexta* larvae and other hornworm species, when feeding on dark fired and burley types of tobacco (Gilmore 1938 and Jones and Thurston 1970).

In North Carolina, *M. sexta* completes two to three generations per year. A heavy infestation of *M. sexta* larvae typically occurs in late June and early July when tobacco plants are well developed. If control measures are not implemented, larval populations can become extremely destructive, completely removing all leaf material from the plant. However, if control measures are implemented in a timely manner, plants can compensate for some or all injury sustained (Kolodny-Hirsch and Harrison 1982). Late season infestations of *M. sexta* can occur just prior to harvest, resulting in eggs and small larvae on harvested leaves and plants. Although many studies have been conducted on *M. sexta* larval feeding in the field, the amount of damage caused by the presence of the different stages of *M. sexta* larvae to curing burley tobacco is unknown.

In eastern North Carolina *M. sexta* larvae that remain on flue-cured leaves at harvest are killed by the high temperatures in the curing barn, and therefore cause no post-harvest damage to curing tobacco leaves. Harvested burley tobacco is not subjected to high temperatures during curing, but instead is cured in open-air structures; therefore *M. sexta* larvae can continue to feed for an unknown amount of time. This can be especially

problematic for organic tobacco growers who wish to avoid spraying pre-harvest synthetic insecticides in order to control *M. sexta* populations.

To date no published studies have measured the potential for post-harvest hornworm damage. We hypothesized that the presence of *M. sexta* in open-air curing structures will reduce cured plant yield. The following studies were thus conducted to determine the amount of damage as measured by yield loss caused by the different instars of *M. sexta* larvae present in open-air curing burley tobacco structures.

Materials and Methods

Two preliminary studies were conducted in the Method Road greenhouses at North Carolina State University in 2007. Each trial consisted of six treatments: first, second, third, fourth and fifth instars of *M. sexta* larvae and a control with no larvae. Each treatment was applied to five plants. Tobacco plants were obtained from field plots on the Central Crops Research Station in Clayton, NC, on August 10 and August 31. Plants were notched, weighed, and tagged with a label identifying the specific treatment. Plants were then hung on wires that had been attached to the greenhouse support beams, and positioned with ample space between them to prevent movement of *M. sexta* larvae between the plants. After the plants were positioned, ten of the assigned instar of *M. sexta* larvae were introduced to each plant using a paintbrush or soft forceps.

In 2008 and 2009 field curing took place at the Central Crops Research Station. Three trials were conducted in 2008 using the burley tobacco variety NC 7 from the Central Crops Research Station for trials 1 and 2 and burley tobacco variety TN 90 from the Upper

Piedmont Research Station near Reidsville, NC, for trial 3. Three trials were also conducted in 2009 using NC 7 burley tobacco from the Cunningham Research Station and the Central Crops Research Station and TN 90 burley tobacco from the Upper Piedmont Research Station. In 2008 and 2009 each trial consisted of 5 treatments: first, second, third and fourth instars of *M. sexta* larvae and a control with no larvae. We did not include a sixth treatment consisting of fifth stage *M. sexta* larvae in the field trials; we determined in the preliminary studies the fifth stage larvae obtained from the NCSU Insectary varied in the amount of feeding they had completed prior to the assay, and some were nearing pupation. The number of plants per treatment varied between trials, ranging from five to ten plants.

For each field trial, plants of similar size were harvested at ground level, using a machete or weed eater modified with a steel brush blade and removed from the field. Plants were transported to a large open-air curing barn at the Central Crops Research Station where they were separated into the five treatment groups. The barn used was open on its two widest sides; large tarps were attached to cover two thirds of each open side to reduce the effect of severe winds, but still allow for air flow. All plants were notched using a mechanical notching machine, weighed, and tagged with a label identifying the specific treatment. Plants were positioned on wires with ample space between them to prevent movement of *M. sexta* larvae between the plants. If needed, plants were secured using a plastic zip tie. Finally, ten larvae from the assigned treatment were carefully placed onto the leaves of each plant using a paintbrush or soft forceps.

Plants were monitored closely for several days following the *M. sexta* introduction to ensure that the larvae remained attached to the plants. Plants were allowed to cure according

to burley tobacco management recommendations over the next several weeks. When completely cured, plants were reweighed to determine the amount of yield loss. In 2007 and 2008 the entire plant was reweighed at once, while in 2009 reweighing took place in two steps. In 2009, the leaves of each plant were first removed and weighed when the midribs had completely dried and browned; stalks were then moved to a small electric flue-cured curing barn where they were heated at 140 degrees Fahrenheit for three days until completely cured. This was done to account for irregular amounts of green material observed in stalks of different size the previous year. Once stalks were dried they were weighed, and the leaf and stalk weights were combined to create a total cured weight for each plant.

The percent of remaining leaf material was calculated for each plant by dividing the final cured weight by the original green weight. All statistical analyses were completed using SAS® software Version 9.1 (SAS Institute, Cary, NC). Data was subjected to an analysis of variance (ANOVA) procedure. If a significant difference was identified, data was subjected to Fisher's LSD.

Results and Discussion

Results from the two preliminary greenhouse studies indicated that fourth stage *M. sexta* larvae reduced cured plant weight by the greatest amount, with 8.86 % and 11.55 % of plant weight remaining at the end of each trial (Figure 3.1). Trial 1 produced significantly different results ($F = 3.35$, $df_N = 5$, $df_D = 23$, $P = 0.0204$) among the six treatments. Treatments of third and fourth stage larvae were significantly different from the control, but not from treatments of first, second and fifth stage larvae. Trial 2 also produced significantly

different results ($F = 4.27$, $df_N = 5$, $df_D = 24$, $P = 0.0064$) among the six treatments. Loss due to fourth and fifth stage larvae was significantly different from that associated with first stage larvae, but not from that associated with second and third stage larvae or the control. More mature larvae were able to consume greater amounts of tobacco leaf material before the plants became brown and dry from curing, while younger larvae were unable to consume the same amounts of leaf material in this period of time. Some older fifth stage larvae had most likely stopped feeding in preparation for pupation resulting in less leaf material consumed.

Results from the three field studies conducted in 2008 were very similar among all treatments (Figure 3.2), and yielded no significant differences. These results can be attributed to uneven stalk length and thickness and lack of precision in the scale we used to measure plant weights. A scale with a larger surface area was used to facilitate weighing large plants, but this reduced precision. Perhaps more importantly, stalks were not evenly dried when reweighed, resulting in variable weights among treatments.

Results from the three field studies conducted in 2009 (Figure 3.3) again yielded no significant differences. However, treatments of third and fourth stage larvae typically caused the most yield loss, similar to the results from 2007. Parasitism of *M. sexta* larvae by the Braconid wasp, *Cotesia congregata*, was observed during the first trial. Parasitized *M. sexta* larvae exhibit reduced food consumption (Beckage and Riddiford 1978). The rate of parasitism was not recorded.

Manduca sexta larvae present in curing structures causes some yield loss, although this yield loss is not always significant. The parasitic wasp, *C. congregata*, can continue to suppress *M. sexta* activity in open-air burley curing structures. Greatest loss is caused by

larvae that enter the curing structure near the fourth instar. Preliminary greenhouse trials were conducted under more controlled conditions than the field trials, in addition, the insects used in the greenhouse trials were not exposed to parasitism by *C. congregata*. This could explain why clearer results were observed and a significant difference was identified during 2007. Growers should examine plants before they enter the curing barn, and periodically during curing to determine whether post harvest *M. sexta* larvae are present.

Acknowledgements

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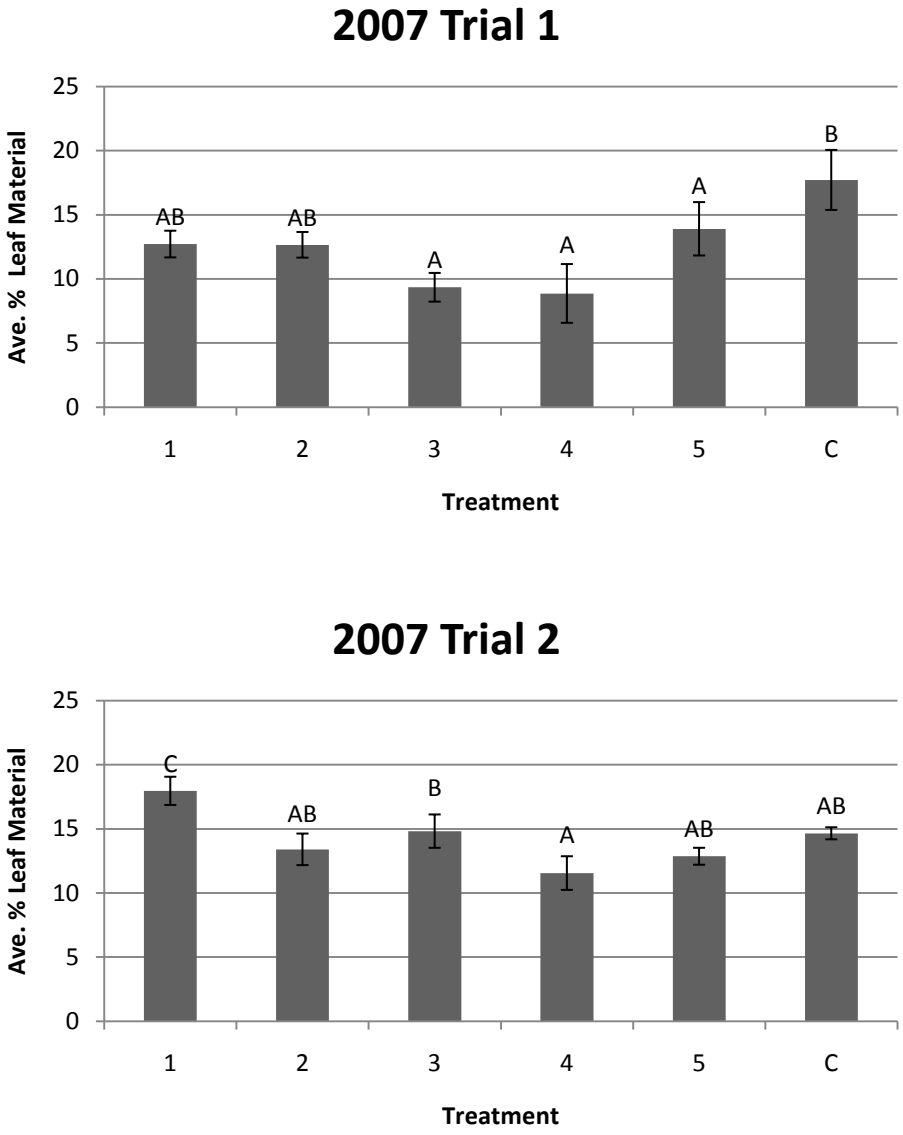

Figure 3.1. Mean ± standard error of % leaf material remaining after cure out and treatment of *M. sexta* larvae from two trials in 2007. Treatments followed by the same letter are not significantly different according to Fisher's LSD.

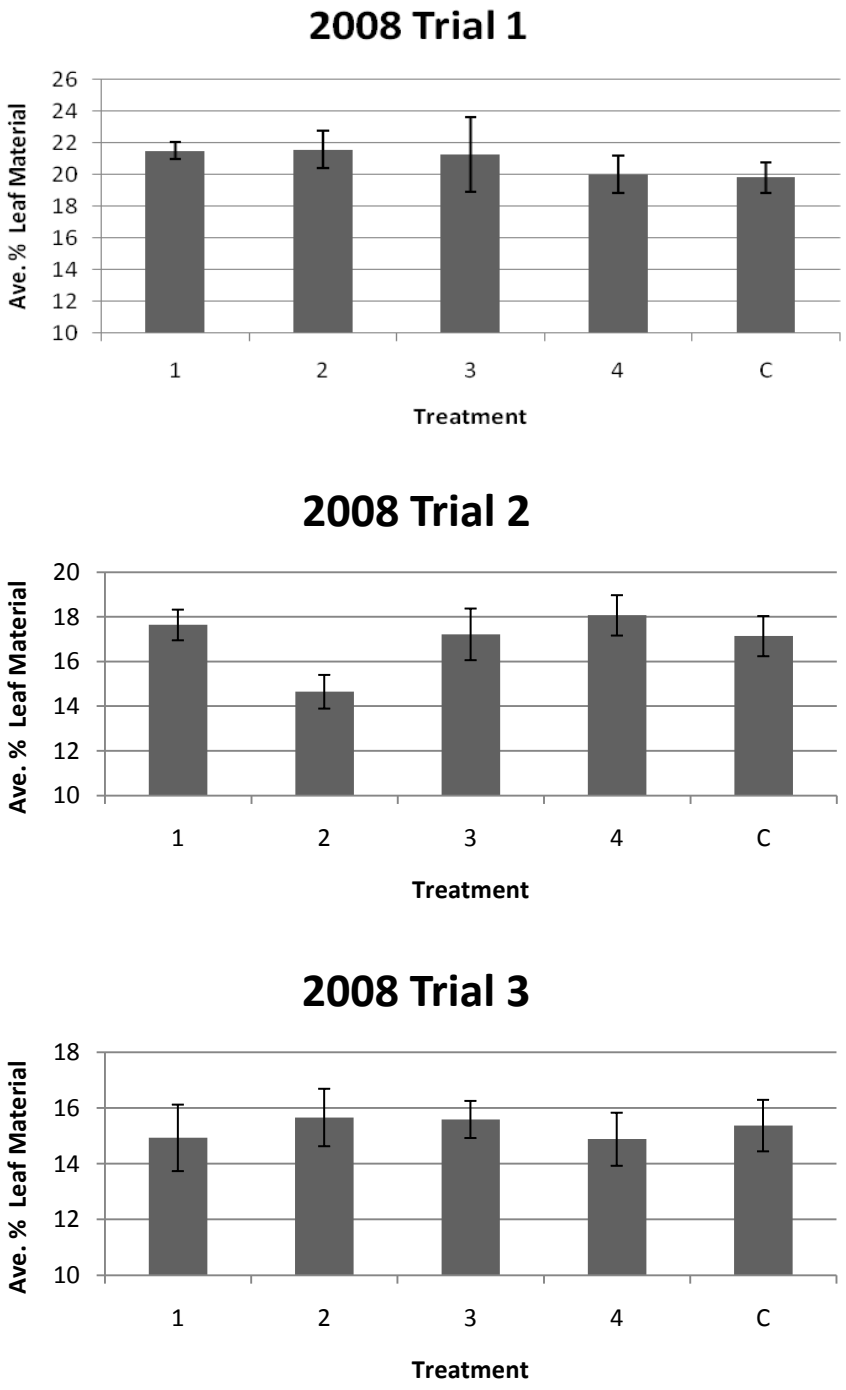

Figure 3.2. Mean ± standard error of % leaf material remaining after cure out and treatment of *M. sexta* larvae from three trials in 2008.

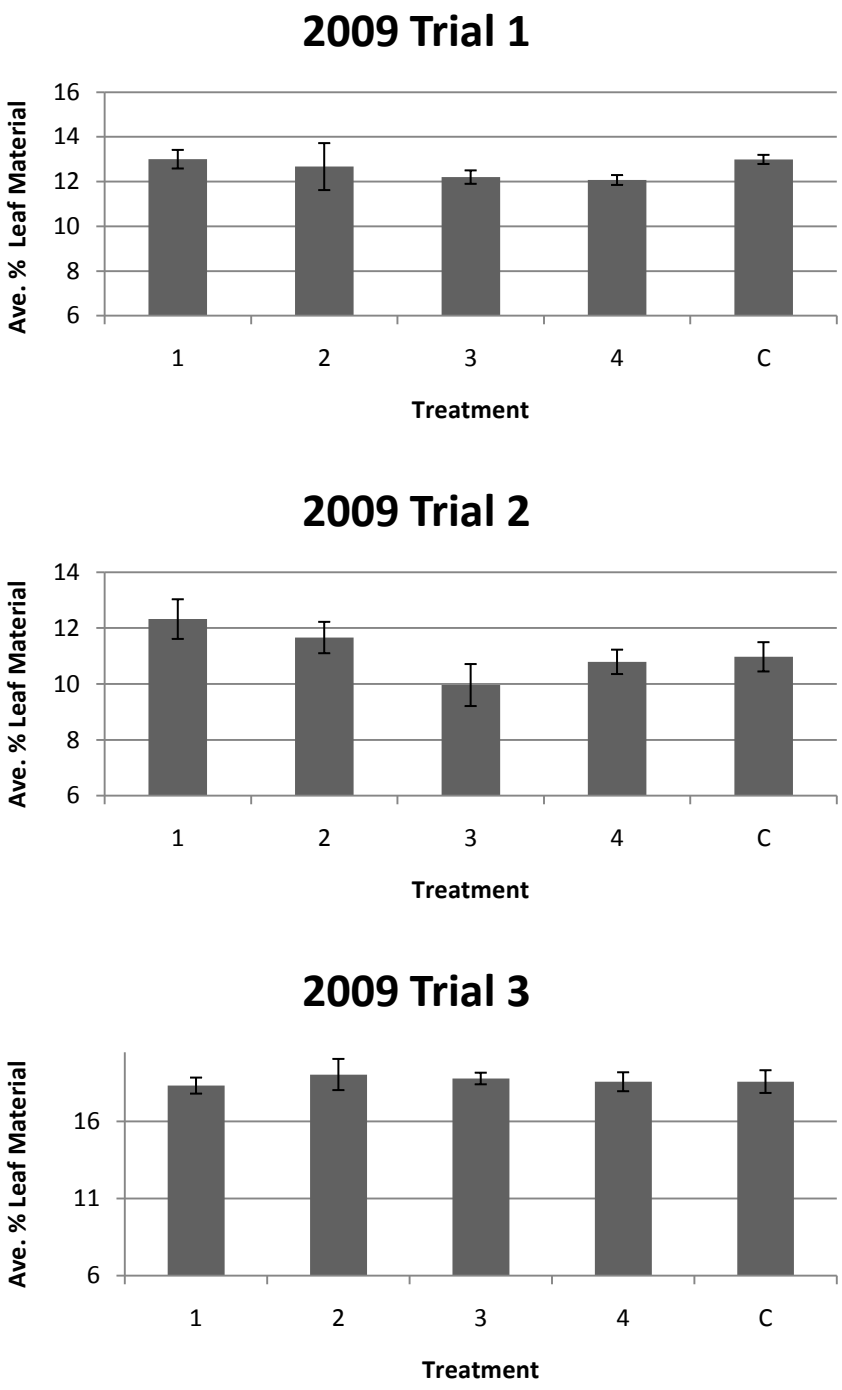

Figure 3.3. Mean ± standard error of % leaf material remaining after cure out and treatment of *M. sexta* larvae from three trials in 2009.