Research Note

SUSCEPTIBILITY OF BURLEY AND FLUE-CURED TOBACCO TYPES TO TOMATO SPOTTED WILT VIRUS TRANSMITTED BY THRIPS (ORDER: THYSANOPTERA)

M.J. Simoneaux[1] and C.E. Sorenson[2*]

Tomato spotted wilt virus (TSWV) is an economically important plant virus, belonging to the family *Bunyaviridae* and genus *Tospovirus,* first identified in the United States in the middle to late 1980s and in North Carolina tobacco in 1988. By 1997 TSWV had been identified in nearly every North Carolina county. TSWV incidence has increased since its introduction. 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. TSWV is transmitted mechanically by 7 thrips species worldwide. The tobacco thrips, *Frankliniella fusca* (Hinds) (Thysanoptera: Thripidae) is the most important vector of TSWV in eastern and central North Carolina, and the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is a locally important vector in the western piedmont and mountainous region of the state. Previous studies have revealed differences in disease progression between burley and flue-cured types of tobacco for some soil-borne diseases, such as black shank and Granville wilt. However, little is known about the differences in TSWV susceptibility between these two types of tobacco. In these field studies TSWV infection rates in burley and flue-cured tobacco were compared side by side under several conditions. Results of the study indicated that burley tobacco is more susceptible to TSWV.

Additional key words: tomato spotted wilt virus, TSWV, thrips, tobacco, burley, flue-cured

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, which was previously confined to the far western regions of the state by the price support program, is a crop of great interest to some of these growers. It is important for growers to understand the different insect and disease pressures they might encounter when planting burley tobacco in the eastern flue-cured tobacco growing regions. Tomato spotted wilt virus (TSWV) is one of the most significant pest threats to tobacco production in the southeastern United States.

TSWV belongs to the family *Bunyaviridae* and genus *Tospovirus*. It was first identified in the United States in the middle to late 1980s (2), 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 (6).

TSWV is transmitted by 7 thrips species worldwide (11). *Frankliniella fusca* (Hinds), the tobacco thrips, is the most important vector of TSWV in eastern and central North Carolina, and *Frankliniella occidentalis* (Pergande), the western flower thrips, is a locally important vector in the western piedmont and mountainous region of the state (3). Additional thrips vectors include *Frankliniella bispinosa* (Morgran), *Frankliniella intonsa* (Trybom), *Frankliniella schultzei* (Trybom), *Thrips tabaci* (Hinds), and *Thrips setosus* Moulton (11). 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 (10). Once the thrips has acquired the virus, it remains a vector for its entire life (9).

Tobacco plants infected with TSWV display a range of symptoms, including wilting and yellowing of leaves, ring spot and 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, 40–75 days after sowing (DAS), are more likely to develop local infections then older plants, at 95–100 DAS. Systemic infection gradually decreases as plant age increases, with plants at 40 DAS being most susceptible, followed by those at 60–75 DAS, and 95–100 DAS (7).

Two agrochemicals 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 (4). Imidacloprid can be applied as a greenhouse float tray overspray, as a soil drench after transplanting, or as a foliar insecticide; this insecticide suppresses TSWV by reducing the frequency and duration of tobacco thrips feeding bouts (5). Acibenzolar-S-methyl (Syngenta Crop Protection, Inc., Wilmington, DE), is a plant activator and is applied in the form of a soil drench. Acibenzolar-S-methyl induces a plant's natural defense mechanisms and has antifungal, antibacterial, and antiviral activity. Past studies have shown that to produce beneficial effects, acibenzolar-S-methyl must

*Corresponding author: C.E. Sorenson; email:clyde_sorenson@ncsu.edu
[1]Louisiana State University AgCenter, P.O. Box 250, Edgard, LA 70049.
[2]Department of Entomology, North Carolina State University, Campus Box 7613, 100 Derieux Place, Raleigh, NC 27695.

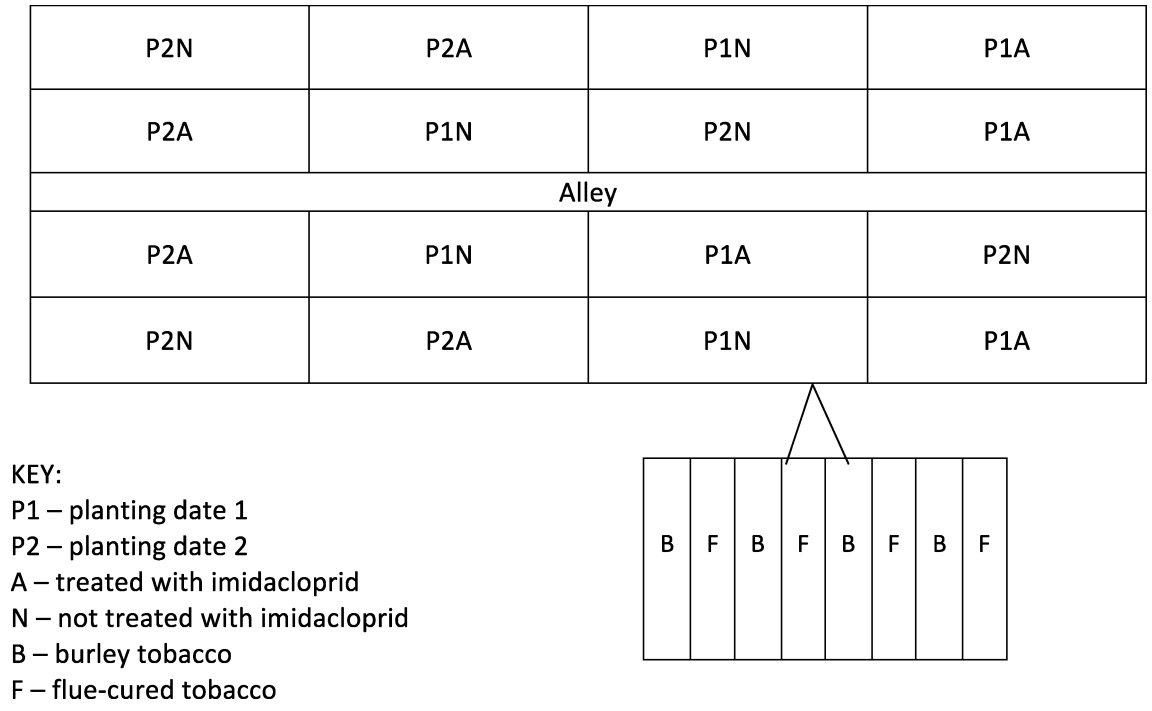


Figure 1. Kinston 2008 field map, showing 4 replications of 8 treatments in a randomized complete block design.

be applied no more than 7–9 days prior to transplanting (1).

The studies described herein were conducted to determine if burley tobacco types are more, less, or equally susceptible to TSWV compared to traditional flue-cured tobacco types grown in eastern North Carolina.

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 2 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 8 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 4 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 288-cell polystyrene float trays in greenhouses dedicated to tobacco transplant production at both locations.

Half of the plants of each tobacco type were pretreated with imidacloprid; 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 fl. oz. of formulated product per 1,000 plants, 2–3 days before transplanting, for appropriate plots.

The field at each location consisted of 16 plots arranged in a 4 × 4 randomized complete block (Figure 1). Each plot measured 16 rows wide and 15.24 m 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 m apart and plants within rows were 0.56 m apart, whereas at the Central Crops Research Station between-row spacing measured 1.14 m and within-row plant spacing measured 0.56 m. Planting date and insecticide treatment were applied to the entire plot. Tobacco type was superimposed within each plot; burley and flue-cured tobaccos were planted in alternating pairs of rows (i.e., 2 rows of burley followed by 2 rows of flue-cured, and so forth, for a total of 8 rows of each tobacco type). This was done to account for any unevenness in thrips distribution and resultant variation in TSWV incidence that might have occurred within the field.

At each location, transplanting was conducted on 2 dates, 2 weeks apart. In 2008, transplanting was done at the Cunningham Research Station on April 23 and May 8; at the Central Crops Research Station tobacco was transplanted 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 field flag; this allowed us to account more accurately for plants dead from TSWV later in the season.

Statistical analyses were completed with the use of SAS® software Version 9.1 (8). Data from the entire season at each location were pooled and subjected to an analysis of variance (ANOVA) procedure (PROC GLM); with means separation through a least-significant-difference (LSD) test (LSMEANS).

TSWV Commercial Surveys. With the help of North Carolina Cooperative Extension county field personnel, 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 3 km. 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 8 fields, 4 of flue-cured and 4 of burley, were surveyed. Fields in Sampson County were surveyed on July 24, and fields in Wilson, Johnston, and Duplin counties were surveyed on August 1. At each field site four 100-plant samples were randomly selected. Samples were surveyed visually, and the number of plants apparently infected with TSWV, as indicated by visual symptoms, was recorded.

In 2008, survey sites were located in Sampson, Wilson, Edgecombe, Johnston, and Duplin counties. A total of 15 fields were surveyed, including 6 flue-cured, 7 burley, and 2 Maryland tobacco fields. 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 surveyed visually, and the number of plants apparently infected with TSWV, as indicated by visual symptoms, was recorded.

In 2009, survey sites were located in Sampson, Wilson, Johnston, and Duplin counties. A total of 19 fields were surveyed, including 9 of flue-cured tobacco and 10 of 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 surveyed visually, and the number of plants apparently infected with TSWV as indicated by visual symptoms was recorded.

Statistical analyses were completed with the use of SAS® software Version 9.1 (8). Individual site data and yearly pooled data were subjected to an analysis of variance (ANOVA) procedure and Fisher's LSD.

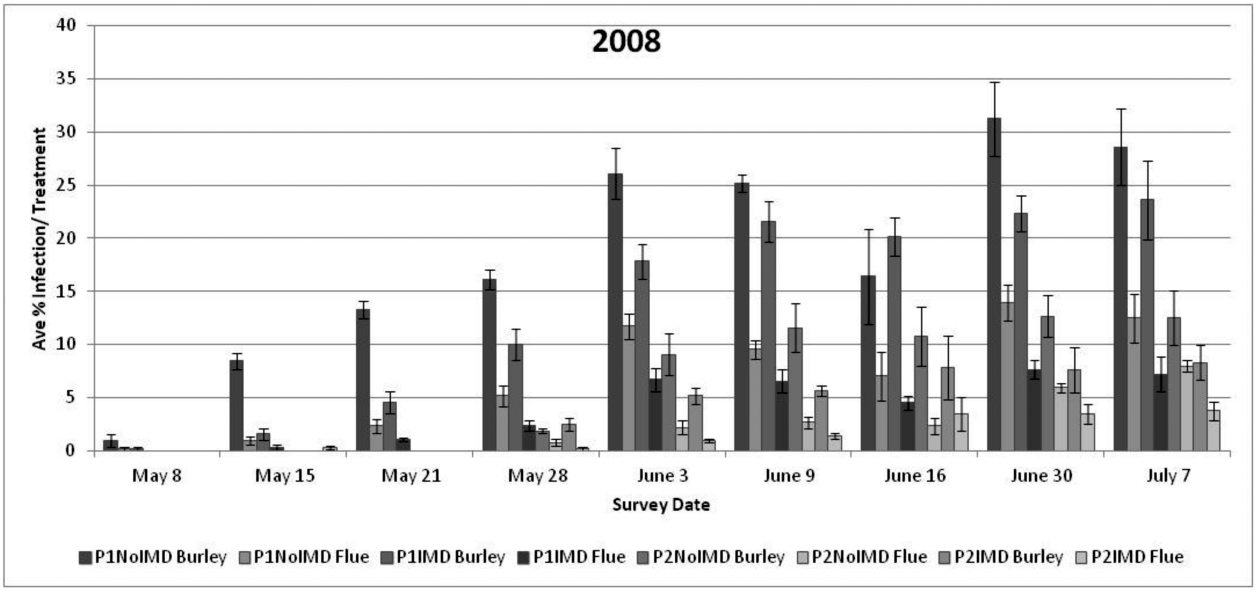
RESULTS

TSWV Incidence in Burley and Flue-Cured Tobaccos. Because of the 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 were generated at the Cunningham Research Station. TSWV infection at Central Crops Research Station was less than 1.6% per plot on 15 July 2008; no data were collected in 2009 because of early-season cold weather injury.

In 2008, plots transplanted early had a higher percentage of infected plants per plot than the plots transplanted 2 weeks later ($F = 278.56$, $df_N = 1$, $df_D = 33$, $P < 0.0001$) (Figure 2). Plots receiving a pretreatment of imidacloprid had a lower percentage of infected plants per plot than plots not receiving imidacloprid ($F = 43.30$, $df_N = 1$, $df_D = 33$, $P < 0.0001$). Burley tobacco plants had a higher percentage of infected plants per plot than the flue-cured tobacco plants ($F = 260.59$, $df_N = 1$, $df_D = 33$, $P < 0.0001$); the infection rate in burley tobacco was approximately twice that 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 2 weeks earlier ($F = 26.40$, $df_N = 1$, $df_D = 29$, $P < 0.0001$). Plots receiving a pretreatment of imidacloprid had a lower percentage of infected plants per plot than plots not receiving imidacloprid ($F = 125.25$, $df_N = 1$, $df_D = 29$, $P < 0.0001$). Burley tobacco plants again had a higher percentage of infected plants than the flue-cured ($F = 282.62$, $df_N = 1$, $df_D = 29$, $P < 0.0001$). Infection in burley tobacco plants was again 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 ($F = 55.21$, $df_N = 1$, $df_D = 10$, $P < 0.0001$), Duplin County ($F = 25.22$, $df_N = 1$, $df_D = 6$, $P = 0.0024$), Wilson County–Parkers BBQ ($F = 19.09$, $df_N = 1$, $df_D = 6$, $P = 0.0047$), and Wilson County–Contentnea Creek ($F = 11.19$, $df_N = 1$, $df_D = 6$, $P = 0.0155$), and between mean incidence of TSWV-infected burley, flue-cured and Maryland tobacco in Edgecombe County–Old ($F = 4.83$, $df_N = 2$, $df_D = 9$, $P = 0.0376$). A significant difference was not identified between mean incidence of TSWV-infected burley and flue-cured tobacco in Johnston County or in Edgecombe



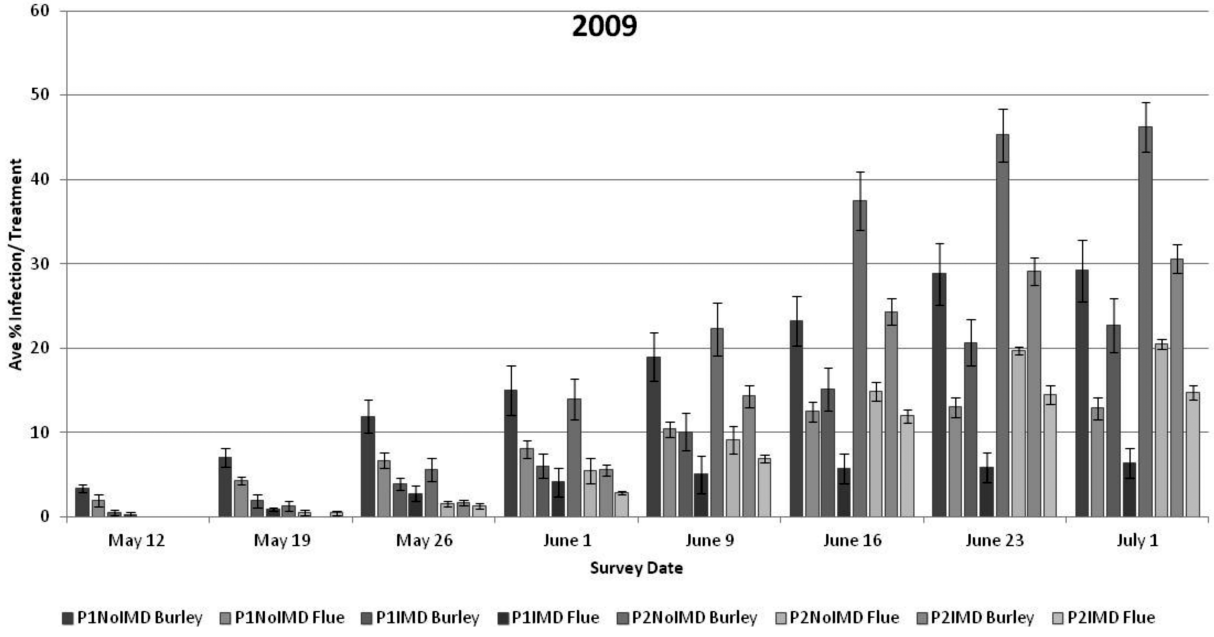


Figure 2. Mean ± standard error of tomato spotted wilt virus incidence by sampling date at Kinston in 2008 and 2009.

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 = 0.0015), Johnston County–Raleigh Road ($F = 18.38$, $df_N = 1$, $df_D = 6$, P = 0.0052), Johnston County–Langdon ($F = 53.57$, $df_N = 1$, $df_D = 6$, P = 0.0003), Sampson County ($F = 83.33$, $df_N = 1$, $df_D = 6$, P < 0.0001), Wilson County ($F = 35.27$, $df_N = 1$, $df_D = 6$, P = 0.0010), Wilson County–water tower ($F = 12.26$, $df_N = 1$, $df_D = 6$, P = 0.0128), Duplin County ($F = 17.47$, $df_N = 1$, $df_D = 7$, P = 0.0019), and Duplin County–Sandridge Road ($F = 66.65$, $df_N = 1$, $df_D = 6$, P = 0.0002). A significant difference was not identified between mean incidence of TSWV-infected burley and flue-cured tobacco in Wilson County–Wilco Road (Table 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.

Table 1. Commercial survey results 2007, 2008, and 2009.[a]

Year	County	Site	Tobacco Type	TSWV Treatment	Transplant Date	Survey Date	Mean % Incidence
2007	Duplin		Burley (KT204)	None	May 10	August 1	41.0
	Duplin		Flue-cured (NC71)	AD, ACT	April 23	August 1	7.0
	Johnston		Burley	Unknown	Unknown	August 1	7.0
	Johnston		Flue-cured	Unknown	Unknown	August 1	1.0
	Sampson		Burley	None	May 15	July 24	26.5
	Sampson		Flue-cured	AD	April 21	July 24	11.8
	Wilson		Burley (KY204 and NC7)	PLT on KY204	May 10 and April 12	August 1	11.6
	Wilson		Flue-cured	Unknown	Unknown	August 1	2.8
2008	Duplin		Burley	AD, ACT	Unknown	August 8	12.4
	Duplin		Flue-cured	AD, ACT	Unknown	August 8	3.9
	Edgecombe	Old	Burley (NC7)	PLT	April 29–May 3	August 1	3.4
	Edgecombe	Old	Flue-cured	Unknown	Unknown	August 1	1.5
	Edgecombe	Old	Maryland (M609)	PLT	April 29–May 3	August 1	1.0
	Edgecombe	Young	Burley (NC7)	PLT	~May 15	August 1	0.6
	Edgecombe	Young	Maryland (M609)	PLT	~May 15	August 1	0.5
	Johnston		Burley	Unknown	Unknown	August 8	0.9
	Johnston		Flue-cured	Unknown	Unknown	August 8	0.9
	Sampson		Burley	AD	Unknown	July 29	13.3
	Sampson		Flue-cured	AD	Unknown	July 29	4.0
	Wilson	Parkers BBQ	Burley	Unknown	Unknown	August 1	5.6
	Wilson	Parkers BBQ	Flue-cured	Unknown	Unknown	August 1	1.8
	Wilson	Contentnea Creek	Burley	Unknown	Unknown	August 1	7.4
	Wilson	Contentnea Creek	Flue-cured	Unknown	Unknown	August 1	2.8
2009	Duplin	Sandridge Road	Burley (KT 204)	AD, ACT	April 23	July 30	19.1
	Duplin	Sandridge Road	Flue-cured (K326)	AD, ACT	April 20	July 30	4.0
	Duplin		Burley (KT204)	AD	April 27	July 30	29.6
	Duplin		Burley (KT204)	AD, ACT	April 27	July 30	27.3
	Duplin		Flue-cured (NC71)	AD	April 20	July 30	4.1
	Johnston	C. Church Road	Burley	Unknown	Unknown	July 23	6.0
	Johnston	C. Church Road	Flue-cured	Unknown	Unknown	July 23	1.5
	Johnston	Raleigh Road	Burley	Unknown	Unknown	July 23	4.8
	Johnston	Raleigh Road	Flue-cured	Unknown	Unknown	July 23	1.3
	Johnston	Langdon	Burley (KT204)	IMD	May 10	July 23	4.0
	Johnston	Langdon	Flue-cured	Unknown	Unknown	July 23	0.9
	Sampson		Burley	Unknown	Unknown	July 15	19.3
	Sampson		Flue-cured	Unknown	Unknown	July 15	6.8
	Wilson		Burley (NC7)	PLT	May 5	July 15	9.4
	Wilson		Flue-cured (CC27)	PLT	Unknown	July 15	2.9
	Wilson	Water tower	Burley (NC7)	PLT	Unknown	July 15	12.4
	Wilson	Water tower	Flue-cured (CC27)	PLT	May 1	July 15	4.5
	Wilson	Wilco Road	Burley (NC7)	AD	May 5	July 15	5.1
	Wilson	Wilco Road	Flue-cured (K326)	PLT	May 4	July 15	3.9

[a] TSWV = tomato spotted wilt virus, AD = Admire, ACT = Actigard, PLT = Platinum, IMD = imidacloprid.

When the total infection rate for each tobacco type during each year was analyzed, a significant difference was identified between tobacco types for 2007 ($F = 13.86$, $df_N = 1$, $df_D = 34$, $P = 0.0007$), 2008 ($F = 11.95$, $df_N = 2$, $df_D = 61$, $P < 0.0001$), and 2009 ($F = 18.57$, $df_N = 1$, $df_D = 72$, $P < 0.0001$).

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, whereas 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 (7).

Plots receiving pretransplant treatments of imidacloprid had a lower mean incidence of TSWV-infected plants, and imidacloprid continued to act as an effective

means of chemical control. In laboratory studies, imidacloprid reduced the number of thrips feedings and the duration time of each feeding (5), and our observations of reduced TSWV may reflect this effect. TSWV incidence reductions of 50% were observed in some imidacloprid-treated plots (Figure 2).

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 of 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 continued to have higher TSWV pressure than the central region of the state.

TSWV Commercial Surveys. Generally, commercial fields of burley tobacco had a higher incidence of TSWV than nearby commercial fields of flue-cured and Maryland tobacco each year. This is most likely a result of greater susceptibility of burley tobacco to TSWV, and/or a greater attraction of thrips to the light green, almost yellow color of the leaves of burley types of tobacco.

Tobacco fields treated with 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 southeastern-most county included in our survey, and historically has high TSWV incidence (C.E.S., personal observation based on annual surveys).

ACKNOWLEDGMENTS

We thank Carol Berger, Amanda Beaudoin, Ramsey Lewis, and Scott Whitley for providing tobacco seed, Loren Fisher and Joe Priest for providing tobacco plants, Consuelo Arellano for statistical assistance, members of the Sorenson lab, the Central Crops Research Station, the Cunningham Research Station, County Extension Agents, and cooperating growers from Duplin, Edgecombe, Johnston, Sampson, and Wilson counties, and the North Carolina Tobacco Foundation for the funding that made this research possible.

LITERATURE CITED

1. Csinos AS, Pappu HR, McPherson RM, Stephenson MG. 2001. Management of tomato spotted wilt virus in flue-cured tobacco with acibenzolar-s-methyl and imidacloprid. Plant Disease 85:292–296.

2. Culbreath AK, Csinos AS, Bertrand AS, Demski PF. 1991. Tomato spotted wilt virus epidemic in flue-cured tobacco in Georgia. Plant Disease 74:483–485.

3. Eckel CS, Cho K, Walgenbach JF, Kennedy GG, Moyer JW. 1996. Variation in thrips species composition in field crops and implications for tomato spotted wilt epidemiology in North Carolina. Entomologia Experimentalis et Applicata 78:19–29.

4. Elbert A, Overbeck H, Iwaya K, Tsuboi S. 1990. Imidacloprid, a novel systemic nitromethylene analogue insecticide for crop protection. Proceedings of the British Crop Protection Conference—Pests and Diseases, Brighton, England. Pp. 21–28.

5. Groves RL, Sorenson CE, Walgenbach JF, Kennedy GG. 2001. Effects of imidacloprid on transmission of tomato spotted wilt tospovirus to pepper, tomato and tobacco by *Frankliniella fusca* Hinds (Thysanoptera: Thripidae). Crop Protection 20(5):439–445.

6. Groves RL, Walgenbach JF, Moyer JW, Kennedy GG. 2002. The role of weed hosts and tobacco thrips, *Frankliniella fusca*, in the epidemiology of tomato spotted wilt virus. *Plant Disease* 86:573–582.

7. Mandal B, Wells ML, Martinez-Ochoa N, Csinos AS, Pappu HR. 2007. Symptom development and distribution of tomato spotted wilt virus in flue-cured tobacco. Annals of Applied Biology 151:67–75.

8. SAS Institute Inc. 2009. Version 9.1, SAS Institute Inc., Cary, NC.

9. Ullman DE. 1996. Thrips and tospoviruses: Advances and future directions. Acta Horticulture 431:310–324.

10. Van de Wetering F, Goldbach R, Peters D. 1996. Tomato spotted wilt tospovirus ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. Phytopathology 86:900–905.

11. Whitfield AE, Ullman DE, German TL. 2005. Tospovirus–thrips interactions. Annual Review of Phytopathology 43:459–489.