

## ABSTRACT

CHERRY, KATHRYN. Investigation of In Season Progression of *Tomato spotted wilt virus* and its Management in Flue-cured Tobacco Fields of North Carolina. (Under the direction of Asimina Mila).

*Tomato spotted wilt virus* continues to be a serious problem on tobacco production in the southeast United States. The current control recommendation calls for acibenzolar-S-methyl, which is known by growers in North Carolina to have unacceptable phytotoxicity. In an attempt to reduce the amount of phytotoxicity while retaining similar levels of control as the current recommendation, on-farm tests were conducted in North Carolina to refine the application rate, timing and method of acibenzolar-S-methyl application, with or without an insecticide. The lowest disease incidence and highest phytotoxicity were recorded in treatments consisting of greenhouse drench applications of imidacloprid or thiamethoxam and acibenzolar-S-methyl, as compared to the untreated control. TSW severity and temporal progress results suggest that certain treatments delay the onset of symptoms. Furthermore, supplementary greenhouse experiments showed that timing the clipping relative to the timing of applications of the aforementioned chemicals had no effect on phytotoxicity. Both the order in which the chemicals were applied and applying the second chemical at varying times relative to the first had no consistent effect upon the phytotoxicity measured in the field. Overall, applying acibenzolar-S-methyl with an insecticide in the greenhouse provided the best control among the treatments tested.

The temporal and spatial distribution of Tomato spotted wilt (TSW) was studied in a total of 25 naturally infested fields in 2006, 2007, and 2008. Sections within the fields were monitored that ranged in size from 76 x 11 meters to 137 x 10 meters. TSW incidence was measured on a weekly basis. TSW temporal progression data was fit to a logistic regression model with cumulative degree days and past TSW field history as explanatory variables. Analyses revealed that both variables are significant explanatory factors of the temporal progression of TSW on tobacco. Cooling degree-days were calculated with four different bases, and a base temperature of 10°C, corresponding to thrips development, resulted in the

best fitted model. TSW spatial distribution was investigated in 8 locations using universal kriging interpolations on TSW incidence at 5 and 9 weeks post transplanting. Although isolated disease clusters were present, the overall spatial pattern was random, which has been previously observed with TSW incidence in other crop systems in southeast US. These findings suggest that when thrips move into a field infections occur randomly. Further work is needed in order to determine the dispersal of thrips within a field, which will help refine management practices.

Investigation of In Season Progression of *Tomato spotted wilt virus* and its Management in  
Flue-cured Tobacco Fields of North Carolina

by  
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## **BIOGRAPHY**

Kathryn Renee Cherry grew up in Stanley, NC. After finishing high school she traveled to Raleigh, NC to attend North Carolina State University. After a couple years studying computer science she decided she wanted a job that would get her outside more, so she began studying botany. Once she graduated with her degree she found a job nearby in the RTP where she learned a lot during the several months she was there. However, she knew that she was not done with her education and thus headed back to NC State. While she was exploring her options within the Plant Pathology Department, Dr. Dave Shew hired her to take care of the lab and help his aspiring Ph.D. student. Liking what she saw, the paperwork was soon completed to get her started pursuing a master's degree. Now, after eight years in Raleigh, she's ready to start her career as a plant pathologist.

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## TABLE OF CONTENTS

LIST OF TABLES .....	v
LIST OF FIGURES .....	vi

CHAPTER 1. Effects of chemical controls on Tomato Spotted Wilt Virus occurrence and phytotoxicity on tobacco in North Carolina.....	1
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Abstract.....	1
Introduction.....	2
Materials and Methods.....	5
Results.....	9
Discussion.....	12
Literature Cited .....	16

CHAPTER 2. Temporal and spatial distribution of Tomato Spotted Wilt Virus incidence in flue-cured tobacco in North Carolina .....	30
---	----

Abstract.....	30
Introduction.....	31
Materials and Methods.....	33
Results.....	38
Discussion.....	41
Literature Cited .....	45

## LIST OF TABLES

### Page

### Chapter 1

Table 1.	Treatments of imidacloprid (IMD, Admire Pro 4.2F), acibenzolar-S-methyl (ASM, Actigard 50 WG), thiamthoxam (Platinum or T-Moxx) applied in 3 locations in 2006, 2 in 2007, and 3 in 2008 .....	20
Table 2.	Percent of TSWV incidence 9 weeks after transplanting tobacco in a field with different combinations of imidacloprid, thiamethoxam, and ASM in 3 locations in 2006, 2 in 2007 and 3 in 2008 .....	21
Table 3.	Leaf width (mm) 4 weeks after transplanting tobacco in the field with different combinations of imidacloprid, thiamethoxam, and ASM in 3 locations in 2006, 2 in 2007 and 3 in 2008 .....	22
Table 4.	Effect of different combinations of IMD, thiamethoxam (Platinum or T-Moxx), and ASM on TSWV symptom onset and rate of progression in three tobacco fields in 2006 in North Carolina .....	23
Table 5.	Effect of different combinations of IMD, thiamethoxam (Platinum or T-Moxx), and ASM on TSWV symptom onset and rate of development in three tobacco fields in 2007 in North Carolina. ....	24
Table 6.	Effect of different combinations of IMD, thiamethoxam (Platinum or T-Moxx), and ASM on TSWV symptom onset and rate of development in three tobacco fields in 2008 in North Carolina. ....	25

Table 7.	Mean green weight of tobacco seedlings in the greenhouse 15 days after the first chemical application for Trial 1 and 18 days for Trial 2 of the greenhouse studies .....	26
Table 8.	Leaf width at four weeks after transplanting of seedlings subjected to different timing of chemical applications from the greenhouse studies .....	27
Table 9.	Percent (%) TSWV incidence at nine weeks after transplanting the greenhouse studies .....	28

## Chapter 2

Table 1.	Rates and brand names of imidacloprid used in each location and the date on which the tobacco was transplanted at each location.....	49
Table 2.	Locations in 2006, 2007, and 2008 from which data was collected for spatial distribution analysis and a description of the immediately surrounding terrain .....	50
Table 3.	Criteria of goodness of fit of TSWV incidence model I and model II for eastern North Carolina .....	51
Table 4.	Parameter estimates of the logistic regression analysis used to quantify TSWV incidence by using degree-days (DD) and disease history as explanatory variables in tobacco fields in eastern NC from 2006 to 2008.....	52
Table 5.	Classes of TSWV incidence predicted by model II, using the four different lower thresholds, and compared to the observed class nine weeks after transplant.....	53



Table 6.	P-values of the linear regression model between the observed and the predicted values of the four semivariogram models used for the spatial analysis.....	54
Table 7.	Parameter values of the circular, spherical, exponential, and gaussian semivariogram models and their associated cross validation regression equations for the locations in 2006 .....	55
Table 8.	Parameter values of the circular, spherical, exponential, and gaussian semivariogram models and their associated cross validation regression equations for the locations in 2007 .....	56
Table 9.	Parameter values of the circular, spherical, exponential, and gaussian variogram models and the associated cross validation regression equations for the locations in 2008 .....	57

## LIST OF FIGURES

	Page
<b>Chapter 1</b>	
Figure 1. TSWV severity 9 weeks after transplanting with different combinations of imidacloprid, thiamethoxam, and ASM in 1 location in 2007 and 3 in 2008..	
.....	29
<b>Chapter 2</b>	
Figure 1. Maps of the normalized percentage of TSWV incidence in each field in (a) 2006 Duplin County at week 5, 2007 Sampson County at week 5, 2008 Craven County week 5 and (b) 2006 Sampson County at week 9, 2007 Jones County at week 9, 2008 Beaufort County at week 9. ....	58

# **CHAPTER 1. Effects of chemical controls on Tomato Spotted Wilt Virus occurrence and their potential phytotoxicity on tobacco in North Carolina**

## **ABSTRACT**

*Tomato spotted wilt virus* continues to be a serious problem on tobacco production in the southeast United States. Current control recommendation calls for the use of an insecticide along with acibenzolar-S-methyl, which is known by growers in North Carolina to have unacceptable phytotoxicity on tobacco. In an attempt to reduce the phytotoxicity while retaining similar levels of control as the recommendation, on-farm tests were conducted in North Carolina to refine the application rate, timing and method of acibenzolar-S-methyl application, with or without an insecticide. The lowest TSWV incidence and highest phytotoxicity as compared to the untreated control were recorded in treatments of greenhouse drench applications of imidacloprid or thiamethoxam together with acibenzolar-S-methyl. Furthermore, investigation of TSWV symptom severity and temporal progress suggest that the treatments mentioned above delay the onset of symptoms. Simultaneously, greenhouse experiments showed that timing the clipping of tobacco seedlings relative to the timing of applications of the aforementioned chemicals had no effect on phytotoxicity. Both the order in which the chemicals were applied and applying the second chemical at varying times relative to the first had no consistent effect upon the phytotoxicity measured in the field four weeks after the treated plants were transplanted. Overall, in North Carolina applying acibenzolar-S-methyl with an insecticide in the greenhouse provided the best control among the treatments tested due to prevention or delay of TSWV infections the first few weeks after transplant.

## INTRODUCTION

*Tomato spotted wilt virus* (TSWV), the causal agent of Tomato spotted wilt (TSW), has been an important virus worldwide for several decades. It has caused serious economical damage on several crops in the southeastern United States, including tobacco (*Nicotiana tabacum* L.). The virus belongs to the family *Bunyaviridae* and the genus *Tospovirus* [25]. The pathogen has a very wide host range and is transmitted by several species of thrips (*Thrips* and *Frankliniella* spp.) [29,36]. Managing the disease is complicated by the fact that both the pathogen and the vector have wide host ranges and also because the severity of the disease varies unpredictably from year to year.

The thrips species mostly responsible for the spread of TSWV on tobacco in eastern and central North Carolina is the tobacco thrips, *Frankliniella fusca* (Hinds) [9]. TSWV has not been reported in greenhouse tobacco seedlings, except for rare cases, and therefore is not considered a greenhouse production problem. Based on the available evidence, thrips and TSWV overwinter on weeds and eventually a migration occurs of thrips from the weeds to the crops [9,13,14]. The timing of the migration depends upon several variables, including weather and thrips population growth on winter weeds, and thus the peak of the migration varies between both locations and years [13,14]. The migration of thrips from winter weeds to crops can coincide with the transplanting date. This means that tobacco seedlings can be infected during the time they are most susceptible, which is soon after they have been transplanted [3]. Therefore, protecting the plants before they are transplanted has been proven to be the most beneficial strategy [6,20].

While there has been some success in managing TSWV with resistant cultivars in crops such as peanut and tomato, there has not been success breeding a resistant tobacco cultivar so far [2,21,24]. Other alternative methods of managing TSWV, such as varying the transplant date, have also proven to be largely inapplicable against suppressing the level of TSWV in tobacco [22,27] despite the fact that in 2008 Nischwitz *et al.* [27] demonstrated that the age of the tobacco seedlings at the time of transplant may influence TSWV incidence.

Thus the application of pesticides has been the only successful tactic to manage TSWV on tobacco. In North Carolina, the recommended pesticides include imidacloprid and acibenzolar-S-methyl [3,23,24]. Imidacloprid (IMD) is an insecticide that is labeled for use against flea beetles and aphids, but has been shown to be useful in acting as a feeding deterrent to some thrips species [12,16]. According to Groves *et al.* [12] IMD is capable of reducing the number and duration of feeding of *Frankliniella fusca* on tobacco. Similar results were recorded using *F. fusca* by Joost *et al.* [16] on tomato. Similar effectiveness of suppressing TSWV with IMD has been documented in tomato and lettuce [6,28]. On the other hand, use of IMD in experiments in Greece was not effective against another vector, *Thrips tabaci*, although the authors state this may be due to the low rates used in the study [4].

Acibenzolar-S-methyl (ASM) is a plant activator that has been reported to suppress fungal, bacterial, and viral diseases in several hosts and thus it has been considered a potential tool of crop protection [1,8,11,15,18,28,30,34]. ASM works by activating the systemic acquired resistance (SAR) pathway [10,11,17] of the plant. ASM activates the same defense responses as the natural signal molecule salicylic acid does in the biological activation of SAR [31,33]. In a previous study by Tosi *et al.* [34], ASM was shown to provide protection against *Plasmopara helianthi* in sunflower plants. Another study by Louws *et al.* reported a reduction in bacterial fruit spot and speck incidence in tomato.

ASM and IMD have been shown to reduce the incidence of TSWV in the southern United States, especially in Georgia [7,20,27,28] when they are applied in the greenhouse a few days before the tobacco is planted in the field. So far in North Carolina the adaptation of ASM for TSWV control has not been as successful as in Georgia for two reasons: (i) TSWV has not been as widespread and severe as in Georgia and (ii) growers have noticed phytotoxicity, in this case slow growth of the treated plants, when ASM is used. This is prominent during the first few weeks after transplanting and although plants recover eventually, growers do not like this initial delay in growth because it interferes with other aspects of crop production. This limits the recommendable options to ASM application only

in cases that TSWV incidence is so high that the control provided by ASM offsets the undesirable phytotoxicity [3].

The objectives of this study were to: (i) investigate different methods of application of ASM alone or in combination with an insecticide in managing TSWV on flue-cured tobacco under field conditions in North Carolina; (ii) investigate how these applications affect TSWV progress and severity during the season; and (iii) evaluate phytotoxicity associated with the application of chemical controls on tobacco and management practices that potentially could reduce the phytotoxic effect.

## MATERIALS AND METHODS

**Field studies.** Field studies were conducted in three fields in southeastern North Carolina located in Duplin, Onslow, and Sampson counties in 2006, in two fields located in Jones and Sampson Counties in 2007 and three fields located in Beaufort, Craven, and Duplin Counties in 2008. All fields had a history of TSWV occurrence. Pesticides applied for disease control included ASM (Actigard<sup>®</sup> 50 WG, Novartis Crop Protection, Inc., Raleigh, NC) alone or in combination with the insecticides IMD (Admire Pro<sup>®</sup> 4.2 F Bayer Corporation, Kansas City, MO) or thiamethoxam (Platinum<sup>®</sup> Syngenta Crop Protection, Greensboro, NC or T-Moxx<sup>®</sup> Syngenta Crop Protection, Greensboro, NC).

Tobacco cultivars CC 27 (in all locations in 2006), NC 71 (in Jones County 2007) and K 326 (in Sampson County 2007 and in all locations in 2008) were produced in greenhouse float beds in each corresponding county. The applied treatments for each location are presented in Table 1. For the treatments applied in the greenhouse, once the plants had been sprayed they were drenched with water to rinse the chemicals into the root ball, except for the ASM (float) treatment in Craven and Duplin Counties in 2008 that was applied to the float water. For this treatment, ASM was first diluted in a small quantity of water and then added to the float bed water.

Applications were made with a CO<sub>2</sub> powered back sprayer at 30 psi, except for the float water treatments. Plants were transplanted into all fields 5-13 days after chemical applications, depending on the farmer's schedule. The plots were 50 ft. in length and consisted of four 48 in. wide rows, except in Sampson County in 2006 where the rows were 46 in. wide. In each location, the experimental design was a complete randomized block with four replications per treatment. The field treatment was a foliar application of ASM (Actigard 50 WG at 2.87 g a.i. per 1 hectare) and was done on the same day as transplanting, immediately after the plants were planted, except in Duplin County in 2008 which was done two days after transplanting (Table 1). The sprayer that was used in the greenhouse applications was also used for the foliar applications at 30 psi.

**Disease assessment.** In order to assess TSWV incidence in the field studies, the number of new TSWV symptomatic tobacco plants in each plot was evaluated weekly,

beginning three weeks post transplanting and ending nine weeks post transplanting, in all locations. The symptomatic plants were flagged on the date of evaluation and remained flagged until the end of the evaluations at week nine. Phytotoxicity was assessed by measuring the widest leaf on individual plants at three weeks post transplanting. Measuring the widest leaf width was chosen to measure phytotoxicity because the plants were too small to gather height or node-to-node measurements. Previous research has shown that leaf width provides a good representation of the phytotoxicity [23]. Ten plants were arbitrarily selected from each plot and those measurements were then averaged for each plot.

In one location in 2007 (Sampson County 2007) and 3 locations in 2008, TSWV symptoms were also recorded at nine weeks post transplanting on ten random symptomatic tobacco plants from each plot, or on all symptomatic plants in plots that contained less than 10 symptomatic plants. A code consisting of numbers 1 through 7 was devised to describe the various TSWV symptoms on a tobacco plant. Each number corresponded with a description of symptoms: 1=dead; 2=plant smaller than average size; 3=symptoms located in top third of the plant; 4=symptoms located in middle third of the plant; 5=symptoms located in bottom third of the plant; 6=symptoms located on all of plant; 7=symptoms are one-sided. Up to two codes could be recorded for each plant to describe TSWV symptoms.

**Greenhouse studies.** In 2007 (trial 1) and 2008 (trial 2) greenhouse trials were established in a greenhouse at North Carolina State University in Raleigh, NC. The trials were a split-plot design with the pesticide treatments as the main plot and the time delay between chemical applications as a subplot. There were three replications of each treatment. Plots consisted of a polystyrene tray (195 cells per tray of 35 cm x 37.5 cm). Each cell was filled with a soil-less potting mix and seeded with pelletized tobacco seeds of cultivar K 326. The polystyrene trays were then placed into large black trays filled with water in order to simulate the float bed system used by tobacco growers in greenhouses. The greenhouse treatments were applied to 60-day old seedlings, and for both trials consisted of combinations of IMD (Admire Pro<sup>®</sup> 4.2F at 13.11 g a.i. per 1,000 plants) and ASM (Actigard<sup>®</sup> 50 WG at 0.28 g a.i. per 1,000 plants). The treatments were: IMD; ASM; IMD plus ASM; IMD applied 24 hours before ASM; IMD applied 72 hours before ASM; ASM applied 24 hours



before IMD; and ASM applied 72 hours before IMD. All treatments were applied as foliar sprays and then drenched with a sufficient amount of water to move the pesticides to the root zone. The seedling green weights were measured at 5, 10, and 15 days after the first chemical application for trial 1 and at 15 days after the last chemical application for trial 2. Green weight measurements were conducted by weighing 10 individual seedlings per replication per treatment. These weights were then averaged.

The tobacco seedlings of trial 1 were transplanted on a farm in Duplin County 8 days after the chemical applications and those of trial 2 were transplanted on North Carolina's Upper Coastal Plain Research Station (UCPRS) located in Rocky Mount, NC 15 days after the chemical applications. The farm in Duplin County has a history of high TSWV pressure and the UCPRS has a history of low TSWV pressure. The trials were arranged as randomized complete blocks; plots consisted of four 48 in. wide rows, 50 ft in length with three replications. TSWV incidence was evaluated at 9 weeks post transplanting using the same method used in the field studies, and leaf width measurements were taken four weeks post transplanting by measuring the widest leaf on individual plants. For leaf width, 10 plants were measured in each plot and then averaged.

**Statistical analysis.** For the field studies, data on TSWV incidence, leaf width, and severity were analyzed using SAS, version 9 (SAS Institute, Cary, NC). The TSWV incidence data from 9 weeks post transplanting was chosen for analysis in order to investigate how effective the treatments are near the end of the growing season. TSWV incidence data was arcsine transformed and then analyzed with an analysis of variance (PROC GLM). Mean comparisons were conducted using Fisher's least significant difference (FLSD,  $P \leq 0.05$ ) test.

The effect of different treatments on initiation and rate of development of TSWV incidence [26] was investigated with PROC LOGISTIC using SAS, version 9 (SAS Institute, Cary, NC). Cumulative weekly TSWV incidence was the dependent variable. Treatments were the input (independent) variable used in the form of an indicator variable with the untreated control selected as the reference group, so that the effects of other treatments were estimated relative to the effects of the untreated control. Also, the number of weeks after

transplant and their interaction with treatments were input as explanatory variables of TSWV cumulative incidence. If the indicator variable for treatments is significant, that means that a specific treatment delays or accelerates TSWV symptom initiation relative to the untreated control. If the interaction between weeks and treatment is significant then that implies that different treatments affect the rate TSWV symptoms develop on a tobacco plant relative to the untreated control. *F* tests were used to determine statistical significance of input variables.

The percentage of each TSWV severity code recorded was averaged across each treatment. These mean percentages were arcsine transformed and a Bartlett's test for homogeneity of variances was performed to evaluate the possibility of combining the data from all four locations. Comparisons of mean frequencies of TSWV severity codes in each treatment were conducted using the general linear model procedure of SAS (PROC GLM; LSMEANS).

For the greenhouse study, the first step taken was conducting a Bartlett's test for homogeneity of variances to evaluate the possibility of combining data from both trials for green weight, leaf width, and TSWV incidence. The percent TSWV incidence was analyzed using analysis of variance (PROC GLM) and the means were separated with Fisher's least significant difference test (FLSD,  $P \leq 0.05$ ).

## RESULTS

**Field studies.** Six locations (Duplin County 2006, Onslow County 2006, Jones County 2007, Beaufort County 2008, Craven County 2008, and Duplin County 2008) had high TSWV pressure (greater than 15% at 9 weeks post transplanting). Two locations (Sampson County 2006 and Sampson County 2007) had low TSWV pressure (less than 15% at 9 weeks post transplanting) (Table 2). In all locations, except Sampson County 2006, the untreated control had the highest TSWV incidence. In areas of high TSWV pressure, when ASM was used in conjunction with an insecticide there was significantly less TSWV incidence than the untreated control. However, this was not the case when the method of application of ASM was as a foliar spray in the field, in which case the results were inconsistent between locations (Table 2). The treatment resulted in one of the statistically lowest TSWV incidences in some locations, but in other locations it did not. When imidacloprid or thiamethoxam were applied alone TSWV incidence was statistically different than the untreated control in most locations where TSWV pressure was high (Table 2). In the two locations mentioned above that had low TSWV pressure, using ASM with an insecticide resulted in significantly lower percentages of TSWV symptoms than the untreated plants.

Analysis of the leaf width data showed that all locations had similar results relative to one another. In other words, the untreated control had the largest leaf width in all locations (Table 3). Using ASM as a drench application at the higher rate (Actigard 50 WG at 0.28 g a.i. per 1000 plants) in the greenhouse plus IMD resulted in the smallest leaf width in all locations. Using IMD plus ASM in a drench application at the lower rate (Actigard 50 WG at 0.07 g a.i. per 1,000 plants) in the greenhouse resulted in numerically, although not statistically, larger leaf widths than when using the higher rate of ASM plus IMD. In the greenhouse, using ASM alone or with an insecticide causes significantly smaller leaf widths than an insecticide alone or applying IMD in the greenhouse followed by a foliar application of ASM in the field (Table 3). Furthermore, the treatments of IMD, thiamethoxam, or IMD in the greenhouse plus a foliar application of ASM in the field varied between locations with regards to the leaf width measurements they resulted in when compared to the untreated control (Table 3).

For the TSWV severity data, the Bartlett test showed that the four locations could be analyzed together. Analysis of the TSWV severity data showed that different treatments had a significant effect on the percentage of the different TSWV symptoms observed (Figure 1). Plant death soon after transplant was the most common symptom recorded among all treatments except IMD plus ASM applied at the higher rate, IMD plus ASM applied at the lower rate, and IMD plus ASM applied to the float water (Figure 1). Furthermore, these three treatments had more plants showing symptoms on only one side and had a smaller overall size than non-symptomatic plants than any other treatment. In general, IMD plus ASM had the most plants showing symptoms on the whole plant, no matter what the method of application was for ASM.

**Effect of treatments on TSWV progress.** These results are based on cumulative weekly TSWV incidence from the field studies from 2006 to 2008. In 2006, the treatments of IMD plus ASM applied as a drench in the greenhouse and IMD followed by a foliar spray of ASM in the field right after transplant delayed the onset of symptoms significantly longer than the untreated control in areas of high TSWV pressure (Duplin and Onslow Counties) (Table 4). In Sampson County, an area of lower TSWV pressure, applications of IMD plus ASM at the higher rate in the greenhouse was the only treatment to significantly delay the onset of symptoms.

In 2007, IMD followed by a foliar application of ASM, an insecticide plus ASM as a drench application at the higher rate in the greenhouse, and IMD alone delayed the onset of symptoms significantly longer (by 1 to 2 weeks) than the untreated control in Jones County, an area of high TSWV pressure (Table 5). In Sampson County, where the field had low TSWV pressure, an insecticide plus ASM and ASM alone delayed the onset of symptoms longer than the untreated control (Table 5).

In 2008, IMD plus ASM at the higher rate significantly delayed the onset of symptoms longer than the untreated control in all locations (Table 6). In Duplin and Craven Counties, applying IMD plus ASM at the lower rate or IMD plus ASM applied in the float water also significantly delayed the onset of symptoms longer (by about 1 week) than the untreated control. Beaufort County was the only location where the rate of symptom

development was significantly different between treatments. An application of IMD followed by a foliar application of ASM in the field had significantly slower symptom development than the untreated control. No other treatment in any year or location had a significant effect on the rate TSWV symptoms developed (Tables 4,5,&6).

**Greenhouse studies.** According to the results of the Bartlett test, data could not be analyzed together from the two greenhouse trials. Trial 1 showed that timing the sprays either 24 or 72 hours apart did not significantly affect the green weight (Table 7), leaf width (Table 8), or disease incidence (Table 9). The order in which the chemicals were applied did not result in any significant differences either. The untreated control had the lowest weight in the green house, but had the largest leaf width in the field at four weeks post transplant (Table 8). When the untreated control is not included in the analysis the correlation between green weights and leaf widths is high ( $r = 0.81$ ). Also, the leaf width was highly correlated with disease incidence ( $r = 0.91$ ); small leaf widths were observed in the same treatments where disease incidence was low.

In trial 2, timing the sprays either 24 or 72 hours apart did not have a significant effect upon the green weight (Table 7), leaf width (Table 8), or disease incidence (Table 9). The order in which the chemicals were applied only had a significant effect upon leaf width, or phytotoxicity, and not upon the green weight or TSWV incidence. Applying IMD before ASM resulted in significantly smaller leaf widths than applying ASM before IMD. The IMD treatment had a larger average green weight than the untreated control, which was likely due to the uneven hand clipping of the seedlings that was done to simulate mowing done by the growers, but in the field the untreated control had a larger leaf width (Table 8). When the IMD treatment was not included in the analysis, the correlation between green weights and leaf widths is high ( $r = 0.63$ ). There was not a strong correlation between leaf width and disease incidence ( $r = 0.38$ ).

## DISCUSSION

Findings from the present study demonstrated the effectiveness of ASM with IMD under North Carolina weather conditions and under different levels of TSWV incidence. Different insecticides with and without ASM, varying rates of ASM, and varying modes of application were all tested. In addition, greenhouse experiments were done to gain insight into how ASM is most effective and less phytotoxic on tobacco.

Previous research has shown that the use of ASM and IMD in the greenhouse provides consistent suppression of TSWV on tobacco [7,20,23]. Our multi-year field studies documented that this is the case under the North Carolina weather conditions as well: all field tests showed that using an insecticide and ASM together best suppresses TSWV symptoms. When an insecticide plus ASM were applied as a drench or in the float water in the greenhouse, there was significantly higher suppression of symptoms. These results are in agreement with previously reported results from Georgia [7,28].

Applying IMD in the greenhouse plus ASM as a foliar application in the field provided control of the disease, but the results were variable, which is supported by a previous study [7]. There are a few possible explanations for this variability, probably associated with the fact that ASM is a plant activator that triggers the tobacco's systemic acquired resistance (SAR) pathway [10]. Mandal *et al.* [20] reported that activation of resistance was observed within 2 days after treatment with ASM and a high level of resistance was observed at 5 days onward. Thus a foliar application of ASM in the field may not allow for sufficient time between application and SAR activation especially in the incidences in which thrips are already flying in the area, and thus moving TSWV into the crop.

Phytotoxicity, in the form of stunting, is a documented side effect of ASM [5,27]. Applying ASM alone resulted in significantly higher phytotoxicity than the untreated control in all but one location, whereas applying IMD alone did not result in statistically different phytotoxicity than the untreated control in most of the tests. Therefore most of the phytotoxicity seen in the IMD plus ASM treatments is likely attributed to ASM. Based on previous findings [20,27,28] and results from our study, it appears that slow growth of

tobacco plants treated with ASM is rather universal and not affected by local environmental conditions, although the amount of phytotoxicity may be variable from year to year and location to location implying that other circumstantial factors may be involved as well.

Walters and Heil [35] suggest that there is a fitness cost, such as a decrease in plant growth and development, from using a pesticide that induces SAR. The IMD plus ASM applied as a foliar spray treatment was variable not only in the amount of control it provided, as discussed earlier, but also in the amount of phytotoxicity it showed. Overall, there appears to be a correlation between the disease incidence and the phytotoxicity. The treatments that showed the most phytotoxicity also showed the lowest occurrence of TSWV symptoms. This trend was seen in all three years of field studies and in the phytotoxicity tests. The negative correlation between ASM rate and TSWV incidence and the positive correlation with phytotoxicity had also been reported in other states [20,27]. This may suggest that phytotoxicity is an unavoidable side effect, a cost that growers have to pay for the TSWV suppression they gain with the application of ASM. Given the fact that a small number of counties in North Carolina are under consistent TSWV risk [24], the wide adaptation of ASM in North Carolina is difficult. However, states like Georgia, where TSWV risk is consistent in most counties, ASM application has gained popularity [7,28].

No information is available about the importance of the order in which ASM and IMD are applied in the greenhouse with regards to phytotoxicity. Thus our greenhouse studies are the first documented study on this aspect and demonstrated that the order of applying the pesticides did not significantly affect the disease incidence observed in the field. Nevertheless, another factor became apparent. Trial 1 was transplanted into an area of high disease pressure and trial 2 was transplanted into an area of low disease pressure. In both locations, phytotoxicity was apparent in the treatments where ASM was applied, but the correlation between TSWV incidence and phytotoxicity was significant only in the location with the high disease pressure. This suggests that in areas of low disease pressure only IMD may be needed [12]. Otherwise, unnecessary phytotoxicity is introduced into the crops. However, this issue imposes a significant challenge for controlling TSWV season after season given that it is almost impossible to know which fields will have low or high TSWV

pressure. Furthermore, an interesting observation was that in trial 2 the seedlings that did not receive a treatment were the smallest seedlings in the greenhouse, but after three weeks in the field they had grown enough to become the largest. This is a good example of how resilient tobacco can be.

The TSWV severity data revealed that treatments of IMD plus ASM applied in the greenhouse had the fewest dead plants due to TSWV compared to the other treatments. Nonetheless, these treatments had more plants that were smaller overall than a normal plant and symptoms observed on one side of the plant than other treatments. It is important to know that the age at which plants are infected influences the symptoms that appear [7,20,22]. For example, if a plant is infected at a young age then the plant will most likely die, but if the plant is at an age where it is flowering then the symptoms will not spread as quickly [20,22]. Therefore, since dead plants were less frequent and one-sided symptoms were more frequent in treatments of IMD plus ASM, applied in the greenhouse, a likely assumption is that these treatments prevent early season infections or their progression. ASM has been shown to restrict TSWV replication and movement, and as a result reduce systemic infection [20].

The treatments that included IMD plus ASM delayed onset of TSWV symptoms significantly longer than the untreated control, according to the disease progression results. When IMD and ASM are applied together, with ASM applied as a drench or float water application, the delay in development of TSWV symptoms is consistent. However, this is not the case if AMS is applied as a foliar spray at transplant; the results are variable. So the trend seen in the field of low disease incidence early in the season followed by lower disease incidence later in the season is likely due to the treatments preventing or delaying early season infections, which account for the majority of the infection since early season infections of TSWV play a larger role than secondary infections [3,14]. This conclusion complements the results from the TSWV severity studies, mentioned earlier in this study and results reported by Mandal *et al.* [20] on ASM activity against TSWV.

Csinos *et al.* [7] have demonstrated in previous studies that seedlings are most vulnerable in the field during the first few weeks following transplanting. This is likely the reason why a treatment of IMD and ASM, applied in the greenhouse, was the most effective



way to control TSWV in a tobacco field and it is the recommended way to control TSWV on tobacco in North Carolina [24]. An alternative insecticide, thiamethoxam, was investigated in our field trials, but it did not exhibit a similar efficacy to IMD in suppressing TSWV. Similar results were reported by Coutts and Jones [6]. IMD has been demonstrated to reduce the length of probing of thrips on tobacco leaves and thus reducing the probability of TSWV transmission on a tobacco plant [16]. Thiamethoxam kills the vector [19]; however it is apparent from our field studies that this mode of action is not sufficient to suppress TSWV incidence on tobacco in North Carolina as some thrips may not be killed and thus will be able to infect the crop.

This study provided detailed information into the currently recommended TSWV management practices for eastern North Carolina. Given the undesirable phytotoxic effect of ASM, different strategies should be recommended for different locations based on the amount of disease pressure they expect to experience. Thus more information on TSWV expected incidence is still needed to complement the management recommendations. The amount and spread of TSWV is variable from year to year, and growers need to know the TSWV risk for the upcoming season so that they can appropriately protect the tobacco seedlings before they are transplanted in the field. A prediction system is under development that will complement and refine the recommended management based on findings from the present study.

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**Table 1.** Treatments of imidacloprid (IMD, Admire Pro 4.2F), acibenzolar-S-methyl (ASM, Actigard 50 WG), thiamthoxam (Platinum or T-Moxx) applied in 3 locations in 2006, 2 in 2007, and 3 in 2008

<b>Treatment<sup>a</sup></b>	<b>Duplin 06</b>	<b>Onslow 06</b>	<b>Sampson 06</b>	<b>Jones 07</b>	<b>Sampson 07</b>	<b>Beaufort 08</b>	<b>Craven 08</b>	<b>Duplin 08</b>
IMD	+ <sup>b</sup>	+	+	+	+	+	+	+
Platinum	- <sup>c</sup>	+	+	-	+	-	-	-
T-Moxx	-	-	-	+	+	-	-	-
ASM	+	+	+	+	+	+	+	+
ASM + Platinum	-	-	-	-	+	+	-	-
ASM + T-Moxx	-	-	-	+	+	-	-	-
ASM (.28) + IMD	+	+	+	+	+	+	+	+
ASM (fol) + IMD	+	+	+	+	+	+	+	+
ASM (float) + IMD	-	-	-	-	-	-	+	+
ASM (.07) + IMD	-	-	-	-	-	+	+	+

<sup>a</sup> IMD = imidacloprid at 12.64 g a.i. per 1,000 plants; ASM (.28) = ASM at 0.28 g a.i. per 1,000 plants; ASM (float) = ASM at 0.005 g/L a.i. applied in the float water bed; ASM (.07) = ASM at 0.07 g a.i. per 1,000 plants; T-Moxx<sup>®</sup> = thiamethoxam at 7.94 g a.i. per 1,000 plants; Platinum = thiamethoxam at 9.38 g a.i. per 1,000 plants; ASM (fol) = ASM at 2.87 g a.i. per hectare applied as foliar application immediately after planting tobacco in the field.

<sup>b, c</sup>: + applied, - not applied

**Table 2.** Percent of TSWV incidence 9 weeks after transplanting tobacco in a field with different combinations of imidacloprid, thiamethoxam, and ASM in 3 locations in 2006, 2 in 2007 and 3 in 2008

<b>Treatment<sup>a</sup></b>	<b>Duplin 06</b>		<b>Onslow 06</b>		<b>Sampson 06</b>		<b>Jones 07</b>		<b>Sampson 07</b>		<b>Beaufort 08</b>		<b>Craven 08</b>		<b>Duplin 08</b>	
Untreated	43.5	a	26.3	a	9.8	ab	32.4	a	14.7	a	19.8	a	20.1	a	37.8	a
IMD	41.0	a	15.4	bc	9.3	ab	17.7	c	11.3	ab	6.8	b	11.3	b	9.8	b
Platinum	- <sup>b</sup>	-	27.1	a	12.0	a	-	-	11.0	abc	-	-	-	-	-	-
T-Moxx	-	-	-	-	-	-	26.0	b	11.3	ab	-	-	-	-	-	-
ASM	37.5	ab	20.1	ab	9.3	ab	19.1	c	6.0	cd	4.8	bc	5.6	cd	8.9	b
ASM + Platinum	-	-	-	-	-	-	-	-	6.7	bcd	4.6	bc	-	-	-	-
ASM + T-Moxx	-	-	-	-	-	-	16.7	c	5.9	cd	-	-	-	-	-	-
ASM (.28) + IMD	25.8	bc	10.2	c	6.6	b	15.3	c	4.5	d	2.6	c	2.9	d	4.1	c
ASM (fol) + IMD	24.2	c	16.6	bc	8.1	b	18.6	c	12.3	abc	6.9	b	8.4	bc	9.5	b
ASM (float) + IMD	-	-	-	-	-	-	-	-	-	-	-	-	4.6	d	3.9	c
ASM (.07) + IMD	-	-	-	-	-	-	-	-	-	-	4.0	bc	3.7	cd	5.0	bc

<sup>a</sup> IMD = imidacloprid at 12.64 g a.i. per 1,000 plants; ASM (.28) = ASM at 0.28 g a.i. per 1,000 plants; ASM (float) = ASM at 0.005 g/L a.i. applied in the float water bed; ASM (.07) = ASM at 0.07 g a.i. per 1,000 plants; T-Moxx<sup>®</sup> = thiamethoxam at 7.94 g a.i. per 1,000 plants; Platinum = thiamethoxam at 9.38 g a.i. per 1,000 plants; ASM (fol) = ASM at 2.87 g a.i. per hectare applied as foliar application immediately after planting tobacco in the field.

<sup>b</sup> Treatment not included in the specific county, year.

**Table 3.** Leaf width (mm) 4 weeks after transplanting tobacco in the field with different combinations of imidacloprid, thiamethoxam, and ASM in 3 locations in 2006, 2 in 2007 and 3 in 2008

<b>Treatment<sup>a</sup></b>	<b>Duplin 06</b>	<b>Onslow 06</b>	<b>Sampson 06</b>	<b>Jones 07</b>	<b>Sampson 07</b>	<b>Beaufort 08</b>	<b>Craven 08</b>	<b>Duplin 08</b>
Untreated	132.9 a	101.5 a	113.3 b	147.6 a	175.1 a	82.4 a	171.5 a	137.0 a
IMD	106.5 b	98.8 a	110.0 b	130.7 b	157.0 ab	73.3 b	164.8 a	129.5 a
Platinum	<sup>b</sup> -	106.5 a	130.0 a	- -	112.2 bc	- -	- -	- -
T-Moxx	- -	- -	- -	134.1 b	128.4 abc	- -	- -	- -
ASM	104.0 b	100.9 a	98.8 b	- -	102.4 c	59.8 cd	125.6 bc	111.1 c
ASM + Platinum	- -	- -	- -	- -	112.6 bc	54.2 cd	- -	- -
ASM + T-Moxx	- -	- -	- -	102.1 c	126.2 abc	- -	- -	- -
ASM (.28) + IMD	97.6 b	88.3 a	79.5 c	108.1 c	101.0 c	51.4 d	109.7 c	109.9 c
ASM (fol) + IMD	109.2 ab	99.1 a	105.1 b	133.2 b	161.6 ab	71.8 b	141.8 ab	128.6 ab
ASM (float) + IMD	- -	- -	- -	- -	- -	- -	124.6 bc	108.7 c
ASM (.07) + IMD	- -	- -	- -	- -	- -	61.3 c	116.8 bc	112.2 bc

<sup>a</sup>IMD = imidacloprid at 12.64 g a.i. per 1,000 plants; ASM (.28) = ASM at 0.28 g a.i. per 1,000 plants; ASM (float) = ASM at 0.005 g/L a.i. applied in the float water bed; ASM (.07) = ASM at 0.07 g a.i. per 1,000 plants; T-Moxx<sup>®</sup> = thiamethoxam at 7.94 g a.i. per 1,000 plants; Platinum = thiamethoxam at 9.38 g a.i. per 1,000 plants; ASM (fol) = ASM at 2.87 g a.i. per hectare applied as foliar application immediately after planting tobacco in the field.

<sup>b</sup> Treatment not included in the specific county, year.



**Table 4.** Effect of different combinations of IMD, thiamethoxam (Platinum or T-Moxx), and ASM on TSWV symptom onset and rate of progression in three tobacco fields in 2006 in North Carolina.

Treatment <sup>a</sup>	Estimate <sup>b</sup>	Location		
		2006 Duplin	2006 Onslow	2006 Sampson
Untreated	a	-5.81	-7.19	-6.29
	b			
IMD	a	<b>-5.57<sup>c</sup></b>	-7.26	-6.17
	b	0.61	0.60	0.41
Platinum	a	** <sup>d</sup>	<b>-6.73</b>	<b>-6.01</b>
	b	**	0.60	0.41
T-Moxx	a	**	**	**
	b	**	**	**
ASM	a	<b>-5.69</b>	-7.26	-6.30
	b	0.61	0.60	0.41
ASM + Platinum	a	**	**	**
	b	**	**	**
ASM + T-Moxx	a	**	**	**
	b	**	**	**
ASM (.28) + IMD	a	<b>-6.25</b>	<b>-7.81</b>	<b>-6.66</b>
	b	0.61	0.60	0.41
ASM (fol) + IMD	a	<b>-6.23</b>	<b>-7.41</b>	-6.35
	b	0.61	0.60	0.41
ASM (float) + IMD	a	**	**	**
	b	**	**	**
ASM (.07) + IMD	a	**	**	**
	b	**	**	**

<sup>a</sup> IMD = imidacloprid at 12.64 g a.i. per 1,000 plants; ASM (.28) = ASM at 0.28 g a.i. per 1,000 plants; ASM (float) = ASM at 0.005 g/L a.i. applied in the float water bed; ASM (.07) = ASM at 0.07 g a.i. per 1,000 plants; T-Moxx® = thiamethoxam at 7.94 g a.i. per 1,000 plants; Platinum = thiamethoxam at 9.38 g a.i. per 1,000 plants; ASM (fol) = ASM at 7.09 g a.i. per acre applied as foliar application immediately after planting tobacco in the field.

<sup>b</sup> Estimates were calculated using logit transformation:  $\ln(X/(1 - X)) = a * \text{treatment} + b * \text{treatment}$ . The week \* treatment was not significant. Treatment was input as an indicator variable with the untreated control as the reference group. Thus significance of estimates is relative to the untreated control.

<sup>c</sup> bold numbers correspond to estimates that were statistically significant ( $P < 0.05$ ).

<sup>d</sup> No available data

**Table 5.** Effect of different combinations of IMD, thiamethoxam (Platinum or T-Moxx), and ASM on TSWV symptom onset and rate of development in three tobacco fields in 2007 in North Carolina.

Treatment <sup>a</sup>	Estimate <sup>b</sup>	Location	
		2007 Jones	2007 Sampson
Untreated	a	-5.43	-7.10
	b		
IMD	a	<b>-5.57<sup>c</sup></b>	<b>-6.61</b>
	b	0.50	0.56
Platinum	a	<b>**<sup>d</sup></b>	<b>-6.87</b>
	b	<b>**</b>	0.56
T-Moxx	a	<b>-5.00</b>	<b>-6.74</b>
	b	0.50	0.56
ASM	a	-5.46	<b>-7.50</b>
	b	0.50	0.56
ASM + Platinum	a	<b>**</b>	<b>-7.59</b>
	b	<b>**</b>	0.56
ASM + T-Moxx	a	<b>-5.78</b>	<b>-7.55</b>
	b	0.50	0.56
ASM (.28) + IMD	a	<b>-6.00</b>	<b>-7.84</b>
	b	0.50	0.56
ASM (fol) + IMD	a	<b>-5.58</b>	<b>-6.85</b>
	b	0.50	0.56
ASM (float) + IMD	a	<b>**</b>	<b>**</b>
	b	<b>**</b>	<b>**</b>
ASM (.07) + IMD	a	<b>**</b>	<b>**</b>
	b	<b>**</b>	<b>**</b>

<sup>a</sup> IMD = imidacloprid at 12.64 g a.i. per 1,000 plants; ASM (.28) = ASM at 0.28 g a.i. per 1,000 plants; ASM (float) = ASM at 0.005 g/L a.i. applied in the float water bed; ASM (.07) = ASM at 0.07 g a.i. per 1,000 plants; T-Moxx<sup>®</sup> = thiamethoxam at 7.94 g a.i. per 1,000 plants; Platinum = thiamethoxam at 9.38 g a.i. per 1,000 plants; ASM (fol) = ASM at 7.09 g a.i. per acre applied as foliar application immediately after planting tobacco in the field.

<sup>b</sup> Estimates were calculated using logit transformation:  $\ln(X/(1 - X)) = a * \text{treatment} + b * \text{treatment}$ . The week \* treatment was not significant. Treatment was input as an indicator variable with the untreated control as the reference group. Thus significance of estimates is relative to the untreated control.

<sup>c</sup> Bold numbers correspond to estimates that were statistically significant ( $P < 0.05$ ).

<sup>d</sup> No available data

**Table 6.** Effect of different combinations of IMD, thiamethoxam (Platinum or T-Moxx), and ASM on TSWV symptom onset and rate of development in three tobacco fields in 2008 in North Carolina.

Treatment <sup>a</sup>	Estimate <sup>b</sup>	Location		
		2008 Beaufort	2008 Craven	2008 Duplin
Untreated	a	-6.61	-5.47	-6.00
	b			
IMD	a	<b>-5.87<sup>c</sup></b>	<b>-4.66</b>	<b>-5.71</b>
	b	0.40	0.81	0.29
Platinum	a	** <sup>d</sup>	**	**
	b	**	**	**
T-Moxx	a	**	**	**
	b	**	**	**
ASM	a	-7.21	<b>-5.78</b>	-5.92
	b	0.51	0.81	0.29
ASM + Platinum	a	-7.51	**	**
	b	0.53	**	**
ASM + T-Moxx	a	**	**	**
	b	**	**	**
ASM (.28) + IMD	a	<b>-8.89</b>	<b>-6.59</b>	<b>-6.95</b>
	b	0.62	0.81	0.29
ASM (fol) + IMD	a	<b>-5.61</b>	<b>-5.10</b>	<b>-5.81</b>
	b	0.37	0.81	0.29
ASM (float) + IMD	a	**	<b>-6.11</b>	<b>-6.99</b>
	b	**	0.81	0.29
ASM (.07) + IMD	a	-7.04	<b>-6.01</b>	<b>-6.64</b>
	b	<b>0.46</b>	0.81	0.29

<sup>a</sup> IMD = imidacloprid at 12.64 g a.i. per 1,000 plants; ASM (.28) = ASM at 0.28 g a.i. per 1,000 plants; ASM (float) = ASM at 0.005 g/L a.i. applied in the float water bed; ASM (.07) = ASM at 0.07 g a.i. per 1,000 plants; T-Moxx® = thiamethoxam at 7.94 g a.i. per 1,000 plants; Platinum = thiamethoxam at 9.38 g a.i. per 1,000 plants; ASM (fol) = ASM at 2.87 g a.i. per hectare applied as foliar application immediately after planting tobacco in the field.

<sup>b</sup> Estimates were calculated using logit transformation:  $\ln(X/(1 - X)) = a * \text{treatment} + b * \text{treatment}$ . The week \* treatment was significant only in the ASM (fol) + IMD treatment in Beaufort County 2008. Treatment was input as an indicator variable with the untreated control as the reference group. Thus significance of estimates is relative to the untreated control.

<sup>c</sup> Bold numbers correspond to estimates that were statistically significant ( $P < 0.05$ ).

<sup>d</sup> No available data

**Table 7.** Mean green weight of tobacco seedlings in the greenhouse 15 days after the first chemical application for Trial 1 and 18 days for Trial 2 of the greenhouse studies

<b>Treatments<sup>b</sup></b>	<b>Mean green weights (g)<sup>a</sup></b>	
	<b>Trial 1</b>	<b>Trial 2</b>
Untreated	1.4 d	2.8 a
IMD	1.6 bcd	2.1 c
ASM	1.8 a	2.3 bc
IMD + ASM	1.6 abc	1.9 c
IMD + ASM (24 hrs later)	1.7 ab	2.0 c
IMD + ASM (72 hrs later)	1.6 abcd	2.1 c
ASM + IMD (24 hrs later)	1.6 bcd	2.7 ab
ASM + IMD (72 hrs later)	1.4 cd	2.0 c

<sup>a</sup>Means within columns followed by same letter do not significantly differ (P=0.05).

<sup>b</sup>IMD = imidacloprid at 13.11 g a.i. per 1,000 plants; ASM = ASM at 0.28 g a.i. per 1,000 plants; (24 hrs later) = pesticide was applied 24 hrs after first listed pesticide in the treatment; (72 hrs later) = pesticide was applied 72 hrs after first listed pesticide in the treatment.

**Table 8.** Leaf width at four weeks after transplanting of seedlings subjected to different timing of chemical applications from the greenhouse studies

<b>Treatments<sup>b</sup></b>	<b>Leaf Width (mm)<sup>a</sup></b>			
	<b>Trial 1</b>		<b>Trial 2</b>	
Untreated	100.8	a	138	b
IMD	90.2	bc	153.5	a
ASM	96.1	ab	118.1	e
IMD + ASM	84.2	cd	125.9	cde
IMD + ASM (24 hrs later)	94.8	ab	121.3	e
IMD + ASM (72 hrs later)	88.9	bc	122.8	de
ASM + IMD (24 hrs later)	92.1	abc	134.4	bc
ASM + IMD (72 hrs later)	78.7	d	132.1	bcd

<sup>a</sup>Means within columns followed by same letter do not significantly differ (P=0.05).

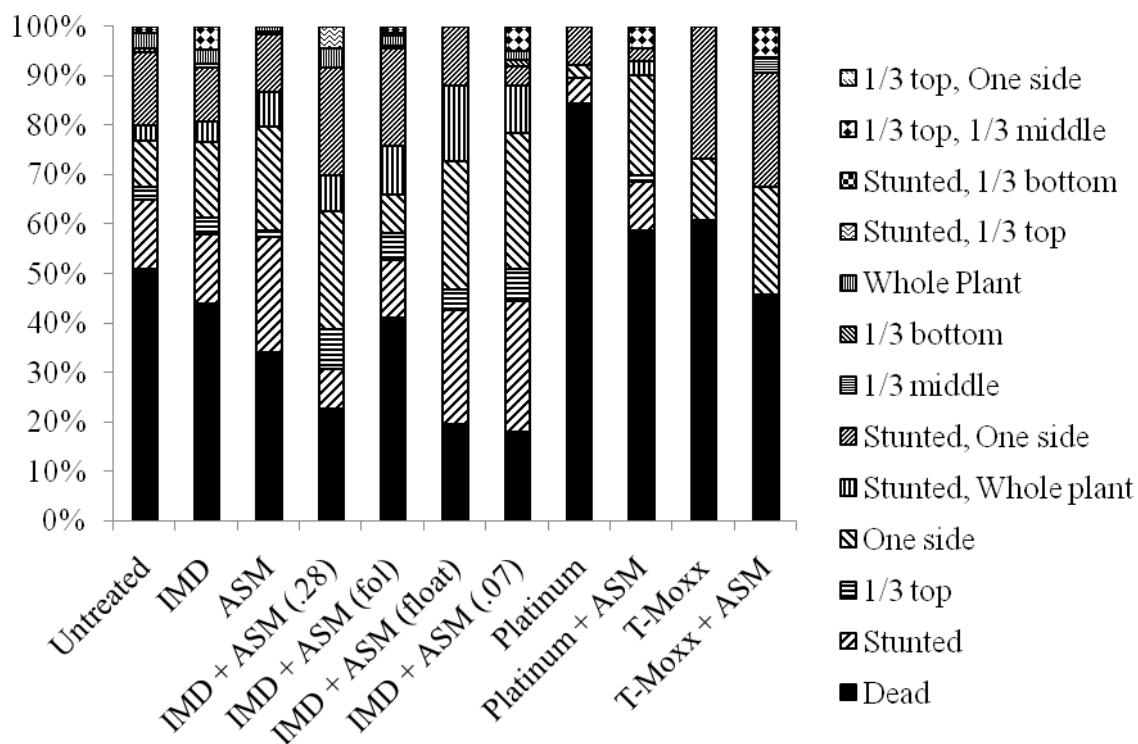
<sup>b</sup>IMD = imidacloprid at 13.11 g a.i. per 1,000 plants; ASM = ASM at 0.28 g a.i. per 1,000 plants; (24 hrs later) = pesticide was applied 24 hrs after first listed pesticide in the treatment; (72 hrs later) = pesticide was applied 72 hrs after first listed pesticide in the treatment.

**Table 9.** Percent (%) TSWV incidence at nine weeks after transplanting the greenhouse studies

<b>Treatments<sup>b</sup></b>	<b>Disease Incidence (%)<sup>a</sup></b>	
	<b>Trial 1</b>	<b>Trial 2</b>
Untreated	51.7 a	4.8 a
IMD	27.9 bc	1.4 b
ASM	42.2 ab	2.7 ab
IMD + ASM	24.4 c	2.6 ab
IMD + ASM (24 hrs later)	35.6 abc	2.2 b
IMD + ASM (72 hrs later)	28.6 bc	2.9 ab
ASM + IMD (24 hrs later)	33 bc	1.8 b
ASM + IMD (72 hrs later)	22.2 c	2.3 b

<sup>a</sup>Means within columns followed by same letter do not significantly differ (P=0.05).

<sup>b</sup>IMD = imidacloprid at 13.11 g a.i. per 1,000 plants; ASM = ASM at 0.28 g a.i. per 1,000 plants; (24 hrs later) = pesticide was applied 24 hrs after first listed pesticide in the treatment; (72 hrs later) = pesticide was applied 72 hrs after first listed pesticide in the treatment.



**Figure 1.** TSWV severity 9 weeks after transplanting with different combinations of imidacloprid, thiamethoxam, and ASM in 1 location in 2007 and 3 in 2008. Severity was expressed based on different TSWV symptoms and up to two different symptoms had been attributed to an infected tobacco plant

## **CHAPTER 2. Temporal and spatial distribution of Tomato Spotted Wilt Virus incidence in flue-cured tobacco in North Carolina**

### **ABSTRACT**

The temporal and spatial distribution of *Tomato spotted wilt virus* (TSWV) was studied in a total of 25 and 8, respectively, naturally infested tobacco fields in 2006, 2007, and 2008. Sections within the fields were monitored that ranged in size from 76 x 11 meters to 137 x 10 meters. TSWV incidence was recorded on a weekly basis. TSWV temporal progression data was fit to a logistic regression model with cumulative degree-days and past TSWV field history as explanatory variables. Degree-days were calculated with four different thresholds corresponding to thrips or tobacco growth stages. Analyses revealed that both variables are significant explanatory factors of the temporal progression of TSWV on tobacco. TSWV spatial distribution was investigated using universal kriging interpolations on TSWV incidence at 5 and 9 weeks post transplanting. Although isolated disease clusters were present, the overall spatial pattern was random, which has been previously observed with TSWV incidence in other crop systems in southeast US. These findings suggest that when thrips move into a field infections occur randomly. Further work is needed in order to determine the dispersal of thrips within a field, which will help refine management practices.



## INTRODUCTION

*Tomato spotted wilt virus* (TSWV) is an economically important virus and is the causal agent of Tomato spotted wilt (TSW). It was first documented in the United States in the 1930's and in North Carolina in 1989. TSWV has a wide host range including tobacco, pepper, peanut, and tomato crops [3,12,24], and it is vectored by several species of thrips (*Frankliniella* and *Thrips* spp.) [28]. On tobacco, the primary vector in eastern and central North Carolina is the tobacco thrips, *Frankliniella fusca* (Hinds), but the western flower thrips, *Frankliniella occidentalis* (Pergande), and the onion thrips, *Thrips tabaci* (Lindeman), may also play an important role in some areas [10,15]. The adult thrips, which can be viruliferous for life, are primarily responsible for spreading TSWV [28]. In order for the thrips to be capable of transmitting the virus they must acquire the virus by feeding on an infected host during their larval stage [1]. A plant needs to be susceptible to systemic infection by TSWV and also be able to support thrips reproductive populations in order to act as a source for spread of TSWV [13]. Volunteer plants and local weeds are likely places for the thrips and the virus to overwinter [5,14,15].

When spring comes, the temperatures rise and the winter weeds die. During this time the thrips migrate from the weeds to the crops, and therefore so does the virus [15]. Thus, temperature is a major factor in determining thrips development and movement and also tobacco growth. For tobacco thrips the lower developmental threshold is estimated at 10.5°C [21] whereas tobacco, a plant that thrives in high temperatures, has a reported lower threshold of 26°C for optimum growth [16]. Furthermore, a lower threshold of 20°C is associated with thrips flight [20], which is important because of the migration of thrips between local weeds and crops. Olatinwo *et al.* [23] reported an improvement of the TSWV risk index developed for peanuts when weather variables were included, especially average daily temperature of April. In North Carolina, the dispersal pattern of tobacco thrips in April and May was well correlated to degree-days summed from January 1 to May 10 [22].

Evidence suggests that the spread of TSWV within a crop, such as tomato, pepper, lettuce, or tobacco, is predominantly monocyclic [2,6,12]. It has been reasoned that secondary spread is limited due to the fact that only adult thrips that acquire the virus during

the larval stage are able to transmit the virus [12]. It is generally accepted that vectors arriving from weed hosts at the margins of fields produce a gradient of diseased plants from the edge of fields and insect vectors coming from sources that are more distant typically produce a random pattern of disease incidence [25]. Groves *et al.* [13] reported that the majority of *F. fusca* captured in April, May, and June in North Carolina were close to the infection source and there was no gradient present when the virus source was 35 m away. Coutts *et al.* [6] showed that when there is an external infection source adjacent to pepper and lettuce fields, clusters of infection occur near the TSWV source and TSWV incidence declines as the infection source is located increasingly further away from the fields, with only isolated clusters occurring distantly from the TSWV source. Similarly, Gitaitis *et al.* [12] observed disease gradients in tomato fields when the source was directly adjacent to the field but not when the source was 200 m away. A barrier of 15 m wide fallow or non-host planting was sufficient to slow TSWV spread in lettuce [6].

Overall, despite the general agreement on the TSWV dispersal pattern in several crops little has been done to investigate the disease's spatial pattern as it is related to the surrounding landscape and the simultaneous quantification of the temporal TSWV progression in the same fields. Due to the complexity of the movement of TSWV in tobacco, the amount of disease that occurs varies greatly from year to year. Therefore, understanding the epidemiology of TSWV, especially the temporal and spatial distribution of incidence, will help us understand more about epidemics of the disease and how to better manage it. Presently, control of TSWV in flue-cured tobacco is limited to chemical control [8,24]. The purpose of this study is to investigate the temporal and spatial distribution of TSWV incidence in flue-cured tobacco fields in North Carolina.

## MATERIALS AND METHODS

**Temporal progression.** *Data collection.* In 2006, 2007, and 2008 a total of 25 tobacco fields in eastern North Carolina were monitored for TSWV incidence. Each location was naturally infested and growers implemented their typical production practices throughout the season. The planting dates for each location can be found in Table 1. In 17 of the 25 locations, four plots that were four rows wide and about twenty five plants long were arbitrarily marked off in each location so that each plot contained approximately 100 tobacco plants. The other eight locations were part of chemical management studies conducted by Cherry *et al.* (*unpublished*). In each of these eight locations, data was collected from the four plots in which only imidacloprid had been applied. These plots were four rows wide and 15.24 m long, resulting in about 100 plants total within each plot. In each of the 25 locations the cumulative number of TSWV symptomatic tobacco plants in each plot was evaluated weekly beginning three to six weeks post transplanting and the data from the four plots were averaged for each location. Evaluations consisted of visually assessing the tobacco plants for foliar symptoms that were characteristic of TSWV, and if these were present the plant was counted and flagged.

*Input variables.* The dependent variable in the analysis was the cumulative weekly percent disease incidence. Next, cumulative degree-days (DD) were added to the analysis as an independent variable in order to determine if temperature influences TSWV progression within a season. The field's TSWV history was included next as another independent explanatory variable.

DD are an expression of thermal time using a lower threshold, which can have a specific association with a biological factor. DD has been extensively used with success for pest management in crops [4,11,29]. In the present study, the lower thresholds used for these analyses were: 10.5°C corresponding to *F. fusca* development [21], 18°C corresponding to standard DD calculated by the North Carolina Climate office and readily available online, 20°C corresponding to thrips flight threshold [20], and 26°C corresponding to optimum tobacco growth [16]. A lower threshold corresponding to TSWV symptom expression was not included because the associated lower thresholds range from 18°C to 30°C [19] and this

range was indirectly investigated with temperature bases associated with thrips flight and tobacco growth.

TSWV history was included in the logistic regression model in order to determine whether or not there is evidence that a prior history of TSWV has an effect upon the TSWV incidence a field will experience in subsequent years. The field's TSWV history was incorporated as a categorical variable with the following incidence levels: Very Low (<5%), Low (5-10%), Medium (11-20%), High (21-30%), or Very High (>30%). These levels correspond to current TSWV control recommendations, which involve applications of imidacloprid and/or acibenzolar-S-methyl in the greenhouse [2]. Based on previous research conducted in North Carolina [2], imidacloprid application would provide a 20-40 percent disease control. The percent increases to 30-60 percent when acibenzolar-S-methyl is used together with imidacloprid. Thus, in a field with Very Low or Low levels of TSWV incidence, imidacloprid at the highest rate is not necessary for control of TSWV. In these fields an application of imidacloprid in the greenhouse at a low rate, which is appropriate for insect management, would be recommended. At Medium levels a field would need imidacloprid at a high rate, while areas experiencing a High level would benefit greatly from a high rate of imidacloprid and an application of acibenzolar-S-methyl. Once a field is experiencing Very High levels of TSWV incidence the same recommendation as High is given but greater losses are expected, and therefore it may be suggested that the grower should not plant tobacco in this location due to the level of expected TSWV incidence that is associated with economic yield loss. TSWV history data was based on anecdotal evidence from the growers and county agents about the levels of TSWV incidence typically seen in that field during the past five years. For the statistical analyses, field history was used as an indicator variable with the Very Low level used as the reference group. Therefore, significance of the other field history categories is relative to the Very Low level.

Weather data were obtained online from the National Oceanic and Atmospheric Administration (NOAA) through the National Climatic Data Center (NCDC) in Asheville, NC. Data that was retrieved online was mean daily maximum temperature and mean daily minimum temperature for the months of April, May, June, and July for the years 2006, 2007,

and 2008. These daily data were averaged for each day and then a lower threshold was subtracted in order to estimate daily degree-days, and if the value estimated was negative it was set to zero. This process was repeated using all four of the lower thresholds chosen for this study. To calculate the DD for each location, daily degree-days were summed for each week, with the first week beginning at the transplant date, through 9 weeks post transplanting, which was the length of time that TSWV incidence was recorded in each location. For locations where the transplant date was unknown, Lenoir County 2007 and W. Bertie County 2008, the transplant date of the nearest location from that same year was used, S. Craven County 2007 and E. Bertie County 2008 respectively. For each location the weather data were obtained from the nearest weather station, making it a total of 15 weather stations from which data was retrieved. Seventy-five percent of the locations were located less than 20 km away from the nearest weather station. Seven locations were at least 22 km away from the nearest weather station (Sampson County 2007, North Sampson County 2007, South Craven County 2007, North Craven County 2007, Lenoir County 2008, Johnston County 2008, and East Bertie County 2008).

*Statistical analysis and model verification.* TSWV progression within a season and the factors associated with it were investigated using a logistic regression model (PROC LOGISTIC, SAS, version 9, SAS Institute, Cary, NC). Initially the model only included DD (Model I). A linear regression model of observed versus predicted values was calculated to determine how well the model predicted the observed data. The c value from the logistic regression model (i.e. the equivalent of the  $R^2$  value of a linear regression) and the  $R^2$  value from the linear regression model were used to determine which lower threshold used to calculate DD was most appropriate to describe TSWV progression within a season. The field's TSWV history was added (Model II) and the c value and the  $R^2$  value were recalculated.

The models were then verified by investigating whether or not the TSWV class predicted at 9 weeks after transplanting in each field was the same as the one observed. TSWV classes were the same as TSWV field history levels described above and they were used due to their usefulness for TSWV management purposes [2].

**Spatial distribution.** Eight locations were used to investigate the spatial distribution of TSWV in a field. Areas from which data was collected ranged in size from 76 x 11 meters to 137 x 10 meters. These fields were the same locations where chemical management studies by Cherry *et al.* (*unpublished*) were established in 2006, 2007 and 2008. Each of these fields was divided into plots that were four rows wide and fifty feet long. Plots were treated with different chemical treatments and each treatment was replicated four times. Due to the application of chemicals that influenced the observed TSWV incidence, spatial analyses were conducted after an initial normalization of the data. Normalization was done by subtracting the overall mean of TSWV incidence, which was calculated by averaging the TSWV incidence recorded in every plot, and the treatment mean of TSWV incidence, which was calculated by averaging across four replications of the treatment, from the cumulative TSWV incidence recorded in each plot (replication) of the same treatment.

Semivariogram analyses were conducted on the spatial patterns of TSWV incidence from the fifth and ninth week post transplanting. These two weeks were chosen in order to determine if patterns are present early in the season (week 5), and if so whether or not they persist over several weeks (week 9). Data subjected to spatial analysis was the average TSWV incidence of a plot. Plots were 1.26 m x 15.2 m and for analysis purposes the average TSWV incidence was assumed a point in the center of the plot. Thus analyzed points were 1.26 m x 15.2 m apart. Parameters used to characterize the semivariogram plot were: the nugget, which represents independent error, measurement error, or microscale variation at spatial scales that are too fine to detect; the range, which represents a distance beyond which there is little or no autocorrelation among variables; and the sill, which represents a value that the variogram tends toward when distances become greater than or equal to the range [7]. There are a few accepted mathematical methods of fitting semivariogram models [7], but visual inspection of a fit to a few standard models is often satisfactory [17]. Therefore, a visual check of several models was done to determine which models fit the variogram points. Four models were chosen for visual assessment because they are commonly used to describe spatial patterns of biological processes. The semivariogram models that were used are circular, exponential, linear, and spherical models using a nonlinear, least squares

optimization weighted by the number of distance pairs. The model that fit the best of the four chosen was selected based upon how well it predicted the data. This was determined by using the models to predict the observed data and then using a linear regression model to determine how well the predicted values correlated with the observed values. TSWV spatial analyses were accomplished using ArcMap, Geographic Information Systems (GIS), version 9.1 (Environmental Systems Research Institute, Redlands, CA). The linear regression model analyses were done using SAS, version 9 (SAS Institute, Cary, NC).

## RESULTS

**Temporal progression.** Overall, DD and the field's TSWV history are significant explanatory variables of the in-season TSWV incidence. In Model I the inclusion of DD as an explanatory variable resulted in good concordant % and c value (Table 3). Furthermore, the inclusion of TSWV field history (Model II) improved the model's power as demonstrated by the higher values of criteria of goodness of fit (Table 3). Model II had a higher c value associated with the logistic regression model and a higher  $R^2$  value associated with the linear regression model than Model I (Table 3). Interestingly, Model I had very poor predictability of the observed TSWV incidence, although DD was a significant explanatory variable (Table 3). On the contrary, inclusion of TSWV field history increased explanatory power and predictability (Model II).

When estimating DD for both Models I and II, using a lower threshold of 10.5°C resulted in the highest c value associated with the logistic regression model and the highest  $R^2$  value associated with the linear regression model (Table 3) of observed versus predicted TSWV incidence. Estimating DD using lower thresholds of 18°C and 20°C resulted in similar c values and  $R^2$  values. A lower threshold of 26°C resulted in the lowest c values and  $R^2$  values when it was used to estimate DD. In both Models, DD was significant regardless of which lower threshold was used. These results were the same for both Model I and II (Table 3). In Table 4 the parameter estimates of Model II are presented for the DD with the four different lower thresholds. The effect of DD was always positive but the absolute value increased as the lower threshold increased (Table 4). The analysis of maximum likelihood estimates showed that each level of TSWV history is significantly different from the reference group with regards to effect on TSWV incidence based on the parameter estimate values (Table 4). All of the parameter estimates for TSWV history were negative but their absolute effect increased significantly when TSWV history increases from Very Low to Very High levels (Table 4).

Model II, which includes both DD and the field's TSWV history, was used for further verification by investigating if it predicted the TSWV class at 9 weeks after transplanting in each field to be the same as the observed. The procedure was followed for all four DD lower



thresholds. Out of the total 25 locations that TSWV incidence was predicted for, 10 (40%) were predicted correctly (i.e. predicted the same TSWV incidence class as the one observed) when DD was estimated using 10.5°C (Table 5). The rest were either predicted higher (6 locations) or lower (9 locations) than the observed TSWV incidence (Table 5). All but one of the locations for which TSWV incidence was predicted incorrectly was placed into an adjacent TSWV field history level; one was placed into TSWV history level that was two levels lower than the observed levels (Table 5). For example, if a location was observed to have a Medium level of TSWV incidence the model may have predicted it to have a Low level of TSWV incidence, and thus was wrong by one level.

When 18°C and 20°C were used as the lower thresholds, 10 (40%) of the locations were predicted correctly (Table 5). The remainder of the locations was either predicted higher (4 locations) or lower (11 locations) than the observed TSWV incidence (Table 5). All but three of the locations for which TSWV incidence was predicted incorrectly were placed into an adjacent TSWV history level; three were predicted to be two levels lower than their observed levels (Table 5).

When the DD were estimated using 26°C as the lower threshold, 13 (52%) of the locations were predicted correctly (Table 5). The remainder of the locations was either predicted higher (1 location) or lower (11 locations) than the observed values (Table 5). All but five of the locations for which TSWV incidence was predicted incorrectly were placed into adjacent TSWV history level; five were predicted to be two levels lower than their observed levels (Table 5).

**Spatial distribution.** The immediate surrounding terrain of the eight fields subjected to spatial analysis was very diverse (Table 2). Five of the fields were surrounded by tobacco crop on at least two out of the four sides. Half of them were next to one or two public roads and/or woods or a meadow (Table 2). Based on visual assessment, none of the semivariogram models that were examined fit the observed values well. This was largely supported by the semivariogram analyses; the analyses suggest that the data from most weeks do not fit the semivariogram models (Table 6). TSWV incidence occasionally appears in

segregated clusters within a field (Figure 1a), but otherwise shows no spatial patterns in the tobacco fields investigated (Figure 1b).

Spatial aggregations of TSWV incidence were detected in: 2006 in Duplin County at both 5 and 9 weeks post transplanting; 2007 in Sampson County at 5 weeks post transplanting; 2008 in Beaufort County at 9 weeks post transplanting; and 2008 in Craven County at 5 weeks post transplanting. In 2006 in Duplin County TSWV aggregation reduced towards the woods but it was high towards the crop (left side of Fig. 1a). There was a small path on this side where weeds were present for the first few weeks after transplanting due to frequent rain. In 2007 in Sampson County TSWV aggregation reduced towards the meadow, there was a farm path between the meadow and the field that could have served as a barrier. In Craven County in 2008 TSWV aggregation increased towards the public road where weeds were present. In Beaufort County in 2008 TSWV aggregation increased towards the woods where there was also a dirt path where weeds were growing. Nevertheless, based on visual inspection no semivariogram model fit the data from any week well. Parameters of the four semivariogram models are listed in Table 7 for 2006, Table 8 for 2007, and Table 9 for 2008.

## DISCUSSION

TSWV continues to be a major disease in the southeastern United States in several crops. The complexity of the pathosystem along with the unpredictable occurrence of the disease makes its management difficult. Considerable research has been conducted on the biology and epidemiology of one or more contributors of this disease, however little quantified findings are available on thrips development or TSWV progress [22,23,30].

When quantifying the seasonal TSWV incidence for a field of flue-cured tobacco in eastern North Carolina, degree-days and the field's TSWV history are important variables. Regardless of the lower threshold used to estimate DD, Model II had the best explanatory power and predicted the observed values reasonably well. As the lower threshold decreased the model fit the data and predicted the observed values more accurately. Schoeny and Lucas [26] were successful in developing a model using DD to describe take-all epidemics on wheat. Leclercq-Le Quillec, *et al.* [18] also developed a model using DD to describe *Barley yellow dwarf virus*, a virus that is similar to TSWV as it is also vectored by insects. Both studies used a lower threshold of 0°C based on different biological associations. Shoeny and Lucas [26] used it because of its association with wheat growth and Leclercq-Le Quillec, *et al.* [18] chose this lower threshold due to its association with aphid development. This study also found that a lower threshold associated with the development of the vector was appropriate which is in accordance with our findings. It is possible that there is a lower threshold below 10.5°C that would allow the model to fit the data and predict the observed values even more accurately. Further analysis is needed to determine this and what biological association, if any, the lower thresholds have.

Morsello, *et al.* [22] demonstrated that degree-days were the sole best explanatory variable of seasonal patterns of dispersing *F. fusca* in North Carolina. In our study degree-days was also found to be significant in explaining TSWV incidence on tobacco crops and that could be directly related to its effect on thrips and/or other undocumented biological aspects. Similarly, Olantinwo, *et al.* [23] successfully developed a predictive model that integrated localized weather information into the existing TSWV risk index for peanut. They used a variety of explanatory variables, including minimum and maximum temperature,

accumulated rainfall, and number of chilling hours (lower threshold of 7.2°C). In the case of peanuts, the inclusion of weather variables increased the explanatory power of the model from 0.23 to 0.54 [23]. Twelve input variables were necessary to accomplish this explanatory power, whereas in our study a similar increase of the explanatory power was obtained only with the inclusion of degree-days, an indication that TSWV incidence on tobacco may be a simpler case than in other crops.

TSWV history was included in the temporal progression model on the premises that the virus overwinters in the same area [5,13], and the amount of virus that overwinters could possibly affect TSWV incidence in the following season. Indeed when TSWV history was added to the model it greatly improved the model's power of prediction; when the model only included DD and was used to predict the observed values the corresponding  $R^2$  value was 0.08, but when TSWV history was added to the model the  $R^2$  value was 0.52. The significance of TSWV field history indicates that, although TSWV is a weather driven disease, it has an established history in different regions of North Carolina that influences the incidence observed in subsequent years.

Precipitation variables for the months of April and May have been found to be important explanatory variables in other studies [22,23]. Given that the explanatory power of the model in which both DD and TSWV history were included was 0.52, there is evidence that prior field history provides some explanation, but not all, of the expected TSWV incidence in a tobacco field. Considering and testing more explanatory variables, such as precipitation, may improve the model's power of prediction. On the other hand, precipitation patterns are very arbitrary in the southeast United States [27] and thus values obtained by the network of the state climate office may not be representative of all areas, even when the weather stations are in close vicinity to the field of interest. Thus, these variables may be site-specific, and thus difficult to obtain, and therefore likely to produce a model with good biological explanatory power that is too complex to be used for prediction purposes.

Our spatial analysis did not reveal any pattern for most locations. Only random clusters were observed. The random clusters suggest that the thrips population came from within the field [25], perhaps via volunteer plants, weeds present in the field, or from far

away via the wind which allowed them to enter the field away from the edges. If the patterns of spatial distributions had shown a gradient from the edge of the field then it could have been assumed that the thrips were flying from weeds adjacent to the field [25]. All of this agrees with what other research has found when studying TSWV spatial patterns in other crop systems [6,12,13].

A few locations revealed a pattern during the fifth week (early season), ninth week (late season), or both weeks. All of the locations that showed a spatial pattern had at least two sources of weeds (road, meadow, woods) that were both adjacent and distant. For instance, in Duplin County 2006 there was a small path adjacent to the side of the field where the segregated TSWV incidence was observed. Due to heavy rain during the first few weeks after transplant the path was full of weeds that were less than 5m away from the tobacco plants. Groves *et al.* [13] reported that there was no gradient present when the virus source was 35 m away. Similarly, Gitaitis *et al.* [12] reported gradients when the source was directly adjacent to the field. Therefore all of the locations in this study had the chance to develop random clusters, which arise from thrips populations present in or adjacent to the field. Wind could have blown the thrips from any of these virus sources and into the field. However, the other locations did not show a pattern but were bordered by similar potential virus sources. Perhaps the adjacent areas were not harboring virus or the virus in the surrounding areas was not vectored into the field. Either way, this indicates that TSWV spatial progression is variable between locations.

When summarizing the temporal and spatial analyses together it becomes obvious that our results from these two scales are complimentary. TSWV spatial field patterns were random, indicating that the virus moved into the crops from the adjacent weeds only occasionally. Thus, the virus likely moved into the crops from further distances via wind. Yet an established inoculum potential should be present in different regions given that TSWV history is an important explanatory factor for temporal disease progression. TSWV epidemics on tobacco were first documented in Georgia in 1989 [9] and 13 years later in North Carolina [2]. Currently, TSWV epidemics in North Carolina are predominately in the eastern part of the state [2]. Within the past thirteen years the virus has progressively moved north via

thrips, wind movement, and weather favorable for establishment and persistence among seasons [22,23].

The temporal progression of TSWV incidence, based on degree-days and TSWV field history, which we developed in this study may provide sufficient information for management purposes (i.e. expected TSWV incidence class) but not about the absolute expected TSWV incidence, for which in this case more site-specific information may be needed. Further investigation is underway to clarify the predictability of this model for TSWV management on tobacco crops.

## LITERATURE CITED

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**Table 1.** Rates and brand names of imidacloprid used in each location and the date on which the tobacco was transplanted at each location

<b>Location/Year</b>	<b>Rate of Imidacloprid<sup>a</sup></b>	<b>Transplant Date</b>
Duplin 06	Admire Pro 0.8 oz/1000 cells	5/4/2006
Onslow 06	Admire Pro 0.8 oz/1000 cells	4/28/2006
Sampson 06	Admire Pro 0.8 oz/1000 cells	4/18/2006
Jones 07	Admire Pro 0.8 oz/1000 cells	4/24/2007
Sampson 07	Admire Pro 0.8 oz/1000 cells	4/23/2007
N. Craven 07	NuPrin 1.8 oz/1000 cells	4/13/2007
S. Craven 07	Admire Pro 0.6 oz/1000 cells	4/18/2007
Johnston 07	Admire Pro 0.8 oz/1000 cells	4/28/2007
Lenoir 07	unknown	unknown
N. Sampson 07	Admire Pro 0.7 oz/1000 cells	4/17/2007
Beaufort 08	Admire Pro 0.8 oz/1000 cells	5/5/2008
Craven 08	Admire Pro 0.8 oz/1000 cells	5/2/2008
Duplin 08	Admire Pro 0.8 oz/1000 cells	4/30/2008
W. Bertie 08	Admire Pro 0.8 oz/1000 cells	unknown
E. Bertie 08	NuPrin 1.2 oz/1000 cells	5/1/2008
Edgecombe 08	Admire Pro 0.6 oz/1000 cells	4/26/2008
W. Greene 08	no imidacloprid	4/19/2008
E. Greene 08	Admire Pro 1.0 oz/1000 cells	4/18/2008
Johnston 08	Admire Pro 0.8 oz/1000 cells	4/23/2008
Lenoir 08	no imidacloprid	4/28/2008
Martin 08	Admire Pro 0.8 oz/1000 cells	4/25/2008
N. Duplin 08	Admire Pro 0.8 oz/1000 cells	4/18/2008
N. Sampson 08	Admire Pro 0.8 oz/1000 cells	4/29/2008
Pitt 08	Admire Pro 0.8-1.0 oz/1000 cells	4/22/2008
Wilson 08	Admire Pro 0.4 oz/1000 cells	5/8/2008

<sup>a</sup> Applied in the greenhouse as a drench application

**Table 2.** Locations in 2006, 2007, and 2008 from which data was collected for spatial distribution analysis and a description of the immediately surrounding terrain.

<b>Location/Year</b>	<b>Areas bordering plots</b>			
Duplin 06	woods	dirt path	road	crop
Onslow 06	crop	crop	woods	road
Sampson 06	crop	crop	crop	crop
Jones 07	crop	dirt path	crop	dirt path
Sampson 07	crop	crop	road	meadow
Beaufort 08	woods	woods	crop	crop
Craven 08	road	crop	crop	meadow
Duplin 08	crop	meadow	crop	meadow

**Table 3.** Criteria of goodness of fit of TSWV incidence Model I and Model II for eastern North Carolina

Criteria	Model I <sup>a</sup>				Model II <sup>b</sup>			
	DD (10.5°C)	DD (18°C)	DD (20°C)	DD (26°C)	DD (10.5°C)	DD (18°C)	DD (20°C)	DD (26°C)
Concordant (%) <sup>c</sup>	61.2	54.4	54.7	31.7	73.3	72.2	72.2	69.2
Discordant (%) <sup>c</sup>	35.5	39.9	39.4	40.5	24.9	26.1	26.1	25.1
c <sup>d</sup>	0.63	0.57	0.58	0.46	0.74	0.73	0.73	0.72
R <sup>2</sup> <sup>e</sup>	0.08	0.02	0.02	0.00	0.52	0.43	0.43	0.38

<sup>a</sup> Logistic regression for Model I: Cumulative disease incidence = a \* degree-days.

<sup>b</sup> Logistic regression for Model II: Cumulative disease incidence = a \* degree-days + b \* TSWV history. TSWV history was input as an indicator variable with the Very Low level as the reference group. Thus significance of estimates is relative to Very Low.

<sup>c</sup> Measurements assess the association of estimated probabilities and observed frequencies.

<sup>d</sup> Indices computed from the two first measurements. A model with higher values for these indices have better predictive ability than a model with lower values.

<sup>e</sup> R<sup>2</sup> value corresponds to the linear regression model: observed values = predicted values.

**Table 4.** Parameter estimates of the logistic regression analysis used to quantify TSWV incidence by using degree-days (DD) and disease history as explanatory variables in tobacco fields in eastern NC from 2006 to 2008

Variable <sup>ab</sup>	DD (10.5°C)		DD (18°C)		DD (20°C)		DD (26°C)	
	Parameter Estimate	p-value	Parameter Estimate	p-value	Parameter Estimate	p-value	Parameter Estimate	p-value
Intercept	-3.70	<.0001	-3.48	<.0001	-3.38	<.0001	-3.04	<.0001
DD	0.0024	<.0001	0.0036	<.0001	0.0046	<.0001	0.0105	<.0001
L	-3.92	0.0413	-3.72	0.0286	-3.62	0.0265	-3.28	0.028
M	-3.93	0.0027	-3.76	0.0004	-3.66	0.0004	-3.30	0.0009
H	-3.34	<.0001	-3.05	<.0001	-2.95	<.0001	-2.62	<.0001
VH	-2.57	<.0001	-2.30	<.0001	-2.21	<.0001	-1.88	<.0001

<sup>a</sup> Classes of TSWV incidence: VH = Very High (>30%), H = High (20%-30%), M = Medium (10%-20%), L = Low (5%-10%).

<sup>b</sup> Intercept = indicator variable in this model was Very Low (<5%); DD = Degree-days (°C)

**Table 5.** Classes of TSWV incidence predicted by model II, using the four different lower thresholds, and compared to the observed class nine weeks after transplant

<b>Location/Year</b>	<b>Observed<sup>a</sup></b>	<b>Predicted (10.5°C)<sup>a</sup></b>	<b>Predicted (18°C)<sup>a</sup></b>	<b>Predicted (20°C)<sup>a</sup></b>	<b>Predicted (26°C)<sup>a</sup></b>
Duplin 06	VH	H	M*	M*	M*
Onslow 06	M	L	L	L	L
Sampson 06	M	L	L	L	VL*
N. Craven 07	M	M	M	M	M
S. Craven 07	H	M	M	M	M
Lenoir 07	L	M	L	L	L
Johnston 07	VL	VL	VL	VL	VL
N. Sampson 07	L	L	VL	VL	VL
Jones 07	M	M	L	L	L
Sampson 07	M	L	L	L	VL*
E. Bertie 08	VL	VL	VL	VL	VL
W. Bertie 08	VL	VL	VL	VL	VL
N. Duplin 08	M	VL*	VL*	VL*	VL*
Edgecombe 08	VL	VL	VL	VL	VL
E. Greene 08	L	VL	VL	VL	VL
W. Greene 08	M	L	L	L	L
Johnston 08	VL	L	L	L	VL
Lenoir 08	VH	H	M	M	M*
Martin 08	VL	L	L	L	VL
Pitt 08	L	L	L	L	L
N. Sampson 08	VL	VL	VL	VL	VL
Wilson 08	VL	L	L	L	L
Beaufort 08	L	L	L	L	L
Craven 08	M	H	H	H	M
Duplin 08	M	H	M	M	M

<sup>a</sup> Classes of TSWV incidence: VH = Very High (>30%), H = High (20%-30%), M = Medium (10%-20%), L = Low (5%-10%), VL = Very Low (<5%).

\*Predicted two classes apart from observed classes

**Table 6.** P-values of the linear regression model between the observed and the predicted values of the four semivariogram models used for the spatial analysis

<b>Location/Year</b>	<b>WPT<sup>a</sup></b>	<b>Circular</b>	<b>Spherical</b>	<b>Exponential</b>	<b>Gaussian</b>
		<b>model</b>	<b>model</b>	<b>model</b>	<b>model</b>
		<b>equation</b>	<b>equation</b>	<b>equation</b>	<b>equation</b>
Duplin 06	5	0.0001	0.0003	0.0005	0.0002
	9	<.0001	<.0001	<.0001	<.0001
Onslow 06	5	0.1842	0.1775	0.1461	0.2039
	9	0.7017	0.6958	0.6807	0.6977
Sampson 06	5	0.4923	0.4989	0.4951	0.4814
	9	0.8666	0.8607	0.8118	0.8897
Jones 07	5	0.7538	0.7555	0.7565	0.759
	9	0.1798	0.1791	0.1738	0.1857
Sampson 07	5	<.0001	<.0001	0.0001	<.0001
	9	0.1461	0.1461	0.1607	0.1317
Beaufort 08	5	0.57	0.57	0.57	0.57
	9	0.006	0.0061	0.0064	0.0064
Craven 08	5	0.0037	0.0032	0.0016	0.0075
	9	0.0492	0.0516	0.0539	0.049
Duplin 08	5	0.2357	0.2407	0.263	0.2172
	9	0.1763	0.1793	0.1716	0.1885

<sup>a</sup> WPT = Weeks post transplanting



**Table 7.** Parameter values of the circular, spherical, exponential, and gaussian semivariogram models and their associated cross validation regression equations for the locations in 2006

Location/Year	WPT <sup>a</sup>	Model	Semivariogram variables			Cross validation regression equation
			Nugget	Range	Sill	
Duplin 06	5	Circular	6.6163	162.02	2.5578	$0.305 * x + -4.049$
		Spherical	4.0582	45.454	4.3148	$0.312 * x + -3.848$
		Exponential	2.4527	41.118	5.9123	$0.331 * x + -3.839$
		Gaussian	4.9465	40.64	3.4136	$0.310 * x + -4.057$
	9	Circular	23.768	162.02	24.607	$0.328 * x + -20.551$
		Spherical	23.353	162.02	22.951	$0.328 * x + -20.538$
		Exponential	20.298	162.02	24.488	$0.310 * x + -21.007$
		Gaussian	26.739	162.02	22.939	$0.322 * x + -20.708$
Onslow 06	5	Circular	0.9116	119.65	0.2425	$0.080 * x + -0.571$
		Spherical	0.9071	119.65	0.2266	$0.080 * x + -0.571$
		Exponential	0.85	119.65	0.2828	$0.086 * x + -0.570$
		Gaussian	0.951	119.65	0.2029	$0.076 * x + -0.572$
	9	Circular	17.631	34.683	8.2	$0.005 * x + -18.434$
		Spherical	17.411	38.481	8.4134	$0.018 * x + -18.182$
		Exponential	15.046	37.428	10.856	$0.022 * x + -18.032$
		Gaussian	19.127	35.184	6.7717	$0.004 * x + -18.518$
Sampson 06	5	Circular	0.4805	134.52	0.0388	$-0.037 * x + -0.594$
		Spherical	0.4807	134.52	0.0344	$-0.037 * x + -0.594$
		Exponential	0.4825	134.52	0.0261	$-0.037 * x + -0.594$
		Gaussian	0.4835	134.52	0.0411	$-0.037 * x + -0.594$
	9	Circular	7.5023	48.015	1.4192	$0.075 * x + -8.368$
		Spherical	7.4891	54.428	1.4349	$0.075 * x + -8.370$
		Exponential	7.2317	53.644	1.6795	$0.044 * x + -8.734$
		Gaussian	7.683	45.902	1.2471	$0.077 * x + -8.353$

<sup>a</sup> WPT = Weeks post transplanting

**Table 8.** Parameter values of the circular, spherical, exponential, and gaussian semivariogram models and their associated cross validation regression equations for the locations in 2007

Location/Year	WPT <sup>a</sup>	Model	Semivariogram variables			Cross validation regression equation
			Nugget	Range	Sill	
Jones 07	5	Circular	6.4272	176.74	3.0007	-0.028 * x + -5.251
		Spherical	6.3933	176.74	2.7629	-0.028 * x + -5.251
		Exponential	6.1532	176.74	2.7371	0.005 * x + -5.321
		Gaussian	6.7342	176.74	2.9466	-0.027 * x + -5.249
	9	Circular	17.157	119.74	5.9877	0.119 * x + -20.287
		Spherical	16.926	120.59	5.8954	0.119 * x + -20.290
		Exponential	15.746	120.59	6.9948	0.119 * x + -20.278
		Gaussian	17.453	86.854	5.0241	0.118 * x + -20.311
Sampson 07	5	Circular	1.223	58.475	1.9241	0.385 * x + -1.054
		Spherical	1.281	58.475	1.637	0.386 * x + -1.054
		Exponential	1.0082	58.475	1.9168	0.376 * x + -1.058
		Gaussian	1.2576	58.475	2.3042	0.387 * x + -1.100
	9	Circular	13.701	59.784	8.9306	0.127 * x + -0.265
		Spherical	13.581	59.784	8.2905	0.127 * x + -0.265
		Exponential	12.792	59.784	8.5149	0.209 * x + 0.235
		Gaussian	14.799	59.784	8.3145	0.131 * x + -0.266

<sup>a</sup> WPT = Weeks post transplanting

**Table 9.** Parameter values of the circular, spherical, exponential, and gaussian variogram models and the associated cross validation regression equations for the locations in 2008

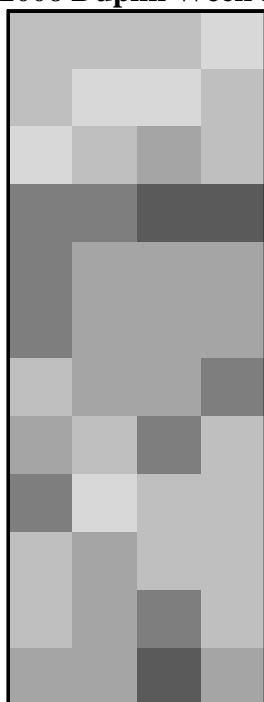
Location/Year	WPT <sup>a</sup>	Model	Semivariogram variables			Cross validation regression equation
			Nugget	Range	Sill	
Beaufort 08	5	Circular	1.5357	41.62	0	-0.001 * x + -3
		Spherical	1.5357	41.62	0	-0.001 * x + -3
		Exponential	1.5357	41.62	0	-0.001 * x + -3
		Gaussian	1.5357	41.62	0	-0.001 * x + -3
	9	Circular	1.3166	32.694	2.7576	0.299 * x + -5.077
		Spherical	1.2626	36.048	2.7923	0.278 * x + -5.293
		Exponential	0.918	43.453	3.2849	0.309 * x + -5.017
		Gaussian	1.6986	32.943	2.4834	0.243 * x + -5.605
Craven 08	5	Circular	2.2579	42.13	5.7246	0.333 * x + -3.156
		Spherical	2.1262	42.13	5.42	0.340 * x + -3.118
		Exponential	1.4414	42.13	5.848	0.368 * x + -2.995
		Gaussian	3.0457	42.13	5.1532	0.303 * x + -3.297
	9	Circular	5.4663	42.13	5.3407	0.197 * x + -6.853
		Spherical	5.296	42.13	5.1275	0.197 * x + -6.845
		Exponential	4.5324	42.13	5.7158	0.202 * x + -6.811
		Gaussian	6.1395	42.13	4.9118	0.208 * x + -6.654
Duplin 08	5	Circular	23.166	36.333	38.351	0.177 * x + -4.998
		Spherical	22.799	39.895	38.251	0.177 * x + -5.016
		Exponential	19.57	53.569	44.85	0.172 * x + -4.836
		Gaussian	28.416	36.745	34.585	0.180 * x + -4.967
	9	Circular	11.544	32.001	7.8702	-0.065 * x + -12.678
		Spherical	11.223	34.728	8.1449	-0.064 * x + -12.666
		Exponential	10.18	41.546	9.6179	-0.064 * x + -12.684
		Gaussian	12.459	30.074	6.9984	-0.062 * x + -12.643

<sup>a</sup> WPT = Weeks post transplanting

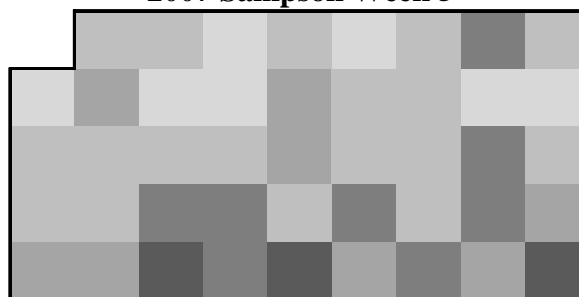
**Fig. 1.** Maps of the normalized percentage of TSWV incidence in each field in (a) 2006 Duplin County at week 5, 2007 Sampson County at week 5, 2008 Craven County week 5 and (b) 2006 Sampson County at week 9, 2007 Jones County at week 9, 2008 Beaufort County at week 9.

(a)

**2006 Duplin Week 5**



**2007 Sampson Week 5**



-4.37 – -3.35

-3.34 – -1.83

-1.82 – -0.67

-0.66 – 0.65

0.66 – 2.26

**2008 Craven Week 5**



-11.44 – -9.22

-9.21 – -6.65

-6.64 – -4.39

-4.38 – -1.95

-1.94 – 0.79

-9.4 – -7.8

-7.7 – -5.7

-5.6 – -4.4

-4.3 – -1.4

-1.3 – 1.4

(b)

