EFFECT OF SEEDLING SOURCE ON INCIDENCE OF TOMATO SPOTTED WILT IN FLUE-CURED TOBACCO

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Tobacco seedlings from 16 different outdoor seedbeds in 11 counties in Georgia were assayed by ELISA for tomato spotted wilt virus (TSWV). Only one asymptomatic seedling out of more than 1,200 was positive for TSWV. Additional seedlings from the 16 sources were transplanted into fields in each of six different counties, and incidence of spotted wilt was monitored in all fields throughout the season. Significant ($P \leq 0.05$) field location effects were found for final, cumulative incidence of spotted wilt, but no significant effects were found for seedling source or field location X seedling source interaction. There were no consistent differences in final, cumulative incidence of spotted wilt among transplant sources. However, across sources of seedlings, incidence of spotted wilt varied greatly among field sites, with average incidence for the six fields ranging from 2.3% to 12.7%. Final, cumulative spotted wilt incidence was affected more by field location than by transplant source.

Additional key words: Tomato spotted wilt virus, TSWV, disease incidence, plant beds, *Nicotiana tabacum*.

INTRODUCTION

Spotted wilt, caused by tomato spotted wilt virus (TSWV), occurs in all tobacco producing counties of Georgia (4), and over the past three years it has accounted for annual losses in stand and yield estimated at 6-8% (1,2,3). TSWV is vectored by thrips (7,8), but little is known about the relationships between vector, virus, and host-plant interactions in the southeastern U.S.A.

The source of initial inoculum of TSWV in Georgia tobacco has not been identified. Tobacco seedlings often are grown in outdoor beds for transplanting into the field, and they harbor thrips species that are potential vectors of TSWV (5). Seedlings with symptoms of spotted wilt can be found in seed beds, but disease incidence is typically very low. First symptoms of spotted wilt may develop in the field within 3 weeks after transplanting, but it is not known whether those plants became infected in the seed beds or in the field after transplanting because the same thrips vectors are present in both locations (6). The objective of this study was to survey seed beds for asymptomatic plants of tobacco infected with TSWV, and to determine the relative effects of transplant source and field location on incidence of spotted wilt in tobacco.

MATERIALS AND METHODS

Seedling transplants of cultivar 'K-326' with no symptoms of spotted wilt were obtained from 16 commercial farms in 11 counties in a linear southwest-northeast range of approximately 265 km in the southern Georgia tobacco-producing region. Seventy-five asymptomatic plants were collected from each source on 3-5 April, 1991, and each was assayed individually for TSWV by ELISA. Sap was extracted from the root tissue with a stainless steel, double-roller, plant sap extractor (Model 1, Ravenel Specialties, Seneca, SC), and buffered with a phosphate buffered saline (PBS)-Tween extraction buffer (pH = 7.0). Double sandwich ELISA was performed with commercial antiserum to the common or "Lettuce" strain of TSWV (Agdia Inc., Elkhart, IN). The antiserum was diluted 1:500 in PBS-Tween buffer. Peroxidase labeled conjugate was used in all cases. All test plates contained positive control wells, negative control wells, and blank wells. ELISA results were read spectrophotometrically at 490 nm using a Dynatech Minireader II (Dynatech Laboratories Inc., Alexandria, VA). A sample was considered TSWV-positive if contents of the well turned a pronounced yellow after addition of the substrate, and its A_{490nm} value was greater than twice the mean A_{490nm} value for samples of roots that exhibited no yellow color.

Field experiments were conducted at one farm site in each of six Georgia counties (Appling, Berrien, Brooks, Jeff Davis, Tattnall and Ware) in 1991. In each location, 16 treatments that corresponded to the 16 transplant sources previously described were arranged in a completely randomized design. Boxes of transplants were selected for each site at random from the six boxes from each transplant source.

Six replications were used at the Jeff Davis site. Each replication contained three or four (30.5 m long) rows of each treatment. Four replications were used at the Brooks Co. site, and plots were two rows wide and 25 m long. At the other four locations, plots consisted of two parallel rows, 50 m long, and were replicated four to six times. Seedlings with no symptoms were collected from each of the plant beds on 4 April, transported to the field sites and transplanted on 5-8 April. The farms used in Appling, Berrien, Jeff Davis and Ware counties also were sources of transplants. Incidence of spotted wilt was determined six times at 2-week intervals at each location with initial evaluations on 17 and 18 April, and final evaluations on 25 and 26 June. Diagnosis was based upon symptoms (e.g., concentric ringspots, and veinal chlorosis and necrosis), and was confirmed in arbitrarily chosen symptomatic leaves by means of ELISA techniques described previously.

Final cumulative incidence was calculated as percent infected plants of the total initial plant population per plot. Data were subjected to analysis of variance (9) and Fisher's Protected Least Significant Difference (LSD) (9).

RESULTS

Only one of 1,200 seedling root samples tested positive for TSWV. All negative controls, blank wells, and positive control wells gave expected results.

There were significant ($P \leq 0.01$) field location effects on final incidence of spotted wilt. Across locations, there were no significant ($P > 0.05$) seedling source or location X seedling source effects. When averaged across field locations, the final incidence among the 16 seedling sources ranged from 4.3 to 7.5% (**Table 1**). The highest numerical ranking of final disease incidence was observed in plants from five different seedling sources for the six test locations (**Table 1**). Numerical rank of the transplant sources based on final incidence of spotted wilt in the various locations was not consistent ($P \leq 0.05$).

Final disease incidence averaged across seedling sources was highest at the Jeff Davis location (12.7%). Disease

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Table 1. Effect of 16 seedling sources on final cumulative percent incidence of spotted wilt in K-326 tobacco planted in six Georgia counties, 1991.

	Disease incidence (%)[a]						
	Field Location (County)						
Seedbed Source	Tattnall	Appling	Jeff Davis	Ware	Berrien	Brooks	Average
Appling Co. (A)[b]	2.4	3.5	12.7	1.9	3.1	4.9	4.8
Appling Co. (B)	1.6	4.1	10.9	4.1	7.2	4.1	5.3
Berrien Co.	1.6	3.8	10.5	2.4	4.1	3.5	4.3
Coffee Co. (A)	2.6	5.6	15.2	3.4	6.0	4.1	6.2
Coffee Co. (B)	2.9	6.5	18.8	3.7	4.8	6.6	7.2
Coffee Co. (C)	3.6	5.3	10.9	2.5	5.9	3.4	5.3
Colquitt Co.	2.1	5.1	13.4	2.6	6.5	6.3	6.0
Cook Co.	1.5	5.4	8.6	3.2	5.1	4.4	4.7
Evans Co.	2.7	6.5	11.7	2.9	6.5	5.6	6.0
Jeff Davis Co.	3.2	6.3	17.4	4.4	8.3	4.8	7.4
Mitchell Co. (A)	1.6	8.0	10.5	2.5	3.2	2.5	4.3
Mitchell Co. (B)	2.4	5.2	12.9	1.4	3.6	5.5	5.2
Pierce Co.	3.0	7.1	16.1	3.0	7.7	8.2	7.5
Tift Co.	2.4	7.4	11.5	2.9	5.8	4.7	5.8
Ware Co. (A)	0.7	6.3	10.7	3.3	4.2	5.1	5.5
Ware Co. (B)	1.8	4.8	11.9	1.5	4.4	6.7	5.2
LSD ($P \leq 0.05$)[c]							NS

[a] Final cumulative total incidence of spotted wilt after six evaluations.

[b] Letters in parentheses indicate separate seedling sources within the same county.

[c] NS indicates that no significant ($P \leq 0.05$) planting source effects were indicated by analysis of variance.

incidence was similar in the Appling (5.7%), Berrien (5.8%), and Brooks Co. (5.0%) fields. Low incidence of spotted wilt was observed at the Tattnall (2.3%) and Ware Co. (2.8%) sites.

DISCUSSION

ELISA results from all transplant sources indicate that incidence of TSWV infections in asymptomatic plants is very low. Symptomatic plants were found in the seed beds, but these plants were readily identified as infected and were pulled and discarded before transplanting. The number of plants assayed relative to the number planted in the six fields also was relatively small. Thus, the possibility exists that a low incidence of infected but asymptomatic plants could have gone undetected. However, it is doubtful that 12% incidence would be observed in the field at the Jeff Davis location from asymptomatic plants that had been inoculated exclusively in the seed bed. The very low incidence of infections among asymptomatic seedlings also indicates that location effects observed in the field experiments were not due to environmental effects on expression of symptoms, but were due to differences in incidence of infection that occurred after tobacco seedlings were transplanted to the field.

The lack of differences in final incidence of spotted wilt among the seedling sources also indicates that infection in the seed bed currently is less important than infection in the field in Georgia. These results do not rule out the possibility that plants from seed beds may be an important inoculum source in the future. Our results indicate that control measures directed toward reduction of TSWV inoculum and/or thrips vectors should be focused on those sources associated with the tobacco fields.

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