Evaluation of Tobacco Germplasm for Seedling Resistance to Stem Rot and Target Spot Caused by *Thanatephorus cucumeris*

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ABSTRACT

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Stem rot and target spot of tobacco, caused by *Rhizoctonia solani* and its teleomorph *Thanatephorus cucumeris*, respectively, can cause serious problems in production of tobacco (*Nicotiana tabacum*) seedlings. Previous screens for genetic resistance in tobacco have been limited. The objective of this study was to evaluate 97 genotypes composing several classes of tobacco and related *Nicotiana* spp. for seedling resistance to stem rot and target spot. Significant differences in disease incidence initially were observed among the genotypes for both stem rot and target spot; however, resistance to target spot was not observed when disease pressure was high. Partial resistance to stem rot was observed in several genotypes in repeated tests. These accessions may be useful as a source of resistance to *R. solani* in future breeding efforts.

Rhizoctonia solani J.G. Kühn (teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk) can cause serious disease problems in both transplant and field production of tobacco. This pathogen became more of a problem on seedlings in the 1990s as tobacco growers largely switched from producing transplants in outdoor plant beds to production in greenhouse float systems. In this system, tobacco is seeded on a soilless growth medium in polystyrene trays which then are floated on a reservoir containing a nutrient solution (18). This produces transplants with very good quality, but the high plant density, high humidity, and shared water reservoir associated with this type of production is very favorable for disease development (17). Reused polystyrene float trays also can harbor *R. solani* sclerotia and hyphae from previous years and can be a primary source of inoculum (5). The fungicide mancozeb (Dithane DF) has been labeled for use to control damping off and target spot in greenhouse production and provides moderate control of these diseases. However, in a comparison of the activity of several fungicides against *R. solani*, it was demonstrated that mancozeb was the least active of the fungicides tested (3). In this test, iprodione provided excellent control for stem rot and target spot symptoms in seed beds but is not currently registered for

use in tobacco greenhouses. Currently recommended controls include proper disinfection of float trays by steam treatments or methyl bromide before reuse and removal of plant debris near tobacco greenhouses (5). Growers also may choose to use new float trays each year, although this does increase transplant production costs.

R. solani causes damping-off and stem rot in young transplants and a disease of the lower stem and root called sore shin in older field plants (7,15,19). This disease can result in seedling death in the greenhouse and moderate to severe stunting and death of plants in the field. Transplanting infected seedlings is a major cause of sore shin in field plants; however, infection also can be caused by *R. solani* that is already present in the field.

Target spot is a relatively new foliar disease that first appeared in the United States in the 1980s and is now an economically important disease in tobacco production (8,14). This disease is caused by infection with basidiospores of *T. cucumeris* which are produced on hymenia that form on the soil surface or infected plant tissue (14,16). Symptoms begin as small water-soaked lesions on the leaves which can expand to large circular spots with concentric rings. This disease can occur on tobacco seedlings in greenhouse environments and usually is observed after the leaves of the seedlings have grown close enough to form a canopy, forming a high-humidity environment that favors disease development. In severe cases, the pathogen may grow from the leaf tissue into the stem, resulting in plant death. Plants with leaf lesions that are transplanted into the field also may provide an inoculum source for later infec-

tion, because a second cycle of target spot can occur on large field plants (17). The disease develops after the leaves are large enough to shade the soil, which provides an area of high relative humidity and promotes the production of basidiospores from hymenia on the soil surface. The spores are dispersed onto the lower leaves of the plants and the disease progresses up the plant. Isolates of *R. solani* associated with stem and root rot symptoms have been characterized by anastomosis group as AG-1, AG-2-2, and AG-4, whereas target spot in the greenhouse and the field is associated with AG-3 (20).

Resistance to *R. solani* in tobacco cultivars would be highly desirable but previous screens for resistance in tobacco germplasm have been limited to a small number of commonly used cultivars. However, evaluation of germplasm and identification of resistance to *R. solani* has been successful in other crops such as peanut, bean, rice, sorghum, and sugar beet (4,9-12). The United States *Nicotiana* germplasm collection maintained by North Carolina State University contains approximately 1,900 *N. tabacum* accessions as well as 59 *Nicotiana* spp., and is a valuable resource for the discovery of new traits (6).

The objective of this work was to screen a diverse sample of the accessions in the *Nicotiana* collection as well as several elite cultivars representing various market types in order to identify potential sources of seedling resistance to both target spot and stem rot. Improved seedling resistance would reduce direct greenhouse losses due to these diseases. This could reduce crop losses as well by reducing the potential of transplanting infected seedlings into the field.

MATERIALS AND METHODS

Plant material. Ninety-seven accessions chosen to represent a wide range of *Nicotiana* spp. diversity were evaluated for resistance. The *N. tabacum* accessions are categorized according to market types and included 2 cigar binder, 11 cigar filler, 11 cigar wrapper, 14 burley, 26 flue-cured, 16 oriental, and 1 each of dark, Maryland, and air-cured types (Table 1). In addition, 14 *Nicotiana* spp. were evaluated. Accessions chosen were a set of tobacco genotypes collected from around the world, and included popular U.S. cultivars and a selection of tobacco introductions (TIs). The

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TIs were chosen based on diversity of geographic origin. These accessions were obtained from the U.S. *Nicotiana* Germplasm Collection maintained in Oxford, NC.

Inoculum production. An AG-3 target spot isolate of *T. cucumeris* and an AG-4 stem rot isolate of *R. solani* were isolated from flue-cured tobacco in North Carolina. The isolates were grown on potato dextrose agar at room temperature (22 to 25°C) for 5 days. Colonized agar plugs were removed and transferred to 250-ml Erlenmeyer flasks containing autoclaved rice grains (16). The pathogen was allowed to thoroughly colonize the rice grains for approximately 2 weeks at room temperature prior to use in experiments.

Inoculation and disease evaluation. Experiments were conducted in walk-in growth chambers in the North Carolina State University Southeastern Plant Environmental Laboratory (Phytotron), Raleigh.

The initial resistance screen was conducted in a randomized complete block design with four replications for the target spot experiment and three replications for the stem rot experiment. Because of space limitations, only one replication (24 seedlings per accession) could be placed into a phytotron chamber at one time. Therefore, the experiment was replicated over time.

The tobacco accessions were germinated and grown in Carolina's Choice tobacco potting mix (Carolina Soil Company, Kinston, NC). For each accession, 2-week-old seedlings were transferred to a 30-cell section of a polystyrene tray (5 by 6 cells, each cell 2.3 by 2.3 by 6.2 cm) that was cut from a 288-cell float tray from Carolina Soil Company. Each tray served as a replication, with 24 plants and six evenly spaced cells that were left unplanted for introduction of the inoculum. Transplanted trays were floated in polyethylene pans filled with a fertilizer solution of Peters 20-20-20 with nitrogen at 150 ppm to mimic the greenhouse float-tray system used by tobacco growers. Transplants were grown with a 12-h photoperiod at 28°C day and 20°C night temperatures. Relative humidity levels in the chamber were typically between 70 and 85%. Distilled water was added to the pans as needed.

Tobacco seedlings were inoculated with either the stem rot or target spot pathogen 2 days after transplanting. Infested rice (approximately six grains) was placed onto the surface of the soil in each of the six unplanted cells and the pathogen was allowed to colonize the trays. Some plants were bordered by more than one inoculation site (cell), but no differences in the amount of disease were observed on the plants in those cells compared with other plants. Plants were evaluated for disease symptoms starting at 1 week after inoculation. Two phases of disease development for both diseases were observed. For the

Table 1. Reaction of *Nicotiana* accessions to stem rot isolate of *Rhizoctonia solani*

Genotype name	PI number ^a	Type ^b	Arcsine incidence (%) ^c	AUDPC ^d
TI 1316	PI 405604	CF	28.7	162.1
TI 1311	PI 239655	O	39.7	264.5
<i>Nicotiana rustica</i>	PI 555554	Sp.	40	217
TI 428	PI 112830	CF	41.8	130.4
Burley 21	PI 552363	B	42.3	181.4
TI 1029	PI 119162	CF	42.7	198.8
L8	PI 551280	B	42.7	121.9
Ky 14	PI 552477	B	43.5	295.6
Xanthi	PI 552780	O	43.6	205.7
TI 1605	PI 415008	B	43.8	134.6
TI 835	PI 118113	CF	46.2	343.5
<i>N. sylvestris</i>	PI 555570	Sp.	46.5	286.3
TI 1451	PI 292205	CF	47.1	173.9
TI 1315	PI 405603	O	47.2	175.6
TI 232	PI 112227	CF	47.7	165.8
TI 1280	PI 405600	O	48.7	235.5
<i>N. knightiana</i>	PI 555527	Sp.	48.7	531
TI 1555	PI 378072	O	49	234.6
TI 109	PI 105954	FC	50	236.5
NC 2326	PI 552453	FC	50.7	209.8
TI 395	PI 112783	O	52.2	187.5
TI 123	PI 404976	CF	52.9	176.2
TI 1269	PI 405590	O	53.1	192.7
TN 90	PI 543792	B	53.7	473.9
TI 165	PI 405002	CF	55.4	245.5
TI 1524	PI 370290	FC	56.5	431.5
Conn. Broadleaf	PI 552619	CW	56.6	228.9
TI 501	PI 113985	FC	56.8	266.4
Samsun	PI 552748	O	56.9	471.9
TI 1449	PI 292203	B	57.2	313.1
<i>N. rotundifolia</i>	PI 555553	Sp.	57.4	684.4
Kavala	PI 552668	O	57.7	222.7
Havana 307	PI 552348	CB	57.9	303.7
TI 79	PI 67720	CF	57.9	184.5
<i>N. glutinosa</i>	PI 241768	Sp.	58	600.3
TI 716	PI 116144	FC	58.6	290.3
<i>N. velutina</i>	PI 244638	Sp.	59.2	601.3
TI 88	PI 404956	O	59.4	370
NC 297	...	FC	59.7	427.1
TI 819	PI 117674	B	59.8	306.2
Speight G70	PI 552497	FC	59.9	261.2
TI 1414	PI 405662	B	60.1	289.3
TI 1379	PI 286820	O	60.2	288.5
TI 672	PI 114634	CF	60.8	434.2
MD 609	PI 552452	M	61.3	304
TI 1577	PI 372918	CF	62.7	290.2
Pennleaf 1	PI 552403	CF	62.7	308.8
TI 1500	PI 329206	FC	63.7	296.9
NC 95	PI 552380	FC	63.7	300.7
TI 1222	PI 405555	O	63.9	315.2
Florida 301	PI 552629	CW	64.1	322.3
TI 1589	PI 390128	CF	64.1	251.1
K 326	PI 552505	FC	64.2	388
TI 1247	PI 405577	O	65.3	282
TI 1241	PI 405571	CF	65.7	117.1
Speight 168	...	FC	66.1	436.9
Havana 503	PI 551281	CB	66.9	280.1
NC 2002	...	B	67.1	602.3
Clay 402	...	B	67.1	512.6
TI 158	PI 404994	O	68.2	542
<i>N. longiflora</i>	PI 555531	Sp.	68.6	355
TI 186	PI 112205	B	69.8	345.4
TI 1279	PI 405599	CF	69.9	317
TI 1462	PI 304901	FC	70	762.8
<i>N. langsdorffii</i>	PI 42337	Sp.	70.9	583.4
TI 1569	PI 349332	B	71	473.1

(continued on next page)

^a Plant introduction number.

^b Tobacco types are B, burley; CB, cigar binder; CF, cigar filler; CW, cigar wrapper; FC, flue-cured; O, oriental; DFC, dark fire-cured; AC, air-cured; M, Maryland; and Sp., *Nicotiana* sp.

^c Arcsine-transformed percentage of disease incidence.

^d Area under the disease progress curve.

^e Least significant difference.

^f Coefficient of variation.

Table 1. (continued from preceding page)

Genotype name	PI number ^a	Type ^b	Arcsine incidence (%) ^c	AUDPC ^d
McNair 944	PI 552494	FC	72.7	461.5
TI 1330	...	CF	73.8	311.1
OX 414	...	FC	74.1	475
K 346	PI 549110	FC	74.2	250.2
Coker 371 Gold	...	FC	74.3	409.6
NC 5	...	B	74.5	349.1
NC 4	...	B	74.8	565.6
TI 1306	PI 241414	O	75.7	526.2
<i>N. debneyi</i>	PI 503323	Sp.	76.6	1008.1
TI 1224	PI 405557	FC	77.1	448.2
R7-11	...	B	77.8	442.5
Speight H20	...	FC	77.9	229.6
Speight G28	PI 551318	FC	83.1	530.3
TI 1512	PI 370278	FC	83.4	436.2
TI 1480	PI 405678	CF	85.6	436.8
TI 1558	PI 355073	FC	86.4	374.4
NC 71	...	FC	88.1	406.7
TI 1616	PI 418593	FC	90.1	265.2
Hicks	PI 552373	FC	91.5	389.5
Tom Rosson Madole	PI 552764	DFC	91.5	558.1
NC 72	...	FC	92.5	368
<i>N. nudicaulis</i>	PI 555540	Sp.	96.5	911.1
TI 1734	...	AC	97.8	715.8
TI 1518	PI 370284	CF	97.9	624.1
<i>N. repanda</i>	PI 555552	Sp.	100.3	691.2
<i>N. tomentosiformis</i>	PI 555572	Sp.	100.3	651.5
Pennbel 69	PI 552404	CF	103.3	239.4
TI 119	PI 404974	CF	106.1	344.3
SA 1214	...	FC	122	1192.3
<i>N. alata</i>	PI 42334	Sp.	150.2	1513.9
<i>N. africana</i>	PI 555472	Sp.	157.1	2815.5
Mean	...	...	67.56	413.23
LSD (0.05) ^e	...	...	42.47	435.26
CV (%) ^f	...	...	38.9	66.1

Table 2. Comparison of genotypes with high and low stem rot incidence over multiple experiments

Genotype	Class ^b	Incidence (%) ^a			
		Exp 1	Exp 2	Exp 3	Average
TI 1605	l	43.8	NT	38.4	41.1
TI 1029	l	42.7	30.6	50.3	41.2
TI 1311	l	39.7	49.2	56.0	48.3
Xanthi	l	43.6	50.1	57.2	50.3
TI 1316	l	28.7	55.4	76.5	53.5
<i>Nicotiana rustica</i>	l	40.0	61.1	59.8	53.6
TI 428	l	41.8	58.6	64.5	54.9
Burley 21	l	42.0	77.0	63.7	60.9
L8	l	42.7	91.4	62.7	65.6
KY 14	l	43.5	87.3	66.6	65.8
K326	m	64.2	82.7	82.9	76.6
TI 1518	h	97.9	93.7	51.7	81.1
TI 1734	h	97.8	82.8	70.2	83.6
Hicks	h	91.5	100.7	68.7	87.0
TI 119	h	106.1	76.8	81.6	88.2
Pennbel 69	h	103.3	97.2	67.8	89.4
NC 72	h	92.5	110.1	82.1	94.9
SA 1214	h	122.0	106.2	74.2	100.8
<i>N. alata</i>	h	150.2	107.3	107.3	121.6
LSD (0.05) ^c	...	...	26.07	22.58	...
CV (%) ^d	...	...	19.63	20.20	...

^a Arcsine-transformed percentage of disease incidence for three experiments (Exp); NT = not tested.

^b Initial classification. Genotypes with low (l), moderate (m), or high (h) stem rot disease incidence in the initial experiment.

^c Least significant difference.

^d Coefficient of variation.

stem rot isolate, the pathogen would overcome and kill the small seedlings up to about two weeks after inoculation. After that time, little additional disease development occurred until about 35 days

postinoculation (dpi), at which time stem rot lesions at the base of the stem began to develop. Disease incidence, which included the percentage of plants exhibiting seedling death and stem rot, was evaluated

every other day for about 2.5 weeks post inoculation and then twice a week up to 42 dpi. For the target spot isolate, the pathogen also caused a small amount of seedling death up to about 2 weeks after inoculation. After this time, very little symptom development was observed until after 50 dpi, when target spot leaf lesions began to appear. The percentage of seedling death was evaluated every other day up to 2 to 2.5 weeks post inoculation and, at 8 weeks, the percentage of plants exhibiting target spot symptoms was evaluated.

Several accessions with the highest and lowest levels of disease were retested in additional experiments in order to confirm previous results. The stem rot test was repeated two more times with the selected accessions. Each of these tests consisted of three replications arranged in a randomized complete block design, with all three replications in a single phytotron chamber. The target spot test was repeated one additional time in an experiment with three replications. For the target spot isolate, several accessions were retested using a modification of the original protocol in order to evaluate resistance under higher disease pressure. In this test, the relative humidity was maintained at 90 to 98% by misting for 20 s every 5 min until 46 dpi, when target spot lesions first developed. Each replication was tented with plastic sheeting to prevent water from settling on the plants. The percentage of seedling death and plants with target spot was calculated as before. In addition, the diameter of each target spot lesion was measured to calculate the leaf area covered by target spot. The total area was divided by the number of living plants to determine the lesion area per plant.

In 2005, a heavy target spot outbreak was observed at the Border Belt Tobacco Research Station in Whiteville, NC. Official variety tests for flue-cured and burley cultivars had been planted at this site and both tests had significant disease. Target spot leaf lesions occurred most heavily on the lowest portions of the plants and declined toward the top the plants. The amount of leaf area with over 10% damage was recorded for plots of several burley and flue-cured cultivars over the three replications in these tests.

Statistical analysis. An arcsine transformation was performed on all percent incidence data before statistical analysis in order to improve homogeneity of variance. For the stem rot test, the area under the disease progress curve (AUDPC) was calculated using the formula $\sum_{i=1}^{n-1} [(y_i + y_{i+1})/2](t_{i+1} - t_i)$, where n = number of disease assessments, i = observations from 1 to n , y_i is the disease incidence on the i th rating date, and t_i is the day of the i th rating (1,13).

Analysis of variance was performed with PROC GLM and means were compared using Fisher's protected least sig-

nificant difference test ($P = 0.05$) using SAS statistical software (SAS Institute, Inc., Cary, NC). Pearson's correlation coefficient was calculated to compare disease measurements in different tests.

RESULTS

Stem rot. Significant differences in overall disease incidence and AUDPC were observed between genotypes inoculated with the stem rot isolate of *R. solani*, although no accession was completely resistant (Table 1). The percentage of disease incidence ranged from 12.5 to 100% (28.7 to 157.1% after arcsine transformation). Percent incidence and AUDPC were well correlated in this test, with a correlation coefficient of $r = 0.74$ ($P < 0.0001$), but AUDPC had a much higher coefficient of variation. Among the U.S. cultivars, two burley cultivars, Burley 21 and Ky 14, were observed to have relatively low amounts of disease.

Because the least significant difference covered a wide range in this test, this experiment was repeated twice with several of the accessions with the lowest and highest disease ratings to determine whether there was a repeatable difference in the level of resistance of different cultivars (Table 2). There was a significant test-genotype interaction for disease incidence; therefore, the data for each test was analyzed separately. The disease incidence data from the first test was correlated with the second ($r = 0.74$, $P = 0.0005$) and third test ($r = 0.62$, $P = 0.0045$). The incidence for all three tests was averaged and compared with the original classifications. All of the accessions that were classified as having low disease incidence in the first experiment had a lower average disease incidence than the most susceptible accessions, although there was enough variation in individual tests so that some accessions did not match the original classification in all tests. The accession with the lowest disease incidence was TI 1605, which is a burley accession from Japan named Shiroenshu 202.

Target spot. Highly significant differences in disease incidence were observed among genotypes inoculated with an isolate of *T. cucumeris* that causes target spot (Table 3). The most common symptom produced by the target spot isolate was foliar target spot lesions; however, this disease also caused a low level of seedling death. The observed leaf area affected by target spot was low and target spot lesions developed late in the test. Disease incidence ranged from 6.2 to 97.9% (21.4 to 149.7% in arcsine-transformed data). TI 1605 also had the lowest disease incidence in this test, exhibiting no seedling death and a low percentage of seedlings with target spot. Among the cultivars tested, several burley cultivars had a relatively low disease incidence, including TN 90, NC 2002, Burley 21, and Clay 402, whereas

Table 3. Reaction of *Nicotiana* accessions to target spot isolate of *Thanatephorus cucumeris*

Genotype name	Arcsine (%)		
	Seedling death	Target spot	Total incidence
TI 1605	0	21.4	21.4
<i>Nicotiana nudicaulis</i>	9	20.9	26.3
<i>N. africana</i>	22.6	12.5	28.7
<i>N. glutinosa</i>	27	14.7	34.1
<i>N. longiflora</i>	15.3	32.1	37.2
TN 90	10.2	34.5	37.6
<i>N. knightiana</i>	21.4	24.3	38.3
NC 2002	7.3	37.1	39.3
TI 1524	5.1	34.6	39.7
<i>N. debneyi</i>	12.4	31.7	40.5
TI 819	10.3	38.1	41.6
Clay 402	23.7	32	42.7
Burley 21	9	34.2	43.2
<i>N. repanda</i>	22.9	32.1	45.1
TI 1449	10.3	43.2	45.7
TI 186	23.7	35.6	45.9
TI 1734	19.9	39.1	46.2
L8	19.2	40.3	47.6
TI 165	12.4	44	47.6
NC 4	13.2	41.8	48.5
TI 716	0	48.5	48.5
<i>N. rotundifolia</i>	14.6	43.6	48.7
Ky 14	17.6	44.3	49.6
TI 1029	17.5	44.7	49.9
<i>N. alata</i>	14.1	45.6	50.6
R7-11	14.6	44.8	50.8
<i>N. sylvestris</i>	23.7	41.5	51.5
TI 428	24.8	44.8	52.7
Pennleaf 1	22.9	44.5	52.8
TI 672	19.6	46.2	52.9
TI 1569	5.1	52.2	53.4
<i>N. tomentosiformis</i>	40.8	22.7	54.4
<i>N. rustica</i>	30.9	33.5	54.5
TI 1555	5.1	53.7	54.9
TI 109	20.4	45.3	54.9
TI 1518	5.1	54.3	55.3
TI 835	23.7	46.3	55.5
NC 5	21.6	48.9	56.2
Speight 168	19.7	49.9	56.3
Tom Rosson Madole	15.3	52.9	56.6
TI 1279	17.5	52.1	56.9
TI 1269	7.3	55.2	57.6
TI 1316	0	58.3	58.3
MD 609	5.1	58.1	59.2
Pennbel 69	20.5	47.3	59.4
TI 501	17.6	54.9	59.4
TI 232	9	56.9	60.3
Havana 307	0	61	61
Havana 503	0	62.4	62.4
TI 1247	23.2	54.6	62.5
McNair 944	12.4	59.2	62.5
SA 1214	43.4	40.1	62.9
TI 158	30.1	48	63.2
TI 1330	10.2	61.2	63.3
TI 1512	7.3	62.5	64.6
Speight G28	24.2	54.5	64.7
Conn. Broadleaf	10.2	62.6	64.7
TI 1306	12.4	62.2	65.3
TI 119	26	53.5	65.5
TI 1500	17.5	60.6	65.7
TI 1222	0	65.8	65.8
TI 1558	5.1	64.8	65.8
TI 123	19.2	61.1	66.5
TI 1414	10.2	64.7	66.8
TI 1315	0	67.9	67.9
TI 79	5.1	66.9	67.9
TI 1241	12.4	65.2	68.3
TI 1462	10.2	66.8	68.9
TI 1451	22	61.4	69
<i>N. velutina</i>	24.1	55.3	69.4

(continued on next page)

^a Least significant difference.

^b Coefficient of variation.

Table 3. (continued from preceding page)

Genotype name	Arcsine (%)		
	Seedling death	Target spot	Total incidence
TI 1589	0	70	70
Kavala	0	70.5	70.5
TI 1224	7.3	69	71.1
<i>N. langsdorffii</i>	22.6	63.1	71.1
TI 1616	19.2	67.7	73
Speight G70	0	73.1	73.1
TI 1379	7.3	71.2	73.3
Coker 371 Gold	26	65.7	75.1
NC 71	17.5	71.8	76.2
Xanthi	12.4	73.2	76.4
Florida 301	10.2	74.3	76.9
TI 1577	12.4	75.4	78.5
K 346	20.6	72.2	78.6
TI 88	29.7	69.7	80
Hicks	25.1	71.8	80.7
TI 1311	28.8	70.5	83
K 326	10.2	77.7	83.8
Samsun	45.4	63.6	84.9
TI 1480	27.4	77.4	85.5
NC 297	12.4	83.5	86.6
TI 1280	12.5	83.8	87
NC 95	7.7	88.6	96.3
NC 2326	10.2	91.9	98.1
NC 72	12.4	95	98.2
Speight H20	23.8	89.4	113.2
OX 414	24.8	112.2	127.9
TI 395	15.3	137.8	149.7
Mean	15.1	55.9	62.4
LSD (0.05) ^a	21.2	23.3	22.8
CV (%) ^b	100.1	29.6	26

Table 4. Comparison of selected genotypes infected with target spot in a high-humidity environment

Genotype	Class ^a	Seedling death ^b	Target spot ^b	Total incidence ^b	Lesion area per plant (cm ²)
TI 1605	l	7.0	17.0	20.0	0.01
NC 2002	l	30.6	6.9	32.4	0.02
NC 2326	h	34.9	13.7	38.5	0.02
TN 90	l	32.8	12.1	38.8	0.06
TI 1311	h	35.0	20.9	41.7	0.08
NC 95	h	34.9	24.1	43.1	0.35
K 326	h	23.2	32.5	43.6	0.27
Burley 21	l	39.6	16.6	44.7	0.10
NC 4	l	35.6	29.2	47.2	0.18
TI 819	l	37.0	24.1	47.9	0.09
NC 72	h	32.9	23.4	48.4	0.19
Hicks	h	37.0	32.4	51.6	0.52
K 346	h	47.7	24.3	58.5	0.06
TI 1280	h	36.4	43.4	58.8	0.29
KY 14	l	55.4	9.8	59.7	0.01
L8	l	56.0	16.6	60.2	0.19
Speight H-20	h	45.6	35.9	62.0	0.28
TI 1316	m	34.0	47.0	65.0	0.72
TI 1480	h	55.8	25.6	66.4	0.18
OX 414	h	55.6	40.5	74.2	0.44
LSD (0.05) ^c	...	23.0	NS	NS	NS

^a Initial classification. Genotypes with high (h) and low (l) disease incidence in initial experiment.

^b Data shown is arcsine-transformed percentage of disease incidence.

^c Least significant difference; NS = no significant difference.

most of the flue-cured cultivars had a high disease incidence.

The target spot experiment also was repeated with several accessions that had the highest and lowest disease incidence (Table 4). Because the leaf area affected by target spot lesions was small, the experiment was modified to provide a high-humidity environment that was thought to increase the development of target spot. However, this environment

was very favorable for disease development and resulted in a higher frequency of seedling death. In this environment, there was a significant difference between cultivars for seedling death, with TI 1605 exhibiting significantly less seedling death than almost all of the other accessions. There were no significant differences for target spot incidence, total incidence, or lesion area per plant in this experiment.

Table 5. Field observations of target spot severity in burley and flue-cured cultivars

Type, cultivar	Leaf area affected (%)	Standard deviation
Burley		
NC 2002	30.0	0.0
TN 90	32.5	5.0
NC 5	32.5	5.0
Flue-cured		
K 326	36.7	8.2
K 346	43.3	5.0
NC 71	40.0	10.0

The lower incidence of target spot symptoms in seedlings of burley cultivars that was observed in the first experiment was not confirmed in the second growth-chamber experiment. However, an opportunity to compare target spot symptoms in mature field-grown plants occurred in 2005, when a heavy target spot outbreak was observed at the Border Belt Tobacco Research Station in Whiteville, NC (Table 5). Official variety tests for flue-cured and burley cultivars had been planted in adjacent plots, and six cultivars in this test also had been tested in the growth-chamber experiment. Although the burley cultivars had a slightly lower level of leaf area affected, this level was not substantially different compared with the flue-cured cultivars.

DISCUSSION

Significant differences in disease incidence and AUDPC were observed among tobacco genotypes evaluated for resistance to stem rot. Variation in disease development required that accessions be tested several times to identify genotypes that were consistently resistant. Several accessions, including TI 1605, TI 1029, TI 1311, and 'Xanthi,' consistently had low disease incidence in multiple tests. Among U.S. cultivars, the burley cvs. Burley 21 and Ky 14 had a low average disease incidence, but this was not consistently observed in repeated tests. In contrast, the flue-cured cvs. Hicks and NC 72 had a high incidence of disease.

Partial resistance to target spot was not consistently identified in these tests. Although there were significant genotype differences in seedling death, target spot, and total incidence in the initial observations, these differences were not observed when the test was conducted under a high-humidity environment designed to increase disease severity. Although the development of target spot lesions was increased, this environment also substantially increased seedling death. One genotype, TI 1605, had a significantly lower incidence of seedling death than almost all of the other accessions. TI 1605 also was one the genotypes that was most resistant to the stem rot isolate.

Based on the initial growth-chamber experiment, it appeared that several burley

cultivars had a significantly lower target spot disease incidence than flue-cured cultivars. However, this observation was not confirmed in the second growth-chamber experiment or in a natural field infection. Shew and Melton noted that differences in target spot disease severity have been observed among different genotypes in field and growth-chamber tests, but that this level of resistance was not high enough to provide control of the disease when environmental conditions were favorable (17). The results from the second growth-chamber experiment appear to confirm this observation. Csinos and Stephenson reported that several commonly used flue cultivars, including NC 71, NC 72, K326, Coker 371-Gold, Speight G-70, and Speight G-168, had low to moderate disease levels in a natural field epidemic compared with several breeding lines that had not yet been accepted as cultivars (3). These cultivars did not appear to have resistance to target spot in the growth-chamber experiments or in the field epidemic we observed. However, the breeding lines that were present in their test were not available for our experiment, and may represent highly susceptible genotypes.

It also is possible that there are differences between the North Carolina and Georgia target spot populations which might account for the lower disease levels observed in the Georgia epidemic. However, genetic diversity in tobacco target spot isolates collected throughout North Carolina has been examined by comparison of molecular marker profiles and somatic compatibility (2). This study showed that the tobacco isolates had lower diversity compared with isolates from potato, and that four clones represented 22% of the total population. Two of these clones were recovered in more than one field in widely separated (by 220 km) counties. The relatively low diversity of tobacco isolates over a widespread area in North Carolina suggests that target spot populations across the southeastern tobacco-growing region are similar.

Partial resistance to *R. solani* diseases has been described in several crops, and resistance often is expressed in the form of a reduction in disease severity or rate of infection. Two dominant major resistance

genes have been identified as conferring partial resistance to *R. solani* in rice (10). In peanut, it has been suggested that some genotypes may have partial resistance due to an erect growth habit that allows more rapid leaf drying and reduced soil contact. However, resistance also has been identified in genotypes with a spreading growth habit and, for these genotypes, it was hypothesized that differences in resistance were due to physiological or biochemical responses (4). In our study, all of the seedlings were grown in a high-density hydroponic environment; therefore, the resistance observed could not be explained by differences in plant architecture.

This study identified genotypes with partial resistance to damping-off and stem rot caused by an AG-4 stem rot isolate of *R. solani* but did not identify resistance to foliar symptoms caused by an AG-3 target spot isolate. Of particular interest is the accession TI 1605, which appears to be resistant to damping-off and stem rot symptoms caused by both isolates. The accessions identified in this work may be useful as sources of disease resistance to *R. solani* in tobacco-breeding programs. In addition, there are a large number of accessions that could be tested in the future.

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